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Morpho-anatomical adaptations of *Schoenoplectus lacustris* (L.) Palla along the salinity gradients in Sargodha, Pakistan

Muhammad Hassan*
Department of Botany, University of Sargodha, Sargodha, Pakistan
Email: muhammadhassanmalik0786@gmail.com & muhammad852hassan@gmail.com

Muhammad Imran
Department of Botany, University of Sargodha, Sargodha, Pakistan
Email: imran.yousaf@uos.edu.pk

Iqra Qamer
Department of Botany, University of Sargodha, Sargodha, Pakistan
Email: iqraqamar019@gmail.com

Moattar Zaman
Department of Botany, University of Sargodha, Sargodha, Pakistan
Email: moattarzaman39@gmail.com

Aneeqa Ali
Department of Botany, University of Sargodha, Sargodha, Pakistan
Email: aneeqaali2000@gmail.com

Badar Mujahid
Department of Botany, University of Sargodha, Sargodha, Pakistan
Email: badarmujahid7@gmail.com

Rubbab Zahra
Department of Botany, University of Sargodha, Sargodha, Pakistan
Email: rubabzahra2018@gmail.com

ABSTRACT

بائیوٹک اور اے۔بائیوٹک سٹریسز پودوں کو اندرونی اور بیرونی تبدیلیاں لانے پر مجبور کرتی ہیں۔یہ تحقیقی کام شینو پلکٹس لکسٹرس کا سالٹ سٹریس کے حوالے سے اندرونی اور بیرونی تبدیلیوں کا مطالعہ کرنے کے لیے کیا گیا۔اس تحقیق میں کئی ساختیاتی تبدیلیوں کا مطالعہ کیا گیا۔نتائج سے پتہ چلا کہ یہ پودا زیادہ نمکین حالات میں بہترین نشوونما کرتا ہے۔اس کی لمبائی، بائیوماس، جڑ اور پتے زیادہ نمکین مٹی میں بڑھ جاتے ہیں۔ساختیاتی تبدیلیوں میں تنے کا کم قُطری رقبہ، موٹی اینڈوڈرمس، بڑی زائلم نالیاں، بڑے پیرنکائما خلیات اور زیادہ ایرنکائما شامل ہیں۔اس کی جڑیں، تنے اور پتے نمک کے مطابق ڈھل جاتے ہیں، یہ پاکستان کی نمکین زمینوں کو قابل کاشت بنانے کے لیے ایک موزوں امیدوار ہے۔

*Correspondence to: muhammadhassanmalik0786@gmail.com

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Abstract

Salinity is a critical environmental stressor that significantly impairs plant productivity, rendering approximately 800 million hectares of agricultural land globally unfit for cultivation. This study investigates the morpho-anatomical adaptations of *Schoenoplectus lacustris (L.) Palla*, a halophytic sedge from the Cyperaceae family, which dominates the saline wetlands of the Sargodha region. This research represents a novel approach, as such an investigation had not been previously conducted for this species. Plant samples of *Schoenoplectus lacustris* were collected from natural habitats exhibiting varying levels of salinity. The corresponding soil samples were categorized into highly saline, moderately saline, and less saline based on their physicochemical properties. The species demonstrated optimal growth under highly saline conditions, underscoring its potential as a highly efficient halophyte. Morphological parameters, including plant height, shoot and root biomass (fresh and dry weight), root length, leaf length, and leaf area, were found to be highest in highly saline soils and lowest in less saline conditions. Anatomical adaptations of *Schoenoplectus lacustris* to salinity stress included reductions in stem cross-sectional area and modifications in root structure. These included increased endodermis thickness, broader xylem vessels, larger parenchymatous cells, and enhanced aerenchyma development. In addition, *Schoenoplectus lacustris* exhibited complementary responses such as an increase in Epidermal cell area, reduced stomatal density, intensified Sclerification, enlarged Vascular bundle, Metaxylem, Phloem, and Bulliform cell areas. These morpho-anatomical modifications enable *Schoenoplectus lacustris* to thrive under hypersaline conditions, making it a promising candidate for the rehabilitation of saline soils in Pakistan for both food and fodder production.

Keywords: *Schoenoplectus lacustris*, Morpho-anatomical plasticity, Salinity stress, Sedge, Cyperaceae, Salt tolerance mechanism

Introduction

Modern society is confronted with numerous global challenges, among which soil salinity stands out as a critical concern. This issue significantly hampers agricultural productivity by adversely impacting crop growth and yield (El-Ramady et al., 2024). Elevated soil salinity profoundly influences plant development and growth (Zhao et al., 2024). The ability of plants to tolerate salinity represents a highly complex process encompassing a multitude of modifications across various biological scales, including molecular, organelle, cellular, tissue, and whole-plant levels. Moreover, halophytic species exhibit diverse adaptive strategies, which extend beyond morphological and structural traits to encompass metabolic and physiological adjustments, thereby equipping them to endure and thrive in severely saline conditions (Hameed et al., 2010). Sedges, commonly found in swamps and marshes, are frequently exposed to significant physiological stress. The simultaneous occurrence of salinity and waterlogging poses a considerable challenge to plant growth, as the combined impact of these stressors is often more detrimental than individual stress factors. Consequently, sedges inhabiting saline and waterlogged environments have developed specialized adaptive strategies to withstand these harsh conditions (Ashraf, 2014; Mishra et al., 2016; Grigore & Toma, 2021). Rising salinity

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triggers significant anatomical modifications at both cellular and tissue levels (Kaleem et al., 2024). These morphological and anatomical adaptations in plant structures effectively mitigate the adverse impacts of salt stress (El Yamani & Cordovilla, 2024). Morpho-anatomical adaptations denote the specialized structural and anatomical tactics developed by halophytes to cope with saline environmental conditions (Iqbal et al., 2024; Leal, 2021). Among the notable anatomical adaptations are the pronounced succulence observed in roots, stems, and leaves, the presence of a multilayered epidermis, the development of specialized parenchymatous tissues for water storage, the substantial widening of the casparian strip, and the enhanced structural development of the root endodermis (Rančić et al., 2019; Zahedi et al., 2024) Salinity stress frequently induces an increase in root thickness, a physiological adaptation that may improve the plant's capacity to uptake water and nutrients under saline conditions (Srivastava, 2022). All these structural adaptations exhibit remarkable effectiveness in coping with extreme environmental stresses (Khan et al., 2024).

Among halophytic plants, members of the family Cyperaceae hold significant ecological importance due to their ability to thrive in a wide variety of habitats. These species are capable of growing in environments ranging from arid, barren soils to hyper-saline wetlands and acidic soils (Öztürk et al., 2023; Turcios et al., 2021). The genus *Schoenoplectus* (family Cyperaceae) is characterized by its halophytic species, which are well-adapted to hyper-saline environments.

In the Sargodha region, *Schoenoplectus lacustris (L.) Palla* is a dominant species within such habitats. This species, along with other members of the genus, demonstrates a range of physiological and morphological adaptations that facilitate survival in saline conditions, thereby playing a crucial role in the structure and function of the local ecosystem (Dagar & Gupta, 2024; Hassan et al., 2020). The investigation of salt-tolerant plant species has become a prominent research focus globally, driven by its importance for agricultural resilience and environmental sustainability in saline-affected regions (Zhang & Shi, 2013). Wetlands, particularly salt marshes, face continuous and significant threats due to their rapid degradation (Giuliani & Bellucci, 2019; White & Kaplan, 2017). Consequently, the species inhabiting the marshes are near to be extinct. It is imperative to explore new species, such as *Schoenoplectus lacustris (L.) Palla*, which remain unstudied for their anatomical adaptations in response to salinity tolerance. The research focused on examining the structural adaptations of *Schoenoplectus lacustris (L.) Palla* to diverse saline environments. Therefore, it was hypothesized that *Schoenoplectus lacustris (L.) Palla* may have evolved some specific anatomical traits to adapt to highly saline habitats, and that these adaptations enhance the plant's ability to tolerate saline conditions. Furthermore, the characterization of morphological and anatomical traits linked to salinity tolerance is instrumental in advancing the development of salt-tolerant crop species.

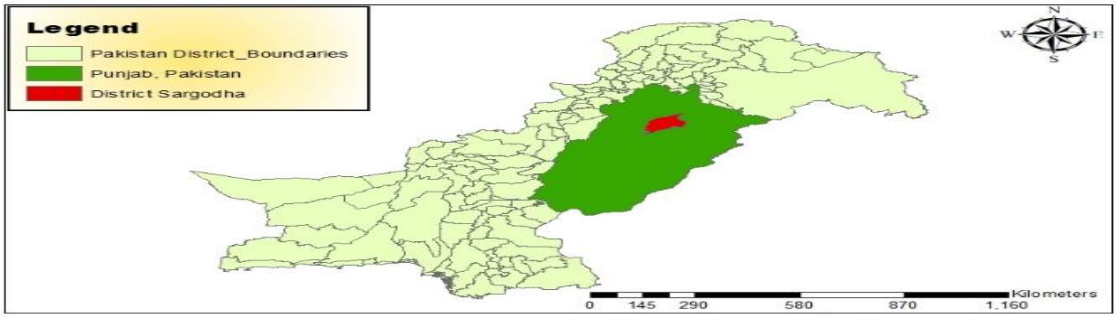
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Materials and methods

Halophytes are extraordinary plants that have evolved to flourish in highly saline environments. Through remarkable morphological and anatomical modifications, they possess the unique ability to withstand and complete their life cycles under conditions that prove inhospitable for the majority of plant species. To investigate these adaptive



1. Study area map of Sargodha, Pakistan

traits of *Schoenoplectus lacustris (L.) Palla*, populations were sampled from diverse habitats specially sedges dominating, i.e. marshes, stagnant waterbodies in the Sargodha region, Punjab Pakistan (fig.1.). These habitats spanned a gradient of salinity, ranging from lesser saline to hypersaline conditions and from Sargodha and its vicinities to border line of Sargodha with other district i.e. Khushab. Site*1 (Soil pH 7.5, ECe 32.8 (mScm-1), Organic matter (%) 1.09, Available Phosphorus 3.33 (mg Kg-1), Available Potassium 719.3 (mg Kg-1), Saturation (%) 48, Depth 16 (cm), Soil Texture: Clay Loam) was (Highly saline). Site*2 (Soil pH 9.0, ECe 8.81 (mScm-1), Organic matter (%) 1.39, Available Phosphorus 21.3 (mg Kg-1), Available Potassium 616 (mg Kg-1), Saturation (%) 47, Depth 16 (cm), Soil Texture: Clay Loam) was (Moderately saline). Site*3 (Soil pH 8.4, ECe 6.4 (mScm-1), Organic matter (%) 1.2, Available Phosphorus 19.4 (mg Kg-1), Available Potassium 1693 (mg Kg-1), Saturation (%) 48, Depth 16 (cm), Soil Texture: Loam) was (Less saline). Site*4 (Soil pH 8.3, ECe 3.78 (mScm-1), Organic matter (%) 0.67, Available Phosphorus 0.47 (mg Kg-1), Available Potassium 93.3 (mg Kg-1), Saturation (%) 20, Depth 16 (cm), Soil Texture: Sandy Loam) was (Less saline). Site*5 (Soil pH 7.87, ECe 3.69 (mScm-1), Organic matter (%) 0.90, Available Phosphorus 16.3 (mg Kg-1), Available Potassium 113 (mg Kg-1), Saturation (%) 23, Depth 16 (cm), Soil Texture: Loam) was (Less saline). Several field trips were conducted to collect samples from various salt-stressed habitats. Rhizospheric soil samples were also obtained from each collection site to conduct a comprehensive physicochemical analysis of the soil. The collected plant specimens were carefully transported from the field to the laboratory using bags, adhering strictly to the established methodologies outlined by (Smith & Chinnappa, 2015). A brief overview of the collection sites is presented in (Table 1). After being preserved and mounted, the plant specimens were deposited in the herbarium of Sargodha University. The identification of these specimens was carried out using the Flora of Pakistan (Kukkonen, 1990) and the Flora of China as key references.

Morphological traits such as shoot length (cm), shoot fresh weight, total plant fresh weight (g), total plant dry weight (g), plant height (cm), leaf area, leaf length (cm),

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leaf width, root length (cm), root fresh weight (g), and root dry weight (g) were carefully measured and recorded to ensure a comprehensive analysis of the specimens.

Double-stained permanent slides were carefully prepared to study anatomical features, employing the freehand sectioning technique. A 2 cm segment from the root-shoot junction of the thickest root was selected for anatomical analysis, while a 1 cm section from the midrib region of the leaf's center was chosen for examination. Initially, the specimens were preserved in FAA (formalin acetic alcohol) solution for 48 hours, comprising 5% formalin, 10% acetic acid, 50% ethanol, and 35% distilled water. For prolonged preservation, the samples were transferred to an acetic alcohol solution containing 25% acetic acid and 75% ethanol. Measurements were obtained using an ocular micrometer, and photographic documentation was facilitated through a microscope equipped with a Carl Zeiss camera.

The anatomical parameters analyzed for the stem including the stem cross-sectional area (mm^2), epidermal cell area (μm^2), sclerenchyma thickness (μm), vascular bundle area (μm^2), metaxylem area (μm^2), phloem area (μm^2), cortical cell area (μm^2), and stem aerenchyma cell area (μm^2). For leaf anatomy, the focus was on parameters such as upper epidermis thickness (μm), lower epidermis thickness (μm), vascular bundle area (μm^2), cortical cell area (μm^2), and metaxylem area (μm^2). In root anatomy, the studied parameters included root cross-sectional area (μm^2), epidermal cell area (μm^2), endodermis thickness (μm), aerenchyma thickness (μm), parenchyma cell area (μm^2), metaxylem cell area (μm^2), and the number of metaxylem vessels.

Statistical Analysis

From each study site, three plants were randomly chosen for anatomical analysis, serving as replicates. The data were analyzed using a one-way analysis of variance (ANOVA) under a completely randomized design, followed by Welch's test to assess differences. between means. Statistical processing was carried out with Statistix 10, while mean values were computed using MS Excel 2016. Results for each dataset were expressed as the mean (n=3) accompanied by the standard error.

Table 1. Collection sites of Schoenoplectus lacustris (L.) Palla along with habitat descriptors

Sites denotion	Sites name	Salinity level	Habitat descriptor
Site*1	Behak maken	Highly saline	Highly saline salt marsh dip, waterlogged area seasonally dried, near Tehsil Sahiwal of District Sargodha
Site*2	Jura Sakesar	Moderately saline	Moderately saline waterlogged area with stagnant waste water near Tehsil Bhalwal of District Sargodha.
Site*3	Nabi Shah Bala	Less saline	Road side stagnant waterlogge habitat near lakk morr, Tehsil and District Sargodha
Site*4	Wegowal	Less saline	Brackish water receiving effluents from factories, Tehsil Shahpur District Sargodha
Site*5	Khushab Lake, Punjab	Less saline	Khushab lake, waterlogged site at the border line between District Sargodha and Khushab District

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Results

Morphological traits:

Schoenoplectus lacustris (L.) exhibited a steady and progressive increase in shoot length and overall plant height with rising salinity levels across various sites. Notably, at higher salinity levels, these parameters showed a significant increase compared to the non-saline site. Among the locations studied, Behak Maken (Site*1), (70 cm) recorded the highest values, followed by Jura Sakesar (Site*2), (67.1 cm) and Nabi Shah Bala (Site*3), (59.99 cm). The fresh weight of shoot and whole plant in *Schoenoplectus lacustris* initially showed a significant and steady increase with rising salinity levels. However, beyond a certain threshold, further salinity increments led to a marked reduction in both parameters. Growth peaked in the moderately saline conditions of Jura Sakesar (Site*2), (6.04 g) but declined in the highly saline soils of Behak Maken (Site*1), (5.48 g), however least plant fresh weight was recorded for Khushab Lake, Punjab (Site*5), (3.35 g). A parallel response was shown for plant dry weight, that increased consistently up to moderately saline site, and then falls abruptly for highly saline site. In *Schoenoplectus lacustris*, a notable rise in leaf sheath length, width, and area was observed as salinity levels increased, progressing from the less saline Khushab Lake to the highly saline Behak Maken in Punjab. These parameters showed a consistent and gradual enhancement corresponding to the escalating salinity across distinct habitats. Root length exhibited a variable response to salinity levels. It increased with rising salinity up to moderately saline conditions but showed a slight decline in highly saline soils. While this reduction was not highly significant, it was noticeable. The longest root length was observed at Nabi Shah Bala (Site*3), followed by a decrease at B. Maken (Site*1). Root fresh and dry weights increased with rising salinity levels, peaking at Nabi Shah Bala (Site*3) before slightly declining under higher salt stress. The lowest measurements for both parameters were recorded at Khushab Lake, Punjab (Site*5).

Anatomical modifications:

Stem anatomical features:

A notable expansion in stem cross-sectional area was observed at Khushab Lake, Punjab (Site*5), followed by a similar increase at the Wegowal (Site*4). In contrast, a marked decline in this trait was recorded at Behak Maken (Site*1), while a slight increase was detected at Jura Sakesar (Site*2). The other sites displayed minimal variation in stem cross-sectional area. The study revealed a notable and consistent enlargement in the area of epidermal cells with rising salt concentrations across different locations. At Jura Sakesar (Site*2), this increase was particularly pronounced, whereas the smallest epidermal cell area was recorded at Khushab Lake, Punjab (Site*5). These findings highlight a clear positive relationship between salinity levels and the expansion of epidermal cell area. The thickest sclerenchyma was noted at the Wegowal (Site*4), whereas the thinnest was recorded at Behak Maken (Site*1) and Jura Sakesar. A modest increase in thickness was observed at Khushab Lake in Punjab (Site*5). The largest vascular bundle area was identified at the Wegowal (Site*4), whereas the smallest was recorded at Behak Maken (Site*1). A subsequent rise in vascular bundle area was noted at Khushab Lake, Punjab (Site*5). At lower

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salinity levels, an increase in vascular bundle area was observed, followed by a slight decline at Nabi Shah Bala (Site*3). The metaxylem area was notably larger at Khushab Lake, Punjab (Site*5), where it reached its maximum. In contrast, the smallest metaxylem area was recorded at Behak Maken (Site*1). Other sites exhibited intermediate values, reflecting moderate variations in metaxylem area across the locations. The smallest phloem area was observed at Behak Maken (Site*1), which gradually increased at Khushab Lake in Punjab (Site*5). The maximum phloem area was recorded at Jura Sakesar, with Nabi Shah Bala (Site*3) ranking second. Wegowal (Site*4) exhibited a moderate phloem area in comparison. In *Schoenoplectus lacustris (L.) Palla*, the cortical cell area showed a significant increase at Khushab Lake, Punjab (Site*5), with a slightly lower value observed at Nabi Shah Bala (Site*3). A moderate cortical cell area was measured at Wegowal (Site*4), while the smallest value was recorded at Behak Maken (Site*1), followed by a subsequent rise at Jura Sakesar (Site*2). In *Schoenoplectus lacustris (L.) Palla*, the stem aerenchyma cell area reached its highest value at Khushab Lake, Punjab (Site*5). The lowest value was recorded at Nabi Shah Bala (Site*3), which was statistically comparable to Behak Maken (Site*1). In contrast, the remaining study sites exhibited intermediate values for this trait.

Root anatomical modifications

The root traits of *Schoenoplectus lacustris (L.) Palla* were analyzed, focusing on key anatomical features: root cross-sectional area (μm²), epidermal cell size (μm²), endodermis thickness (μm), aerenchyma thickness (μm), parenchyma cell size (μm²), metaxylem cell size (μm²), and the number of metaxylem vessels.

All root anatomical traits were found to be statistically significant. A notable increase in root cross-sectional area was observed at Khushab Lake, Punjab (low salinity) (Site*5), with a subsequent decrease recorded at Wegowal (low salinity) (Site*4). The smallest root cross-sectional area was identified at Nabi Shah Bala (low salinity) (Site*3), which was followed by an increase at Behak Maken (high salinity) (Site*1). The epidermal cell area exhibited a consistent and notable increase with rising salinity levels across various locations. The largest cell area was observed in the moderately saline environment of Jura Sakesar (Site*2), while the smallest was recorded at the less saline Khushab Lake (Site*5). A comparable trend was noted in the endodermis thickness of *Schoenoplectus lacustris (L.) Palla*. The thickness showed a consistent and marked increase with rising salinity levels across various locations, ranging from the less saline Khushab Lake (Site*5) to the highly saline Behak Maken (Site*1). These variations were statistically significant. The greatest aerenchyma thickness was observed at the less saline Khushab Lake (Site*5), while the lowest was recorded at Jura Sakesar (Site*2), a site with moderate salinity. Intermediate values were found at the remaining study locations. The parenchyma cell area was found to increase progressively with rising salinity levels. The maximum cell area was observed at Behak Maken (Site*1), a highly saline site, while the minimum was recorded at the less saline Khushab Lake (Site*5). The largest metaxylem cell area was recorded at Wegowal (Site*4), a less saline site, while the smallest was observed at Khushab Lake(Site*5), which is also less saline. The other locations exhibited moderate values for this parameter, with a consistent number of vessels across all sites.

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Leaf sheath anatomical features

The study evaluated several anatomical features of the leaf sheath in *Schoenoplectus lacustris (L.) Palla*, including leaf lamina thickness, upper and lower epidermal cell thickness, sclerenchyma thickness, mesophyll cell thickness, and dimensions of vascular structures. Specifically, it measured vascular bundle area, metaxylem area, phloem area, bulliform cell area, and aerenchyma cell area.

The leaf sheath exhibited its greatest thickness at Jura Sakesar (Site*2), a moderately saline site. Moderate thickness was observed at Wegowal (Site*4), a less saline location, with a further decrease noted at Nabi Shah Bala (Site*3), another less saline site. The thinnest leaf lamina was recorded at Khushab Lake (Site*5), also characterized by lower salinity. These findings suggest a trend where leaf sheath thickness increases at less saline sites but diminishes in highly saline conditions. A notable reduction in the thickness of the upper epidermal cells was observed as salt levels increased across various locations. The thinnest cells were found at Khushab Lake (Site*5), (which has lower salinity), followed by a decrease at Wegowal (Site*4) (also less saline). The smallest cell thickness, however, was recorded at Behak Maken (Site*1), which is highly saline. The thickness of the lower epidermal cells exhibited a similar trend across different locations. The greatest thickness was observed at Khushab Lake (Site*5) (low salinity), followed by a slight reduction at Nabi Shah Bala (Site*3) (also low salinity). The lowest thickness was recorded at Behak Maken (Site*1), a highly saline site. The remaining locations displayed moderate values for this parameter. The highest sclerification was observed at Jura Sakesar (Site*2), a moderately saline site. The lowest sclerenchyma thickness was recorded at Khushab Lake, Punjab(Site*5), which has lower salinity. A moderate level of sclerification was noted at Behak Maken (Site*1), a highly saline location, with a slight reduction in thickness at Nabi Shah Bala (Site*3), another less saline area. The thickest mesophyll cells were observed at Jura Sakesar (Site*2) (moderately saline), while the thinnest cells were found at Behak Maken (Site*1) (highly saline). A moderate mesophyll cell thickness was noted at Wegowal (Site*4) (less saline), with a slight reduction at Nabi Shah Bala (Site*3) (less saline). The largest vascular bundle area was found at Nabi Shah Bala (Site*3) (low salinity), followed by a reduction at Jura Sakesar (Site*2) (moderate salinity). The smallest area was recorded at Behak Maken (Site*1) (high salinity). A moderate vascular bundle area was observed at Jura Sakesar (Site*2), with a decrease at Wegowal (Site*4) (low salinity). The highest meta xylem area was found at Wegowal (Site*4) (low salinity), while the lowest was at Khushab Lake(Site*5) (also low salinity). Other sites exhibited moderate values. A similar trend was observed for phloem area, with the largest value at Nabi Shah Bala (Site*3) (low salinity) and the smallest at Behak Maken (Site*1) (high salinity). The remaining sites showed moderate phloem area values. The bulliform cells, which exhibited the largest cell area, were most prominent at Jura Sakesar (Site*2) (moderately saline), followed by a reduction at Nabi Shah Bala (Site*3) (less saline), which was similar to the values at Behak Maken (Site*1) (highly saline). The smallest cell area was observed at Khushab Lake, Punjab (Site*5) (less saline). The remaining locations displayed intermediate values for this histological characteristic. A substantial increase in the area of aerenchyma cells was observed at Jura Sakesar (Site*2)

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(moderately saline), while a decline was noted at Nabi Shah Bala (Site*3) (less saline). In contrast, the leaf sheath of *Schoenoplectus lacustris (L.) Palla* collected from Khushab Lake, Punjab (Site*5) (less saline) exhibited the smallest value for this histological feature.

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Plant	Sites	S.L (cm)	P.H (cm)	S.F. wt. (g)	P.F. wt. (g)	P.D. wt. (g)	L.L (cm)	L.W (cm)	L.A (cm^2)	R.L (cm)	R.F. wt. (g)	R.D. wt. (g)
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
S. lacustris L. (palla)	**B.M**	79 ± 5.568	82.5 ± 5.568	3.98 ± 0.119	5.48 ± 0.119	3.48 ± 0.119	10.87 ± 0.296	0.86 ± 0.015	9.27 ± 0.12	19.52 ± 0.5	4.74 ± 0.145	3.54 ± 0.145
	J.S	67.1 ± 1.43	70.6 ± 1.43	4.54 ± 0.082	6.04 ± 0.082	4.04 ± 0.082	10.11 ± 0.757	0.6 ± 0.058	6.11 ± 0.85	18.41 ± 0.4	3.04 ± 0.37	1.84 ± 0.37
	N.S	59.99 ± 1.308	63.49 ± 1.308	3.49 ± 0.301	4.99 ± 0.301	2.99 ± 0.301	8.42 ± 0.334	0.62 ± 0.016	5.22 ± 0.20	20.12 ±0.44	4.85 ± 0.20	3.65 ± 0.20
	W.W	56 ± 2.082	59.5 ± 2.082	3.24 ± 0.352	4.74 ± 0.352	2.74 ± 0.352	7.94 ± 0.297	0.69 ± 0.009	5.43 ± 0.21	16.34 ± 0.4	2.36 ± 0.09	1.16 ± 0.09
	K.L	42.34 ± 1.453	45.84 ± 1.453	1.85 ± 0.144	3.35 ± 0.144	1.35 ± 0.144	6.07 ± 0.146	0.35 ± 0.029	2.4 ± 0.349	15.75 ± 0.4	2.22 ± 0.10	1.02 ± 0.10

Table 2 Mean ± SE values of morphological attributes of selected Schoenoplectus lacustris from distinct localities of Sargodha, Pakistan

"Where, B.M= Behak maken, L.M= Lak morr, W. W= Wegowal, K. L= Khushab Lake, Punjab, 71 N. B= 71 N.B Canal Road, N. P= Nawab Pur Road, J. S= Jura Sakesar and N. S= Nabi Shah Bala"

"SL = Shoot length, PH= Plant height, SF= Shoot fresh weight, PF= Plant fresh weight, PD= Plant dry weight, L.L= Leaf length, L.W= Leaf width, L.A= Leaf area, R.L= Root length, RF= Root fresh weight, RD= Root dry weight, SCA= Stem cross-sectional area, ECA= Epidermal cell area, ST= Sclerenchyma thickness, VB= Vascular bundle, MXA= Metaxylem cell area, PA= Phloem area, CCA= Cortical cell area, SA= S. Aerenchyma. "

Plants	Sites	S.C.A (mm^2)	E.C.A (μm^2)	S.T (μm^2)	V.B. (μm2)	M.X.A (μm^2)	P.A (μm^2)	C.C.A (μm^2)	S.A. (μm^2)
S. lacustris (stem)	**B.M**	6.17 ± 0.088	754.33± 2.33	26.34± 0.88	18002 ± 1.201	1054 ± 2.081	3004 ± 2.645	3005.3 ± 2.728	75000 ± 1.666
	J.S	6.47 ± 0.033	995 ± 1.732	31.67± 1.76	28802 ± 1.452	1401.7± 10.1	9003 ± 1.527	4503.3 ± 2.027	133400 ± 0.00
	N.S	7.23 ± 0.095	442.67± 1.45	35 ± 1.527	29402 ± 1.66	2028.7 ± 1.8	6303.3 ± 2.4	7203.7 ± 2.33	75000 ± 1.667
	W.W	7.26 ± 0.075	154 ± 2.081	40.34 ± 1.2	39200 ± 0.333	2000 ± 0.00	5403.3 ± 2.0	7203.7 ± 2.34	252000 ± 0.00
	K.L	7.37 ± 0.068	130 ± 1.732	32.34 ± 1.4	30800 ± 0.00	3575 ± 0.00	4802.3 ± 1.4	9003.3 ± 1.66	671500 ± 0.00

Table 3 Mean ± SE values for stem anatomical values of S. lacustris collected from distinct habitats of Sargodha, Pakistan

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Table 4 Mean values for root anatomical values of S. lacustris collected from distinct habitats of Sargodha, Pakistan

Plants	Sites	R.C.A (mm2)	E.C.A (µm2)	E.T (µm2)	A.T (µm2)	P.A (µm2)	M.A (µm2)	M.V.N (µm2)
S. lacustris (root)	B.M	977517 ± 8.819	92.34 ± 1.452	43 ± 1.732	200.33 ± 0.881	1052.7 ± 1.33	1563 ± 1.5275	1.00 ± 0.00
	J.S	990017 ± 8.819	108.67 ± 1.763	34.34 ± 0.881	147 ± 1.527	902.67 ± 1.452	1681.3 ± 0.881	1.00 ± 0.00
	N.S	532518 ± 9.279	70 ± 2.886	30 ± 0.577	233 ± 0.527	645.33 ± 0.881	931.67 ± 0.881	1.00 ± 0.00
	W.W	1016000 ± 11.68	72 ± 0.577	22.34 ± 1.452	203.33 ± 2.02	502 ± 1.527	2752.7 ± 1.763	1.00 ± 0.00
	K.L	1540000 ± 10.83	32.67 ± 1.452	14.67 ± 0.881	506 ± 2.081	397 ± 0.577	661.67 ± 0.881	1.00 ± 0.00

"Where, B.M= Behak maken, L.M= Lak morr, W. W= Wegowal, K. L= Khushab Lake, Punjab, 71 N. B= 71 N.B Canal Road, N. P= Nawab Pur Road, J. S= Jura Sakesar and N. S= Nabi Shah Bala"
" RCA= Root cross-sectional area, ECA= Epidermal cell size, ET= Endodermis thickness, AT= Aerenchyma thickness, PA= Phloem area, MA= Metaxylem area, MVN= Metaxylem vessel number".

Table 5 Mean values for leaf sheath anatomical values of S. lacustris collected from distinct habitats of Sargodha, Pakistan

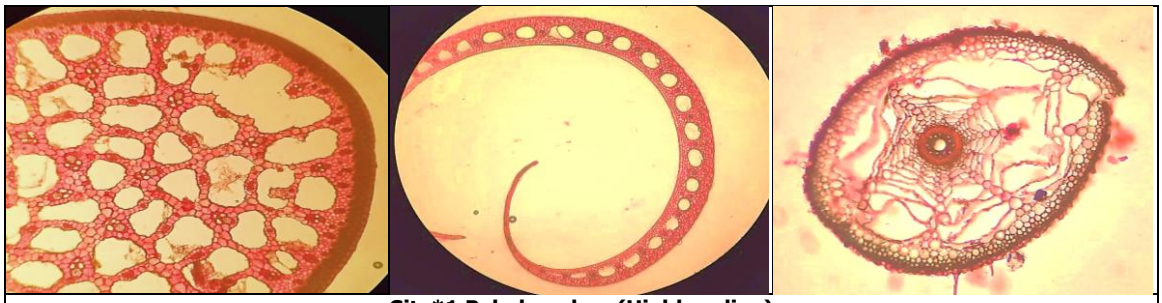
Plants	Sites	L.S.T (mm^2)	U.E.T (μm^2)	L.E.T (μm^2)	S.T (μm2)	M.T (μm^2)	V.B.A (μm^2)	M.X.A (μm^2)	P.A (μm^2)	B.C.A (μm^2)	A.C.A (μm^2)
Schoenoplectus lacustris (leaf)	B.M	345 ± 2.88	8 ± 0.577	16 ± 0.577	38 ± 1.154	24.67± 0.8	7704 ± 2.3	74 ± 1.15	721.3 ± 1.33	563 ± 1.527	31500 ± 2.33
	J.S	705 ± 2.64	13.5 ± 0.2	20.67± 1.2	47.67 ± 1.4	74.34± 0.6	12105± 2.4	122.67± 1.4	873.3 ± 0.66	662.67 ± 1.45	200000 ± 0.0
	N.S	575 ± 2.51	14.67± 0.8	32.67± 1.4	34.34 ± 0.8	37.67± 0.8	14404± 2.3	126.33 ±1.4	1052.3 ± 1.20	563.67 ± 2.02	83200 ± 2.18
	W.W	601.3± 0.8	17 ± 0.577	32 ± 1.00	28 ± 0.577	39 ± 0.57	11704± 2.6	135.67± 0.6	961.3 ± 0.88	415.33 ± 0.88	37500 ± 2.33
	K.L	291 ± 1.00	22 ± 0.577	35.67± 1.2	23.34 ± 0.8	51.67± 0.3	9754 ± 2.3	18.33 ± 0.8	855 ± 2.88	239 ± 0.577	15600 ± 1.15

"Where, B.M= Behak maken, L.M= Lak morr, W. W= Wegowal, K. L= Khushab Lake, Punjab, 71 N. B= 71 N.B Canal Road, N. P= Nawab Pur Road, J. S= Jura Sakesar and N. S= Nabi Shah Bala"
"LST= Leaf sheath thickness, UET= Upper epidermis thickness, LET= Lower epidermis thickness, ST= Sclerenchyma thickness, MT= Mesophyll thickness, VBA= Vascular bundle area, PA= Phloem."

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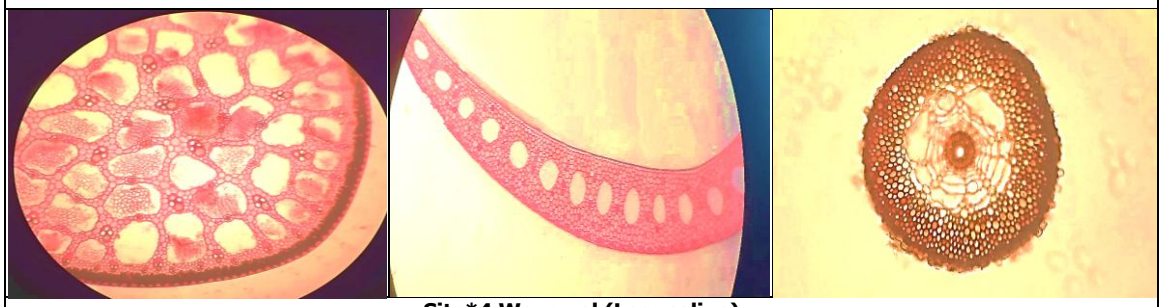
Site*1 Behak maken (Highly saline)



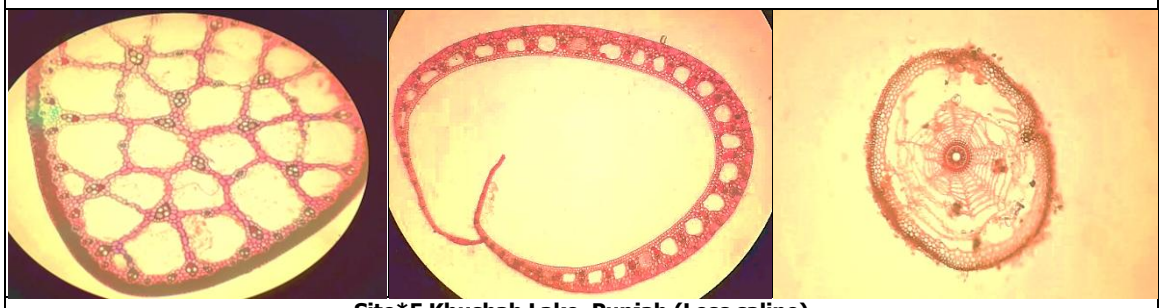
Site*2 Jura Sakesar (Moderately saline)



Site*3 Nabi Shah Bala (Less saline)



Site*4 Wegowal (Less saline)



Site*5 Khushab Lake, Punjab (Less saline)

Stem	Leaf sheath	Root

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One-way ANOVA (CRD Design)

Table 6 Stem Anatomical parameters of Schoenoplectus lacustris (L.) Palla

Anatomical parameters	Mean squares	Error	P_value
Stem cross sectional area (mm2)	0.87553***	0.01709	0.0000
Epidermal cell area (µm2)	427124***	11	0.0000
Sclerenchyma thickness (µm)	78.2667***	5.8667	0.0005
Vascular bundle area (µm2)	1.711E+08***	3.86667	0.0000
Meta xylem area (µm2)	2800246***	66	0.0000
Phloem area (µm2)	1.458E+07***	12.8000	0.0000
Cortical cell area (µm2)	1.722E+07***	15.1333	0.0000
Stem aerenchyma cell area (µm2)	1.891E+11***	3.33333	0.0000

*=ns, **= significant, ***= highly significant

Table 7 Root Anatomical parameters of Schoenoplectus lacustris (L.) Palla

Anatomical parameters	Mean squares	Error	P_value
Root cross sectional area (µm2)	3.828E+11***	297.333	0.0000
Epidermal cell area (µm2)	2444.93***	9.60	0.0000
Endodermis thickness (µm)	356.433***	4.200	0.0000
Aerenchyma thickness (µm)	60572.9***	8.3	0.0000
Parenchyma cell area (µm2)	224587***	4.0	0.0000
Metaxylem cell area (µm2)	1972649***	5.0	0.0000
Metaxylem vessel number	0.00000***	0.00000	0.0000

*=ns, **= significant, ***= highly significant

Table 8 Leaf sheath Anatomical parameters of Schoenoplectus lacustris (L.) Palla

Anatomical parameters	Mean squares	Error	P_value
Leaf lamina thickness (µm)	94173.3***	14.1	0.0000
Upper epidermal cell thickness (µm)	78.2667***	1.1167	0.0000
Lower epidermal cell thickness (µm)	219.400***	3.800	0.0000
Sclerenchyma thickness (µm)	264.233***	3.200	0.0000
Mesophyll cell thickness (µm)	1055.27***	1.47	0.0000
Vascular bundle area (µm2)	1.922E+07***	17.4000	0.0000
Meta xylem area (µm2)	7289.23***	4.07	0.0000
Phloem area (µm2)	46017.2***	7.7	0.0000
Bulliform cell area (µm2)	81853.2***	5.8	0.0000
Aerenchyma cell area (µm2)	1.688E+10***	10.2000	0.0000

*=ns, **= significant, ***= highly significant

Discussion

Plants in their natural environments are continuously exposed to a variety of biotic and abiotic challenges (Syvertsen & Garcia-Sanchez, 2014). Among these, soil salinity stands out as a critical environmental stressor, significantly hindering plant growth and agricultural productivity (Zhao et al., 2020). This issue is widespread, affecting both irrigated lands and non-irrigated agricultural regions, as well as rangelands across the globe (Pessarakli & Szabolcs, 2019). The effects of salinity on plants are profound, manifesting as inhibited growth and development. This is often reflected in reduced root and shoot lengths, lower biomass accumulation, and altered morphological, anatomical, and physiological traits (Mbarki et al., 2018). A diverse

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range of ecological sites was carefully selected for the collection of sedge specimens. These sites were categorized into three salinity levels—high, moderate, and low—based on the outcomes of soil physicochemical analysis. Parameters such as soil electrical conductivity (ECe, mS cm^{-1}), pH, organic matter content (%), available phosphorus (mg kg^{-1}), available potassium (mg kg^{-1}), soil saturation (%), texture, and other key edaphic factors were systematically recorded.

According to established criteria, soils with an ECe of 2.1 to 4.0 dS m^{-1} are considered mildly saline, while those ranging from 4.0 to 8.0 dS m^{-1} fall into the moderately saline category. Highly saline soils exhibit an ECe of 8.1 to 16.0 dS m^{-1}, and soils exceeding 16.0 dS m^{-1} are classified as extremely saline. For reference, the salinity threshold for halophytes is approximately 200 mM NaCl, corresponding to an ECe of around 20 dS m^{-1} (Ievinsh, 2023; Santos et al., 2016).

In *Schoenoplectus lacustris (L.) Palla*, moderate salinity in the soil was found to enhance growth and other morphological characteristics. However, exposure to highly saline conditions significantly suppressed these traits, leading to a marked reduction in overall plant development. All species within the *Schoenoplectus* genus demonstrated a notable capacity to tolerate moderate salinity stress (up to 200 mM NaCl), a trait commonly observed in halophytic or salt-tolerant plants. These species are able to incorporate potentially harmful ions, such as Na+ and Cl−, into their metabolic processes, thereby promoting growth, particularly under conditions of low to moderate salinity (Hameed et al., 2014). Similar findings have been reported in other studies. For instance, Bayuelo-Jiménez et al. (2002) evaluated salinity tolerance during germination and early seedling development in 24 accessions of four wild Phaseolus species, concluding that salinity stress inhibited germination to varying degrees across accessions.

Salinity's effects on plant growth, especially at low to moderate levels, are primarily osmotic in nature. While root systems may experience reductions in both length and volume under such conditions, they often adjust morphologically, becoming either thinner or thicker depending on the species. The rate of plant maturation may also shift—delayed or accelerated—based on salinity levels and plant species (Shannon & Grieve, 1998). Notably, salinity-induced growth reductions vary widely among plant species and even between different variants of the same species. Shoot biomass is generally more sensitive to salinity stress than root biomass, resulting in more pronounced limitations on above-ground growth and development. This disparity is thought to arise from the roots' greater capacity for osmotic regulation under saline conditions (Wang et al., 2020).

In the current study, the shoot length and biomass of *Schoenoplectus lacustris* were significantly reduced at the highly saline B. Maken site. Conversely, root length and biomass showed a notable increase under these same conditions, highlighting an adaptive response to elevated salinity levels. Environmental factors, such as high salinity in the growth medium, can profoundly affect plant development. While reduced growth may seem disadvantageous, it is a crucial survival strategy for halophytic plants, as prioritizing survival over rapid growth enhances resilience under stress (Nadeem et al., 2013). A related study on the halophyte *Phragmites australis* revealed that increased salinity significantly reduced both shoot and root dry weight

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(Gorai et al., 2011). Morpho-anatomical characteristics are analyzed with the understanding that a plant's resilience to drought and salinity depends significantly on its ability to adapt to a variety of environmental stresses (De Micco & Aronne, 2012). Salt-tolerant species, in particular, display a range of adaptations across morphological, anatomical, metabolic, and physiological domains. These morpho-anatomical modifications include xeromorphic features such as a thicker epidermis, the presence of sclerenchyma, well-developed bulliform cells, increased trichome density, and the capacity for higher moisture retention achieved through larger cell sizes and expanded vacuoles. Additionally, the development of specialized excretory structures, such as vesicle hairs and salt glands, plays a critical role in facilitating salt tolerance. From a physiological perspective, these plants demonstrate improved ion selectivity, increased tissue succulence, effective osmotic adjustment, and mechanisms to exclude toxic ions from cellular systems (Hameed et al., 2010). The presence of extensive sclerification within the root cortex enhances mechanical strength and reduces radial water loss, contributing to the plant's structural integrity (Mumtaz et al., 2021). Anatomical analysis of *Cyperus haspans* stems revealed a marked increase in sclerenchyma tissue. Additionally, a thinner epidermal layer in the stem was observed to promote water evaporation, facilitating the establishment of a concentration gradient that helps manage water retention in saline soils. In contrast, other species of *Cyperus* lacking these stem modifications exhibited more restricted development. In *Schoenoplectus lacustris (L.) Palla*, an increase in stem cortical cell area was noted with rising salinity levels in moderately saline soils, although this trend reversed in highly saline conditions. The larger cortical cells, often equipped with expansive vacuoles, demonstrated an improved capacity for water storage (Nawaz et al., 2020). Aerenchyma tissue development in plants exposed to saline and waterlogged conditions typically forms a continuous channel extending from the roots to the stem. This structure plays a crucial role in oxygen transport, enabling plant survival in anaerobic and saline waterlogged environments (Shimamura et al., 2010; Singh et al., 2018). In the current study, all observed sedge species exhibited a notable increase in aerenchyma cell formation under such conditions. These findings underscore the critical importance of aerenchyma in the growth, development, and survival of *Schoenoplectus lacustris (L.) Palla*. This tissue not only aids in oxygen diffusion but also contributes to salt transport and the sequestration of harmful ions in saline habitats (Kaleem et al., 2022).

In *Schoenoplectus lacustris (L.) Palla*, the vascular bundle area was observed to diminish significantly in soils with high salinity levels. Additionally, under conditions of limited soil moisture, vascular tissue tends to reduce in size. This adjustment appears to function as an adaptive strategy, enabling the plant to better manage the combined stresses of salinity and drought, a phenomenon supported by research findings (Acosta-Motos et al., 2017; Iqbal et al., 2020). This study revealed key insights into the anatomical responses of sedge leaf sheaths under varying salinity conditions. The epidermal cell area exhibited a variable pattern, increasing as salinity levels rose up to moderately saline soils, followed by a decline under extreme salinity conditions. These findings align with previous observations reported by Kataria and Verma (2018). In *Schoenoplectus lacustris (L.) Palla*, significant reductions were observed in the vascular bundle area, metaxylem area, and phloem area, particularly

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in highly saline environments. These results corroborate those documented by Kaleem et al. (2022). Similarly, the cortical cell area of the leaf sheath demonstrated a comparable trend, with a marked increase at lower salinity levels, followed by a notable decrease under extreme salinity stress. This decline in cortical cell area under high salinity is consistent with earlier findings by Evert (2006).

Root cross-sectional area demonstrated a significant increase with rising salinity levels, reaching its peak in moderately saline soil. However, a decline was observed in highly saline conditions. This trend aligns with findings by Bahaji et al. (2002), who identified an expanded root area as a characteristic trait of plants with moderate salt tolerance. Similar observations were reported by Batool and Hameed (2013).

The enlargement of root cross-sectional area is believed to play a critical role in optimizing water retention and utilization under saline stress (Niknam & McComb, 2000). Additionally, the study highlighted a notable increase in root epidermal cell area as salinity progressed from low to moderate levels. This layer serves a dual function, providing structural protection while facilitating the regulated transport of water and nutrients through the root system (Naz et al., 2014). The thickness of the endodermis also exhibited a consistent increase across all three ecotypes, mirroring the pattern observed in the epidermal cell area. Furthermore, the phloem and metaxylem areas expanded significantly in the most salt-tolerant sedge species as salinity levels rose. This vascular expansion, particularly in xylem vessels, enhances the plant's ability to transport water and nutrients to aerial tissues, a vital adaptation during physiological droughts induced by salinity. Notably, an enlarged xylem vessel area has been directly linked to improved root hydraulic conductivity (Vercambre et al., 2002). Plants growing in saline, waterlogged environments exhibit a distinctive adaptation in the form of root aerenchyma cells. In *Schoenoplectus lacustris (L.)*, a remarkable and consistent increase in the root aerenchyma cell area was observed as soil salinity levels rose, with the largest increase occurring under highly saline conditions, highlighting the species' exceptional tolerance.

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