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# Structural and Functional Determinants of Physiological Pliability in *Kyllinga brevifolia* Rottb. for Survival in Hyper-Saline Saltmarshes

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**Abstract** The role of morpho-anatomical adaptations of six *Kyllinga brevifolia* populations in successfully invading hyper-saline environments was investigated. Physiological and anatomical characteristics showed a high degree of plasticity indicating its adaptability potential to a variety of environmental conditions. The population from hyper-saline saltmarsh Sahianwala was exposed to physiological drought for a long time and its survival relied on the prevention of water loss attained by decreased stomatal density and area, lignin deposition in the inner and outer cortical region, especially outside vascular tissue. Larger cells of cortical storage parenchyma aided in water storage and wide metaxylem vessels in better conduction of solutes. Higher accumulation of shoot $Ca^{2+}$ in this habitat protected neutralized the impact of the enhanced shoot and root $Na^{+}$ ion uptake. Organic osmoprotectants like total free amino acid, proline, soluble proteins, and sugars accumulated in a higher quantity that contributed towards an osmotic adjustment in Sahianwala population. Population from seasonal inundation (Treemu Headworks) showed larger root aerenchyma to supply sufficient oxygen for respiration, broader xylem vessels for better water and nutrient conduction, and greater density of leaf stomata for better transpiration. Maximum shoot and root length, total leaf area, and water potential were observed in the least saline Chinyot population indicating its best growth potential in a slightly saline aquatic environment. Each population showed specific physiological and anatomical modifications to colonize their respective habitats.

**Keywords** Aerenchyma · Bulliform cells · Environmental heterogeneity · Plasticity · Sclerification

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## 1 Introduction

Environmental heterogeneity is the distinctive feature of landscape significantly contributing to an increase in richness of biodiversity (Field et al., 2009). The major proportion of the Punjab region comes under the arid and semi-arid zone, however, seasonal inundations during monsoon season and extensive canal systems resulted in the formation

of many water bodies like saline waterlogged areas, salt marshes and freshwater ponds (Hanif et al., 2010). These water-bodies support only specific floral composition, which has some specific modifications to colonize aquatic wetlands (Dítě et al., 2019).

Colonizing of plant species to extremely stressed habitats are supported by the development of certain structural adaptations like the development of aerenchyma in root, stem and leaves for the supply of oxygen required for respiration (Takahashi et al., 2014). Gas exchange ability also increases by high stomatal density and size of stomatal complex (Muhlenbock et al., 2007). Bulliform cells development results in leaves rolling for maintenance of moisture content under physiological drought conditions because of high salinities (Alvarez et al., 2008). Ion secretion from glands is a strategy to reduce the salt contents in halophytes of the family Cyperaceae (Grigore & Toma, 2007). Water loss during transpiration is handled by thick and well-developed epidermis (Carignato et al., 2019).

In general, all growth phases of plants are affected by soil type that enables plants to modify morpho-anatomically and physiologically under variable environmental conditions. Most members of family Cyperaceae face severe stresses as they grow in aquatic habitats and salt marshes (Bernhardt & Kropf, 2006). *Kyllinga brevifolia* is distributed all over the temperate and tropical regions. Like many other species of family Cyperaceae, it is generally inhabitants of saline wetlands (Mishra et al., 2015). This species flourishes mostly in aquatic and cold habitats of the world. It colonizes in the form of the mat and is spread by stolons. This species have a high invasive potential, and invades in rice fields and saline waterbodies (Les, 2020).

No work has previously been reported on *Kyllinga* regarding the degree of salt tolerance and adaptive components to cope with stressful environments like saline waterlogged areas. It was hypothesized that there must have been flexibility in structural and functional features of this species that enables it to adopt saline aquatic environments. This study was conducted to explore those anatomical and physiological modifications in natural populations of *Kyllinga brevifolia*, and to investigate the mechanism of adaptation that plays a critical role in this species to successfully colonize in aquatic habitats.

## 2 Materials and Methods

### 2.1 Collection Sites

Six populations of *Kyllinga brevifolia* Rottb. along with soil samples were collected from different aquatic habitats in the Punjab Province varying in their level of soil salinity (Table 1). The collection sites were (from high to low salinity gradient) as: Sahianwala (hypersaline saltmarsh), Treemu Headworks (seasonal inundations), Baloki Headworks (seasonal water body), Changa Manga (artificial forest plantation), Khanki Headworks (waterlogged area), and Chinyot (riparian vegetation). Pictorial view of the collection sites along with coordinates are presented in Fig. 1. Seasonal weather and soil physicochemical characteristics of the collection sites of *Kyllinga brevifolia* are outlined in Table 1.

### 2.2 Soil Physicochemical Characteristics

Soil samples were collected from all collection sites of *Kyllinga brevifolia* to analyze the soil physicochemical attributes. Soil samples were taken at the depth of 15 to 25 cm, at the distance of 50 cm away from the plant roots at all four directions (north, south, east and west) and then average was used for data analysis. To calculate the saturation percentage, ECe and pH, a soil saturation paste was prepared by taking 200 g of dry soil. Soil ECe and pH of soil extract was computed by ECe/pH meter (WTW series Ino LAB pH/Cond 720, USA). Soil sodium ($Na^+$), potassium ($K^+$) and calcium ($Ca^{2+}$) ions were determined by using a flame photometer (PFP-7, Jenway, UK). Soil $Cl^-$ was analyzed by using chloride meter (Model-926, Sherwood Scientific Limited Cambridge, UK).

### 2.3 Collection of Plant Samples

Five average-sized plants were collected from each population during August 2017. Plants were carefully uprooted using soil auger (15 cm dia.), washed and weighed immediately on a portable digital balance. The samples were then put in plastic zipper bags (24 × 12 cm), sealed carefully and kept in an

**Table 1** Seasonal weather, soil physico-chemical characteristics, and ionic conic contents of the collecting sites of *Kyllinga brevifolia*

| | Sahianwala (Sah) | Treemu (HT) | Baloki (HB) | Changa Manga (CM) | Khanki (HK) | Chinyot (Chi) | *F*-ratio |
|---|---|---|---|---|---|---|---|
| Habitat type | | | | | | | |
| Annual rainfall (mm) | 375 | 159 | 379 | 364 | 578 | 336 | |
| Ave. Max. Temp. (°C) | 32 | 37 | 34 | 36 | 33 | 35 | |
| Ave. Min. Temp. (°C) | 15 | 11 | 17 | 16 | 15 | 15 | |
| Elevation (m a.s.l.) | 186.5 | 147.8 | 191.1 | 200.2 | 218.2 | 178.8 | |
| pH | 8.3a | 7.2b | 6.9bc | 7.3b | 8.3a | 8.4a | 9.11** |
| ECe (dS $m^{-1}$) | 27.4a | 20.2b | 12.9c | 8.5d | 5.3e | 1.9f | 16.61*** |
| Saturation percentage (%) | 34.2a | 30.7bc | 27.4c | 29.5b | 32.1bc | 29.4b | 86.76*** |
| $Na^+$ (mg $L^{-1}$) | 2786.1a | 2143.6b | 1313.6c | 834.5d | 588.6e | 123.1f | 288.43*** |
| $K^+$ (mg $L^{-1}$) | 301.8a | 261.6b | 161.9c | 63.9e | 144.2d | 155.7 cd | 112.34*** |
| $Ca^{2+}$ (mg $L^{-1}$) | 166.8b | 198a | 112.7d | 62.1e | 108.4de | 133.9c | 72.28*** |
| $Cl^-$ (mg $L^{-1}$) | 1633.7a | 1397.4b | 821.9c | 622.1d | 387.6e | 144.5f | 215.76*** |

**, ***Significant at $p \leq 0.01$ and 0.001, respectively. Ionic content were determined from saturation paste extract

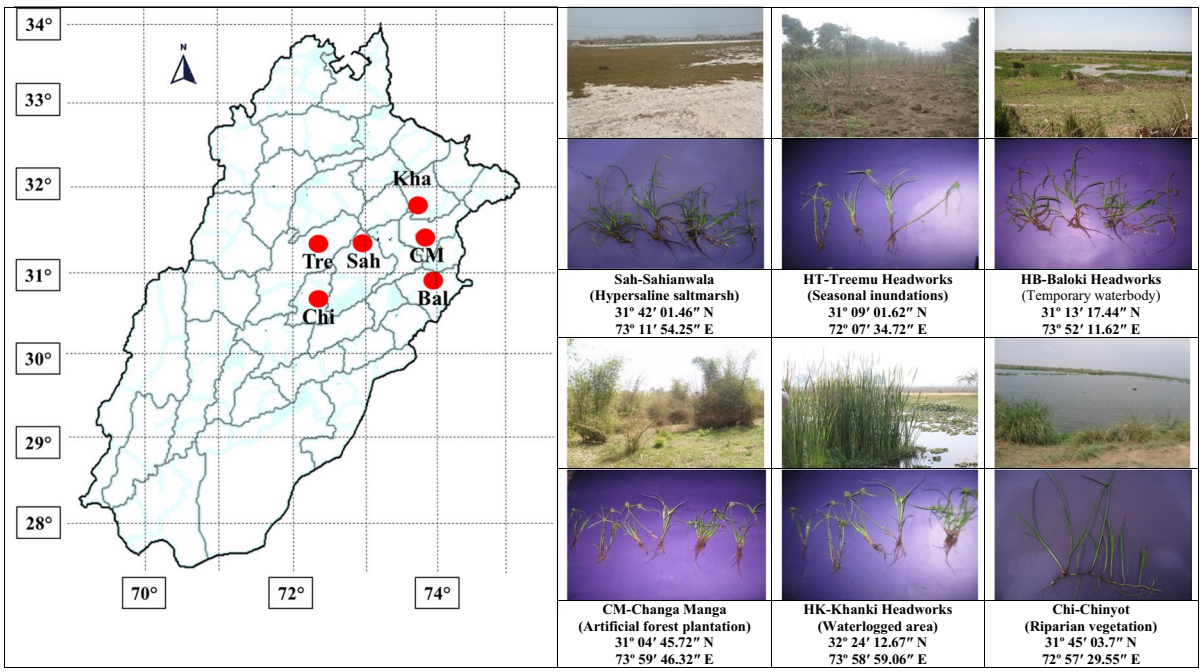


**Fig. 1** Pictorial presentation of collection sites of *Kyllinga brevifolia* on map of the Punjab Province

icebox. The plants were then brought to research laboratory for detailed morpho-anatomical and physiological analysis.

### 2.4 Morphological Data

Plant height and root length were measured with a scale. The number of leaves and bracts per plant were counted and total leaf area per plant was calculated by the following formula:

Number of leaves × average length of all leaves × average width of all leaves × 0.75

The plant samples were then oven-dried for root and shoot dry weight at 60 °C until the constant weight achieved.

### 2.5 Gas Exchange Parameters

$CO_2$ assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (*gs*), sub-stomatal $CO_2$ concentration (*Ci*), and water use efficiency (WUE) were measured immediately after uprooting plants from their natural habitats by using LCA-4 ADC portable IRGA (Analytical Development Company, Hoddesdon, England). The third leaf from the top of the plant was selected for data recording. Data for these parameters were recorded from 9:00 a.m. to 11:00 a.m. (chamber temp. 28.4 to 32.4 °C, the molar flow of air per unit leaf area 403.3 mmol $m^{-2}$ $s^{-1}$, ambient $CO_2$ conc. 352 µmol $mol^{-1}$, ambient temp. 22.4 to 27.9 °C, atmospheric pressure 99.9 kPa, the water vapor pressure of chamber 6.0 to 8.9 mbar, PAR 1711 µmol $m^{-2}$ $s^{-1}$).

### 2.6 Water Potential

Portable Scholar-type pressure chamber (1505D, PMS Instrument Company, Albany, USA) was used to determine water potential. Frozen samples (− 20 °C) for one week were used to analyze the solute potential of shoots by vapor pressure osmometer (Wescor 5500, USA). An indirect method was used to calculate turgor potential by deducting the value of solute potential from water potential.

### 2.7 Plant Ionic Contents

Plant material (both shoot and root) from selected samples (0.1 g) were oven dried and then subjected to grinding. The plant material was digested with concentrated $H_2SO_4$ according to Wolf (1982) technique to determine quantity of $Na^+$, $K^+$, and $Ca^{2+}$ by using a flame photometer (Jenway, PFP-7, UK).

### 2.8 Osmoprotectants

Moor and Stein (1948) method was used for the quantification of total amino acids. Proline content of samples was determined by following the method of Bates et al. (1973). Soluble proteins were determined by the method of Lowry et al. (1951). Anthrone method was used for the determination of total soluble sugars (Yemm & Willis, 1954).

### 2.9 Anatomical Parameters

Average-sized stem, the thickest root, largest leaves and bracts selected were rinsed with water and then kept in formalin acetic alcohol solution. Permanent anatomical slides were prepared by the free-hand sectioning technique. Thin transverse sections were treated with a series of ethanol percentages for dehydration. Safranin and fast green stains were used to distinguish between lignified and other tissues according to the method designed by Ruzin (1999). Stomata were studied by the epidermis peel method. The epidermis was removed carefully by the double-edge razor blade and placed on glass side, dehydrated by a series of ethanol grades and stained with safranin for improvement of contrast. Photographs of transverse sections were taken by a camera-equipped compound microscope (Nikon 104, Japan). Anatomical data were recorded by ocular micrometer calibrated with a stage micrometer (Fig. 2).

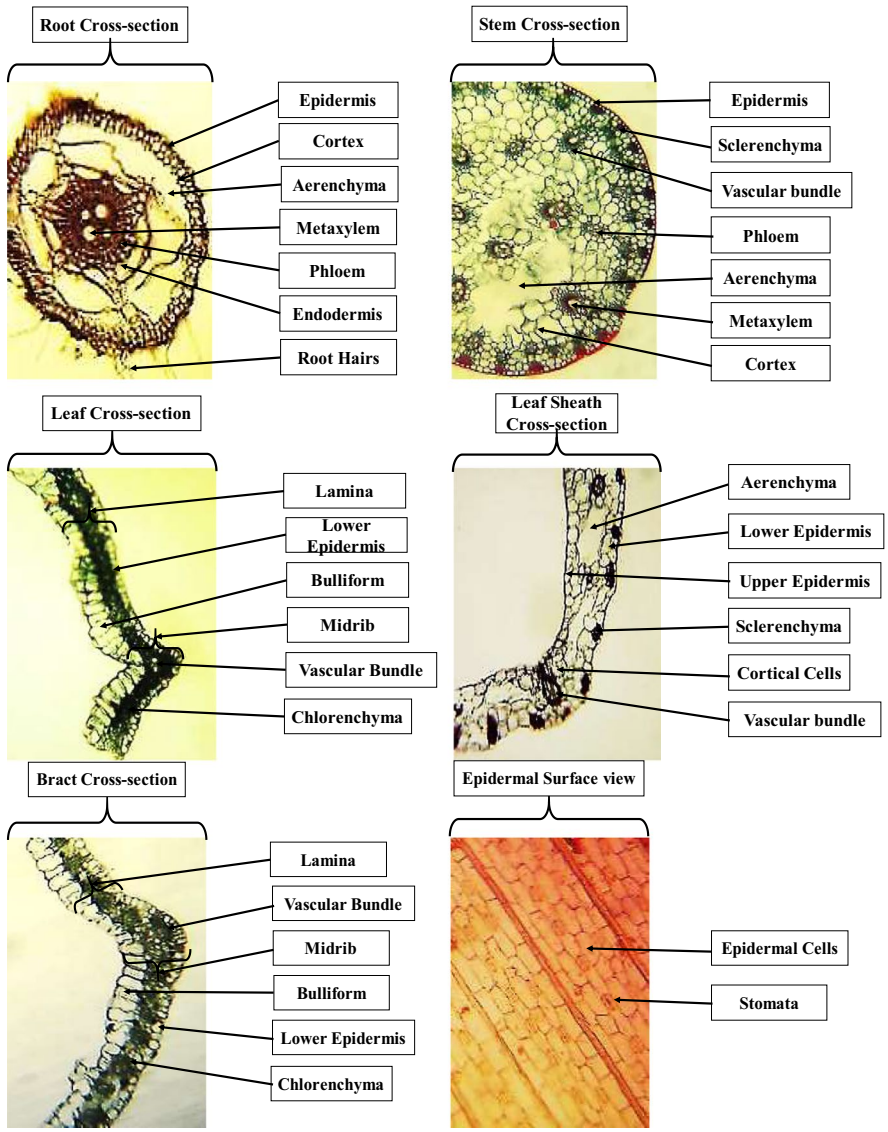


**Fig. 2** Measurement detail of root, stem, leaf, and bract anatomical characteristics of *Kyllinga brevifolia*

### 2.10 Statistical Analysis

The data were subjected to analysis of variance (one-way ANOVA) using the completely randomized design by Microsoft Excel (v. 2010) and multivariate redundancy analysis (RDA) done by XLSTAT (v. 2014) statistical software. Means were compared by the least significant difference (LSD) test at a 5% level of significance.

## 3 Results

### 3.1 Soil Physicochemical Attributes

Soil samples collected from highly saline habitat Sahianwala surpassed all the habitats regarding soil ECe, saturation percentage, $Na^+$, $K^+$, and $Cl^-$ content. The maximum soil pH was recorded at Chinyot while the highest soil $Ca^{2+}$ at Treemu Headworks.

**Table 2** Morpho-physiological characteristics of *Kyllinga brevifolia* collected from different ecological regions of Punjab

| | Sahianwala (Sah) | Treemu (HT) | Baloki (HB) | Changa Manga (CM) | Khanki (HK) | Chinyot (Chi) | *F*-ratio |
|---|---|---|---|---|---|---|---|
| Morphological traits | | | | | | | |
| Plant height (cm) | 14.3bc | 17.3ab | 15.2b | 10.1c | 17.1ab | 19.2a | 189.75*** |
| Root length (cm) | 8.2bc | 10.2ab | 9.1b | 6.1c | 10.1ab | 11.3a | 136.04*** |
| Number of leaves ($plant^{-1}$) | 5.6d | 6.7 cd | 7.1c | 8.3bc | 8.9b | 11.2a | 125.42*** |
| Total leaf area ($cm^2$ $plant^{-1}$) | 6.5d | 8.5 cd | 10.2c | 14.5bc | 15.3b | 22.3a | 115.58*** |
| Total bract area ($cm^2$ $plant^{-1}$) | 3.2c | 3.5bc | 3.5bc | 3.9b | 4.3ab | 5.0a | 10.34*** |
| Shoot fresh weight (g $plant^{-1}$) | 4.1d | 9.5ab | 7.6 cd | 6.0c | 10.4a | 8.4b | 193.00*** |
| Root fresh weight (g $plant^{-1}$) | 0.94d | 1.3b | 1.6a | 1.1c | 1.1c | 0.89de | 8.82*** |
| Shoot dry weight (g $plant^{-1}$) | 0.92 cd | 1.8ab | 1.3bc | 1.1c | 2a | 1.5b | 7.89*** |
| Root dry weight (g $plant^{-1}$) | 0.22c | 0.53a | 0.25bc | 0.24bc | 0.47b | 0.21c | 1.09*** |
| Gas exchange attributes | | | | | | | |
| $CO_2$ assimilation rate (μmol $m^{-2}$ $s^{-1}$) | 9.3ab | 9.5ab | 12.7a | 7.3bc | 8.9b | 6.6c | 3.62* |
| Transpiration rate (mmol $m^{-2}$ $s^{-1}$) | 1.7c | 2a | 1.8b | 1.7c | 1.4d | 1.7c | 1.63 $^{NS}$ |
| Stomatal conductance (mmol $m^{-2}$ $s^{-1}$) | 96de | 135c | 105d | 131c | 164a | 150b | 0.57 $^{NS}$ |
| Sub-stomatal $CO_2$ conc. (μmol $mol^{-1}$) | 292.2d | 340.3a | 320.3b | 338.9a | 312.5bc | 307.5c | 143.73*** |
| Water use efficiency (WUE) | 4.7c | 5.3ab | 5.5a | 4.4d | 5.2b | 3.9e | 8.02*** |
| Water potential | | | | | | | |
| Shoot water potential (-M Pa) | 1.31d | 1.40c | 1.50b | 1.49b | 1.61a | 1.61a | 5.24* |
| Shoot osmotic potential (-M Pa) | 0.73d | 0.92c | 0.53f | 0.63e | 1.22a | 1.09b | 3.71*** |
| Shoot turgor potential (M Pa) | 0.57c | 0.53bc | 0.97a | 0.87b | 0.41d | 0.51bc | 2.15*** |
| Plant ionic contents | | | | | | | |
| Shoot $Na^+$ (mg $g^{-1}$ d.wt.) | 72.1a | 60.2b | 53.5c | 41.7d | 33.5e | 19.3f | 10.90*** |
| Root $Na^+$ (mg $g^{-1}$ d.wt.) | 47.0a | 42.5b | 33.2c | 27.5d | 19.8d | 14.2e | 4.59* |
| Shoot $Ca^{2+}$ (mg $g^{-1}$ d.wt.) | 20.8a | 16.3bc | 17b | 13.3c | 19.7ab | 19.7ab | 3.34* |
| Root $Ca^{2+}$ (mg $g^{-1}$ d.wt.) | 4.03a | 4.2a | 3.6bc | 2.9c | 3.9b | 3.9b | 1.66 $^{NS}$ |
| Shoot $K^+$ (mg $g^{-1}$ d.wt.) | 9.7b | 13a | 7.5c | 8.9bc | 9.3b | 7.3c | 9.39** |
| Root $K^+$ (mg $g^{-1}$ d.wt.) | 27.8bc | 32.4a | 25.7c | 28.8b | 25.7c | 20.7d | 9.33** |
| Organic osmoprotectants | | | | | | | |
| Total free amino acids (μg $g^{-1}$ f. wt.) | 1425.7a | 1186.8b | 906.7c | 740.6d | 624.7e | 298.5f | 45.56*** |

**Table 2** (continued)

| | Sahianwala (Sah) | Treemu (HT) | Baloki (HB) | Changa Manga (CM) | Khanki (HK) | Chinyot (Chi) | *F*-ratio |
|---|---|---|---|---|---|---|---|
| Proline (μmol $g^{-1}$ f. wt.) | 325a | 290b | 220c | 198d | 194d | 150e | 4.71* |
| Total soluble proteins (μg $g^{-1}$ f. wt.) | 928a | 864b | 860b | 776c | 700d | 711d | 5.02* |
| Total soluble sugars (mg $g^{-1}$ d. wt.) | 21.1a | 19.2ab | 19.5ab | 18.2bc | 17.8 cd | 16.7d | 3.95* |

*Significant at $p < 0.05$, **significant at $p < 0.01$, ***significant at $p < 0.001$, *NS* not significant

Changa Manga soil possessed the least soil $K^+$ and $Ca^{2+}$. Soil collected from the Chinyot possessed the minimum ECe, $Na^+$, and $Cl^-$ content (Table 2).

### 3.2 Morphological Characteristics

All the morphological characteristics varied significantly in *K. brevifolia* populations collected from different habitats of Punjab (Table 2). The Chinyot population surpassed all other habitats regarding plant height (19.2 cm) and root length (11.3 cm). The number of leaves, total leaf and bract area was the maximum in population collected from Chinyot while the minimum was recorded from the population of Sahianwala saltmarsh. Root fresh and dry weights were the maximum in the Chinyot population. The maximum shoot fresh and dry weights were recorded in the Khanki population. Plant height and root length were the minimum in the population collected from Changa Manga. Root fresh weight was the maximum in the Baloki population. Population collected from Sahianwala exhibited the lowest shoot fresh weight, and, shoot and root dry weight.

### 3.3 Gas Exchange Parameters

Net $CO_2$ assimilation rate was the highest in the Baloki population, while the minimum in the Chinyot population (Table 2). Population collected from Treemu exhibited the highest sub-stomatal $CO_2$ concentration, while least was observed in the Sahianwala population. Transpiration rate and stomata conductance varied non-significantly among the *K. brevifolia* populations. The Baloki population possessed the maximum water use efficiency and the minimum was observed in the Chinyot population.

### 3.4 Water Relation Parameters

Population collected from Chinyot had the minimum shoot water potential (1.61 MPa), while the maximum (1.31 MPa) was reported in the Sahianwala population (Table 2). Khanki population depicted the highest shoot osmotic potential and the lowest shoot turgor potential. Baloki population exhibited the minimum shoot turgor potential shoot osmotic potential.

### 3.5 Plant Ionic Content

Shoot $Na^+$ varied significantly ($p < 0.001$) among different populations of *K. brevifolia* (Table 2). The Sahianwala population surpassed all other populations regarding shoot $Na^+$, whereas the least was noted in the Chinyot population. The Sahianwala population accumulated the maximum root $Na^+$ and shoot $Ca^{2+}$. Root $Na^+$ varied from 14.2 mg $g^{-1}$ in the Chinyot population to 47.0 mg $g^{-1}$ in the Sahianwala population. The minimum shoot $Ca^{2+}$ (13.3 mg $g^{-1}$) was reported in the Changa Manga population. Variation regarding root $Ca^{2+}$ were non-significant. The Treemu population depicted the highest concentration of shoot and root $K^+$, while the minimum was observed in the Baloki population.

### 3.6 Organic Osmolytes

The maximum accumulation of all osmolytes (free amino acids, proline, soluble proteins and soluble sugars) was observed in the Sahianwala population (Table 2). Total free amino acids varied from 1425.7 μg $g^{-1}$ in the Sahianwala population to 198.5 μg $g^{-1}$ in the Chinyot population.

**Table 3** Anatomical characteristics of *Kyllinga brevifolia* collected from different ecological regions of Punjab

| | Sahianwala (Sah) | Treemu (HT) | Baloki (HB) | Changa Manga (CM) | Khanki (HK) | Chinyot (Chi) | F-ratio |
|---|---|---|---|---|---|---|---|
| Root anatomical traits | | | | | | | |
| Root thickness (µm) | 187.9bc | 334.9a | 191.9bc | 171.5c | 212.4b | 318.6ab | 229.92*** |
| Epidermal thickness (µm) | 7.3c | 10.6ab | 8.2bc | 8.2bc | 11.4a | 8.9b | 11.3*** |
| Cortical thickness (µm) | 73.5b | 130.7a | 36.8c | 49.0bc | 77.6b | 138.9a | 791.0*** |
| Cortical cell area ($\mu m^2$) | 113.6d | 141.9c | 69.5e | 63.3e | 165.5b | 199.6a | 132.6*** |
| Endodermal thickness (µm) | 8.2 cd | 10.6b | 7.3d | 7.3d | 8.9c | 12.2a | 17.3*** |
| Metaxylem area ($\mu m^2$) | 52.8e | 288.8a | 94.7 cd | 69.6d | 118.3c | 231.1b | 191.1*** |
| Vascular region thickness (µm) | 24.5bc | 40.0a | 31.9b | 20.4c | 32.7b | 40.8a | 299.3*** |
| Aerenchymatous area ($\mu m^2$) | 315.0d | 6634.7a | 1652.4bc | 996.8c | 1888.4b | 1573.7bc | 2877.5*** |
| Stem anatomical traits | | | | | | | |
| Stem thickness (µm) | 359.5c | 531.0b | 506.5bc | 547.4ab | 531.0ab | 669.9a | 443.3*** |
| Epidermal thickness (µm) | 8.2b | 8.9a | 7.3c | 8.2b | 6.5d | 7.3c | 3.3* |
| Cortical cell area ($\mu m^2$) | 629.7d | 787.0 cd | 1310.9b | 755.5 cd | 1835.9a | 813.2c | 290.5*** |
| Vascular bundle area ($\mu m^2$) | 1919.8b | 1468.8c | 2307.9a | 629.7f | 1154.1d | 918.1e | 393.0*** |
| Metaxylem area ($\mu m^2$) | 63.3d | 262.6a | 208.0b | 90.0 cd | 94.7 cd | 115.7c | 60.9*** |
| Sclerenchyma thickness (µm) | 8.2d | 17.9a | 13.9b | 14.7ab | 8.9 cd | 12.2c | 60.9*** |
| Chlorenchyma thickness (µm) | 31.9ab | 25.3c | 32.7a | 29.4b | 20.4d | 23.7 cd | 106.4*** |
| Leaf anatomical traits | | | | | | | |
| Leaf midrib thickness (µm) | 1912.5bc | 1448.4d | 2368.8a | 2316.3ab | 1576.9c | 1930.9b | 117.3** |
| Leaf lamina thickness (µm) | 73.5a | 68.6ab | 57.2c | 64.5bc | 66.9ab | 65.4b | 130.1*** |
| Bulliform thickness (µm) | 32.7a | 24.5b | 31.0ab | 17.9d | 20.4c | 24.5b | 149.3*** |

**Table 3** (continued)

| | Sahianwala (Sah) | Treemu (HT) | Baloki (HB) | Changa Manga (CM) | Khanki (HK) | Chinyot (Chi) | F-ratio |
|---|---|---|---|---|---|---|---|
| Epidermal thickness (µm) | 8.9b | 9.8a | 8.2bc | 8.9b | 9.8a | 7.3c | 4.1* |
| Vascular bundle area (µm$^2$) | 671.6 cd | 1759.9a | 629.7d | 1290.5b | 991.6c | 1023.0bc | 235.3*** |
| Metaxylem area (µm$^2$) | 29.7a | 13.5bc | 15.0b | 6.6c | 22.4ab | 13.5bc | 5.6** |
| Chlorenchyma thickness (µm) | 24.5bc | 28.6a | 25.3b | 23.7c | 28.6a | 26.1ab | 19.2*** |
| Stomatal area (µm$^2$) | 734.6b | 944.4a | 613.9c | 795.9ab | 315.0d | 618.1c | 96.0*** |
| Stomatal density | 18c | 26b | 25b | 34a | 24b | 19c | 99.2*** |
| Bract anatomical traits | | | | | | | |
| Bract midrib thickness (µm) | 89.9a | 73.5c | 80.9ab | 77.6bc | 80.1b | 81.7bc | 1.3$^{NS}$ |
| Bract lamina thickness (µm) | 65.4b | 66.9bc | 81.7a | 64.5c | 72.7b | 73.5ab | 191.6*** |
| Bulliform thickness (µm) | 26.1ab | 14.7c | 22.9b | 16.3bc | 32.7a | 24.5ab | 196.8*** |
| Epidermal thickness (µm) | 8.2bc | 12.2a | 11.4ab | 8.9c | 10.6b | 7.3d | 16.8*** |
| Vascular bundle area (µm$^2$) | 1049.3c | 805.9 cd | 1333.5b | 1510.8a | 787.0d | 1442.6ab | 123.0*** |
| Metaxylem area (µm$^2$) | 9.8 cd | 13.5c | 17.1b | 26.0a | 17.1b | 8.7d | 3.6* |
| Chlorenchyma thickness (µm) | 32.7ab | 22.9d | 26.1c | 36.8a | 31.9b | 26.1c | 121.2*** |
| Stomatal area (µm$^2$) | 734.6a | 472.3bc | 387.4c | 369.0 cd | 554.2b | 367.5 cd | 57.2*** |
| Stomatal density | 28a | 22b | 26ab | 15c | 26ab | 15c | 99.6*** |
| Leaf sheath anatomical traits | | | | | | | |
| Leaf sheath thickness (µm) | 73.5b | 81.7a | 77.6ab | 49.0d | 57.2c | 80.9ab | 836.0*** |
| Upper epidermal thickness (µm) | 17.1a | 12.2bc | 16.3ab | 9.8c | 16.3ab | 15.5b | 37.7*** |
| Lower epidermal thickness (µm) | 16.3a | 15.5ab | 12.2b | 16.3a | 8.2c | 12.2b | 46.5*** |

**Table 3** (continued)

| | Sahianwala (Sah) | Treemu (HT) | Baloki (HB) | Changa Manga (CM) | Khanki (HK) | Chinyot (Chi) | F-ratio |
|---|---|---|---|---|---|---|---|
| Cortical Cell area ($\mu m^2$) | 189.1b | 503.8a | 94.7c | 179.7bc | 150.3bc | 471.8ab | 161.8*** |
| Aerenchyma area ($\mu m^2$) | 0 | 2412.8ab | 755.6c | 1416.4bc | 2570.2a | 1678.6b | 706.8*** |
| Vascular bundle area ($\mu m^2$) | 2216.2a | 429.9 cd | 393.7d | 623.9bc | 461.9c | 787.0b | 829.6*** |
| Metaxylem area ($\mu m^2$) | 38.1a | 19.2bc | 23.9bc | 29.7ab | 16.0c | 26.0b | 3.4* |

*Significant at $p < 0.05$, **significant at $p < 0.01$, ***significant at $p < 0.001$, *NS* not significant

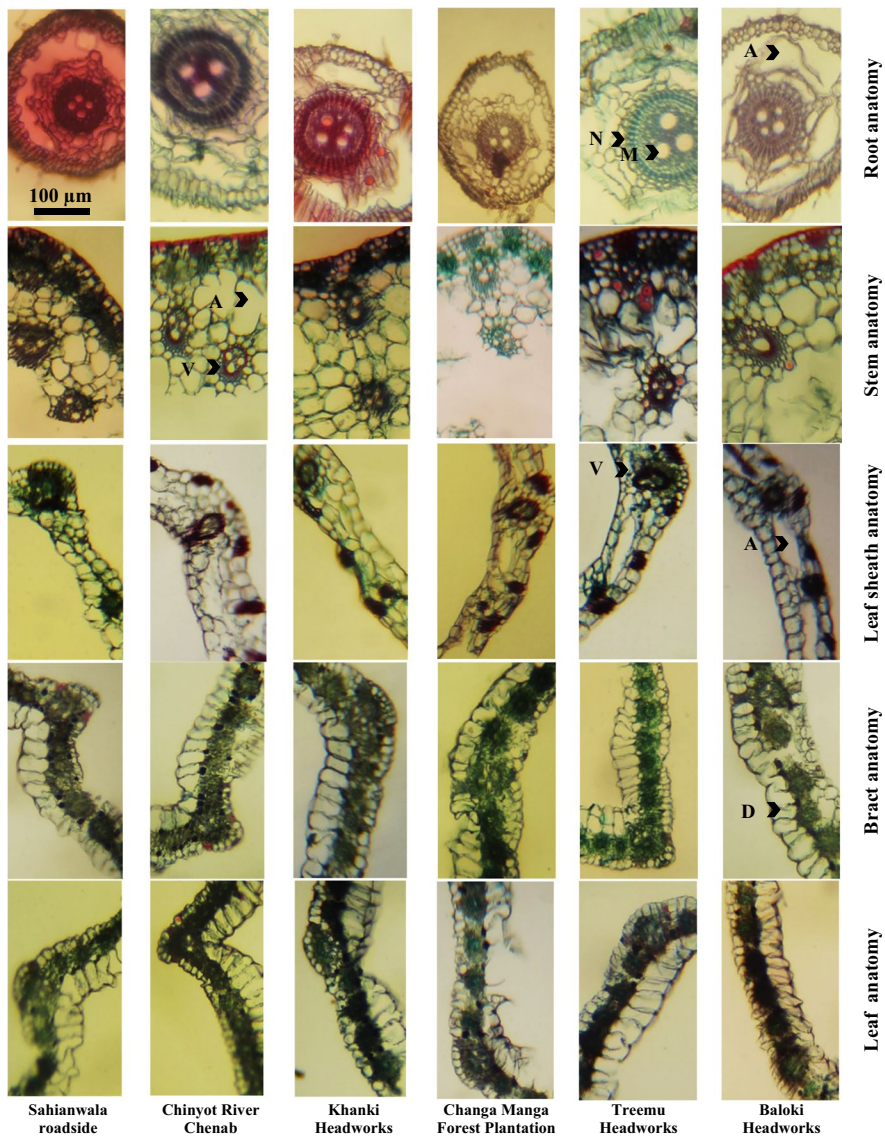


**Fig. 3** Root, stem, bract, and leaf transverse sections of *Kyllinga brevifolia* collected from different habitats in the Punjab. A, aerenchyma; D, epidermis (adaxial); M, metaxylem; N, endodermis; V, vascular bundle. All photographs were taken on same resolution (40×)

The highest accumulation of proline was observed in the Sahianwala population and least in the Chinyot population. The maximum accumulation of total soluble proteins was recorded in the population collected from Sahianwala, while the Khanki population possessed the minimum concentration. The concentration of total soluble sugars was the highest in the Sahianwala population and the minimum was observed in the Chinyot population.

### 3.7 Root Anatomical Characteristics

Root anatomical parameters varied significantly in *K. brevifolia* populations collected from different vegetation zones in the Punjab (Table 3, Fig. 3). The Treemu population showed higher values for most of the anatomical parameters like root thickness, vascular region thickness, aerenchymatous area and metaxylem area. The Chinyot population showed the maximum cortical region thickness and cellular area, and endodermis thickness.

Population collected from Khanki had the maximum epidermal thickness, whereas cortical cell area, vascular region thickness and aerenchymatous area were also higher than other populations except that collected from Chinyot. The Sahianwala population showed the minimum epidermal thickness, metaxylem area and aerenchymatous area, while Changa Manga population showed minimum root thickness, cortical cell area, endodermal thickness and vascular region thickness.

### 3.8 Stem Anatomical Characteristics

Significant variation was observed in all populations of *K. brevifolia* regarding stem anatomical characteristics (Table 3, Fig. 3). The proportion of storage parenchyma was relatively high in the populations collected from Chinyot and Changa Manga. The Treemu population showed the maximum epidermal thickness, sclerenchyma thickness and metaxylem area. The Baloki population showed the highest chlorenchyma thickness and vascular bundle area, while cortical cell and metaxylem areas were greater than all other populations except that of Chinyot population.

The Khanki population showed the maximum cortical cell area, but chlorenchymatous thickness was the minimum in this population. The Sahianwala population had the minimum stem thickness, cortical cell area, metaxylem area, and sclerenchymatous area. Vascular bundle area was the minimum in Changa Manga population, while epidermal and sclerenchyma thicknesses were higher than other populations except for the Treemu population.

### 3.9 Leaf Anatomical Characteristics

Leaf anatomical characteristics showed significant variation among different populations of *K. brevifolia*. Sahianwala population showed the maximum leaf lamina thickness, bulliform thickness, and metaxylem area, while stomata density was the minimum in this population (Table 3, Fig. 3). The Treemu population showed the highest leaf anatomical characteristics like epidermal thickness, vascular bundle area, chlorenchymatous thickness, and stomatal area. The Changa Manga population showed high midrib thickness, epidermal thickness, vascular bundle area and stomatal area. Bulliform thickness, metaxylem area and chlorenchyma thickness were the minimum in Changa Manga population.

The Khanki population was characterized by thick epidermis, thick chlorenchymatous region and broad metaxylem vessels, while the stomatal area was the minimum. The Baloki population showed the maximum thickness of leaf midrib, but lamina thickness was the minimum.

### 3.10 Bract Anatomical Characteristics

Bract anatomical characteristics varied significantly among *K. brevifolia* populations collected from diverse habitats (Table 3, Fig. 3). Bract midrib thickness, stomatal area, and stomatal density were the maximum in the Sahianwala population. This population showed more bulliform and chlorenchymatous thicknesses. The Changa Manga population had greater vascular bundle area, metaxylem area and chlorenchymatous thickness. Lamina thickness and stomatal density was the minimum in this population. Lamina thickness was the maximum in the Baloki population, which also showed high epidermal thickness, metaxylem area, and stomatal density (Fig. 4).

The Treemu population showed the highest epidermal thickness, whereas midrib thickness,

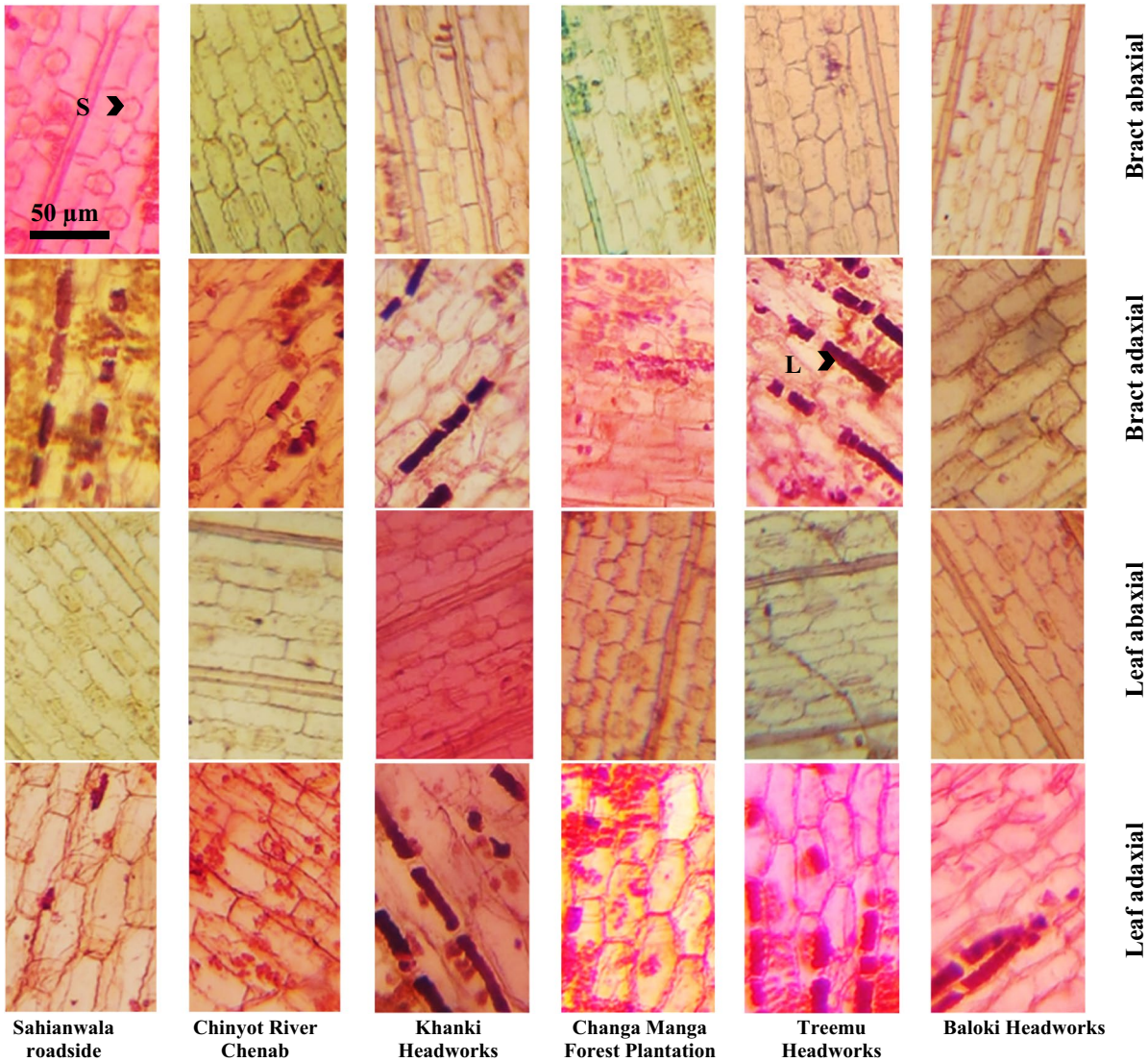


**Fig. 4** Epidermal surface view of bracts and leaves of *Kyllinga brevifolia* collected from different ecological regions of the Punjab. L, phytoliths; S, stomata; all photographs were take on same resolution (40×)

bulliform thickness, and chlorenchymatous thickness were the minimum. The Chinyot population showed the minimum bract anatomical characteristics like epidermal thickness, metaxylem area, stomatal area and stomatal density. Midrib and lamina thicknesses and vascular bundle area were the second higher in this population. Bulliform thickness was the maximum in the Khanki population, while vascular bundle area was the minimum in this population.

### 3.11 Leaf Sheath Anatomical Characteristics

The Sahianwala population had the highest adaxial and abaxial epidermal thicknesses, vascular bundle area and metaxylem area (Table 3, Fig. 3). Leaf-sheath thickness and cortical cell area was the maximum in the Treemu population, which also showed high abaxial epidermal thickness and aerenchymatous area. Leaf sheath thickness, cortical cell area and vascular

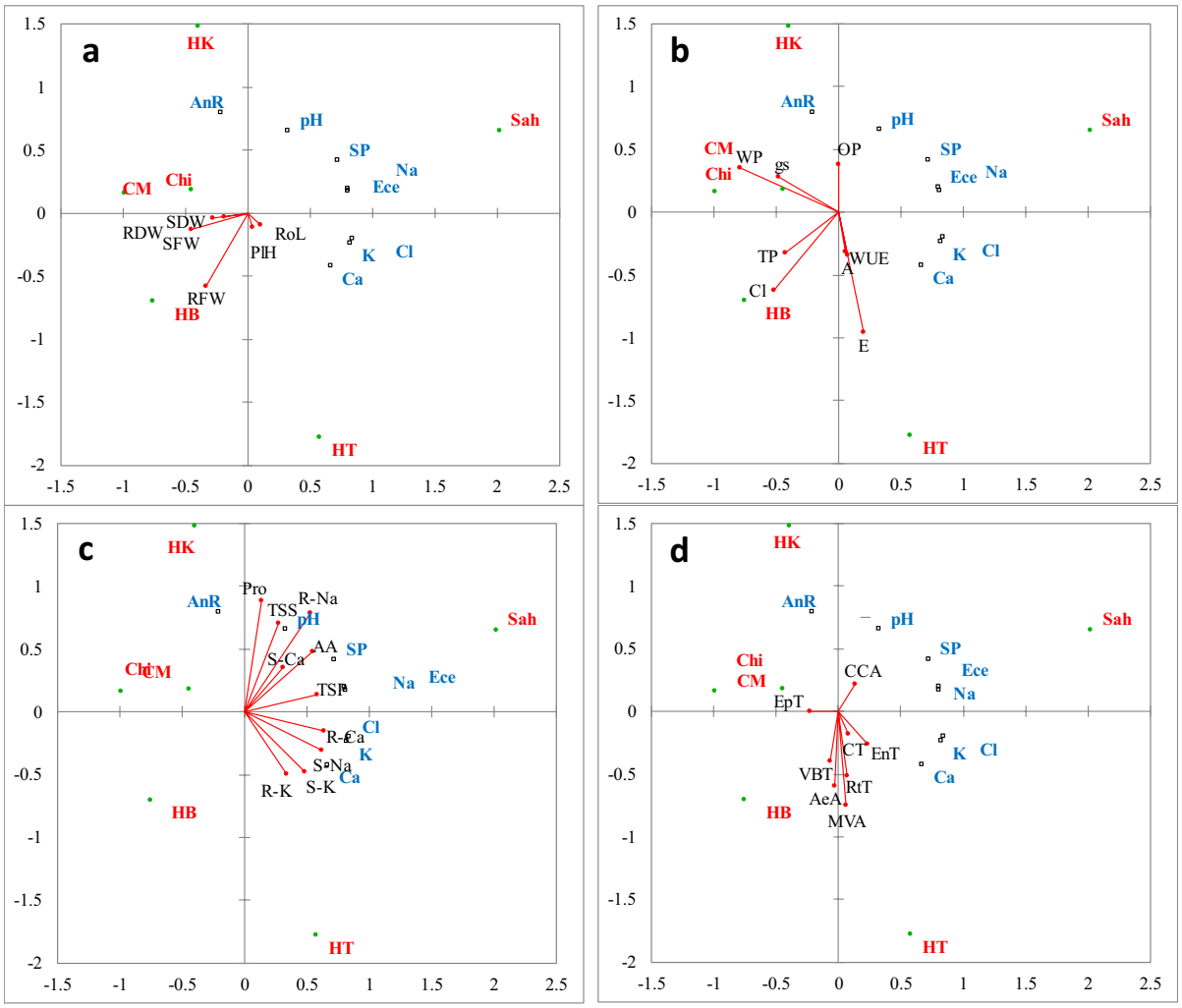


**Fig. 5** RDA ordination triplot showing influence of soil's physicochemical characteristics (blue labels) of different habitats (red labels) on **a** morphological, **b** water relation and gas exchange parameters, **c** root and shoot ionic content, and **d** root anatomical characteristics of *Kyllinga brevifolia*. Abbreviations: Sah, Sahianwala; HT, Treemu Headworks; HB, Baloki Headworks; CM, Changa Manga; HK, Khanki Headworks; Chi, Chinyot; AnR, annual rainfall; PlH, plant height; SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight; RDW, root dry weight; *A*, $CO_2$ assimilation rate; *E*, transpiration rate; *gs*, stomatal conductance; *Ci*, sub-stomatal $CO_2$ conc.; WUE, water use efficiency; WP, shoot water potential; OP, shoot osmotic potential; TP, shoot turgor potential; S-Na, shoot Na+; R-Na, root Na+; S-Ca, shoot Ca2+; R-Ca, root Ca2+; S-K, shoot K+; R-K, root K+; AA, total free amino acids; Pro, proline; TSP, total soluble proteins; TSS, total soluble sugars; RtT, root thickness; EpT, epidermal thickness; CT, cortical thickness; CCA, cortical cell area; EnT, endodermal thickness; MVA, metaxylem area; VBT, vascular region thickness; AeA, aerenchymatous area

bundle area was the second-best in the Chinyot population. The Baloki population showed the minimum cortical cell area, aerenchymatous area and vascular bundle area. Leaf sheath and epidermal thicknesses were the minimum in the Changa Manga population. Aerenchyma development was more prominent in the Khanki population, which also showed the minimum abaxial epidermal thickness and metaxylem area.

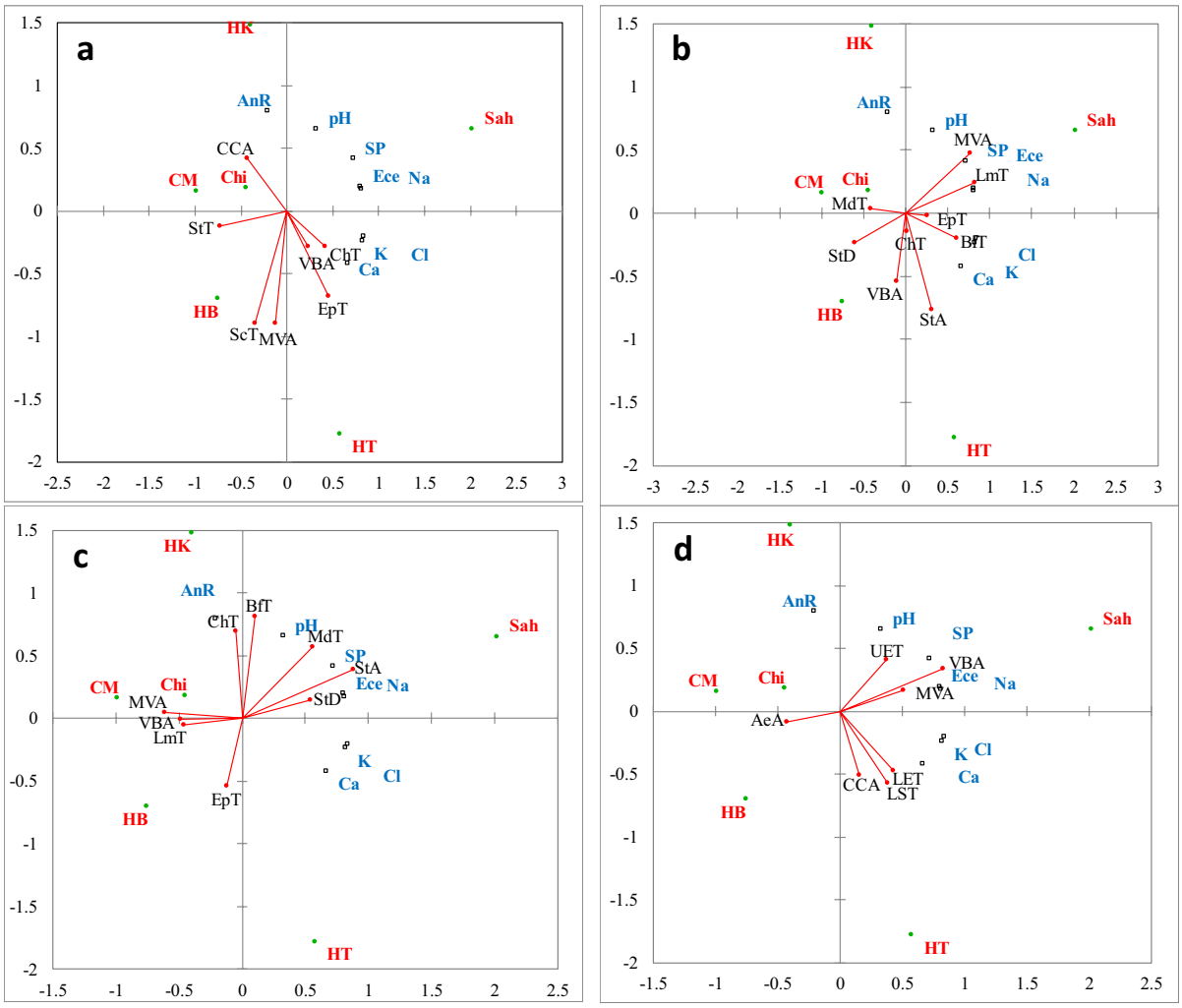


**Fig. 6** RDA ordination triplot showing influence of soil's physicochemical characteristics (blue labels) of different habitats (red labels) on **a** stem, **b** leaf, **c** bract, and **d** leaf sheath anatomical characteristics of *Kyllinga brevifolia*. Sah, Sahianwala; HT, Treemu Headworks; HB, Baloki Headworks; CM, Changa Manga; HK, Khanki Headworks; Chi, Chinyot; AnR, annual rainfall; StT, stem thickness; EpT, epidermal thickness; CCA, cortical cell area; VBA, vascular bundle area; MVA, metaxylem area; ScT, sclerenchyma thickness; ChT, chlorenchyma thickness; MdT, leaf midrib thickness; LmT, leaf lamina thickness; BfT, bulliform thickness; StA, stomatal area; StD, stomatal density

### 3.12 Relationship Between Soil and Plant Morpho-physiological and Anatomical Parameters

Redundancy analysis (RDA) ordination triplot showed the effect of soil physicochemical characteristics of different habitats on morpho-physiological and anatomical parameters of *K. brevifolia* (Figs. 5 and 6). In the Treemu population, plant height and root length were influenced by soil $K^+$, $Ca^{2+}$ and $Cl^-$. Stomatal conductance and water potential had a strong effect on Chinyot and Changa Manga populations. Turgor potential and sub-stomatal $CO_2$ concentration were associated with Baloki population. Root $Ca^{2+}$, shoot $Na^+$, and shoot and root $K^+$ were strongly linked with soil $Ca^{2+}$, $K^+$ and $Cl^-$. Free amino acids, root $Ca^{2+}$, root $Na^+$, total soluble sugars and proline were strongly influenced by soil pH and saturation percentage.

Root epidermal thickness depicted a strong relationship with the Changa Manga population. Root vascular bundle thickness and aerenchymatous area

were influenced by soil physicochemical properties of Baloki population. Stem cortical cell area was strongly linked with the Chinyot population. Stem vascular bundle area and chlorenchymatous thickness had a strong influence on soil $Ca^{2+}$ and $K^{+}$. Leaf midrib thickness possessed a strong association with the Chinyot population. Leaf stomatal density and vascular bundle area were played a key role in adaptability of the Baloki population. Leaf bulliform thickness was strongly influenced by soil $Ca^{2+}$, $K^{+}$ and $Cl^{-}$. Leaf lamina thickness and metaxylem vessel area were strongly affected by soil saturation percentage, EC and $Na^{+}$.

Bract and lamina thickness, vascular bundle area and metaxylem vessel area possessed a strong influence of soil properties of the Changa Manga and Chinyot populations. Bract midrib thickness, stomatal density and stomatal area of the Sahianwala population showed a strong relationship with soil pH, EC, saturation percentage and $Na^{+}$ soil. Chlorenchymatous thickness was mainly affected by annual rainfall. Adaxial epidermal thickness, vascular bundle area and metaxylem vessel area of the Sahianwala population were strongly linked to the soil pH, EC, saturation percentage and $Na^{+}$.

Heatmaps were constructed to show relationship between soil physicochemical attributes of collection sites and morpho-anatomical traits of *K. brevifolia* (Figs. 7 and 8). Soil $K^{+}$ and $Ca^{2+}$ were grouped with root $Ca^{2+}$ and total soluble proteins. Soil $Na^{+}$, Cl, ECe and saturation percentage were clustered in a separate group. Soil pH influenced proline and total free amino acids in *K. brevifolia*. Total soluble salts in soil were associated with root-shoot $Na^{+}$ and root $Ca^{2+}$. Annual rainfall influenced total soluble salts, root $Na^{+}$ and shoot $Ca^{2+}$. Soil $Na^{+}$ was associated with the root fresh weight, shoot turgor potential, net $CO_2$ assimilation rate and

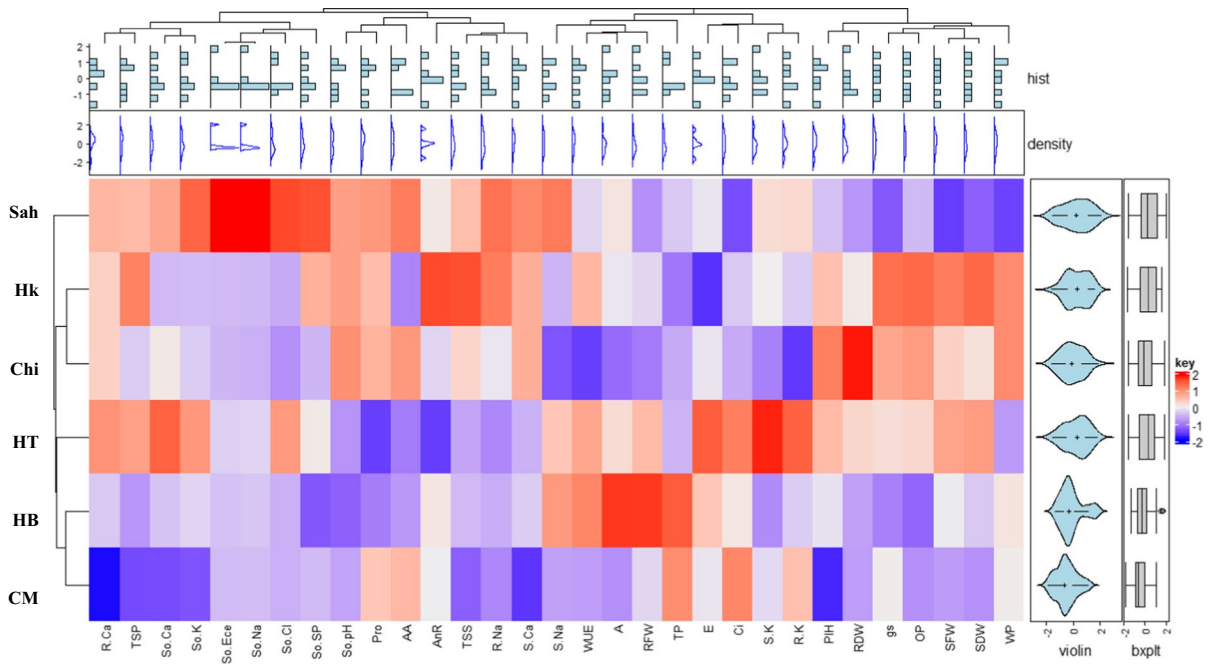


**Fig. 7** A heatmap constructed to draw the relationship between soil's physicochemical characteristics of different habitats and growth, water relations, organic osmolytes, and photosynthetic attributes of *Kyllinga brevifolia*. Abbreviations: Sah, Sahianwala; HT, Treemu Headworks; HB, Baloki Headworks; CM, Changa Manga; HK, Khanki Headworks; Chi, Chinyot; AnR, annual; So-pH, soil pH; So-ECe, soil ECe, So-SP, soil saturation %; So-Na, soil Na; So-K, soil K; So-Ca, soil Ca; So-Cl, soil Cl; PlH, plant height; SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight; RDW, root dry weight; *A*, $CO_2$ assimilation rate; *E*, transpiration rate; *gs*, stomatal conductance; *Ci*, sub-stomatal $CO_2$ conc.; WUE, water use efficiency; WP, shoot water potential; OP, shoot osmotic potential; TP, shoot turgor potential; S-Na, shoot Na+; R-Na, root Na+; S-Ca, shoot Ca2+; R-Ca, root Ca2+; S-K, shoot K+; R-K, root K+; AA, total free amino acids; Pro, proline; TSP, total soluble proteins; TSS, total soluble sugars

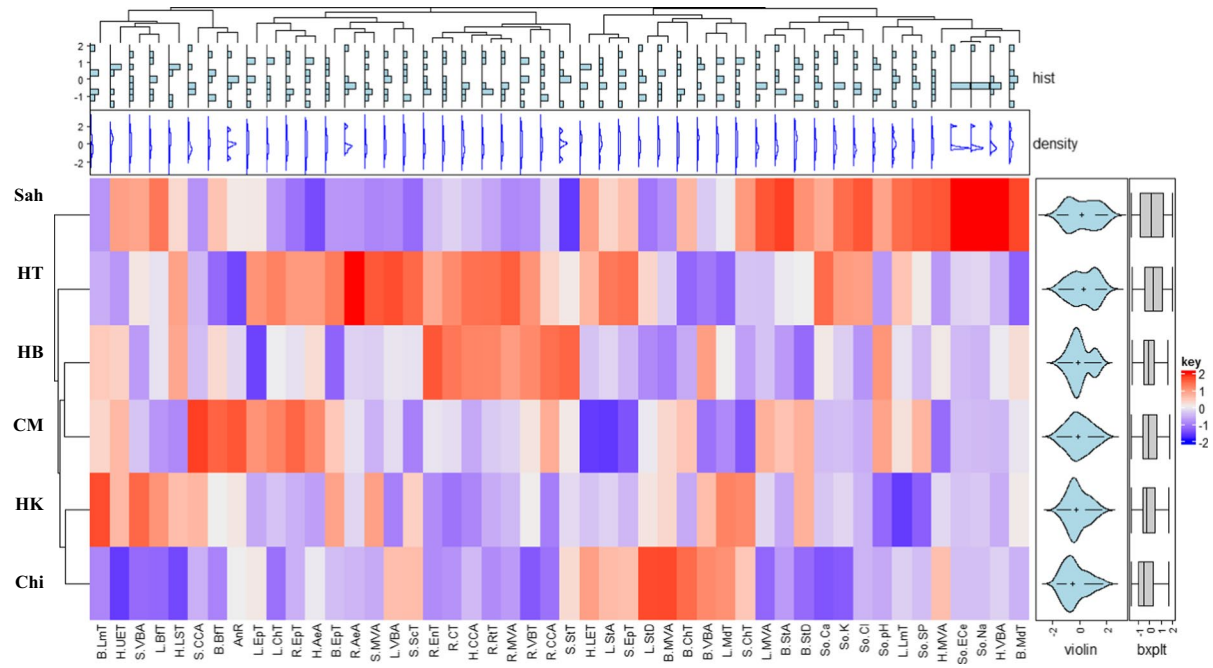


**Fig. 8** A heatmap constructed to draw the relationship between soil's physicochemical characteristics of different habitats and leaf sheath, leaf blade, stem, and root anatomical attributes of *Kyllinga brevifolia*. Abbreviations: Sah, Sahianwala; HT, Treemu Headworks; HB, Baloki Headworks; CM, Changa Manga; HK, Khanki Headworks; Chi, Chinyot; AnR, annual; So-pH, soil pH; So-ECe, soil ECe; So-SP, soil saturation %; So-Na, soil Na; So-K, soil K; So-Ca, soil Ca; So-Cl, soil Cl; RtT, root thickness; EpT, epidermal thickness; CT, cortical thickness; CCA, cortical cell area; EnT, endodermal thickness; MVA, metaxylem area; VBT, vascular region thickness; AeA, aerenchymatous area; StT, stem thickness; EpT, epidermal thickness; CCA, cortical cell area; VBA, vascular bundle area; MVA, metaxylem area; ScT, sclerenchyma thickness; ChT, chlorenchyma thickness; MdT, leaf midrib thickness; LmT, leaf lamina thickness; BfT, bulliform thickness; StA, stomatal area; StD, stomatal density (R-, root; S-, stem; L-, leaf; H-, sheath)

water use efficiency (Fig. 7). Transpiration rate was associated with sub-stomatal $CO_2$ concentration, root and shoot $K^+$. Plant height, root dry weight, root-shoot fresh weight showed a relationship with stomatal conductance, shoot osmotic potential and water potential.

Heatmap (Fig. 8) showed a relationship between root, stem, leaf, and bract anatomical characteristics in five major clusters. The first cluster had 4 sub-clusters, where leaf metaxylem area was associated with stomatal density and area of bract. Soil $Cl^-$ influenced soil $Ca^{2+}$ and $K^+$. Soil pH had a relationship with soil saturation percentage and leaf lamina thickness. The last sub-cluster showed an association of soil ECe and $Na^+$ with leaf sheath vascular bundle area and metaxylem area and bract midrib thickness. The second cluster had 3 sub-clusters, where stem thickness, leaf sheath epidermal thickness and leaf stomatal area was closely associated. Leaf stomatal density was related with metaxylem area and chlorenchymatous thickness in bract. Leaf midrib thickness, bract vascular bundle area and stem chlorenchymatous thickness showed a strong association. In the third cluster, root thickness was associated with endodermal thickness, cortical thickness and its cell area, metaxylem area and vascular bundle thickness in root, stem thickness, and bract cortical cell area. In the fourth cluster, leaf epidermal thickness was associated with leaf chlorenchymatous thickness and the root epidermal thickness. Bract epidermal thickness was associated with root aerenchymatous area, stem metaxylem area and sclerenchymatous thickness and leaf vascular bundle area. In the fifth cluster, two sub-clusters were noticed. Leaf sheath thickness was related to leaf sheath adaxial epidermal thickness, stem vascular bundle area, leaf bulliform thickness and bract lamina thickness.

## 4 Discussion

Members of the family Cyperaceae are among few plant families that can grow in almost every kind of habitat, e.g., cool alpine regions, deserts and semi-deserts, fresh and saltwater marshes, river and canal banks, roadsides and wastelands, and as weeds in agricultural fields (Uddin et al., 2006). The large number of species of family Cyperaceae colonizes moist and aquatic habitats, especially they are well adapted to saline wetlands (Piwpuan et al., 2013). Other than physio-morphological modifications, sedges have modified anatomical features that enable them to grow under saline waterlogged conditions (Abulfatih, 2003). Aerenchyma development in roots and stem is one of striking anatomical features that facilitate oxygen diffusion towards root for respiration (Benz et al., 2007). Modification in endodermis and bundle sheath cells, which surround vascular tissue in root and leaves respectively, significantly contribute towards stress tolerance by preventing radial water loss (Hoque et al., 2018).

In the present study, differently adapted population showed a differential response to the degree of salinity of their respective habitats. The Sahianwala population was collected from a saltmarsh that was highly saline (ECe 27.4 dS $m^{-1}$). Hyper-saline soils of Sahianwala restricted growth of root and stem in *K. brevifolia* population, as was previously reported by several authors like Batool et al. (2013) and Younis et al. (2014). Accumulation of $K^+$ ion was observed in this population, which dilutes the toxic effect of $Na^+$ and $Cl^-$ accumulation to some extent and contributes towards the continuation of growth (Tavakkoli et al., 2012). This is an important feature of aquatic plants inhabiting saltmarshes (Kafi et al., 2021). Organic osmolytes like total free amino acids, total soluble sugars, total soluble proteins and proline contents increased significantly in this population, and this is the protective mechanism to prevent cell collapse under physiological drought caused by high salinities (Atreya et al., 2009; Hayat et al., 2011).

Leaf and leaf bract are the main photosynthetic organ in *K. brevifolia*. Any increase in chlorenchymatous thickness (cells containing chloroplast) will regulate the photosynthetic efficiency. This is critical as high salinity slows down many physiological processes (Stoeva & Kaymakanova, 2008). Thick leaf was a characteristic feature if Sahianwala population. Increased succulence in terms of leaf thickness has earlier been reported in halophytic species (Hameed et al., 2009). Additionally, larger bulliform cells in the leaves and bracts ease leaf rolling, which controls water loss due to transpiration and enhance survival under stressful conditions (Alvarez et al., 2008). Leaf anatomical modifications like well-developed bulliform cells, thicker leaves (midrib and lamina) and large stomatal area were observed as the important traits for the growth and survival of the Sahianwala population in highly saline habitats (Grigore et al., 2014; Shimamura et al., 2010). Leaf sheath surrounds the stem base, and hence critically important for stress tolerance, growth, and survival (Kokkonen et al., 2005). An increase of leaf sheath epidermal thickness, vascular bundle area and metaxylem area was noted in the Sahianwala population, which ensured the survival in hyper-saline environments.

The Treemu population was collected from hypersaline seasonal inundation (ECe 20.2 dS $m^{-1}$). Soil $Ca^{2+}$ was the maximum at this site, and this is an important cation that significantly contributed to root dry weight (Bonomelli et al., 2019). Transpiration rate (and sub-stomatal $CO_2$ concentration) was the maximum in this population, which is a general feature of aquatic species like *K. brevifolia* when sufficient water is available (Hanson et al., 2016). The higher accumulation of $K^+$ and $Ca^{2+}$ in root and shoot along with high $Na^+$ is critical for salt-tolerant species like *K. brevifolia* (Ahmad et al., 2014). This neutralizes the toxic effect of $Na^+$ accumulation by maintaining turgor potential under high salinities (Hussain et al., 2009). The notable modifications in the stem was intensive sclerification (particularly outside vascular bundles), which is vital for preventing water loss (Endo et al., 2008; Hameed et al., 2012b). Broader metaxylem vessels in root and stem can facilitate water conduction in the Treemu population (Smith et al., 2013). The stomatal size was exceptionally large in this population, which is certainly advantageous when water availability is sufficient enough (Xu & Zhou, 2008). Leaf sheath was the thickest, primarily due to the high proportion of cortical parenchyma. This increases the storage capacity in leaf sheath, and is a significant modification under highly saline conditions (Hameed et al., 2012a).

Head Baloki is a moderately saline habitat; the population was collected from a temporary water body. Photosynthetic rate and water use efficiency was notably high in this habitat because higher

photosynthetic activity leads towards increased water use efficiency (Omamt et al., 2006). Endodermis was relatively thin in this population, which seemed not efficient enough to prevent the influx of solutes. Endodermis is a layer present outside of vascular bundle that acts as a barrier against water and ions influx like $Na^+$ and $Cl^-$ (Moller et al., 2009). Stem growth was comparatively better in this population. The Baloki habitat seemed ideal for the development of storage parenchyma that was the main tissue observed in *K. brevifolia* stem (Grigore & Toma, 2007). This tissue is capable of storing large amount of water, and therefore, critically important when plant faces water deficit conditions (Bell & O'Leary, 2003). Leaf and bract thickness were also higher in this population, and this might be due to less stressful growth conditions (Hameed et al., 2009).

Changa Manga is moderate to low-saline habitat. The population was collected from forest plantations that receive irrigation water occasionally. Root and shoot growth was negatively affected, possibly due to dry salinity and low water availability. $Ca^{2+}$ content in roots and shoots of this population was the minimum that is the possible reason for retarded growth of this population (Jiang et al., 2013). Root thickness, cortical cells and vascular region thickness decreased in Changa Manga due to inappropriate supply of water for the development of conducting and storage tissues (Younis et al., 2014). Development of stem sclerenchyma might be very beneficial as a plant can survive for much longer periods of water deficit environments by minimizing water loss (Farooq et al., 2009). Population of Changa Manga forest plantation had broader vessels but vascular region thickness decreased, which is directly related to hydraulic conductivity and water conduction efficiency (Smith et al., 2013). Leaves contain dense and smaller stomata that can regulate stomatal opening and closing more efficiently and hence minimizes water loss through transpiration (Bray & Reid, 2002).

Khanki population was collected from the low-saline waterlogged areas near the Headworks. High osmotic potential and maximum shoot fresh and dry weight was recorded in this population. Osmotic potential is directly related to better growth and development (Benzarti et al., 2014). Thicker root in this population was mainly due to larger proportion of cortical cells and extensive aerenchymatous formation. Aerenchyma in the roots enables a plant to maintain their oxygen requirements (Barrett-Lennard, 2003). Large cortical cells generally have larger vacuoles, and this proves beneficial as waterlogged conditions create physiological drought (Nawaz et al., 2012). Smaller-sized stomata as observed on leaves and large bulliform cells in bract ensure water conservation (Camargo & Marenco, 2011; Grigore et al., 2014).

Chinyot population represented better plant height and root growth parameters due to enough availability of river water. Plenty of water and non-saline soil yielded maximum plant growth because morphological characteristics have a negative correlation with salinity as reported by Alam et al (2015) and Qados (2015). Thick stem with extremely thin epidermis provides minimum protection and is an indicator of enough water availability. Desiccation tolerant plants like halophytes and xerophytes are generally equipped with thicker epidermis that is coated with thick waxy layer (Kosma et al., 2009; Liu et al., 2015). Low stomatal density and area in this population can be related to the growth condition as this population might not be best suitable for riverine habitat (Camargo & Marenco, 2011).

## 5 Conclusion

*Kyllinga brevifolia* populations showed specific physiological and anatomical modifications at their specific habitats. Growth and development were better in populations from low saline areas. The Sahianwala population collected from hypersaline saltmarsh relied on the high accumulation of $Ca^{2+}$ and organic osmolytes in shoots for high degree of salinity tolerance. Among anatomical traits, leaves and bracts were thicker and xylem tissue of leaf sheath was larger in this population. The Treemu population collected from saline seasonal inundation accumulated high concentration of $K^+$ and $Ca^{2+}$ in root and shoot accompanied with thicker roots, broader metaxylem vessels in root and stem, and larger cortical parenchymatous cells. The Baloki population collected from moderately saline temporary water body had the maximum $Co_2$ assimilation rate, water use efficiency, shoot turgor potential, leaf midrib thickness and bract lamina thickness. The Changa Manga population (from artificial forest plantation)

showed larger vascular bundle area, metaxylem area and chlorenchymatous area in bracts. The Khanki population showed higher stomatal conductance, stem cortical cell area, root and leaf epidermal thickness, bract bulliform thickness and leaf sheath aerenchymatous area. The Chinyot population from the least saline riparian area showed root modifications like larger cortical region and cell area, endodermal area and vascular region thickness. Stem area was the maximum in this population.

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**Author Contribution** Sahar Mumtaz conducted the experiment. Mansoor Hameed and Farooq Ahmad supervised the research work. Muhammad Sajid Aqeel Ahmad analyzed the data statistically and performed the multivariate analysis. Iftikhar Ahmad and Muhammad Hamzah Saleem were involved in the preparation of the manuscript. Muhammad Ashraf proofread is the group leader and finally edited the scientific and English language.

**Data Availability** The herbarium samples used for identification of plant species deposited to the Herbarium Collection of the Department of Botany, University of Agriculture Faisalabad.

**Declarations**

**Ethics Approval** The manuscript was submitted solely to Water, Air and Soil Pollution and no part was published or submitted elsewhere. All ethical guidelines set by parent institution(s) were observed during sampling and analysis.

**Consent to Participate and Publish** All authors equally participated in the execution of the experiment and unanimously agreed to publish in Environmental Science and Pollution Research.

**Competing Interests** The authors declare no competing interests.

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