Structural and functional traits for salinity tolerance in differentially adapted ecotypes of *Phragmites australis*

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Abstract

Phragmites is a well-known genus of the family Poaceae, in Pakistan, and this genus consists of only two species, i.e., *Phragmites australis* and *Phragmites karka*. In the present work, structural and functional modifications of *Phragmites* were evaluated under salt stress. Different populations of *Phragmites australis* were collected from fifteen different habitats to appraise the morpho-anatomical and physiological modifications in terms of salinity tolerance. By using Minitab statistical software, the data were analyzed for the Analysis of variance for comparison of mean values. Between morpho-anatomical and physiological characteristics the Correlation coefficient was drained. In *Phragmites australis* populations from high saline habitats limited the uptake of Na^+ supplemented by a high uptake of K^+ and Ca^{2+} in the root and shoot while there was a maximum accumulation of total free amino acids, proline and total soluble sugars. Extensive aerenchyma formation in the cell of the leaf epidermal area, and the region of the cortex also contributed to salinity tolerance. Under salt stress, bulliform cells in leaves were highly developed, so leaves could roll to reduce water and accumulate excess amounts of toxic salts. The root and shoot dry weight, the number of stomata, stomatal size, shoot length, epidermal thickness, phloem area, and chlorophyll contents were decreased due to salt stress. However, an increasing effect of salt stress was observed on sclerenchymatous thickness, aerenchyma cells, cortical cell area, metaxylem area, bulliform cell area, trichomes length and density, stomatal density and area. These species can be effectively used to re-vegetate salt-affected areas, as well as for the phytoremediation of salt-affected lands. It was concluded that *Phragmites* are a widespread perennial grass and have the potential to survive in all heterogenic environments by making variations in their structural and functional features. In the future number of genetically identical markers can be isolated from this species to improve the crop productivity and tolerance level.

Keywords: Abiotic factors; C_4 grass; Distributional pattern; Structural modifications; Species composition

Abbreviations: Soil attributes: ECe-electrical conductivity, pH-soil pH, OM-organic matter, SP-saturation percentage, Ca-soil calcium, K+-soil potassium, Na+-soil sodium, Cl– soil chloride, NO3-nitrate, PO4-phosphate. SCL = sandy clayey loam, SL = sandy loam, L = loam, CL = clayey loam, S = sandy, L = loam, LS = loamy sand, 1-Bombanwala (BO), 2–Head Baloki (HB), 3-Head Khanki (HK), 4-Nandipur (NA), 5-Lahore Model Town (LM), 6-Wapda city (WC), 7-Nala Palkho (NP), 8-Gatwala canal (GC), 9-Shorkot Cantt (SC), 10- Gojra (GO), 11-Rasool chowki (RC), 12-Kalar Kahar Lake (KKL), 13- Sahianwala Lake (SL), 14- Pakka Aana (PA), 15-Lal Suhanra (LS).

1. Introduction

Salt stress is an immense concern in Pakistan, which is located in a semi-arid to dry environment. About 6.28 million hectares of agricultural land are salinized, out of a total of 22 million hectares (Sharif *et al*., 2019). Salinization degrades 40,000 Mha of land every year (Sharif *et al*., 2019). Human activities, soil erosion, and inefficient irrigation practices, as well as traditional agriculture methods, are the main causes of salinity in Pakistan (Aslam., 2016). Pakistan is the eighth most salinized country in the world, with around 6174.5 thousand hectares salinized. Salt stress affects predominantly irrigated fields in Pakistan, with a total of 16.795 Mha. In Pakistan, somewhat saline-affected areas account for roughly 10%, moderately saline for 4%, highly saline for 7%, and non-salinized areas for 73 % (Gorji *et al*., 2017).

Soil salinity causes changes in physio-biochemical functions in plants primarily by osmotic stress and later ionic stress (Majeed and Muhammad, 2019). The osmotic imbalance incites water deficit and reduces new leaf production and shoot growth (Hassan *et al.,* 2021) though the older leaves cause early senescence due to high Na^+ ions accumulation ionic stress (Munns, 2002). The stomata are closed early (Zhang *et al.,* 2022), reduces CO_2 intake (Shahzad et al., 2019), decrease the chlorophyll contents (Saddiq *et al.,* 2019), photosynthetic efficiency (Zahra *et al.,* 2022) relative water contents due to osmotic and ionic stresses (Sarwar and Shahbaz, 2019) and ions uptake (Iqbal *et al.,* 2019), which divert the balance between reactive oxygen species and antioxidants so ultimately execute harmful effects on membranes of different organelles (Huang *et al.,* 2021).

Under stressful conditions, halophytic species demonstrate morphological modifications, such as above-average intensity of stomata on the upper leaf surface (Kwon., *et al*., 2019). Halophytes tend to take in and accumulate Na^+ in their vacuoles under high saline conditions, and utilise it as an osmoticum (Shahzad., *et al*., 2022). Salt stress raises Na^+ levels and lowers K^+ and the K^+/Na^+ proportion (Gul *et al*., 2019).

Halophytes have adapted physiological mechanisms to extreme salinity and flourish by employing two strategies i.e. salt tolerance and salt avoidance. In general, halophytes use three salt tolerance mechanisms i.e. reduced Na+ influx, compartmentalization, and sodium ion excretion (Kotula *et al.,* 2020). Sodium salts, particularly sodium chloride, cause salt stress in plants (Grigore *et al*., 2019). The halophytes generally grow in soil that becomes more saline as a consequence of the rapid vaporization of water during the summer season, resulting in a higher soil salinity on the surface (Fu *et al.,* 2020). Seeds of halophytes grow optimum in non-saline surroundings, and their sprouting rate declines as salinity increases (Alhaddad *et al*., 2021). The halophytes show various responses to Na^+ and Cl^- accumulations throughout their development (Kumar *et al.,* 2018).

Poaceae, the fifth biggest plant family, of grasses has about 12000 species and 668 genera with a wide range of distribution. (Singh *et al*., 2022). Approximately 20% of the land is protected by grasses and they are the main component of the rangelands (Kellogg, 2014). Poaceae species can be found in a variety of environments, including wetlands, marshy swamps, saline marshes, steep foothills, and seasonal inundations throughout the global biome (Christenhusz and Byng, 2016). Various cereal crops i.e. *Triticum aestivum, Zea mays, Avena sativa, Oryza sativa, Saccharum officinarum, Sorghum bicolor*, and *Pennisetum glaucum* are all members of this family. Improving the invention of these crops is a crucial need in the contemporary era to fulfil the food necessities of an ever-rising population (De Wrachien *et al*., 2021).

Phragmites (Adans.) *Phragmites australis* (Cav.) Trin. ex Steud. is a widely distributed genus with phenotypic plasticity and a wide variety of morphological and genetic variants (Saltonstall, 2016 #19). *Phragmites* is a halophytic grass in the Poaceae family that can be found in saline wetlands, along with briny environments like river reservoirs and lakes. The plant is up to 6 meters tall and can be found in temperate regions along the littoral regions of lakes, rivers, irrigation vessels, and clean water bogs (Batty, 2021). *Phragmites australis* may thrive in a

variety of saline environments because its roots release a poisonous acid known as 3, 4, 5-trihydroxybenzoic acid (gallic acid), which disintegrates the structural protein of neighbouring plants. *Phragmites australis* is known to hinder the growth of other species (Rudrappa *et al.*, 2021).

Worldwide, the common reed (*Phragmites australis* Cav.) is one of the most widely spread species of promising plant (Errico et al., 2019). The adaptive features of the common reed show its modest character, due to high intraspecific diversity, along with its phenotypic flexibility, the plant shows a wide ecological range (Milke et al., 2020). Furthermore, the plant shows a high capacity for adaptation to climatic conditions which are considered antagonistic (Reijers et al., 2019). This plant has been used for many years in phytoremediation to purify various types of wastewater (Dias et al., 2021). In the current study, we systematically explored the modifying effect of salinity on morphology, growth and osmolytes in different common reed ecotypes.

It is expected that they may respond variably to different soil and environmental conditions. The response of each species must be different and specific, which may depend on their adaptability potential. Soil physio-chemical characteristics may alter morpho-anatomical and physiological characteristics in these species Poaceae, which enable these species to colonize a variety of environmental conditions. The present research aimed to explore the role of functional and structural traits in modifications in *P. australis* and elucidate the degree of salinity tolerance, the role of morpho-anatomical physio-chemical features of *P. australis* populations that enabled them to flourish in saline wetlands. Another objective laid out for this study was to evaluate the feasibility of this species as a candidate for phytoremediation of saline marshes and marginal wetlands.

2. Material and Methods

Frequent surveys were conducted in the spring season for collections of naturally adapted populations of species of *Phragmites*. The current study aimed to examine the adaptive features of species of *Phragmites* (Poaceae Family) Naturally adapted populations of *Phragmites* were collected from 15 different habitations of Punjab province. All of the average-size plants were selected randomly from each study site and considered as replicates. Collection was made based on soil physicochemical characteristics and habitat types ***Phragmites australis*** populations were collected from 15 different sites i.e. Bombanwala, Head Baloki, Head Khanki, Nandipur, Lahore

Model Town, Wapda City, Nala Palkho, Gatwala canal, Shorkot Cantt, Gojra, Rasool chowki, Kalar Kahar Lake, Sahianwala Lake, Pakka Aana, Lal Suhanra. (Fig. 1).

2.1.Soil properties

The samples of soil were collected from the plant rhizospheric portion at 15cm depth. Individually soil sample was preserved in polythene bags and tagged and then used to examine the soil's physic-chemical properties. The hydrometer technique was used to determine the soil texture. In a glass container (400 ml), a soil sample (100 g) was collected. It was then mixed with distilled water (200 mL) and a solution of 1% sodium hexametaphosphate (125 mL). The samples were soaked overnight. Finally, the samples were taken to a dispersion cup and thoroughly blended with an electric mixer for (five minutes). The contents were taken in a sedimentation cylinder (1L), and the volume was increased to 1L by pouring distilled water. The Bouycous-hydrometer was used to calculate the silt and clay fractions, whereas the sand fraction was calculated by subtraction. The texture class of each soil sample was determined using a textural triangle (Brady et al., 2008).

2.2. Saturation percentage

To make the saturation paste of 200g, in the oven soil was dried (at 70°C). The saturation percentage was calculated using the following formulas:

SP (%) =Amount of water added (g) / Mass of oven-dried soil (g) x [100-Pw]

Where SP% is saturation percentage, PW is known as water content.

2.3. Soil pH and electric conductivity (ECe)

The soil extract was used to measure the ECe (electrical conductivity) and pH by using a pH/EC metre (WTW series InoLab pH/Cond 720).

2.4. Organic matter (Walbley)

The litter was separated from the sample, organic matter was measured using Walkley and Black's titration technique (Walkley and Black, 1934), and then the readings were obtained in percentage. The following formula was used for the final calculation:

The percentage of organic matter = S-T x 6.7/S

here, S= blank reading, T= $FeSO_4$ used volume

2.5. Soil inorganic content (Flame photometric method)

The soil ionic contents (Ca^{2+}, Na^+ and K^+) were determined using a flame photometer from soil water extract. The extract was used for the determination of Cl^- by mixing the samples of plants (100 mg each) in 10 ml of water, grinding, and heating at 80ºC until the volume was attained. The extract was then read using a chloride meter (Jenway, PCLM 3).

2.6. Morphological parameters

The height of the Plant (cm), the length of the root (cm), the length of the shoot (cm), the dried and fresh weight of the root and shoot (g), flag leaf area (cm2), and inflorescence length were the morphological characteristics studied (cm). Five plants were chosen from each site for morphological research. The 65°C temperature was used to oven-dry the plants and a steady weight was attained. A digital balance was used to weigh the sample, and a measuring scale was used to record its length.

2.7. Physiological parameters

2.7.1. Photosynthetic pigments

For each sample, 0.5g of fresh leaf material, was crushed and placed into a vial. Each vial was filled with 80% acetone and left overnight to dissolve completely. The chlorophyll contents (Chla, Chlb, and Carotenoids) were determined by using Arnon (1967) technique. The following formulae were used to compute the final value:

Chl. a (mg g-1 f.wt).=12.7OD663- 2.69OD645xV1000x W]
Chl. b (mg g-1 f.wt).=22.9OD645- 4.68OD663xV1000x W]
Carotenoids (mg g-1 f.wt.=12.7OD480- 0.114 OD663-0.638 (OD645)]/2500

2.7.2. Glycine betaine (GB)

The Grieve et al. (1983) protocol was used to determine the glycine betaine content of leaves. Dry leaf material (500 mg) was completely ground in a tissue grinder with 20 mL of distilled water and stored at room temperature overnight. One mL of the extract was extracted from the mixture and dissolved in 2NH2SO4. Following that, 0.5mL was collected in a separate tube and mixed with KI3. For 90 minutes, the test tubes holding the reaction mixture were placed in a cold

bath. In each tube, pre-chilled deionized water and 1,2-dichloroethane were also added. Two layers emerged, with the top one eliminated, and absorbance was noted at 365nm.

2.7.3. Free amino acid contents

The total amino acid content was measured using the method of Hamilton and Van Slyke (1943). The 0.5 g plant material was homogenised in five ml of the buffer of citrate with a pH of 5.0 and 20 g of C6H8O7. Water was dissolved in a 200 mL solution of 1N sodium hydroxide, and the amount was then increased to 500 mL using deionized water. After incubating homogenised material at room temperature for 1 hour, reactants were centrifuged for 10 minutes at 15,000 rpm at 15 °C. Then, in tiny (10 mL) test tubes, 1 mL of aliquot was carefully aspirated and completely reacted with ninhydrin acid (1.25 g of ninhydrin warmed in 20 mL 6M H_3PO_4 and 30 ml CH_3COOH, held for 24 h.).

For one hour at 80 °C, the reaction mixture was immersed in a boiling water bath. The cold water was used to stop the process. Each tube was then filled with 5 mL of diluent (n-propanol; H_2O, 1:1). A UV visible spectrophotometer set to 570 nm was used to measure absorbance. The calibration stock solution was made by dissolving 0.06 g of leucine in 100 ml of deionized water. Different concentration ranges (0.1-0.9 mg/g mL) were utilised to plot a standard curve by dilution.

2.7.4. Total soluble sugar contents

The soluble sugar content was quantified using the technique developed by (Yemm and Willis, 1954). A 0.5g plant sample was crushed in ten ml of eighty per cent ethanol. The extracted material was centrifuged for 10 minutes at 4000 rpm (revolution per minute) before being diluted with 80 per cent ethanol to a volume of 15 ml. A 0.1 ml aliquot was obtained and by applying dist. water the volume of one ml was maintained. In a boiling water bath, the five ml of anthrone reagent was reacted with supernatant (made from dilute H_2SO_4 and 0.2 % anthrone conc.). The test tubes were immersed in cold water to completely halt the reaction. At 620 nm, absorbance was measured using a UV 20 visible spectrophotometer. Total soluble sugar contents were calculated using a standard curve as mg. g1 FW.

2.7.5. Proline determination

The procedure to determine the proline contents was disclosed by Bates *et al*. in 1973. Fresh shoot weight weighing 0.5 g was homogenised with 10 ml of 3% sulfo-salicylic acid. Whatman No. 2 filter paper was then used to filter the extract. In a test tube at 100 C for one hour, 2 mL of

the filtered plant material was combined with 2 mL of glacial acetic acid, 2 mL of acid ninhydrin solution (1.25 g ninhydrin in 30 mL glacial acetic acid), and 20 mL of 6 M orthophosphoric acid. Then an ice bath was used to store these test tubes. This reaction mixture was extracted using 4 ml of toluene and quickly stirred for 1-2 minutes with air streams running through it. Toluene was used as a blank to measure the absorbance at 520 nm after the toluene-containing chromophore was extracted from the aqueous phase. The following formula was used to calculate the concentration of proline using fresh weight and the standard curve.

$$\text{Proline } (\mu\text{mol } g^{-1} \text{ fresh weight}) = \frac{\mu \text{ g proline } (ml^{-1}) \times \text{ml of toluene}}{15.5 \text{ sample weight (g)}}$$

2.8. *Anatomical parameters*

A tiny 2-cm portion of plant material leaf blade was used for anatomical studies. It was preserved in a solution of formalin and acetic acid (FAA) (five per cent formalin, ten per cent acetic acid, 35% distilled water and fifty per cent ethyl alcohol). Following that, samples were transferred in vials comprising the solution of acetic alcohol (one part acetic acid and 3 parts ethyl alcohol) for long-term preservation. The portions were cut using a Free-hand sectioning technique with the plant portions by double-edged blade. By serial dehydrating the ethanol, substantial slides were formed (Ruzin, 1978). The sections were stained by succeeding the conventional double staining method of safranin and fast green (1% safranin for mechanical tissues and primary tissues 1% fast green). Photographs were captured from permanent slides using a digital microscope connected to a camera. All tissue measurements were taken with an ocular micro-metre and calibrated with a stage micro-metre.

The formula for calculating area was obtained from the circle formula, which is as follows:

$$\text{Area} = \text{Maximum length (cm)} \times \text{maximum width (cm)} \times 22/28$$

2.9. Statistical analysis

The data for all morpho-anatomical and physiological characteristics were recorded in three replicates, whereas for soil physicochemical characteristics of rhizospheric soil of three plants of each population were taken. *Phragmites australis* species from 15 different ecozones of Punjab were selected for determining the morpho-physiological and anatomical response to soil physico-chemical characteristics. The data was used for statistical analysis by using Microsoft Excel software and Minitab statistical software for one-way analysis of comparison of mean values and

analysis of variance (ANOVA) and of each characteristic for each species separately. Moreover, the data was also used for cluster analysis, multivariate analysis (PCA), and heat map and using an R statistical software(R Core Team, 2019) to determine the correlation between studied traits.

3. Results

3.1. Soil Physico-Chemical Characteristics

All populations of *Phragmites* showed enormous variations in soil physio-chemical characteristics, collected from different habitations of Punjab Pakistan. Soil ECe was maximum in LS high saline while minimum in BO non-saline habitat (Table 2). Soil pH was maximum in BO non-saline habitat while it was minimum in SC high-saline habitat (Table 2). The highest concentration of Ca^{2+} was in SC, KKL and PA high saline sites while the lowest was recorded in HB non-saline habitat (Table 2). Soil phosphate (PO_4^{3-}) was the maximum in GO high saline, while the minimum in the NA non-saline site (Table 2). The high concentration of soil NO_3^- was recorded in the KKL high saline habitat, whereas the lowest was in the HB and NA non-saline habitat (Table 2).

3.2. Morphological characteristics

3.2.1. Dry weight of shoot / fresh weight of Shoot

Maximum shoot dry weight was in the SC population from high saline habitat and NP population from low saline habitat, while its minimum was in BO population from a non-saline habitat (Table 3). The fresh weight of the shoot was maximum in SC population from high saline habitat and WC and NP populations from low saline habitats while minimum in BO population from non saline habitats (Table 3).

3.2.2. Dry weight of root/ fresh weight root

The dry weight of the root was maximum in the NA population from non-saline habitat and minimum in GC population from low saline habitat (irrigated land) (Table 3). Root fresh weight was maximum in GC population from low saline habitat and RC population from high saline habitat while it was minimum in LM population from low saline habitat and HB population from non saline habitat (Table 3).

3.2.3. Flag leaf area

Leaf area was maximum in BO population from non-saline habitat and minimum in GC population from low saline and KKL from high saline habitat respectively (Table 3).

3.3. Physiological parameters

3.3.1. Organic osmolytes

Proline showed a variable pattern among all the populations of *Phragmites*. The maximum amount of proline was in the population SL from high saline habitat while its least quantity was in the BO population from non saline habitat and WC and GC from low saline habitat (Table 4).

Total soluble sugar showed variable patterns among all the populations of *Phragmites australis*. The maximum amount was recorded in the population of NP population from low saline habitats and RC population from high saline habitat while its least quantity was recorded in the LM and WC populations from low saline habitats (Table 4). In *Phragmites australis* the maximum amount of glycine betaine was recorded in the population SL from high saline habitat while its least quantity was recorded in the HK population from non saline habitat and WC from low saline habitat (Table 4). Amino acid concentration showed variation among all the populations of *Phragmites australis*. The maximum amount of amino acids was recorded in the population SL and LS populations from high saline habitats, while its least quantity was recorded in the BO from non-saline habitats and LM populations from low saline habitats (Table 4).

3.3.2 Photosynthetic Pigments

Chlorophyll amount showed a variable pattern among all the populations of *Phragmites australis*. The maximum Chl. *a* content was recorded in RC population from the high saline habitat while its least quantity was recorded in GC population from the low saline habitat (Table 3). Chlorophyll b content showed variation in different populations of *Phragmites australis*. The maximum amount of Chl *b* was recorded in the GO and KKL populations from high-saline habitats while its least quantity was recorded in GC population from low saline habitat and HB from non-saline habitat (Table 3).

The amount of carotenoids varied in all the populations of *Phragmites australis*. GO and SL populations from high saline habitats showed the maximum amount of carotenoids while its least quantity was recorded in the BO population from non-saline habitat and the WC population from low saline habitat (Table 3).

3.4. Ionic contents

In *Phragmites australis*, shoot K^+ was higher in population SL and LS from high saline habitats. The population GC from low saline habitat had a minimum accumulation of K^+ (Table 4). Highest content of Na^+ in the shoot was in SL from a high saline habitat. This parameter was minimal in NA population from non-saline habitat (Table 4). The highest shoot Ca^{2+} was analyzed in KKL population from the high saline habitat, while the minimum was in LM population from low saline habitat (Table 4).

3.5. Anatomical characteristics

3.5.1. Leaf anatomical characteristics

There was significant variation in lamina thickness of different *Phragmites australis* populations. Maximum lamina thickness was recorded in the KKL population from high saline habitat, whereas the minimum thickness was in the GC population from low saline habitat (Table 5, Fig. 3). Sclerification was the most diverse characteristic found in all populations. The maximum sclerification was noted in a population of SL from high saline habitat, whereas the population of BO from non saline habitat, GC and WC from low saline habitat had the minimum sclerification (Table 5, Fig. 3). Bulliform cell area was highest in the KKL population from high saline habitat, whereas it was lowest in the BO and HB populations from non saline habitat (Table 5, Fig. 3). The maximum epidermal thickness was noted in GO population from high saline habitat followed by WC and LM populations from low saline habitat. The minimum thickness was in PA population from high saline habitat (Table 5, Fig. 3). The populations of HB from non-saline habitat had the maximum vascular bundle area VBA compared to the rest of the populations, while the GC population from low saline habitat and KKL and LS population from high saline habitat had the minimum vascular bundle area (Table 5, Fig. 3). All populations of *Phragmites* exhibited enormous variability in metaxylem area. The populations KKL from high saline habitat had the maximum metaxylem area, whereas HK population from non-saline habitat and GC population from low saline habitat had the minimum metaxylem area (Table 5, Fig. 3). Phloem area was maximum in the HB population from non-saline habitat, whereas it was minimum in SL and LS populations from high saline habitat (Table 5, Fig. 3).

3.5.2. Epidermal features

The adaxial stomatal density was highest in NP population from low saline habitat, whereas it was lowest in LS population from the high saline habitat (Table 6). The populations NA from non saline habitat showed maximum stomatal density, while minimum stomatal density was observed in the WC and LM populations from low saline habitats (Table 6). The adaxial area of stomata was maximum in the GC and NP populations from low saline habitats whereas minimum in the LS population from high saline habitats (Table 6, Fig.5.). The abaxial stomatal area was maximum in LM population from low saline habitat and HK population from non saline habitat. On the other hand, this parameter was minimal in SL population from high-saline habitat (Table 6, Fig. 4).

3.6. Multivariate analysis

3.6.1. Principal component analysis

Principal component analysis (PCAs) had shown a noteworthy effect of soil physiochemical contents on the anatomical characteristics of a leaf of *Phragmites* spp. (*Phragmites australis*). The first and second PCAs explained 36.2% and 16.7% (52.9%) variability among the soil and leaf traits. Principal component analysis (PCAs) noticed a major effect of soil physiochemical contents on the physiological traits of *Phragmites* spp. (*Phragmites australis*) (Fig. 6). The first and second PCAs explained 39% and 17.2% (56.2%) variability among the soil and physiological traits. Major contributors to the high saline habitat were Proline and Glycine betaine contents (Fig. 6). Principal component analysis (PCAs) revealed a significant effect of soil physiochemical contents on the physiological traits of *Phragmites* spp. (*Phragmites australis*) (Fig.6). The first and second PCAs explained 30.6% and 14.8% (45.4%) variability among the soil and Morphology and leaf traits. Major contributors to the high saline habitat were SDW and AdSD traits. Principal component analysis (PCAs) showed variation among the soil physiochemical contents on the morphological traits of *Phragmites* spp. (*Phragmites australis*) (Fig. 6). The first and second PCAs explained 36.5% and 17.7% (64.2%) variability among the soil and Morphological parameters. Major contributors to the high saline habitat were SDW and RFW traits (Fig. 6).

3.6.2. Clustered heatmap and Pearson correlation matrix

A clustered heatmap was constructed to evaluate variability and association between traits and collection sites. A moderate positive association was assessed between RFW, NO, SP, PO, S.Ca, S.K, ECe, S.Na, S.Cl and collection sites KKL, RC, PA, LS, SL, while these traits were negatively linked with sites NA, HB, LM, BO, HK, and WC (Fig. 7). A strong positive influence was assessed between traits ScT, VBA, and PhA with HB and LM sites, while the least positive linkage was shown with NP, RC, PA, NA, HK and WC. The traits TSS, AbSA, AdSD, Ca, Na, A.A, OM, K, Chl *a* and *b* were positively associated with GC, NP, HK, and BO. The site BO negatively corresponded to SDW and SDW while strong positive influence on LA of the plants (Fig. 7).

The traits undertaken in this study showed a significant correlation ($p \leq 0.05$) in plants collected from various ecozones. The K showed a positive correlation with RFW, Chl *a*, *b*, S.Ca, S.K, S.Na, S.Cl, NO, PO, while Na possesses a negative correlation with all of these traits (Fig. 8). The RFW was significantly and positively correlated with organic osmolytes such as Pro, GB, TSS, and S.Ca, S.K, S.Na, S.Cl, NO, and PO. The AA, Chl *a*, *b*, Cart, LtK, EpT, ScT, and VBA strongly positive and negative correlation with S.Ca, S.K, S.Na, S.Cl, NO, PO. AbSD and pH have also shown the same negative relation with all of these traits (Fig. 8).

5. Discussion

In the present study, *Phragmites* species responded clearly to salt stress levels by showing adaptations in structural and functional features. *Phragmites* are adapted to soil with high salinity, therefore, they can be considered the best grass that is salt tolerant among all grass species. *Phragmites* is a cosmopolitan genus of Poaceae with a wide range of morphological and genetic variations as well as phenotypic plasticity (Saltonstall, 2016). *Phragmites australis* has a wide distribution and thrives in both freshwater and brackish environments due to environmental heterogeneity and phenotypic plasticity (Eller et al., 2017). *Phragmites australis* can grow in extremely saline areas due to its wide adaptations to varied soil conditions (Liu et al., 2018).

5.1. Morphological characteristics

The plants growth and development is diversely affected by salinity (Saddiq et al., 2021a; Saddiq et al., 2021b), and the morpho-anatomical adaptations are severely affected by salinity (De Micco et al., 2021). Under salt stress, deleterious effects (plant death or decrease in

productivity) are observed at the whole-plant level (Saddiq et al., 2021a; Saddiq et al., 2021b; Riaz et al., 2022). Halophytes are those plants that are capable of tolerating high levels of salinity (Hafeez et al., 2021).

In the present research, *Phragmites* species showed adjustable responses to the stress of salinity. All the populations of *Phragmites australis* from Punjab province responded differently to agro-morphological attributes in different habitat types. The populations NA collected from non-saline habitats and RC from high saline habitats showed a maximum of all growth attributes like length of shoot, flag area of leaf and inflorescence length (dry and fresh weight). An increase in biomass production and overall growth attributes is an attribute of species tolerance (Mohsin et al., 2023). All of these features play a significant role in the wide distribution and survival of a species like *P. antidotale* across a heterogenic environment (Hussain et al., 2019).

Elongated roots of *Phragmites australis* help it to extract water from a deeper layer of soil, which ensures its survival under severe stress conditions. (Ahkami et al., 2017). Water use efficiency increases in *Phragmites* species as the salt stress increases, but may be critical under limited moisture availability that is caused by physiological drought. Due to this tolerant mechanism, *Phragmites* can grow under hazards environmental (Klink et al., 2019),

5.2. Physiological characteristics

Plants adopt a defensive strategy by accumulating organic osmolytes to avoid plant desiccation and folding of living tissue (Madani et al., 2019). Maximum proline was found in the SL population from high saline habitat and amino acid in the LM population from a non-saline habitat. All halophyte plants have almost the same morpho-anatomical adaptations against slat stress as halophyte plants can accumulate high concentrations of organic osmotica, mainly glycine betaine, proline, and free amino acids. Plants use different strategies to mitigate various kinds of stresses among these strategies accumulation of organic acids is useful for the successful survival of plants (Zia et al., 2021). To detoxify the lethal effect of toxic ions plants used limited uptake of noxious ions with beneficial ions (Gupta et al., 2023). The plants having increased concentrations of salt have high levels of Na^+ and Cl^- and less concentration of K^+, Ca^{2+} and Mg^{2+} (Łuczak et al., 2021). Toxic levels of Na^+ in plants can cause to decrease in the Ca^{2+} concentration in plants (Zhao et al., 2021), but in a recent study, *Phragmites australis* showed an increased level of Ca^{2+} in a shoot in the KKL population from high saline habitat. Under

unfavourable environmental conditions, this might play a crucial role in the survival of these species

The plasma membrane in the root is depolarized by salt stress. This depolarization occurs due to potassium leakage (Tian et al., 2021). In many plants, such ion leakage occurs including halophytes and glycophytes (Khandare et al., 2023). Similarly, Na+ and Cl- content in the root of this plant were positively related with most of the morphological parameters confirming the halophytic nature of this plant. This is also reported by (Moghaddam et al., 2023) in *Salicornia persica* and (Toscano et al., 2022) in *Callistemon citrinus* (Curtis).

All the populations significantly responded to their environment in the form of altered physiological traits. Salinity affects thylakoids and grana lamella resulting in the reduction of chlorophyll pigments (Feng et al., 2021). In *Phragmites australis* Chl *a* concentration was maximum in the RC population from high saline habitat. In salt-tolerant plants degradation occurs, and Chl *b* is converted into Chl *a* increasing by increasing, Chl *a* content (Guo et al., 2022). In *Sesuvium sp.* (a halophytic plant) there is a significant increase in carotenoids, Chl *a*, and Chl *b* under salt stress. It is speculated that higher light-harvesting capacity results in more efficient photosynthetic activity (Peng et al., 2019). Another strategy of *Phragmites australis* populations was the accumulation of organic osmolytes such as total soluble proteins, free amino acid, soluble sugars, glycine betaine and proline to prevent tissue desiccation and collapse (Mann et al., 2023). In this study maximum value of Na^+ and K^+ in SL and LS populations from high saline habitats. The negative impact of toxic ions on roots is membrane depolarization resulting in the restricted uptake of beneficial ions (Peng et al., 2019).

5.3. Anatomical characteristics

Anatomical features are more susceptible to ecological conditions and hence, strongly responsive to multiple environmental stresses (Mohamed et al., 2020). An important strategy of plants adapted to drought or any other stress conditions is water conservation. To conserve water in such an environment, plants alter their physiological and morpho-anatomical attributes (De Micco et al., 2021). Under a restricted supply of water, they conserve water either by an increasing proportion of parenchymatous tissues, widening of metaxylem vessels, and mechanical strengthening of tissues (Amitrano et al., 2021).

The anatomical structures vary greatly in different plant species and are significantly important. In leaves, stem and root, phloem, size and nature of cortex and sclerenchymatous tissues have significant importance (Ahmad et al., 2023), and play an important role in the tolerance of abiotic stress particularly under salinity and aridity (Ahmad et al., 2023; Wasim et al., 2020).

5.3.3. Leaf blade anatomy

Maximum lamina thickness was observed in the KKL from the population that was collected from high-saline habitat. It is mainly due to epidermal thickness, sclerenchyma thickness and enlarged bulliform cells, and therefore is an important mechanism to survive in harsh environments (Wasim et al., 2020). Maximum sclerification was noted in population SL from high saline habitat. Sclerification in tissues is an abrupt response of those plants that are subjected to drought and salt stress that provide mechanical strength to soft tissues and also protect from excessive loss of water (Luo et al., 2021). Bulliform cell area was highest in the KKL population collected from high saline habitat. The formation of large bulliform cells is extremely important for water conservation when water is a vital commodity (Tufail et al., 2023). Leaf micro-structural adaptations like trichomes and stomatal aperture size and density are involved in controlling of transpiration rate, thereby reducing the impact of temperature and wind speed on the surface of a leaf. The adaxial area of stomata was maximum in GC and NP populations from non-saline habitats. All of the plants have been found with very few and smaller stomata on the adaxial leaf surface. This stomatal orientation on the abaxial surface is a special adaptation to overcome the maximum transpirational rate (das Neves et al., 2022). Vascular bundle area significantly decreased (Zhang et al., 2021) in almost all populations during salt stress, whereas the area of the metaxylem increased in population KKL collected from high saline habitats. It ratifies its better tolerance that other populations with wider vessels can translocate more water (Wasim et al., 2020) and that's why it has a vital role in the limited availability of moisture (Humphrey et al., 2021).

Conclusion

It is concluded that species of *Phragmites (P.australis)* can easily survive in non-saline and low-saline habitats, however, within some populations, growth was reduced under the

highest concentration of salt. The species of *Phragmites* showed varied responses under salinity and adopted different mechanisms to combat salinity by morpho-anatomical and physiological modifications. Salt-tolerant genes from these species can be incorporated into cultivated crops to improve salinity tolerance. These species can also be used to rehabilitate salt-affected barren lands and for phytoremediation.

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Author contribution statement

This is a thesis work for the Ph.D. of Sana Maqsood who has done the experiments under the supervision of Farooq Ahmad. Farooq Ahmad and Muhammad Sajid Aqeel Ahmad designed the experiment and analyzed the data. A final draft is reviewed and edited by Sana Maqsood, Farooq Ahmad, Muhammad Sajid Aqeel Ahmad and Muhammad Shahbaz Naeem.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Table 1. Showing habitats and coordinates of differently adapted populations of *Phragmites spp.* collected from various ecozones of Punjab province

Ecozones	Districts	Collection sites	Habitat	Longitude (N)	Latitude (E)	Altitude (m.a.s.I)
Phragmites australis						
Bombanwala	Sialkot	BO	Along canal bank	°32'21.34"	74°18'12."	234
Head Baloki	Qasur	HB	Bank of river	31°11'37.96"	73°52'34.65"	195
Head Khanki	Gujranwala	HK	Bank of river	32°23'50."	73°58'51."	222
Nandipur	Gujranwala	NA	Along the lake	32°15'24.65"	74°15'51.44"	232
Lahore Model Town	Lahore	LM	Marshy place	31°28'58.99"	74°20'03.58"	229
Wapda city	Faisalabad	WC	Canal bank	31°30'04."	73°13'10."	190
Nala Palkho	Wazirabad	NP	Waterlogged area	32°26'07.72"	74°07'30.57"	232
Gatwala canal	Faisalabad	GC	Irrigated land	31°30'04."	73°13'10."	190
Shorkot Cantt	Jhang	SC	River bank	30°47'00.82"	72°14'32.03"	145
Gojra	Toba Tek Singh	GO	Water pond	31°08'46.59"	72°40'53.96"	165
Rasool chowki	Mandi Bahauddin	RC	Bank of river	32°41'42.56"	73°33'59.22"	235
Kalar Kahar Lake	Chakwal	KKL	Near the lake	32°46'33.77"	72°43'29.89"	652
Sahianwala Lake	Faisalabad	SL	Salt marsh	31°38'33.40"	73°14'03.89"	192
Pakka Aana	Faisalabad	PA	Saline area	31°14'39.94"	72°47'46.31"	173
Lal Suhanra	Bahawalpur	LS	Along the road	29°26'27.77"	71°59'02.89"	129

Table. 2. Showing soil physiochemical attributes of river/canal bank populations of *Phragmites sp.*collected from Punjab province

Phragmites australis

			ECe	pH	OM	SP	Ca^{2+}	K^+	Na^+	Cl^-	NO_3	PO_4	ST
Non-saline	Bombanwala-Sialkot	BO	1.2	8.5	1	32	8.6	41	38.9	34.5	2.2	4.4	SL
	Balloki Headworks-Qasur	HB	1.3	8.1	0.7	31	7.4	28	44.8	38.6	1.6	4.9	CL
	Khanki Headworks-Gujjranwala	HK	1.4	8.4	0.7	33	15	35	71.5	65.8	2.1	5.6	L
	Nandipur-Gujranwala	NA	2.3	7.3	0.8	40	11	38	105	95.5	1.6	1.7	L
Low saline	Lahore-Model Town	LM	4.5	8.3	0.8	38	50	99	233	223	3.6	6.1	SCL
	WAPDA City-Faisalabad	WC	5.5	8.2	0.6	34	40	82	371	332	3.6	7.4	L
	Nala Palkho-Wazirabad	NP	6.1	8.2	0.6	34	40	82	465	455	3.6	7.4	S
	Gatwala Canal-Faisalabad	GC	6.3	7.4	0.8	39	27	75	497	468	3.8	9.6	L
High saline	Shorkot Cant-Jhang	SC	7.3	7.1	0.8	37	71	135	576	486	8.8	16.8	SL
	Gojra-Toba Tek Singh	GO	7.5	8.2	0.8	39	67	136	715	596	4.5	18.2	CL
	Rasool Choki-Mandi Bahauddin	RC	8.1	8.5	0.9	40	72	138	894	731	3.3	13.2	CL
	Kalar Kahar Lake-Chakwal	KKL	9.4	8.4	0.9	36	71	142	1042	944	9.3	3.3	SCL
	Sahianwala Lake	SL	27.5	8.5	0.8	39	74	148	2861	2575	4.6	3.9	SL
	Pakka Anna-Faisalabad	PA	29.1	8.4	0.9	36	71	141	3067	2634	4.8	15.9	SL
	Lal Suhanra-Bahawalpur	LS	30.1	8.3	0.8	34	69	138	3161	2712	2.5	6.4	L

Table legends: ***Phragmites australis*** 1-Bombanwala;2–Head Baloki;3-Head Khanki;4-Nandipur;5-Lahore Model Town;6-Wapda city;7-Nala Palkho;8-Gatwala canal;9-Shorkot Cantt;10- Gojra;11-Rasool chowki;12-Kalar Kahar Lake;13- Sahianwala Lake;14- Pakka Aana;15-Lal Suhanra. **Soil attributes:** ECe(dS m^{-1}) electrical conductivity, pH-soil pH, OM-organic matter(%), SP-saturation percentage(%), Ca-soil calcium Ca^{2+} (mg kg^{-1}), K+-soil potassium K^+ (mg kg^{-1}); Na- Soil Na^+ (mg kg^{-1}); Cl-Soil chloride Cl^- (mg kg^{-1}); NO- Soil NO_3^- (mg kg^{-1}); PO- Soil PO_4^{3-} (mg kg^{-1}) **Soil texture:** SCL = sandy clayey loam, SL = sandy loam, L = loam, CL = clayey loam, S = sandy, L = loam, LS = loamy sand

Table 3. Growth traits and photosynthetic pigments of *Phragmites australis* ecotypes collected from different area of Punjab, Pakistan.

Habitats	Ecotypes	SDW	SFW	RDW	RFW	LA	Chl *a*	Chl *b*	Cart.
Non-saline									
	BO	30.93±2.60k	284.12±0.76k	0.38±0.01e	3.62±0.15e	335.69±2.03a	1.43±0.01j	0.81±0.01d	0.22±0.01l
	HB	48.33±0.92f	484.63±0.82d	0.23±0.02m	1.43±0.12m	260.15±1.73d	0.71±0.00c	0.22±0.01l	0.42±0.02f
	HK	53.05±2.63a	505.53±1.50a	0.40±0.04d	3.43±0.08f	195.75±1.72k	1.43±0.01h	0.62±0.01f	0.53±0.04e
	NA	52.71±3.26b	493.66±0.81c	0.51±0.03a	2.41±0.05i	242.97±0.89g	0.42±0.01i	0.31±0.00i	0.25±0.03j
Low saline									
	LM	46.65±2.33h	444.86±1.80h	0.34±0.01i	1.31±0.14n	231.69±1.21h	0.81±0.02b	0.42±0.01h	0.31±0.01i
	WC	54.09±2.57a	515.61±0.66a	0.21±0.03n	1.66±0.18k	178.09±1.46l	1.62±0.01d	0.82±0.01c	0.23±0.02k
	NP	54.67±3.33a	513.41±0.80b	0.36±0.02f	2.66±0.18g	273.12±1.73c	1.52±0.01g	0.71±0.00e	0.62±0.01c
	GC	44.97±2.64j	424.41±1.11i	0.17±0.01o	5.31±0.05a	157.42±1.45n	1.93±0.01a	0.21±0.00m	0.21±0.00m
High saline									
	SC	54.77±3.23a	516.73±1.67a	0.36±0.04g	3.91±0.06d	282.27±1.46b	0.51±0.01g	0.41±0.00h	0.32±0.01h
	GO	47.14±2.56g	445.41±0.55g	0.35±0.02h	1.56±0.19lk	259.99±1.72e	0.61±0.02f	0.92±0.01b	0.85±0.01a
	RC	49.09±2.63e	465.63±1.05f	0.25±0.03j	5.31±0.04a	252.83±1.73f	1.93±0.01a	0.30±0.02k	0.22±0.02l
	KKL	50.61±3.31d	472.66±1.12e	0.43±0.04b	2.46±0.21h	171.38±0.89m	0.31±0.00j	0.95±0.01a	0.43±0.01f
	SL	45.15±3.57i	414.86±1.14j	0.25±0.02k	2.31±0.05j	223.57±0.87i	0.22±0.01k	0.62±0.02f	0.84±0.03b
	PA	50.26±2.99d	502.63±0.40b	0.25±0.03l	4.31±0.03b	214.24±0.88j	0.61±0.01e	0.52±0.01g	0.42±0.02g
	LS	52.71±3.26c	493.66±0.81c	0.41±0.03c	4.21±0.06c	242.97±0.89g	0.42±0.01i	0.31±0.00j	0.61±0.01d

Means provided with error bars. Small letter indicates significant ($p \leq 0.05$) difference between traits.
Abbreviations are given in the start of the manuscript.

Table 4. Organic osmolytes and shoot ionic contents of *Phragmites australis* ecotypes collected from different area of Punjab, Pakistan.

Habitats	Ecotypes	Pro	TSS	GB	A.A	Ca^+	K^+	Na^+
Non-saline								
	BO	27.86±0.34m	5.53±0.27f	31.95±0.33k	1.67±0.01m	6.57±0.20h	27.39±1.16l	15.61±0.22e
	HB	35.86±0.65f	5.38±0.26g	38.09±0.32f	2.37±0.03i	7.69±0.03c	25.71±1.04n	10.12±0.41m
	HK	34.19±0.31g	6.56±0.28e	26.72±0.70n	2.66±0.01d	6.66±0.13g	26.67±1.63m	14.61±0.22f
	NA	37.92±0.99d	6.70±0.29c	45.65±0.53b	2.81±0.03b	5.15±0.18m	30.8±1.21k	9.71±0.28n
Low saline								
	LM	39.86±0.34b	4.82±0.25l	37.59±0.29g	2.10±0.02l	3.53±0.15n	32.22±0.74i	18.68±0.27e
	WC	28.85±0.64l	4.73±0.27m	27.06±0.66	2.37±0.31i	6.67±0.14f	31.71±1.23j	13.77±0.28i
	NP	32.58±0.47i	5.03±0.29k	28.65±0.32l	2.69±0.02c	7.95±0.15b	32.51±0.96h	14.17±0.47g
	GC	29.08±0.26k	6.09±0.27	33.58±0.29j	2.52±0.03g	5.77±0.08j	20.81±1.19o	17.98±0.41d
High saline								
	SC	36.76±0.65e	5.24±0.26i	41.06±0.33d	2.27±0.03j	5.55±0.18k	36.92±1.40e	19.96±0.34c
	GO	33.64±0.57h	6.65±0.28d	40.26±0.34e	2.48±0.02h	7.39±0.28d	44.32±1.04c	12.07±0.41j
	RC	31.95±0.34j	6.98±0.26b	28.52±0.59m	2.58±0.01f	6.46±0.04i	41.11±1.09d	10.23±0.47l
	KKL	34.40±0.33g	5.29±0.29h	35.82±0.30h	2.17±0.03k	8.73±0.12a	33.55±0.51g	11.54±0.17k
	SL	45.65±0.53a	6.18±0.27d	48.98±0.55a	2.92±0.02a	6.73±0.08e	47.11±1.83a	29.42±0.18a
	PA	36.23±0.99e	5.20±0.26j	33.57±0.33i	2.64±0.03e	5.49±0.12l	34.25±0.76f	13.77±0.28h
	LS	38.82±0.98c	7.28±0.27a	42.52±0.01c	2.94±0.02a	5.62±0.11j	45.85±1.08b	25.89±0.29b

Means provided with error bars. Small letter indicates significant ($p \leq 0.05$) difference between traits.
Abbreviations are given in the start of the manuscript.

Table 5. Leaf structural traits of *Phragmites australis* ecotypes collected from different area of Punjab, Pakistan.

Habitats	Ecotypes	LT	ET	Scl.T	BL.A	VBA	Met.A	P.A
Non-saline								
	BO	516.99±4.79f	27.33±1.73h	53.09±1.85o	30121.71±2977.35o	373100.56±6970.86f	10273.32±2051.79h	25893.79±911.06i
	HB	770.34±4.71b	25.50±0.46i	127.24±1.70g	30122.71±3074.08n	870480.48±15525.57a	24174.21±1973.49c	92876.00±5212.63a
	HK	389.40±4.73k	24.41±1.08j	70.32±1.46l	80415.35±4832.29h	317814.98±7789.40i	4362.53±130.24n	27121.71±918.60g
	NA	418.88±4.40h	17.41±2.25m	75.40±1.65j	113730.81±5650.39c	334441.87±8072.49	5395.86±135.44l	24560.45±1586.04j
Low saline								
	LM	752.81±5.23c	35.97±1.77c	163.70±1.14f	160701.59±6906.26b	649639.38±13419.20b	23840.88±1702.76d	43443.70±1199.93c
	WC	542.99±10.54d	36.13±1.44b	55.08±4.40n	92876.00±5115.75e	484561.21±7222.39d	21507.55±2595.44e	29560.45±1143.00e
	NP	317.45±5.23m	19.33±1.16k	74.77±1.25k	69954.70±4548.85i	496021.86±7473.21c	9913.99±1765.81i	26788.37±1283.23h
	GC	273.31±5.23o	29.42±1.11f	56.72±1.31m	81415.35±4832.29g	27336.65±31281.23m	4495.86±191.60m	18543.36±1137.19m
High saline								
	SC	353.73±4.18l	30.56±0.71f	124.64±1.45i	59432.36±4111.052j	344561.21±3967.66h	6371.64±101.75j	23893.79±1101.29k
	GO	527.26±6.31e	39.15±1.51a	164.70±1.14e	92876.26±5215.67f	357894.54±13921.72g	14413.99±467.70g	22560.45±1310.62l
	RC	510.33±3.36g	28.41±0.57g	218.73±1.09b	29599.37±2442.84m	282256.04±8858.33k	24383.15±1682.21b	28188.20±1484.78f
	KKL	890.52±4.40a	18.82±0.81l	165.70±1.14d	317576.30±9489.97a	27822.30±32211.85l	32875.99±826.79a	32875.99±826.79d
	SL	417.88±4.41i	34.54±0.51d	396.97±1.21a	57365.86±4046.50k	319642.80±9457.64j	6338.30±90.63k	15404.70±1344.91n
	PA	400.74±4.42j	16.62±0.91n	126.24±1.70h	104336.65±5399.21d	466662.40±8607.15e	18180.66±602.55f	53365.86±1356.66b
	LS	299.76±4.40n	33.67±2.31e	217.73±1.09c	51071.45±3859.87l	26669.98±31943.74	14329.19±133.42g	15210.02±1455.73o

Means provided with error bars. Small letter indicates significant ($p \leq 0.05$) difference between traits.
Abbreviations are given in the start of the manuscript.

Table 6. Leaf epidermal traits of *Phragmites australis* ecotypes collected from different area of Punjab, Pakistan.

Habitats	Ecotypes	AD.SD	AB.SD	AD.SA	AB.SA
Non-saline					
	BO	42.33±0.88m	94.33±0.87h	3394.33±298.88n	6467.12±299.56c
	HB	43.66±0.89l	102.33±0.88f	4102.33±270.89l	5466.26±296.35f
	HK	73.66±0.87e	84.12±1.15j	5384.21±251.15h	6799.25±131.15b
	NA	54.21±1.15h	116.66±0.89a	5816.66±260.88d	5366.52±131.24g
Low saline					
	LM	44.11±1.14k	64.33±1.45m	5264.33±271.45i	6865.92±261.13a
	WC	48.31±1.13i	63.66±0.88n	5963.66±250.88c	4566.52±685.23i
	NP	105.21±0.57a	113.66±0.87c	6313.66±230.87a	5260.11±299.24h
	GC	96.33±0.88c	115.33±0.89b	6216.33±240.89b	3899.59±298.25k
High saline					
	SC	46.23±1.15j	82.33±1.20l	5484.33±121.20g	6266.78±296.32d
	GO	58.33±0.88g	83.66±0.88k	5684.66±320.88e	3699.85±935.12l
	RC	65.24±0.57f	95.66±0.87g	3294.66±340.89o	4366.52±344.26j
	KKL	103.66±0.88b	105.22±0.57d	4105.21±420.57m	2333.27±657.28n
	SL	42.33±0.89m	104.33±0.88e	5504.33±320.88f	2133.79±612.38o
	PA	75.11±0.57d	86.24±1.73i	5186.21±121.73j	6167.04±296.38e
	LS	33.66±0.88n	56.14±1.15o	4356.11±231.15k	2933.44±317.28m

Means provided with error bars. Small letter indicates significant ($p \leq 0.05$) difference between traits.

Abbreviations are given in the start of the manuscript.

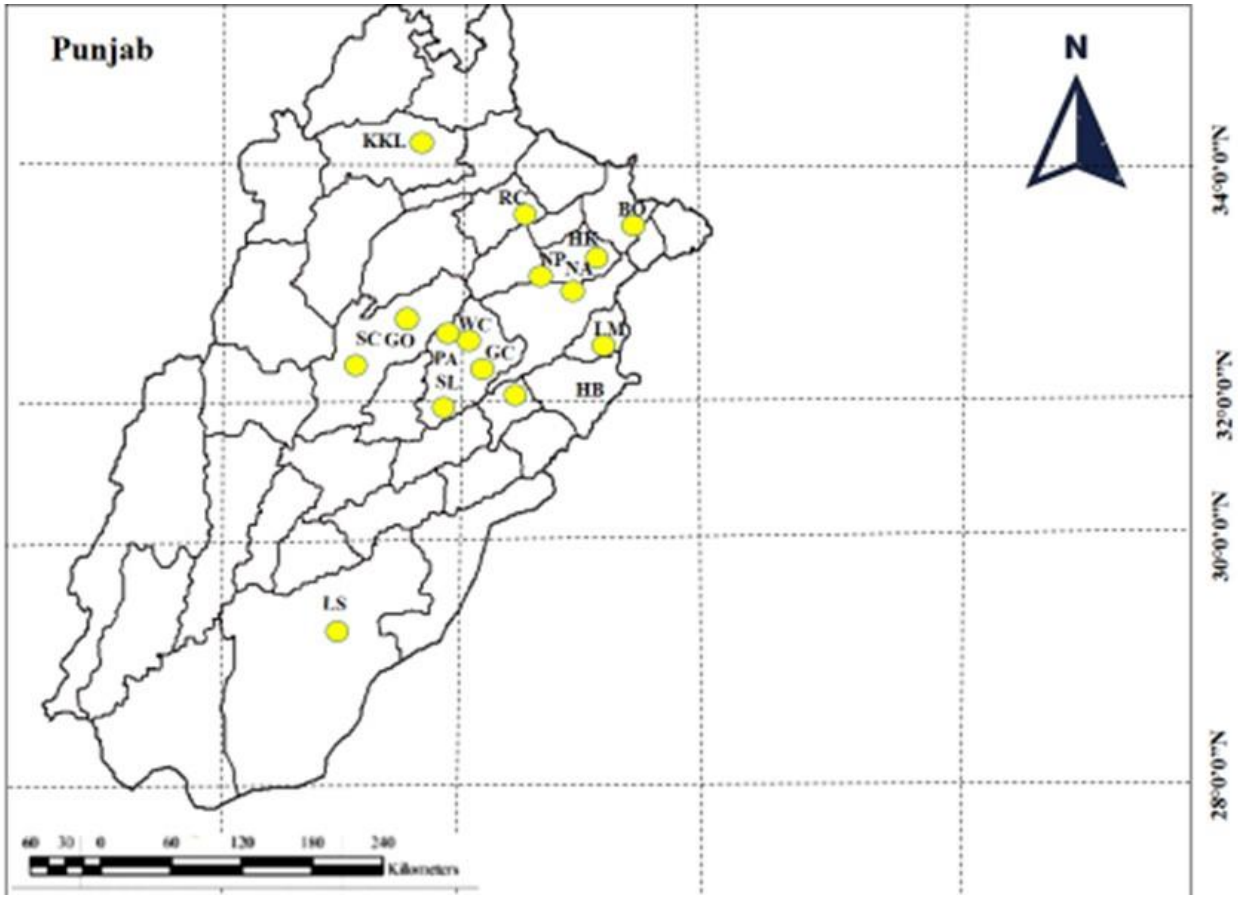
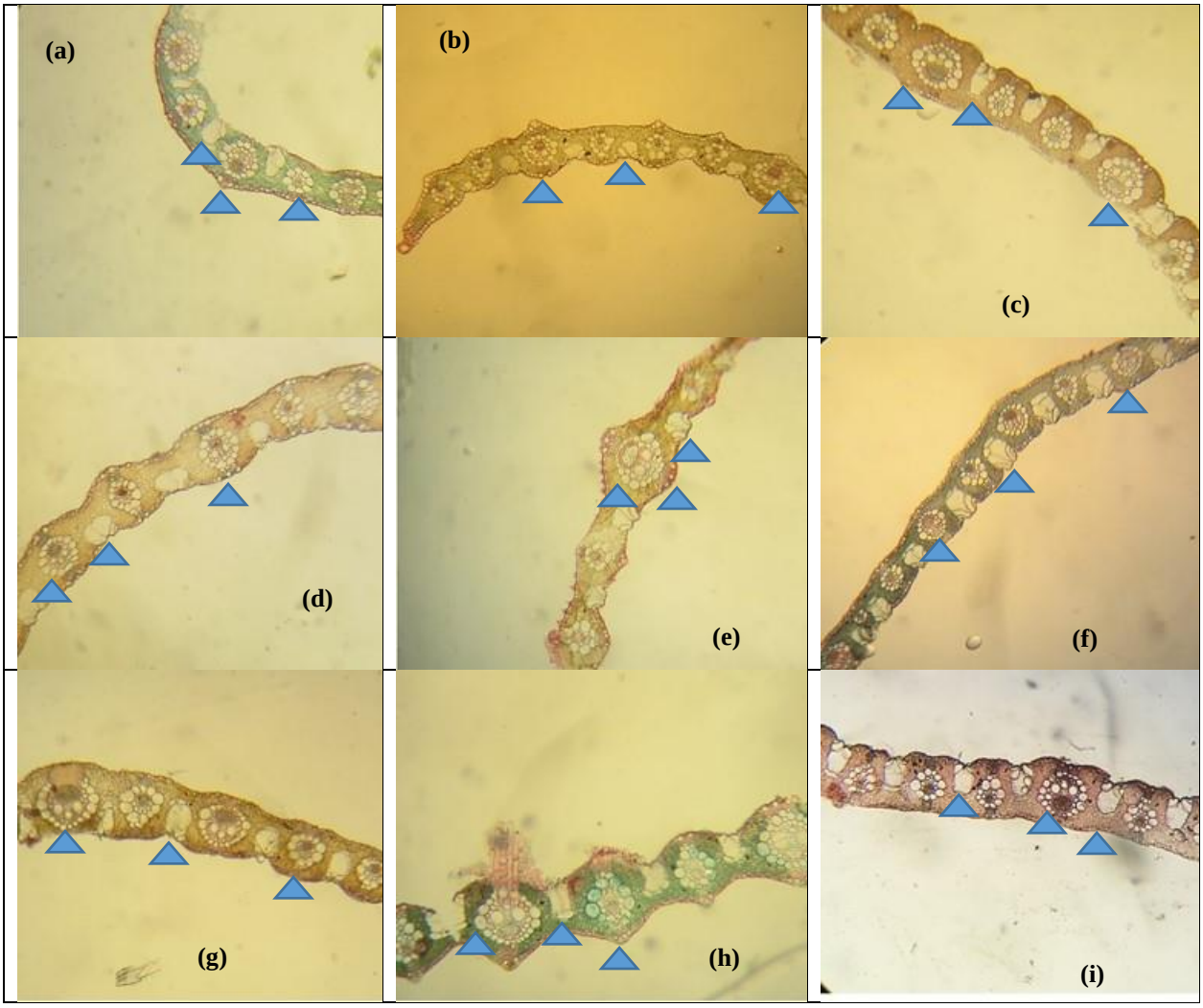


Fig 1. Map of Punjab province showing collection sites of *Phragmites australis.*

Figure legends: 1-Bombanwala (BO), 2–Head Baloki (HB), 3-Head Khanki (HK), 4-Nandipur (NA), 5-Lahore Model Town (LM), 6-Wapda city (WC), 7-Nala Palkho (NP), 8-Gatwala canal (GC), 9-Shorkot Cantt (SC), 10- Gojra (GO), 11-Rasool chowki (RC), 12-Kalar Kahar Lake (KKL), 13- Sahianwala Lake (SL), 14- Pakka Aana (PA), 15-Lal Suhanra (LS).

		
Bombanwala	Head Baloki	Head Khanki
		
Nandipur	Lahore	Wapda City gate 1
		
Nala Palkho	Gatwala Canal	Shorkot Cantt
		
Gojra	Rasool chowki	Kalar Kahar Lake
		
Sahianawala Lake	Pakka Aana	Lal Suhanra

Fig.2. Pictorial description of collection sites of *Phragmites* australis in the Punjab Pakistan.



Bombanwala: a. Enlarged bulliform cells.Enlarged vascular bundles.Abaxial surface sclerified. **Head Baloki: b.** Vascular bundles sclerified and enlarged.Small bulliform cells.Lamina reduced. **Head Khanki: c.** Enlarged bulliform cells. Abaxial surface sclerified. Vascular bundles sclerified. Metaxylem prominent. **Nandipur: d.** Enlarged lamina Enlarged bulliform cells.Sclerified phloem. **Lahore: e.** Enlarged and round shape vascular bundles. Small bulliform cells. **Wapda City gate 1: f.** Highly sclerified vascular bundles. Abaxial thick and sclerified. Small bulliform cells. **Nala Palkho: g.** Small bulliform cells. Enlarged vascular bundles. Prominent metaxylem **Gatwala Canal: h.** Small and enlarged vascular bundles Prominent metaxylem. Enlarged bulliform cells.Bundle sheath cells closely packed around the vascular bundles. **Shorkot Cantt: i.** Vascular bundles arranged in a specific pattern. Fan shape bulliform cells. Phloem sclerified. Adaxial sclerified

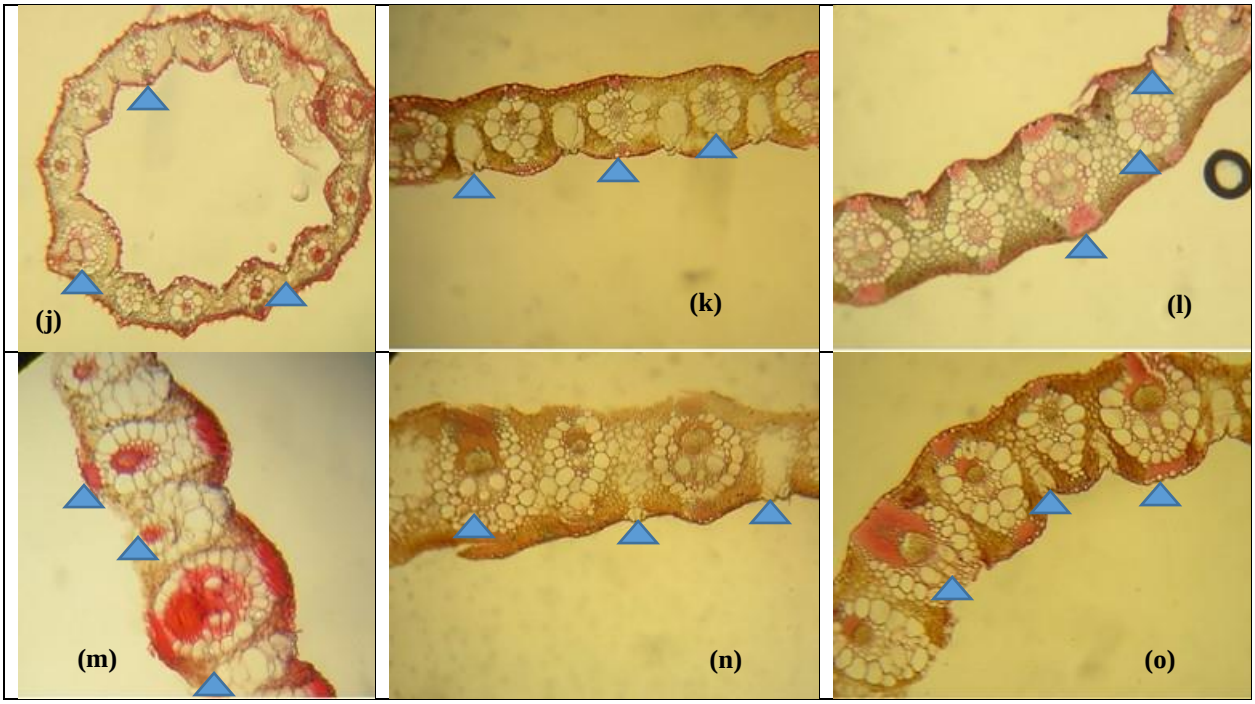
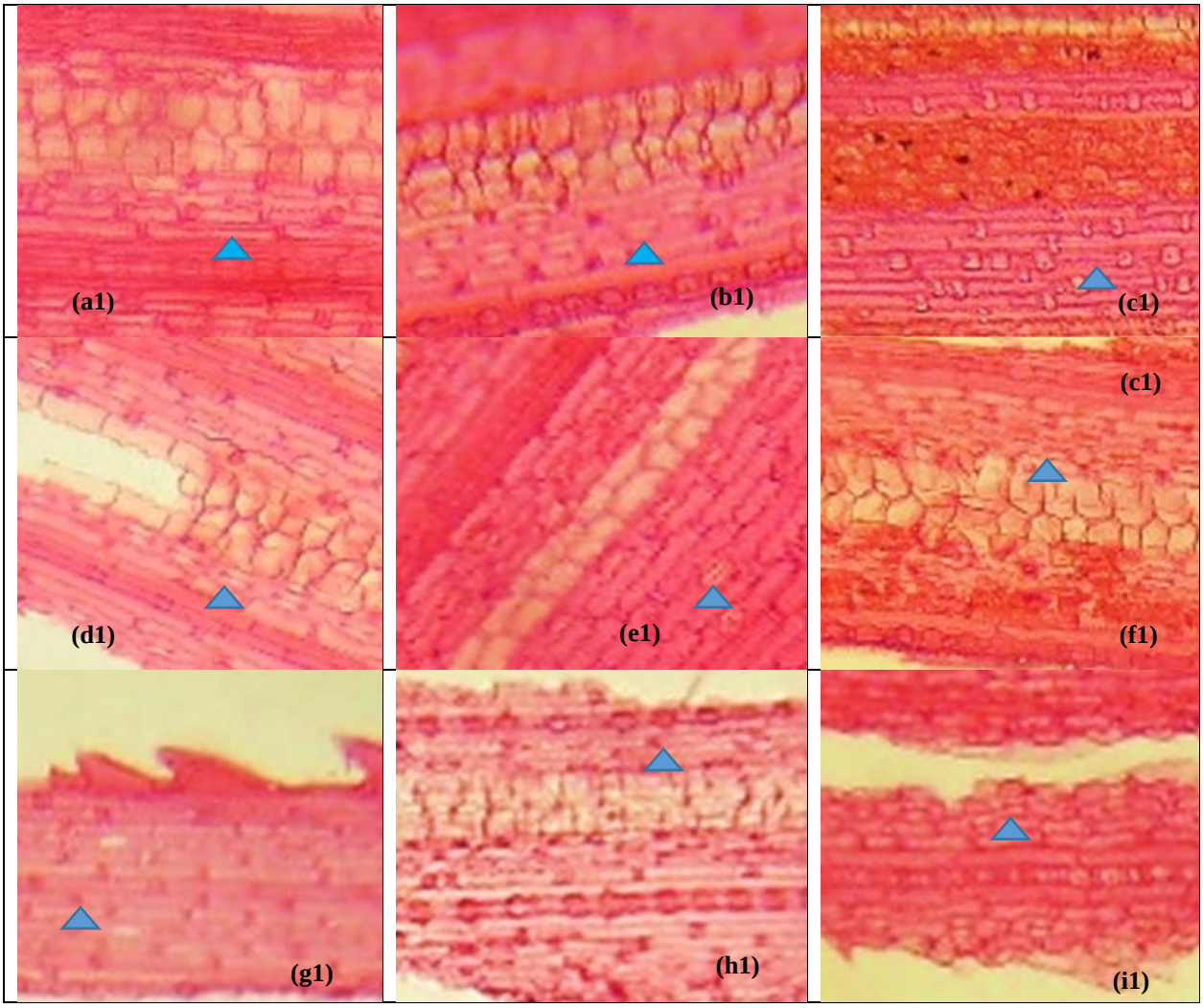


Fig 3. Leaf transverse sections of differently adapted population of (*Phragmites australis)* collected from different ecozones of Punjab Pakistan.

Gojra: j. Round shape leaf Sclerification in and outside the epidermis. Small and enlarged vascular bundles. **Rasool chowki: k.** High sclerification around vascular bundles Enlarged metaxylem. Enlarged bulliform cells. **Kalar Kahar Lake: l.** Enlarged vascular bundles sclerified Abaxial and Adaxial surface. Fan shape bulliform cells surrounded vascular bundles. **Sahianawala Lake: m.** Adaxial highly sclerified.Elongated vascular bundles.Small parenchymatous tissue.Bundle sheath cells closely packed around the vascular bundles **Pakka Aana: n.** Adaxial sclerified.Small bulliform cells around the vascular bundles.small parenchymatous storage tissues in the lamina **Lal Suhanra: o.** Sclerified Abaxial and adaxial epidermis. Fan shaped bulliform cells. Enlarged metaxylem. sclerification around vascular bundles.



Bombanwala: a1. Numerous dumble shape stomata Epidermal cells irregular wavy shaped. **Head Baloki: b1.** Epidermal cells rectangular. **Head Khanki: c1.** Epidermal cells long eliptic. **Nandipur: d1.** Epidermal cells rectangular and small. **Lahore: e1.** Epidermal cells large wavy shaped. **Wapda City gate 1: f1.** Epidermal cells rectangular and large. **Nala Palkho: g1.** Angular prickels present. **Gatwala Canal: h1.** Epidermal cells small more or less eliptic. **Shorkot Cantt: i1.** Epidermal cells irregular in size and shape.

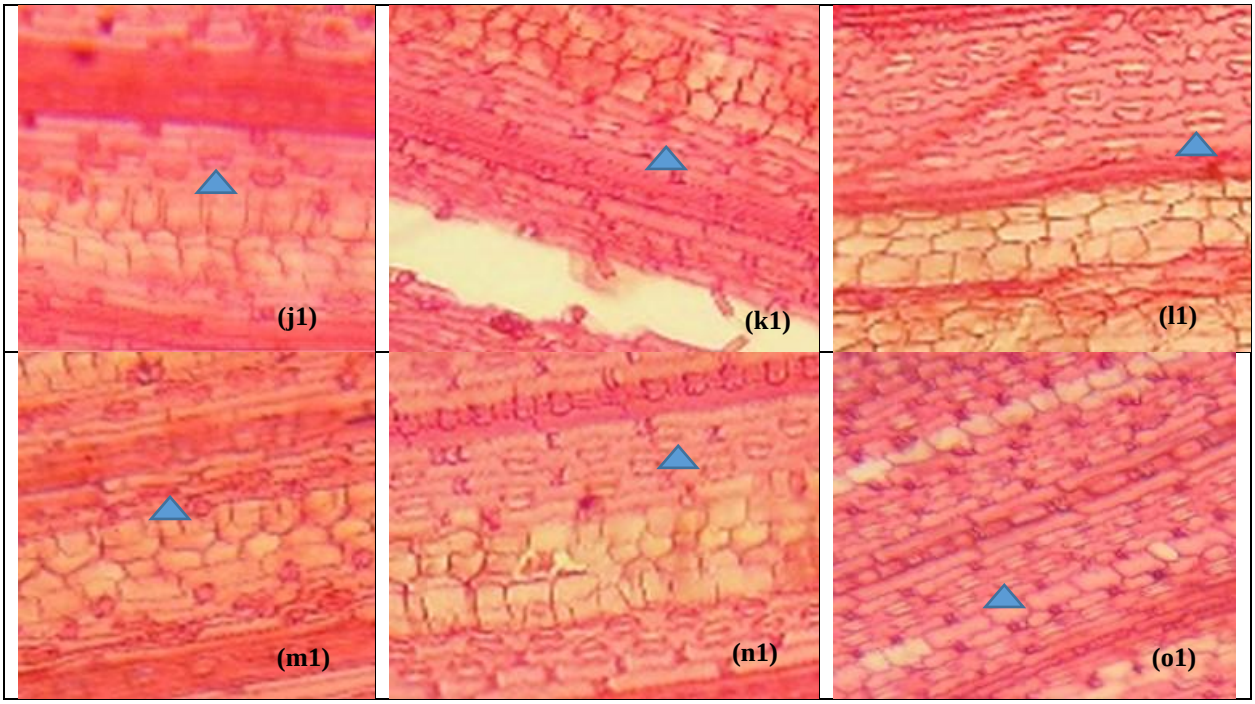
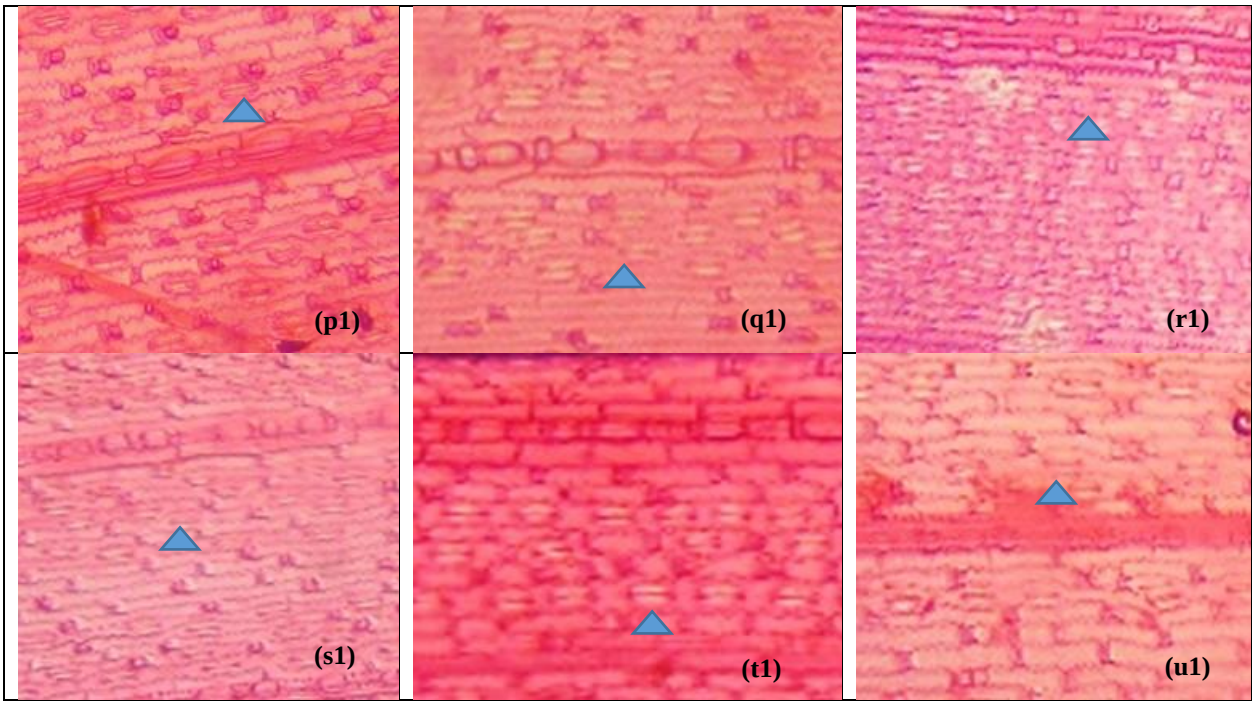
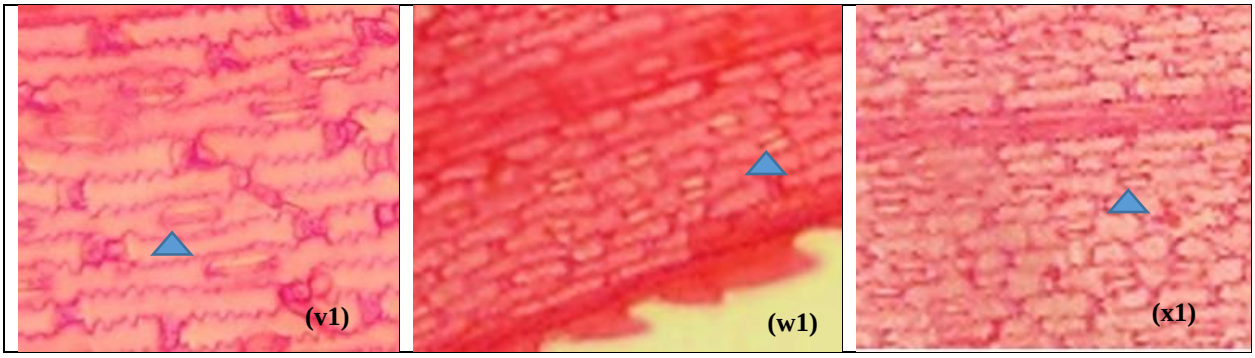


Fig 4. Leaf abaxial epidermal surface of differently adapted population of (*Phragmites australis*) collected from different ecozones of Punjab Pakistan.

Gojra: j1. Epidermal cells rectangular and small. **Rasool chowki: k1.** Epidermal cells rectangular and large.**Kalar Kahar Lake: l1.** Numerous dumble shape stomata small deeply sinous epidermal cells. **Sahianawala Lake: m1.** Epidermal cells rectangular and large. **Pakka Aana: n1.** Stomatal number increase long cells epidermal cells. **Lal Suhanra: o1.** Epidermal cells parallel in size and shape.





Bombanwala: p1. Numerous dumble shape stomata, small deeply sinous epidermal cells. **Head Baloki: q1.** Stomatal number increase long cells epidermal cells. **Head Khanki: r1.** Stomata greater in number. deeply sinous epidermal cells. **Nandipur: s1.** Epidermis walls slightly wavy. More stomata.**Lahore**. **: t1.** Epidermis cells irregular. **Wapda City gate 1: u1.** Epidermis walls slightly wavy. **Nala Palkho: v1.** Epidermal cells large deeply sinous **Gatwala Canal: w1.** Angular prickels present. **Shorkot Cantt: x1.** Epidermal cells irregular.

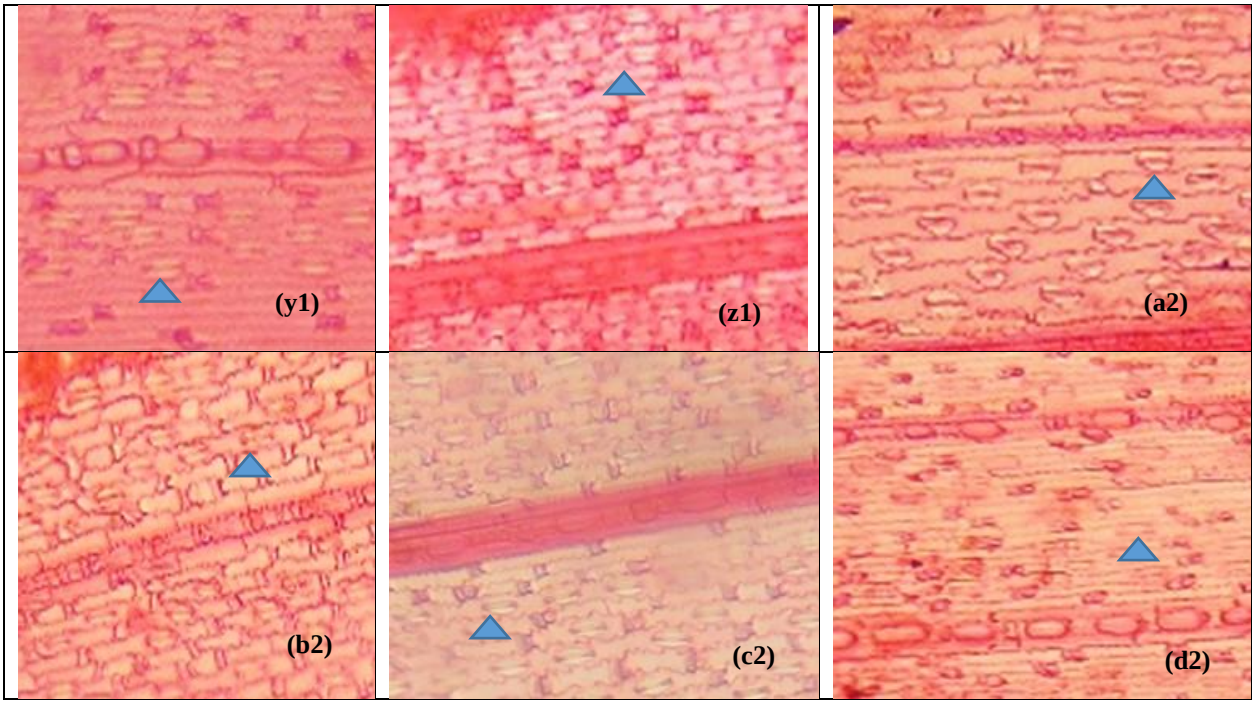


Fig 5. Leaf adaxial epidermal surface of differently adapted population of (*Phragmites australis*) collected from different ecozones of Punjab Pakistan.

Gojra: y1. Epidermal cells large wavy shaped. **Rasool chowki: z1.** Epidermal cells small more or less eliptic. **Kalar Kahar Lake: a2.** Epidermal cells long slightly sinuos. **Sahianawala Lake: b2.** Epidermal cells irregular **Pakka Aana: c2.** Epidermal cells small. **Lal Suhanra: d2.** Epidermal cells long eliptic.

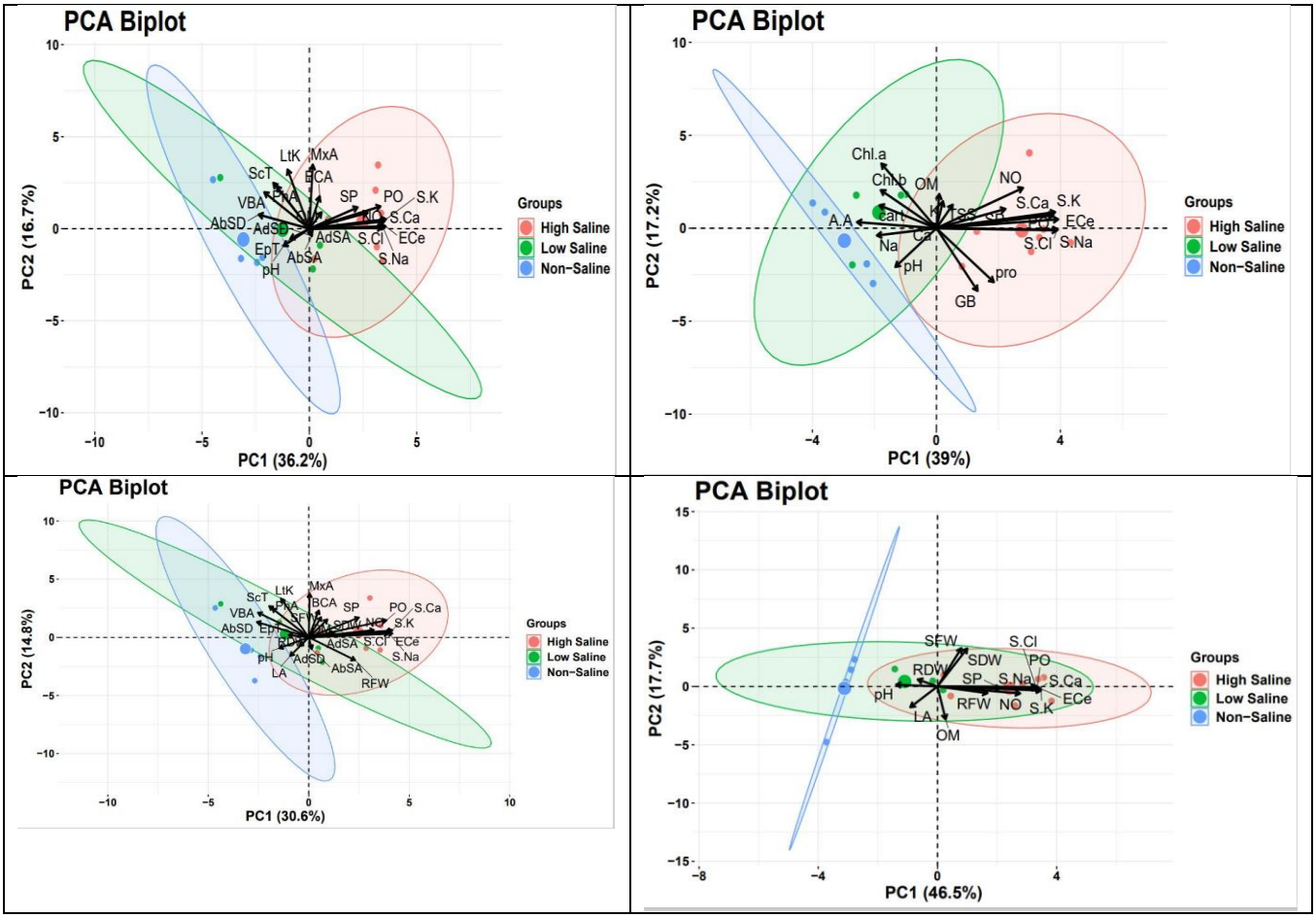


Fig. 6. PCA biplot analysis for morphological, physiological and anatomical traits with soil physiochemical characteristics populations of Phragmites spp. collected from different ecological zones Punjab

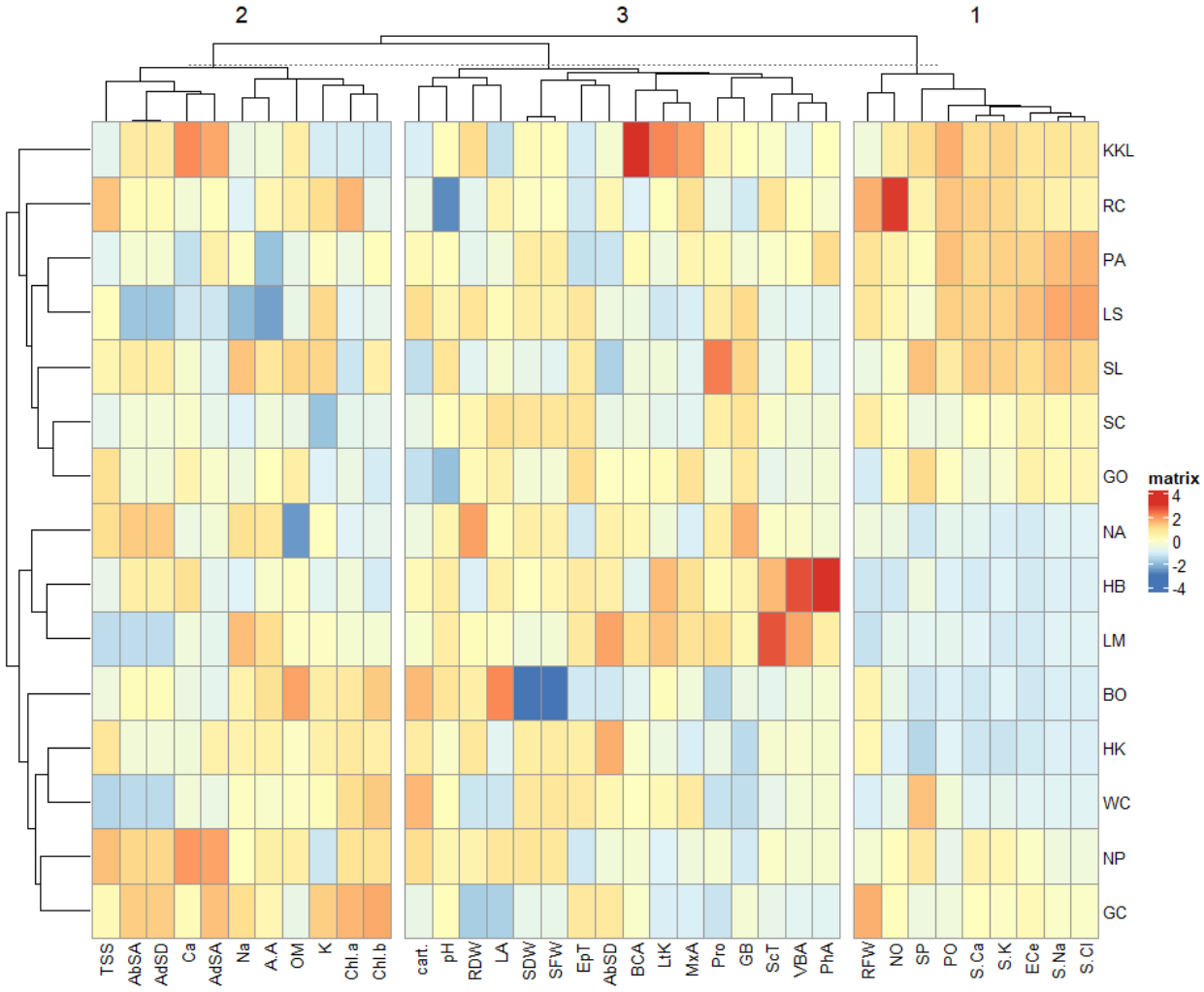


Fig.7. Clustered heatmap representing the traits involved in salinity tolerance in differently adapted populations of Phragmites spp. collected from different ecological zones Punjab **LtK**-lamina thickness, **EpT**-epidermal thickness, **ScT**-sclerenchyma thickness, **VBA**-vascular bundles area, **MxA**-metaxylem vessel area, **PhA**-phloem area, **BCA**-bulliform cell area, **AdSA**-adaxial stomatal area, **AbSA**-abaxial stomatal area. , **AdSD**-adaxial stomatal density, **AbSD**-abaxial stomatal density **ECe**-electrical conductivity, **pH**-soil pH, **OM**-organic matter, **PO**-available phosphorus, **SP**-saturation percentage, **S.Ca**-soil calcium, **S.K**+-soil potassium, **S.Na**+-soil sodium, **S.Cl**$^{-}$ soil chloride, **NO**- soil nitrate. potassium (K+); Calcium (Ca+); Sodium (Na+) **TSS**-total soluble sugars, **GB**-glycine betaine, **Pro**-proline, **Chl a**-chlorophyll a, **Chl b**-chlorophyll b, **Cart**-carotenoids. **SDW**-shoot dry weight, **SFW**-shoot fresh weight, **RDW**-root dry weight, **RFW**-root fresh weight ,**LA**-leaf area,

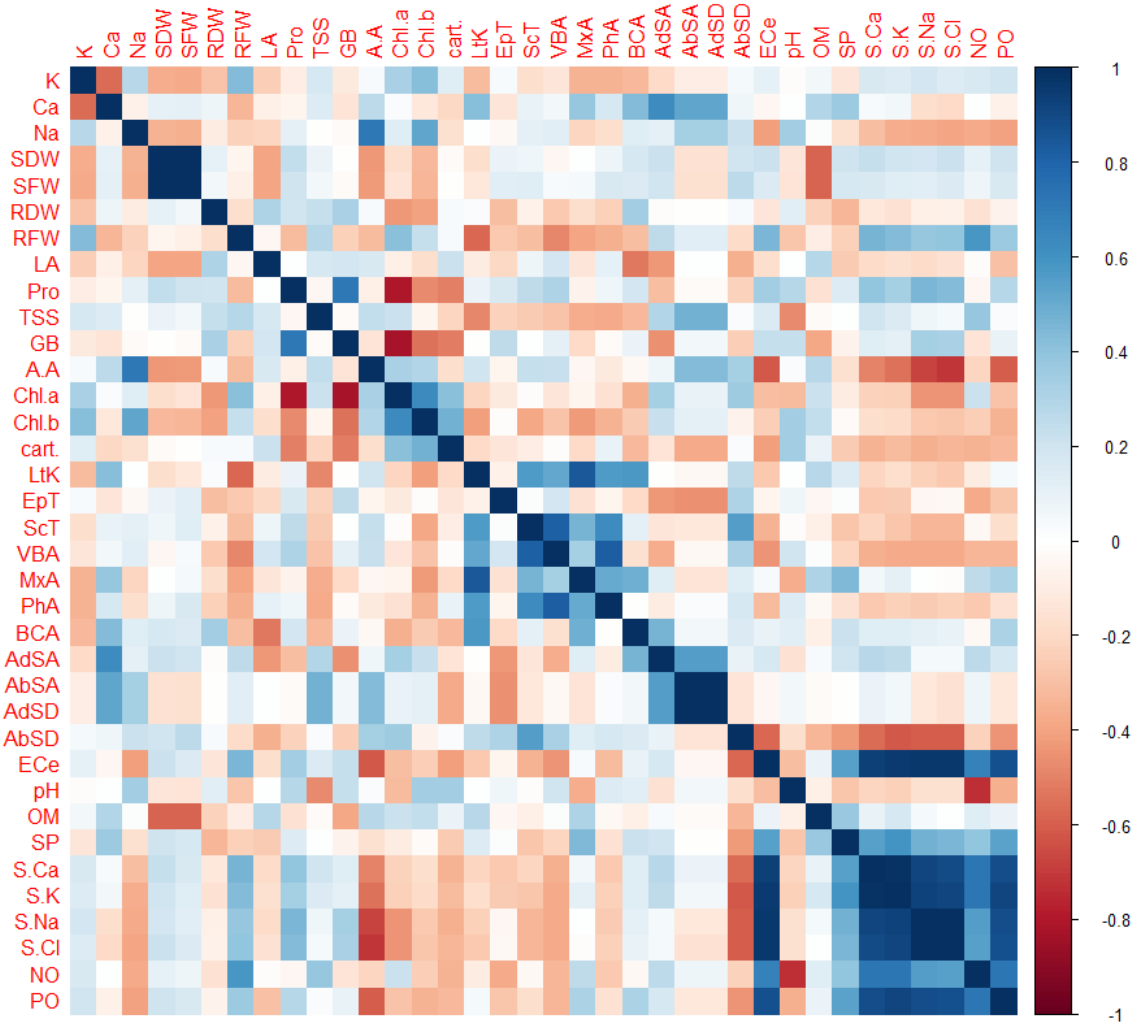


Fig. 8. Correlation among morphological, physiological and anatomical traits.

Figure legends: **LtK**-lamina thickness (um), **EpT**-epidermal thickness (um), **ScT**-sclerenchyma thickness (um), **VBA**-vascular bundles area (um^2), **MxA**-metaxylem vessel area (um^2), **PhA**-phloem area (um^2), **BCA**-bulliform cell area (um^2), **AdSA**-adaxial stomatal area (um^2), **AbSA**-abaxial stomatal area (um^2) , **AdSD**-adaxial stomatal density, **AbSD**-abaxial stomatal density, **ECe**-electrical conductivity (dS m^{-1}), **pH**-soil pH, **OM**-organic matter (%), **PO**-available phosphorus (mg kg^{-1}), **SP**-saturation percentage, **S.Ca**-soil calcium (mg kg^{-1}), **S.K**+-soil potassium (mg kg^{-1}), **S.Na**+-soil sodium (mg kg^{-1}), **S.Cl**$^-$ soil chloride (mg kg^{-1}), **NO**- soil nitrate (mg kg^{-1}). **Potassium K**$^+$ (mgg^{-1} d.w.t), **Calcium Ca**$^{2+}$ (mgg^{-1} d.w.t), **Sodium Na**$^+$ (mgg^{-1} d.w.t), **A.A**-Amino acid (umg^{-1}), **TSS**-total soluble sugars (mgg^{-1}), **GB**-glycine betaine (umg^{-1}), **Pro**-proline (umg^{-1}), **Chl a**-chlorophyll a (mgg^{-1}FW), **Chl b**-chlorophyll b (mgg^{-1}FW), **Cart**-carotenoids. **SDW**-shoot dry weight (g/plant), **SFW**-shoot fresh weight (g/plant), **RDW**-root dry weight (g/plant), **RFW**-root fresh weight (g/plant), **LA**-leaf area (um^2)