

STRUCTURAL AND FUNCTIONAL PLASTICITY IN THE ROOT AND STEM OF *DICHANTHIUM ANNULATUM* (FORSSK.) STAPF UNDER SALT STRESS

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Abstract

Naturally grown populations of Halophytic grass *Dichanthium annulatum* were collected from saline habitats for the evaluation of structural and functional adaptations in saline soils. Different populations of *D. annulatum* showed various adaptations i.e. decreased shoot length and shoot fresh weight, increased root length and root fresh weight in highly saline habitats. Increased epidermal thickness and cell area, enlarged storage parenchyma, broader metaxylem vessels, enhanced pith and phloem area observed in roots of highly saline populations. Thickest stem, well developed metaxylem vessels and broader cortical region was seen under severe salinity. Accumulation of organic osmotica i.e. Glycine betaine, proline, total soluble sugars and total soluble proteins contributed significantly to endure harmful consequences of saline conditions. This grass exhibited increased uptake of toxic ions like Na^+ , Cl^- which is very harmful for growth under salt affected habitats.

Key words: Halophyte, Salt accumulation, Cortical parenchyma, Organic osmolyte, Osmotic adjustment.

Introduction

Soil salinity is one of the most challenging factors, which affect plant growth and yield (Zorb *et al.*, 2019). Plant growth is limited in saline conditions due to the existence of excessive concentration of soluble salts. Plant development is inhibited by osmotic and ion specific effects of salts linked with the deposition of excessive sodium (Na) and chloride (Shrivastava & Kumar, 2015). Salinity involved not only in the reduction of agricultural productivity, but it also has impact on physico chemical characteristics of soil. Reduced agricultural production, limited socioeconomic returns, and soil degradation are some of the consequences of salt stress (Hu & Schmidhalter, 2002). Soil salinity cause oxidative and osmotic stress, nutritional deficiency (N, Ca, K, P, Fe, Zn), and limited water absorption from the soil (Bano & Fatima, 2009). Halophytes have an exceptional ability to complete their life cycle in saline environments (Song and Wang, 2015). They had evolved a variety of ways to cope with injurious effects of salts accumulation through structural, functional, and metabolic changes during the evolutionary process (Rozentsvet *et al.*, 2017).

Specific anatomical and physiological changes in plants exposed to harsh situations may allow them to flourish in such conditions (Basu *et al.*, 2016). Salinity stress is tolerated by halophytes through the formation of certain anatomical features such as succulence in the stem and midrib, development of aerenchyma, enlarged vascular bundles, higher phloem and metaxylem area, and extensive sclerification (Imran *et al.*, 2019). Stomatal area, density, and orientation also play important roles in the salt tolerance (Mohamed *et al.*, 2020b). Halophytes can withstand greater salt concentrations and thrive in stressful environments due to the storage of several essential ions and osmolytes (Usman *et al.*, 2018). Higher Ca^{2+} concentrations in plants developing under salt stress preserve membrane permeability, K^+/Na^+ selectivity, and osmoregulation (Safdar *et al.*, 2019; Yaseen *et al.*, 2020). Soluble proteins, sugars, and other solutes have important roles in osmotic adjustment, such as increased water intake and storage, preservation of macromolecule that

can be disrupted under salinity stress (Jabeen & Ahmad, 2017; Saleem *et al.*, 2020). The best way to combat with salinization is to retain sustainability of cultivated field and landscape by introducing salt-tolerant plants (Beltrão *et al.*, 2009). Because of salt accumulation in soil and seawater intrusion into groundwater, salt tolerant cultivars are now becoming highly significant in many parts of the globe (Uddin *et al.*, 2011). *Dichanthium annulatum* is a halophytic grass which can tolerate soil salinity up to 300-500 mM NaCl and mostly cultivated in salt affected soil and potentially used as forage crop (Cope, 1982). Therefore, this investigation was conducted to study the effects of salinity on the morphological and anatomical traits of *Dichanthium annulatum*.

Material and methods

Eleven different populations of *Dichanthium annulatum* were collected from various salt affected area of Punjab Pakistan. The highly saline sites were Ladam Sir (LS), Salluwanli (SW) and Sahiwanla (SH); Moderately saline sites were Khanewal (KH), Gutwala (GW), Banjusa Lake (BL) and Salmani Adda (SA); Non saline sites were Jabba (JB), Karana Hill (KR), Rawalakot (RK) and Khanpur (KP).

Soil physicochemical characteristics: Soil samples were collected at depth of 15 to 25 cm from each site for the determination of different soil physicochemical properties. For the determination of soil saturation percentage (SP %) soil sample were crushed into small piece and oven dried at 70°C to fully dried and 200g soil was used to measure saturation percentage. Suction pump was used for the extraction of soil water from the paste and it was used for the estimation of soil pH and ECE with a pH/EC meter (pH/Cond 720, WTW series InoLab, USA). Flame photometer (PFP-7, Jenway, UK) was used for the analyses of cations (Ca^{2+} , K^+ and Na^+) from extracted soil. Chloride ions was analyzed by means of digital chlorimeter (Model 926, Sherwood Scientific Ltd. Cambridge, UK) (Table 1).

Table 1. Soil physico-chemical characteristics of *D. annulatum* collected from different Saline habitats.

Sites	ECe (dS m ⁻¹)	pH	OM (%)	NO ₃ ⁻¹ mg/Kg	PO ₄ ⁻³ mg/Kg	K ⁺ mg/Kg	SP (%)	Na ⁺ mg/Kg	Cl ⁻ mg/Kg	Ca ²⁺ mg/Kg
LS	39	8.1	0.9	0.04	5.2	240	30	3776.6	1550	2014
SW	30.5	8.1	0.69	0.03	3.6	180	31	2958.6	978.3	1690
SH	11.23	9.6	0.58	0.02	2.1	140	32	830.3	712	804
KH	9.25	8.2	0.76	0.038	3.6	160	31	762.1	675.2	772
GW	8.72	7.9	0.83	0.04	4	220	29	753.2	619	672
BL	7.34	7.4	0.76	0.03	2.9	140	34	686.5	445.2	596
SA	6.49	8.3	0.97	0.04	2.3	160	34	615	301.1	422
JB	1.79	8.2	0.97	0.04	3.2	180	30	79.5	39.5	376
KR	1.79	8.1	0.91	0.04	2.1	220	30	75.9	39	284
RK	2.05	7.8	0.83	0.04	3.1	160	29	68.8	30	210
KP	0.65	7.8	0.97	0.48	5.9	200	31	29.9	15	56

Ligands: OM-Organic matter, SP-Saturation percentage

Morphological attributes: Morphological attributes of each population like shoot and root length was measured with measuring scale from the main tillers. Shoot and root fresh weight was measured directly on a digital loading balance (ISO 9001, Household Electronic Co., Ltd., Guangdong, China) however, for dry weight plant samples were oven dried at 65°C until we got final weight.

Plant ionic contents: Crushed shoot dry material (0.5 g) was placed in a flask having 5 ml conc. of H₂SO₄ and leave it for overnight. The sample was digested on a hot plate (350°C) and wait until the solution become clear by the addition of H₂O₂ as demonstrated by Wolf (1982). Flame photometer (Model 410, Sherwood Scientific Ltd., and Cambridge, UK) was used for the estimation of Cations (Ca²⁺, Na⁺ and K⁺).

Compatible solutes: Leaf samples were collected from uppermost plant shoot and stored in an icebox for determination of proline, glycine, total soluble sugars and total soluble proteins. For the analysis of TSP (total soluble proteins) fresh leaf sample (0.2g) was minced in 5 ml of phosphate buffer and grinded carefully. The extract collected then centrifuge for 5 min at 5000 × g. The supernatant was removed for quantification of protein following by Lowry *et al.*, (1951). For the estimation of proline, fresh leaves (0.5 g) were placed in sulfo-salicylic acid and homogenized in ninhydrin. The mixture was placed at 100°C for period of 1 hour and absorbance was recorded on spectrophotometer at 520 nm (Model 220, Hitachi, Japan). For the determination of GB (glycine betaine) fresh leaf sample (0.5 g) were crushed and kept in deionized water (20 ml) for one day at 25°C. The extract was made and analyzed according to the procedure of Grattan and Grieve (1998). Total soluble sugars were analyzed by following the protocols of Dubois *et al.*, (1951).

Anatomical studies: For anatomical studies root and stem were separated from the plants and placed in formalin acetic alcohol (FAA) solution by following Ruzin (1999). Free hand sectioning techniques was used for the preparation of permanent slides. Various alcohol grades were used for the dehydrations of sections and 2 dyes named safranin and fast green were used for escalating difference among different tissue systems. Photography was made by using camera equipped compound microscope (Nikon 104, Japan). Ocular micrometer was used for readings.

Statistical analysis

Tukey pairwise comparison test and one way analysis of variance (ANOVA) was used for comparison of means by using software Minitab 19 (Minitab, LLC, and State College, PA, USA). Redundancy analysis and response curve between different traits were obtained by using of GLM (Generalized linear model) in CANOCO version 5 for windows.

Results

Morphological attributes: The maximum length of plant shoot was observed in KR population which is non-saline habitat and it was minimum in LS and SW population in highly saline group. LS population showed greater root length from highly saline and minimum root length was noticed in KP population from non-saline group. The maximum shoot fresh weight was depicted in the population of KR from non-saline and and minimum was seen in LS population from highly saline sites. KR population from non0 saline sites showed maximum shoot dry weight and it was minimum in LS population from highly saline sites. The maximum root fresh and weight was observed in SH and LS populations respectively from highly saline group and minimum value was recorded in JB and GW population from non- saline and moderately saline respectively (Fig. 1).

Root anatomy: The maximum root epidermal thickness was possessed by LS from highly saline population while least thickness was observed in KP from non-saline population. LS population also showed maximum epidermal cell area and lowest was recorded in RK population from non-saline group. The maximum cortical cell area and metaxylem area was depicted in LS population from highly saline sites and its minimum value was recorded in KP population from non-saline sites. The maximum endodermal thickness and cell area was noticed in LS population from highly saline group while its least value was recorded in RK population from non- saline sites. LS population showed maximum phloem area and pith cell area while its least value was recorded in GW population from moderately saline sites and KR population from non-saline sites respectively (Figs. 2 and 3).

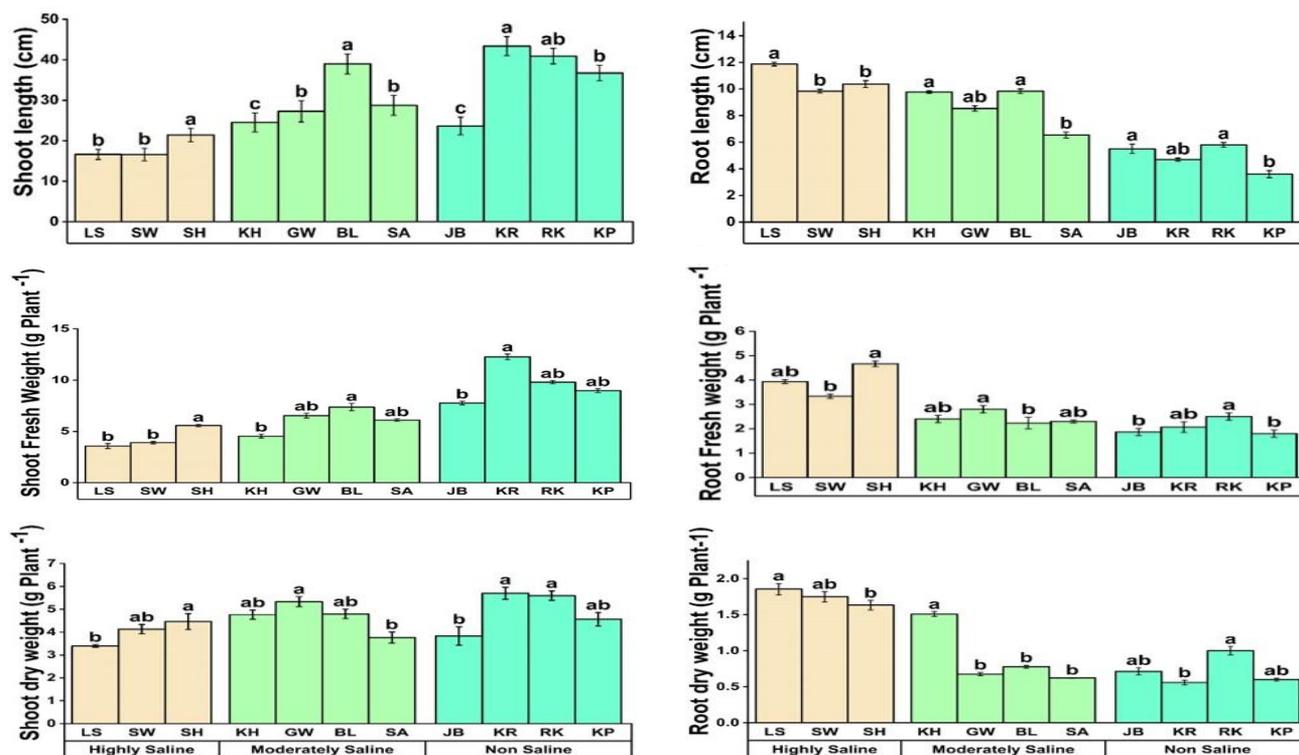


Fig. 1. Morphological attributes of *Dichanthium annulatum* collected from different saline habitats.

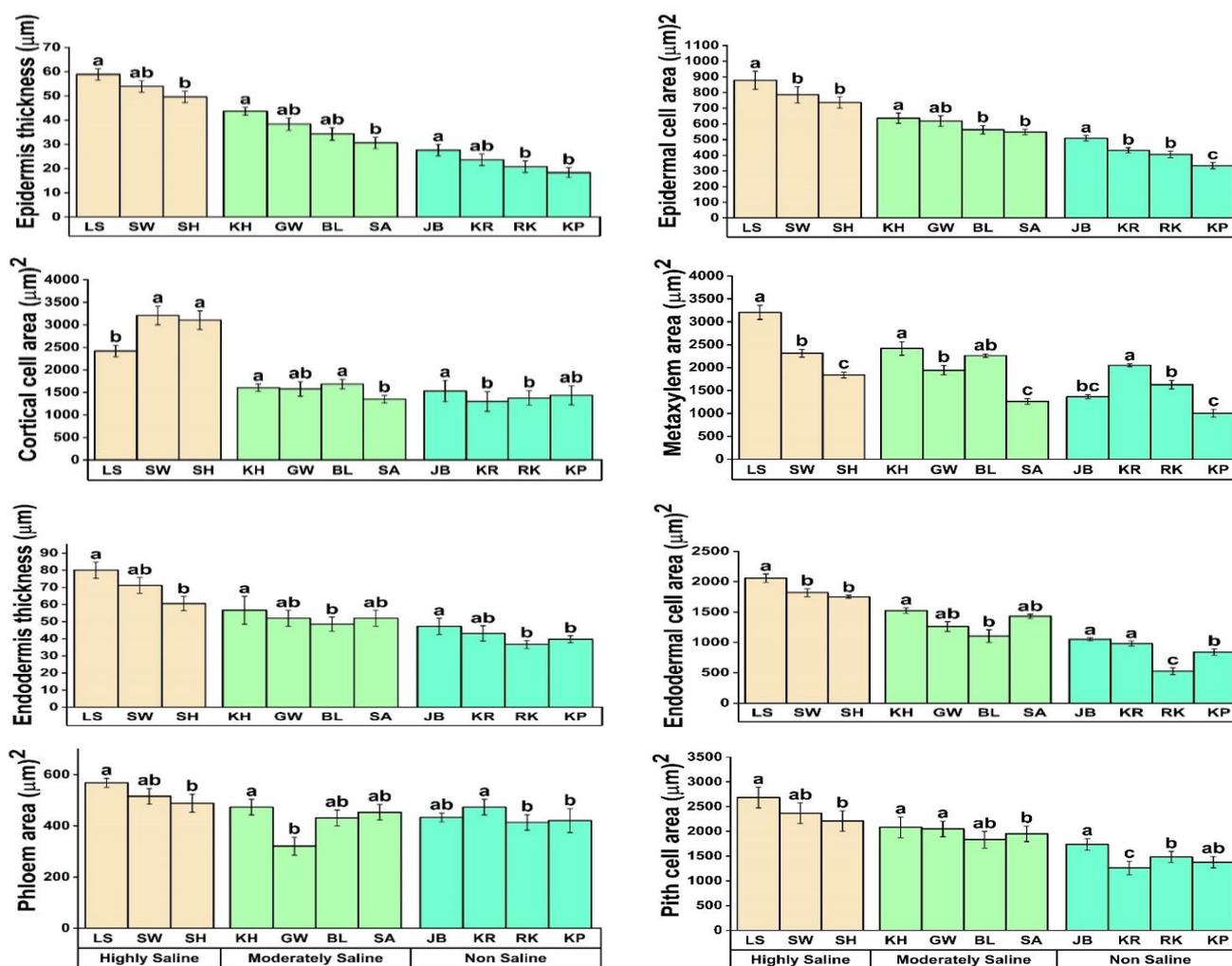


Fig. 2. Root anatomical characteristics of *Dichanthium annulatum* collected from different saline habitats.

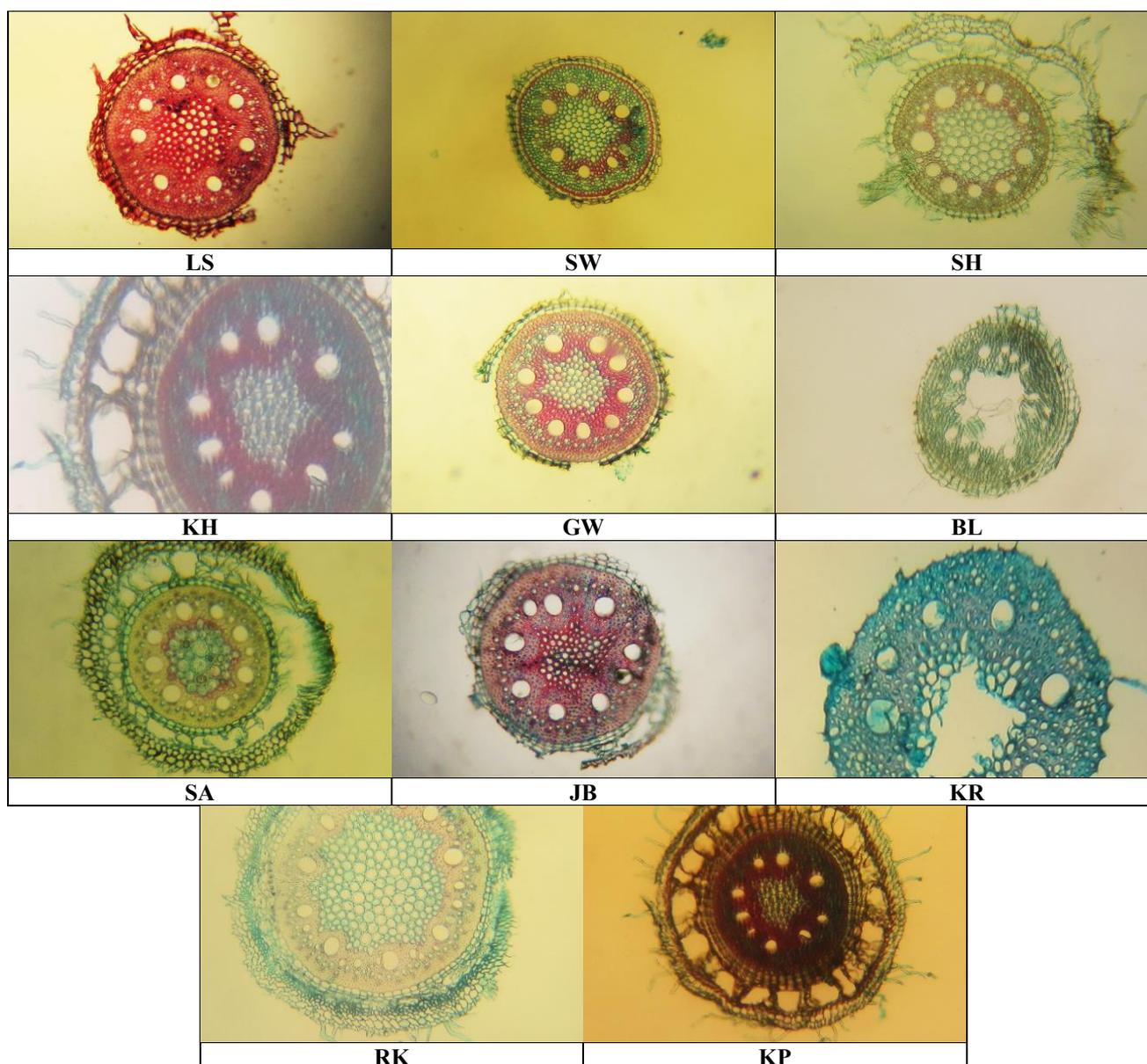


Fig. 3. Root transverse sections of different populations of *D. annulatum* collected from different saline conditions.

Stem anatomy: The thickest epidermis and epidermal cell area was possessed by LS population from highly saline group and thinnest epidermis was seen in KP population from non-saline sites. The metaxylem vessels were larger in LS population from highly saline and it narrow vessels was seen in KP population. The phloem area was maximum in SH population from highly saline and lowest in KR from non-saline sites. The highest cortical cell area was depicted in SW population from highly saline sites and lowest was recorded in KR population from non-saline sites. The vascular bundle area was maximum SW population from highly saline and it was minimum in KP population from non-saline (Figs. 4 and 5).

Physiological parameters: The maximum concentration of Glycine betaine was recorded in SW population from highly saline sites and lowest concentration was recorded in RK population from non-saline sites. The highest concentration of proline was depicted in LS population from highly saline

and KR population showed minimum value of proline contents. Total soluble sugars and total soluble proteins concentration was maximum in LS population from highly saline group and minimum value was recorded in SH and KR population respectively (Fig. 6).

Plant ionic contents: The maximum value of shoot and root sodium was possessed by LS population from highly saline group and its least concentration was seen in KP population from non-saline sites. The shoot calcium contents were maximum in LS population and minimum was seen in KP population. The concentration of calcium contents was maximum in roots of KH population from moderately saline sites. The maximum concentration of shoot potassium was seen in SH population from highly saline and RK population from non-saline. Root potassium was maximum in SW population from highly saline and its value was minimum in GW population from moderately saline group (Fig. 7).

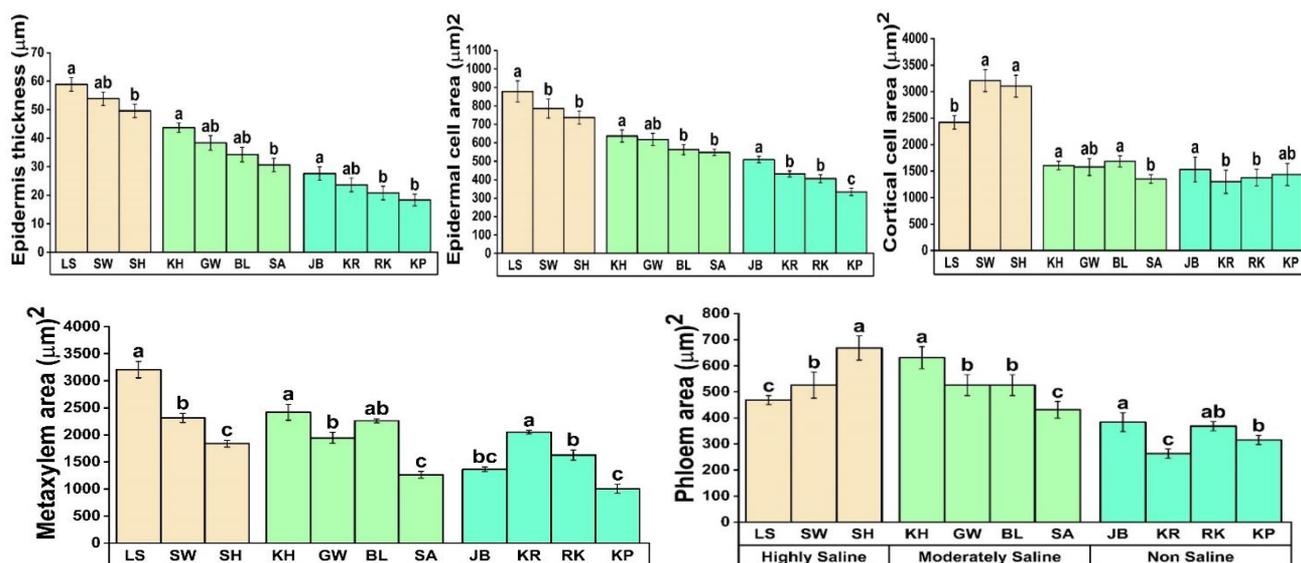


Fig. 4. Stem anatomical characteristics of *Dichanthium annulatum* collected from different saline habitats.

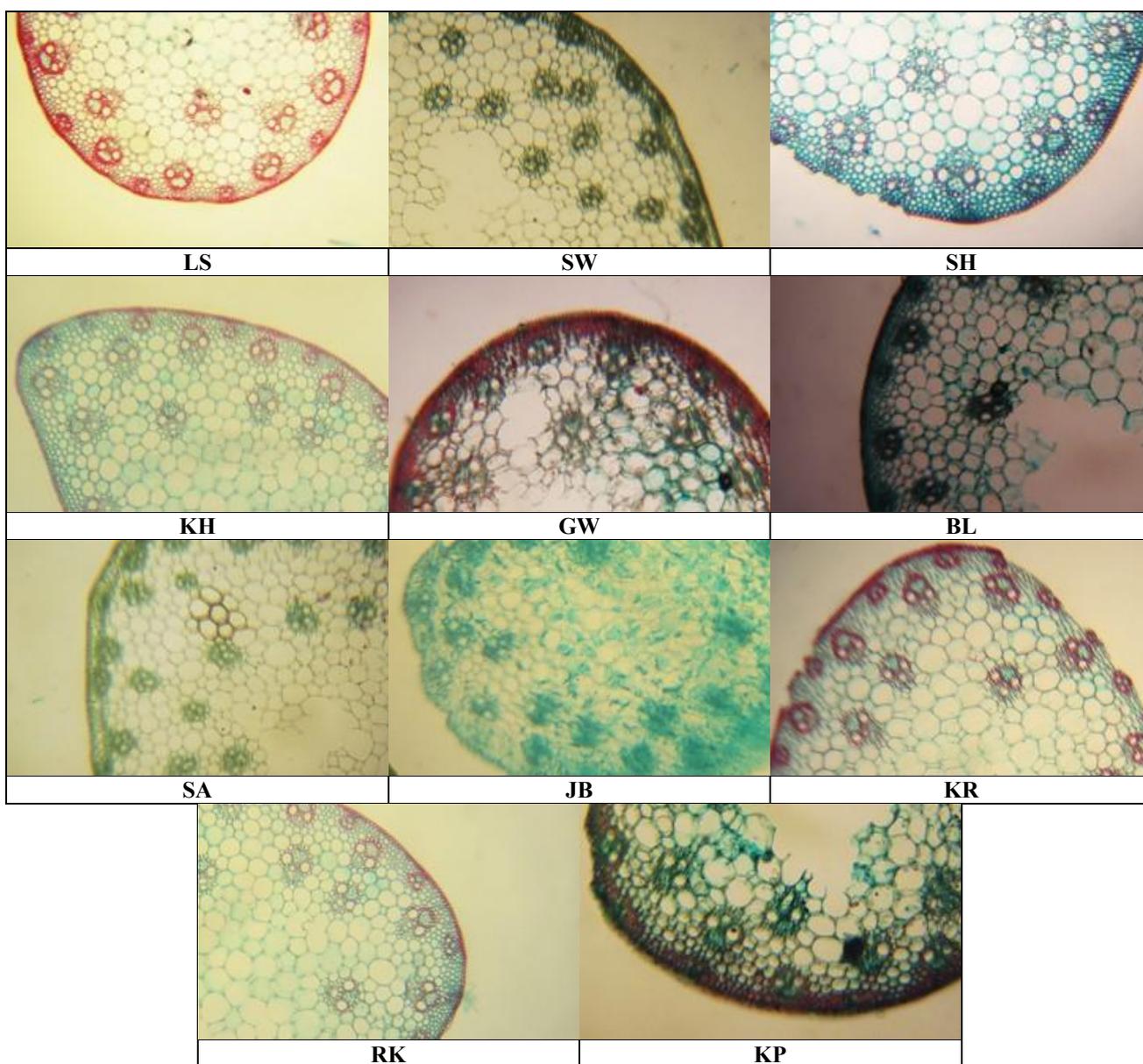


Fig. 5. Stem transverse sections of different populations of *D.annulatum* collected from different saline conditions.

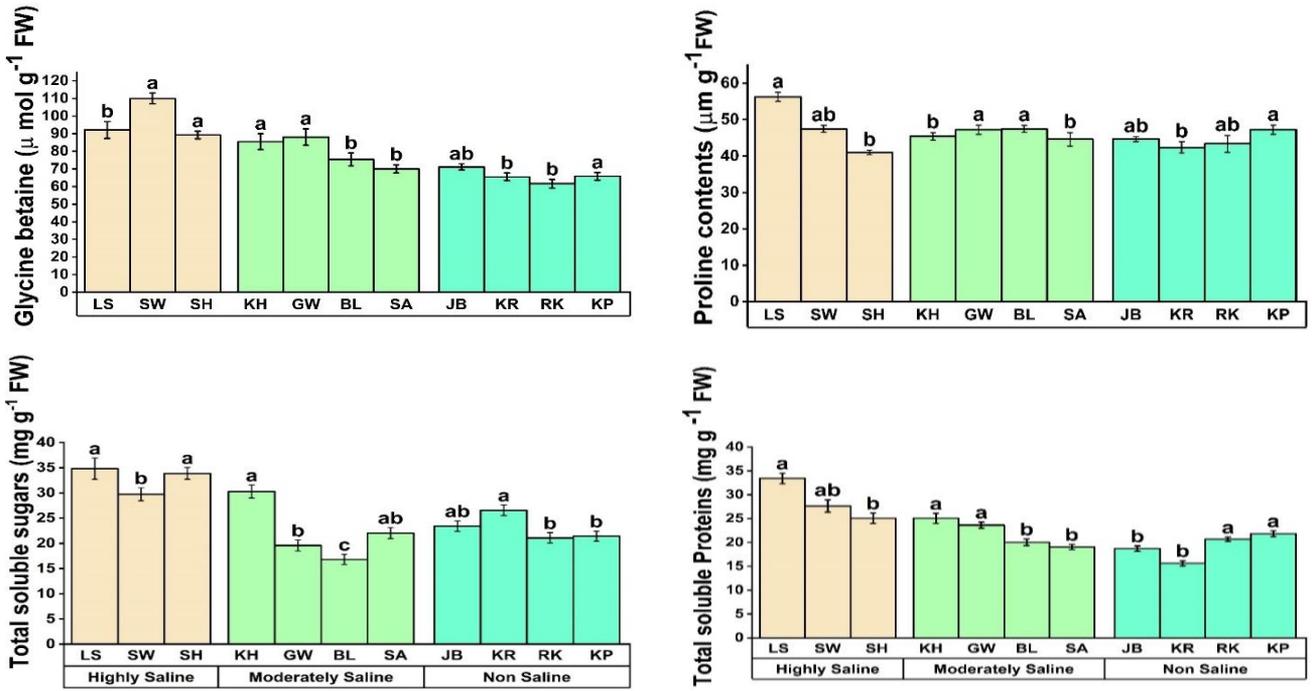


Fig. 6. Organic osmotica in *Dichanthium annulatum* collected from different saline habitats.

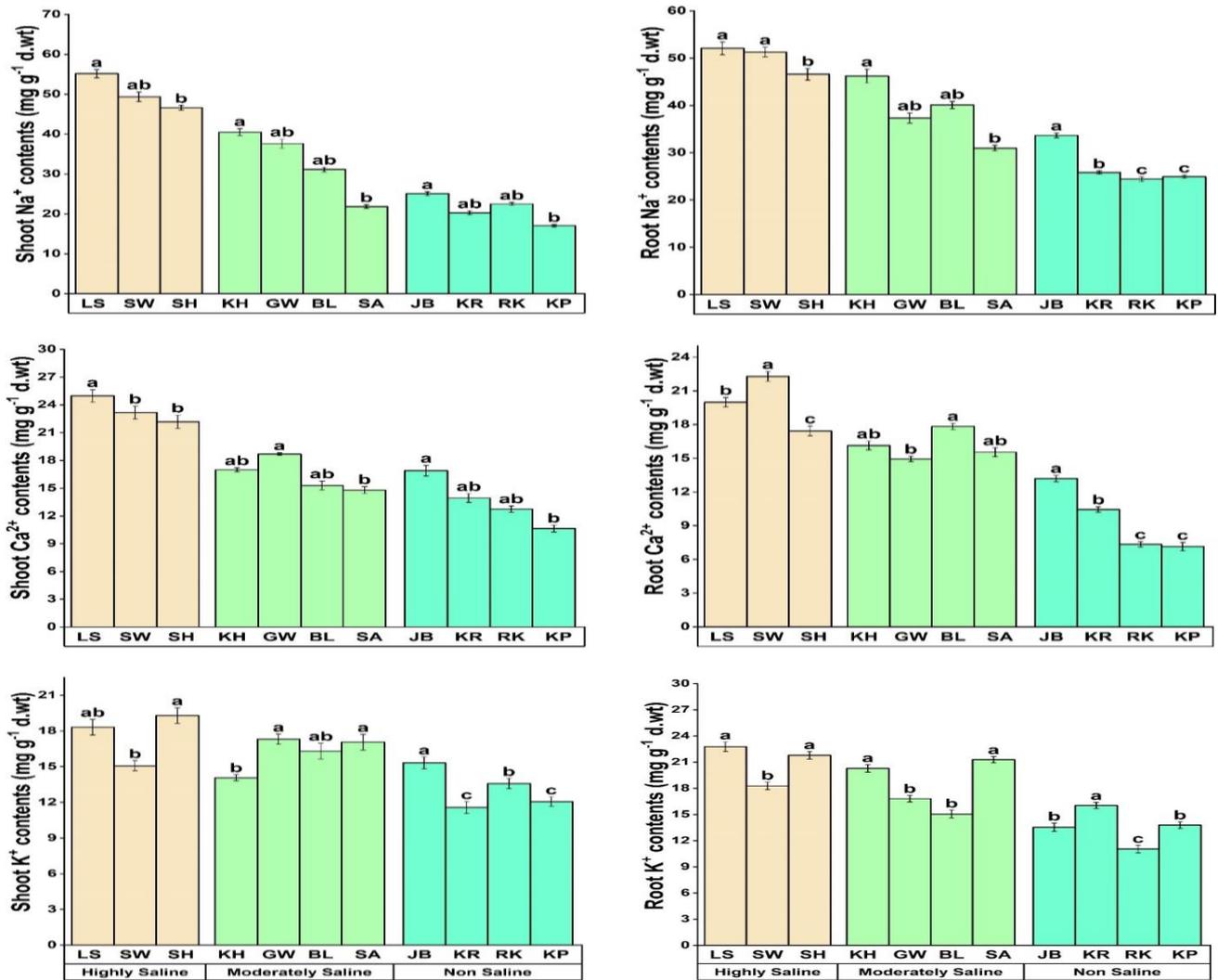


Fig. 7. Plants ionic contents of *Dichanthium annulatum* collected from different saline habitats.

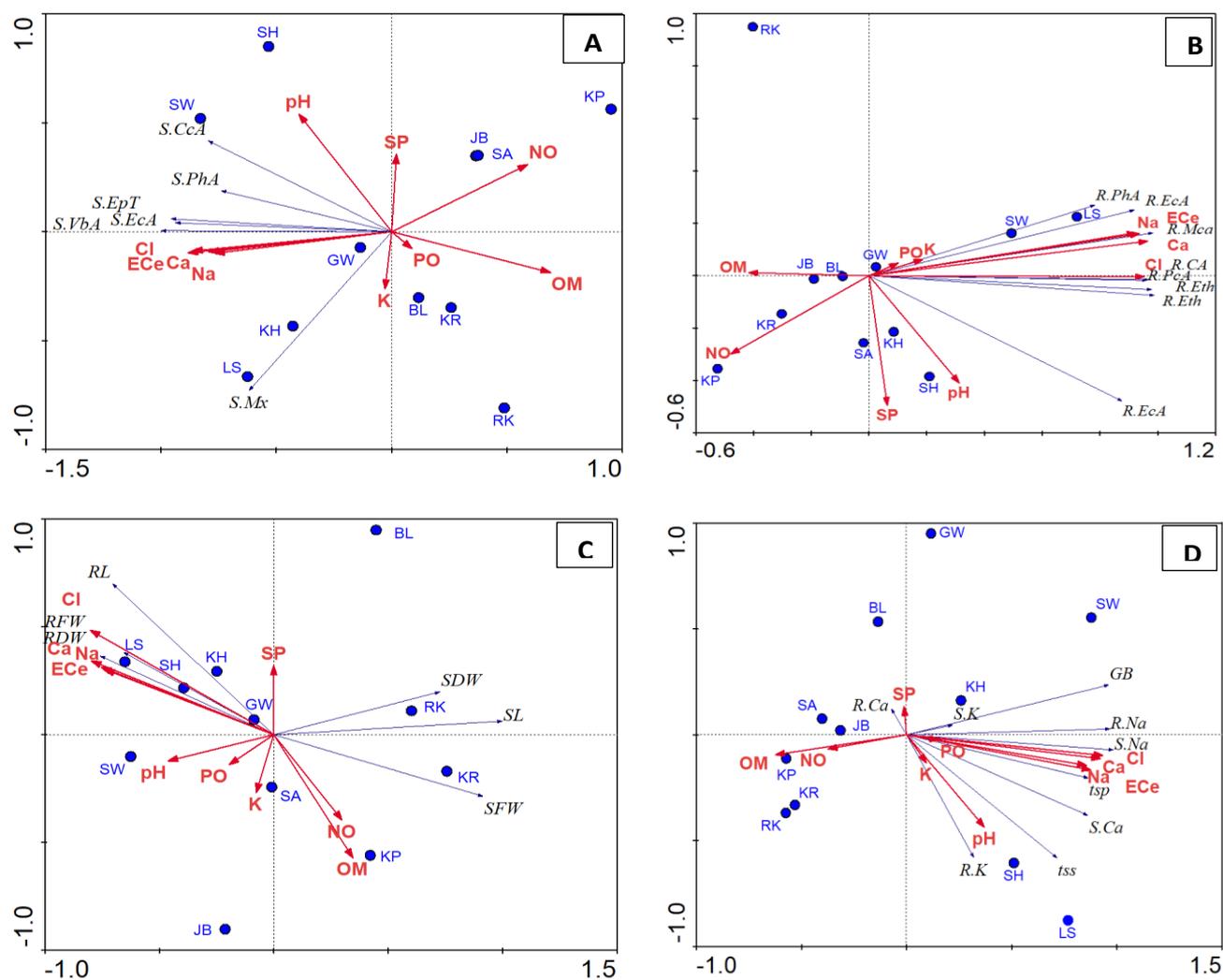


Fig. 8. A) RDA between stem anatomy and soil characteristics B) RDA between root anatomy and soil characteristics C) RDA between growth parameters and soil D) RDA between plant ionic contents and organic osmolytes.

Association between soil and plant morpho-physiological & anatomical parameters: The impact of soil properties from various habitats on physiological and morpho-anatomical attributes of *D. annulatum* was demonstrated using a redundancy analysis (RDA) biplot. Root fresh and dry weight showed closed association with soil ECe, Na⁺ and Ca²⁺ at Ladam Sir and Salluwanli population. Root epidermal cell area and phloem area showed closed association with soil K⁺ and phosphate at Ladam sir and Salluwanli population. Root length was closely associated with Cl⁻ at Khanewal population. Root pith cell area and cortical cell area were closely associated with soil chloride ions. Soil saturation percentage and pH had great impact at Sahiwanla, Karana Hill and Salmani adda populations. Total soluble sugars had close association with soil pH at Salluwanli population and shoot potassium showed closed association with soil saturation percentage at Karana Hill population (Fig. 8).

Response of differently adapted populations to salinity gradients: Response of *Dichanthium annulatum* to different salt affected habitat was represented in GLM model. Root epidermal thickness, endodermal thickness, metaxylem area, pith cell area and phloem area increased as soil salinity increased. A sharp increase was recorded in stem epidermal thickness with the increase of soil salinity.

Stem vascular bundle area, cortical cell area and epidermal cell area showed increase with the increase of salinity. Root length, root fresh and dry weight increased as soil salinity increased but shoot length, shoot dry weight and shoot fresh weight reduced with the increase of soil salinity. Root and shoot sodium increased with increase of salinity and organic osmolytes also increased against salinity gradient (Fig. 9).

Discussion

Salinity had a significant impact on the growth of both glycophytes and halophytes. The degree of salinity tolerance varies greatly among plant species. Halophytes are often more salt tolerant than glycophytes (Kosová *et al.*, 2011). Perennial grasses like *Dichanthium annulatum* are more abundant among 150 halophytes flourished in Pakistan. During the growth of this species, it exposed to various degree of temperature, humidity and salt stress due to the unreliable monsoon rains leading to adaptation of diverse strategies to exploit their fitness (Saeed *et al.*, 2011). The tolerance ability of different species to cope with environmental hazards in the hot and dry saline habitats was shown by significant changes in anatomical and physiological characteristics against the salinity gradient (Naz *et al.*, 2015).

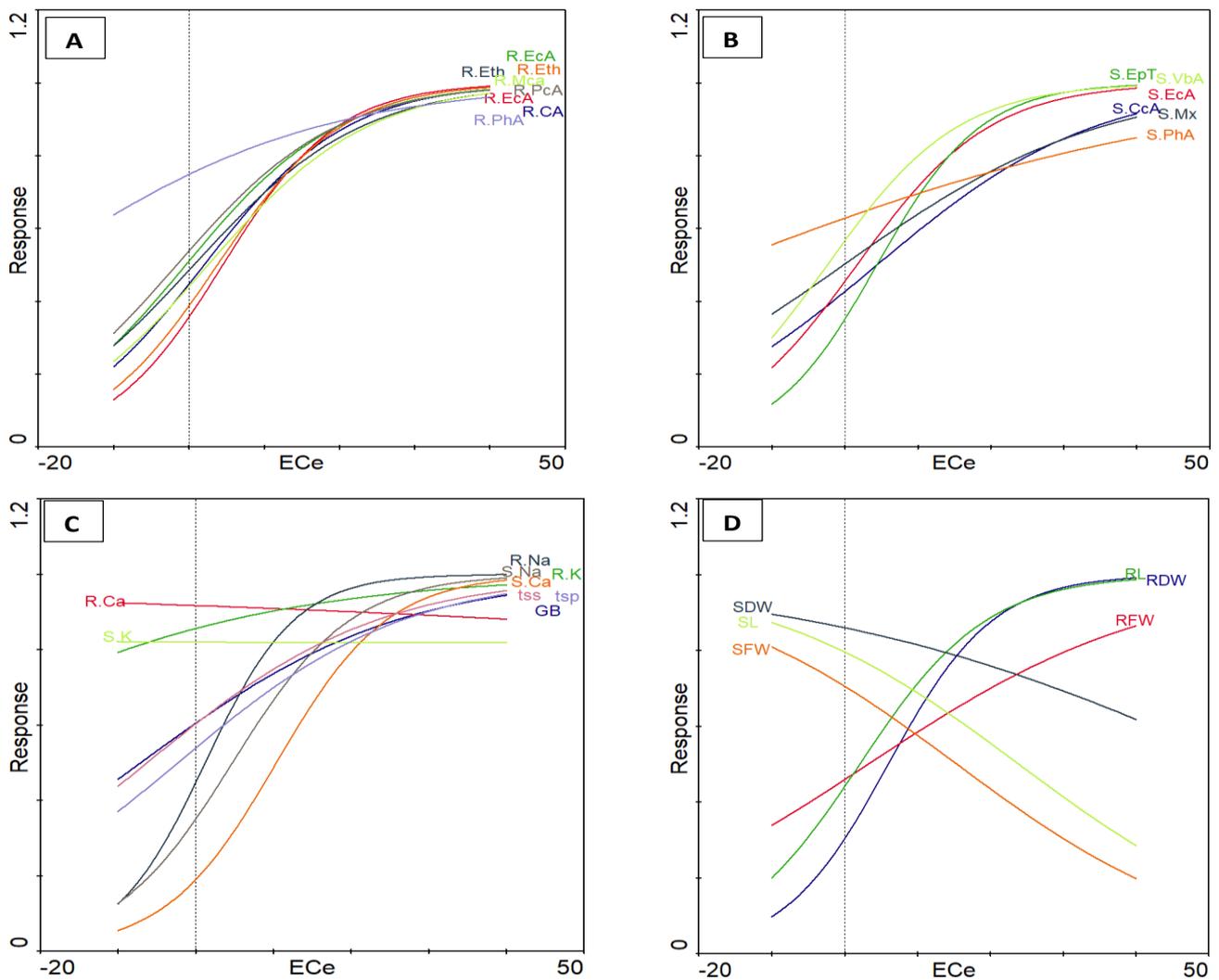


Fig. 9. GLM (Generalized linear model) showing response curve of *Dichanthium annulatum* against salinity gradient A) Root anatomical traits B) Stem anatomical traits C) Physiological and ionic contents D) Growth parameters.

Soil parameters: SP: saturation percentage, OM: Organic matters, Na: Soil sodium, SP = soil saturation percentage, ECe = Electric conductivity, NO= nitrate content, PO= Phosphate, Cl: Chloride contents, Na: soil Na contents: **Stem anatomy:** S.CcA: stem cortical cell area, S.PhA: Stem phloem cell area, S.EpT: Stem epidermis thickness, S.EcA: Stem epidermal cell area, S.VbA: stem vascular bundle area. **Root anatomy:** R.Eth: Root epidermis thickness, R.PhA: root phloem area, R.PcA: root pith cell area, R.EcA: Root epidermal cell area, R.CA: Root cortical cell area, R.Mx: root metaxylem area. **Morphology:** SL: Shoot length, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, SDW: Shoot dry weight, RDW: Root dry weight. **Physiology:** GB: Glycine betaine, TSS: Total soluble sugars, TSP: total soluble proteins, S.Na: Shoot sodium, R.Na: Root sodium, S.K: Shoot Potassium, R.K: Root potassium, S.Ca: Shoot calcium, R.Ca: Root Calcium

Growth characteristics are regarded as the most essential criteria for weeding out salt-tolerant plants and determining their degree of salinity tolerance (El-Hendawy *et al.*, 2017). In present work, the shoot length and shoot fresh weight was decreased at highly saline habitats. Maximum reduction in shoot length and shoot fresh weight was observed in LS and SW population. Salinity decreased shoot length due to restricted growth, decreased leaf area (Läuchli and Epstein, 1990), and leaf expansion (Jafri & Ahmad, 1995), as well as shrinkage in cell content and membrane disruption. The decrease in shoot length might possibly be owing to the toxic effects of Na^+ and Cl^- on metabolic processes, which generate some sticky substance on cell walls, reducing cell flexibility and expansion. As a result, new cells formed quickly and the shoot remained dwarf (Ashraf, 2002;

Ibrahim, 2003). Similarly, a decrease in shoot fresh weight occurs as a result of excessive salt absorption by the root and decreased water uptake (Saqib *et al.*, 2002). This was supported by the findings of other authors as Noor *et al.*, (2001), Iqbal *et al.*, (2013), Abbas *et al.*, (2011) Akhter & Azhar (2001) on cotton that clearly stated the considerable decrease in shoot growth at increased salinity stress. Root length was increased at highly saline habitats i.e. LS population, increased in root length under high salinity was earlier demonstrated in *Sporobolus ioclados* by (Naz *et al.*, 2016).

The primary functional modifications in plants under salinity is to endure osmotic adjustment by two mechanisms: accumulation of toxic ions in vacuole and production of organic osmolytes in cytosol (Li *et al.*, 2010). Therefore, salt stress instigate changes in levels of

different organic osmolyte as proline, glycine betaine, total soluble proteins and total soluble sugars were examined to elucidate the role of these compounds against salt stress in *D. annulatum*. In our study the total soluble sugars was significantly increased at highly saline population i.e., LS and SH. The increased total soluble sugars are possibly due to inter-conversion of starch-sugars to provide more sugar for osmo protection for salinity tolerance (Parida & Jha, 2013; Slama *et al.*, 2015). The present study showed maximum proline concentration at high and moderate saline habitats i.e., LS population, proline concentration increased in response salinity has been noted in many plants Parida *et al.*, 2004b; Koyro, 2006; Rajaravindran & Natarajan, 2012; Zakery-Asl *et al.*, 2014).

Modifications in plants to salt stress induce changes in uptake and transportation of inorganic ions to regulate the cellular homeostasis. The osmotic and turgor pressure of halophytic plants shoot was maintained by using minerals ions (Na^+ , Cl^- and K^+) under salt stress while glycophyte accomplish this by the production of organic osmolytes (Shabala, 2013; Shabala & Pottosin, 2014). As noticeable from our research work, accumulation of sodium ions increased by the increase of NaCl in stem and root of *D. annulatum*. Halophytes like *crystallinum* and *Salicornia bigelovii* accumulate greater concentration of Na^+ in tissues and this accumulation involve in the metabolic process as well as improved growth, as halophyte demands NaCl for better success (Tran *et al.*, 2020).

Plants undergo structural adaptations in root and stem as a result of salt stress (Barberon *et al.*, 2016). Roots are the first line of defense due to the direct encounter with the saline soil solution (Rewald *et al.*, 2013). Epidermis is a defensive layer and inhibits internal tissue system from direct exposure to environmental hazards. Thicker and well-developed epidermis can merely guard a plant from dehydration, and therefore, the LS population subsisted successfully in physiological drought triggered by High salt stress (Akram *et al.*, 2011). Increased endodermal thickness may regulate the radial flow of water through the stellar cell, as in most salt tolerant population i.e., LS. This was in accordance with other authors conclusions like Fatima *et al.*, (2021) in *Cymbopogon jwarancusa* and Wasim & Naz (2020) in *Cenchrus ciliaris*. Increased storage parenchyma tissues (pith area) were founded in highly saline population (LS), which may improve the water retention capacity of this population to relieve the oxidative stress as a result of low water potential of the soil (Kaleem & Hameed, 2021). In stem most conspicuous adaptation were the development of larger metaxylem and phloem in highly saline populations i.e., LS and SH, because they develop most successful mechanism for the translocation of minerals and water under high salinity because they provide protection from the accumulation of salts by increasing translocation through stem to outside of plant body (Reginato *et al.*, 2016).

Conclusion

In conclusion, all three populations of *D. annulatum* from highly saline habitats showed greater degree of salinity tolerance by adaptation in structural and

functional traits. Population SH and LS showed greater performance and rated as high tolerant as compared to other populations. Accumulation of toxic Na^+ in shoot tissues indicated high phytoremediation potential, mainly of the highest tolerant LS population.

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