

SALT TOXICITY IN A NATURAL HABITAT INDUCES STRUCTURAL AND FUNCTIONAL MODIFICATIONS AND MODULATE METABOLISM IN BERMUDA GRASS (*CYNODON DACTYLON* [L.] PERS.) ECOTYPES

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(Received 22nd Apr 2020; accepted 13th Aug 2020)

Abstract. We investigated salt toxicity effects and related physiological as well as anatomical changes for adaptation in *Cynodon dactylon*. Eleven ecotypes from different areas of Pakistan were examined for their survival capacity in a controlled environment. The experiment was designed in a two factor Completely Randomized Design (ecotypes and 4 salt levels 0, 100, 200, 300 mM NaCl). Differently adaptive populations displayed specific structural and functional adaptations with respect to photosynthesis and photosynthetic pigments to withstand salinity. The ecotypes from saline and waterlogged areas exhibited higher Photosynthetic rate to same level of increase in salinity level. Transpiration rate of all ecotypes showed variations with different salinity levels and stomatal conductance increased in all ecotypes with increasing salinity. Similarly, all ecotypes responded significantly as far as chlorophyll contents were concerned. Most ecotypes consistently showed high leaf blade thickness and sclerenchyma thickness with elevated salinity except for plants collected from saline, waterlogged and salt affected wasteland. Different salt levels decreased the metaxylem cell area while phloem area increased in the ecotypes correspondingly. *Cynodon* ecotypes possessed low abaxial stomatal density at 300 mM salinity. Overall, physiological and anatomical adaptations to saline environments appeared very specific in grasses and supported life cycle under salt stress.

Keywords: *anatomical changes, Bermuda grass, photosynthetic pigments, salinity, sclerification*

Introduction

Plants, as sessile organisms, are often subjected to various environmental stresses i.e. biotic and abiotic causing massive structural and functional changes (Khalid et al., 2020; Mickelbart et al., 2015; Pandey et al., 2017). Growth and all other plant activities depend on photosynthesis, photosynthetic pigments and photosynthetic machinery (Ashraf and Harris, 2013; Cardona et al., 2018). But these cellular systems are damaged by abiotic and biotic stresses (Gururani et al., 2015; Mohanty et al., 2006) especially the degradation of chlorophyll pigmentation due to alteration in gene expression (Dutta et al., 2009; Noman and Aqeel, 2017; Sharma et al., 2019) that limits photosynthesis but also reduce plant growth and yield (Ali and Ashraf, 2011; Arshad et al., 2016; Khalid et al., 2020, 2018; Thuynsma et al., 2016). The salinization of irrigated lands in arid/semi-arid areas is one of the major causes of low crop production in many parts of the world (Araus et al., 2010; Hussain et al., 2019).

Cynodon dactylon (L.) Pers. (Bermuda grass, locally known as Khabbal grass) is distributed abundantly in tropical and warm temperate areas (Shi et al., 2012) throughout the world between 45°N and 45°S (Ling et al., 2015). It tolerates high temperatures, drought, and salinity (Chen et al., 2015; Hu et al., 2015). It has C₄ photosynthesis pathway (Edwards et al., 2004) which is a complex adaptation evolved from C₃ photosynthesis (Carmo-Silva et al., 2008). The C₄ adaptation is extremely successful in the monocotyledonous families i.e., Poaceae and Cyperaceae (Besnard et al., 2009; Sage, 2004). Salinity tolerance, especially in grasses, depends on plant morpho-physiological features (Bahrani et al., 2010; Volkov and Beilby, 2017). In different *C. dactylon* ecotypes salinity ranges from 6 to 10 dS m⁻¹, and the different species are classified as semi-tolerant to tolerant to drought stress (Uddin et al., 2012).

Physiological adaptations are important criteria for salt tolerance selection (Ashraf, 2004; Gupta and Huang, 2014; Hernández, 2019), but anatomical modifications under high salinities are also crucial (Naz et al., 2014; Noman et al., 2017, 2012; Ye et al., 2015). Photosynthesis is affected by leaf anatomy and CO₂ exchange. Structural features of the leaf contributing to the maintenance of the high CO₂ concentration in the chloroplast stroma may have been selected during evolution (Terashima et al., 2011). Stomata and stomatal activities are much important for the photosynthetic activities and reduction in net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration, quantum efficiency of PSII, and non-photochemical quenching in *C. dactylon* is directly linked to non-stomatal activities (Bhuiyan et al., 2015).

A typical respond to salt stress is the development of thick leaves due to the thickening of the cortex and mesophyll cells (Boughalleb et al., 2009; Geldner, 2013). The structural alterations in vascular bundles, the nature of lignified tissues are all thought to be very helpful in allowing the plant to fight against various environmental stresses (Noman et al., 2014; Qaderi et al., 2019; Zwieniecki et al., 2003). The emptying of the vacuoles of large bulliform cells induces leaf curling and the rolling of leaves that can be distinctively considered as an important adaptation against salinity-induced physiological drought stress for the water conservation and lowering the rate of transpiration (Alvarez et al., 2008; Balsamo et al., 2006; Hameed et al., 2013). Besides, the increased density and size of trichomes may also provide the additional benefit of enabling a plant to withstand environmental stresses like drought and salinity. Increasing the density of micro-hairs under conditions of increased salt may be one of the most effective mechanisms for salinity tolerance in Bermuda grass (Hu et al., 2015).

It was hypothesized that differently adaptive populations might have some specific structural and functional adaptations with respect to photosynthesis and photosynthetic pigment to withstand salt stress condition. The investigation was focused on the leaf anatomical changes as dermal tissues (epidermis, stomatal density and area, trichome length, and leaf hairiness), vascular tissues (large metaxylem vessels), and mechanical tissues (sclerenchyma) that influence photosynthesis and changes in chlorophyll pigments and gas exchange characteristics under salt stress conditions.

Materials and methods

Selected ecotypes of Cynodon dactylon

Various ecotypes of *C. dactylon* were selected from the Punjab, Pakistan from different ecological regions as reported by Tufail et al. (2017). The ecotypes (DF-SD) and (S-HS) were selected from saline arid regions. Ecotypes (KL-HS), (UL-HS), and (KKL-S) were collected from saline lakes in the Salt Range. Ecotypes (S-SW) and (T-SW) were from saline waterlogged areas. Two ecotypes were from salt-affected wasteland, (PA-HS) and (PA-RF). Two ecotypes were from non-saline well irrigated habitats, (MG-RB) and (BG-NS) (*Table 1; Fig. 1*).

Table 1. Various ecotypes of *Cynodon dactylon* L. from the Punjab, Pakistan

| Sr. No. | Sampling sites | Ecotypes | Latitude (N) | Longitude (E) |
|---------|----------------------------|----------|--------------|---------------|
| 1 | Derawar Fort-Saline Desert | DF-SD | 28° 75' | 71° 33' |
| 2 | Muzaffargarh-River Bank | MG-RB | 30° 08' | 71° 19' |
| 3 | Khabbeki Lake-Hyper Saline | KL-HS | 32° 29' | 72° 34' |
| 4 | Uchali Lake-Hyper Saline | UL-HS | 32° 32' | 72° 11' |
| 5 | Kalar Kahar Lake-Saline | KKL-S | 32° 47' | 72° 42' |
| 6 | Treemu-Saline Wetland | T-SW | 31° 31' | 72° 33' |
| 7 | Sahianwala-Saline Wetland | S-SW | 31° 38' | 73° 14' |
| 8 | Sahianwala-Hyper Saline | S-HS | 32° 13' | 73° 29' |
| 9 | Pakka Anna Hyper Saline | PA-HS | 31° 14' | 72° 48' |
| 10 | Pakka Anna Reclaimed Field | PA-RF | 31° 98' | 72° 99' |
| 11 | Botanic Garden-Non Saline | BG-NS | 31° 36' | 73° 35' |

Cynodon dactylon culture and salt treatment

Naturally adapted populations of *C. dactylon* from several regions of the Punjab, Pakistan were established in the Botanic Garden, University of Agriculture, Faisalabad, in non-saline soil. The plants were kept under full sunlight and irrigated daily up to their establishment in Faisalabad environment. Randomly selected ramets from each ecotype of equal size (with two mature up tillers) were detached, fixed and grown in aerated hydroponics using half-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) for eight weeks. Air pumps were used for aeration of hydroponic culture system for about 12 h daily. Ten ramets from each population were selected for each replication and fixed in the pores of thermophores (mineral fiber) sheets for each experimental unit, which was placed on hydroponic culture solution.

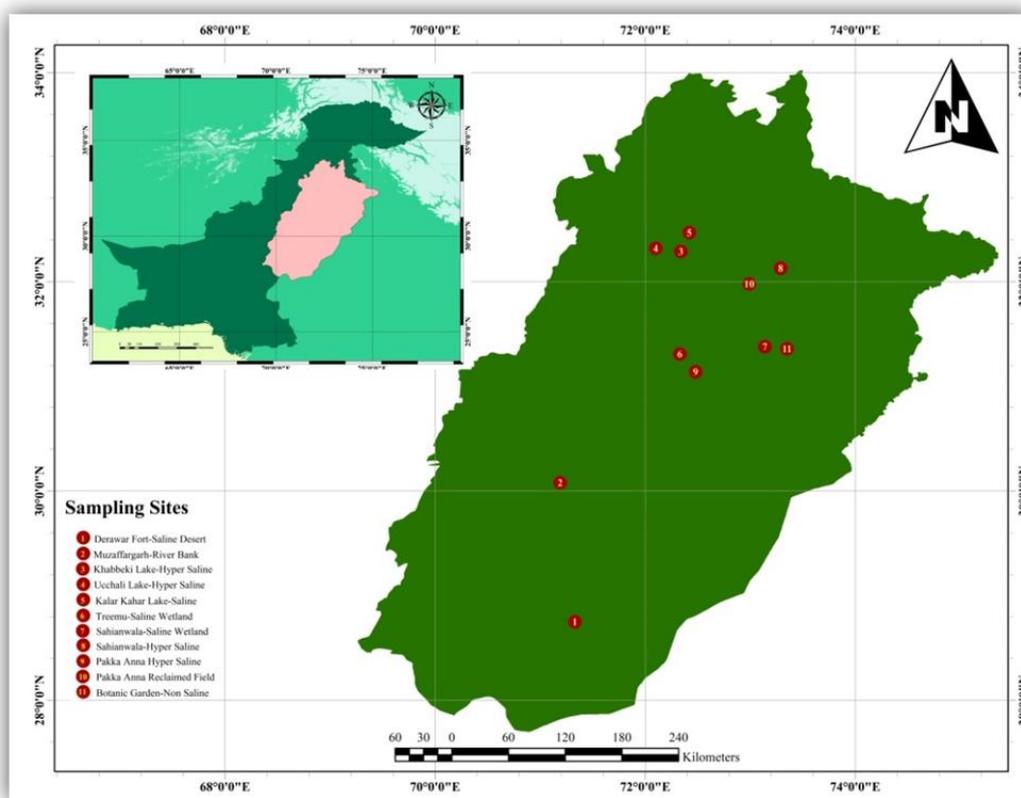


Figure 1. Map of the Punjab, Pakistan showing ecotypes of *Cynodon dactylon* from different sampling sites

Four salinity levels were maintained during the experiment viz. control (no salinity treatment), 100, 200 and 300 mM of NaCl salinity in solution culture up to flowering stage. After 8-weeks, plants were washed thoroughly and used for studying the various anatomical characteristics.

The ecotypes were subjected to salt stress after their establishment in the Faisalabad environment. The experiment for salt stress was arranged in two factor-factorial (ecotypes and salt levels) completely randomized design with ten replications and 0 (control), 100, 200, and 300 mM NaCl levels in aqueous culture media containing half-strength Hoagland's medium.

Leaf anatomical studies

Transvers sections of the newly grown leaves from three plants as replicates were made by the free hand sectioning by serial dehydrations in ethanol using double standard staining (Safranin and fast green) technique. Plant material was preserved in the Formalin Acetic Alcohol (FAA) fixative for 48 h and subsequently transferred to acetic alcohol (v/v acetic acid 25%, and ethanol 75%) solution for long term storage.

Photographs were taken by a camera-equipped light microscope (Meiji Techno: MT4300H USA). Parameters were recorded as: Leaf blade thickness (μm), Epidermal cell area (μm^2), Sclerenchymatous thickness (μm), Bulliform cell area (μm^2), Vascular Bundle area (μm^2), Metaxylem area (μm^2), Phloem area (μm^2), Trichome length (μm) and density, Stomatal area (μm^2) and density.

Photosynthetic parameters

Measurements of net CO₂ Assimilation Rate (*A*), Transpiration (*E*) and Stomatal Conductance, Sub Stomatal CO₂ concentration and Water Use Efficiency (calculated as WUE = *A*/*E*) were recorded by using LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). The gas exchange measurements were performed *in situ* from 10:30 a.m. to 12:30 p.m. with specific specifications/adjustments as reported by Schiavon et al. (2016) and Tamayo et al. (2001).

Photosynthetic pigments

The chlorophyll a, b and carotenoids were determined according to the method of Arnon (1949).

Statistical analysis

The data were subjected using analysis of variance in completely randomized design. Redundancy analysis (RDA) was performed using Conoco 4.5 computer software.

Results

Photosynthetic attributes

Gas exchange attributes

The net CO₂ assimilation rate (*A*) decreased in the ecotypes of *C. dactylon* KL-HS, UL-HS, KKL-S, S-HS, PA-RF and BG-NS (*Table 1*) with increase in the concentration of salinity. The ecotypes from T-SW and S-SW responded the best to the maximum salinity level with higher net CO₂ assimilation rates (*Fig. 2*).

The transpiration rate (*E*) of ecotypes of *C. dactylon* from S-HS, PA-RF, and BG-NS decreased as the salt stress level increased (*Fig. 2*). Ecotypes from DF-SD, KKL-S, T-SW, S-SW showed slightly decreased transpiration rate at salinity levels (100 and 200 mM NaCl) but increased at 300 mM NaCl. The transpiration rate for the PA-HS ecotype peaked at 100 mM NaCl but decreased at higher levels of salt stress (*Fig. 2*).

The stomatal conductance of ecotypes from the DF-SD, M-RB, KL-HS, KKL-S, and BG-NS increased as the salinity level increased (*Fig. 2*). There was no significant variation in sub stomatal CO₂ concentration, for many of the experimental ecotypes, except from DF-SD, KL-HS, UL-HS and PA-RF exhibited reduced sub stomatal CO₂ concentrations at the highest experimental salinity level (300 mM NaCl) (*Fig. 2*).

Ecotypes from the DF-SD, M-RB, KL-HS, UL-HS, and BG-NS exhibited a quadratic response of water use efficiency to salinity. S-SW, S-HS, PA-HS exhibited quadratic responses and exhibited the highest levels of water use efficiency at 300 mM NaCl (*Fig. 2*).

Photosynthetic pigments

DF-SD, S-HS, and BG-NS exhibited slightly increased chlorophyll a levels at the 100 and 200 mM NaCl levels, and exhibited maximum reduction in the pigment at 300 mM NaCl (*Fig. 2*).

The chlorophyll b response was, for many of the experimental ecotypes, higher at the 100 or 200 mM NaCl levels. M-RB exhibited increasing chlorophyll b levels as salinity increased. KL-HS, UL-HS, and S-SW exhibited peak levels of chlorophyll b at 100 mM

NaCl (Fig. 2). DF-SD, PA-RF, T-SW, S-HS, PA-HS, and BG-NS exhibited maxima at the 200 mM NaCl level (Fig. 2).

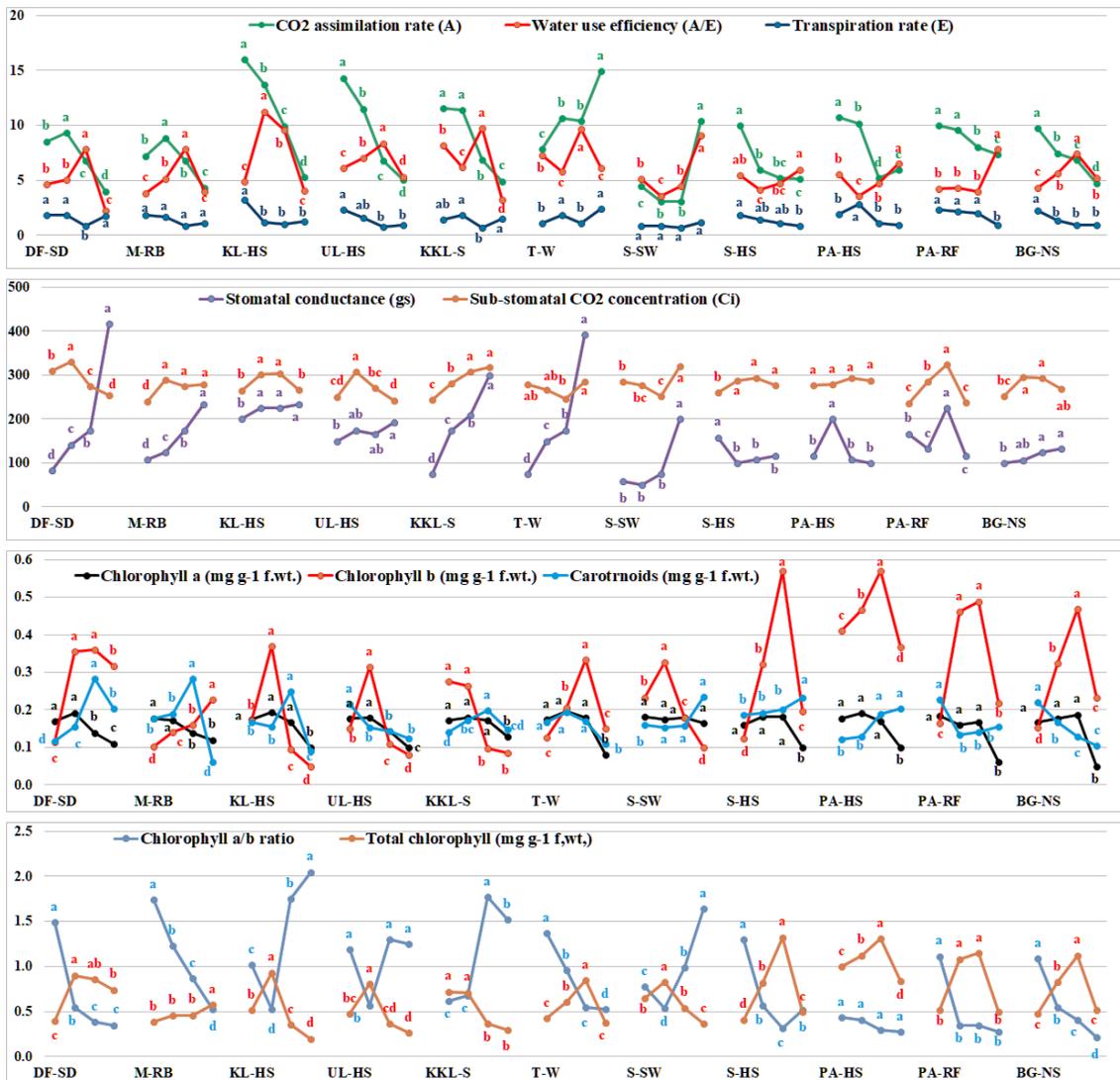


Figure 2. Series plots of gas exchange characteristics and photosynthetic pigments under four salt levels (0, 100, 200, 300 mM) NaCl. DF-SD (Derawar Fort-Saline Desert), MG-RB (Muzaffargarh-River Bank), KL-HS (Khabbeki Lake-Hyper Saline), UL-HS (Uchali Lake-Hyper Saline), KKL-S (Kalar Kahar Lake-Saline), T-SW (Treemu-Saline Wetland), S-SW (Sahianwala-Saline Wetland), S-HS (Sahianwala-Hyper Saline), PA-HS (Pakka Anna Hyper Saline), PA-RF (Pakka Anna Reclaimed Field) and BG-NS (Botanic Garden-Non Saline)

The amount of leaf carotenoid pigments indicated different patterns of response in the experimental ecotypes at the experimental levels of salinity. While ecotypes from S-SW, S-HS and PA-HS exhibited increasing leaf carotenoid compounds due to salinity. DF-SD, M-RB, and KL-HS responded with higher amounts of carotenoids at 200 mM NaCl (Fig. 2).

Chlorophyll a and b ratio also decreased with increase in salinity level in ecotypes of *C. dactylon* from DF-SD, M-RB, T-SW, PA-HS, PA-RF and BG-NS (Fig. 2). Furthermore KL-HS, UL-HS and S-SW exhibited higher amounts of total chlorophyll at

100 mM NaCl. Ecotypes from T-SW, S-HS, PA-HS, PA-RF and BG-NS exhibited higher amounts of total chlorophyll at 200 mM NaCl (Fig. 2).

Leaf anatomy

Many of the ecotypes of *C. dactylon* exhibited consistently increased leaf blade thickness with increased salt stress, except ecotypes from S-SW, S-HS, and PA-HS, which exhibited gradual increases of leaf blade thickness up to 200 mM NaCl stress but reduced leaf thickness at the maximum experimental salinity level. In the ecotypes from BG-NS, the maximum leaf blade thickness was observed at 100 mM NaCl, and increasing levels of salinity reduced leaf blade thickness (Fig. 3).

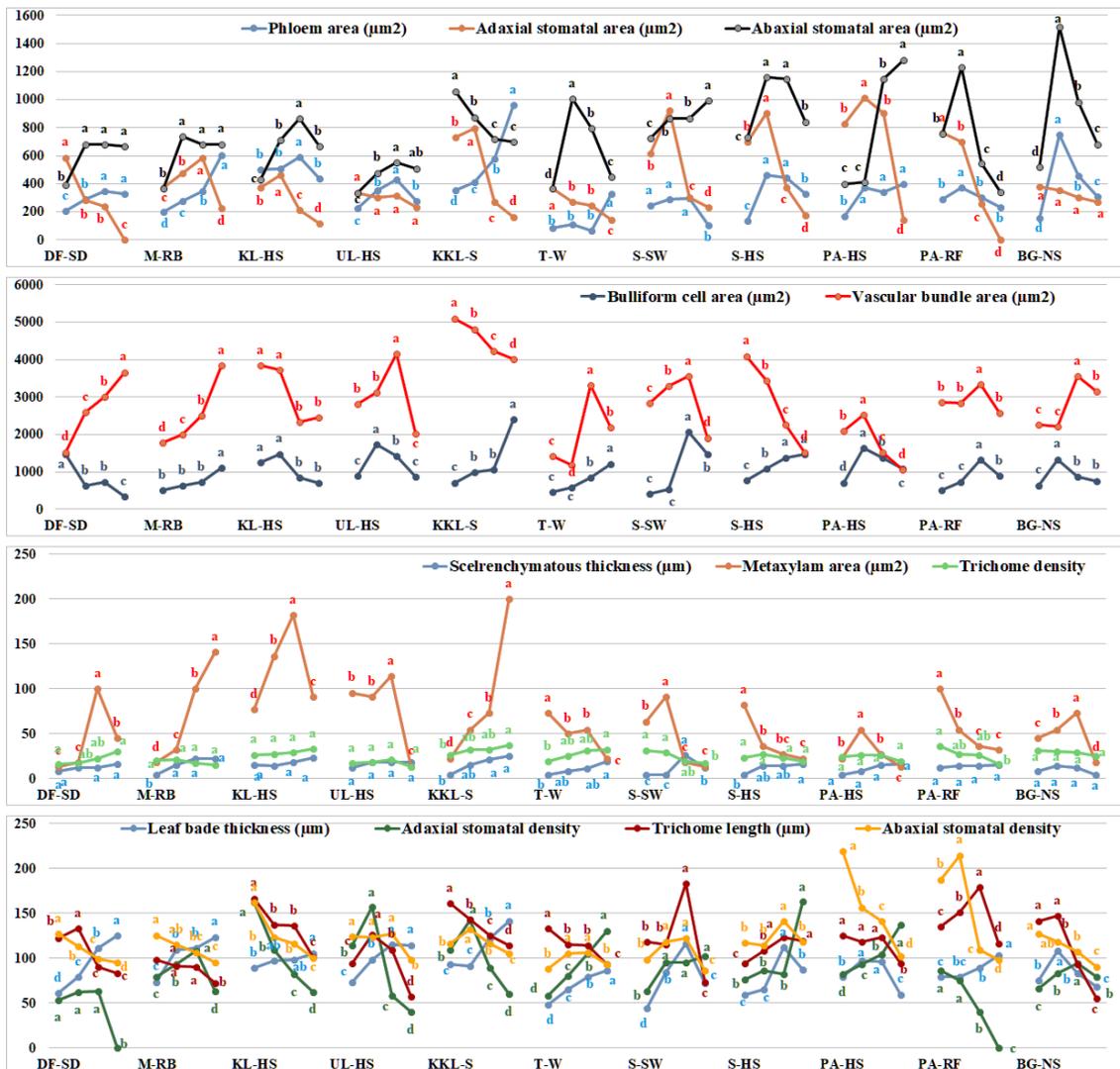


Figure 3. Series plots of leaf anatomical attributes under four salt levels (0, 100, 200, 300 mM) NaCl. DF-SD (Derawar Fort-Saline Desert), MG-RB (Muzaffargarh-River Bank), KL-HS (Khabbeki Lake-Hyper Saline), UL-HS (Uchali Lake-Hyper Saline), KKL-S (Kalar Kahar Lake-Saline), T-SW (Treemu-Saline Wetland), S-SW (Sahianwala-Saline Wetland), S-HS (Sahianwala-Hyper Saline), PA-HS (Pakka Anna Hyper Saline), PA-RF (Pakka Anna Reclaimed Field) and BG-NS (Botanic Garden-Non Saline)

Sclerenchyma thickness was consistently high where plants were subjected to 300 mM NaCl, except in the ecotypes from BG-NS, where the maximum sclerenchyma thickness was observed at 100 mM NaCl. With the increase of salinity a reduction of sclerenchyma thickness was observed, but S-SW exhibited a gradual increase of sclerenchyma thickness up to 200 mM NaCl stress, but reduced sclerenchyma thickness at 300 mM NaCl (*Fig. 3*).

The ecotypes of *C. dactylon* from M-RB, KKL-S, T-SW, and S-HS exhibited that bulliform cell area increased as salinity increased while bulliform cell area of DF-SD were described by a third order polynomial or sinusoid (*Fig. 3*). Ecotypes from KL-HS, UL-HS, PA-HS and BG-NS showed increased bulliform cell area at 100 mM NaCl stress, but above the 100 mM NaCl level, bulliform cell area decreased with increase in salinity. Furthermore, ecotypes from S-SW and PA-RF exhibited a quadratic response of bulliform cell area that peaked at 200 mM NaCl and decreased at the maximum experimental salinity level (*Fig. 3*).

Ecotypes from DF-SD and M-RB exhibited Vascular Bundle (VB) area that increased as salinity increased, while a negative linear component described the KL-HS, KKL-S and S-HS VB area in response to salinity. Ecotypes from UL-HS, T-SW, S-SW, PA-RF, and BG-NS exhibited marked induction of VB area at 200 mM NaCl stress, but reduced areas at 300 mM NaCl. Ecotypes from PA-HS exhibited a quadratic response for VB area to salt stress that peaked at 100 mM NaCl (*Figs. 3, 4*).

Metaxylem cell area decreased in response to salinity, in the ecotypes from T-SW, S-HS and PA-RF, while M-RB and KKL-S exhibited increasing metaxylem area with the increasing salinity. The metaxylem area of ecotypes DF-SD, KL-HS, UL-HS, and BG-NS peaked at 200 mM NaCl stress. The metaxylem area of ecotypes from S-SW and PA-HS peaked at 100 mM NaCl (*Figs. 3, 4*).

Ecotypes from M-RB, KKL-S, T-SW, and PA-HS exhibited higher phloem areas at the highest experimental salinity level (300 mM NaCl). The phloem area of ecotypes from PA-RF and BG-NS peaked at 100 mM NaCl (*Figs. 3, 4*).

Most of the ecotypes of *C. dactylon* showed reduced stomatal density at the 300 mM NaCl level, (*Figs. 3, 5*). Stomatal cell area decreased in response to salinity in ecotypes from DF-SD, M-RB, KL-HS, and PA-HS. S-HS exhibited peak induction of stomatal density at 200 mM NaCl, and T-SW and PA-RF exhibited peak stomatal density at 100 mM NaCl stress (*Figs. 3, 5*).

Trichome length decreased in response to salinity in the ecotypes from M-RB, KL-HS, KKL-S, and T-SW (*Figs. 3, 6*). Trichome density increased in response to salinity in the ecotypes from DF-SD, KL-HS, KKL-S, and T-SW but decreased due to salinity in the ecotypes from M-RB, S-SW, PA-RF and BG-NS (*Figs. 3, 6*).

Redundancy (RDA) ordination

RDA ordination biplot for leaf anatomy

The RDA ordination biplot (*Fig. 7*) showed DF-SD showed the association with BCA at control and with Trichome Length (TRL), Abaxial Stomatal Density (ASD), Vascular Bundle Area (VBA), Trichome Density (TRD), Adaxial Stomatal Area (ADA), Sclerenchymatous Thickness (SCT), Leaf Blade Thickness (LBT), Adaxial Stomatal Density (ADD) and Metaxylem Area (MXA) at minimum salinity level 100 mM NaCl while from M-RB showed the same association up to maximum salinity level 300 mM NaCl and with Phloem area (PHA) at moderate salinity level

200 mM. Ecotypes from KL-HS showed association with PHA up to maximum salinity level 300 mM, however from UL-HS showed association with TRL, LBT, SCT and TRD at moderate salinity level 200 mM and with VBA, MXA at maximum salinity level 300 mM. Ecotype from KKL-S showed association with VBA, MXA at 0, 200 mM, with TRL, ASD, VBA, TRD, ADA, SCT, LBT, ADD and MXA at minimum salinity level 100 mM. Ecotype from T-SW showed association with ADA at control (Fig. 7).



Figure 4. Leaf blade transverse sections of *Cynodon dactylon* ecotypes collected from the Punjab, Pakistan

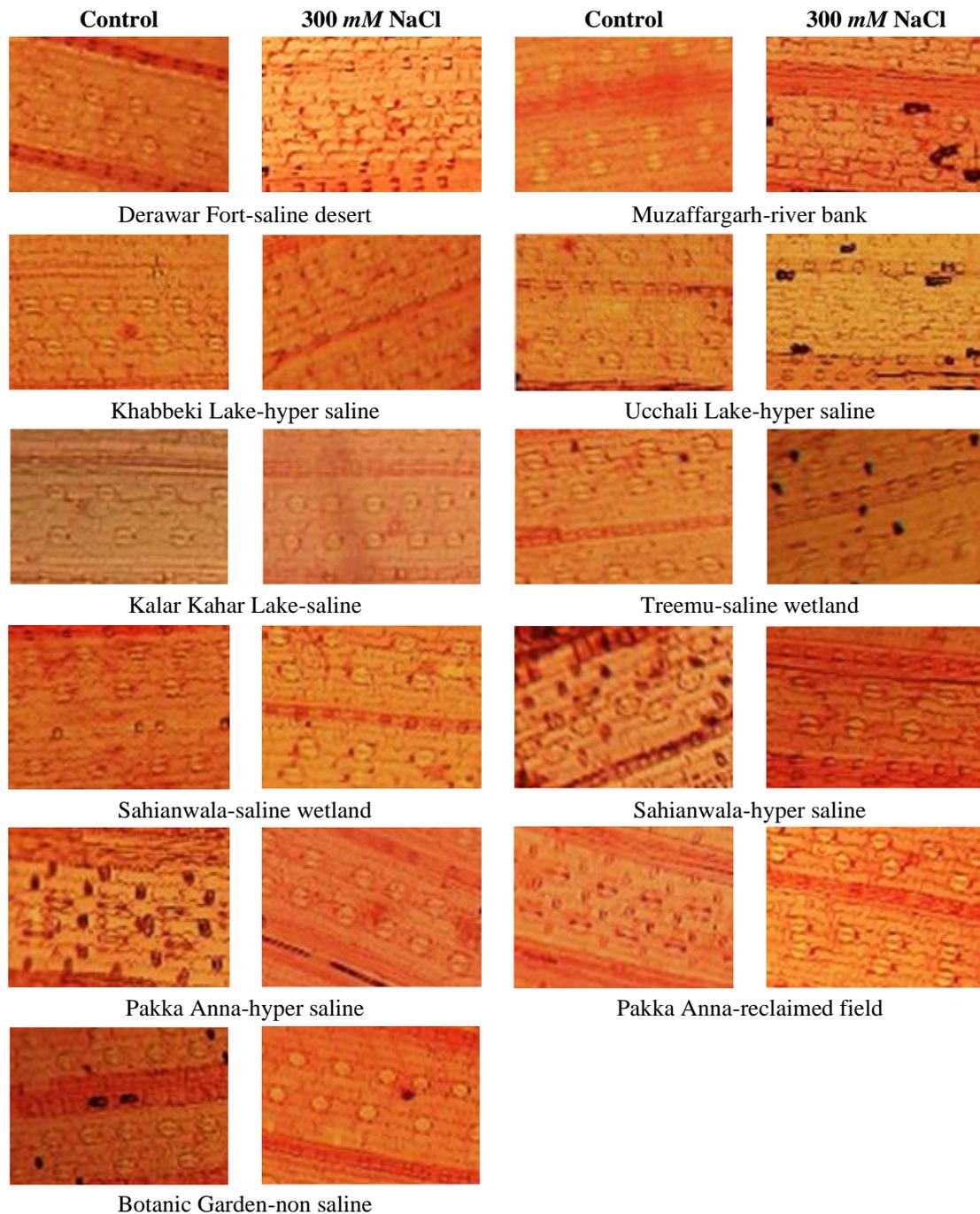


Figure 5. Surface view of leaf epidermis of *Cynodon dactylon* ecotypes collected from the Punjab, Pakistan

Ecotypes from S-SW showed association with ASD at control 0, 100 mM NaCl, with TRL at minimum salinity level 100 mM, with BCA at maximum salinity level 300 mM, however from S-HS showed association with VBA at control 0 mM NaCl BCA while up to maximum salinity level 300 mM showed association with BCA. Ecotypes from PA-HS showed association with ADA and ASA up to maximum salinity level 300 mM while PA-RF showed association with PAR at 0 mM salinity and with VBA, MXA and ADA at maximum salinity level 300 mM salinity. Ecotypes from BG-NS showed

association with PHA, ADD, LBT and SCT at control 0 mM NaCl and with PHA up to maximum 300 mM salinity level (Fig. 7).



Figure 6. Leaf margins of *Cynodon dactylon* ecotypes collected from the Punjab, Pakistan

RDA ordination biplot for photosynthetic characteristics

RDA ordination biplot (Fig. 8) showed the effect of photosynthetic characteristics on the ecotypes of *C. dactylon*. The ecotypes from DF-SD and S-SW showed the

association with Chlorophyll-a (Chl-a), Chlorophyll-b (Chl-b), Carotenoid (CAR), Total Chlorophyll (Chl-T) and Transpiration rate (E) at control while the ecotypes from DF-SD and KL-HS with gs at moderate salinity level 200 mM. Ecotypes from M-RB and UL-HS showed no noticeable association along salinity gradient. Ecotypes from KKL-S showed association with A at 0 mM NaCl, with gs at minimum salinity level 100 mM however from T-SW showed association with A/E and Ci at control. Ecotypes from S-SW showed association with A/E at minimum 100 mM salinity level and with A and Ci at moderate salinity level 200 mM. However from S-HS showed association with gs at control 0 mM NaCl, with A and Ci at minimum salinity level 100 mM NaCl, with Chl-T, E , Chl-a and Chl-b at moderate salinity level 200 mM NaCl while at maximum salinity level 300 mM showed association with A/E (Fig. 8).

Ecotypes from PA-HS showed association with A at control 0 mM NaCl, with Chl-T, E , Chl-a and Chl-b at moderate salinity level 200 mM NaCl and with Chl-T, Chl-a Chl-b, CAR, A , E and chlorophyll a/b at maximum salinity level 300 mM NaCl, while PA-RF showed association with A/E at maximum salinity level 300 mM NaCl, however from BG-NS showed association with Ci at control 0 mM NaCl, with A , Ci at minimum salinity level 100 mM NaCl (Fig. 8).

Discussion

Across the world, increasing soil salinity is a serious threat for the plants (Habib et al., 2016) with respect to modifications in their physiological, biochemical and anatomical attributes. Ecotypes are the species that have long term genetic variations among their populations (Sridevi et al., 2012) and showed structural, functional and geographical variations that leads to genetic variance (Johnson, 2010; Phillips et al., 2015). Morphological, anatomical, physiological and biochemical adaptive markers are of prime importance to study the adaptive mechanism in differentially adapted ecotypes against abiotic and biotic stress (Hameed et al., 2011; Naz et al., 2009). Therefore, ecotypes of *Cynodon dactylon* were collected from various highly saline, moderately saline, marshy, waterlogged and non-saline areas to evaluate adaptation strength and extent of involvement of these adaptations in plant survival.

C. dactylon is also considered as halophytic grass due to strong resistance against salinity (Marcum et al., 2005; Pessarakli, 2015). In view of stresses “*escape*” or “*tolerance*” is the most prevailing phenomenon in adaptation (Sunkar, 2010; Witcombe et al., 2008). Salt tolerant grasses can minimize the detrimental effects of high salinity by showing a series of structural and functional modifications in anatomical and physiological characteristics of plants (Zhou et al., 2015) similar to our findings e.g. as lignification around the vascular region (Alam et al., 2015), increased thickness (succulence) of epidermis, midribs and cortical parenchyma (Vijayan et al., 2011), increased sclerenchyma in the leaves (Noor et al., 2015), reduced leaf area (Monteverdi et al., 2008), greater density of salt secreting glands and hairs on the leaf surface (Farooq et al., 2015), enlarged bulliform cells that help in leaf rolling to avoid water loss (Alvarez et al., 2008), increased stomatal density with decreased stomatal size (Hameed et al., 2013).

Our findings of adaptive responses get advocacy from the subrized exodermis and endodermis, thick sclerenchymatous tissues around vascular bundle, large mesophyll cells and large cortical aerenchyma cells that are reportedly developed against stress (Noman et al., 2014; Noman et al., 2012; Yang et al., 2011). Tolerance level of *C. dactylon* to environmental stresses varies from highly sensitive to tolerant grass. Increased succulence

for water conservation and toxic ion accumulation, excretory structures like micro-hairs for toxic ion exclusion (Colmer and Flowers, 2008), sclerification for desiccation tolerance and minimizing water loss, and wider metaxylem vessels for efficient water and nutrient conduction (Ali et al., 2009; Naz et al., 2014; Noman et al., 2017).

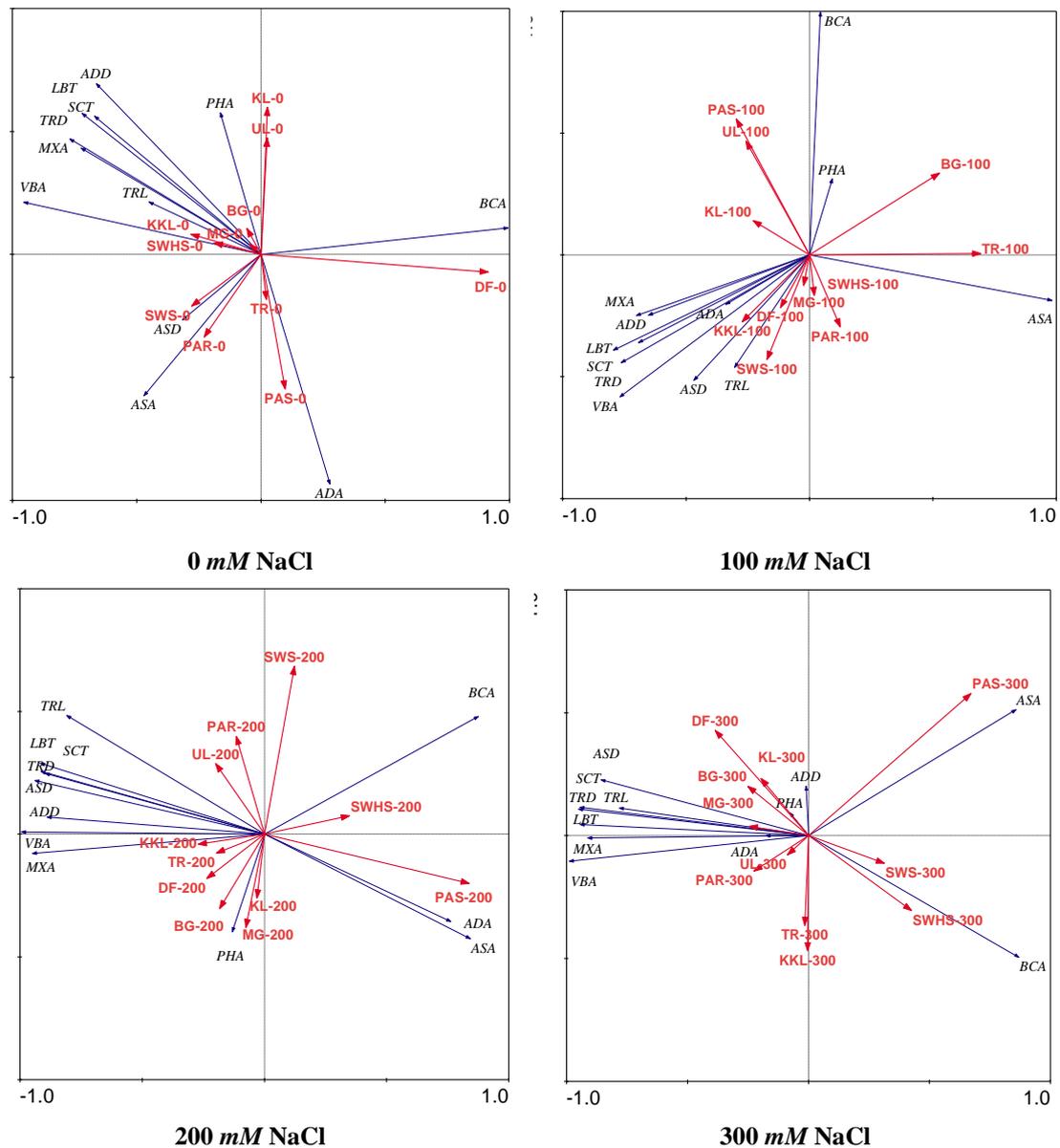


Figure 7. RDA ordination biplot showing the effect of leaf anatomical characteristics on the ecotypes of *Cynodon dactylon* from various region of Punjab. DF (Derawar Fort), MG (Muzaffargarh), KL (Khabbeki Lake), UL (Uchali Lake), KKL (Kalar Kahar Lake), T-SW (Treemu-Saline Wetland), SWS (Sahianwala-Saline Wetland), SWHS (Sahianwala-Hyper Saline), PAS (Pakka Anna Saline), PAR (Pakka Anna Reclaimed) and BG (Botanic Garden). Leaf anatomical characteristics are abbreviated as LBT: Leaf blade thickness, SCT: Sclerenchymatous thickness, BCA: Bulliform cell area, VBA: Vascular bundle area, MXA: Metaxylem area, PHA: Phloem area, TRL: Trichome length, TRD: Trichome density, ASA: Abaxial stomatal area, ASD: Abaxial stomatal density, ADA: Adaxial stomatal area and ADD: Adaxial stomatal density

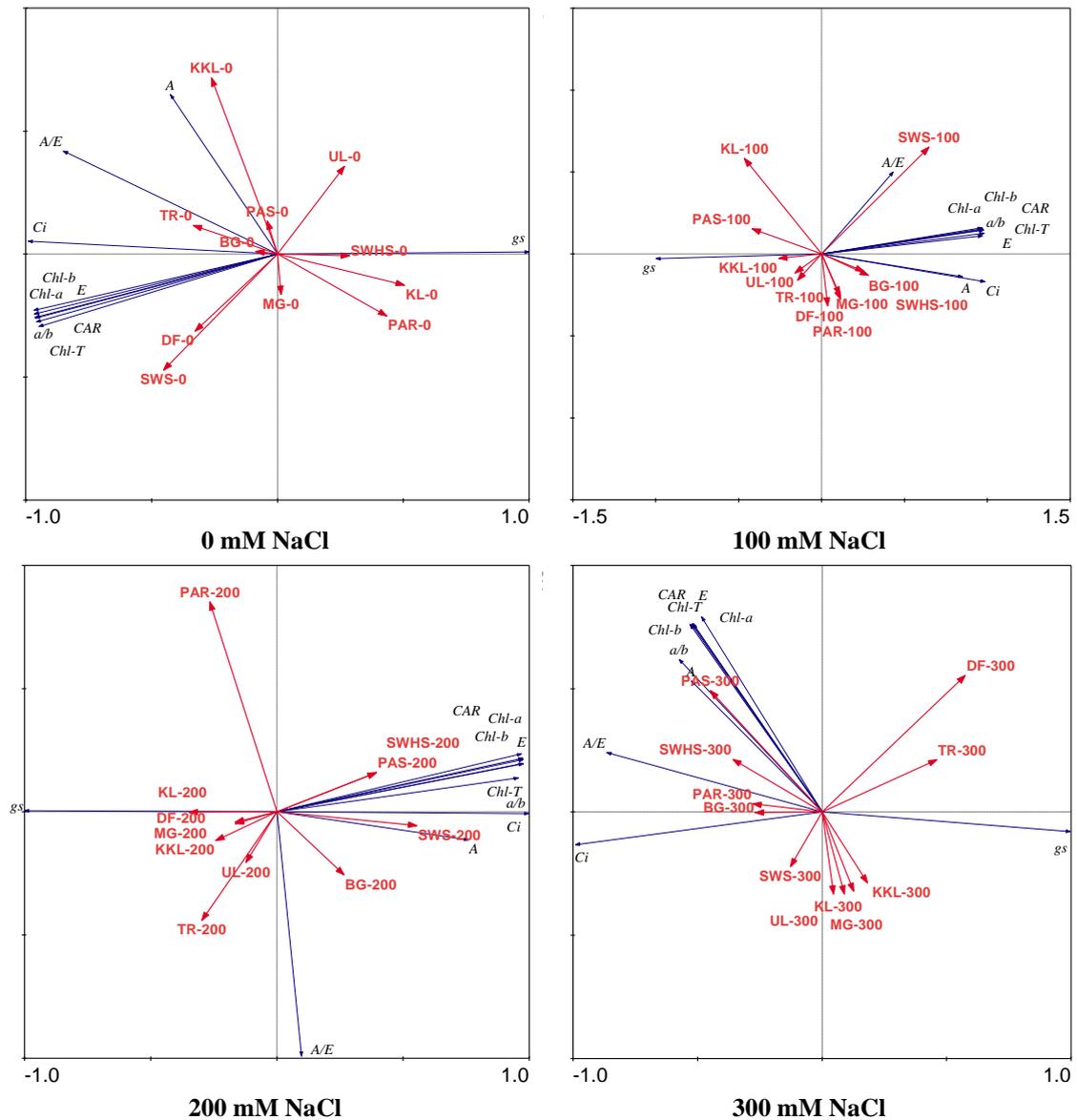


Figure 8. RDA ordination biplot showing the effect of photosynthetic characteristics on the ecotypes of *Cynodon dactylon* from various region of Punjab. DF (Derawar Fort), MG (Muzaffargarh), KL (Khabbeki Lake), UL (Uchchali Lake), KKL (Kalar Kahar Lake), T-SW (Treemu-Saline Wetland), SWS (Sahianwala-Saline Wetland), SWHS (Sahianwala-Hyper Saline), PAS (Pakka Anna Saline), PAR (Pakka Anna Reclaimed) and BG (Botanic Garden).

Photosynthetic characteristics are abbreviated as: A: Net- CO_2 assimilation rate, E: Transpiration rate, gs: Stomatal conductance, Ci: Sub-stomatal CO_2 concentration, A/E; Water use efficiency, Chl-a, Chlorophyll a, Chl-b, Chlorophyll b, a/b: Chlorophyll ratio a/b, Chl-T: Total Chlorophyll and CAR: Carotenoids

Leaf blade thickness is a critical adaptation against harsh environment (Hameed et al., 2011). We noticed invariably increased in this characteristic among all ecotypes of *C. dactylon* under salt stress, mainly due to increased sclerification and bulliform cell area. Therefore, our finding is in accordance with the facts that sclerification is the immediate response of plants when exposed to moisture limiting environments. This process provides mechanical strength to soft and delicate tissue avoiding from collapse

(Al-maskri et al., 2013; Leroux et al., 2015) and at the same time preventing undue water loss through leaf surface (Toon et al., 2015) and hence important for water conservation.

Additionally, we recorded increasing Metaxylem area with increased along with increased salinity levels, at least in more tolerant ecotypes of *C. dactylon*. This adaptive feature has earlier been reported for different plants facing salt stress, e.g., *Alternanthera bettzickiana* (Younis et al., 2013), *Juncus* species (Al Hassan et al., 2015) and Cattail (Correa et al., 2015).

Increased density of micro-hairs with increasing salt level may be one of the most effective mechanism for salinity tolerance in this salt excretory halophyte (Hu et al., 2015). A substantial increase in trichome density and length was recorded in relatively more tolerant ecotypes of *C. dactylon*, which is regarded as an important ecological adaptation. The increased density and size of trichomes in this case provide additional benefit to a plant to cope with environmental stresses like drought and salinity. Stomatal area on abaxial leaf invariably increased under salinity in all cases except in ecotype from KKL-S but density decreased on both surfaces. In *C. dactylon*, abaxial leaf surface is directly exposed to sun and in that condition stomatal regulation is very important in controlling transpiration rate.

Photosynthetic parameters were severely affected in all grasses (Alam et al., 2015; Ali et al., 2015) and particularly in ecotypes collected from low and moderate salinities. Photosynthetic parameters, in particular net CO₂ assimilation rate, transpiration rate, water use efficiency decreased in most of ecotypes up to maximum salinity level 300 Mm except T-W. Stomatal conductance was observed higher in most of ecotypes with the increase of salinity except S-SW and some reports (Manuchehri and Salehi, 2015; Noman et al., 2018; Yu et al., 2013) have already mentioned these attributes supportive in halophytic or highly salt tolerant grasses.

Reduction in chlorophyll contents as a result of high salinity is well documented by many researchers (Amareh et al., 2015). However, stability in these parameters (chl a, chl b and carotenoids) in more tolerant ecotypes might have contributed to increased salt tolerance. Stimulated concentration of photosynthetic pigments chl b was observed in most of the ecotypes except UL-HS, KKL-S and S-SW and chlorophyll a and carotenoids showed reduction in most of ecotypes and showed increase Chl a pigment in sensitive ecotypes T-W and BG-NS and carotenoids in MG-RB at 200 mM NaCl indicates the better adaptation of these populations as also reported by (Takahashi and Badger, 2011)

Physiological mechanisms such as repression in transpiration loss, improved water-use efficiency, maintenance of turgor potential, deep root system, upregulation of antioxidants, stomatal regulation, and photosynthetic rate at low water potential, and synthesis of osmolytes/osmoregulation help plants to sustain growth and biomass production under stress condition as reported by Farooq et al. (2012), Khalid et al. (2019), Shafiq et al. (2014) and Zafar et al. (2016).

Conclusion

Cynodon dactylon is a widespread grass that has an excellent potential to inhabit a variety of habitats. Differently adapted ecotypes of this grass are independently evolved during the long evolutionary history and it is confirmed by their specific adaptive mechanism for salinity tolerance under similar controlled environments. The changes in

structural (Sclerenchyma bulliform cells, trichomes, broad metaxylem) and physiological (repression of transpiration, stability in chlorophyll contents) attributes confirms its potential to thrive well in different conditions and laid the basis for future investigations. *Cynodon dactylon* being considered as best halophytes and have mechanistic ability to tolerance salinity, it is recommended for farmers to use it as an alternative fodder crop for salt effected areas/land.

Acknowledgements. The authors extend their appreciation to the Deanship of Scientific Research, King Khalid University for funding this work through research groups program under grant number R.G.P. 2/101/41.

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