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GROWTH PERFORMANCE AND STOMATAL BEHAVIOR IN RELATION TO ECOTYPIC ADAPTATIONS IN *CYNODON DACTYLON* (L.) PERS.

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Abstract

Evolution has great ecological significance in terms of plant morphological and stomatal characteristics that must have been genetically fixed during the long evolutionary period. Impact of environmental conditions on growth and stomatal features of twelve ecotypes of *Cynodon dactylon* that were collected from ecologically different habitats in the Punjab, Pakistan were evaluated. The collected ecotypes Derawar Fort-saline desert (DF-SD), Muzaffar garh-River bank (M-RB), Khabbeki Lake-hyper saline (KL-HS), Ucchali Lake-hyper saline (UL-HS), Kalar Kahar Lake-saline (KKL-S), Treemu-saline wetland (T-SW), Sahianwala-saline wetland (S-SW), Sahianwala-hyper saline (S-HS), Pakka Anna-hyper saline (PA-HS), Pakka Anna-reclaimed field (PA-RF), Botanic Garden-non saline (BG-NS) and Gatwala-saline semiarid (G-SSA) were grown in controlled environments at University of Agriculture, Faisalabad till their acclimatization to evaluate genetically fixed characteristics. After 6-month growth in soil, the plants were transferred to half-strength Hoagland's nutrient medium. There was a huge variation in all morphological characteristics recorded during the investigation, which were due to environmental heterogeniety to which these ecotypes were originally adapted. An exclusive feature of the DF-SD ecotypes is the long and numerous roots, and tillering capacity that surpassed all other ecotypes. Leaves per plant were also exceptionally high that may improve the photosymthetic efficiency of the plant. It showed a good potential of overall growth and biomass production. The robust growth was also recorded in the KKL-S ecotypes, and this can be related to the complete dominance of these two ecotypes in their respective habitats. Small stomata were recorded in the three ecotypes (DF-SD, KL-HS and PA-HS), which are of great ecological significance. Stomatal shape, however, is different in different ecotypes, but its contribution towards stress tolerance is still to be investigated.

Key words: *Cynodon dactylon,* Ecotypes, Stomata, Evolution, Environmental heterogeniety.

Introduction

Geographical distribution of species depend on a specific set of environment that might has fixed during the long evolutionary history (Lowery *et al*., 2014). These ecotypes have specific structural, functional and geographical variations (Johnson, 2010; Phillips *et al*., 2015) and these variations was reflected into their morpho-anatomical and physio-biochemical characteristics (Sri-Devi *et al*., 2012). These adaptive markers have principal importance to study the adaptive mechanism in differentially adapted ecotypes (Hameed *et al*., 2011). The grasses occupying extreme adverse environmental conditions, which are extremely unfavorable to plant growth and development (Arshad *et al*., 2008). But against environmental heterogeneity xeromorphic characters develops to reduce water loss as thickness of dermal tissue, decreased stomatal area and density on adaxial leaf surface (Hameed *et al*., 2013), extensive root system (Hameed & Ashraf, 2008), reduced leaf area (Monteverdi *et al*., 2008).

A significant proportion of land is covered by grasses that constitutes rangelands all over the world (Kellogg, 2011, Wang *et al*., 2015). Bermuda grass [*Cynodon dactylon* (L.) Pers.], which is naturally occur in Indo-Pak subcontinent (Kim *et al*., 2008), can grow best and gets the maximum coverage and growth at 20-25°C. It is stoloniferous, fast growing grass with deep rhizomes and high percentage of seed production. It is extensively planted all over the world as a turf grass (Omezine & Harzallah, 2011).

Cynodon dactylon is extremely drought tolerant and riparian grass that plays a fundamental role in controlling soil erosion by developing deep root and creeping stems (Chen *et al*., 2015a). Soil-root system expressively correlates to the plant and soil features as plant species (Stokes *et al*., 2009; Fan & Lai, 2014), root configuration and spreading (Ji *et al*., 2012; Ghestem *et al*., 2014).

Stomata are small perforated structures (Hetherington & Woodward, 2003) that act as a vital gate between plant and atmosphere (Nilson & Assmann, 2007). These are strongly influenced by environmental impact (Niu *et al*., 2008), and key factor in taxonomic delimitation (Babu & Savithramma, 2014). These involve in gaseous interchange (Franks & Farquhar, 2007) and photosynthesis aptitude that ultimately induce or reduce plant growth (Franks *et al.*, 2009). *Cynodon dactylon* has paracytic type of stomata (Abid *et al*., 2007) and arranged in parallel rows in the epidermis with silica bodies on the leaf epidermal surface.

The study was focused on the evolution of ecological significance of plant morphological and stomatal characteristics that must have been genetically fixed during the long evolutionary period. Since soil characteristics and environmental factors greatly effect plant growth and stomatal characteristics (Shrivastava & Kumar, 2015), the study was designed to correlate the impact of environmental conditions to growth and stomatal features of differently-adapted ecotypes of *C. dactylon* all over the Punjab. The main objective of the study was to evaluate ecological response of differently adapted ecotypes of *C. dactylon* based on morphological and stomatal characteristics.

Material and Methods

Collection sites: Twelve ecotypes of *Cynodon dactylon* were collected from ecologically different habitats in the Punjab, Pakistan (Fig. 1). Selection was based on environmental conditions of the collection site. The selective sites were: DF-SD (Derawar Fort-saline desert, ECe 25.1 dS m^{-1}, Na^+ 4253.4 mg Kg^{-1}, Cl^- 2351.9 mg Kg^{-1}) from the Cholistan desert, soil is sandy to loamy sandy, compact and heavily salt-affected. MG-RB (Muzaffargarh-river bank, ECe 2.9 dS m^{-1}, Na^+ 320.4 mg Kg^{-1}, Cl^- 320.5 mg Kg^{-1}) was collected from non-saline sandy river soil, which was in direct contact with river water. Three ecotypes, KL-HS (Khabbeki Lake-hyper saline, ECe 11.5 dS m^{-1}, Na^+ 2336.4 mg Kg^{-1}, Cl^- 1086.7 mg Kg^{-1}), UL-HS (Ucchali Lake-hyper saline, ECe 19.4 dS m^{-1}, Na^+ 4035.1 mg Kg^{-1}, Cl^- 2021.6 mg Kg^{-1}) and KKL-S (Kalar Kahar Lake-saline, ECe 5.1 dS m^{-1}, Na^+ 1052.9 mg Kg^{-1}, Cl^- 471.7 mg Kg^{-1}) were collected from banks of salt marshes in the Salt Range, which were exposed to saline waters. Two ecotypes were from saline wetlands, T-SW (Treemu-saline wetland, ECe 12.5 dS m^{-1}, Na^+ 2895.2 mg Kg^{-1}, Cl^- 1148.2 mg Kg^{-1}) and S-SW (Sahianwala-saline wetland, ECe 13.3 dS m^{-1}, Na^+ 2719.7 mg Kg^{-1}, Cl^- 1229.7 mg Kg^{-1}), were collected from saline waterlogged soils. G-SSA (Gatwala-saline semiarid, ECe 19.2 dS m^{-1}, Na^+ 3946.2 mg Kg^{-1}, Cl^- 2015.3 mg Kg^{-1}) and S-HS (Sahianwala-hyper saline, ECe 4.4 dS m^{-1}, Na^+ 947.2 mg Kg^{-1}, Cl^- 446.8 mg Kg^{-1}) and PA-HS (Pakka Anna-Hyper saline, ECe 6.7 dS m^{-1}, Na^+ 1320.6 mg Kg^{-1}, Cl^- 656.2 mg Kg^{-1}) were collected from dryland salinities, whereas PA-RF (Pakka Anna-reclaimed field, ECe 2.2 dS m^{-1}, Na^+ 461.7 mg Kg^{-1}, Cl^- 232.7 mg Kg^{-1}), where the soil was reclaimed by salt-excretory species and now is cultivable for many glycophytic crop species. BG-NS (Botanic Garden-non saline, ECe 1.0 dS m^{-1}, Na^+ 81.6 mg Kg^{-1}, Cl^- 312.2 mg Kg^{-1}) was collected from the University of Agriculture, Faisalabad and treated as the control.

Soil analysis: Soil from rhizosphere was taken from each habitat to analyze the physico-chemical characteristics at 16 cm depth. The soil extract was used to determine the pH and ECe using pH/EC meter (WTW series InoLab pH/Cond 720). Sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) contents were determined with a flame photometer (Jenway, PFP-7), whereas Cl^- content was determined with a chloride meter (Model 926; Sherwood Scientific Ltd., Cambridge, UK). Available phosphorus in soil was determined following Bray and Kurtz (1945) method and magnesium (Mg^{2+}) was determined by the method of Richards (1954) with an atomic absorption spectrophotometer (Model Analyst 3000; Perkin Elmer, Norwalk, CT).

Morphological parameters: The collected ecotypes were grown in controlled environments at Botanic Garden Research Area in University of Agriculture, Faisalabad till their acclimatization to evaluate genetically fixed characteristics during long evolutionary history. After 6-month growth in soil, the plants were transferred to half-strength Hoagland's nutrient medium. For dry weight, plants were oven-dried at 65°C until constant weight was achieved and growth parameters i.e., shoot length (cm), root length (cm), internode length (cm), number of tillers $plant^{-1}$, number of leaves $plant^{-1}$, flag leaf area per plant (cm^2), number of adventitious roots $plant^{-1}$, fresh weight (g $plant^{-1}$) and dry weight (g $plant^{-1}$) was recorded.

Stomatal attributes: For the stomatal studies, leaves from the all ecotypes were preserved in formalin acetic alcohol fixative for 48 h and then transferred to 75% solution of ethyl alcohol solution. Permanent slides were prepared by peeled off method (Eckerson, 1908; Weyers & Travis, 1981) then by serial dehydrations in ethanol using Safranin as stain. Photographs were taken by a camera-equipped light microscope (Meiji Techno: MT4300H USA) using an ocular micrometer, which was calibrated with a stage micrometer.

Statistical analysis: The data was analyzed for analysis of variance in completely randomized design with three replications. The data was also subjected to redundancy analysis (RDA) using Conoco 4.5 computer software. The data was also subjected to multivariate cluster analysis

Results

Morpho-agronomic characteristics: Various ecotypes of *Cynodon dactylon* (L.) Pers. from the Punjab region responded differently for morpho-agronomic characteristics in several soil types from differently adapted ecotypes of *C. dactylon.* Plant height was significantly higher in ecotype collected from UL-HS than the second best from KKL-S and KL-HS (Table 1). The smallest plants were recorded from S-SW, BG-NS and PA-RF ecotype. The maximum root length was recorded in DF-SD ecotype, followed by that in UL-HS and KKL-S ecotypes. The minimum value for root length was recorded in the KL-HS ecotype, which was significantly lower than the second minimum from BG-NS. Internode length was the maximum in KKL-S ecotype, followed by the G-SSA ecotype and its minimum was observed in the S-SW ecotype.

The DF-SD ecotype from saline desert showed the maximum of tillers per plant, leaves per plant, number of seminal roots and inflorescence. Tillering capacity in also high in the M-RB and S-SW, whereas leaves per plant in PA-HS and S-HS. Number of roots were relatively higher in MG-RB and G-SSA, while number of inflorescence was also high in KL-HS and UL-HS (Table 1).

Leaf area was the maximum in the ecotype S-HS, which was followed by that in PA-HS. The lowest value of leaf area was recorded in two ecotypes, M-RB and S-SW. Root fresh and dry weights were the maximum in ecotype from KKL-S, closely followed by that in DF-SD and MG-RB. The minimum value for root fresh and dry weights were observed in the UL-HS and PA-RF ecotypes (Table 1).

Stomatal density and size was the maximum in the PA-HS and S-HS ecotypes, while the minimum values for stomatal density and stomatal area were recorded in the PA-HS and T-SW ecotypes (Table 1). Stomatal shape was almost circular in the KL-HS ecotype, whereas the S-SW, KKL-S, PA-HS and BG-NS had broadly elliptic stomata. The DF-SD and PA-RF showed rectangular stomata, while the others has typically elliptic stomata (Fig. 2).

Fig. 1. Pictorial representation of the collection sites of *Cynodn dactyon* (L.) Pers. from the Punjab, Pakistan.

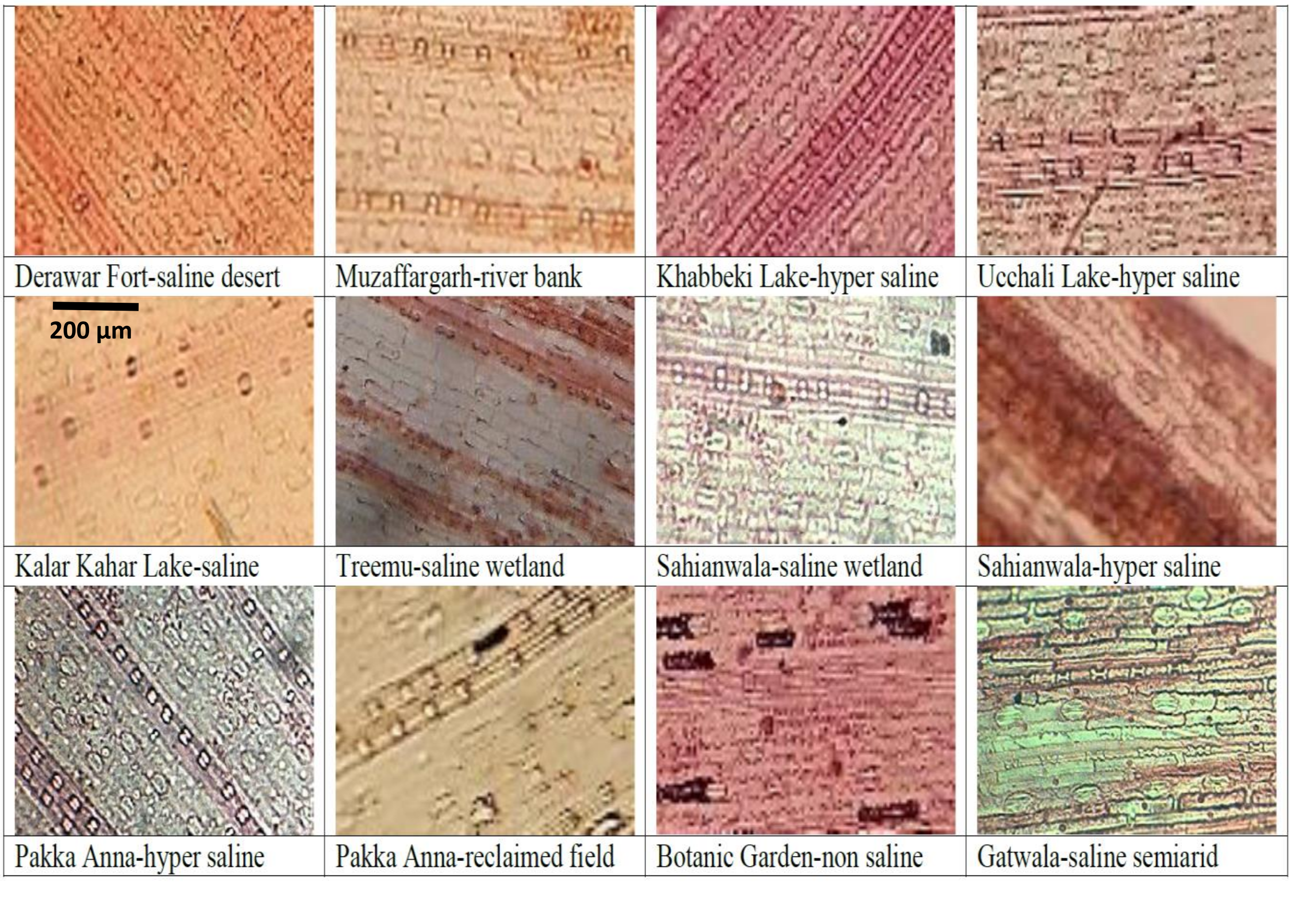

Fig. 2. Leaf surface view of *Cynodon dactylon* (L.) Pers. ecotypes collected from the Punjab, Pakistan.

Table 1. Plant growth and stomatal behavior of *Cynodon dactylon* (L.) Pers. ecotypes from selected habitats in the Punjab, Pakistan.

Characteristics	Saline-arid	River water	Salt marshes			Saline wetlands		Dry-land salinity			Reclaimed soil	Non-saline control
	DF-SD	M-RB	KL-HS	UL-HS	KKL-S	T-SW	S-SW	G-SSA	S-HS	PA-HS	PA-RF	BG-NS
Morphology												
Shoot length (cm)	49.9 bcd	45.7 cde	57.5 abc	69.4 a	62.6 ab	43.1 cde	30.4 e	43.5 cde	43.1 cde	43.1 cde	37.2 de	33.8 de
Root length (cm)	17.7 a	10.1b	9.3 b	14.4 ab	14.3 ab	9.3 b	7.6b	9.8b	10.1b	11.0 b	11.8 b	8.4 b
Roots per plant	59.3 a	53.3 b	28.8 e	14.6 g	45.3 c	34.6 d	36.3 d	51.5 b	44.6 c	24.1 f	18.3 g	35.3 d
Internode length (cm)	3.3 a	4.3 a	4.9a	4.6a	5.9a	4.2a	3.1a	4.9a	4.9a	4.2 a	3.6 a	3.3 a
Tillers per plant	44.3 a	25.4 b	18.6 c	15.1 c	15.0 c	17.6 c	24.7 b	23.5 b	15.3 c	8.6 d	13.3 c	17.2 c
Leaves per plant	369.1 a	155.5 f	125.8 g	193.2 d	244.5 c	260.1 b	75.0 i	154.6 f	171.2 e	125.7 g	105.2 h	109.7 a
Single leaf area (cm^2)	0.26 abc	0.21 c	0.36 abc	0.45 abc	0.35 abc	0.45 abc	0.37 abc	0.21 c	0.50 a	0.48 ab	0.29 abc	0.26 abc
Total leaf area (cm^2)	95.9 g	32.5 bc	45.6 d	86.8 f	85.4 f	117.3 g	32.3 bc	35.2 c	85.5 f	60.1 e	30. 4b	27.7 a
inflorescence per plant	4.0 a	1.6 cd	3.3 ab	2.1 bcd	1.3 cd	1.3 cd	1.1 d	2.5 bcd	1.1 d	1.1 d	1.1 d	3.2 abc
Root fresh weight (g)	5.4 a	4.3 b	4.8 ab	1.1 e	5.4 a	2.8 cd	3.2 cd	4.2 b	3.0 cd	4.3 b	2.6 d	3.4 c
Root Dry weight (g)	0.91 bc	1.12 b	0.53 cde	0.41 de	1.44 a	0.68 bcde	0.75 bcde	0.94 bc	0.68 bcde	0.92 bc	0.34 e	0.86 bcd
Shoot fresh weight (g)	7.5 c	3.3 h	4.6 g	8.2 b	10.3 a	5.7 e	5.6 ef	2.8 h	6.8 d	4.9 efg	5.5 ef	4.8 fg
Shoot dry weight (g)	3.4 a	1.3 d	2.4 bc	3.4 a	3.1 ab	2.6 abc	2.5abc	1.1 d	3.0 ab	1.9 cd	1.9 cd	1.5 d
Stomata												
Stomatal area (mm^2)	0.016 cd	0.021cd	0.017 c	0.026 abc	0.027 abc	0.023 cd	0.024 bcd	0.024 bcd	0.034 a	0.015 d	0.033 ab	0.020 cd
Stomatal density/mm^2	14.3 c	15.2 b	11.7 e	10.5 f	10.8 f	8.6 g	13.1 d	11.6 e	10.0 f	22.4 a	13.6 d	11.2 e
Stomatal shape	Rect	Ellip	Cir	Ellip	B-Ellip	Ellip	B-Ellip	Ellip	Ellip	B-Elip	Rect	B-Ellip

DF-SD: Derawar Fort-saline desert, M-RB: Muzaffar garh-River bank, KL-HS: Khabbeki Lake-hyper saline, UL-HS: Ucchali 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, BG-NS: Botanic Garden-non saline, G-SSA: Gatwala-saline semiarid

B-Ellip: Broadly elliptic, Cir: Circular, Ellip: Elliptic, Rect: Rectangular

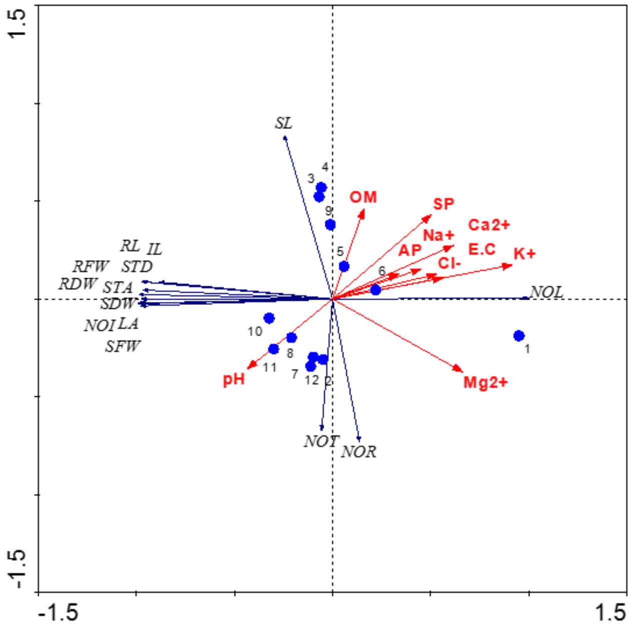

Fig 3. RDA ordination triplot showing correlation of soil physico-chemical, morphological characteristics, abaxial stomatal characteristics of *Cynodon dactylon* (L.) Pers. ecotypes collected from the Punjab, Pakistan

1 Derawar Fort-saline desert, 2 Muzaffar garh-River bank, 3 Khabbeki Lake-hyper saline, 4 Ucchali Lake-hyper saline, 5 Kalar Kahar Lake-saline, 6 Treemu-saline wetland, 7 Sahianwala-saline wetland, 8 Sahianwala-hyper saline, 9 Pakka Anna-hyper saline, 10 Pakka Anna-reclaimed field, 11 Botanic Garden-non saline, 12 Gatwala-saline semiarid. Soil characteristics are abbreviated as AP: Available Phosphorus, SP: Saturation (%), OM: Organic matter. Morphological characteristics were abbreviated as SL: Shoot length, RL: Root length, IL: Internode length, NOT: Number of tillers, NOL: Number of leaves, LA: Leaf area, NOR: Number of roots, NOI: Number of inflorescence, RFW: Root fresh weight, RDW: Root dry eight, SFW: Shoot fresh weight, SDW: Shoot dry weight, STA: Stomatal area, STD: Stomatal density

RDA analysis: The RDA ordination triplot of soil physicochemical characteristics generally showed a weak association with the morphology characteristics of the ecotypes (Fig. 3). Among morphology attributes, only number of leaves of the ecotype T-SW was interconnected with soil K^+ and Cl^-. Shoot length had a weak correlation with the KL-HS, UL-HS and PA-HS ecotypes. Other morphology characteristics like total tillers and roots showed a weak association with the M-RB, S-HS and G-SSA ecotypes (Fig. 3). Among soil characteristics, soil Mg^{2+} was associated with DF-SD and organic matter with KKL-S. Soil pH was strongly correlated with S-SW and BG-NS ecotypes (Fig. 3).

Cluster analysis: Dendrogram of morphological characteristics (Fig. 4) showed some specific clustering of the studied ecotypes. The DF-SD was different from all other ecotypes regarding to root characteristics that responded independently. Other ecotype clustered into two major group, the first containing 4 ecotypes, in which M-RB and G-SSA closely clustered, as were KKL-S and S-HS. In the second group, T-SW closely clustered with S-SW and BG-NS, whereas KL-HS clustered with PA-HS and UL-HS with PA-RF.

The DF-SD ecotype again clustered independently of all other ecotypes among stem morphological characteristics. The other ecotypes clustered into two major groups, one containing 7 ecotypes and the other 4 ecotypes. In the first group, the M-RB ecotypes closely clustered with G-SSA and S-SW, whereas KL-HS with PA-HS and PA-RF with BG-NS. In second cluster, UL-HS closely clustered with S-HS and KKL-S with T-SW.

Dendrogram for stomatal characteristics (Fig. 4) showed isolated behavior for PA-HS, whereas other ecotypes clustered into two major groups. The first group consisted of 9 ecotypes and the second of 2 ecotypes (S-HS and PA-RF). The first group showed 3 sub-clusters, in which DF-SD clustered independently, while KL-HS clustered with BG-NS. Six ecotypes clustered into a separate group, in which UL-HS closely clustered with KKL-S and S-SW with G-SSA.

Discussion

Cynodon dactylon is a C_4 perennial grass and it is warm season turf grass (Shi *et al*., 2012) that can acquire a variety of habitats including high salinities (Chen *et al*., 2015b), waterlogging (Xie *et al*., 2015; Lukacs *et al*., 2015), arid and semi-arid regions (Akram *et al*., 2015; Malik *et al*., 2015), river and canal beds (Soliman *et al*., 2015; Chirebvu and Chimbari, 2015. Zwerts *et al*., 2015), forests (Joubert *et al*., 2015; Rasool *et al*., 2015.), high altitudes (Acic *et al*., 2015; Faizul-Haq *et al*., 2015), wastelands (Nowak *et al*., 2016; El-Ghani *et al*., 2015), grasslands (Radutoiu, 2015; Srivastava *et al*., 2015), etc.

There was a huge variation in all morphological characteristics recorded during the investigation, which were due to environmental heterogeniety to which these ecotypes were originally adapted. Since these ecotypes were evaluated under controlled conditions, therefore, characteristics that are genetically fixed expressed (Mojica *et al*., 2012; Paccard *et al*., 2013), which are the representatives of their respective habitats.

Growth in terms of shoot length (and internode length) was relatively high in ecotypes that were collected from salt marshes (Khabbeki, Ucchali and Kalar Kahar lakes) in the Salt Range. The Salt Range is a unique biome (Ahmad *et al*., 2012), and therefore it is expected that evolutionary forces acted similarly on the Salt Range ecotypes, and as a result, these populations are relatively taller than the others. The ecotypes that were collected from either no salinity (Botanic Garden) or low salinity (Pakka Anna-reclaimed field) were shorter in length. The ecotypes collected from stressful condition when grown in normal climatic conditions may show stimulated growth (Bita & Gerates, 2013; Hu *et al*., 2015), whereas those from non-stressed environmats responded normally.

An exclusive feature of the DF-SD ecotypes is the long and numerous roots, which is of great ecological significance (Pessarakli, 2015). Environmental conditions in the Cholistan desert are extremely harsh facing multiple stresses like aridity, salinity, heat, nutrient non-availability, etc., and longer roots are capable of extracting water from deeper soil layer (Gao *et al*., 2016), hence can play a vital role in the survival of the Cholistan ecotype. Generally, the ecotypes from salt marshes also showed longer root than other ecotypes, where the water is hyper-saline, but number of adventitious roots were not as high as was recorded in the Derawar Fort (Cholistan) ecotype. Under such conditions, longer root are very useful that can extract less saline filtered water from deeper soil layers (Hu *et al*., 2015).

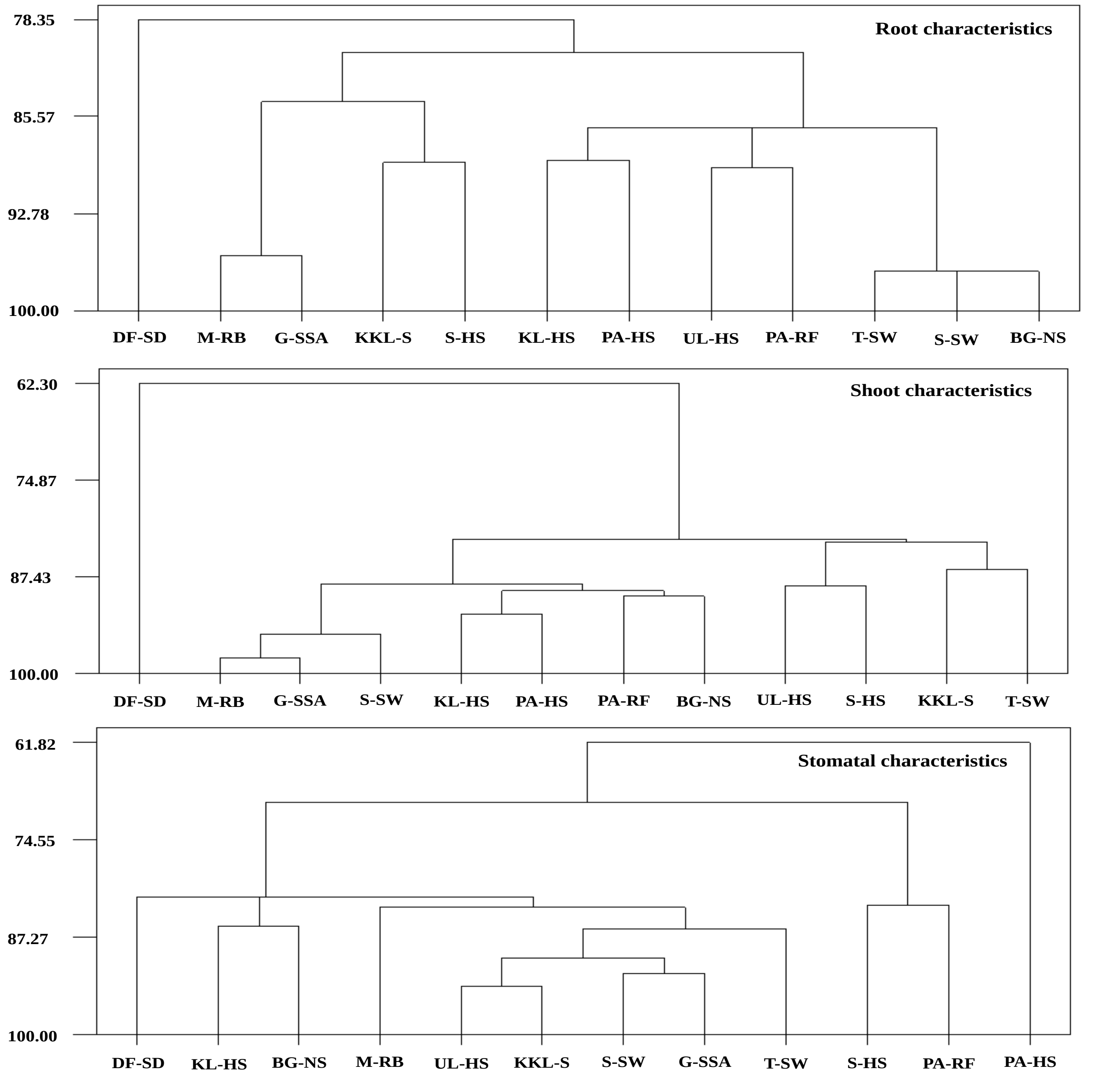

Fig. 4. Dendrogram of morphological and stomatal characteristics of *Cynodon dactylon* (L.) Pers. ecotypes collected from the Punjab, Pakistan. 1 Derawar Fort-saline desert, 2 Muzaffar garh-River bank, 3 Khabbeki Lake-hyper saline, 4 Ucchali Lake-hyper saline, 5 Kalar Kahar Lake-saline, 6 Treemu-saline wetland, 7 Sahianwala-saline wetland, 8 Sahianwala-hyper saline, 9 Pakka Anna-hyper saline, 10 Pakka Anna-reclaimed field, 11 Botanic Garden-non saline, 12 Gatwala-saline semiarid

Tillering capacity is the most dominant feature of *C. dactylon* that controls its vegetative propagation and spread along the ground (Mobasheri, 2011; Rangani *et al*., 2016). The Cholistan ecotype surpassed all other ecotypes regarding tillers per plant, almost 2-fold greater that the second maximum. Nutrien avaiability is restricted in paches in desert soils (Hodge, 2006; He *et al*., 2015), a this kind of verticle spread to longer distance enables a plant to utilize maximum nutrient resources (Irving, 2015).

Leaves per plant were exceptionally high in the DF-SD population, but much smaller in size. Large number of leaves may certainly improves the photosymthetic efficiency of a plant (Weraduwage *et al*., 2015), but smaller leaves may increase water use efficiency by controlling transpiration rate (Medrano *et al*., 2015), which is a vital commodity to survive in harsh saline desert conditions. Treemu saline wetland is expected to fluctuate considerably regarding salinity level, as fresh water from a canal is added seasonally to tha saline soil, hence droping down the salinity level. Number of leaves as well as leaf area per plant were significantly higher in this ecotype.

Inflorescence number was again significantly higher in the DF-SD ecotypes, and this might be due to better growth and tillering in this ecotype. The success of *C. dactylon* in dominating an environmental hyterogeniety may be due to a variety of propagation means, i.e., via seeds, suckers, runners or stolons (Rita *et al*., 2012), and this might be the reason of its survival under extremely harsh climatic condition like the Cholistan desert.

Fresh and dry biomass production is a good parameter to judge stress tolerance (Jaleel *et al*., 2009; Khosroshahi *et al*., 2014). The DF-SD ecotype showed a good potential of overall growth. The robust growth was also recorded in the KKL-S ecotypes, and this can be related to the complete dominance of these two ecotypes in their respective habitats. Root parameters like length, number, fresh and dry weights has earlier been related with drought and /or physiological drought by Wang and Bughrara (2008) in several Fescue grasses, Xu *et al*. (2010) in various plants, Talukdar (2013) in lentil and grass pea genotype and Ye *et al*. (2015) in *C. dactylon*.

Stomatal parameters like size, number and shape are of great ecological significance (Jian *et al*., 2012) and extremely important in stress tolerance (Xu & Zhou, 2008; Zheng *et al*., 2013) Small stomata were recorded in three ecotypes (DF-SD, KL-HS and PA-HS), which can be related to more efficient regulation, as less turgor is required for their opening and closing. Stomatal regulation, in addition to size and shape is also very important for increasing water use efficiency of a plant (Hameed *et al*., 2002; Lawson & Blatt, 2014). Moreover, stomatal density again improves the photosynthetic efficiency of a plant species, and therefore ecotypes like PA-HS, M-RB and SF-SD can be rated as the better. Stomatal shape, however, is different in different ecotypes, but its contribution towards stress tolerance is still to be investigated.

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