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ROLE OF LEAF MICRO-STRUCTURAL AND TOPOGRAPHICAL TRAITS IN ECOLOGICAL SUCCESS OF SOME ARID ZONE GRASSES

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Abstract

Water deficit influences the phenotypic appearance of leaf hairs and stomata which plays a critical role in adaptability of plants growing in such habitats. Four grass species *Aristida adscensionis, Cenchrus ciliaris, Desmostachya bipinnata* and *Heteropogon contortus* were collected along with soil sample from six moisture deficit areas. Soil attributes including soil pH, ECe, moisture content, saturation percentage, organic matter, P, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl, NH_4-N, NO_3-N were recorded. Leaf topographical traits such as density and area of adaxial hairs, and microstructural traits including adaxial and adaxial stomatal density and area measured. Moisture deficit affected morpho-anatomical traits such as growth traits and development of leaf hair and stomata. Populations from hyper-arid regions relied particularly upon smaller leaf area, decreased fresh to dry biomass ratio for both root and shoot tissue, and, longer roots to extract water from soil deeper layers. Among leaf micro-structural traits, more stomata were found to be oriented on leaf abaxial as compared to the adaxial surface in hyper-arid populations of all grasses. Adaxial leaf surface covered with long hairs possibly helped survive in these environments by decreasing leaf transpirational losses in most of the species under study.

Key words: Hairs, Stomatal density, Adaptability, Arid zone, Grasses.

Abbreviations: Ad HD: adaxial hair density, Ad HA: adaxial hair area, Ad SD: adaxial stomatal density, Ad SA: adaxial stomatal area, Ab SD: abaxial stomatal density, Ab SA: abaxial stomatal area, NeW: Neelawahn, SoV: Soonvalley, KKr: Kalarkahar, KaM: Kathamountains, CsD: Choasaidan shah, ChK: Chakwal, PaK: Pakanna, HaP: Hasilpur, PiR: Pirowal, Cho: Cholistan, NpT: Noorpurthal

Introduction

Leaf topological traits such as development of hairs show a vital eco-physiological role since these are involved in plant protection against abiotic stress such as moisture deficit and heat. Leaf hair density is under environmental control and shows tremendous plasticity (Chaudhari *et al*., 2014; Bibi *et al*., 2015). Leaf hair density varies sometimes even among different populations of the same species growing under differential environmental conditions indicating a highly plastic response in their adaptability towards environmental adversities (Amada *et al*., 2017; Javaid *et al.*, 2020).

Soil water content varies in arid habitats depending upon the rainfall pattern and mean annual temperatures. This exerts a variable degree of stress in plants growing in diverse habitats and therefore induces appearance of certain morphological features which otherwise would not be conspicuous under normal conditions. Appearance of leaf hairs are among such leaf topographical attributes that play a vital role in alleviating the effects of stresses imposed in arid areas to a variable degree (Muhammad *et al*., 2019). In some studies, leaf hairs and stomatal density has been shown to be correlated with water loss as small leaves with fewer stomata, but with more hairs, reduce water loss by decreasing leaf temperature (Karabourniotis *et al*., 2019).

Poaceae (grass family) is widely distributed around the world and considered the most successful group among flowering plants, having the ability to tolerate diverse climatic conditions. *Aristida adscensionis* is found growing worldwide in dry and shallow soils (Khan *et al*., 2017). *Cenchrus ciliaris* is a perennial forage plant able to grow in hyper-arid environments (Mansoor *et al*., 2019). *Desmostachya bipinnata* is commonly found on arid and semi-arid regions receiving an annual rainfall of 250-750 mm. It even flourishes well in dry hot conditions forming big tussocks in dry sandy areas (Subramaniam & Sivasubramanian, 2015). *Heteropogon contortus* is perennial grass found in tropical and warm temperate regions in sandy to clay loam soil, on hillsides and among rocks (Wang *et al*., 2016). Due to superior aridity tolerance, these grasses are used to enhance the productivity of degraded soils and re-vegetate the arid region around the world (Ullah *et al*., 2015).

Since arid zone grasses exhibits the potential to cope with the stresses prevailing in these regions by adopting various morpho-anatomical strategies, it is expected that differential development of certain leaf epidermal features such as hairs might play a key role in their survival and success in such environments. In this context, it was hypothesized that exploration of variation in leaf micro-structural and topographical attributes i.e. leaf hairs and stomata might provide the key to understand the mechanisms involved in growth maintenance and survivorship of all these grasses in hyper arid environments facing extreme moisture deficit.

Materials and Methods

The role of variability in micro-structural traits of some arid zone grasses towards ecological success in arid areas of Punjab, Pakistan was evaluated in this study. Plants from each study area were uprooted with soil auger. Rhizospheric soil samples were collected for all populations and various soil attributes like pH, ECe, saturation percentage, moisture content, ionic content (Na, K, Ca, P, NO_3-N, NH_4-N, P, Cl and organic matter) were recorded using the methods as described below. Meteorological and soil data regarding habitat are presented in Tables 1 and 2, respectively.

Table 1. Meteorological attributes of collection sites of different grasses from the Punjab, Pakistan.

Parameters	NeW	SoV	KKr	KaM	PaK	NpT
Aridity level (D)	8.40	8.81	8.99	10.71	13.29	10.90
Latitude (N)	32°39’55.39”	32°43’13.0”	32°36’00.1”	32°35’00.62”	31°15’11.7”	31°58’02.88”
Longitude (E)	72°37’11.70”	72°58’00.2”	72°26’52.7”	72°27’31.32”	72°48’41.2”	72°12’46.05”
Elevation	833	592	506	525	172	187
Annual Rainfall (mm)	519	459	485	407	328	400
Min. Temp. (°C)	14	16	22	24	22	27
Max. Temp. (°C)	37	36	31	33	35	39
Humidity (%)	15	16	18	20	21	22
Wind speed (Km h^{-1})	12	11	10	9	8	12
Soil texture	SL	SCL	SL	SCL	SL	SL
Habitat description	Steep slope, fresh water streams	Steep slope (60%), dry mountains	Bank of hyper-saline lake	Dry mountains	Hyper-saline wasteland	Sand dunes, Thal desert
Parameters	**HaP**	**PiR**	**Cho**	**ChK**	**CsD**	
Aridity level (D)	26.2	26.5	30.4	8.71	8.50	
Latitude (N)	29°37’08.5”	30°20’08.1”	28°41’56.6”	32°55’46.64’’	32°43’16.96’’	
Longitude (E)	72°22’50.9”	72°01’11.4”	71°19’00.8”	72°52’12.53’’	72°58’59.95’’	
Elevation	137	136	103	498	676	
Annual Rainfall (mm)	166	164	143	457	447	
Min. Temp. (°C)	29	30	17	15	16	
Max. Temp. (°C)	41	40	42	27	24	
Humidity (%)	32	38	36	18	17	
Wind speed (Km h^{-1})	14	16	18	12	13	
Soil texture	L	SCL	SCL	SL	SCL	
Habitat description	Margin of Cholistan Desert	Artificial forest plantation	Inter-dune flats	Plain area along roadside	Sand stone hills, slope 45%	

SL; Sandy loam, SCL; Sandy clay loam, L; Loam, NeW; Neelawahn, SoV; Soonvalley, KKr; Kalarkahar, KaM; Kathamountains, CsD; Choasaidan shah, ChK; Chakwal, PaK; Pakanna, HaP; Hasilpur, PiR; Pirowal, Cho; Cholistan, NpT; Noorpurthal

Table 2. Soil physico-chemical attributes of collection sites of all species from the Punjab, Pakistan.

Soil parameters	NeW	SoV	KKr	KaM	PaK	NpT
pH	7.6	7.8	8.8	8.1	8.6	7.8
ECe (dS m^{-1})	5.6	4.8	7.3	6.8	4.3	6.5
Na^+ (mg g^{-1} dw)	26.1	27.2	51.2	49.2	128.0	51.3
K^+ (mg g^{-1} dw)	4.0	3.0	24.0	22.0	10.0	12.0
Ca^{2+} (mg g^{-1} dw)	12.0	15.0	20.0	11.0	24.0	18.0
Mg^{2+} (mg g^{-1} dw)	0.34	0.44	0.29	0.22	0.36	0.37
Cl^- (mg g^{-1} dw)	1.7	1.8	2.4	2.5	2.8	2.9
Organic matter (%)	0.55	0.59	0.33	0.34	0.38	0.56
Moisture content (%)	3.4	3.5	3.4	3.6	2.5	2.4
Saturation percentage (%)	36	35	45	46	20	25
NH_4-N (mg L^{-1})	0.31	0.30	0.23	0.29	0.2	0.22
NO_3-N (mg L^{-1})	0.04	0.05	0.48	0.47	0.08	0.34
Soil parameters	**HaP**	**PiR**	**Cho**	**ChK**	**CsD**	
pH	7.8	8.7	9.1	7.8	7.8	
ECe (dS m^{-1})	9.6	10.7	10.7	7.7	7.5	
Na^+ (mg g^{-1} dw)	52.8	17.6	30.4	28	27	
K^+ (mg g^{-1} dw)	19.0	42.0	61.0	18	19.0	
Ca^{2+} (mg g^{-1} dw)	23.0	27.0	27.0	19	17.0	
Mg^{2+} (mg g^{-1} dw)	0.34	0.39	0.41	0.48	0.36	
Cl^- (mg g^{-1} dw)	3.8	2.6	4.2	2.3	2.8	
Organic matter (%)	0.16	0.35	0.49	0.35	0.37	
Moisture content (%)	2.0	3.7	3.0	3.3	3.7	
Saturation percentage (%)	40	50	30	28	25	
NH_4-N (mg L^{-1})	0.50	0.46	0.23	0.32	0.46	
NO_3-N (mg L^{-1})	0.32	0.08	0.08	0.07	0.21	

NeW; Neelawahn, SoV; Soonvalley, KKr; Kalarkahar, KaM; Kathamountains, CsD; Choasaidan shah, ChK; Chakwal, PaK; Pakanna, HaP; Hasilpur, PiR; Pirowal, Cho; Cholistan, NpT; Noorpurthal

Soil analysis: Soil moisture content was calculated as the difference between final weight and initial weight divided by initial weight of sample (Sparks *et al*., 1996). Soil texture was recorded by using USDA textural triangle while organic matter according to the method of Walkley (1947). A saturation paste of the soil was prepared and used to analyze various attributes of soil such as pH and ECe by using a combined pH and EC meter (WTW series InoLab pH/Cond 720, USA). The concentration of various ions such as Na^+, K^+ and Ca^{2+} determined from saturation paste by flame photometer (Jenway, PFP–7, UK). Mg^{2+} content determined using atomic absorption spectrophotometer (Perkin Elmer/AA-300, Germany) while Cl^- content estimated via Mohrs' titration method (Mohrs, 1856). Nitrogen content was estimated by micro-Kjeldahl method using the UDK-132 semiautomatic ammonia distillation unit (NIB-B (3)-DSU-003 Italy). Soil phosphorus was determined by using the protocol of Wolf (1982).

Morphological traits: Plants were uprooted from the soil, washed and data for both shoot and root tissue fresh biomass recorded using electronic digital balance. The plants were kept in oven at 65ºC to record the dry biomass. Fresh to dry biomass ratio was calculated by dividing the fresh tissue weight to dry tissue weight. Root and shoot length was measured by measuring tape. Ratio of root to shoot length was calculated by dividing the root length to shoot length.

Leaf area was calculated using the formula (Lopes *et al*., 2016).

Total leaf area = Maximum leaf length × maximum leaf width × C.F.
C.F. = Correction factor = 0.68

Anatomical traits: All populations of selected grasses were collected and washed with tap water followed by distilled water before measuring anatomical parameters. A piece measuring 1.5 cm of flag leaf separated from the leaf-base adjoining to leaf sheath and preserved for anatomical studies. A formalin acetic alcohol (v/v formalin 10%, acetic acid 5%, ethyl alcohol 50 % and distilled water 35%) was used for fixation and acetic alcohol (v/v acetic acid 25%, and ethanol 75%) solution for preservation. Epidermal slides were prepared using a safranin stain to enhance the background contrast. Micrographs of stained slides were taken on a stereo-microscope (Nikon 104) equipped with a digital camera (Nikon FDX-35). All measurements were taken by ocular micrometer calibrated with stage micrometer. Means were compared using LSD ($p>0.05$). The Redundancy Analysis (RDA) of anatomical traits performed using CANOCO for windows (v. 4.5). The response curves as attribute plot were plotted with CanoDraw (v 4.0) supplied with CANOCO (v. 4.5).

Results

Morphological traits: Data regarding the morphological traits are given in the Table 3. Root to shoot length increased significantly in almost all species (*Aristida adscensionis*, *Cenchrus ciliaris, Desmostachya bipinnata,* and *Heteropogon contortus*) collected from hyper-arid areas (Cho). Longer roots of *Cenchrus ciliaris* (*Cc*) and *Desmostachya bipinnata* (*Db*) observed in more arid habitat (i.e. Cho). Fresh to dry biomass ratio for both root and shoot decreased significantly as the aridity increased in all species. Populations of *Aristida adscensionis* from hyper-arid habitat (Cho, HaP and NpT) showed reduced leaf area as compared to the lesser arid habitats (NeW, KKr and KaM). *Cenchrus ciliaris* populations collected from hyper-arid habitat (Cho) exhibited smaller leaf area as compared to lesser-arid NeW and SoV. Small leaf area observed for Cho populations of *Desmostachya bipinnata* and CsD and ChK populations of *Heteropogon contortus* (Table 3).

Leaf epidermal anatomy: Adaxial leaf hair density increased significantly along the increasing moisture deficit for populations of *Aristida adscensionis, Desmostachya bipinnata* and *Heteropogon contortus* while this trait remained unaffected in *Cenchrus ciliaris* populations. Higher adaxial hair density was recorded for hyper-arid HaP and Cho populations of *Aristida adscensionis*, PiR and Cho populations of *Desmostachya bipinnata* and CsD and ChK populations of *Heteropogon contortus*. Adaxial leaf hair area showed non-significant variation for the *Aristida adscensionis, Cenchrus ciliaris* and *Heteropogon contortus* populations while *Desmostachya bipinnata* (PiR and Cho) populations exhibited large leaf area as compared to other less arid SoV, KKr and PaK populations (Fig. 1; Fig. 4).

Adaxial stomatal density decreased as the moisture deficit increased. More number of stomata observed in populations of *Aristida adscensionis* (NeW, KKr, KaM), *Cenchrus ciliaris* (NeW, SoV, KKr), *Desmostachya bipinnata* (SoV, KKr) and *Heteropogon contortus* (NeW, SoV), respectively. Adaxial stomatal area increased along the moisture deficit for *Aristida adscensionis, Cenchrus ciliaris* and *Heteropogon contortus* species except *Desmostachya bipinnata* which showed decreased adaxial stomatal area at hyper-arid (Cho) populations (Fig. 2; Fig. 3; Fig. 5).

Abaxial stomatal density and area increased as the moisture deficit increased for all populations. More number of abaxial stomata and large stomatal area was recorded for hyper-arid populations of all species (Fig. 2; Fig. 3; Fig. 6).

RDA analysis: Leaf epidermal traits RDA triplots for each species are given in the Fig. 7. Ad HA was significantly influenced in KaM habitat. Ad SA influenced by the saturation percent of NpT habitat while Ab SA with the soil ECe and pH with the Ad HD, Ab SD, Ad SD of HaP in *Aristida adscensionis* (Fig. 7a). In *Cenchrus ciliaris*, Ad HD, Ab SD, Ad SD influenced by the soil K and Mg content of Cho. Ad HA was associated with soil MC of NeW habitat (Fig. 7b). Organic matter of soil influenced Ad HD, Ab SD, Ad SD in *Desmostachya bipinnata* specie (Fig. 7c). In comparison, Cl content of soil influenced the Ad HD, Ab SD, Ad SD in *Heteropogon contortus* (Fig. 7d).

Table 3. Morphological data of all species collected from arid areas.

Habitat	*Aristida adscensionis*						Habitat	*Desmostachya bipinnata*					
	NeW	**KKr**	**KaM**	**NpT**	**HaP**	**Cho**		**SoV**	**KKr**	**PaK**	**HaP**	**PiR**	**Cho**
R:SL	0.07	0.09	0.14	0.16	0.34	0.41	R:SL	0.07	0.08	0.18	0.2	0.19	0.3
Rf:dw	2.73	2.53	1.83	1.39	1.17	1.16	Rf:dw	3.58	2.61	2.08	1	1.26	1.04
Sf:dw	1.44	1.31	1.34	1.23	1.18	1.13	Sf:dw	1.66	1.31	1.35	1.3	1.15	0.66
LA	0.96	0.89	0.8	0.7	0.57	0.40	LA	12.39	9.67	8.87	6.77	5.27	5.83
Habitat	*Cenchrus ciliaris*						**Habitat**	*Heteropogon contortus*					
	NeW	**SoV**	**KKr**	**NpT**	**PaK**	**Cho**		**NeW**	**SoV**	**KKr**	**KaM**	**CsD**	**ChK**
R:SL	0.33	0.54	0.72	0.56	0.5	0.37	R:SL	0.11	0.15	0.16	0.17	0.19	0.2
Rf:dw	1.06	1.23	1.08	1.06	1.04	0.33	Rf:dw	1.69	1.64	1.46	1.48	1.25	1.26
Sf:dw	2.4	2.04	1.65	1.21	1.27	1.17	Sf:dw	1.86	1.61	1.49	1.54	1.24	1.25
LA	3.53	3.53	3.03	2.35	1.77	1.52	LA	2.59	2.44	1.8	1.69	1.36	0.74

NeW; Neelawahn, SoV; Soonvalley, KKr; Kalarkahar, KaM; Kathamountains, CsD; Choasaidan shah, ChK; Chakwal, PaK; Pakanna, HaP; Hasilpur, PiR; Pirowal, Cho; Cholistan, NpT; Noorpurthal

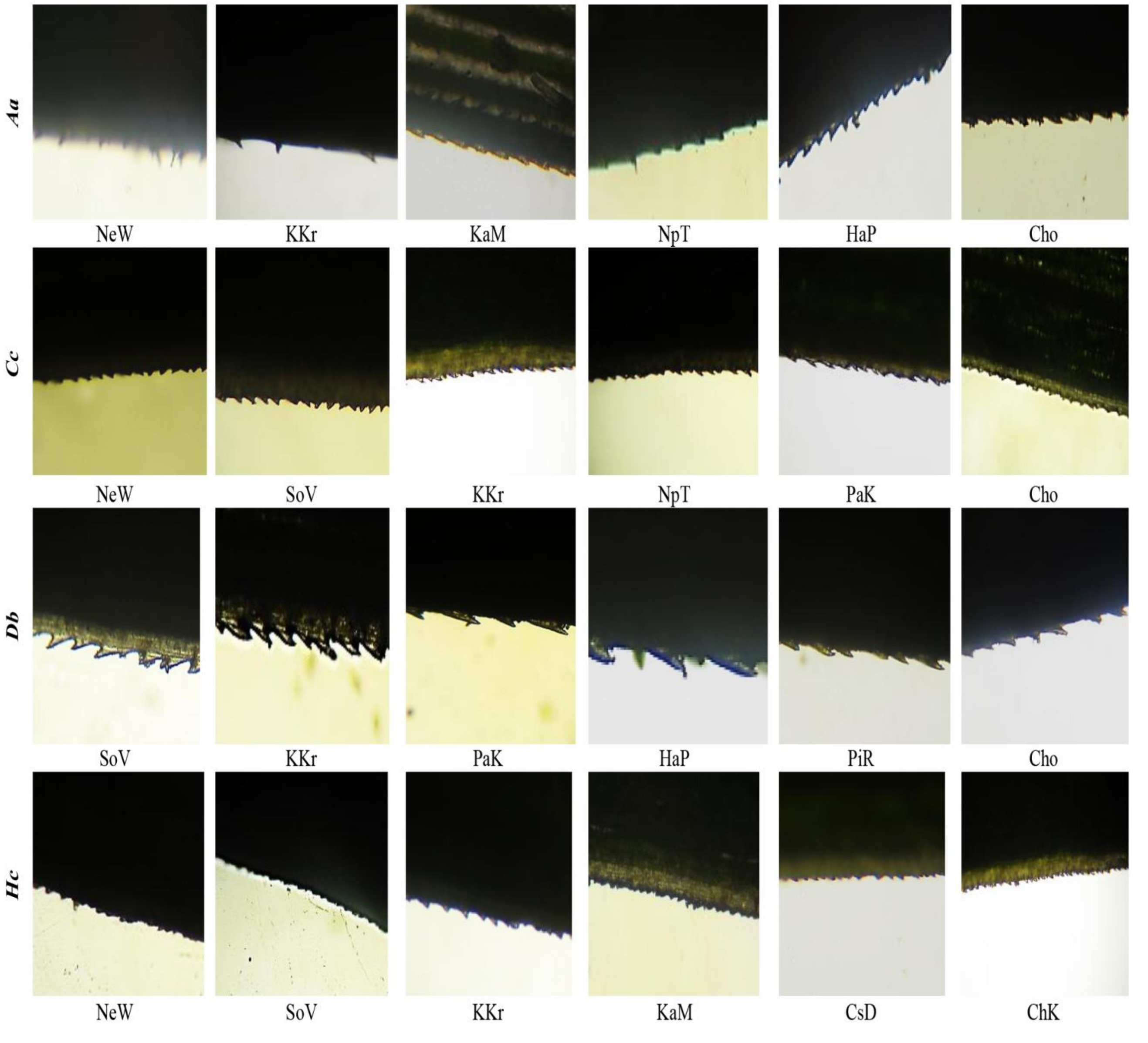

Fig. 1. Differential development pattern of hairs on adaxial leaf surface in different grass populations belonging to four species collected from various arid habitats (NeW; Neelawahn, SoV; Soonvalley, KKr; Kalarkahar, KaM; Kathamountains, CsD; Choasaidan shah, ChK; Chakwal, PaK; Pakanna, HaP; Hasilpur, PiR; Pirowal, Cho; Cholistan, NpT; Noorpurthal).

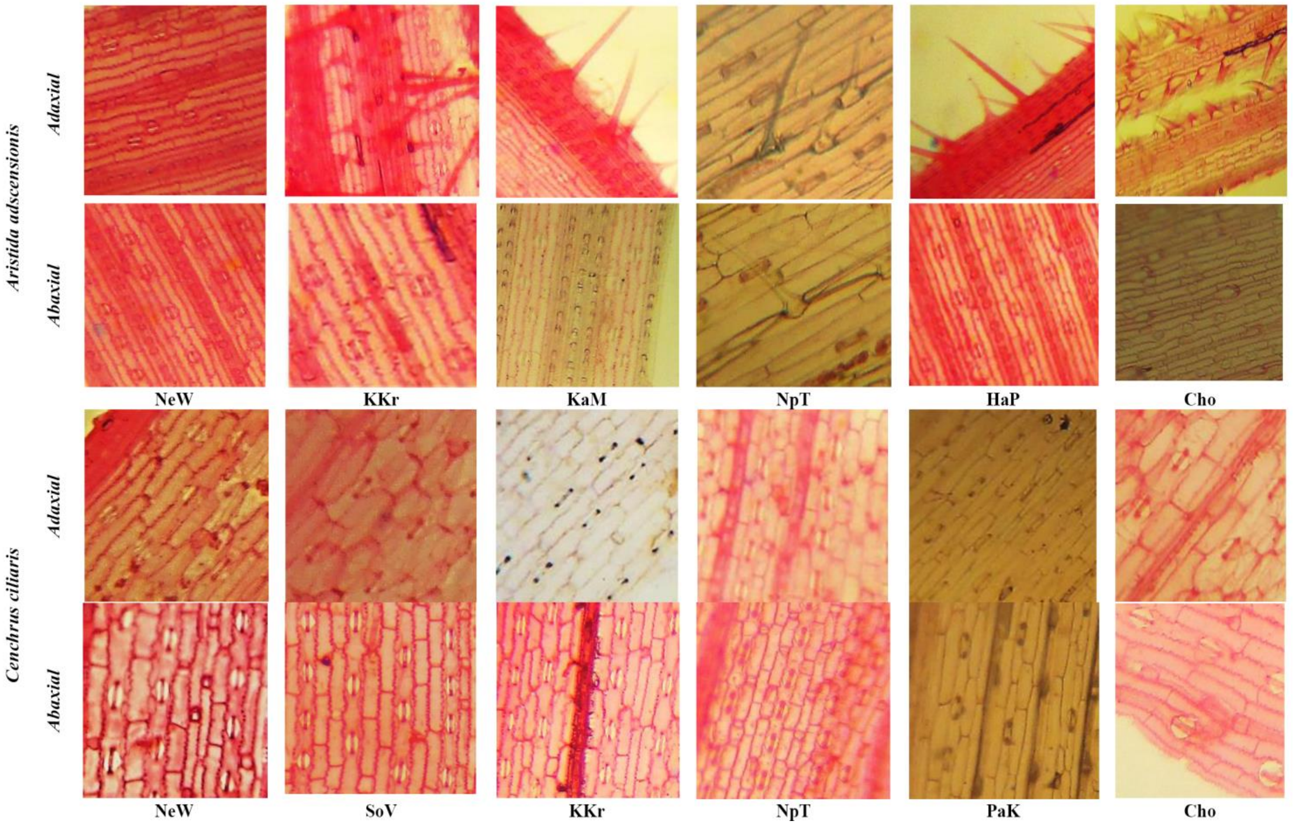

Fig. 2. Leaf adaxial and abaxial epidermal surface of *Aristida adscensionis* and *Cenchrus ciliaris* populations collected from various arid habitats (NeW; Neelawahn, SoV; Soonvalley, KKr; Kalarkahar, KaM; Kathamountains, CsD; Choasaidan shah, ChK; Chakwal, PaK; Pakanna, HaP; Hasilpur, PiR; Pirowal, Cho; Cholistan, NpT; Noorpurthal).

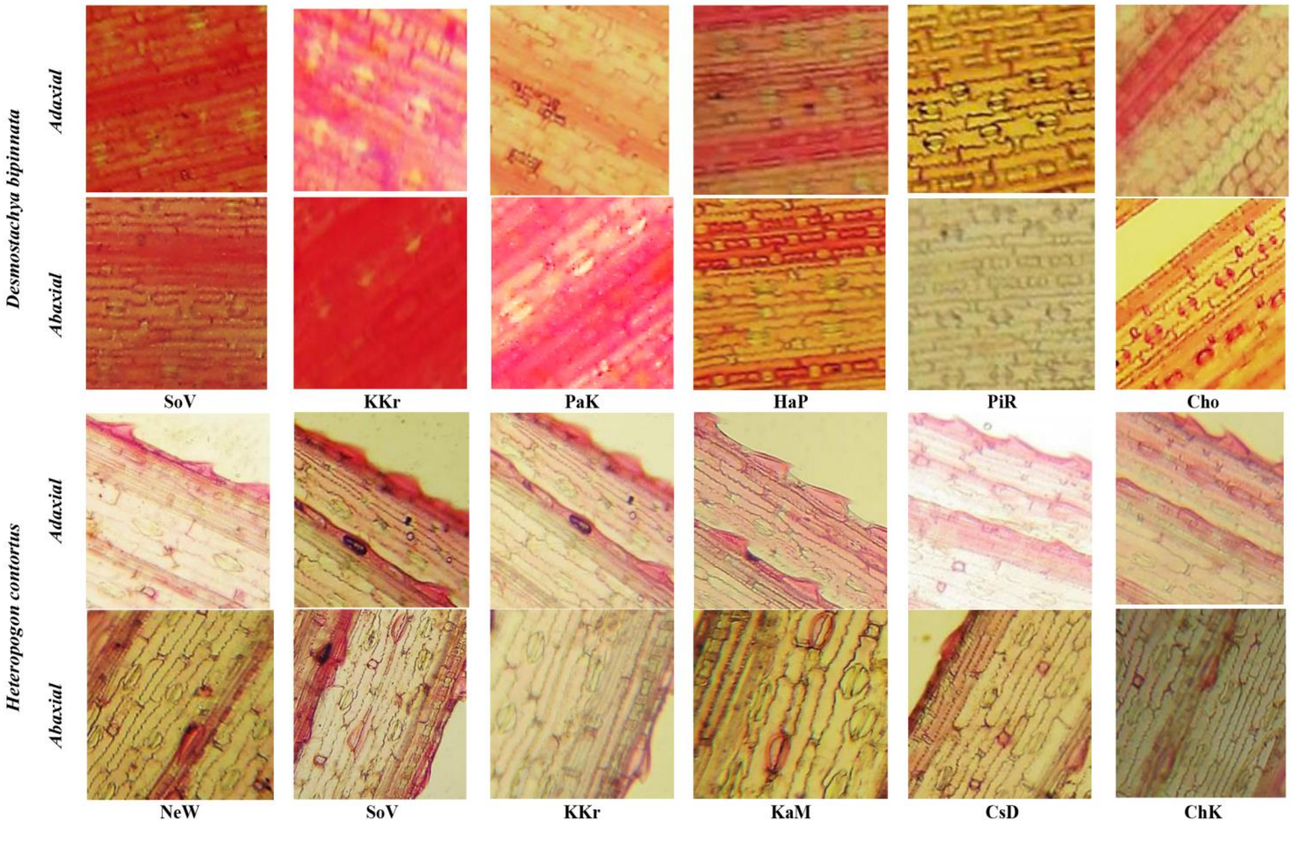

Fig. 3. Leaf adaxial and abaxial epidermal surface of *Desmostachya bipinnata* and *Heteropogon contortus* populations collected from various arid habitats (NeW; Neelawahn, SoV; Soonvalley, KKr; Kalarkahar, KaM; Kathamountains, CsD; Choasaidan shah, ChK; Chakwal, PaK; Pakanna, HaP; Hasilpur, PiR; Pirowal, Cho; Cholistan, NpT; Noorpurthal).

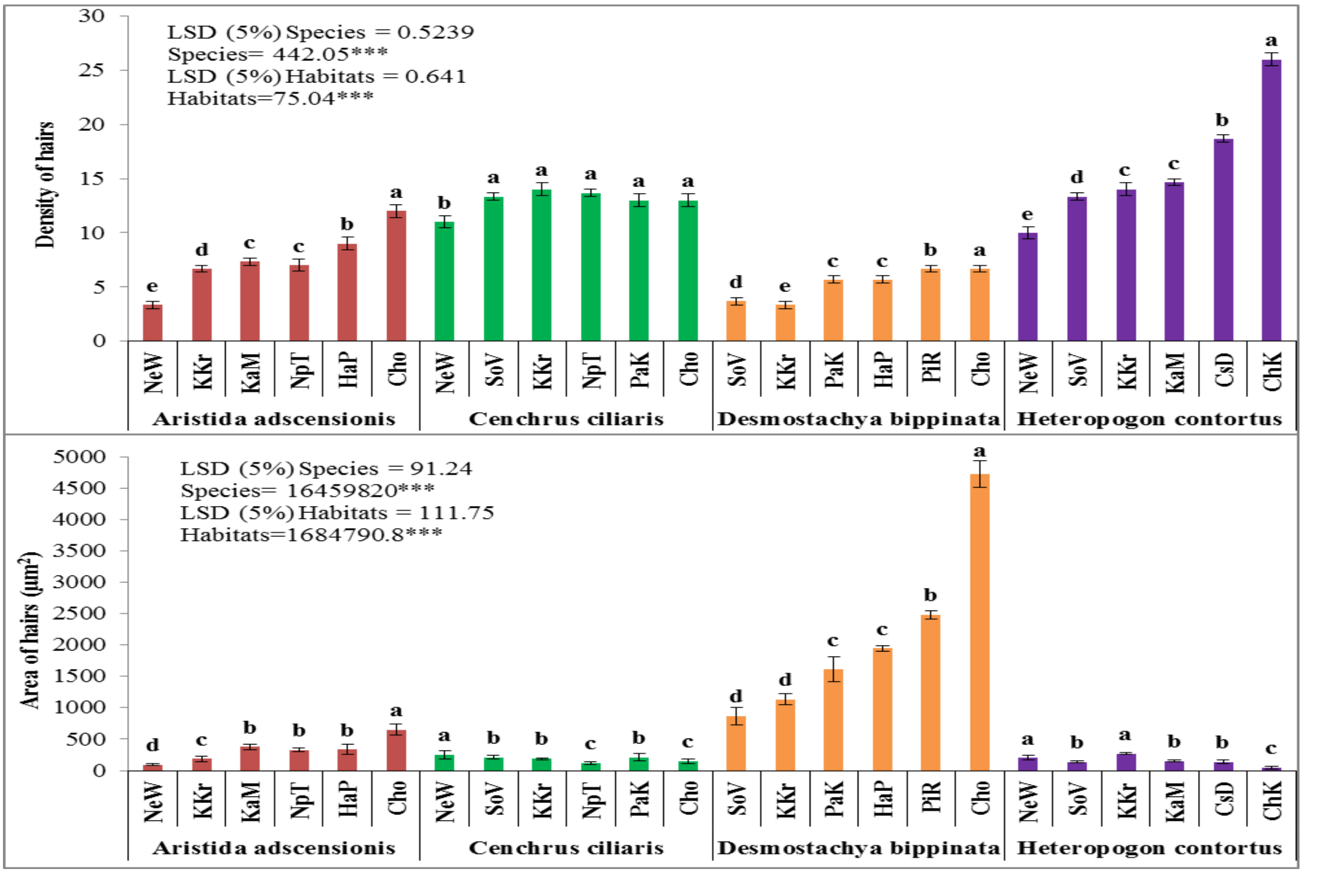

Fig. 4. Leaf hair density and stomatal area of the *Aristida adscensionis, Cenchrus ciliaris, Desmostachya bipinnata* and *Heteropogon contortus* populations collected from arid habitats. Habitats arranged according to increasing soil moisture deficit.

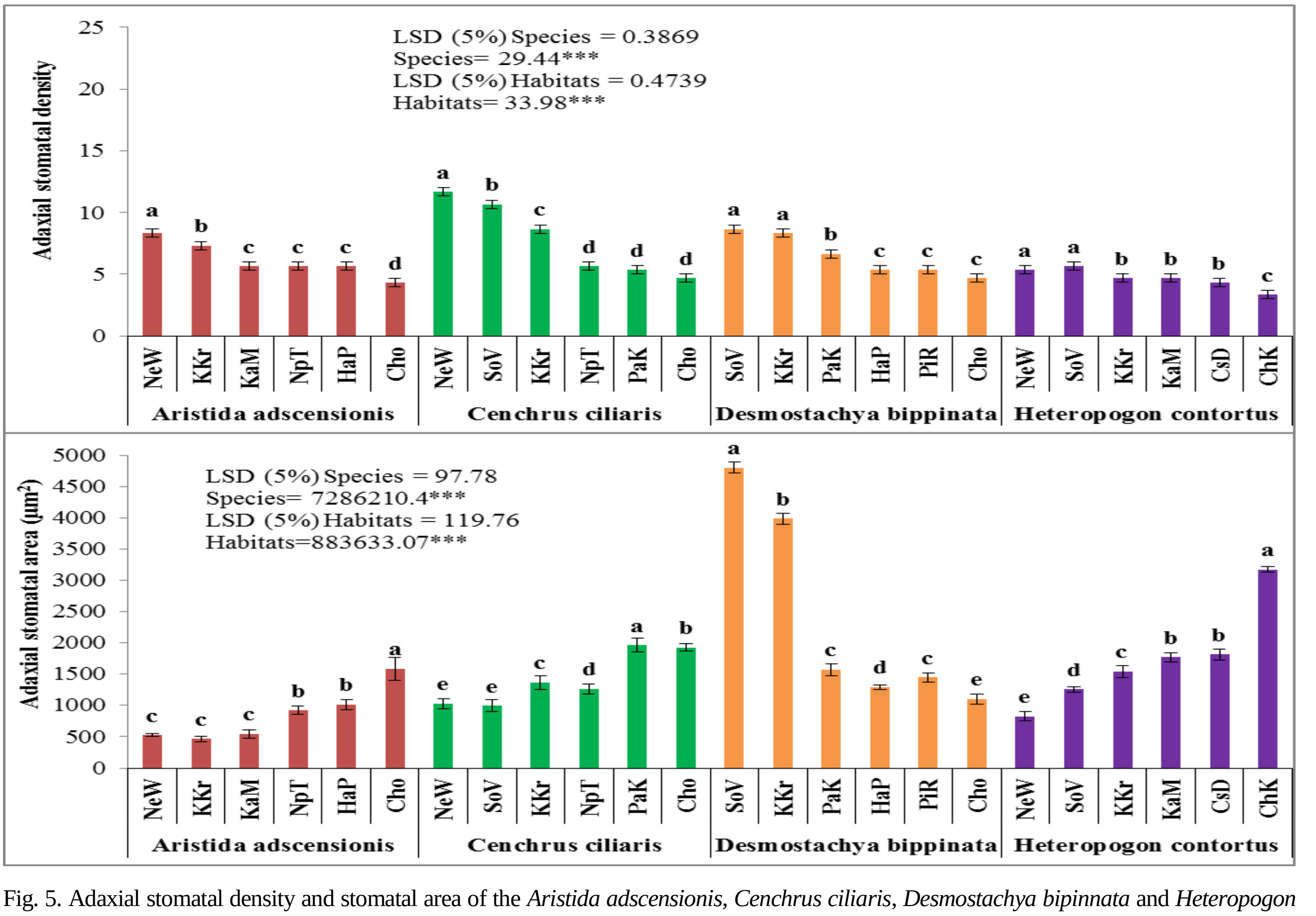

Fig. 5. Adaxial stomatal density and stomatal area of the *Aristida adscensionis, Cenchrus ciliaris, Desmostachya bipinnata* and *Heteropogon contortus* populations collected from arid habitats. Habitats arranged according to increasing soil moisture deficit.

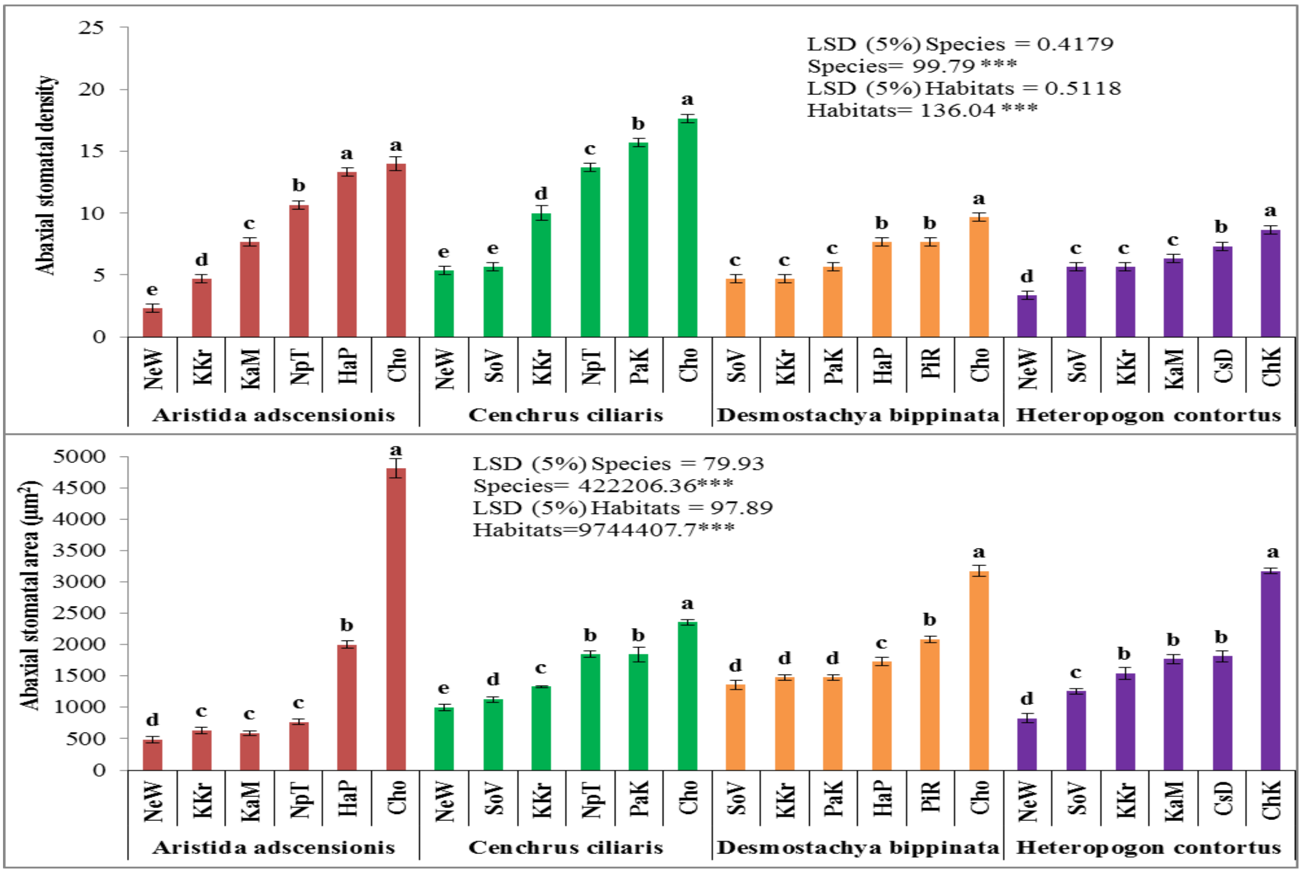

Fig. 6. Abaxial stomatal density and stomatal area of the *Aristida adscensionis, Cenchrus ciliaris, Desmostachya bipinnata* and *Heteropogon contortus* populations collected from arid habitats. Habitats arranged according to increasing soil moisture deficit.

Response curves (RC): Response curves for leaf epidermal traits of each species shown in the Fig. 8. The leaf epidermal traits such as Ad HA, Ad HD, Ab SD, Ab SA and Ad SA of *Aristida adscensionis* showed positive relation while Ad SD showed negative relation to the MC (Fig. 8a). In comparison, *Cenchrus ciliaris, Desmostachya bipinnata* and *Heteropogon contortus* leaf epidermal traits like Ad HA, Ad HD, Ab SD and Ab SA showed positive relation while Ad SD and Ad HA showed negative correlation to the MC (Fig. 8b, 8c, 8d).

Discussion

Arid region plants utilized energy in effective way for their growth under prevailing stress conditions. These plants have the ability to change their morphological traits in response to ecological variations. This morphological plasticity plays a vital role for utilization of resources by plants. Plants alter their growth form to adjust in changing environment. Decrease in shoot and root fresh and dry weights under water deficit had been mostly credited to their small leaf area (Zhang *et al.*, 2018). Current study show that leaf morphological traits of different grasses (*Aristida adscensionis*, *Cenchrus ciliaris*, *Desmostachya bipinnata*, and *Heteropogon contortus*) such as fresh to dry biomass ratio for both root and shoot decreased significantly with increase in aridity. A parallel decrease in leaf area for *Aristida adscensionis* populations from Cho, HaP and NpT, *Cenchrus ciliaris* and *Desmostachya bipinnata* populations collected from Cho, and, CsD and ChK populations of *Heteropogon contortus* observed (Zhang *et al.*, 2020).

Plants when confronted with moisture deficit in rhizospheric soil shows inhibited growth of aerial parts. Higher moisture deficit stimulates root elongation thereby increases root: shoot ratio in many species (Zhang *et al.*, 2020). As seen in this study, root: shoot length increased significantly in almost all populations of *Aristida adscensionis, Cenchrus ciliaris, Desmostachya bipinnata, Heteropogon contortus* collected from hyper-arid areas of Cholistan desert. Longer roots of *Cenchrus ciliaris* and *Desmostachya bipinnata* also observed in more arid Cholistan desert to extract water from soil deeper layers (Rahat *et al.*, 2019).

In current study, adaxial stomatal density decreased as the moisture deficit increased. More number of stomata observed in populations of *Aristida adscensionis* (NeW, KKr, KaM), *Cenchrus ciliaris* (NeW, SoV, KKr), *Desmostachya bipinnata* (SoV, KKr) and *Heteropogon contortus* (NeW, SoV), respectively. Abaxial stomatal density and area also increased as the moisture deficit increased. More number of abaxial stomata with larger area was recorded for hyper-arid populations of all species of *Aristida adscensionis* (Cho, HaP and NpT), *Cenchrus ciliaris* and *Desmostachya bipinnata* (Cho) and *Heteropogon contortus* (CsD and ChK). Variation in structure of morphological attributes among various species reflected their adaptation to the ecological selection pressures. Plants avoid aridness by regulating stomatal and leaf topographical features for minimizing transpirational losses. In shrubs and perennial grasses, stomatal density and area had been shown to decrease along with increasing moisture deficit as reported by Guo *et al*., (2017). Lower stomatal densities in grasses under water stress helps to minimize water loss via transpiration along concurrent enhancement in WUE (Guo *et al*., 2017). Leaf epidermal micro-structural modifications such as hairs and stomata density control transpiration by reducing leaf temperature and wind speed to control water loss. Arid plants have been shown to exhibit smaller and fewer stomata present on abaxial surface of leaf (Xu & Zhou, 2008). In many leaves of arid zone grasses, stomata oriented on abaxial leaf surface as compared to the adaxial side had been shown to effectively decrease in transpiration rate (Soares *et al*., 2008). In current study as well, *Cenchrus ciliaris* exhibited more number of stomata on abaxial leaf surface that is of significant ecological impact as discussed earlier (Obidiegwu *et al*., 2015).

In current study, adaxial leaf hair density increased significantly along the increasing moisture deficit for populations of *Aristida adscensionis*, *Desmostachya bipinnata* and *Heteropogon contortus* while this trait remained stable in *Cenchrus ciliaris* populations. Higher adaxial hair density was recorded in the HaP and Cho populations of *Aristida adscensionis*, PiR and Cho populations of *Desmostachya bipinnata*, and, CsD and ChK populations of *Heteropogon contortus*. Adaxial leaf hair area showed non-significant variation for the *Aristida adscensionis*, *Cenchrus ciliaris* and *Heteropogon contortus* populations while *Desmostachya bipinnata* (PiR and Cho) populations exhibited large leaf area as compared to other SoV, KKr and PaK populations of the same species. Development of hairiness is a characteristics feature to resist excessive water loss and to protect leaves from drying out. Arid plants possessed dense and long hairs to minimize water loss via leaf surface as they are involved in reflecting solar radiations and developing a moist boundary layer near leaf surface (Dolatabadian *et al*., 2011).

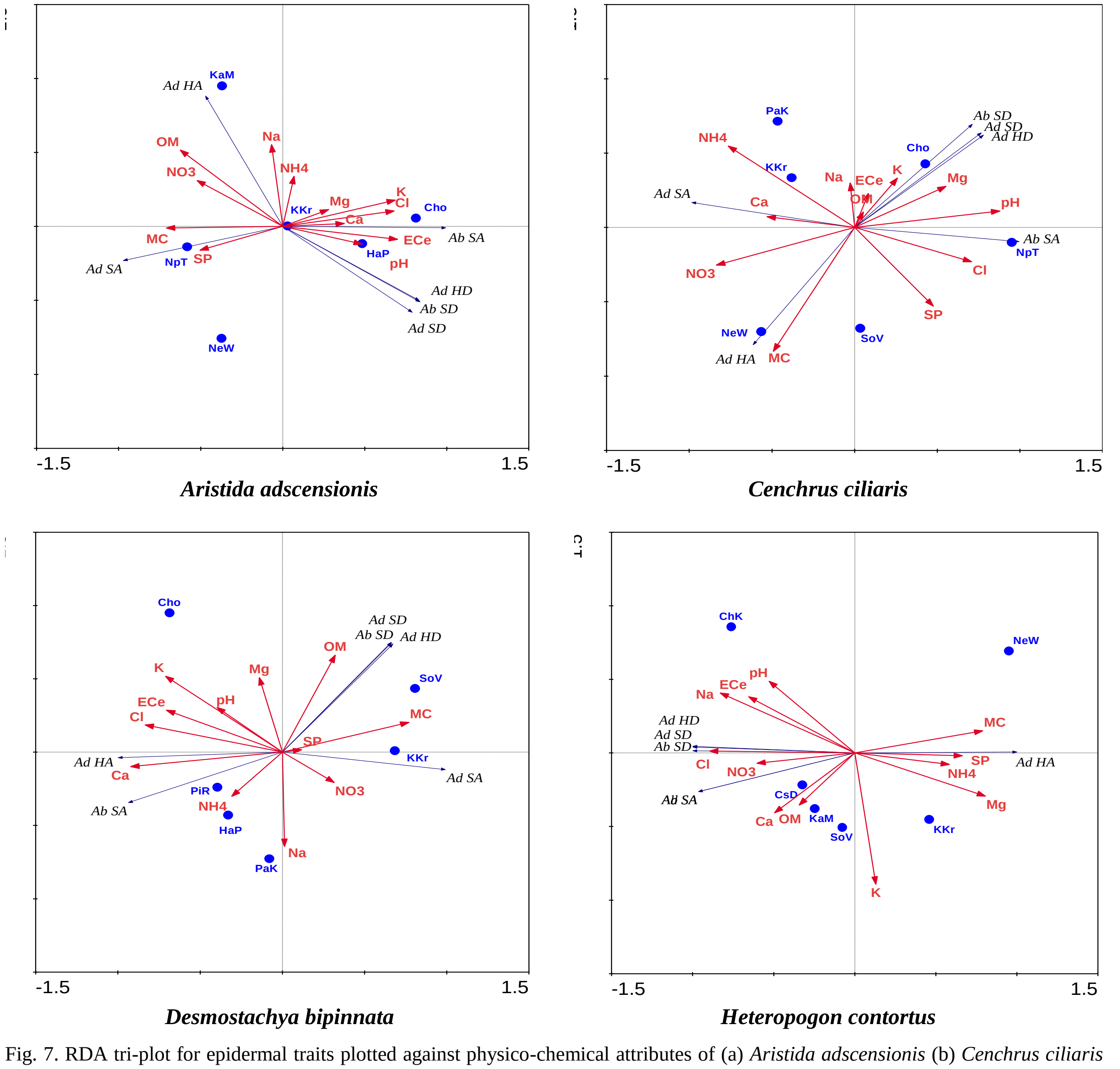

Fig. 7. RDA tri-plot for epidermal traits plotted against physico-chemical attributes of (a) *Aristida adscensionis* (b) *Cenchrus ciliaris* (c) *Desmostachya bipinnata* (d) *Heteropogon contortus* rhizospheric soil collected from various arid habitats.

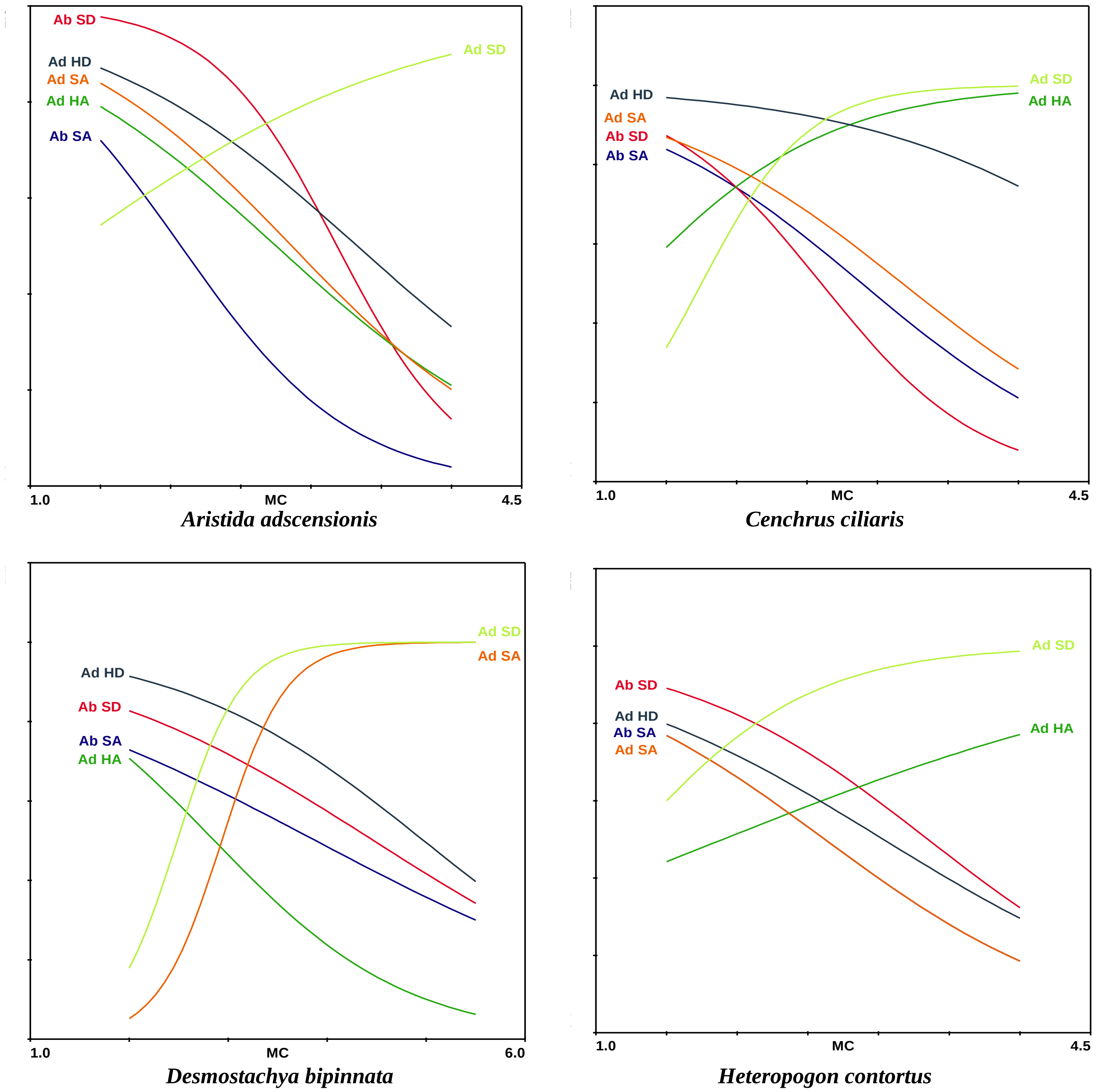

Fig. 8. Response Curves (RC) for epidermal traits plotted against increasing moisture content (left to right) of rhizospheric soil of (a) *Aristida adscensionis* (b) *Cenchrus ciliaris* (c) *Desmostachya bipinnata* (d) *Heteropogon contortus* collected from various arid habitats.

Conclusion

Moisture deficit affected morpho-anatomical traits such as growth and leaf hair and stomata. Populations from hyper-arid regions relied upon small leaf area and decreased fresh to dry biomass ratio for both root and shoot tissue, longer roots to extract water from soil deeper layers. Among leaf micro-structural traits more number of stomata oriented on leaf abaxial as compared to the adaxial surface in hyper-arid populations of all grasses along with adaxial surface covered with long hairs possibly helped survive in these environments by decreasing leaf transpirational losses in most of the species under study.

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