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Relationships between gas-exchange characteristics and stomatal structural modifications in some desert grasses under high salinity

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Abstract

Two populations, one from lesser saline Derawar Fort (DF) and the other from highly saline Ladam Sir (LS) in the Cholistan desert, for each of the five grass species, *Aeluropus lagopoides, Cymbopogon jwarancusa, Lasiurus scindicus, Ochthochloa compressa,* and *Sporobolus ioclados* were examined to investigate the influence of salinity on structural and functional characteristics of stomata. Salinity tolerance in *A. lagopoides* mainly depended on controlled transpiration rate (E) and high water-use efficiency (WUE), which was found to be regulated by fewer and smaller stomata on both leaf surfaces as well as stomatal encryption by epidermal invaginations. *C. jwarancusa* had sunken stomata on the abaxial surface only, which largely reflected a reduced E, but less affected stomatal conductance (g_s) or WUE. *L. scindicus* had fewer but larger stomata along with hairs/trichomes which may function to avoid water loss through transpiration, and hence, to attain a high WUE. In *O. compressa* stomata were found only on the abaxial surface and these were completely encrypted by epidermal invaginations as well as a dense covering of microhairs, which was associated with a low E and high WUE under salinity stress. In *S. ioclados*, the traits of increased stomatal density and decreased stomatal area may be critical for stomatal regulation under salt-prone environments. High stomatal regulation depended largely on stomatal density, area, and degree of encryption under salinity, which is of great ecophysiological significance for plants growing under osmotic stresses.

Additional key words: desert grasses; stomatal regulation; stomatal structural modifications; transpiration rate; water-use efficiency.

Introduction

The Cholistan Desert of Pakistan (coordinates: 27º42' and 29º49' N, 69º52' and 75º42 E) is a vast hot sandy desert spreading over an area of 2.6 million ha (Akhter and Arshad 2006). Mean annual rainfall in the desert ranges from 100 to 250 mm (mostly during the monsoon season from July to September). Mean temperature ranges from 46.5°C (absolute maximum 51ºC) during June to 6.5ºC during December and January (Arshad *et al.* 2008). Vegetation of the area is typical of xeric type with grasses, such as *A. lagopoides, L. scindicus, O. compressa, C. jwarancusa* and *S. ioclados,* dominating the interdunal saline flats between high sand dunes of about 100 m (Hameed *et al.* 2002). The distribution pattern and relative dominance of these grasses depend on the salinity level of the habitats in the Cholistan desert. With respect to this degree of salinity tolerance, the five grass species used in the present study can be rated in the following sequence: *A. lagopoides* (most important), *S. ioclados, O. compressa, C. jwarancosa*, and *L. scindicus* (least important) (Naz *et al.* 2009).

The harsh and fluctuating desert environment has a strong impact on vegetation structure and distribution (Arshad *et al.* 2008). Plant interactions with their surrounding environment result in diverse strategies and mechanisms in plants for their successful survival under stressful conditions (Soukup *et al.* 2004). The natural plants are highly adapted to their native climatic and edaphic conditions, and therefore, have some specific structural and physiological adaptations to environmental stresses (Hameed *et al.* 2009, Naz *et al.* 2009).

Leaf growth is more responsive to high salinities than root growth, and reduction in photosynthetic area (leaf

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Abbreviations: ANOVA – analysis of variance; DF – Derawar Fort; E – transpiration rate; ECe – electrical conductivity of soil saturation extract; g_s – stomatal conductance; LS – Ladam Sir; LSD – least significant difference; P_N – net CO_2 assimilation rate; PAR – photosynthetically active radiation; PPFD – photosynthetic proton flux density (maximum light intensity); SAR – sodium absorption ratio; WUE – water-use efficiency.

size) cannot support well the continued growth (Munns and Termaat 1986). Adverse environmental conditions like salinity and drought are known to directly affect photosynthesis (Naumann *et al.* 2007), as well as stomatal conductance (Souza *et al.* 2004), which ultimately limit CO_2 diffusion into the leaf tissues (Centritto *et al.* 2003). High flexibilities in stomatal behaviour in response to plant water status may be positively associated with photosynthesis and water-use efficiency (Xu and Zhou 2008). Thus, photosynthetic parameters have been considered as the potential targets for selection for salinity tolerance in grasses (Lee *et al.* 2004).

Plant stomata play a crucial role under different environmental conditions by acting as vital gates between plants and the atmosphere (Nilson and Assmann 2007). As the inhabitant species of the Cholistan desert are exposed to multiple stresses, stomatal behaviour may play an important role in their distribution, survival and adaptation to such harsh climatic conditions. Many researchers have reported the function of stomata in resisting various environmental stresses such as heat (Beerling and Chaloner 1993), salt (Zhao *et al.* 2006), and drought (Galmés *et al.* 2007). Stomatal density and distribution along with other leaf epidermal features may significantly affect key gas-exchange parameters in response to different environmental factors (Nilson and Assmann 2007). Stomatal morphology, distribution, and behaviour are important indicators of resistance to a variety of environmental conditions (Hetherington and Woodward 2003). Stomatal behaviour is very important in controlling different gas-exchange parameters. For example, opening and closing of stomata and their orientation on leaf surfaces may prove very vital (Nejad *et al.* 2006). Especially, the complete absence of stomata from upper leaf surfaces is critical for maintaining plant water relations under water-limiting environments as has been observed previously in *Festuca novae-zealandiae* (Abernethy *et al.* 1998).

Sodium chloride (NaCl) salinity has a direct effect on the photosynthetic apparatus independent of its impact on stomates (Brugnoli and Lauteri 1992), as has also been reported in many halophytic and nonhalophytic species (Ball and Farquhar 1984, Seemann and Sharkey 1986). However, stomatal closure in response to salinity has been reported in different plant species, *e.g.*, *Aster tripolium* (Perera *et al.* 1995, Kerstiens *et al.* 2002), *Oryza sativa* (Sultana *et al.* 1999), and *Vicia faba* (Melesse and Caesar 2008), which in turn reduces the CO_2 conduction. Stomatal closure is also known to reduce internal CO_2 concentration and assimilation rate in plants under salt stress (Dionisio-Sese and Tobita 2000, Netondo *et al.* 2004).

Stomatal regulation in halophytes or salt-tolerant species has received little consideration even though the transpiration stream is thought to be an important factor in ion regulation, especially under high salinities (Robinson *et al.* 1997). In view of these reports it is evident that gas-exchange characteristics in leaves depend on stomatal form and structure in different plants exposed to saline regimes. Therefore, we hypothesized that these two attributes bear a positive relationship with photosynthetic capacity in the five grass species differentially adapted to salt-affected soils of the Cholistan desert.

The present study was conducted with a principal objective to appraise the relationships of gas-exchange characteristics (function) with stomatal modifications (form) in the plants growing under saline regimes of the Cholistan desert. In fact, the fundamental problem of form and function in leaves is quite an active area of research in these days.

Materials and methods

Five salt-tolerant grasses [*Aeluropus lagopoides* (L.) Trin. ex Thw., *Cymbopogon jwarancusa* (Jones) Scult., *Lasiurus scindicus* Henrard, *Ochthochloa compressa* (Forssk.) Hilu, and *Sporobolus ioclados* (Trin.) Nees] were collected from salt-affected habitats differing in salt content in the Cholistan Desert, Pakistan. Derawar Fort (DF) was the low salt-affected habitat (coordinates 29º24'31.95" N, 71º27'32.83" E; pH 8.35; electrical conductivity of soil saturation extract (ECe) 15.21 dS m^{-1}; sodium adsorption ratio (SAR) 2,049.27; Na^+ 3,236.56 mg l^{-1}; Cl^- 1,493.11 mg l^{-1}). Ladam Sir (LS) was the highly salt-affected site (coordinates 30º53'26.47" N, 72º64'25.08" E, pH 8.38; ECe 49.18 dS m^{-1}; SAR 2,795.57; Na^+ 5,139.30 mg l^{-1}; Cl^- 2,637.73 mg l^{-1}). Plants from the two populations were collected in selected habitats in the Cholistan desert and established in a netting house in the Botanic Garden, University of Agriculture, Faisalabad for a period of one year.

Ramets of equal size (with two mature tillers) were detached and grown in aerated hydroponics in half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) for eight weeks during February-April in the netting house under ambient climatic conditions [photoperiod 11 to 12 h, light (average PAR 1,258 ± 32 μmol m^{-2} s^{-1}), day and night temperatures were 38 ± 4°C and 26 ± 3°C, relative humidity 44.6 to 59.4%]. Air pumps were used for aeration of hydroponic culture system for about 12 h daily. Ten plants were grown on a thermopore (mineral fibre) sheets.

The experiment was arranged in a completely randomized design with ten replications and three main factors, *e.g.*, grasses, populations, and salinity levels. The salt levels used in the experiment were 0 and 300 mM NaCl in half-strength Hoagland's nutrient solution. The data were subjected to statistical analysis using *Microsoft Excel* software and *Minitab* statistical software for analysis of variance (*ANOVA*) and LSD for comparison of mean values.

Net CO_2 assimilation rate (P_N), transpiration rate (E) and stomatal conductance (g_s) were measured on one fully expanded youngest leaf from each plant using an open system *LCA-4 ADC* portable infrared gas analyzer (*Analytical Development Company*, Hoddesdon, England). The data were recorded on May 15, 2009 from 9.00 to 11.00 h with molar flow of air per unit leaf area 403.3 mmol m^{-2} s^{-1}, atmospheric pressure 99.9 kPa, maximum light intensity (PPFD) at leaf surface 1,711 µmol m^{-2} s^{-1}, leaf temperature ranging from 28.4 to 32.4ºC, ambient temperature from 22.4 to 27.9ºC, and ambient CO_2 concentration of 352 µmol mol^{-1}.

For stomatal studies, 1-cm piece from the leaf used for gas-exchange measurements was taken and fixed in FAA (formalin 5%, acetic acid 10%, ethanol 50%, and water 35%) solution for 48 h. The material was then transferred to acetic-alcohol (v/v, acetic acid 25%, and ethanol 75%) solution for long-term storage. Stomatal density, area, and orientation were studied by scratching the leaf surface. The basal portion of the leaf (1 cm long) was selected for stomatal studies. Measurements and photographs were taken with the help of a camera-equipped light microscope (*Nikon 104*, Japan).

Results

Photosynthetic parameters: The populations of all five grasses from both lesser saline DF and highly saline habitat LS showed very little differences in P_N under the control treatment, *i.e.*, 0 mM NaCl (Fig. 1). In general, *S. ioclados* showed higher P_N than the other grasses studied, but *A. lagopoides* was the solitary case in which the LS population showed an increase in net CO_2 assimilation rate under high salinity.

Transpiration rate was severely affected in all grasses due to salt stress (Fig. 1). *C. jwarancusa* had significantly lower E at 0 mM NaCl than that in the other grasses and this characteristic was markedly less affected in this species by high salinity level than that recorded in the other grasses.

g_s decreased significantly under high salinity level in all five grasses, but the populations of all five grasses from DF were relatively more affected than those from the highly saline LS (Fig. 1). The populations of *C. jwarancusa* and *L. scindicus,* in general, showed much reduced g_s at both salt regimes, *i.e.*, 0 and 300 mM NaCl, as compared to the populations of other selected grasses.

Substomatal CO_2 concentration was significantly affected due to high salinity in all the grass populations (Fig. 1). However, the LS populations of all grasses showed significantly higher substomatal CO_2 concentration at 300 mM NaCl than that recorded in the populations from DF.

WUE (calculated as P_N/E) was significantly higher in all populations of five grasses under high-salinity stress (Fig. 1). Both populations of each of the three grasses, *S. ioclados, A. lagopoides* and *C. jwarancusa*, showed greatly increased water-use efficiency under 300 mM NaCl.

Stomatal density and size: The DF populations of all grasses showed a significant decrease in adaxial stomatal density under high salinity (300 mM NaCl) of the external medium (Fig. 2). The LS populations of *S. ioclados* had increased stomatal density at high salinity level. Both populations of *C. jwarancusa* and the LS population of *O. compressa* had no stomata on adaxial leaf surface. Stomatal density on abaxial leaf surface, in contrast, showed quite variable response to high salt level. It decreased significantly under high salinity in both populations of *L. scindicus.* The LS populations of *C. jwarancusa, O. compressa,* and *S. ioclados* showed increased density at high salinity.

Stomatal area on adaxial leaf surface generally decreased under high salt level. The population of *A. lagopoides* from LS generally had a very much reduced stomatal area as compared to all other grasses as well as its counterpart from DF (Fig. 2). Stomatal area on abaxial leaf surface was significantly reduced due to salt stress in the LS populations of all grasses. The LS population of *C. jwarancusa* was the worst affected as a decrease of more than half was recorded in its stomatal area. The DF populations were generally less affected due to salinity stress as compared to LS populations, where this parameter was increased in *O. compressa* and *S. ioclados* at high salinity level.

Shape and orientation of epidermal cells and stomata on abaxial leaf surface: *A. lagopoides* population from DF had relatively large elliptic stomata. At higher salt level, stomatal size was greatly reduced and the stomata were more or less round in shape and relatively closer to one another (Fig. 3). In the LS population (Fig. 3), the abaxial leaf surface was densely hairy by the presence of both trichomes and vesicular hairs (micro-hairs) at 0 mM NaCl. At 300 mM NaCl, the margins of epidermal cells were very wavy and they covered most of the stomatal aperture.

In *C. jwarancusa*, the population from DF was little affected by salinity with respect to the size and shape of stomata, but at high-salinity level, the stomata were much more apart from one another than that recorded at 0 mM level. The stomatal shape in the populations from LS was more or less round, but under 300-mM salt level, the size of epidermal cells was greatly reduced and stomata were relatively close one to another.

Stomatal size in both populations of *L. scindicus* was little affected by the addition of salt to the growth

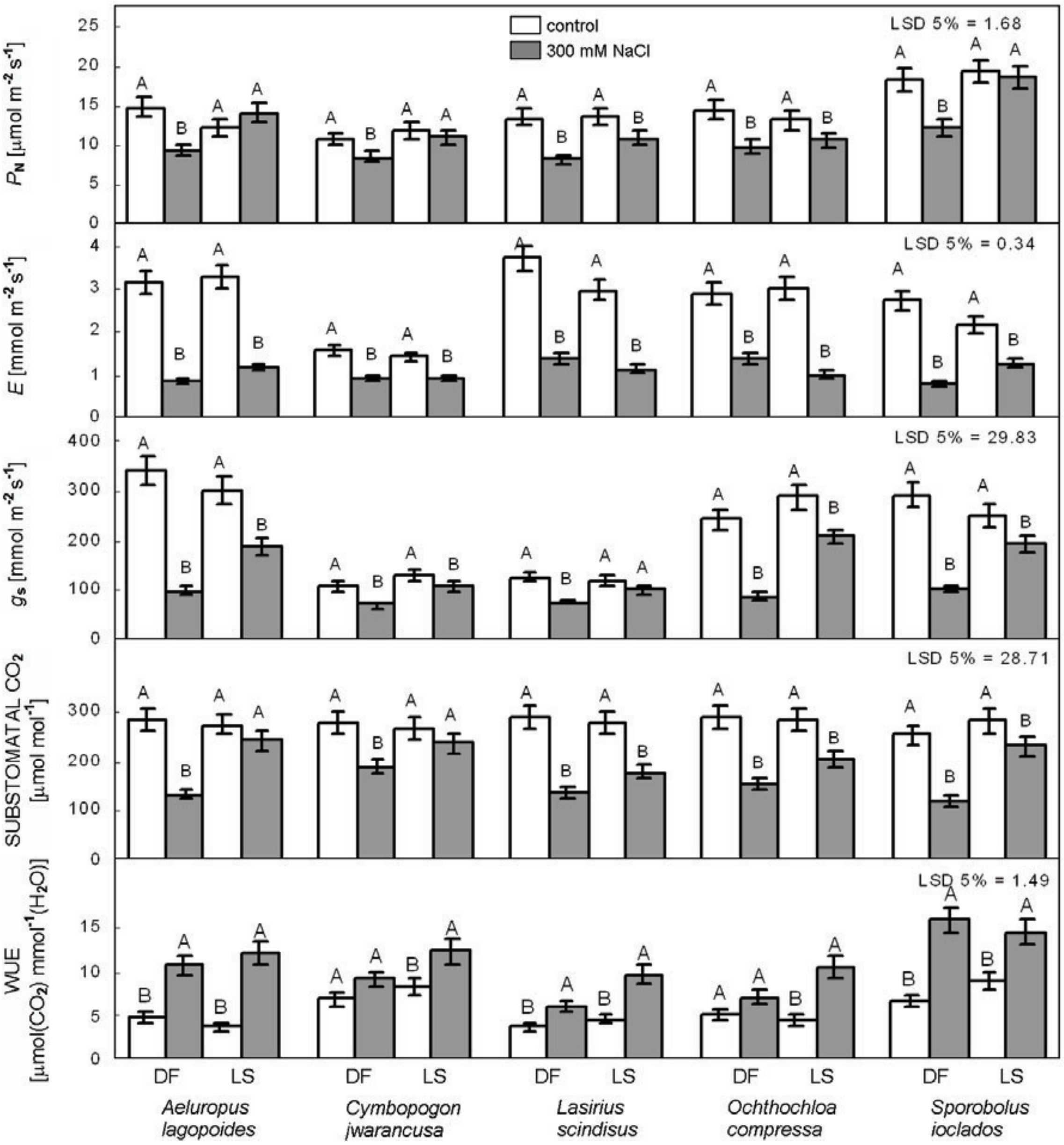

Fig. 1. Gas-exchange characteristics in five grass species from the Cholistan desert under two salt regimes in hydroponics (means ± SE; n = 10; DF – Derawar Fort; LS – Ladam Sir). P_N – net CO$_2$ assimilation rate; E – transpiration rate; g_s – stomatal conductance; WUE – water-use efficiency.

medium. The LS population showed wavy margins at the epidermal cells at 0 and 300 mM NaCl. However, the waviness of epidermal cells was more prominent at high salt level.

In *O. compressa,* both populations showed increased waviness in their epidermal cells under salt stress. However, this waviness in the LS population was very much distinct and the epidermal margins appeared to have covered the stomata partially (Figs. 4, 5). In addition, stomatal size was greatly reduced by salt stress and the shape of stomata was more or less round.

Stomatal orientation was minimally affected by salt stress in both populations of *S. ioclados.* The LS population showed greatly increased waviness in epidermal cells under 300 mM NaCl of the growth medium. However, the stomatal shape in the populations from both DF and LS were round in shape under salt stress than that recorded at 0 mM NaCl.

Correlation between stomatal (form) and gas-exchange (function) characteristics: Salinity-induced changes in transpiration relate to stomatal characteristics. However, different types of grasses showed different associations of the two attributes (Fig. 6). Under normal environmental conditions (nonsaline) both stomatal density and stomatal area positively and significantly

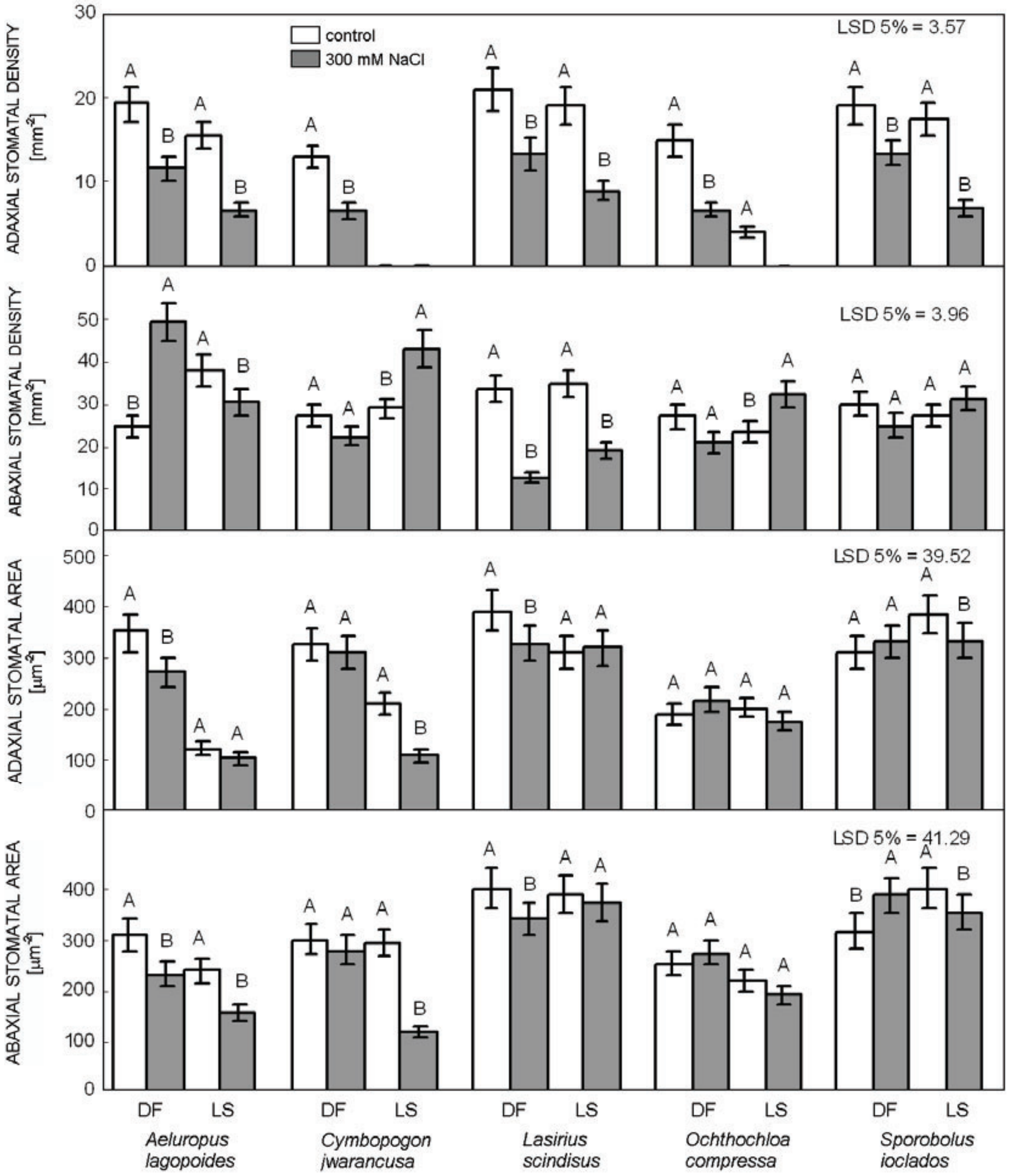

Fig. 2. Stomatal density and area in five grass species from the Cholistan desert under two salt regimes in hydroponics (means ± SE; *n* = 10; DF – Derawar Fort; LS – Ladam Sir).

correlated with P_N (Table 1). However, the density and area on abaxial leaf surface was not correlated with any of the gas-exchange characteristics. *E*, on the other hand, negatively and significantly correlated to WUE. Under saline conditions, stomatal density on adaxial leaf surface correlated significantly and positively with P_N, but stomatal density on the abaxial leaf surface showed a strong negative correlation with substomatal CO_2. Stomatal density on abaxial leaf surface correlated significantly and negatively with *E*, but significantly and positively with WUE. Stomatal area on both leaf surfaces correlated negatively and significantly with substomatal CO_2. However, most of the gas-exchange parameters had significant and positive correlations among themselves under saline environments, except for *E*, which was negatively correlated with WUE.

Discussion

Naturally adapted salt-tolerant plants can be used effectively for investigating adaptative mechanisms to counteract high levels of salts. Since genetically based variation in natural populations of plants has not been

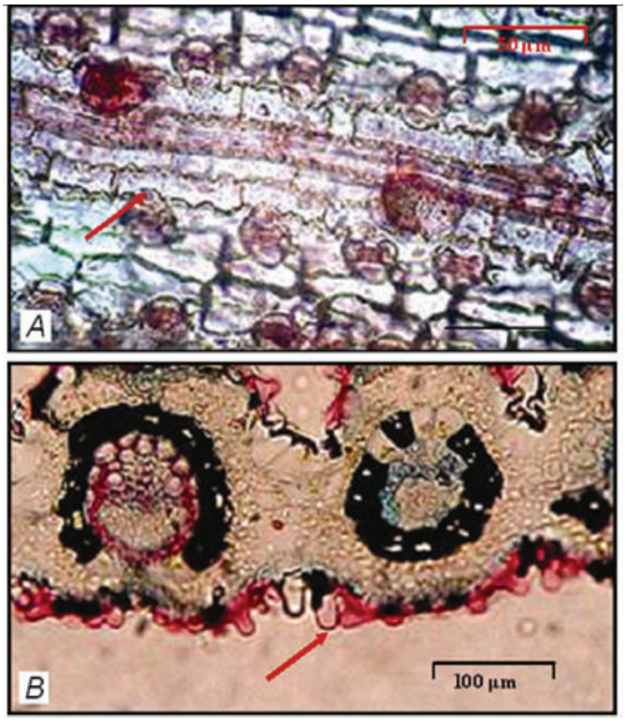

Fig. 3. Leaf transverse section and stomatal orientation in *Aeluropus lagopoides* from the Cholistan desert under two salt regimes in hydroponics.

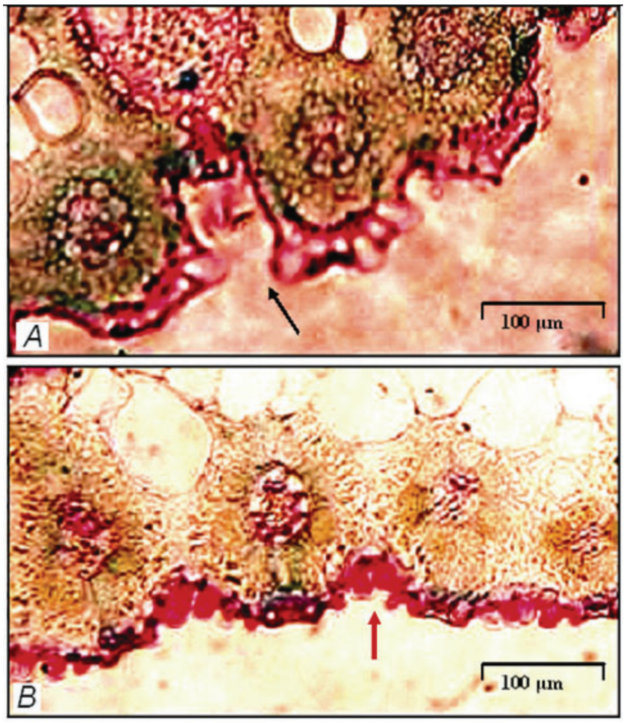

Fig. 4. Leaf transverse section and stomatal orientation in *A*: *Cymbopogon jwarencusa* (hairiness considerably increased and stomata in distinct grooves protected by trichomes) and *B*: *Ochthochloa compressa* from the Cholistan desert under two salt regimes in hydroponics (distinct grooves on adaxial surface covered with dense layer of microhairs).

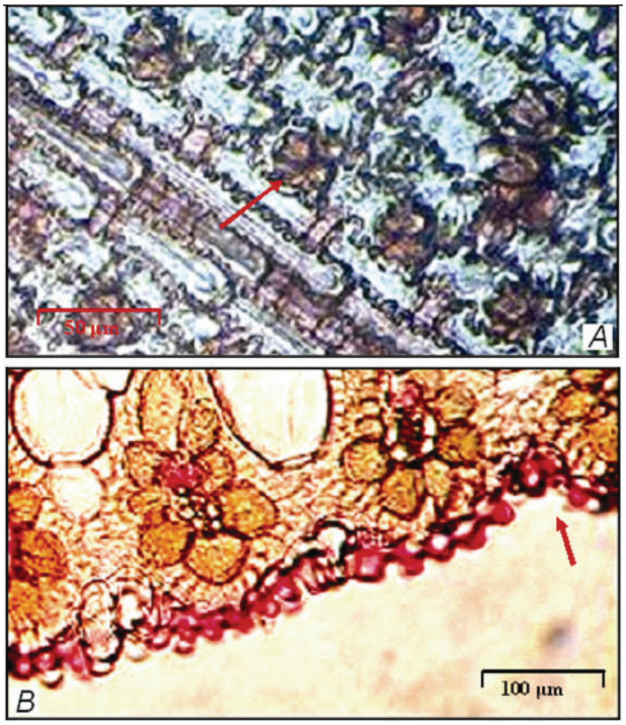

Fig. 5. Leaf transverse section and stomatal orientation in *Ochthochloa compressa* from the Cholistan desert under two salt regimes in hydroponics. *A*: Stomata partially covered by epidermal invaginations. *B*: Stomata in distinct grooves, covered with dense layer of microhairs.

much investigated, it was considered valuable to examine the anatomical adaptations in the five grass species in relation to stomatal functioning because of the potential value of such genetic resources (Munns *et al*. 2002, Ashraf 2004, Flowers and Colmer 2008, in improving the salinity tolerance of crop plants (Munns and Tester 2008).

Photosynthesis and transpiration are two important processes controlled by stomata. Plant leaves have the ability to adjust the density and patterning of stomata in response to changing environment (Zarinkamar 2006). Under water-limiting conditions, stomatal size may be more useful to improve WUE than the stomatal density (Yang *et al.* 2004). Reduced g_s and *E* help plants to counteract high salinities (Flanagan and Jefferies 1989). Any sort of disturbances in stomatal functioning may prove to be vital for the survival of plants under stressful environments (Robinson *et al.* 1998).

The survival of *A. lagopoides* from lesser saline DF under adverse conditions mainly relied on high stomatal density and markedly reduced stomatal area, which is typical of the xeric plant adaptation (Martinez *et al.* 2007). The major strategy used by this population seems to be the avoidance of transpirational water loss through stomata under adverse climatic conditions. Such a decrease in stomatal area may contribute to regulation of stomatal conductance and transpirational rate, as found in *Populus tremula* (Aasamaa *et al.* 2001) and *Olea*

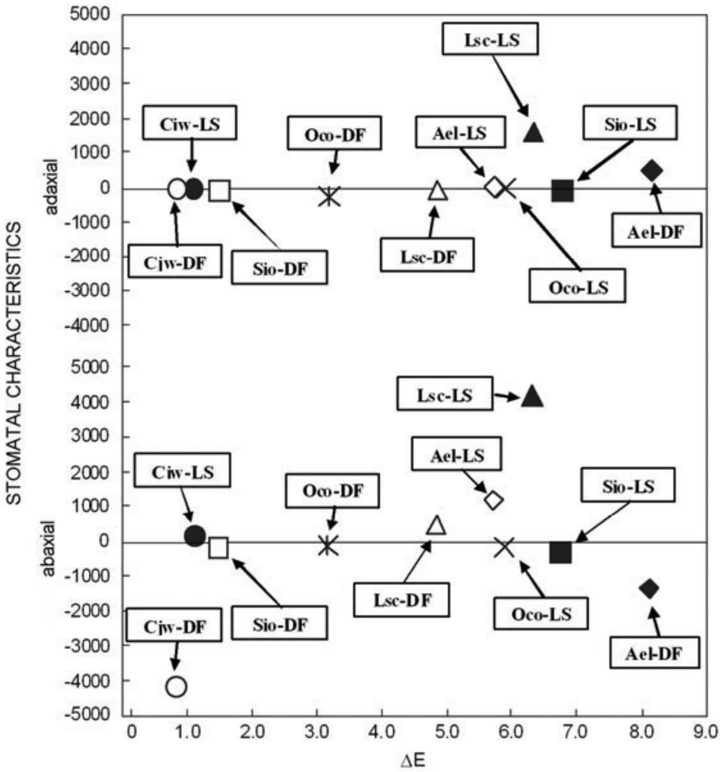

Fig. 6. Transpiration [ΔE (E 0 mM NaCl/E 300 mM NaCl) versus stomatal characteristics {ΔSD × ΔSA × [(SD 0 mM NaCl/SD 300 mM NaCl) × (SA 0 mM NaCl/SA 300 mM NaCl)]} of five grass species from different habitats in the Cholistan desert (Ael – *Aeluropus lagopoides;* Cjw – *Cymbopogon jwarancusa;* Lsc – *Lasiurus scindicus,* Oco – *Ochthochloa compressa;* Sio – *Sporobolus ioclados;* DF – Derawar Fort; LS – Ladam Sir; E – transpiration rate; SD – stomatal density; SA – stomatal area).

europaea (Bosabalidis and Kofidis 2002). In contrast, stomatal behaviour, *i.e.* density, structure and orientation in high saline LS population of *A. lagopoides* was not much affected due to salinity, which indicates that the successful adaptation of this population may be related to the adjustment of photosynthetic parameters and may be due to the effectiveness of stomatal closure rather than the stomatal density and area. However, stomatal orientation might play an important role, as the stomata were found to be covered partially by epidermal invaginations, which may prevent excessive water loss. Large stomata in this population can facilitate CO_2 diffusion into leaves (Parkhurst 1994), and this is mainly dependent on the radius of stomatal pore, which leads to an increased stomatal conductance (Maherali *et al.* 2002).

Photosynthetic parameters were relatively less affected in both populations of *C. jwarancusa* as compared to those in the other grasses, but WUE increased considerably in these two populations. Improved photosynthetic capacity has been considered as an adaptive component of salinity tolerance in plants (Locy *et al.* 1996). However, the LS population performed slightly better as compared to its counterpart from DF. Stomata were completely absent on the adaxial surface in both populations, and this may be the strong reason of decreased transpiration rate in *C. jwarancusa* as compared to that in the other grasses. The LS population showed increased stomatal density, but decreased stomatal area, and this might reflect stomatal regulation through more efficient closing and opening of stomata at relatively less change in turgor. Small guard cells may cause stomata to remain open under water-limiting conditions, which develop a balance in photosynthesis and transpirational water loss. This is an important adaptive response of plant species to dry conditions (Spence *et al.* 1986). Stomatal density was found to be closely linked to carbon fixation and water status in C_3 plants (Van de Water *et al.* 1994) and, therefore, the balance in stomatal density and area may determine the direct effect on various gas-exchange attributes and WUE, and hence is of great ecophysiological significance (Xu and Zhou 2008). Increased stomatal densities may trigger a regulatory mechanism to counteract several concurrently occurring stresses including drought and salinity (Zhang *et al*. 2004). In addition, stomata in the high-saline LS population were in distinct grooves, *i.e.*, sunken, and this may provide complementary support by protecting stomata from exposing directly to the external harsh environments. Sunken (or covered and encrypted) stomata is a characteristic feature under arid environments and responsible for reduced transpiration rate (Jordan *et al.* 2008).

In the high-saline LS population of *L. scindicus*, stomatal density decreased significantly, but area was not affected by salinity treatment at both leaf surfaces. A greater stomatal size under stress conditions can also aid CO_2 diffusion into the leaf (Parkhurst 1994) and this may promote an increase in leaf stomatal conductance (Maherali *et al.* 2002). Moreover, the presence of trichomes and sclerification above the vascular tissues may play a vital role in controlling water loss through transpiration (Abernethy *et al*. 1998, Hameed *et al.*

Table 1. Correlation between structural and functional characteristics of stomata under normal and saline conditions. * – positive and significant at $p<0.05$, ** – positive and significant at $p<0.01$, *** = positive and significant at $p<0.001$. P_N – net CO_2 assimilation rate; E – transpiration rate; g_s – stomatal conductance; WUE – water-use efficiency.

	Adaxial stomatal density	Abaxial stomatal density	Adaxial stomatal area	Abaxial stomatal area	P_N	E	g_s	Substomatal CO_2
Control (0 mM NaCl)								
Abaxial stomatal density	0.480^{*}							
Adaxial stomatal area	0.846^{***}	0.215						
Abaxial stomatal density	0.400	0.257	0.746^{***}					
P_N	0.510^{*}	–0.199	0.700^{**}	0.421				
E	0.695^{**}	0.340	0.520^{*}	0.044	0.123			
g_s	0.402	–0.264	0.182	–0.434	0.474^{*}	0.386		
Substomatal CO_2	0.034	–0.117	0.051	0.021	–0.277	0.330	–0.142	
WUE	–0.363	–0.358	–0.103	0.239	0.372	-0.859^{***}	–0.175	–0.412
Saline (300 mM NaCl)								
Abaxial stomatal density	–0.021							
Adaxial stomatal area	0.791^{***}	–0.273						
Abaxial stomatal density	0.395	-0.622^{**}	0.771^{***}					
P_N	0.516^{*}	0.179	0.241	0.104				
E	0.273	-0.545^{*}	0.310	0.200	0.172			
g_s	0.174	0.288	–0.186	–0.307	0.704^{*}	0.123		
Substomatal CO_2	–0.224	0.279	-0.572^{*}	-0.575^{*}	0.542^{*}	0.101	0.640^{**}	
WUE	0.311	0.456^{*}	0.081	0.023	0.715^{***}	-0.536^{*}	0.454^{*}	0.276

2009), and hence, more efficient water use. Presence of trichomes may enhance leaf surface roughness and ultimately the hydrophobic nature of surfaces (Brewer and Nuñez 2007). As a nonsalt secreting species, it depended mainly on transpirational control, *i.e.* by decreasing density or size of stomata (Shi and Cai 2008).

Stomata on the adaxial surface of the high-saline LS population of *O. compressa* were completely absent, which is an important xeric adaptation (Abernethy *et al.* 1998) and might be a major factor reducing the transpiration rate. However, on abaxial surface the stomata were intensively covered by micro-hairs (basically salt secretary in function) particularly at 300 mM NaCl. This might be the strong reason for its low transpiration rate under adverse water-limiting environments. Stomata are a sensory system to control the amount of salt delivered to the leaf by the transpirational stream (Sibole *et al.* 2003) and this may define the complete mechanism of salt tolerance in combination of stomatal regulation and transpirational control with the help of micro-hairs in excretory grasses such as *O. compressa.*

In the high-saline LS population of *S. ioclados*, stomatal density seems to be not involved in controlling the transpirational water loss from leaf surface, but high stomatal density under salt stress may be responsible for the improved photosynthetic activity accompanied with efficient distribution of CO_2 (Degl'Innocenti *et al.* 2009). Furthermore, greatly increased sclerification and/or thick cuticle deposition all over the leaf surface may have been the major factor in controlling transpiration in the LS population. This might be a crucial phenomenon for controlling g_s for gas-exchange and other related parameters in addition to stomatal size and cuticle deposition on leaves (Boyer *et al.* 1997, Turhan and Eris 2007).

Smaller stomatal area is generally linked to greater control over the stomatal conductance of the plants, thus resulting in higher control of transpiration and ultimately higher WUE in plants (Franks and Farquhar 2007). High-salinity tolerance in *A. lagopoides* seems to be dependent on controlled transpiration and high WUE, which were regulated by fewer and smaller stomata on both surfaces, and particularly the encryption of stomatal pores by micro-hairs. *C. jwarancusa* tolerated the adverse climates by having stomata only on the abaxial surface, as well as the stomatal encryption in specific grooves. *L. scindicus* had fewer, but larger stomata, an increased surface roughness due to the presence of hairs/trichomes, and mainly tolerated the environmental adversaries by preventing excessive transpirational loss. *O. compressa* had no stomata on the adaxial surface but with stomatal pores completely encrypted by the micro-hairs on the abaxial surface, which may be of great ecological significance for maintaining high WUE under saline conditions. In *S. ioclados*, stomatal density increased, but stomatal area decreased, and this is critical for stomatal regulation under salt-prone environments. The present findings of high stomatal regulation, which depend on stomatal density, area, and degree of encryption under salinity, are of great ecophysiological significance, because the grasses used in the present study grow in the region encountering multiple stresses, such as high salinity, aridity, and high temperature *etc.* Specific adaptations in relation to structural and physiological modifications of stomatal apertures are of great importance for our understanding of the mechanisms of plant's acclimatization to long-term environmental changes.

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