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RELATIONSHIP OF PHOTOSYNTHETIC CAPACITY AND PROLINE ACCUMULATION WITH THE GROWTH OF DIFFERENTLY ADAPTED POPULATIONS OF TWO POTENTIAL GRASSES (*CYNODON DACTYLON* (L.) PERS. AND *CENCHRUS CILIARIS* L.) TO DROUGHT STRESS

NUDRAT AISHA AKRAM, MUHAMMAD SHAHBAZ* AND MUHAMMAD ASHRAF

Department of Botany,
University of Agriculture, Faisalabad 38040, Pakistan

Abstract

Response of two populations of each of two grass species viz., *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. to drought stress was assessed in a pot experiment. One population of each grass species was collected from the Salt Range which is known for high salt and drought stress. The other population was collected from a frequently irrigated soil in Faisalabad. Eighty eight days old plants of each population were subjected for 30 d to different water stress levels [control (maintained at field capacity), 75 % or 50 % of field capacity). Although growth of both grass species from two different habitats decreased with increase in water deficit conditions, the Salt Range populations of both grass species produced significantly higher shoot biomass than Faisalabad populations. A significant detrimental effect of water deficit conditions was observed on chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b* ratio, proline contents, photosynthetic rate, transpiration rate, stomatal conductance, sub-stomatal CO_2 concentration and also on C_i/C_a ratio, but not on water use efficiency. Although populations of both species from the Salt Range were more drought tolerant as compared to those from Faisalabad, it was not possible to discriminate among the populations on the basis of biochemical and physiological parameters examined in this study.

Introduction

The scarcity of water and low rainfall are the major factors for converting vast areas into deserts throughout the world. Likewise, in Pakistan large areas including "Salt Range" are uncultivable due to low rainfall and brackish ground water. The Salt Range lies between 71° 00'-74° 00 east and 32° 10'- 33° 15' north with an area of about 10529 km^2 (Afzal *et al*., 1999; Ahmad *et al*., 2002). Vegetation in Salt Range, is very poor and over 40 grass species have been reported. Most of them are highly palatable and nutritious (Chaudhry *et al*., 2001). However, two grass species i.e., *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. are widely occurring species in the Salt Range.

It is now well evident that water stress inhibits plant growth and development. However, water stress is variable in intensity and duration, which is more important to determine the effects produced by water stress. To cope with these water stress conditions, plants use a number of strategies such as drought escape, avoidance and tolerance (Levitt, 1972; Turner, 1986). Naturally occurring drought prone areas impose a considerable selection pressure on the plant species inhabiting there and selectively allow some of plant populations with respect to their ability to grow and produce offspring. Over many generations, significant evolutionary changes may arise. In general, genotypes native from climates are able to adapt to water stress conditions (Pereira & Chaves, 1993, 1995). Since populations of two grass species i.e., *Cynodon dactylon* (L.)

Pers. and *Cenchrus ciliaris* L. have been growing in the Salt Range since very long, it is expected that they must have evolved adaptation to combat severe drought conditions. The primary objective of carrying out the present study was to appraise the extent of adaptability of the two grass species to drought stress and whether their higher degree of adaptability in terms of high growth rate under drought stress is attributable to high photosynthetic rate and other gas exchange attributes. Thus, in the present study parallels were drawn between the growth performance and photosynthetic efficiency of the populations of two grass species from the Salt Range.

Materials and Methods

To explore the mechanism of adaptation, particularly against drought stress, two potential drought tolerant grasses, one prostrate runner species, *Cynodon dactylon* (Bermuda grass locally called Khabbal grass or Dhoob grass) was collected from Ucchali lake (saline area) and the other tussock forming, *Cenchrus ciliaris* (Buffel grass, locally called Anjan ghas) was collected from Kallar Kahar (drought-hit area) of the Salt Range. Ecotypes of both species were also collected from the Faisalabad region which acted as checks.

A pot experiment was conducted in the Botanic Garden of the University of Agriculture Faisalabad, during April to August 2005. The average day and night temperatures were 39.2 ± 4°C and 23.5 ± 5°C, respectively. The relative humidity ranged from 31.6 to 65.8 %, and day length from 11-12 h. Small ramets of uniform size of these grasses from two different habitats (Salt Range and Faisalabad) were transplanted in plastic pots (20 cm diameter and 24 cm depth) containing 8 kg dry sandy loam soil. The saturation percentage of the soil used was 32 and pH, 8.65. The plants were allowed to establish for 88 days before the start of water deficit conditions. Three water deficit treatments were: Field capacity (control), 75% or 50% of field capacity. The plants were clipped so as to maintain uniform plant size before the start of drought stress. Plants were harvested, 30 days after the start of drought stress. Plants were uprooted carefully and washed with distilled water. Plant samples were dried in an oven at 65°C to constant dry weight. However, before harvesting data of the following attributes were recorded:

Chlorophyll contents: The chlorophyll '*a*' and '*b*' contents in fresh leaves were determined according to the method of Arnon (1949). Fresh leaves (0.2 g) were ground in 80% acetone and centrifuged at 10,000 x *g* for 5 minutes. Absorbance of the supernatant was read at 645, 663 and 480 nm using a spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Gas exchange parameters: Measurements of net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i) were made on a fully expanded youngest leaf of each plant using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). These measurements were made from 10:15 am to 12:45 pm with the following specifications/adjustments: leaf surface area 11.35 cm^2, ambient CO_2 concentration (C_{ref}) 352 µmol mol^{-1}, temperature of leaf chamber varied from 31.5 to 37.8°C, leaf chamber gas flow rate (V) 251 µmol s^{-1}, Molar flow of air per unit leaf area (Us) 221.06 mol m^{-2} s^{-1}, ambient pressure 99.2 kPa, water vapor pressure into the chamber ranged from 0.0006 to 0.00089 MPa, PAR (Q_{leaf}) at the leaf surface was maximum up to 1048 µmol m^{-2} s^{-1}.

Proline: Proline in the leaves was measured according to the method of Bates *et al*., (1973) after extraction at room temperature with 3% 5-sulfosalicylic acid solution. The proline concentration was determined from a standard curve and calculated on fresh weight basis.

Statistical analysis of data: Analysis of variance of the data for each attribute was carried out following Steel & Torrie (1980). The mean values were compared with the least significance difference test (LSD) following Snedecor & Cochran (1980).

Results

Water deficit conditions imposed for a period of 30 d to 88 d-old plants of two populations of each of two grass species i.e. *Cynodon dactylon* and *Cenchrus ciliaris* had a significant inhibitory effect on shoot fresh and dry biomass. As difference among the populations of each species was not possible to discern on the basis of mean shoot fresh and dry biomass, so it was not legitimate to compare the populations of each species collected from different habitats. However, while comparing the populations on percent of control basis, it is evident that Each population from the Salt Range was significantly higher than its respective one from Faisalabad in percent shoot fresh and dry mass (Table 1; Fig. 1).

Chlorophyll *a* and *b* pigments of both populations of each species were higher under water deficit conditions as compared to those under well watered conditions and this increase was maximum at 75% field capacity except in the Salt Range population of *C. ciliaris* in which maximum chlorophyll *a* and *b* contents were observed at 50% field capacity. Chlorophyll *a/b* ratios increased in all grass populations with an increase in the levels of drought stress.

Shoot proline concentration of all populations was high under water deficit conditions than that under well watered conditions. The two populations of *C. dactylon* did not differ significantly for leaf proline contents, but in contrast, the Salt Range population of *C. ciliaris* had accumulated significantly lower levels of proline both under well watered and water deficit conditions (Table 1; Fig. 2).

Net CO_2 assimilation rate of the populations of both species decreased significantly with an increase in water deficit. However, *C. dactylon* population from the Salt Range showed much lower net CO_2 assimilation rate than that from Faisalabad under water deficit conditions. In contrast, populations of *C. ciliaris* did not differ significantly in rate of photosynthesis (Table 1; Fig. 3).

The two contrasting populations of *C. dactylon* did not differ significantly in transpiration rate, but in contrast the populations of *C. ciliaris* responded differently to water deficit conditions. The populations of *C. ciliaris* from the Salt Range had a maximum transpiration at 75% field capacity.

Drought stress conditions significantly decreased stomatal conductance of all grass populations. The difference between the populations within each grass species with respect to g_s was possible to discern only under well watered conditions, but not so under water deficit conditions. Although water deficit conditions significantly suppressed C_i and C_i/C_a ratio, variation in these two attributes was not prominent in all the populations of both grass species. Water use efficiency (*A/E*) of *C. dactylon* from Faisalabad was significantly higher than that from the Salt Range under water deficit conditions, but in contrast, the two populations of *C. ciliaris* did not differ significantly in this gas exchange attribute (Table 1; Fig. 3).

Table 1. Mean squares from analyses of variance of data for growth, physiological and biochemical parameters of *Cynodon dactylon* and *Cenchrus ciliaris* when 88 day-old plants of different habitats were subjected for 30 days to various water deficit conditions.

Source of variation	**Degrees of freedom**	**Shoot fresh weight**	**Shoot dry weight**	**Root dry weight**	**Root/shoot ratio**
Drought (D)	2	1400.012**	5088.61***	105.92***	0.239*
Populations (Pop)	1	701.811ns	2376.98**	1.477ns	0.018ns
Species (Sp)	1	1756.194**	2119.75**	54.69**	0.658***
D x Pop	2	572.361ns	1317.27**	6.21ns	0.056ns
D x Sp	2	533.771ns	25.98ns	0.149ns	0.005ns
Pop x Sp	1	108.36ns	655.04ns	70.96**	0.378*
D x Pop x Sp	2	168.636ns	470.08ns	25.48*	0.005ns
Error	36	231.46	234.773	5.715	0.069
		Proline	**Chlorophyll *a***	**Chlorophyll *b***	**Chlorophyll *a/b* ratio**
Drought (D)	2	755.86***	2.244***	0.954***	4.402***
Populations (Pop)	1	66.65***	6.302ns	0.181ns	0.665ns
Species (Sp)	1	134.2***	0.049ns	0.024ns	0.088ns
D x Pop	2	24.50**	0.087ns	0.317*	0.715ns
D x Sp	2	74.41***	0.011ns	0.003ns	0.108ns
Pop x Sp	1	55.25**	0.066ns	3.852ns	0.302ns
D x Pop x Sp	2	18.94*	0.276**	0.131ns	0.133ns
Error	36	4.563	0.043	0.085	0.369
		A	E	g_s	C_i
Drought (D)	2	559.89***	45.07***	287014.7***	17717.9***
Populations (Pop)	1	13.21ns	0.132ns	689.6***	193.48ns
Species (Sp)	1	224.8**	21.63***	2033.1ns	108.69ns
D x Pop	2	26.52ns	1.168ns	1954.2ns	844.63ns
D x Sp	2	41.167ns	14.75***	2307.9ns	123.13ns
Pop x Sp	1	0.487ns	0.255ns	61099.2***	12.98ns
D x Pop x Sp	2	12.131ns	0.613ns	24063.4***	5579.2**
Error	36	29.52	0.773	2595.2	665.24
		C_i/C_a	A/E		
Drought (D)	2	0.142***	8.351ns		
Populations (Pop)	1	0.002ns	7.975ns		
Species (Sp)	1	8.772ns	3.302ns		
D x Pop	2	0.006ns	16.25**		
D x Sp	2	9.94ns	22.08**		
Pop x Sp	1	1.048ns	9.51ns		
D x Pop x Sp	2	0.045**	13.68*		
Error	36	0.005	2.877		

*, **, *** = Significant at 0.05, 0.01, 0.001 levels, respectively.
ns = Non-significant.
A = CO_2 assimilation rate, E = Transpiration rate, g_s = Stomatal conductance
C_i = Intercellular CO_2 conc. C_a = Ambient CO_2 conc. A/E = Water use efficiency

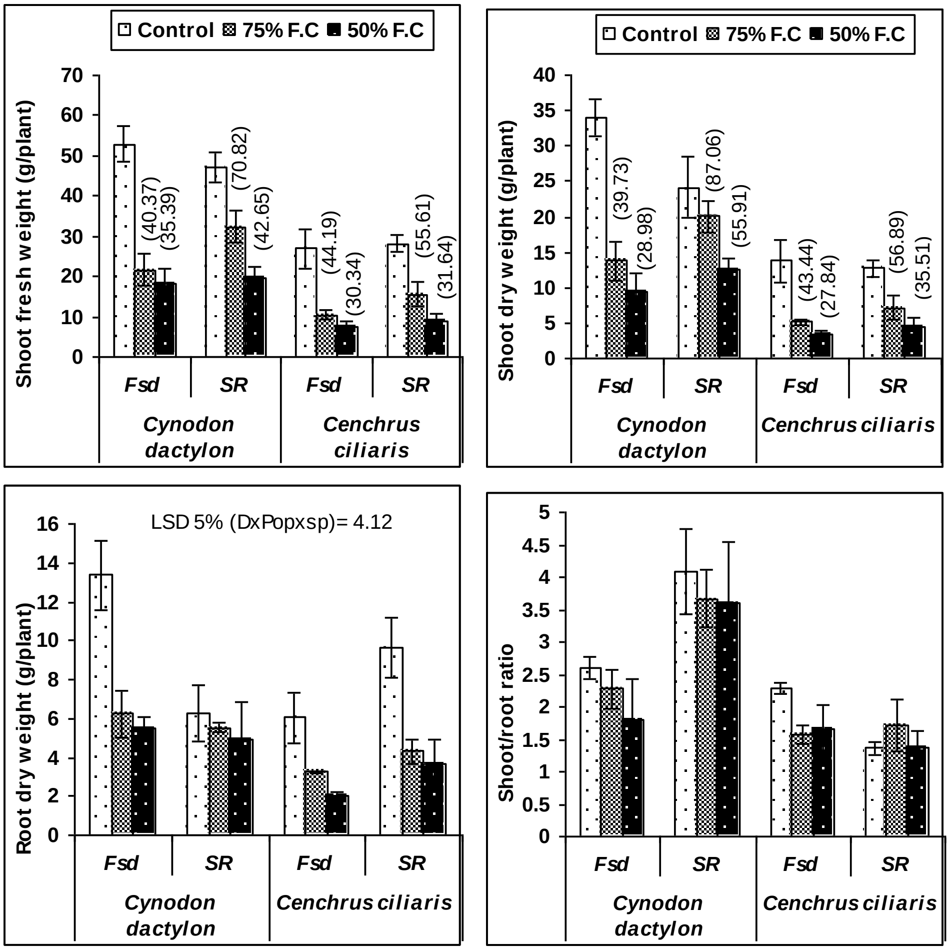

Fig. 1. Growth parameters of *Cynodon dactylon* and *Cenchrus ciliaris* when 88-day old plants from different habitats were subjected for 30 days to well-watered or water deficit conditions. (Figures in paretheses are percent of control), (Fsd = Faisalabad; SR = Salt Range).

Discussion

Increasing water stress conditions decreased the growth of both populations of each species. However, this decreasing effect was more at the lowest water regime. Moreover, this adverse effect of water stress was more on populations of both species from Faisalabad than those from the Salt Range. Thus, the Salt Range populations of both species were relatively more resistant to water stress. The relative higher tolerance of the Salt Range species to water stress is expected in view of the fact that Salt Range populations of both species are inhabitant of the area (Salt Range) which is characterized by acute shortage of water, while sensitivity of the Faisalabad populations is also expected as these populations were collected from the normal soils where water availability is sufficient. These results can be related to the findings of Ashraf *et al*., (1986) who found differences in salt tolerance of natural populations of four grass species collected from different salt hit areas. Symeonidis *et al*., (1985) also observed higher tolerance to heavy metal stress in *Agrostis capillaris*, which came from evolutionary changes through natural selection. These findings can also be explained in view of the arguments of Ashraf (1994) that natural selection pressure is different in different habitats that cause constitutive adaptations (morphological, physiological and biochemical adaptations) in some plant species but not in others, thereby resulting in inter-population variation for stress tolerance. Understanding the biochemical or physiological basis of adaptations may help in exploitation of drought prone areas.

Tissue tolerance to severe dehydration by accumulation of compatible solutes such as proline is one of the key adaptations for successful growth under acute water shortage. However, this adaptation is not common in most of higher plants including crops, but it evolves in species native to dry places (Ingram & Bartels, 1996). In the present study, proline contents along with photosynthetic pigments were increased with increase in water stress in both populations of each species (Figs. 2, 3), which indicates its protective effect on photosynthetic pigments from damage by reactive oxygen species (ROS) (Saradhi *et al*., 1995; Hamilton & Heckathorn, 2001; Ashraf & Foolad, 2007). This protective role of proline is more important than its role in osmotic adjustment (Hare *et al*., 1999). However, increase in leaf proline contents due to water stress was same in both populations of *Cynodon dactylon*. In contrast, drought susceptible *C. ciliaris* population accumulated more proline in their leaves as compared with drought tolerant population. These results clearly show that accumulation of proline in the leaves of both populations of each species was not positively correlated with their ability to withstand severe drought stress. Similar results have already been observed in sorghum (Blum & Ebercon, 1976), and barley (Hanson *et al*., 1977; 1979).

In the present study, photosynthetic rate (*A*) of both populations of each grass species decreased with decrease in stomatal conductance (g_s), sub-stomatal CO_2 (C_i) and transpiration rate (*E*) under water stress conditions, which suggests that reduction in photosynthesis might have been due to stomatal factors. It is now well evident that water deficit caused lower CO_2 (*A*) fixation due to stomatal closure even at moderate water stress (Athar & Ashraf, 2005), because stomatal closure decreases CO_2 availability in the mesophyll. Although moderate water stress reduces photosynthesis due to alterations in photophosphorylation (Tezara *et al*., 1999), it is not widely accepted that this is the most sensitive water-stress component of photosynthesis (Flexas *et al*., 2004). Furthermore, the extent of stomatal conductance can be used as an indicator to assess stomatal or non-stomatal limitations to photosynthesis under water deficit conditions (Chaves & Oliveira, 2004; Athar & Ashraf, 2005). It is now well established that when plant roots are exposed to water deficit conditions, they send chemical signals to their leaves resulting in a reduction in transpiration rate mediated by reduced stomatal conductance (Davies & Zhang, 1991). The decline in growth in many plant species subjected to stressful environment is often associated with a reduction in photosynthetic capacity. However in the present study there was no significant correlation among growth and photosynthetic rate.

In view of the findings in the present study, it can be concluded that the populations of both species from the Salt Range were more drought tolerant, the trait being developed through natural selection. However, a further detailed study is needed to elucidate the underlying physiological and biochemical phenomenon which are responsible for the high drought tolerance of the grass populations from the Salt Range.

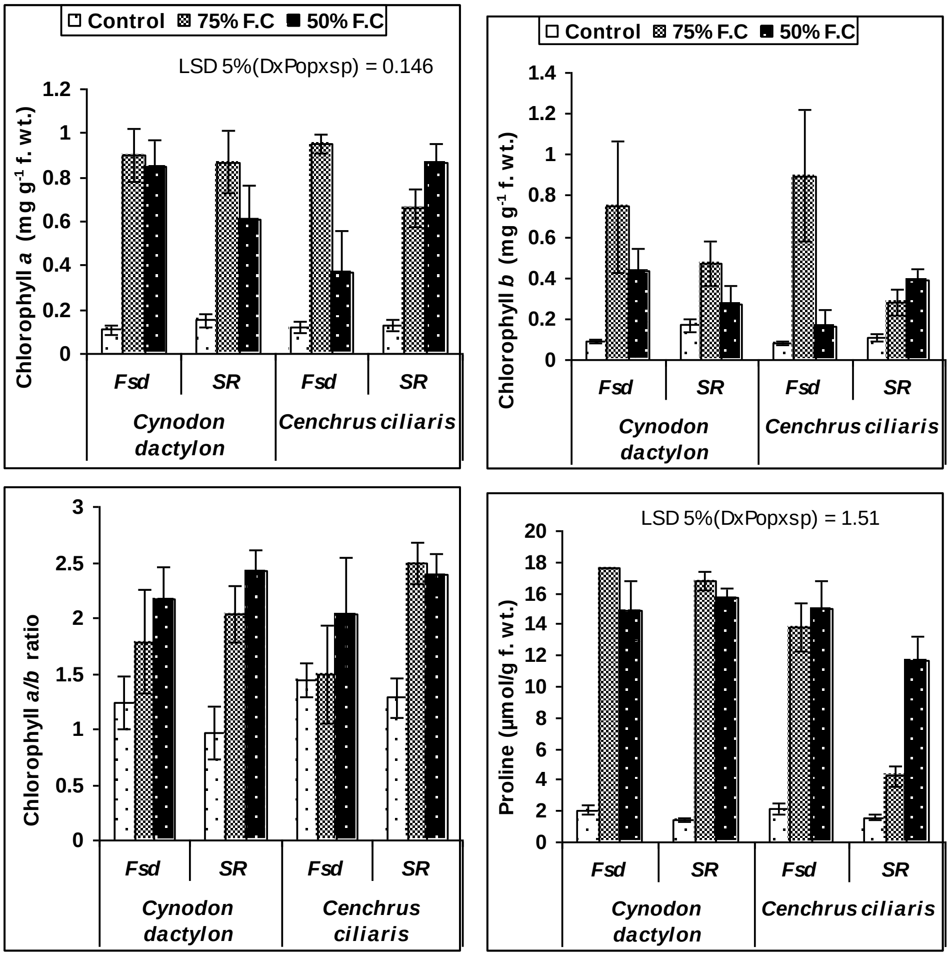

Fig. 2. Chlorophyll and proline contents of *Cynodon dactylon* and *Cenchrus ciliaris* when 88-day old plants from different habitats were subjected for 30 days to well-watered or water deficit conditions.

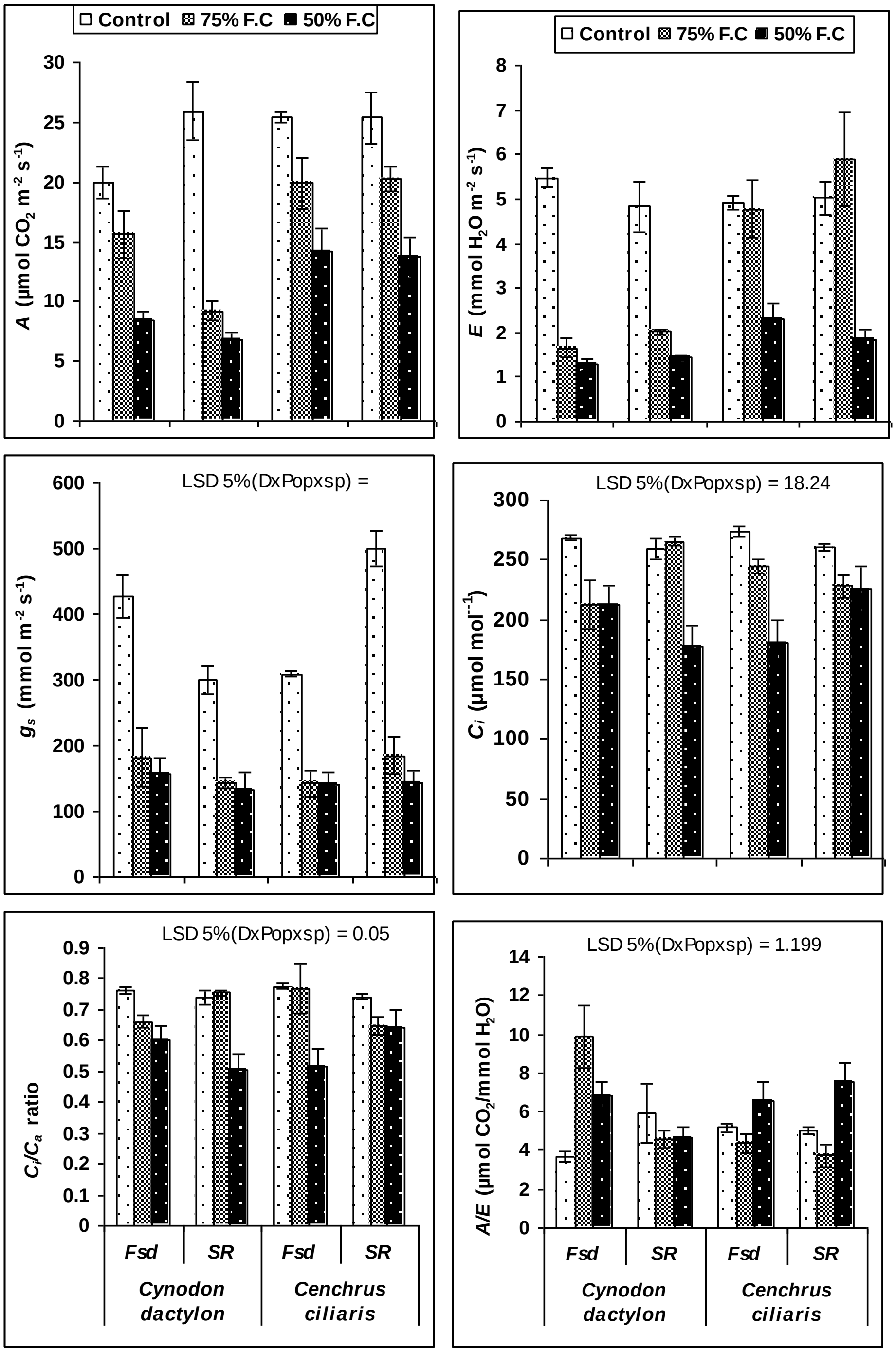

Fig. 3. Gas exchange characteristics of two populations each of *Cynodon dactylon* and *Cenchrus ciliaris* when 88-day old plants from different habitats were subjected for 30 days to well-watered or water deficit conditions.

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