

**MORPHO-PHYSIOLOGICAL RESPONSES OF TWO
DIFFERENTLY ADAPTED POPULATIONS OF *CYNODON
DACTYLON* (L.) PERS. AND *CENCHRUS CILIARIS* L.
TO SALT STRESS**

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Abstract

Two populations each of *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. collected from the Salt Range and Faisalabad were grown under control or saline (150 mM NaCl) conditions. Imposition of salt stress for 30 d decreased shoot and root biomass. However, salt induced reduction was less in the Salt Range populations of each grass species. Better adaptability of the Salt Range populations to salt stress was due to some heritable variation associated with their growth. Although photosynthetic rate along with stomatal conductance and sub-stomatal CO₂ were higher in the salt-stressed plants of the Salt Range population of *C. dactylon*, there was no difference between both populations of *C. ciliaris*. Similarly, leaf proline was also higher in the Salt Range population of *C. dactylon*, while there was no difference between both populations of *C. ciliaris* for proline content. It is suggested that the Salt Range population of *C. dactylon* was better adapted to salt stress due to high accumulation of proline, which might have protected photosynthetic tissue from salt-induced damage because higher photosynthetic rate was recorded in plants with high proline accumulation. However, high salt tolerance of the Salt Range population of *C. ciliaris* might be due to factors other than those examined in the present study.

Keywords: photosynthetic rate, stomatal conductance, chlorophyll, salt tolerance, grasses

Introduction

It is now well established that differences in salt tolerance exist not only among different species, but also within certain species (Ashraf, 1994; 2004; Munns *et al.*, 2006). However, plants native to saline environments have developed adaptations to salt stress by different mechanisms, including morpho-physiological and biochemical adaptations during the evolutionary history of adaptations in such habitats (Ashraf, 1994; 2006). However, plants have to grow on saline habitats for a long period to promote evolution of salt tolerant individuals and populations. However, if plant population is excluded from saline habitat during evolutionary period, it may not develop genetically based variation for salt tolerance. Furthermore, lack of appropriate combination of heritable variation for several adaptive features will lead to lack of salt tolerance. Thus, heritable variation for obligatory adaptational characters is necessary for evolution of salt tolerant individuals (Ashraf, 1994).

Numbers of grasses have been found growing in the Salt Range habitat. Of them *Cynodon dactylon* and *Cenchrus ciliaris* occur predominantly. The Salt Range is located between longitude 71°30'-73°30' east and between latitude 32°23'-33°00' north. It is of

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unique nature in the world with the oldest series referred to as Cambrian (Yeats, *et al.*, 1984; McKerrow *et al.*, 1992). It forms a very prominent feature as it is between the Thar desert in the west and the Potohar Plateau in the north east. The Salt Range has a steep face towards the south and slopes into the Potohar Plateau in the north. The Range extends from Jhelum River up to Kalabagh where it crosses the Indus river and enters the Bannu district and rises to an average height of 750-900 m. Sakesar Peak (1,527 m) is the highest point of the Salt Range (Ahmad *et al.*, 2002).

It is assumed that each population of the two grass species inhabitant to salt affected land for a long period has developed certain adaptive features necessary for salt tolerance. Selection of salt tolerant individuals on the basis of adaptive features will lead to successful re-vegetation of salt affected areas.

The principal objectives of this study were, to determine the morphological and physiological adaptabilities to salt stress of two populations of each of *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. collected from two different localities *i.e.* the Salt Range and Faisalabad. In addition, parallels were drawn between photosynthetic capacity and degree of salt tolerance of differently adapted populations of the two grass species to salinity stress, because photosynthetic capacity is known to be one of the potential physiological selection criteria of salt tolerance of a number of plant species (Ashraf, 2004).

Materials and Methods

A pot experiment was carried out in the Botanic Garden of the University of Agriculture Faisalabad, during April to August 2005, to assess the morpho-physiological responses of two differently adapted populations of each of two grass species, *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. to salt stress. 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 11-12h. *Cynodon dactylon* (dhoob grass) was collected from Uchali Lake and *C. ciliaris* (Buffel grass, locally called Anjan ghas) from Kallar Kahar, saline and drought-hit areas of the Salt Range.

Ecotypes of both species were also collected from the non-saline soils of Faisalabad region for comparison. All the populations were transplanted as small ramets each comprising 2-3 tillers of comparable size into plastic pots (20 cm diameter and 24 cm depth) containing 8 kg dry sandy loam soil. The plants were clipped so as to maintain uniform height of the plants before the start of salt treatment. Two salinity treatments

Table 1. Physical and chemical characteristics of the original soil from Uchali Lake, and Kallar Kahar, Salt Range.

	Characteristics	Uchali lake	Kallar Kahar
1	Saturation percentage	40.26	32.56
2	pH	7.62	8.12
3	Electrical conductivity of soil extract (ECe) dS/m	29.92	34.36
4	Ca ²⁺ mg kg ⁻¹	144.88	169.2
5	Mg ²⁺ mg kg ⁻¹	53	65
6	K ⁺ mg kg ⁻¹	135.46	117.30
7	Na ⁺ mg kg ⁻¹	3034.86	3314.62
8	Cl ⁻ mg kg ⁻¹	2021.32	2467.28

applied were control (2.35) and 15 dS m⁻¹ (\cong 150 mM NaCl). The NaCl concentration was increased step-wise in aliquot of 5 dS m⁻¹ every day until the appropriate treatment was attained. Plants were harvested 30 days after the imposition of salt treatment. Plants were uprooted carefully and washed with distilled water. After measuring fresh biomass, plant samples were dried in an oven at 65°C to constant dry weight.

Gas exchange characteristics: Before harvesting the plants, measurements of net CO₂ assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and sub-stomatal CO₂ concentration (C_i) were made on 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 to 12:45 hours with the following adjustments: leaf surface area 11.35 cm², ambient CO₂ concentration (C_{ref}) 352 μ mol mol⁻¹, temperature of leaf chamber varied from 31.5 to 37.8 °C, leaf chamber gas flow rate (v) 251 μ mol s⁻¹, molar flow of air per unit leaf area (U_s) 221.06 mol m⁻² s⁻¹, ambient pressure 99.2 kPa, water vapor pressure into chamber ranged from 0.0006 to 0.00089 MPa, and PAR (Q_{leaf}) at leaf surface was maximum upto 1048 μ mol m⁻² s⁻¹.

Proline: Proline was determined following the method of Bates *et al.* (1973). Fresh leaf material (0.5 g) was homogenized in 10 ml of 3% sulfosalicylic acid and the homogenate was filtered. The filtrate (2 ml) was treated with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, then with 4 ml of toluene. Absorbance of the colored solution was read at 520 nm using a spectrophotometer (Irmeco U2020) and total amount of free proline was calculated.

Statistical analyses of data: Analysis of variance technique was employed for carrying out statistical analysis of data collected. The mean values were compared with the least significance difference test (LSD) following Snedecor & Cochran (1980).

Results

Salt stress significantly reduced shoot fresh and dry biomass, and root dry weight of each population of both grass species. However, root/shoot dry weight ratio remained unaffected due to salt stress. Furthermore, salt-induced reduction in all these growth attributes was more in the Faisalabad populations as compared to that in the Salt Range populations of both grass species (Fig. 1). Leaf proline concentration of the two populations of both *C. dactylon* and *C. ciliaris* increased ($P \leq 0.001$) due to salt stress. Moreover, the Salt Range population of *C. dactylon* accumulated more proline than that of the Faisalabad population under saline conditions. However, there was no difference in between both populations of *C. ciliaris* in leaf proline (Fig. 1).

Net CO₂ assimilation rate (A) of each population of both species significantly decreased due to salt stress. Although the Salt Range population of *C. dactylon* was higher in A compared with the Faisalabad population under saline conditions, there was no difference in A between both populations of *C. ciliaris*. Similarly, salt-induced reduction in stomatal conductance (g_s), and sub-stomatal CO₂ concentration (C_i) was less in the Salt Range population of *C. dactylon*, while the response of the populations of *C. ciliaris* was similar in these gas exchange attributes. Transpiration rate was also higher in salt stressed plants of the Salt Range population of *C. dactylon* compared with that of the

Faisalabad population. However, both populations of *C. ciliaris* did not differ in transpiration rate under saline conditions. Likewise, there were no significant differences between both populations of each species for C_i/C_a ratio and water use efficiency. However, salt stress decreased C_i/C_a ratio while it increased the water use efficiency in both populations of each grass species (Fig. 2).

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 each of population of both species were subjected for 30 days to salt stress.

Source of variation	df	Shoot fresh weight	Shoot dry weight	Root dry weight	Root/shoot ratio
Salinity (S)	1	2814.4***	746.4***	127.5***	746.4***
Populations (Pop)	1	65.15ns	3.283ns	5.088ns	3.283ns
Species (sp)	1	2673.9***	1420.3***	32.88	1420.3***
S x Pop	1	209.7ns	186.5*	7.624ns	186.6*
S x sp	1	146.3ns	51.94ns	0.021ns	51.94ns
Pop x sp	1	1.256ns	10.02ns	77.44**	10.02ns
S x Pop x sp	1	76.137ns	85.97ns	39.87	85.96ns
Error	24	52.32	24.75	5.669	24.75
		Proline	Chlorophyll a		
Salinity (S)	1	1678.6***	6.224***		
Populations (Pop)	1	0.001ns	0.001ns		
Species (sp)	1	5.19**	2.940ns		
S x Pop	1	2.898*	0.001ns		
S x sp	1	3.760*	7.315ns		
Pop x sp	1	5.788**	1.575ns		
S x Pop x sp	1	5.586**	0.001ns		
Error	24	0.608	0.002		
		A	E	g_s	C_i
Salinity (S)	1	242.1***	73.93***	58118.3***	102487.8***
Populations (Pop)	1	0.183ns	0.004ns	11622.7ns	1128.4ns
Species (sp)	1	216.1***	0.312ns	7624.2ns	809.7ns
S x Pop	1	62.83*	0.714ns	39484.7**	8.030ns
S x sp	1	60.12*	1.155ns	41051.5**	1375.1ns
Pop x sp	1	0.066ns	0.122ns	67688.1***	10.61ns
S x Pop x sp	1	67.62*	0.505ns	36400.1**	1.606ns
Error	24	12.36	0.514	3147.4	657.0
		C_i/C_a	A/E		
Salinity (S)	1	0.827***	189.0***		
Populations (Pop)	1	0.009ns	2.801ns		
Species (sp)	1	0.006ns	10.11ns		
S x Pop	1	6.48ns	21.68ns		
S x sp	1	0.011ns	5.05ns		
Pop x sp	1	8.566ns	7.27ns		
S x Pop x sp	1	1.296ns	36.91ns		
Error	24	0.003	5.444		

* **, *** = 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

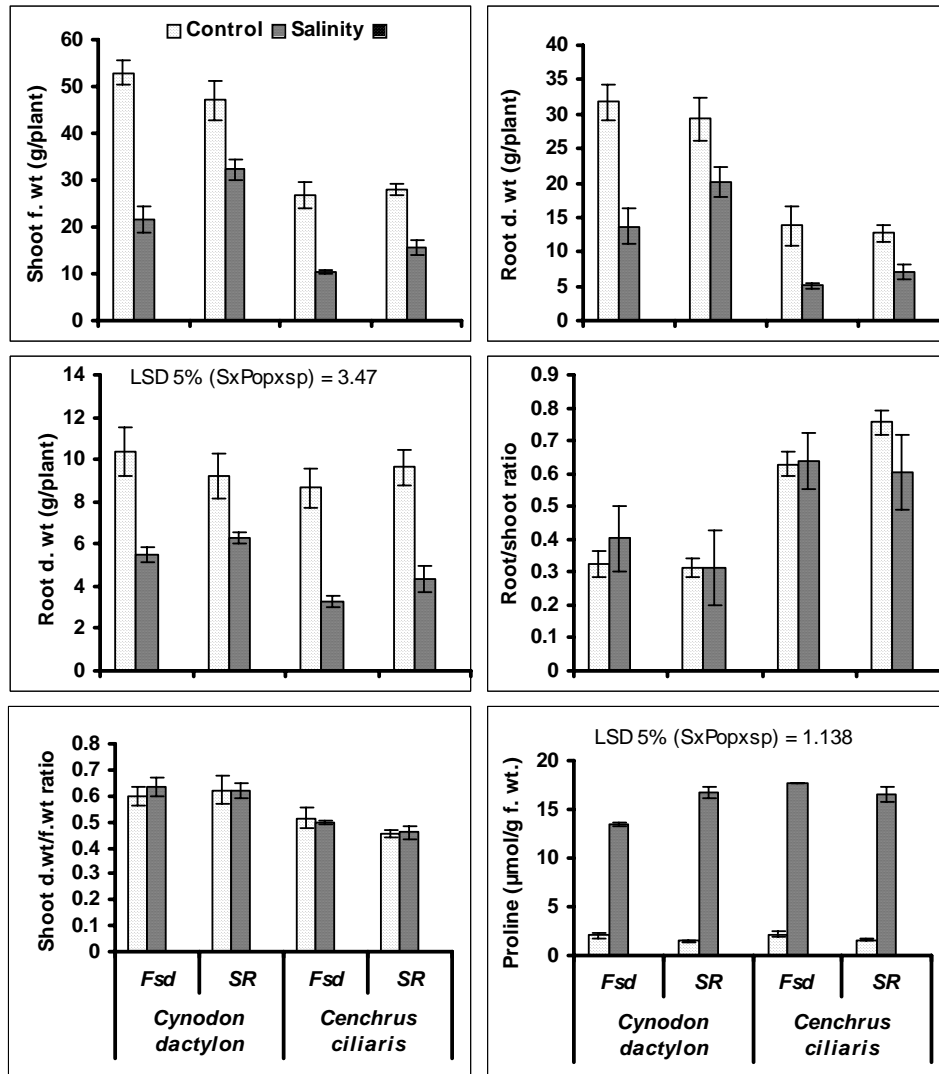


Fig. 10. Growth attributes, chlorophyll a/b ratio and proline content of *Cynodon dactylon* and *Cenchrus ciliaris* when 88-day old plants of different habitats were subjected to salt stress for 30 days.

Discussion

From the results of the present study, salt stress caused a considerable reduction in growth of both populations of each grass species. However, salt stress-induced growth inhibition was less in the Salt Range populations (collected from salt affected area) of each grass species. It is interesting to note that both roots and shoots of both populations of each grass species were equally affected due to salt stress as is evident from the data for root/shoot dry weight ratio (Fig. 1). These results do not conform to those of Marcum

(1999) who reported that root growth of salt tolerant grasses was less affected due to salt stress compared with their shoot growth, although root growth has earlier been considered as one of the important selection criteria of salt tolerance particularly in grasses (Hannon & Bradshaw 1968; Ashraf *et al.*, 1986). Higher salt tolerance of the Salt Range populations of each grass species was expected as these populations were native to saline environments. Furthermore, data for shoot biomass clearly depict that salt-induced reduction was more in the Salt Range population of *C. ciliaris* compared with that of *C. dactylon*. These results can be explained in view of the arguments of Bradshaw (1984) and Ashraf (1994) that evolution of stress tolerance mainly depends on the presence of

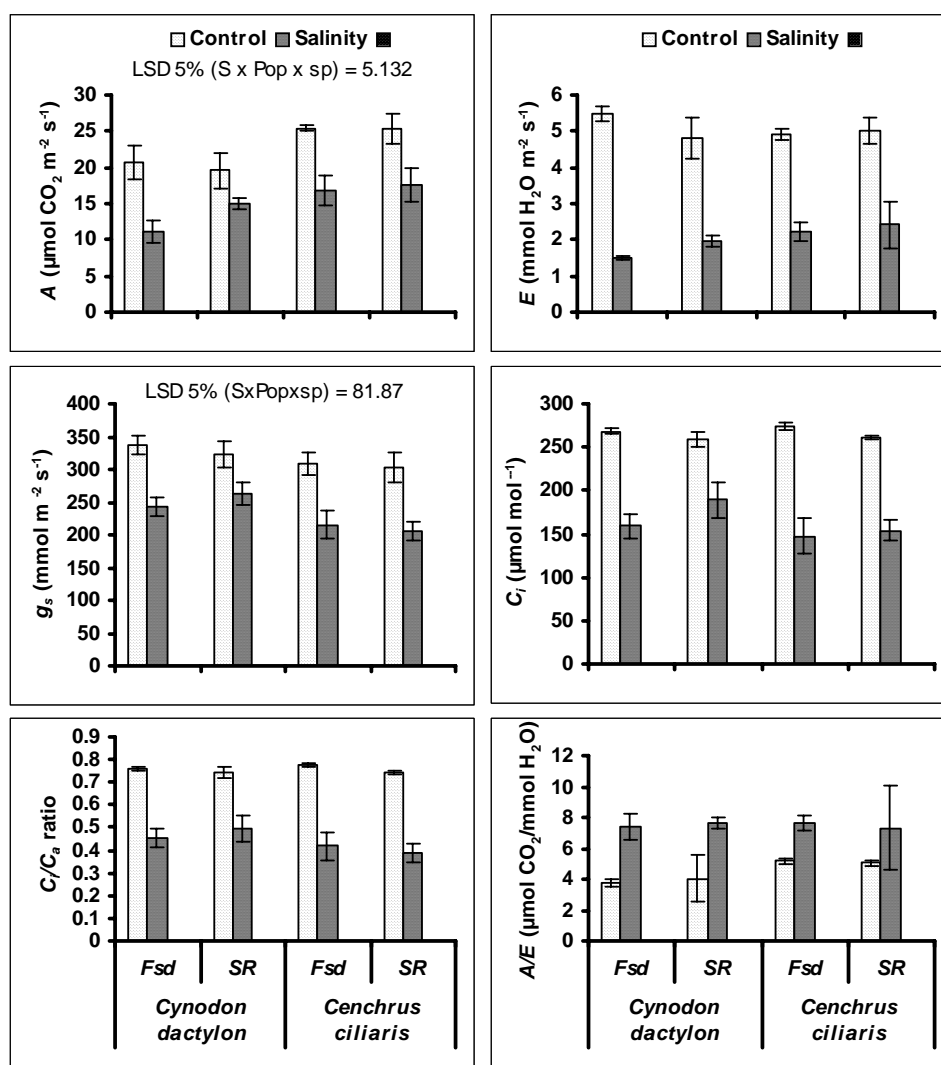


Fig. 2. Photosynthetic characteristics of *Cynodon dactylon* and *Cenchrus ciliaris* when 88-day old plants of different habitats were subjected to salt stress for 30 days.

appropriate gene pool, sufficient time they have been subjected to stress environment, as well as on the existence of suitable selection pressure. For example, in the presence of strong selection pressures, *Agrostis stolonifera* developed tolerance within four years of exposure to copper (Wu *et al.*, 1975) and *Anthoxanthum odoratum* within six years of lime application (Snaydon & Davis, 1972). Thus, natural selection pressure must have worked within that time for inducing stress tolerance. However, from the relatively lower biomass production of the Salt Range population of *C. ciliaris* in the present study, it can be assumed that this population of *C. ciliaris* had not evolved the salt tolerance trait due to lack of any of the factors mentioned earlier.

It is well established that salt-induced osmotic stress causes stomatal closure, which in turn results in lowering photosynthetic activity (Ashraf, 2004; Dubey, 2005). Photosynthetic activity undoubtedly is one of the major factors controlling growth under normal or stress conditions (Athar & Ashraf, 2005; Raza *et al.*, 2006). In the present study, reduced photosynthetic carbon assimilation rate in both grass species was attributed to reduced stomatal conductance, as reported elsewhere (Brugnoli & Lauteri, 1991). It was also noted that the Salt Range population of *C. dactylon* was higher in net CO₂ assimilation rate and stomatal conductance compared with the Faisalabad population. These results can be explained in view of earlier findings of Walker *et al.* (1983) who reported that lower stomatal conductance under saline conditions is generally associated with salt-sensitive species. However, in *C. ciliaris*, such salt-induced reduction in photosynthesis was not associated with stomatal conductance. From these reports, it is clear that genetic differences in rate of photosynthesis exist among different populations of a same species or among different species. In view of the results presented here, it can be suggested that micro-evolution occurred in the traits/genes associated with photosynthetic activity due to natural selection pressure only in the population of *C. dactylon* from the Salt Range.

Both salt-induced osmotic stress and ion toxicity caused reduction in plant growth by reducing photosynthesis (Ashraf, 2004). However, salt induced osmotic effect induces abscisic acid (ABA) accumulation, which can cause stomatal closure, and reduced intercellular CO₂ concentration, and chlorophyll content (Dubey, 2005). Furthermore, plants growing under saline conditions and experiencing salt-induced osmotic stress try to adjust by accumulating compatible solutes of different types. Thus, it can be assumed that the Salt Range population of *C. dactylon* has certain adaptations for osmotic adjustment during its evolutionary history, which resulted in better photosynthetic capacity. Shoot dry/fresh weight ratio can be used as a predictor of water uptake/osmotic adjustment by plants, the ratio being inversely related to water content (Naidoo, 1985). However, in the present study, shoot dry/fresh biomass ratios of both populations of each grass species were not affected due to salt stress (Fig. 1) indicating that both populations of each species had the ability to osmotically adjust to avoid physiological drought. This was further indicated by salt-induced increase in leaf proline in both populations of each grass species. However, higher accumulation of proline in the Salt Range population of *C. dactylon* compared with the Faisalabad population showed its positive role in the salt tolerance of this grass species. However, there is no difference in proline accumulation in the leaves of two populations of *C. ciliaris*. These findings support an earlier argument (Ashraf, 1994; Ashraf & Harris, 2004; Ashraf & Foolad, 2007) that proline accumulation is not an important selection criterion for salt tolerance in some plant species.

In conclusion, population of *C. dactylon* sampled from the Salt Range showed tolerance to salt stress due to natural selection pressure induced-evolution, whereas that of *C. ciliaris* did not show salt tolerance presumably due to lack of genetic variation or insufficient time for sorting of genotypes into that particular environment.

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