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LEAF ANATOMICAL ADAPTATIONS OF *CENCHRUS CILIARIS* L. FROM THE SALT RANGE, PAKISTAN AGAINST DROUGHT STRESS

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Abstract

Drought is one of the most serious environmental hazards that Pakistan is facing at present. It is even more severe to the agricultural crops and only for that single reason vast arid lands remain uncultivated each year. Precipitation ratio, in general, too very low in most of parts of Pakistan, and there is a crying need to hunt suitable germplasm, both from cultivated crops and forages, but also from natural adaptive species. For this purpose the Salt Range can be of inimitable value as native flora seems to be well adaptive to several biotic and abiotic stresses. Biodiversity of the Salt range is of specific importance because many endemic species are adapted to various environmental stresses. .Ecotype of potential drought resistant grass *Cenchrus ciliaris* L. was collected from the drought-hit habitat of the Salt Range, Pakistan. Ecotype of this species was also collected from normally irrigated soils of Faisalabad for comparison. The plants were subjected to three moisture regimes, viz. 100% FC (control), 75 % FC and 50% FC. *Cenchrus ciliaris* from the Salt Range adapted better to moderate and high drought levels. Grass species from the Salt Range showed some specific adaptation against severe drought condition. Increased succulence (leaf thickness), cuticle deposition under adverse climates accompanied by thick epidermal layer was crucially important for maintaining leaf moisture and preventing water loss through leaf surface. Reduced metaxylem area under drought stress was responsible for efficient water transport during adverse climatic conditions. Prevention of water loss under drought stress by highly developed bulliform tissue and reduced stomatal size on adaxial leaf surface make this ecotype excellent selection for arid and semi-arid regions.

Introduction

The Salt Range, Punjab, Pakistan is of unique geographic importance in relation to its geology and biodiversity facing several environmental stresses like drought and salinity *Cenchrus ciliaris* grows and persists well in desert habitats due to its tolerance to extreme drought. It is considered a high-value forage plant (Ramirez, 1999; Ramirez *et al*., 2001). Thus, *Cenchrus ciliaris* was selected for the present studies as it remains green even under extreme drought. Buffelgrass (*Cenchrus ciliaris* L.), locally called Anjan grass, is a perennial cespitose, C_4 grass native to Southern Asia and East Africa. It has been introduced in many countries as a pasture plant, which is considered to be an excellent fodder species for its drought tolerant properties (Arriaga *et al*., 2003).

Increased cuticle deposition, reduced leaf area, xylem differentiation, Pith compactness, which prevents water loss, and the thickness of sclerenchymatous cell layers, which is important for adaptation under water stress (Awasthi & Maurya, 1993). Thick leaves, thick epidermis, larger bulliform cells, and higher stomatal density are the characteristic features of drought stressed plants, as reported by Ristic *et al*. (1991) in *Zea mays,* Silva *et al*., (1999) in common bean, and Sam *et al*. (2000) in tomato. Leaves are

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generally thicker under water stress (Morenosotomayor *et al*., 2002) in *Zea mays* with high density of trichomes, especially on adaxial side (Perez *et al*., 2000 in tomato; Aguirre *et al*., 2002) in *Phaseolus vulgaris*. According to the the Bullifom cells plays an important role in turgour maintenance under water deficit condition (Alvarez *et al*., 2003). Abernethy *et al*. (1998) reported curled leaved forming a compact cylinder in the leaves of *Festuca novae_zelandiae* under water deficit condition.

The present study was emphasized on morphological and anatomical adaptations of *Cenchrus ciliaris*, and more significantly to hunt suitable recourses for severe drought affected areas of Pakistan. More specifically, to identify anatomical features that could be used for future research concerning molecular biology and genetic engineering of drought tolerance.

Materials and Methods

The potential drought tolerant tussock forming *Cenchrus ciliaris* (Buffel grass, locally called 'Anjan ghas') was collected from drought-hit hilly region (coordinates 32º46'35.46"N, 72º10'23.98"E, slope 30 – 45%) near Kallar Kahar, the Salt Range, Pakistan. For comparison ecotype of same species was collected from normal irrigated fields at University of Agriculture, Faisalabad (coordinates 32º76'23.45"N, 72º62'27.58"E). The pot experiment was conducted in the Old Botanical Garden of University of Agriculture Faisalabad. The experiment was conducted in Completely Randomized Design including four repeats and three drought stress levels viz. 100% field capacity (control), 75% field capacity and 50% field capacity.

Plants were carefully grown for one month. After one month the plants were subjected to drought levels, maintained at weight basis (FC).for three weeks. The plants were carefully up rooted for root and shoot fresh and dry weights. For anatomical studies, leaves (2 cm piece from the base) was first fixed in Formalin acetic alcohol solution (formaldehyde solution 5%, acetic acid 10%, ethyl alcohol 50% and distilled water 35%). The material was then transferred to acetic alcohol solution (acetic acid 25% and ethyl alcohol 75%) for long-term preservation. The leaf was then sectioned transversely by free-hand sectioning technique and permanent slides were prepared using standard double-stained technique. Micrographs of stained sections were taken with a digital camera (Nikon FDX-35) equipped stereo-microscope (Nikon 104, Japan). Data was analyzed by using Minitab Statistical Software. Leaf thickness, cuticle thickness, epidermis thickness and its cell area, mesophyll thickness and its cell area, bundle sheath thickness and its cell area, number of vascular bundles and their area, metaxylem area, protoxylem area, phloem area, bulliform cell thickness and its cell area, number of hairs/trichomes, stomatal density and area.

Results and Discussion

Plant biomass: Drought stress imparted significant and adverse effect on *Cenchrus ciliaris* ecotypes, however, the ecotype from the Salt Range showed more tolerance in relation to various morphological characteristics as compared to its counterpart from the Faisalabad region (Table 1, Fig. 1). An increase in root fresh weight was noted in all the cases with increase in drought level, however, the ecotype from the Salt Range possessed the maximum of this characteristic at the highest drought level. It showed an increase from 39.32 g in 100% FC to 51.46 g in 50% FC. The ecotype from the Faisalabad region increased only from 18.88g (100% FC) to 23.04 g (50% FC). Similar trend was observed in case of root dry weight where the ecotype from the Salt Rage increased considerably under severest drought stress(36.64 g) as compared to control ((21.86g). The ecotype from Faisalabad only increased from 13.62 under 100% FC to 15.14 g under 50% FC.

Shoot fresh and dry weights were adversely affected by increasing drought stress. The ecotype from the Faisalabad region, however, more severely affected where almost three-fold decrease in shoot fresh weight was recorded at 50% FC (25.77 g) as compared to 100% FC (47.61 g). The ecotype from the Salt Range, on contrary, decreased its shoot fresh weight from 47.61 (100% FC) to 25.77 g (50% FC). Shoot dry weight, in contrast, was quite severely affected in the ecotype from Faisalabad, where it decreased from 29.83 (100% FC) to 8.02 g (50% FC). The ecotype from the Salt Range only decreased from 21.86 (100% FC) to 13.24 g (50% FC).

Table 1. Leaf morpho-anatomical characteristics of *Cenchrus ciliaris* ecotypes under various regimes of water stress.

		Faisalabad			Salt Range		
		100% FC	75%	50%	100%	75%	50%
		Plant biomass					
Root fresh weight (g $plant^{-1}$)		18.88b	15.93b	23.04a	39.32a	45.17b	51.46c
Root dry weight (g $plant^{-1}$)		13.62a	8.66b	15.14a	21.86a	25.92b	36.64a
Shoot fresh weight (g $plant^{-1}$)		57.15a	23.89b	19.12c	47.61a	37.16b	25.77c
Shoot dry weight (g $plant^{-1}$)		29.83a	10.24b	8.02b	21.86a	17.92b	13.24a
		Leaf anatomy					
Leaf thickness (µ)		310.45a	223.87b	195.23c	214.56c	248.91b	293.45a
Cuticle thickness (µ)		1.96b	4.27a	5.95a	4.41a	4.90a	6.37a
Epidermis thickness (µ)	Adaxial	23.14a	26.31a	24.98a	17.98a	20.91a	25.12a
	Abaxial	13.92a	13.27a	13.01a	14.41a	14.41a	16.17a
Epidermal cell area (μ^2)	Adaxial	681.54c	881.02 a	794.18b	411.45c	556.47b	803.11a
	Abaxial	246.64a	224.12a	215.42a	264.27a	271.32a	332.77a
Bulliform thickness (µ)		42.13b	49.02a	36.36c	21.68c	28.61b	31.69a
Bulliform cell area (μ^2)		2259.01b	3058.31a	1682.60c	598.21c	1041.76b	1278.14a
Mesophyll thickness (µ)		26.5a	22.1b	21.6b	19.4c	22.6b	29.4a
Mesophyll area (μ^2)		572.37a	450.4ab	368.95b	446.60a	467.7a	571.13a
Bundle sheath thickness (µ)		15.39a	14.41b	16.37a	23.43b	25.63a	13.43c
Bundle sheath cell area (μ^2)		319.27a	305.44c	404.37c	517.09a	543.49a	514.14a
Vascular bundle number		32.50a	36.00a	39.00a	27.00a	43.50a	41.25a
Vascular bundle area (μ^2)		82319.11a	75382.29b	58698.65c	68836.9c	71370.34b	82025.00a
Stomata Adaxial)	Number	43.00b	37.00b	64.33a	39.67a	46.00b	88.67b
	Area(μ^2)	8699.59a	6138.04b	4243.58b	5674.36a	3826.80a	3489.22a
Stomata (Abaxial)	Number	58.00a	48.33a	51.33a	38.00a	50.00a	48.67a
	Area(μ^2)	4664.66a	5882.75a	4914.36a	5851.94a	5531.81a	3450.40a
Protoxylem area (μ^2)		496.29a	542.115a	555.45a	251.16a	298.05a	263.98a
Metaxylem area (μ^2)		5928.72a	3669.718b	3870.90b	3447.91a	2852.88b	2292. 0c
Phloem area (μ^2)		4756.70a	3449.3ac	2301.03bc	6363.45a	5715.89a	6161.18ab
Trichome number		20.50bc	21.50ac	26.00a	9.00a	8.00a	11.50a

Dry matter production reduces as a result of soil moisture stress (Tavakol & Pakniyat, 2007). Increased root dry weight has been suggested to be related to drought resistance. Greater root biomass than shoot has been found as a good criterion for assessing drought resistance, as its density increases under irrigation, though its penetration is deeper under moisture stress (Jensen *et al*., 2000). The ecotype from the Salt Range showed considerable increase in its root fresh and dry weights accompanied by much less reduction in shoot fresh and dry weights as compared to that from the Faisalabad region. These finding have been substantiated by significant relationships foliage with fresh and dry matter yield (of shoot, root and their total) under both the water regimes. It is plausible that greater root fresh and dry biomass is crucial sustenance and stable productivity of this grass under longer dry spells. Stable performance of the ecotype from the Salt Range in its dry matter production indicates its potential to withstand against severe droughts and making them suitable stuff for arid or semi-arid regions.

Leaf anatomical characteristics: Under controlled environments (100% FC), the Salt Range ecotype showed considerable high cuticle layer (4.41 µm) as compared to that from Faisalabad, which increased further with the increase in stress level up to 6.37 µm at the highest level (50% FC). Drought-adapted plants generally had thick cuticles (Ali *et al.,* 1999; Boom *et al*., 2005) as was recorded in the ecotype from the Salt Range. These findings seems to be fully justified the better adaptation of the ecotype from the Salt Range to adverse moisture limited conditions as reported by Esau(1977) Under water stress conditions the plants with waxy cuticle in the leaves minimize the loss of water.

Leaf thickness in the ecotype from Faisalabad decreased with increasing drought stress level from 310.45 µm at 100% FC to 195.23 µm at 50% FC. Quite an opposite response of drought stress was recorded in the ecotype from the Salt Range, where leaf thickness was increased from 214.56 µm at 100% FC to 293.45 µm at 50% FC. This clearly indicating its drought tolerant nature as Venora & Calcagno (1991) related drought resistance with thick leaves.

Epidermis thickness and its cell area at both adaxial and abaxial leaf surfaces remained more or less stable with increase in drought stress level in the ecotype from Faisalabad, but gradually increased in that from the Salt Range. Particularly, on adaxial leaf surface, increasing drought stress resulted in significant increase in epidermis thickness, and even more specifically in its cell area in the ecotype from the Salt Range (Table 1, Fig. 1). These findings are in agreement with those reported by ShiLei *et al.* (2002) who related epidermis to selection criteria for drought tolerance.

Bulliform cell thickness and its cell area adversely affected by increasing drought level in the ecotype from Faisalabad, but significantly increased in its counterpart from the Salt Range, particularly at the highest drought level. These significantly enlarged bulliform cell are very crucial under moisture limited environments as these are responsible for leaf curling, and ultimately checking water loss through leaf surface (Abernethy *et al.,* 1998; Alvarez *et al.* 2003).

Mesophyll and its cell area gradually decreased with increase in drought stress level in the ecotype from the Faisalabad region, but these were increased in the ecotype from the Salt Range. A decrease in mesophyll cell size has also been reported by Bosabalidis & Kofidis (2002) where the resistant cultivar of olive was less affected, therefore, the ecotype from the Salt Range showed excellent adaptation in relation to mesophyll as it is capable to produce necessary metabolites during adverse climates by maintaining growth under limited moisture availability.

Bundle sheath cell thickness and area slightly increased in the ecotype from Faisalabad while in that from the Salt Range decreased with increase in drought stress level. The thickness of bundle sheath layer was more responsive than its cell area. Similar findings has been reported by Ali *et al.,* (1999) and ShiLei *et al.* (2002) who reported reduction in cell size of bundle sheath but increased wall thickening.

Number of vascular bundles increased but the area decreased in the ecotype from Faisalabad with increase in drought stress level. However, in the ecotype from the Salt Range, both number and area of vascular bundles increased considerable under drought stress. Pitman *et al.* (1980) and Balog-Nyakas (1988) reported decrease in vascular bundle under drought stress, therefore, the ecotype from the Faisalabad region can be regarded as more sensitive ecotype than that collected from the Salt Range.

Metaxylem area decreased in both the ecotypes under drought stress, but protoxylem increased only in the ecotype from the Faisalabad region. Decreased metaxylem can be immensely important under harsh climates like drought as was reported by Vasellati *et al.* (2001) who reported decreased the diameter of metaxylem vessels, thus lowering the risk of embolisms and increasing water-flow resistance. These plastic responses may confer an ability of the ecotype from the Salt Range to withstand sudden events drought.

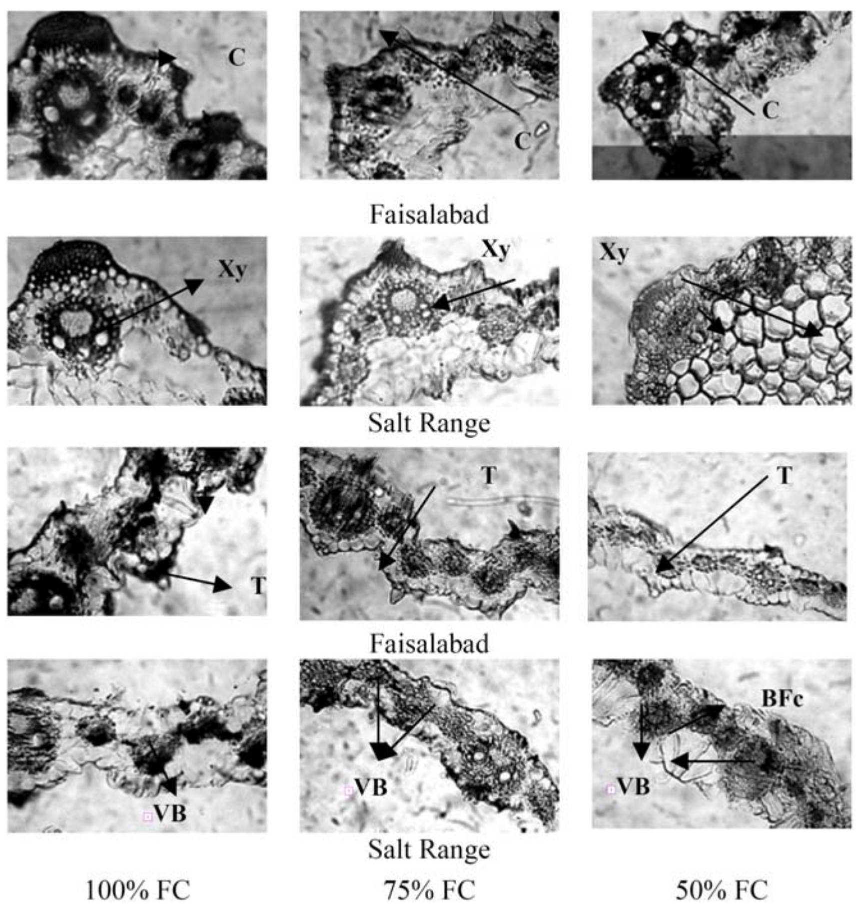

Fig. 1.Transverse sections of leaf (200X) of *Cenchrus ciliaris* ecotypes under various regimes of water stress. [C-Cuticle thickness; T-Trichome; Epi.Thk - Epidermis thickness; Xy-Xylem; BFc-Bulliform cells; FC-Field Capacity].

The ecotype from Faisalabad showed significant and gradual decrease in phloem area with increasing drought stress levels, however phloem area in that from the Salt Range remained more or less unaffected. Burnett *et al*. (2005) reported stability in phloem cross-sectional under drought stress, and hence, it is quite feasible to predict that the ecotype from the Salt Range can maintain transport of photosynthates under harsh environmental conditions.

The result exhibited that the water deficit condition had little effect on trichome density, as also reported by Sam *et al*. (2000). In the present study, both the ecotypes, from Faisalabad and the Salt Range, showed slight increase in trichome density. However, there are several reports on increased trichome density under drought stress (Abernethy *et al.,* 1998; Aguirre-Medina *et al.,* 2002).

Stomatal density, especially on adaxial leaf surface, significantly increased in both the ecotype from the Faisalabad region and the Salt Range. On contrary, stomatal area significantly decreased with increase in drought stress level. Smaller stomatal area accompanied by greater density may be crucially important for maintaining transpiration loss, particularly under limited moisture environments, as their opening and closing is expected to be controlled via less turgor potential. Similar decrease in stomatal area has been cited by several authors, such as Thind (1996), Silva *et al*. (1999), Sam *et al*. (2000), & Aguirre-Medina *et al.* (2002).

Salt Range in Pakistan originating in the Lower Cambrian has a very unique geological history (Butler *et al*., 1987; Burbank and Beck, 1989). Plants inhabiting there should have evolved very specific morpho-anatomical adaptations against several environmental stresses, mainly drought and salinity, in view of the long span of time they have been growing there. The Salt Range has never been explored to assess the extent of adaptation in different grass species inhabiting there. Due to its long geological history and severe drought stress, the grasses inhabiting there must have developed specific drought tolerance/resistance traits.

The ecotype evaluated from the Salt Range should very specific adaptation of leaf anatomical characteristics. These can be summarized as increased leaf thickness (succulence) is a characteristic feature of drought-adapted plants. Thick cuticle deposition accompanied with increased epidermis thickness is crucial for preventing water loss through leaf surfaces. Highly developed bulliform cells are another vital adaptation for arid and semi-arid areas as these are involved in leaf rolling under severe droughts. The reduced vascular tissues, in particular metaxylem and protoxylem cross-sectional area can easily be correlated to efficient water uptake and moisture column maintenance, and therefore, essential under harsh moisture climates. The higher density but reduced area of stomata (especially on adaxial surface of leaf) can be regarded to as critical adaptation for arid and semi-arid regions as smaller and numerous stomata can be maintained by lesser turgor, hence opening and closing according to environmental condition can be regulated efficiently. On these bases, the ecotype from the Salt Range can be regarded as perfectly adapted for semi-arid and arid regions, and therefore, recommended for the desertic habitats of the country with better biomass production and also for future breeding programmes.

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