Pak. J. Bot., 45(2): 351-357, 2013.

MODIFICATIONS IN STOMATAL STRUCTURE AND FUNCTION IN *CENCHRUS CILIARIS* L. AND *CYNODON DACTYLON* (L.) PERS. IN RESPONSE TO CADMIUM STRESS

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Abstract

The effect of cadmium (Cd) on modifications in stomatal structure and function of *Cenchrus ciliaris* and *Cynodon dactylon* was investigated to compare the relative Cd tolerance of these 2 grass species. Each grass species from 3 different habitats were subjected to varying levels of Cd, viz., 0, 30 and 60 mg L^{-1}. Growth of all ecotypes of both grasses decreased under Cd stress. A significant reduction in plant biomass, photosynthetic pigments and gas exchange parameters, such as net photosynthetic rate, transpiration rate, stomatal conductance and sub-stomatal CO_2 concentration was recorded in all ecotypes of both grass species. Cadmium tolerant populations of both grasses from Pakka Anna relied on water conservation mechanism when exposed to metal contaminated environments. These populations showed high net assimilation rate, low transpiration rate, and high water use efficiency, whereas the anatomical parameters they over-expressed were increased leaf thickness, cortical cell area, conducting tissue area, bulliform area, trichome density, and decrease in stomatal density and area.

Introduction

In the entire world agricultural soils are more or less contaminated with heavy metal toxicity. This contamination is mainly due to unwise use of sewage water for irrigation, dust drawn from smelters and poor drainage management practices (Akguc *et al*., 2008). This contamination causes deterioration of soils and damage to biological membranes of plants (Demiral &Turkan, 2005).

Among soil pollutants, cadmium proves to be highly toxic and results in the shoot and root inhibition, affects the uptake of nutrients and their homeostasis, retard growth and decrease overall yield crop (Bhardwaj *et al*., 2009). Cadmium accumulates frequently by the crops and then it enters the food chain with a very high potential to impair health of living beings (Siddiqui *et al*., 2012). The cadmium-induced reduction in biomass could associated with reduced rate of photosynthesis and as well as synthesis of chlorophyll (Kasim, 2005; Shi & Cai, 2008; Raziuddin *et al*., 2011). Elevated levels of cadmium may cause a decrease in uptake of nutrients and alters the function of various enzymes (Kasim, 2005; Siddiqui *et al*., 2012).In vascular plants primary means of gas exchange is stomata. However, cadmium plays a crucial role in stomatal function and structure (Adedeji & Jewoola, 2008; Ozyigit & Akinci, 2009).

Economically *Cynodon dactylon* (L.) Pers. & *Cenchrus ciliaris* L., are very important grasses because they are extensively used as fodder for livestock and resource conservation (Assefa *et al*., 1999). These species are widespread throughout the Punjab that can be established on metal-contaminated polluted soils and industrial wastes if they exhibit considerable tolerance to metal stress. Therefore, the present study was conducted to evaluate the structural and functional modifications of photosynthetic apparatus in these grasses under cadmium toxicity so as to appraise their Cd tolerance.

Materials and Methods

Two grasses species *Cenchrus ciliaris* L. & *Cynodon dactylon* (L.) Pers. were collected from three ecologically different habitats viz., University of Agriculture, Faisalabad (normal well irrigated soil, Cd 1.23 mg kg^{-1}), the Salt Range (moderately polluted, Cd 18.31 mg kg^{-1}) and Pakka Anna (heavily polluted, Cd 30.10 mg kg^{-1}). Soil from the root zone was collected for the determination of Cd concentration of each grass and each habitat. The soil was oven-dried at 70°C, saturation paste prepared and Cd content of the soil extract analyzed using atomic absorption spectrophotometer (Analyst 300, Perkin Elmer, Germany).

The stumps of each grass species from each habitat were first established in the Faisalabad environment for one year. Six ramets of almost equal size were then planted in plastic tubs filled with half-strength hydroponic culture solution. The solution was aerated for 12 h daily using air pumps. Three cadmium levels (0, 30, & 60 mg L^{-1}) were maintained after 30 days of transplantation by applying $CdSO_4$ in the solution culture. The containers were kept in open net-house during April to June with photoperiod 12-13 h, average day temperature 39-45°C, and relative humidity 35 to 46%.

The data for fresh weight of root and shoot, photosynthetic parameters, chlorophyll pigments and leaf anatomy were recorded three months after the application of cadmium stress. The gas exchange characteristics were determined using a portable infra red gas analyzer (IRGA), LCA-4 (Analytical Development Company, Hoddesdon, England). To study the anatomical characteristics, a 2cm piece of the leaves from the leaf base along the midrib of both grass species was taken and kept in formalin acetic alcohol solution containing v/v formalin 10%, acetic acid 5%, distilled water 35% and ethyl alcohol 50%. The material was preserved in the solution for 48 h and then subsequently placed in acetic alcohol solution containing v/v ethyl alcohol 75% and acetic acid 25% for long-term storage. Permanent slides were prepared by the free-hand sectioning technique. Ethanol was used for serial dehydrations of the samples, and 2 stains, fast green and safranine stains were used for staining parenchymatous and lignified tissues, respectively. Anatomical data of the prepared slides were recorded with the help of a light microscope (Anti-Mould, Nikon SE, Japan). A digital camera (Nikon FDX-35) was used to take micrographs of permanent slides.

The experiment was carried out in the Old Botanical Garden, University of Agriculture, Faisalabad using a completely randomized design (CRD) with three factor (grasses, habitats and Cd levels) factorial arrangements with three replications.

Results

Root and shoot fresh weight: Varying Cd levels caused a negative effect on the root and shoot fresh weights of both grasses (Table 1). However, the degrees were more prominent in the Faisalabad population of both *Cenchrus ciliaris* and *Cynodon dactylon*. The population from Pakka Anna of *Cynodon dactylon* was least affected due to Cd stress among all populations of both grasses, which showed a non-significant decrease in the root fresh weight with increase in Cd level of the cultural solution. More or less a similar trend was recorded in the shoot fresh weight, where the Pakka Anna population of both C. *ciliaris* and *C. dactylon* were least affected due to Cd stress, particularly at 30 mg L^{-1}.

Photosynthetic pigments: Induction of Cd in the culture medium resulted in a decrease in photosynthetic pigment (Chlorophyll a & b) in the Faisalabad population of both grasses (Table 1). However, in the Salt Range population of *C. ciliaris* and the Pakka Anna population of *C. dactylon* the pigments significantly increased at 30 mg L^{-1} Cd, and at 60 mg L^{-1}, respectively.

Gas exchange characteristics: Cd stress imposed a negative effect on net assimilation rate (*A*) in all the populations of both grasses (Table 1). However of all grass populations the Pakka Anna population of *C. dactylon* was the least affected due to Cd stress. Transpiration rate (*E*), significantly decreased in all the populations of *C. ciliaris*, but increased in the Faisalabad population of *C. dactylon* at both Cd levels, and that from the Salt Range at 30 mg L^{-1} level only. A variable response in water use efficiency (*A/E*) was observed due to Cd stress, not only in the populations but also in the grass species. In the Faisalabad and the Salt Range populations of *C. ciliaris* and the Pakka Anna population of *C. dactylon*, *A/E* was not non-significantly affected due to Cd stress. However, the Pakka Anna population of *C. ciliaris* showed a significant increase in *A/E*, particularly at the highest Cd regime.

Stomatal conductance (g_s) decreased in all the populations of both grasses with increase in Cd level of the growth medium except the Faisalabad population of *C. dactylon*. In contrast, sub-stomatal CO_2 concentration (C_i) generally decreased with increase in external Cd level. The Faisalabad population of *C. dactylon* was a solitary case in which C_i decreased under Cd stress.

Leaf anatomical parameters: The populations of *C. ciliaris* showed a differential response to Cd stress with respect to midrib thickness (Table 2, Fig. 1). The Faisalabad population showed a decrease in this parameter, whereas the Salt Range population showed an increase with increase in Cd level. However, midrib thickness in Pakka Anna population increased significantly at 30mg L^{-1} Cd level, but thereafter, it decreased at the highest Cd level. In *C. dactylon* populations, this parameter generally increased with increase in Cd level, but the Faisalabad population showed an increase in midrib thickness only at 30 mg L^{-1} level. Lamina thickness, on the other hand, generally increased in all populations with increase in Cd level except that in the Salt Range population of *C. dactylon,* where an increase was recorded at 30 mg L^{-1} Cd.

Epidermal thickness showed an increase in the Faisalabad population of *C. ciliaris* but a decrease in the other populations with increase in Cd levels (Table 2, Fig. 1). In contrast all populations of *C. dactylon* showed increased epidermal thickness at 30 mg L^{-1} Cd and decreased at the highest Cd level. No distinctive hypodermal and cortical tissues were recorded in *C. dactylon*. In *C. ciliaris*, hypodermal thickness was not affected in the Faisalabad population due to Cd stress, but this deceased significantly in the other two populations. Cortical cell area showed an increase in the Faisalabad population, but a decrease in the Salt Range population with increase in Cd level of the growth medium. The Pakka Anna population of this grass showed a significant increase at 30 mg L^{-1} Cd, but a decrease at the highest Cd level.

Vascular bundle area increased in all populations of *C. dactylon* with increase in external Cd level (Table 2, Fig. 1). Vascular bundle area in the Pakka Anna population was not affected due to varying Cd levels, but the Faisalabad population showed an increase and the Salt Range population a decrease with increase in Cd level of the growth medium. Metaxylem area increased in the Faisalabad population of *C. ciliaris* as well as in all the populations of *C. dactylon* with increase in the Cd level. However, the populations of *C. ciliaris* from the Salt Range and Pakka Anna showed a consistent decrease in vascular bundle area with increase in the Cd level. Phloem area was the least altered characteristic due to Cd stress; the *C. ciliaris* population showed a slight decrease in phloem area but the populations of *C. dactylon* showed an increase in this parameter with increase in external Cd level. The Faisalabad populations of *C. ciliaris* was the solitary case that showed a decrease in bulliform cell area due to Cd stress, but in all the other populations this parameter increased significantly by the addition of Cd to the growth medium.

Stomatal number showed a variable response to Cd stress, where it increased in the Salt Range population of *C. ciliaris* and Faisalabad population of *C. dactylon,* but decreased in all the other populations of both grasses with increase in Cd levels. Stomatal area, on the other hand, increased only in the Pakka Anna population of *C. dactylon*, but decreased in the other populations of the grasses due to Cd stress. The Salt Range population of *C. dactylon* showed a substantial increase at 30 mg L^{-1} Cd, but a decrease at 60 mg L^{-1} Cd. No clear-cut trend of trichome number in response to Cd stress was recorded in the populations of both grasses. However, the number was increased in the Pakka Anna population of *C. ciliaris* and the Salt Range population of *C. dactylon* with increase in Cd level. This parameter decreased in all other populations with increase in Cd level; the most adversely affected population was that of *C. dactylon* from the Faisalabad.

Table 1. Fresh weight and photosynthetic parameters of *Cenchrus ciliaris* and *Cynodon dactylon* populations from ecologically different habitats under Cd stress.

		Cd conc. ($mg\ L^{-1}$)	Fresh weight of root ($g\ plant^{-1}$)	Fresh weight of shoot ($g\ plant^{-1}$)	Chlorophyll a ($mg\ g^{-1}$ f.wt.)	Chlorophyll b ($mg\ g^{-1}$ f.wt.)	Net CO_2 assimilation rate (P_n) ($\mu mol\ m^{-2}\ s^{-1}$)	Transpiration rate (E) ($mmol\ m^{-2}\ s^{-1}$)	Water use efficiency (A/E)	Stomatal conductance (g_s) ($mmol\ mol^{-1}$)	Substomatal CO_2 (Ci) ($\mu mol\ mol^{-1}$)
Cenchrus ciliaris	Faisalabad	Control	4.04c	14.21c	1.22c	0.13a	24.34c	9.35b	2.60b	366.75c	223.53a
		30	1.40b	7.95b	1.08b	0.90c	21.05b	7.26a	2.90b	300.04b	405.70b
		60	0.96a	6.40a	0.85a	0.67b	10.44a	6.55a	1.59a	166.74a	413.70c
	Salt Range	Control	5.16c	19.21c	0.82a	0.66a	26.38c	10.74c	2.46a	500.04c	192.63a
		30	2.03b	10.26b	1.98c	2.31c	22.67b	9.56b	2.36a	333.31b	201.40a
		60	1.83a	8.93a	1.36b	1.65b	11.46a	4.32a	2.65a	133.36a	416.87b
	Pakka Anna	Control	4.96c	18.03c	1.16a	1.52b	28.42c	12.81c	2.22a	633.34c	240.40a
		30	3.20b	12.02b	1.78b	1.22a	27.13b	11.61b	2.34a	300.02b	294.13b
		60	2.83a	9.64a	2.72c	3.78c	19.77a	4.27a	4.63c	266.75a	307.83c
Cynodon dactylon	Faisalabad	Control	6.17c	15.51c	2.67b	3.54c	18.69b	9.66a	2.03c	133.38a	469.93c
		30	3.06b	10.40b	2.59b	3.05b	17.26b	12.89b	1.45b	166.78b	314.40b
		60	1.05a	5.303a	1.43a	2.03a	9.60a	12.78b	0.57a	300.07c	280.77a
	Salt Range	Control	5.22c	12.06b	3.43c	3.33c	26.67c	8.10a	3.29c	233.33c	263.17a
		30	4.19b	15.43c	0.76b	1.92b	24.64b	8.24a	2.99b	166.79b	325.27b
		60	2.07a	8.23a	0.49a	1.24a	10.25a	7.93a	1.29a	133.38a	360.07c
	Pakka Anna	Control	5.25b	14.59b	1.41a	0.75a	27.37a	8.55b	3.24a	233.31c	159.47a
		30	4.17a	14.23b	2.45b	1.91c	36.37b	6.67a	3.98b	200.06b	168.70a
		60	4.06a	11.35a	1.29a	1.57b	26.52a	5.83a	3.36a	100.00a	384.53b

Means sharing same letters in each column within each habitat are statistically non-significant

Table 2. Leaf anatomical parameters of *Cenchrus ciliaris* and *Cynodon dactylon* populations from ecologically different habitats under Cd stress.

Species	Habitat	Cd levels (mg L^{-1})	Midrib thickness (µm)	Lamina thickness (µm)	Epidermal thickness (µm)	Hypodermal thickness (µm)	Cortical cell area (μm^2)	Vascular bundle area (μm^2)	Metaxylem area (μm^2)	Phloem area (μm^2)	Bulliform cell area (μm^2)	Stomatal number	Stomatal area (μm^2)	Trichome number
Cenchrus ciliaris	Faisalabad	Control	1781.06a	882.36a	78.89a	236.93a	166.90a	792.78a	24.01a	36.69a	219.26c	80.25c	871.21c	63.28c
		30	1854.59b	908.83a	92.55b	227.78a	191.94b	883.94b	31.10b	34.46a	130.17b	69.12b	600.15b	35.26b
		60	1907.69c	972.23b	115.79c	230.88a	386.44c	980.44c	35.31c	34.02a	111.55a	60.23a	492.35a	27.59a
	Salt Range	Control	2254.92c	759.81a	114.38c	285.95c	561.05c	737.50c	57.77c	30.88b	88.52a	45.01a	620.16c	67.21b
		30	1789.23b	955.89b	89.87b	250.59b	224.68b	550.70b	49.21b	28.42b	145.58b	63.15b	487.27b	61.34a
		60	1219.62a	1037.59c	70.85a	238.89a	66.76a	514.18a	37.70a	20.70a	162.91c	80.26c	370.45a	59.13a
	Pakka Anna	Control	1526.16a	857.85a	89.87c	245.13b	249.71a	781.87a	47.18c	26.42b	278.52a	76.18c	730.64c	71.28a
		30	2295.77b	900.66a	76.21b	236.93b	292.72c	768.53a	34.02b	25.78b	357.77b	73.29b	581.36b	73.59a
		60	1593.81a	1010.7ba	63.53a	183.82a	279.88b	763.46a	28.94a	14.49a	407.20c	59.34a	345.26a	82.53b
Cynodon dactylon	Faisalabad	Control	751.64b	702.62a	32.68b	--	--	365.26a	8.99a	19.90a	146.70a	60.15a	973.52b	15.32b
		30	800.66c	729.09a	57.19c	--	--	585.44b	14.76b	21.41a	158.42b	80.17b	974.16b	19.28c
		60	686.28a	816.67b	24.51a	--	--	667.18c	18.62c	25.41b	232.10c	160.20c	251.45a	5.98a
	Salt Range	Control	604.58a	745.27c	31.05a	--	--	386.94a	18.89a	19.26a	227.92a	55.35c	323.21b	12.31a
		30	639.22a	606.54b	57.19b	--	--	456.63b	21.55b	27.60b	221.18a	43.17b	392.34c	15.27b
		60	731.05b	522.88a	32.68a	--	--	477.03c	27.06c	30.81c	317.97b	39.59a	305.24a	19.26c
	Pakka Anna	Control	559.81a	633.01a	40.85a	--	--	303.48a	19.53a	10.54a	114.44a	41.24c	341.36a	15.36a
		30	657.85b	714.71b	57.19b	--	--	481.45b	21.68a	26.96b	215.89b	36.29b	384.45b	21.56b
		60	763.73c	722.88b	40.85a	--	--	919.40c	25.18b	28.62b	308.67c	32.67a	391.53c	23.48b

Means sharing same letters in each column and within each habitat are statistically non-significant

Fig. 1.Transverse section of leaf of *Cenchrus ciliaris* and *Cynodon dactylon* populations from ecologically different habitats under Cd stress. *Cenchrus ciliaris* midrib (a-f), Faisalabad population under control (a) and Cd stress (b), Salt Range population under control (c) and Cd stress (d), Pakka Anna population under control (e) and Cd stress (f), *Cenchrus ciliaris* lamina (g-l), Faisalabad population under control (g) and Cd stress (h), Salt Range population under control (i) and Cd stress (j), Pakka Anna population under control (k) and Cd stress (l), *Cenchrus ciliaris* midrib (m-r), Faisalabad population under control (m) and Cd stress (n), Salt Range population under control (o) and Cd stress (p), Pakka Anna population under control (q) and Cd stress (r)
(B: bulliform cells, C: Cortex, H: hypodermis, MV: metaxylem vessels, Ph: phloem, T: trichomes, VB: vascular bundle)

Discussion

Cadmium is perhaps the most harmful and widespread among heavy metals that affect agricultural soils in recent years (Wu *et al*., 2005; Ahmad *et al*., 2012), and this is mainly due to industrial emissions, sewage water irrigation and extensive use of phosphate containing fertilizers (Lima *et al*., 2006). Phytoremediation by using Cd hyper-accumulators and usage of tolerant species for the Cd affected soils will be the most effective solution to cope with contaminated soils. For this, the maximum potential was shown by the grasses in the past for the quick improvement of metal affected degraded soils (Carneiro *et al*., 2001). Populations of both *Cenchrus ciliaris* and *Cynodon dactylon* had been growing in their respective habitats for a long time including moderately Cd-affected Salt Range and heavily affected Pakka Anna soils. Both grass species under study are cosmopolitan in distribution and have phyto-potential because of their high biomass production, thus they can be effectively used for the soil improvement.

Cadmium is not part of any biological function, and at the same time it is highly toxic to both animals (including humans) and plants (Vassilev & Yordanov, 1997). It imparts physiological impairment in plants (Krupa & Baszynski, 1995; Ayeni *et al*., 2012). Among heavy metals, mobility in agricultural systems and toxicity to humans was more pronounced by the application of cadmium (Hasan *et al*., 2009).

Induction of cadmium in growth medium adversely affected fresh biomass of all populations of both grass species. Many researchers have also reported a decrease in biomass production due to Cd in different crops, e.g., in *Lemna trisulca* (Prasad *et al*., 2001), *Setaria anceps* & *Paspalum paniculatum* (Melo *et al*., 2007), *Glycine max* (Abdo *et al*., 2012). However, root and shoot fresh weights of the Pakka Anna population of *C. dactylon* were the least affected by Cd stress as compared to the other populations of this grass. The Pakka Anna population of *C. ciliaris* was again less adversely affected than its counterparts particularly at the highest Cd level. Similar findings were reported by Gomes *et al*., (2011) in *Brachiaria decumbens*, a moderately heavy metal tolerant species. This was an indication of its better tolerance of the Pakka Anna populations (in particular that of *C. dactylon*) to Cd as compared to the other populations. The Faisalabad populations of both grasses showed great reduction in their fresh weights under Cd stress. A significant reduction in shoot and root biomass has earlier been reported by Siddiqi & Iqbal (2000) in Cd sensitive *Solanum melongen*a.

Chlorophyll pigments (Chl a & b) were significantly reduced in the Faisalabad populations of both grasses under Cd stress, whereas the Salt Range population of *C. ciliaris* and the Salt Range and Pakka Anna populations of *C. dactylon* showed an increase at 30 mg L^{-1} Cd level only. A slight increase in chlorophyll content at lower Cd level was reported by Bindhu & Bera (2001) in mungbean. The chlorophyll content in Pakka Anna population of *C. ciliaris*, on the other hand, increased consistently up to 60 mg L^{-1} Cd level, which might have increased the photosynthetic activity of this population, and hence its degree of Cd tolerance.

Photosynthetic parameters like net CO_2 assilimiation rate, transpiration rate, water use efficiency and stomatal conductance seems to be related to the degree of tolerance of individual populations. The Faisalabad population of both grasses showed a sharp decrease in net CO_2 assimilation rate and water use efficiency under Cd stress, which may have been due to growth retardation and alteration in different biochemical reaction (Abo-Kassem *et al*., 1995; Vassilev & Yordanov,1997). Net assimilation rate was among the most adversely affected parameters under Cd stress in broad beans (Kasim, 2005; Vassilev & Yordanov, 1997). However, the stability in this parameter was recorded in the Pakka Anna populations, particularly at lower Cd level. This may contribute to enhanced degree of Cd tolerance of this population. Transpiration rate, in contrast, decreased and sub-stomatal CO_2 concentration increased in all the cases with a few exceptions.

Structure and function of the leaves are strongly influenced by Cd toxicity as reported in some previous studies (Lang *et al*., 1995; Sheoran *et al*., 1990). Generaly, leaf thickness (midrib and lamina) increases with increase in Cd level of the culture medium, however, the Salt Range population of *C. ciliaris* showed a decrease in midrib thickness and that of *C. dactylon* in lamina thickness due to Cd stress. Leaf thickness is mainly due to high ratio of physiologically active parenchymatous tissue, and it may play a defensive role in both grasses. However, more Cd-sensitive populations from Faisalabad were more responsive to Cd toxicity regarding the leaf thickness. Gomes *et al*., (2011), in contrast, reported a decrease in leaf thickness in *Brachiaria decumbens*.

Only the Faisalabad population of *C. ciliaris* showed a consistant increase in epidermal thickness due to Cd stress, but the other two populations of this grass showed a significant decrease in epidermal thickness. In *C. dactylon* populations, epidermal thickness increased at lower Cd level, but was markedly reduced at the highest Cd level. Epidermal thickness seemed to be not related to Cd toxicity in the present studies, as it showed a variable response. However, Gomes *et al*., (2011) reported increased epidermal thickness in *Brachiaria decumbens* due to Cd stress.

Thick-walled hypodermis was not recorded in the leaves of *C. dactylon*, as was the thin-walled parenchyma in the midrib. Hypodermal thickness decreased in all populations of *C. ciliaris*, but cortical cell area increased in the Faisalabad and Pakka Anna populations under Cd stress. No relevant literature is available to support these findings, but larger parenchyma may certainly play a key role in Cd tolerance of this species.

Size of the vascular tissue (vascular bundle area, metaxylem area and phloem area) increased in all *C. dactylon* populations under Cd stress, whereas only in the Faisalabad population of *C. ciliaris.* A decrease in vascular bundle, metaxylem and phloem areas has also been reported earlier by El-Ghinbihi & Fatma (2000) in common beans, Kasim (2006) in *Sorghum bicolor* & Gomes *et al*., (2011) in *Brachiaria decumbens* due to Cd stress. A decrease in vascular tissue area may result in reduced hydraulic conductivity (Marchiol *et al*., 1996), but in the present studies, *C. dactylon* proved to be more tolerant than *C. ciliaris.*

Bulliform cells play a decisive role in transpirational control under limited moisture availability (Hameed *et al*., 2012). Size of the bulliform cells increased in the metal comtaminated environments in all cases except in the Faisalabad population of *C. ciliaris.* Gomes *et al*., (2011) also reported increased area of bulliform cells when *Brachiaria decumbens* was exposed to Cd. Stomatal area generally decreased in all *C. ciliaris* populations, but increased in *C. dactylon* under Cd stress, however, there were few exceptions. Chardonnens *et al*., (1998) in *Silene vulgaris,* Baryla *et al*., (2001) in *Brassica napus,* and Shi & Cai (2009) in peanut reported increase in stomatal density under Cd stress.

Plant water relations may be one of the most disturbed physiological parameters in plants exposed to Cd stress (El-Gamal & Hammad, 2003; Rascio *et al*., 2008). The Pakka Anna populations of both grasses, particularly that of *C. dactylon* relied structurally and functionally on water conservation strategies when exposed to metal contaminated environments. Physiological parameters of these populations under Cd stress involved in water conservation were high net assimilation rate, low transpiration rate, and high water use efficiency. Anatomical parameter like increase in leaf thickness, cortical cell area, conducting tissue area, bulliform area, trichome density, and decrease in stomatal density and area may not only increase water storage in parenchyma, but also prevent water loss from the plant body. This could be vital for improving water relations under metal contaminated environments by conserving water inside the plant body.

Conclusion

Structural and functional modifications in both *Cenchrus ciliaris* and *Cynodon dactylon* are critical for maintaining plant water relation under Cd stress. Tolerant populations of both grasses can be effectively used for phytoremediation and revegetation of the metal contaminated soils. Additionally structural and functional markers for heavy metal tolerance can be incorporated in future research programmes.

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(Received for publication 4 January 2012)