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The Bases of *Blepharis* sp. Adaptation to Water-Limited Environment

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Abstract: Plant responses to drought have been extensively investigated, however, little is known about the bases of *Blepharis* sp. adaptation to water-limited environment in North-Western Sudan. The objective of this study was to study the possible mechanisms of drought tolerance in two species of *Blepharis* (Family Acanthaceae) grown under water stress. The results showed that decreasing water potential significantly reduced stomatal conductance, transpiration rate, specific leaf area and stomatal density. In contrast, root length, leaf area ratio and leaf weight ratio increased significantly under water stress. The concentration of inorganic minerals (particularly potassium) increased in response to water stress. These morpho-physiological traits may contribute to drought tolerance mechanisms of these species in water-limited environments. The tested species varied in their responses, although insignificantly, to water deficit. In this respect, *Blepharis linariifolia* (S₁) showed greater stomatal conductance and transpiration rate than *Blepharis ciliaris* (S₂), whereas the later exhibited higher specific leaf weight, stomatal density and solute concentrations. The variations between species in changes-induced by water deficit are of great ecophysiological significance especially under severe water scarcity. Further *in situ* work is needed to gain an insight into how gizzu herbs and grasses acclimatize to long-term water deficit and to assess whether solutes accumulation under water deficit contribute to osmotic adjustment.

Key words: *Blepharis* sp., water stress, osmotic adjustment

INTRODUCTION

Gizzu herbs as described by Tohill (1948) is a collective term used by nomadic Arabs to describe fodder plants that provide food and moisture for grazing in north-western, Sudan. According to the previous studies of plant cover, these plants were commonly reported in areas north of latitude 16°N where the annual rainfall does not exceed 50 mm. Recently, however, due to the desertification elements, they were dragged south to latitude 15°N (Ibrahim, 1984).

Plant responses to drought are complex and different mechanisms have been developed by plants to tolerate drought (Passioura, 2007). Through two major strategies, drought avoidance and drought tolerance, plants may cope with and survive drought conditions and many plants use a combination of both mechanisms (Gaspar *et al.*, 2002). Drought may initially inhibit leaf growth and development, significantly reducing leaf area (Chaves *et al.*, 2003; Gazanchian *et al.*, 2007). The reduction in leaf area and plant growth

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reduce transpiration rate by the reduction of stomatal conductance (Xu and Zhou, 2005; Aguirrezabal *et al.*, 2006). Differences in drought tolerance among genotypes may be associated with osmotic adjustment and in many plants, genotypes with higher drought-induced solute accumulation are more drought-tolerant than genotypes with lower solute accumulation (Munns, 1988; Morgan *et al.*, 1991; Amede *et al.*, 1999).

As reported by Abusam *et al.* (2009), gizzu herbs have adapted their physical structure through special mechanisms to accommodate the scarcity of water. The common adaptation characteristics include reduced leaf surface area as a result of small leaves or no leaves for most of the year. Another important adaptation characteristic is the presence of numerous hairs, spines and prickles and complete or partial dormancy during the dry period. Adaptation to cellular dehydration, modified root systems, decreased plant size or a shorter growing period are also possible drought resistance mechanisms. Thus, it appears that Gizzu herbs are the result of a series of very successful adaptation to local conditions of the desert. Therefore, the objective of this study was to analyze the possible mechanisms of drought tolerance in two species of *Blepharis* (the dominant genus of Gizzu herbs) grown under water stress. The extent to which osmotically active solutes accumulate in leaves during water deficit was also assessed.

MATERIALS AND METHODS

Planting Materials, Study Site and Growth Conditions

Two experiments were conducted under field conditions to study the effect of water stress on physiological characters of two dominant species of Gizzu herbs: namely, *Blepharis linariifolia* (S₁) and *Blepharis ciliaris* (S₂). The experiments were conducted in the Demonstration Farm of the Faculty of Agriculture at Shambat, Sudan (latitude 15°40' N, longitude 32°32' E and altitude 230 m.a.s.l.). The soil of the experimental site is vertisol (Fine montomorillonitic, Isohyperthermic Entic Chromustert) with poor infiltration and alkaline pH of 8.0 to 8.5. The climate of the locality is semi-arid with mean annual rainfall of 150 to 200 mm and maximum temperature of about 42°C in summer and 21°C in winter, but the night temperatures are lower.

Seeds of both species were soaked in tap water for 3 h, thereafter, the seed coats were removed. The uncoated seeds were either sown directly in the soil (field experiment) or in pots, 15 cm in diameter (pot experiment). In both experiments, seeds were sown during the second week of June 2007 and half of the plants were well-watered (WW) and the other half was Water-Stressed (WS). In the field experiment, the well-watered treatment was induced by frequently watering plants every 14 days, whereas in water-stressed treatment, water was withheld from the seedlings until the end of the experiment. In the pot experiment, the well-watered treatment was induced by watering the plants with 600 mL of water, whereas in the water-stressed treatment, the plants were watered with 200 mL every 4 days. In both experiments, watering treatments started one month after seedling emergence. In the field experiment, the treatments were arranged in a split-plot design. The main plots were allotted for the watering treatments and the sub-plots to the species. In the pot experiment a completely randomized block design was used.

Characters Studied

At harvest, four plants from each subplot (pot) were randomly selected and tagged to study the following parameters: Transpiration and stomatal conductance (measurements were made on abaxial surface of fully expanded leaves with a Li-Cor 6400 Portable Photosynthesis System, Lincoln, Nebraska, USA). Water potential was determined using

a portable pressure chamber (Skye SKPM 1400, UK) at midday (11.30-12.30 h) using the youngest, fully expanded leaves to avoid the effects of overnight water recovery (Meiri *et al.*, 2006). Leaf area, leaf dry weight, root length and dry matter production were also determined in all treatments. Leaf area was determined using leaf area meter (Delta-T leaf area meter, LI-300, li-Cor, Lincoln, Nebraska, USA) as outlined by O'Neal *et al.* (2002). Specific leaf area, leaf area ratio and leaf weight ratio were computed from the measured parameters. Light microscope (Leitz-DIALU×20) fitted with camera (EKTAR:125) at magnification ×50 was used to determine stomatal density per unit leaf area as described by Radoglou and Jarvis (1990). Leaf Relative Water Content (RWC) was determined using 10 to 15 fully expanded leaves per plot (pot). Leaves were clipped to determine the Fresh Weight (FW), then placed into small Petri dishes filled with water for approximately 18 h at 4°C and then weighed immediately after excess moisture was removed with paper towels to determine the Turgid Weight (TW). The leaves were then oven dried at 75°C for 72 h to determine Dry Weight (DW). Leaf RWC was calculated as $(FW-DW)/(TW-DW) \times 100$. The concentration of inorganic minerals was determined in mature leaves using Energy Dispersive XRF Spectrometer (Margui *et al.*, 2007).

Statistical Analysis

The data were subjected to analysis of variance, according to Quinn and Keough (2002) and LSD test for mean separation. Correlation coefficients were determined between root length, stomatal conductance, water potential, transpiration rate, leaf area and stomatal density.

RESULTS

Water Potential, Transpiration Rate and Stomatal Conductance

Watering treatments had a significant effect on mean water potential, transpiration rate and stomatal conductance in the pot and field experiments (Table 1). The studied parameters were greatly reduced in water-stressed plants, compared to the well-watered plants (Table 1). Moreover, the water potential (ψ) of the two species was lower (more negative) in the pot experiment than in the field experiment. In contrast, stomatal conductance and transpiration rate were greater in the pot experiment than in the field experiment (Table 1). Although, there were no significant differences between the two

Table 1: Water potential, transpiration rate and stomatal conductance of *Blepharis linariifolia* (S₁) and *Blepharis ciliaris* (S₂) grown under well-watered (WW) and water-stressed (WS) conditions. Values are means of four replicates

Treatment	Water potential (MPa)			Transpiration rate (mmol/m ² /sec)			Stomatal conductance (mmol/m ² /sec)		
	WW	WS	Mean	WW	WS	Mean	WW	WS	Mean
Field experiment									
S ₁	-0.9	-2.4	-1.7	3.1	0.8	2.0	39.5	13.8	26.7
S ₂	-1.4	-2.9	-2.2	2.4	0.5	1.5	35.6	9.1	22.4
Mean	-1.2	-2.7		2.8	0.7		37.6	11.5	
LSD _{0.05} W			0.7			0.9			16.4
LSD _{0.05} S			ns			ns			ns
LSD _{0.05} W×S			ns			ns			ns
Pot experiment									
S ₁	-1.7	-2.6	-2.2	8.0	2.8	5.4	128.3	45.8	87.1
S ₂	-2.1	-2.9	-2.5	3.9	2.2	3.1	61.3	35.0	48.2
Mean	-1.9	-2.8		6.0	2.5		94.8	40.4	
LSD _{0.05} W			0.4			2.2			15.4
LSD _{0.05} S			ns			ns			ns
LSD _{0.05} W×S			ns			ns			ns

ns = Non significant at 5% level of probability

Table 2: Leaf area, root length and dry matter production of *Blepharis linariifolia* (S₁) and *Blepharis ciliaris* (S₂) grown under well-watered (WW) and water-stressed (WS) conditions. Values are means of four replicates

Treatment	Leaf area/plant (mm ²)			Root length (cm)			Dry matter (g)		
	WW	WS	Mean	WW	WS	Mean	WW	WS	Mean
Field experiment									
S ₁	2277	1802	2040	41.6	46.5	44.1	71.6	47.9	59.8
S ₂	3982	2591	3287	49.2	58.5	53.9	124.0	98.5	111.3
Mean	3130	2197		45.4	52.5		97.8	73.2	
LSD _{0.05} W			884			6.6			36.2
LSD _{0.05} S			745			5.3			26.4
LSD _{0.05} W×S			ns			ns			ns
Pot experiment									
S ₁	167	80	124	20.9	24.7	22.8	5.6	3.0	4.3
S ₂	231	120	176	23.0	25.9	24.5	6.6	3.4	5.0
Mean	199	100		22.0	25.3		6.1	3.2	
LSD _{0.05} W			53			3.0			1.2
LSD _{0.05} S			3			1.2			0.8
LSD _{0.05} W×S			ns			ns			ns

ns = Non significant at 5% level of probability

species in mean water potential, transpiration rate and stomatal conductance, there were remarkable drought-induced decreases in these parameters in S₂, both in the field and in the pot experiments (Table 1).

Leaf Area, Root Length and Dry Matter Production

Watering treatments had significant effect on mean leaf area, root length and dry matter production in the field and pot experiments (Table 2). Drought caused substantial decrease in leaf area and dry matter production, whereas root length increased significantly by water stress in both experiments (Table 2). The differences in mean leaf area, root length and total dry matter of the two species were significant and S₂ consistently had higher values than S₁ in both experiments (Table 2).

Relative Water Content and Stomatal Density

Water stress had a significant effect on relative water content and stomatal density in the two experiments (Table 3). They were lower under water stress than in the well-watered treatment. Although, there were no significant differences between the two species with respect to the mean relative water content and stomatal density, S₂ had higher mean values than S₁ in both experiments (Table 3).

Specific Leaf Area, Leaf Area Ratio and Leaf Weight Ratio

In both experiments, specific leaf area was significantly reduced by water stress (Table 4). But, there was insignificant increase in leaf area ratio and leaf weight ratio with water stress in the two experiments. Although there were no significant differences between S₁ and S₂ for mean specific leaf area, leaf area ratio and leaf weight ratio in the field experiment, S₂ had significantly higher values than S₁ in the pot experiment (Table 4).

The Concentration of Inorganic Minerals

The concentration of inorganic minerals, particularly potassium, increased under water deficit treatment (Table 5). *Blepharis ciliaris* (S₂) accumulated higher minerals concentration than *Blepharis linariifolia* (S₁). This indicates that there may be an association between the accumulation of these minerals and the improvement in water relations of plants under water stress conditions.

Table 3: Relative water content and stomatal density of *Blepharis linariifolia* (S₁) and *Blepharis ciliaris* (S₂) grown under well-watered (WW) and water-stressed (WS) conditions. Values are means of four replicates

Treatment	Relative water content (%)			Stomatal density (No. mm ⁻²)		
	WW	WS	Mean	WW	WS	Mean
Field experiment						
S ₁	59	51	55	18.3	12.0	15.2
S ₂	69	47	58	27.5	13.3	20.4
Mean	64	49		22.9	12.7	
LSD _{0.05} W			6			5.8
LSD _{0.05} S			ns			5.3
LSD _{0.05} W×S			ns			ns
Pot experiment						
S ₁	70	61	66	25.8	10.5	18.2
S ₂	81	63	72	42.3	22.0	32.2
Mean	76	62		34.1	16.3	
LSD _{0.05} W			7			10.7
LSD _{0.05} S			ns			ns
LSD _{0.05} W×S			ns			ns

ns = Non significant at 5% level of probability

Table 4: Specific leaf area, leaf area ratio and leaf weight ratio of *Blepharis linariifolia* (S₁) and *Blepharis ciliaris* (S₂) grown under well-watered (WW) and water-stressed (WS) conditions. Values are means of four replicates

Treatment	Specific leaf area (cm ² g ⁻¹)			Leaf area ratio (cm ² g ⁻¹)			Leaf weight ratio (g g ⁻¹)		
	WW	WS	Mean	WW	WS	Mean	WW	WS	Mean
Field experiment									
S ₁	140.0	104.6	122.3	32.7	38.7	35.7	0.23	0.38	0.31
S ₂	162.6	126.7	144.7	34.7	44.4	39.6	0.26	0.29	0.28
Mean	151.3	115.7		33.7	41.6		0.25	0.34	
LSD _{0.05} W			27.8			ns			ns
LSD _{0.05} S			ns			ns			ns
LSD _{0.05} W×S			ns			ns			ns
Pot experiment									
S ₁	77.2	67.9	72.6	25.5	27.9	26.8	0.29	0.32	0.31
S ₂	91.7	86.9	89.3	40.6	41.9	41.3	0.54	0.60	0.57
Mean	84.5	77.4		33.1	34.9		0.42	0.46	
LSD _{0.05} W			6.2			ns			ns
LSD _{0.05} S			13.1			9.6			0.1
LSD _{0.05} W×S			ns			ns			ns

ns = non significant at 5% level of probability

Table 5: Concentrations (ppm) of some inorganic minerals in *Blepharis linariifolia* (S₁) and *Blepharis ciliaris* (S₂) grown under well-watered (WW) and water-stressed (WS)

Treatment	K			Fe			Ca			Zn		
	WW	WS	Mean	WW	WS	Mean	WW	WS	Mean	WW	WS	Mean
S ₁	35541	45234	40388	111	194	153	13092	11128	12110	48	142	95
S ₂	43618	66235	54927	176	170	173	13746	15710	14728	288	400	344
Mean	39580	55735		144	182		13419	13419		168	271	

Correlation Analyses

A highly significant and positive correlation coefficient was obtained between leaf area and root length ($R^2 = 0.85$, Table 6). Similarly, there was strong positive relationship, as expected, between stomatal conductance and transpiration rate and moderate correlation coefficients between leaf stomatal density and stomatal conductance to water vapour ($R^2 = 0.99$ and 0.41 , respectively). In contrast, significant and negative correlation coefficients were obtained between transpiration rate and root length ($R^2 = -0.49$) and between water potential and transpiration rate ($R^2 = -0.36$, Table 6).

Table 6: Correlation coefficients between root length, stomatal conductance, water potential, transpiration rate, leaf area and stomatal density of *Blepharis* sp.

Variables	Root length	Stomatal conductance	Water potential	Transpiration rate	Leaf area
Root length	-	-	-	-	-
Stomatal conductance	-0.47**	-	-	-	-
Water potential	-0.06	-0.38*	-	-	-
Transpiration rate	-0.49**	0.99**	-0.36*	-	-
Leaf area	0.85**	-0.31	-0.32	-0.35*	-
Stomata density	-0.26	0.41**	-0.21	0.42*	-0.14

*, **Significant at 0.05 and 0.01 level of probability, respectively

DISCUSSION

The ability of the plant to absorb available water is critical and various factors are involved in all water relations. The cell water status is normally assessed by the measurement of water potential, which is the water absorbing capacity of a plant. Therefore, plant water stress occurs when low plant water potentials develop and cell turgor begins to fall. In the present study, water stress caused significant reduction in plant water potential under field and pot experiments. This reduction was associated with a significant reduction in stomatal conductance and transpiration rate. This is in line with the findings of Passioura and Fry (1992), who reported that as soil dries up the uptake of water by the root decreases and water potential falls sufficiently to cause stomatal closure leading to a reduction in transpiration rate. The reduction in stomatal conductance depends upon water potential and transpiration rate, because as soil dries and the availability of adequate water is reduced, stomatal conductance declines resulting in lower values of transpiration rate and hence reduced water loss. This is in line with previous studies which reported that stomatal conductance and leaf water potential decrease under water deficit (Cowan *et al.*, 1982). The low water potential (ψ) of the two species in the pot experiment than in the field experiment indicated that drought stress under pot experiment was much more severe due to limited volume of soil. Similarly, stomatal conductance and transpiration rate were greater in the pot experiment than in the field experiment by reason of the more access of roots to available water in the limited volume of the pot.

The reduction in specific leaf area under water stress conditions revealed an adaptation mechanism to the drought as this character was associated with reduction in transpiring surface area. This result agrees with the findings of Aguirrezabal *et al.* (2006) and Gazanchian *et al.* (2007). They reported that the reduction in leaf area and plant growth under drought allows plants to reduce their transpiration rate by the reduction of stomatal conductance. Fotteli *et al.* (2000) recorded that the small leaf area characteristic of plants which grow under drought conditions is an adaptation of xeric sites. Although, many reports indicate that stomatal density was positively correlated with leaf area per plant (Chaves *et al.*, 2003; Galmes *et al.*, 2007), the results of the present study showed that stomatal density was not significantly associated with leaf area suggesting that the changes in leaf size was not responsible for the changes in stomatal density.

The significant reduction in total dry weight under water deficit may be attributed to the reduction in photosynthesis and shedding of leaves. Similar findings were reported by Ravindra *et al.* (1990), who concluded that the reduction in dry matter under drought is associated with reduction in leaf area and size of reproductive sink. Other studies showed that plants that used a finite water should have higher biomass productivity than plants with low water-use efficiency (Jones, 1992). This finding was further substantiated by the positive correlation between leaf area and root length obtained in this study.

The high concentration of inorganic minerals particularly potassium under water deficit is consistent with a putative role in osmotic adjustment. However, drought-induced increase in solutes accumulation is not a reliable indicator of drought tolerance as solutes

accumulation may occur due to water loss and/or sink limitation (Munns, 1988). Therefore, true osmotic adjustment is presumably a function of the accumulation of effective osmotica. The difference in drought-induced solute accumulation between the two species due to water stress indicates that solute accumulation was the result of a concentration effect. This suggestion is substantiated by the great variation between species in shoot biomass as reported by Amede *et al.* (1999).

The positive correlation between stomatal conductance and transpiration rate obtained in this study is in line with the findings of Nambiar and Brown (1997). They reported that water deficit causes a reduction in stomatal conductance with a proportional reduction in transpiration rate. Similar conclusion was also reached by other researchers (Aguirrezabal *et al.*, 2006). The negative correlations between transpiration rate and root length indicate that root length could be considered as a drought tolerance mechanism in these species. Moreover, stomatal density decreased in water-stressed plants constituting another mechanism of adaptation, because decreases in stomatal density increases leaf resistance to water loss. This result is in line with the findings of Fitter and Hay (1993), who reported that increases in stomatal density reduces leaf resistance to water loss.

In conclusion, the results of the present study suggest that stomatal conductance, transpiration rate, specific leaf weight and stomatal density of the two species of *Blepharis* significantly decrease whereas root length significantly increases with soil drying (low water potential). Also, the concentration of inorganic minerals (particularly potassium) increased in response to water stress. These might contribute to drought tolerance mechanisms of these species under water stress conditions. In addition, there were significant differences among the tested species in their response to drought. In this respect, *Blepharis linariifolia* (S₁) showed greater stomatal conductance and transpiration rate than *Blepharis ciliaris* (S₂), whereas the later exhibited higher specific leaf weight, stomatal density and solutes concentrations. The variations between species in changes induced by water deficit may be of great ecophysiological significance especially under severe water scarcity. Further *in situ* work is needed to gain an insight into how gizzu herbs and grasses acclimate to long-term water deficit and to assess whether solutes accumulation under water deficit contribute to osmotic adjustment.

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