

Net photosynthesis and leaf water potential of buffel grass (*Cenchrus ciliaris* L.) accessions, growing in the arid zone of Tunisia

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Received: 4 August 2009

Accepted after revision: 12 January 2010

Four local *Cenchrus ciliaris* L. accessions (P1: Bou Hedma, P2: Tozeur, P3: Raâs Jedir, and P4: Sidi Toui) were planted in a common garden experiment in Tunisia in autumn 2002. Net photosynthesis (Pn), stomatal conductance (gs) and leaf water potential (Ψ) were measured between September 2003 and September 2004. All accessions showed two distinct periods of growth, interspersed by summer and winter dormancy periods. Initiation of the cycle occurred in autumn and appeared to be associated with soil water availability. There were significant differences ($p < 0.001$) between seasonal measures of net photosynthesis (Pn) and stomatal conductance (gs) of each accession. The highest value was observed for accession P3 ($42 \mu\text{mol m}^{-2} \text{s}^{-1}$). P1 and P4 showed the same value of Pn ($35 \mu\text{mol m}^{-2} \text{s}^{-1}$) in spring. Photosynthetic rate (Pn) of all accessions decreased with decreased stomatal conductance (gs). The annual course of predawn water potential (Ψ_{pd}) and minimum water potential (Ψ_{md}) observed during the year, showed that lower water potentials were observed at the end of spring (-3.0 MPa). As would be expected, higher water potentials (-1.0 MPa) were observed during winter, due to the high level of rainfall during that season. It has been observed that seasonal water availability and air temperature influence the seasonal dynamics of photosynthesis and leaf water potential. *Cenchrus ciliaris* accessions originated from different regions of Tunisia, and growing under the same conditions did not differ in their net photosynthesis and water status.

Key words: gas exchange, arid bioclimate.

INTRODUCTION

Studies of the functional mechanisms and behavior of plants are needed, in order to use phylogenetic resources in the rehabilitation and restoration programs of degraded ecosystems.

Indeed, given the scale of disturbance affecting the arid and saharan ecosystems of Tunisia, attempts to improve and restore the ecological balance are deemed necessary (Aronson *et al.*, 1993). However, the success of such operations can only be guaranteed by the use of native species (Chaieb *et al.*, 1992; Ourcival *et al.*, 1994). Several studies have been carried out in the Tunisian arid zones on the water use

dynamics of various perennial species (Floret & Pontanier, 1978; Le Hou  rou, 1984) such as *Cenchrus ciliaris*, *Stipa lagascae*, *Plantago albicans*, *Argyrobolium uniflorum*. In addition to their pastoral qualities, these species are suitable for sand stabilization and combating desertification (Le Hou  rou, 1995). Furthermore, they improve the soil water balance by reducing soil aridity (Floret & Pontanier, 1984). Our contribution towards the process of even partial restoration of the ecological balance in the North African environment in general and in Tunisia in particular, consists an ecophysiological study of one key species of Tunisian arid zones.

Cenchrus ciliaris L. is a perennial buffel grass and C_4 plant, in the grass family (*Poaceae*), invading arid tropical habitats around the globe. It is one of the candidate species for ecological restoration of de-

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graded rangelands in several arid zones of the world. It is present in Australia, Central America, North Africa and South Sahara, Arabia, Madagascar, Indonesia, Northern India and Pakistan (Le Houérou, 1991). In its native range, *C. ciliaris* is common in dry, sandy areas. It can also occur in warm, temperate thornscrub habitats, tropical deserts, and in moist forests. In Tunisia, *C. ciliaris* is a good pastoral species distributed along an important aridity gradient of the country (Emberger, 1954). However, this species is known for its great polymorphism (morphology, photosynthetic capacity, productivity, genetic diversity) (Jessup *et al.*, 2003; Mnif *et al.*, 2005; Saini *et al.*, 2007). In fact, since the initial discovery of C_4 photosynthesis in the late 1960s, a significant progress has been made in elucidating the biochemical and physiological characteristic of this pathway (Percy & Ehleringer, 1984). The higher photosynthetic efficiency of C_4 plants under tropical conditions and their consequently higher growth rate and daily organic production is widely recognised (Hatch, 1999). The study of *C. ciliaris* gas exchange responses and leaf water potential influenced by seasonal environmental change will help to improve our understanding of the strategies developed by this species for favourable growth and biomass production.

The present experiment was a part of three-year study conducted at an experimental site under arid bioclimate of the southern Tunisia. It aims at deter-

mining and comparing the diurnal and seasonal leaf water potential, net photosynthesis and stomatal conductance of four *C. ciliaris* accessions, and thus understanding the eco-physiological mechanisms, the successful resource use, and the fast growth of this species in arid areas. This study is integrated in the framework of restoration and rehabilitation of Tunisian steppes projects. The success of restoration and rehabilitation is, in fact, related to the intimate knowledge of the biological material (Aronson *et al.*, 1993).

MATERIALS AND METHODS

Study site

The experiment was performed at an experimental site in Sfax-Thyna (southern Tunisia 34° 43'N, 10° 41'E) between September 2003 and September 2004. The climate is arid Mediterranean with mild winters (Emberger, 1954). The mean annual precipitation is 234 mm, and the mean air temperatures is 32°C in summer and 19°C in winter, with a maximum in August (38°C) and a minimum in January (5°C). The rainy season lasts from mid-November to April. The most humid months are January and February and the drought period usually extends from May to October. Climatic conditions of the experimental site were obtained from a meteorological station in Thyna (Sfax). Rainfall and air temperatures (minimum and maximum temperatures) during 2003 and 2004 at the experimental site are presented in Fig. 1.

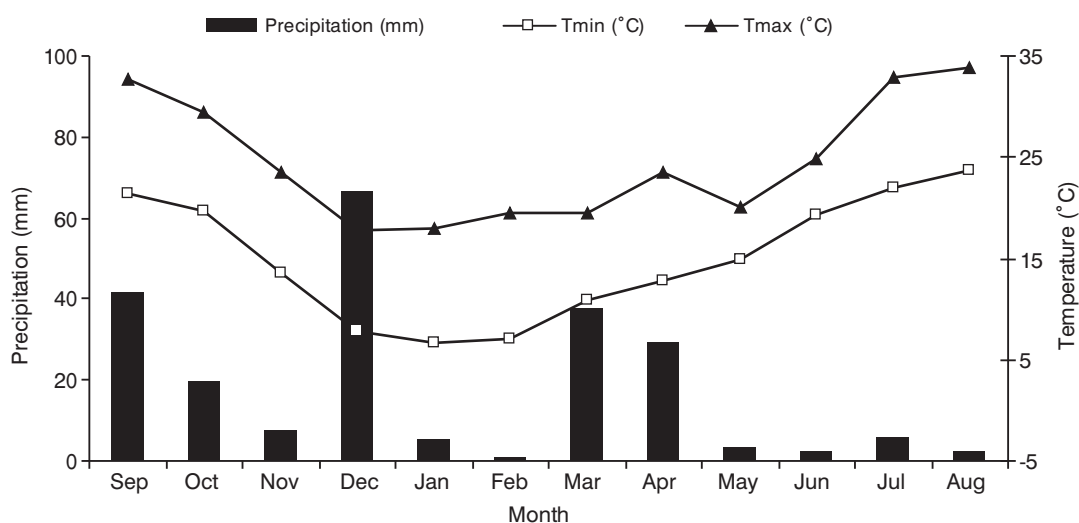


FIG. 1. Mean precipitation and air temperature (Tmax; maximum temperature and Tmin; minimum temperature) at the experimental site, during 2003 and 2004.

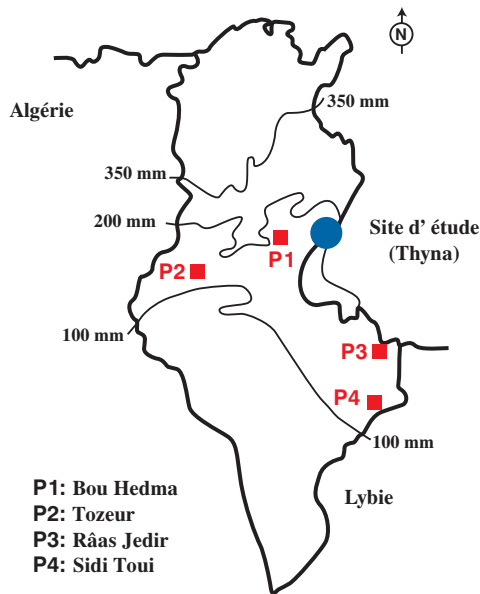


FIG. 2. Geographical locations and the site of the collection of *Cenchrus ciliaris* in Thyna (Sfax, Tunisia).

Plant material

Cenchrus ciliaris is a C_4 perennial grass widely distributed in all continents of the world. It is highly nutritious and is considered excellent for pasture in hot, dry areas and is valued for its production of palatable forage and intermittent grazing during drought periods. Four *C. ciliaris* accessions were collected from different regions of southern Tunisia (Fig. 2), where mean annual rainfall varied between 100 and 200 mm. These accessions are; P1: Bou Hedma, P2: Tozeur, P3: Raâs Jedir and P4: Sidi Toui. Chromosome number of these accessions showed a high degree of aneuploidy with $2n = 18$ for P1, $2n = 27$ for P2 and P4, and $2n = 36$ for P3 (Mnif *et al.*, 2005). According to M'seddi *et al.* (2002) and Mnif & Chaieb (2006), these accessions range from high-growing morphotypes with high dry matter production and important phenological variability.

Experimental design

All studied accessions were established by seedlings throughout autumn 2002. These accessions were planted in 16 plots (4 accessions \times 4 replicates), each 3 m wide and 6 m long and with 48 plants per plot. Despite the homogeneity of the soil of the study site, and in order to have statistically significant results, accessions were randomly distributed within plots. The distance between two plots was 1 m. Plants were chosen in any of the 4 replicate blocks, to present the same phenological stage. Net photosynthesis (Pn) and

stomatal conductance (gs) were measured on clear sunny days, on recently expanded leaves of *C. ciliaris* accessions using a portable gas exchange system (Li-Cor Inc-6200) operated in closed mode. From each plot, six plants were randomly chosen and for each plant, twenty replicate measurements of gas exchange were done for a pair of intact leaves with a combined mean leaf area of 3.5 cm^2 . Leaf water potential (pre-dawn water potential Ψ_{pd} and midday water potential Ψ_{md}) were measured with a Scholander pressure chamber (Scholander *et al.*, 1965) and following the precautions recommended by Turner (1988). For each accession, the diurnal patterns of leaf water potential (Ψ) were measured on a winter day (December 2003) and on a spring day (March 2004). In each plot, six leaves for each of six randomly selected plants were used for the determination of Ψ_{pd} and Ψ_{md} . The time between excision and determination was approximately 60 sec. The differences between predawn and midday leaf water potential ($\Delta\Psi$) were also determined.

Statistical analysis

Analysis of variance was used to evaluate the separate and interactive effects of *C. ciliaris* accessions on response variables related to leaf gas exchange characteristics and leaf water status. We evaluated differences in net photosynthesis, stomatal conductance and leaf water potential by one-way ANOVA. Standard errors of the means and least significant differences were calculated where appropriate. We used Tukey's HDS test to make pair-wise comparisons between accessions. The analyses were conducted with SPSS V.11.0 statistical package.

RESULTS

Seasonal variation in leaf water potential

Diurnal patterns of leaf water potential (Ψ) observed on a winter day (December 2003) and a spring day (March 2004) are illustrated in Fig. 3. The observations of the daily variation of leaf water potential, during the two growing seasons of this species, are precisely exhibited by measurements of Ψ_{pd} and Ψ_{md} . Ψ_{pd} was measured before dawn, while Ψ_{md} was measured between 12:00 and 14:00; the minimum Ψ values were recorded around 12:00-14:00; then, a recovery has been observed. A rapid decrease in Ψ values was observed in the morning during March 2004, as compared to those of December 2003 measurements. It may be related to the change in $\Delta\Psi$. Diurnal pat-

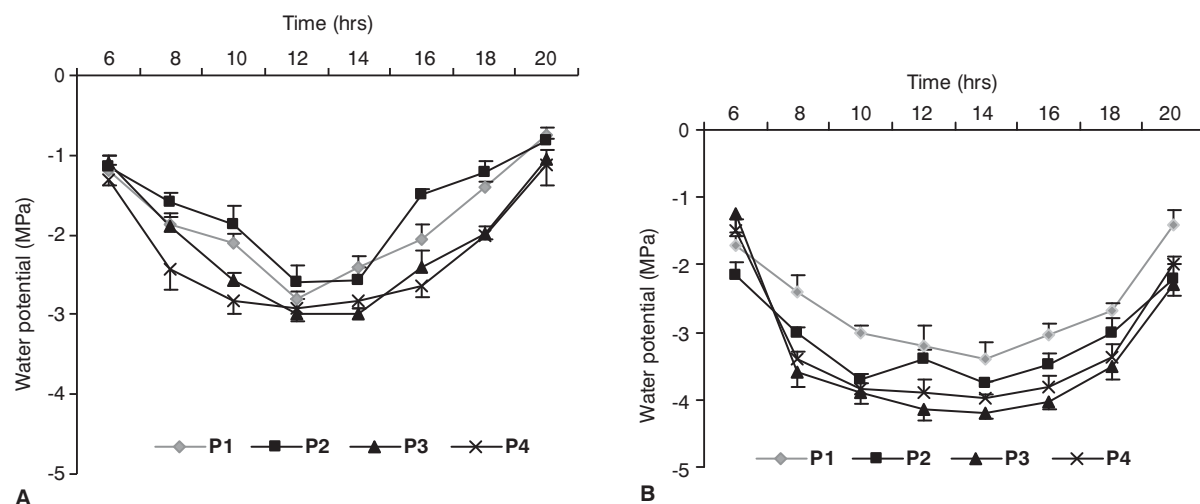


FIG. 3. Diurnal time courses of leaf water potential on (A) a winter day (December 2003) and (B) a spring day (March 2004).

TABLE 1. Net photosynthesis (Pn), stomatal conductance (gs), predawn water potential (Ψ_{pd}) and midday water potential (Ψ_{md}) of *Cenchrus ciliaris* accessions (\pm standard deviation). Different letters within columns indicate significant difference at the 5% level

Accession	Season	Pn ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	gs ($\text{mmol m}^{-2} \text{s}^{-1}$)	Ψ_{pd} (-MPa)	Ψ_{md} (-MPa)
P1	Autumn	14.92 \pm 0.90 ^a	128.00 \pm 12.40 ^a	2.58 \pm 0.25 ^a	2.90 \pm 0.18 ^a
	Winter	9.96 \pm 1.23 ^b	100.33 \pm 13.75 ^{ab}	1.10 \pm 0.23 ^b	1.66 \pm 0.16 ^b
	Spring	27.17 \pm 1.46 ^c	214.83 \pm 22.50 ^b	2.29 \pm 0.32 ^a	2.88 \pm 0.27 ^a
	Summer	9.33 \pm 2.00 ^b	100.00 \pm 17.00 ^{ab}	3.00 \pm 0.15 ^c	3.30 \pm 0.34 ^c
P2	Autumn	13.41 \pm 2.00 ^a	124.00 \pm 17.00 ^a	2.68 \pm 0.22 ^a	2.96 \pm 0.25 ^a
	Winter	7.98 \pm 1.50 ^b	88.83 \pm 12.00 ^b	1.26 \pm 0.26 ^{ab}	1.87 \pm 0.24 ^{ab}
	Spring	24.33 \pm 2.00 ^c	196.00 \pm 22.00 ^c	2.11 \pm 0.27 ^a	2.90 \pm 0.25 ^a
	Summer	6.21 \pm 2.40 ^b	75.00 \pm 3.00 ^d	3.00 \pm 0.50 ^c	3.70 \pm 0.62 ^b
P3	Autumn	12.68 \pm 1.40 ^a	109.85 \pm 16.00 ^a	2.58 \pm 0.23 ^a	2.96 \pm 0.28 ^a
	Winter	13.62 \pm 1.00 ^a	106.47 \pm 20.00 ^a	1.15 \pm 0.26 ^c	1.90 \pm 0.18 ^b
	Spring	24.67 \pm 1.80 ^b	181.00 \pm 22.00 ^b	2.10 \pm 0.50 ^{ab}	3.10 \pm 0.30 ^a
	Summer	3.30 \pm 2.60 ^c	45.00 \pm 14.00 ^c	2.80 \pm 0.40 ^a	3.20 \pm 0.23 ^a
P4	Autumn	14.07 \pm 2.00 ^a	122.00 \pm 20.00 ^a	2.49 \pm 0.22 ^a	3.80 \pm 0.22 ^a
	Winter	8.64 \pm 1.53 ^{ab}	88.21 \pm 18.00 ^{ab}	1.41 \pm 0.70 ^b	2.11 \pm 0.29 ^{ab}
	Spring	26.51 \pm 1.80 ^c	198.00 \pm 21.00 ^c	2.32 \pm 0.88 ^a	3.35 \pm 0.30 ^a
	Summer	6.96 \pm 1.20 ^{ab}	80.00 \pm 8.00 ^{ab}	3.50 \pm 0.60 ^c	4.30 \pm 0.50 ^c
<i>P</i> (season)		***	***	**	**
<i>P</i> (accession)		ns	ns	ns	ns

** $p < 0.001$, *** $p < 0.0001$, ns: not significant

terns in Ψ were similar for all accessions with maxima and minima varying between -1.99 MPa and -2.83 MPa during December, and between -0.94 MPa and -2.83 MPa during March. The differences observed between *C. ciliaris* accessions in diurnal leaf water potential were not significant ($p > 0.05$) (Table 1).

The annual course in leaf water potential found with the four accessions of *C. ciliaris* is shown in Table 1. The observations started (for the studied accessions) early summer and we found that very low values of Ψ_{pd} (i.e. -3.0 MPa) had increased with the precipitation recorded at the experiment site. *Cenchrus ciliaris* seemed to react to the rain that occurred in September 2003 (40 mm) (see Fig. 1). During winter, Ψ_{pd} value is maximum (-1.0 MPa). During February, Ψ_{pd} decreased again, concomitantly with the low precipitations, to reach the very low values observed at the end of summer (-3.0 MPa). Comparisons of the leaf water potential indicated that differences among accessions in the two growing seasons were not statistically significant ($p > 0.05$).

The diurnal change in potential ($\Delta\Psi = \Psi_{pd} - \Psi_{md}$) remained approximately constant throughout the year. The $\Delta\Psi$ increased significantly as the season progressed from winter, spring and decreased in the early summer (June). When predawn values increased around -2.0 MPa in March, the $\Delta\Psi$ values decreased. At low predawn values (-3.0 MPa), $\Delta\Psi$ approached zero.

Seasonal variation in net photosynthesis (Pn) and stomatal conductance (gs)

Data of net photosynthesis (Pn) was collected in all seasons of the year from September 2003 to June 2004. Pn decreased ($p < 0.01$) in all accessions in late spring and early summer (Table 1). However, there were no significant differences in Pn among the four *C. ciliaris* accessions ($p > 0.05$). The highest value of Pn was observed in accession P3 ($42 \mu\text{mol m}^{-2} \text{s}^{-1}$) in March when air temperature was 23°C . The maximum rates of Pn were observed at high temperatures ($> 20^\circ\text{C}$). In winter (December and January), Pn was $< 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ for all accessions. The photosynthetic rate of all accessions decreased with a decline in stomatal conductance (gs). The maximum photosynthetic activity in autumn and spring coincides with the intense vegetative growth phase of the *C. ciliaris* plants. This large decrease in Pn was associated with a cessation of growth and the presence of senescent leaves in this C_4 species, and low temperatures ($< 10^\circ\text{C}$) (Fig. 1).

DISCUSSION

Cenchrus ciliaris is considered to be an excellent grass for pasture in hot dry areas, and it is highly valued for the production of palatable forage and for intermittent grazing during dry periods (Le Houérou, 1991). The life cycle of all accessions would be initiated when the soil water availability during early autumn (September) is adequate (Chaieb *et al.*, 1992). Plant growth responses to arid climate were synchronized in the four accessions, because the species utilize resources in the same way under these experimental conditions. In the arid zone of India, Rao *et al.* (1996) showed that *C. ciliaris* can survive under harsh environmental conditions, with the highest rainfall during the growing season (180 to 250 mm) and in soils with high level of nitrogen. This species has wide ecological amplitude since it can tolerate a variety of climatic conditions, varying from dry sandy areas of Saharans zones to tropical forests (Williams & Baruch, 2000). It responds to high rainfall by increased growth rate and yields. In its natural environment, the vegetative growth of *C. ciliaris* (common for C_4 plants) expands after the first autumn rains (Chaieb *et al.*, 1992). In winter, this species shows a reduction in the vegetative growth with a slight stagnation of leaf elongation and photosynthesis rate. In fact, winter temperatures are very low for C_4 growth and photosynthesis. Wilson & Ludlow (1983) showed that several C_4 grasses were unable to photosynthesise below 10°C . Treharne *et al.* (1971) showed that the optimum temperature for photosynthesis was 35°C for different *C. ciliaris* accessions. In Tunisia, the vegetative growth is intensive for a short period in April, when water reserves are very low. From June, there is a reduction of the main developmental processes that leads to the complete dryness of the aerial parts (Chaieb *et al.*, 1992; Mnif & Chaieb, 2006). Buffel grass grows and persists well in desert habitats due to the species ability to respond with vigorous growth to rainfall events and its tolerance to drought and grazing. The four studied accessions have a bimodal growth in autumn and spring. Autumn growth was much less important for the plant than the spring one. P1 showed an important vegetative growth period and may produce longer shoots and higher leaf area as compared to other accessions (Mnif & Chaieb, 2006). Maroco *et al.* (2000) showed that environmental conditions would be favorable to a C_4 type of photosynthetic metabolism in grasses. However the irregularity of rainfall often results in limitations to soil water availability that

will affect plant growth and survival and may change the length of the growing season.

The *C. ciliaris* accessions did not differ in their photosynthetic responses. In the arid climate of Tunisia, seasonal variations in net photosynthesis (Pn) can be divided into two periods: autumn and spring. *Cenchrus ciliaris* accessions coming from Bou Hedma (P1), Tozeur (P2) Râas Jedir (P3) and Sidi Toui (P4) have a high Pn under the favourable conditions of spring. The growth period (at temperature > 25°C) is also characterized by a high rate of CO₂ assimilation. The decrease in net photosynthesis observed under water deficits is mainly due to the stomatal closure. In the present study, photosynthetic rate (Pn) of all accessions of *C. ciliaris* species decreased with decreasing stomatal conductance (gs), under water stress conditions, which suggests that reduction in photosynthesis might have been due to stomatal factors. It is evident that water deficit caused lower CO₂ (Pn) fixation due to stomatal closure even at moderate water stress, because stomatal closure decreases CO₂ availability in the mesophyll (Athar & Ashraf, 2005). 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). In Pakistan, Akram *et al.* (2007) showed that net CO₂ assimilation rate of the populations of *C. ciliaris* species decreased significantly with an increase in water deficit. However, photosynthetic rates did not differ significantly among populations of *C. ciliaris*. Our results showed that the effect of water and temperature variability on the photosynthetic characteristics of *C. ciliaris* accessions were dependant on season (Table 1). For *C. ciliaris*, Dwyer *et al.* (2007) found a rate of net photosynthesis, 44 µmol CO₂ m⁻² s⁻¹, at a growth temperature of 25°C. Treharne *et al.* (1971) showed that populations of *C. ciliaris* of different origins differed significantly in relative growth rate, net assimilation rate and leaf area ratio.

Similarly to the results with net photosynthesis (Pn), no significant differences between *C. ciliaris* were found in leaf water potential during the growth period, although seasonal variation was quite marked (Table 1). During the whole period of observation, the two water parameters (Ψ_{pd} and Ψ_{md}) showed one period with high values (Ψ_{pd} values of -1.0 MPa and Ψ_{md} values nearly -1.5 MPa) alternating with two periods of low values in which the values of Ψ_{pd} were

about -2.5 MPa and the values of Ψ_{md} were about -3.54 MPa. In this case the Ψ_{pd} and Ψ_{md} curves were positively related to those of the minimum and maximum temperature (Fig. 1). As would be expected, higher water potentials (-1.0 MPa) were observed in winter due to the high level of precipitation that occurred. During the winter period, when leaves are not water stressed through low soil water availability and atmospheric stress, water loss is less due to low temperatures and low leaf to air water vapour pressure deficits. Seasonal water availability was observed to influence the seasonal dynamics of leaf water potential. The small diurnal amplitude in water potential ($\Delta\Psi = \Psi_{pd} - \Psi_{md}$) observed in this study could reflect the ability of *C. ciliaris* to close its stomata and so to control the loss of water by transpiration. It follows that the water potential gradients in the leaf are due to water loss by transpiration. The water potential in the xylem was probably slightly more negative than the pressure chamber value (-7.0 MPa) since recutting the leaf can result in erroneously high water potential values and because the osmotic component of the xylem sap was not taken into account (Ritchie & Hinckley, 1975). These data suggest that these accessions were relatively tolerant, maintaining a substantial positive carbon balance under conditions of severe soil and atmospheric stress. The seasonal trend of the predawn water potential (Ψ_{pd}) and minimum water potential (Ψ_{md}) shows that the four accessions respond quite similarly to the precipitation during the year. In Tunisia, this species is well known for its high adaptive qualities to bioclimatic constraints of the arid and Saharan zones (M'seddi *et al.*, 2002; Mnif *et al.*, 2005). The results of this experiment confirmed that the four *C. ciliaris* accessions coming from different regions and grown under the same conditions, had a similar water and photosynthetic behavior.

CONCLUSIONS

Our results demonstrate remarkably similar physiological responses among *C. ciliaris* accessions of different growth biotopes. During growth period, no significant differences were observed throughout the growing cycle in maximal net photosynthesis rates and stomatal conductance. Our results suggest that *C. ciliaris* accessions will show changes in photosynthetic rates, stomatal conductance and leaf water potential in response to changes in growth temperature and water availability between seasons. There was also correlation between environmental variables (tempe-

perature and water availability), photosynthetic characteristic and water status. *Cenchrus ciliaris* accessions have been developed with increasing growth rates and an increased range of tolerance to different environmental conditions, and for disease resistance. The higher habitual adaptation and tolerance to arid bioclimate, conferred by this *C₄* species is obviously an extremely important feature in arid environments. There is, however a reason to evaluate the water use efficiency (WUE) and to explain differences in productivity and WUE of the four *C. ciliaris* accessions used in this study.

REFERENCES

- Akram NA, Shahbaz M, Ashraf M, 2007. 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. *Pakistan Journal of Botany*, 39: 777-786.
- Aronson J, Kigel J, Shmida A, 1993. Reproductive allocation strategies in desert and Mediterranean populations of annual plants grown with and without water stress. *Oecologia*, 93: 336-342.
- Athar H, Ashraf M, 2005. Photosynthesis under drought stress. In: Pessaraki M, ed. *Handbook of photosynthesis*, 2nd edition. CRC Press, New York: 793-809.
- Chaieb M, Floret C, Le Floc'h E, Pontanier R, 1992. Life History strategies and water-resource allocation in 5 pasture species of the Tunisian arid zone. *Arid Soil Research and Rehabilitation*, 6: 1-10.
- Dwyer SA, Ghannoum O, Nicotra A, Von Caemmerer SV, 2007. High temperature acclimation of C-4 photosynthesis is linked to changes in photosynthetic biochemistry. *Plant Cell and Environment*, 30: 53-66.
- Emberger L, 1954. Une classification biogéographique des climats. *Recueil des Travaux du Laboratoire de Botanique, Univ. Montpellier, Série Botanique*, 7: 3-43.
- Flexas J, Bota J, Cifre J, Escalona JM, Galmés J, Gulías J, Lefi E, Martínez-Cañellas SF, Moreno MT, Ribas-Carbó M, Riera D, Sampol B, Medrano H, 2004. Understanding down regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. *Annals of Applied Biology*, 144: 273-283.
- Floret C, Pontanier R, 1978. *Relation climat-sol-végétation dans quelques formations végétales spontanées du Sud tunisien (production végétale et bilan hydrique des sols)*. Institut des Régions Arides, Médenine. CNRS, Centre d'études phytosociologiques et écologiques, Montpellier et Paris.
- Floret C, Pontanier R, 1984. Aridité climatique, aridité édaphique. *Bulletin de la Société Botanique de France* — *Actualités Botaniques*, 131: 265-275.
- Hatch MD, 1999. *C₄* photosynthesis: a historical overview. In: Sage RF, Monson RK, eds. *C₄ plant biology*. Academic Press, San Diego: 17-46.
- Jessup RW, Burson BL, Burow G, Wang YW, Chang C, Li Z, Paterson AH, Hussey MA, 2003. Segmental allotetraploidy and allelic interactions in buffelgrass (*Pennisetum ciliare* (L.) link syn. *Cenchrus ciliaris* L.) as revealed by genome mapping. *Genome*, 46: 304-313.
- Le Houérou HN, 1984. Rain-use efficiency—a unifying concept in arid land ecology. *Journal of Arid Environments*, 7: 213-247.
- Le Houérou HN, 1991. Forage Species Diversity in Africa: An Overview of the Genetic Resources. *Crop Genetic Resources of Africa*, 1: 99-117.
- Le Houérou HN, 1995. *Bioclimatologie et biogéographie des steppes arides du Nord de l'Afrique: Diversité biologique, développement durable et désertisation*. CIHEAM-IAMM, Montpellier.
- Maroco JP, Pereira JS, Chaves MM, 2000. Growth, photosynthesis and water-use efficiency of two C-4 Sahelian grasses subjected to water deficits. *Journal of Arid Environments*, 45: 119-137.
- Mnif L, Chaieb M, 2006. Comparative phenology and growth of four *Cenchrus ciliaris* L. accessions established under arid bioclimate in Tunisia. *African Journal of Ecology*, 44: 531-536.
- Mnif L, M'seddi K, Chaieb M, 2005. *Cenchrus ciliaris* L. nombres chromosomiques, aptitudes germinatives et reproductives de quelques populations. *Acta Botanica Gallica*, 152: 45-56.
- M'seddi K, Visser M, Neffati M, Rehaul D, Chaieb M, 2002. Seed and spike traits from remnant populations of *Cenchrus ciliaris* L., in South Tunisia: high distinctiveness, no ecotypes. *Journal of Arid Environments*, 50: 309-324.
- Ouercival JM, Floret C, Le Floc'h E, Pontanier R, 1994. Water relations between two perennial species in the steppes of Southern Tunisia. *Journal of Arid Environments*, 28: 333-350.
- Pearcy RW, Ehleringer J, 1984. Comparative ecophysiology of C₃ and C₄ plants. *Plant Cell and Environment*, 7: 1-13.
- Rao AS, Singh K, Wight JR, 1996. Productivity of *Cenchrus ciliaris* in relation to rainfall and fertilization. *Journal of Range Management*, 49: 143-146.
- Ritchie GA, Hinckley TM, 1975. The pressure chamber as an instrument for ecological research. *Advances in Ecological Research*, 9: 165-254.
- Saini ML, Jain P, Joshi UN, 2007. Morphological characteristics and nutritive value of some grass species in an arid ecosystem. *Grass and Forage Science*, 62: 104-108.
- Scholander PF, Bradstreet ED, Hemmingsen EA, Hammel HT, 1965. Sap pressure in vascular plants. *Science*, 148: 339-346.

- Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW, 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature*, 401: 914-917.
- Treharne KJ, Pritchard AJ, Cooper JP, 1971. Variation in photosynthesis and enzyme activity in *Cenchrus ciliaris* L. *Journal of Experimental Botany*, 22: 227-238.
- Turner NC, 1988. Measurement of plant water status by the pressure chamber technique. *Irrigation Science*, 9: 289-308.
- Williams DG, Baruch Z, 2000. African grass invasion in the Americas: ecosystem consequences and the role of ecophysiology. *Biological Invasions*, 2: 123-140.
- Wilson GL, Ludlow MM, 1983. The distribution of leaf photosynthetic activity in a mixed grass-legume pasture canopy. *Photosynthesis Research*, 4: 137-144.