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Morphophysiological Changes in *Cenchrus ciliaris* and *Digitaria commutata* Subjected to Water Stress

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Abstract Among abiotic stresses, water stress is the most environmental constraint for crops, especially in the arid and semi-arid tracts of the world. It reduces growth and development that may cause diverse disturbance in physiological, biochemical and structural integrity of plants. In this view, we assess here drought-induced changes in *Cenchrus ciliaris* and *Digitaria commutata*. Seedlings were grown under two watering regimes for three months. Water stress significantly restricted the photosynthesis and growth activity of both species. Interestingly, water deficit led to a slight decrease of relative water content (RWC). The maximal quantum yield of PSII photochemistry (F_v/F_m) remained unchanged. However, in light-adapted leaves, water deficit reduced the efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m') and the quantum efficiency of the photosystem II (ΦPSII), increased the non-photochemical quenching (q_N) and showed no effects on the photochemical quenching (q_P). These results suggest that *C. ciliaris* and *D. commutata* showed a better aptitude to preserve the PSII functional integrity, therefore a relatively good tolerance to water deficit.

Keywords *Cenchrus ciliaris*, Chlorophyll fluorescence, *Digitaria commutata*, Photosystem, Water stress

1. Introduction

Nowadays, the shortage of water is a serious world-wide problem and it is expected that climate change will accelerate the severity of droughts. UN reports (2006) [1] estimate that one third of world population has been living in areas where the water sources are poor. During the last few decades, water has become an increasingly scarce and precious resource, caused by worldwide climate change and increase in world population, the availability of water will have a greater impact on our ability to produce crops today [2]. Plants, as one of basic food sources, require adequate soil moisture to grow normally and complete its life cycle. It is well known that water stress is one of the most environmental factors limiting crop production worldwide [3]. The water scarcity is the major constraint affecting the survival, growth and plant development [4]. Among the likely mechanisms for decreasing plant development is related to a restriction of photosynthesis, respiration, ion uptake and translocation, as well as the nutrients metabolism and plant growth regulators [5, 6]. Many studies have shown that the photosynthesis reduction under drought can be linked with the biochemical processes disruptions [7]. A previous works demonstrated that water stress has a deleterious effect on the oxygen-evolving complex of photosystem (PSII) [8] and to the PSII reaction centers [9]. PSII photochemistry is hardly affected by water stress [10].

Cenchrus ciliaris and *Digitaria commutata* are perennial grasses common on the arides zones of Tunisia [11]. These species constitute a most important food crop for animals in the pastures. Owing the high degree of complexity in interactions of various factors affecting plant growth, these species would be threatened with extinction. Therefore, it is important to evaluate the impact of water scarcity on plant life.

In this work, we assess the effect of water stress on *Cenchrus ciliaris* and *Digitaria commutata*. A particular attention was paid to plant growth parameters, photosynthetic activity (assessed by gas exchange and chlorophyll fluorescence).

2. Materials and Methods

2.1. Soil Characteristics

The soil used in this study was collected from the horizon 0–20 cm depth from Gafsa, a city in the south-west of Tunisia. The following soil properties were determined: pH (in water) 7.4; K^+ (0.41 μequiv. g^{-1}soil); Na^+ (1.21 μequiv. g^{-1}soil); Ca^{2+} (244.69 μequiv. g^{-1}soil); electric conductivity EC (96.68 μs cm^{-1}); organic matter content (0.87%). Five

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kilogram of dried soil was maintained in an imperforated plastic pot and saturated with tap water for 48 hours. The pot was covered with aluminum foil to prevent loss of water by evaporation. After two days of desiccation in an oven at 100℃, the soil field capacity was measured. The field capacity is the water content held in the soil after 48 hours of dripping of the free water.

2.2. Plant Material and Stress Treatments

In this study, seeds of *Cenchrus ciliaris* and *Digitaria commutata* both species were collected near the National Park of Bouhedma (449 km east of Tunis). Seeds were germinated in plastic pots containing 32 kg of substrate soil (3 plants per pot). Four weeks after germination, the pots were covered with aluminum foil to prevent loss of water by evaporation, thereby accounting for the water lost only by leaf transpiration. Then, 24 plants were subjected to a controlled irrigation treatment (70% of field capacity) for 90 days, which led to substrate water deficit and the other 24 plants to field capacity. The experiment was conducted for a period of three months and it carried out in an open-air area under natural light and ambient temperature, in order as to keep all plants under conditions as similar as possible to those in the field.

2.3. Water Relations

Leaf water potential (Ψ_W) was measured in three randomly selected leaves per plant per treatment, using a pressure chamber model 1000 (PMS Instrument Company, USA). Leaf relative water content (RWC) was measured according to Nauš et al. (2016) [12]. For this purpose, five discs were removed from mature leaves, immediately weighed to obtain fresh mass (MF) and placed under water in the dark for 12 h until full rehydration. The discs were weighed again to obtain the turgid mass (MT) and placed in a forced ventilation oven at 75℃ until constant dry mass (MD). From these variables, the relative water content (RWC) was calculated as:

$$[RWC = ((MF-MD) / (Mt-MD)) x100]$$

Instantaneous and intrinsic water use efficiencies were estimated as the ratios of Pn and E of Pn and g_S, respectively.

2.4. Growth Parameters

The height, total leaf number and leaf area of all plants were evaluated weekly. Individual leaf area (LA) was estimated from sum of measurements of the length of the midrib (L) and maximum width (W) of each leaf, which were used in the equation $LA = (LW)^{0.9660}$ suggested by Pompelli et al. (2012) [13]. The results were summed to obtain the total leaf area.

At the harvest, plants (6 replicates per treatment) were then divided into roots and shoots. Roots were successively rinsed three times with cold distilled water and blotted between two layers of filter paper. The fresh weight was immediately estimated, and the dry weight was measured after 48 h of desiccation in an oven at 60℃.

2.5. Pigment Content

Leaf chlorophyll concentration (6 replicates per treatment) was measured by the absorption spectra of frond extracts using a UV spectrophotometer. Three hundred milligrams of small discs from fresh leaves was extracted in 3 ml 80% acetone and absorbance of extracts was recorded at 470, 646.8 and 663.2 nm [14].

2.6. Photosynthetic Parameter Measurements

2.6.1. Leaf Gas Exchanges

Leaf gas exchange variables were performed at 21, 42, 63 and 90 DAST in fully mature leaves (6 replicates per treatment) using a portable, open-system infrared gas analyzer LCi device under the following conditions: 398 ± 1 ppm CO_2 concentration, 30 ± 0.3℃ leaf temperature, and 1012 mBar atmospheric pressure. Net photosynthetic rate (Pn, µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (g_s, µmol H_2O $m^{-2}s^{-1}$) and the transpiration rate (E, µmol H_2O $m^{-2}s^{-1}$).

2.6.2. Chlorophyll Fluorescence

Chlorophyll fluorescence measurements were also recorded fully mature leaves according to Genty et al. (1989) [15]. Measurements were taken using a portable modulated fluorimeter (PAM-2000, Walz, Germany). Leaves were dark-adapted for at least 20 min using leaf clips. The minimal fluorescence level (F_0) with all PSII reaction centers open was measured by the measuring modulated light which was sufficiently low not to induce any significant variable fluorescence. The maximal fluorescence level (Fm) with all PSII reaction centers closed was determined by a 0.8 s saturating pulse at 8000 mmol m^{-2} s^{-1} in dark-adapted leaves. The leaf disc was then continuously illuminated with white actinic light at an intensity of 180 mmol m^{-2} s^{-1} which was equivalent to growth PPFD of wheat seedlings in the growth chamber. The steady-state value of fluorescence (F_s) was thereafter recorded and a second saturating pulse at 8000 mmol m^{-2} s^{-1} was imposed to determine the maximal fluorescence level in light-adapted leaves (F_m'). The actinic light was removed and the minimal fluorescence level in the light-Plant material and stress treatments adapted state (F_0') was determined by illuminating the leaf disc with 3 s far-red light. Then, the fluorescence parameters determined in both light and dark-adapted were used to calculate the maximal quantum yield of PSII photochemistry, (F_v/F_m), the photochemical quenching coefficient, $q_p = F_m' - F_s/(F_m' - F_0')$ and the non-photochemical quenching coefficient, $q_N = 1 - (F_m' - F_0')/Fm - F_0)$ which is linearly related to heat dissipation [16] the efficiency of PSII reaction centers, F_v'/Fm' and the actual quantum yield of PSII electron transport, ΦPSII [16].

2.7. Statistical Analysis

ANOVA with orthogonal contrasts and mean comparison

procedures were used to detect differences between species and water regimes. Mean separation procedures were conducted using Duncan's multiple range tests with least significant difference (LSD) ($P < 0.05$).

3. Results

3.1. Water Status

A significant reduction ($p<0.05$) of the leaf water potential was observed from 21 days after starting treatment in water-stressed plants of *Cenchrus ciliaris* and *Digitaria commutata* (Fig.1). In both species, the relative water content of leaves water stress decreased slightly (Fig.2).

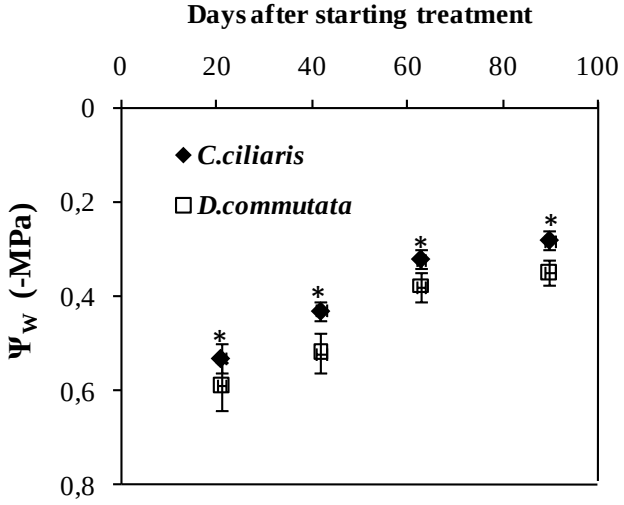

Figure 1. Leaf water potential (Ψw) of *Cenchrus ciliaris* and *Digitaria commutata* plants exposed to water stress during 90 days. Means (n = 6 per treatment ± SE) with at least one same letter are not significantly different at $P \leq 0.05$

3.2. Gas Exchange

The photosynthetic activity of water-stressed *Cenchrus ciliaris* and *Digitaria commutata* plants was adversely impacted as reflected by the significant decline of the main gas exchange parameters (Fig. 3). Net photosynthetic rate (Fig.3A-B), stomatal conductance (Fig.3 C-D) and the transpiration rate (Fig.3 E-F) were significantly ($p<0.05$) reduced in both species by the water stress treatment (Fig.3A-B-C). In *Cenchrus ciliaris*, Pn, g_s and E values recorded at the 63th day were, respectively, 32, 55 and 65% lower than those of the control. For *Digitaria commutata*, water deficit led to Pn, g_s and E reductions of 30, 59 and 56% respectively. There were no significant differences between species for the intrinsic (Pn/g_s) and instantaneous (Pn/E) water use efficiencies (Fig. 4). A significant ($p<0.05$) increase of Pn/g_s was observed in water stressed plants of both species (Figure 4A-B).The Pn/E shows a similar trend for both species, with a significant increase, especially at the 63 and 90th days after starting treatment (Fig. 4C-D).

Water-stressed plants generally displayed different chlorophyll fluorescence emission patterns, which was also dependent on the species investigated. As observed for gas exchange, water-stress related changes were reported (table 1). For both species, the maximal quantum yield of PSII photochemistry (F_v/F_m) measured in dark-adapted remained constant. Though F_v/F_m is frequently used to evaluate the maximal quantum yield of PSII photochemistry and shows no change during water stress, it gives no direct information on the heterogeneity of PSII reaction centers. Table 1 shows that water stress decreased the efficiency of excitation energy capture by open PSII reaction centers (F_v'/Fm') and the quantum yield of PSII electron transport ΦPSII, increased the non-photochemical quenching coefficient (q_N). Whereas, it had no effects on the photo chemical quenching coefficient (q_p).

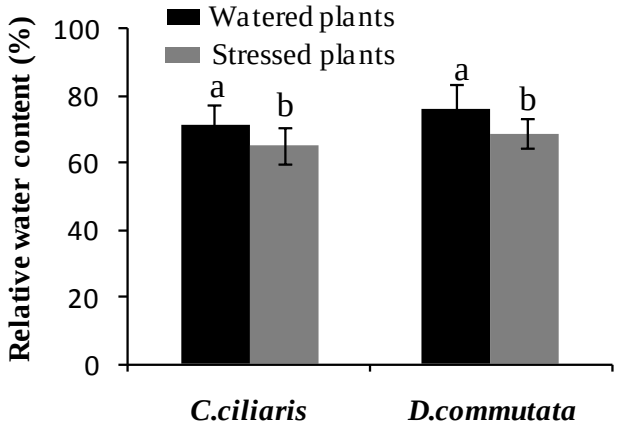

Figure 2. Relative water content (A) and Hydraulic conductivity (B) of *Cenchrus ciliaris* and *Digitaria* submitted to water stress during 90 days. Data refer to mean values of 6 repetitions and the bars indicate the standard error of the mean

Table 1. Effects of water stress on the maximal quantum yield of photochemistry of PSII (*Fv/Fm*), electron transport (*Φ*PSII) of PSII in light-adapted leaves which were illuminated by white actinic light at an intensity of 180 mmol m^{-2} s^{-1}, photochemical (q_P) and non-photchemical (q_N) quenching, the efficiency of excitation capture by open PSII reaction centers (F_v'/F_m') and the actual quantum yield: values are means ±SE of six replicates

Treatment	F_v/F_m	*ΦPSII*	q_P	q_N	*F'v/F'm*
C. ciliaris					
Well-watered	0.82±0.007a	0.62±0.008a	0.79±0.007a	0.34±0.003a	0.76±0.008a
stressed	0.81±0.005ab	0.51±0.004c	0.77±0.006ab	0.39±0.003b	0.68±0.005b
D. commutata					
Well-watered	0.81±0.008a	0.61±0.007a	0.78±0.008a	0.33±0.002a	0.75±0.006a
stressed	0.79±0.006ab	0.53±0.004b	0.79±0.005ab	0.45±0.004c	0.62±0.005c

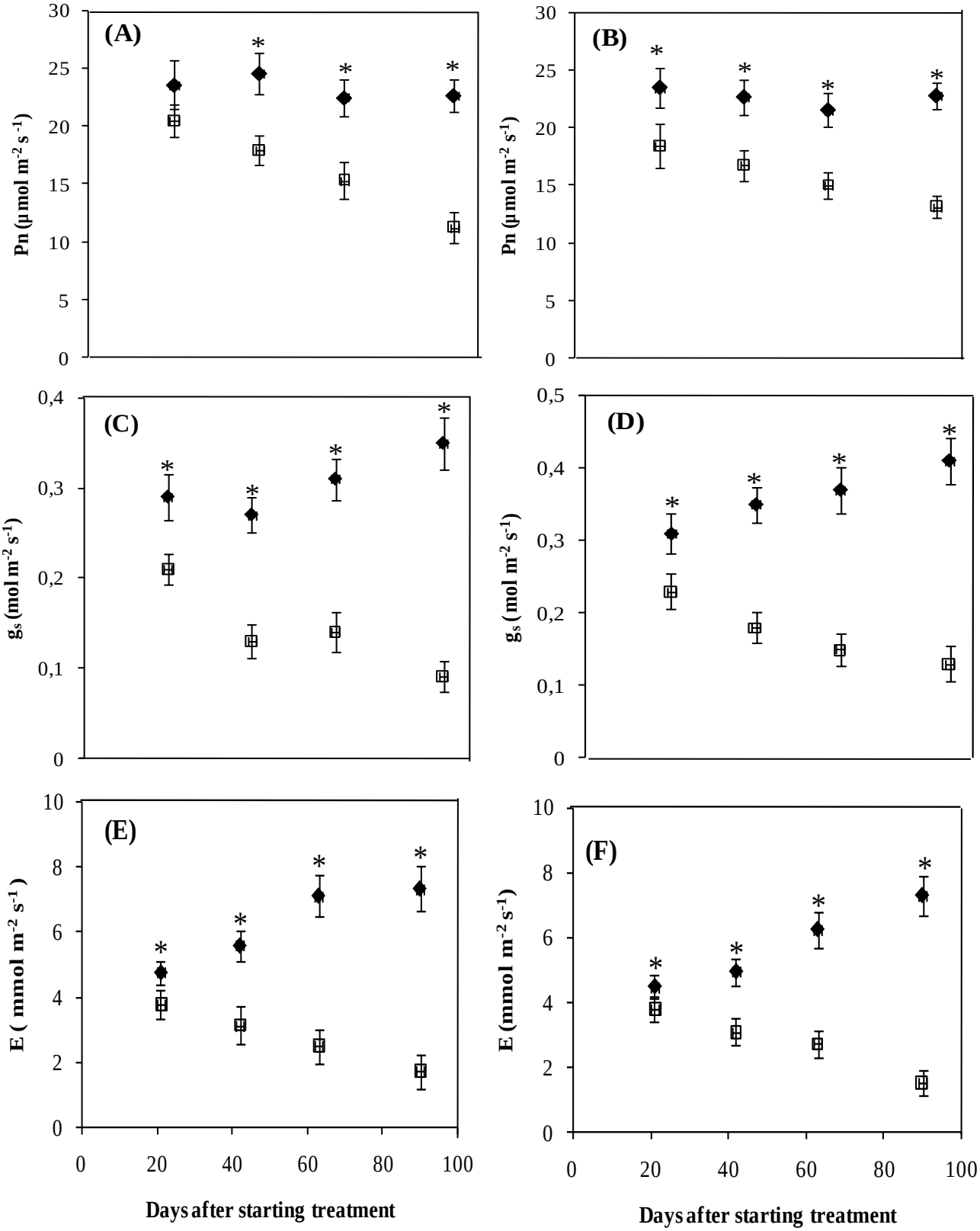

Figure 3. Effect of water stress on the net photosynthesis (A-B), stomatal conductance (C-D), the transpiration rate (E-F) of *Cenchrus ciliaris* (A, C and B) and *Digitaria commutata*. (B, D and F) under control treatment (filled diamonds) and water stress (open diamonds). Means (n = 6 per treatment ± SE) values are means ±SE of six replicates

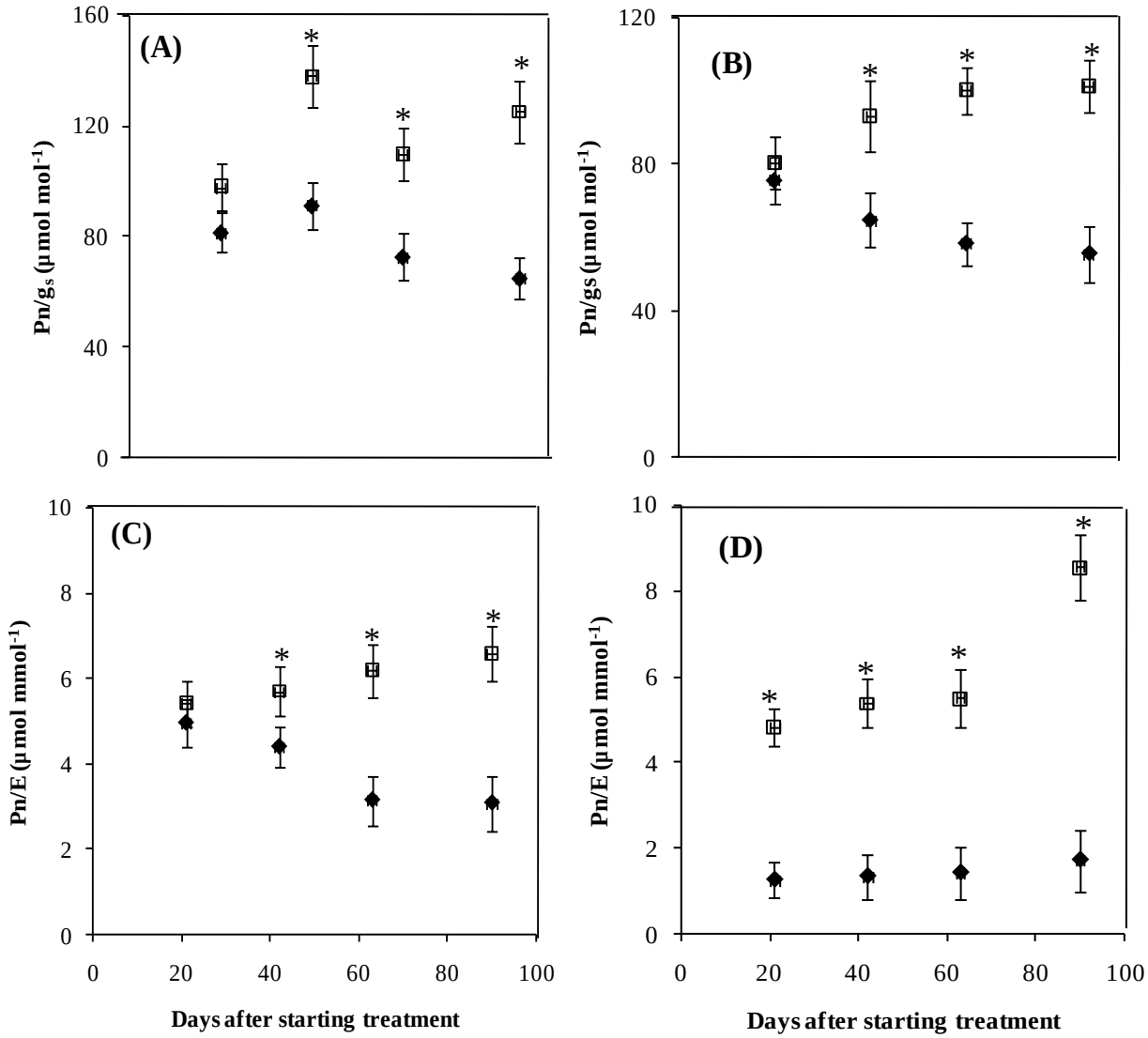

Figure 4. Intrinsic (Pn/g_s) and instantaneous (Pn/E) water use efficiency in *Cenchrus ciliaris* (A-C) and *Digitaria commutata* (B-D) under control treatment (filled diamonds) and water stress (open diamonds). Means (n = 6 per treatment ± SE) with at least one same letter are not significantly different at $P \leq 0.05$

Table 2. Effects of water stress leaf chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (total Chl), and carotenoid (Car) concentrations of *Cenchrus ciliaris* and *Digitaria commutata* plants. Means (n = 6 per treatment ± SE) followed by the same letters are not significantly different at $P \leq 0.05$

Treatment	Ch a (mg /g FW)	Chl b (mg/g FW)	Ch_T (mg/g FW)	Car (mg/g FW)
C. ciliaris				
Well-watered	0.72±0.004a	0.61±0.003a	1.49±0.007a	0.044±0.002a
stressed	0.59±0.002b	0.41±0.004b	1.07±0.005c	0.066±0.003c
D. commutata				
Well-watered	0.64±0.003a	0.58±0.003	1.28±0.009a	0.053±0.002a
stressed	0.55±0.002b	0.38±0.002	0.98±0.006c	0.075±0.004c

3.3. Photosynthetic Pigments

Water stress decreased chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl_T) content in water-stressed *Cenchrus ciliaris* and *Digitaria commutata* plants (Table 2). This was associated with a significant increase of the carotenoid concentration, in both species, especially at the 42 and 63th days after starting treatment.

3.4. Growth

Water stress exposure of *Cenchrus ciliaris* and *Digitaria commutata* plants induced early morpho-phytotoxicity symptoms. In both species, there was a general decrease in the shoot heights of the plants in the different water treatments (Fig. 5A). There was a significant difference ($P<0.05$) between the shoot height of the water-stressed plants and the well-watered plants from the beginning of the experiment until the last day of the experiment. Both root and shoot biomass decreased significantly in both species with increasing water deficit (Fig. 5B-C). As a result, the whole plant biomass production of both species was adversely affected by water stress (Fig. 5D). Numbers of leaves and leaf area were also significantly reduced in both species following water stress (Fig.5E-F).

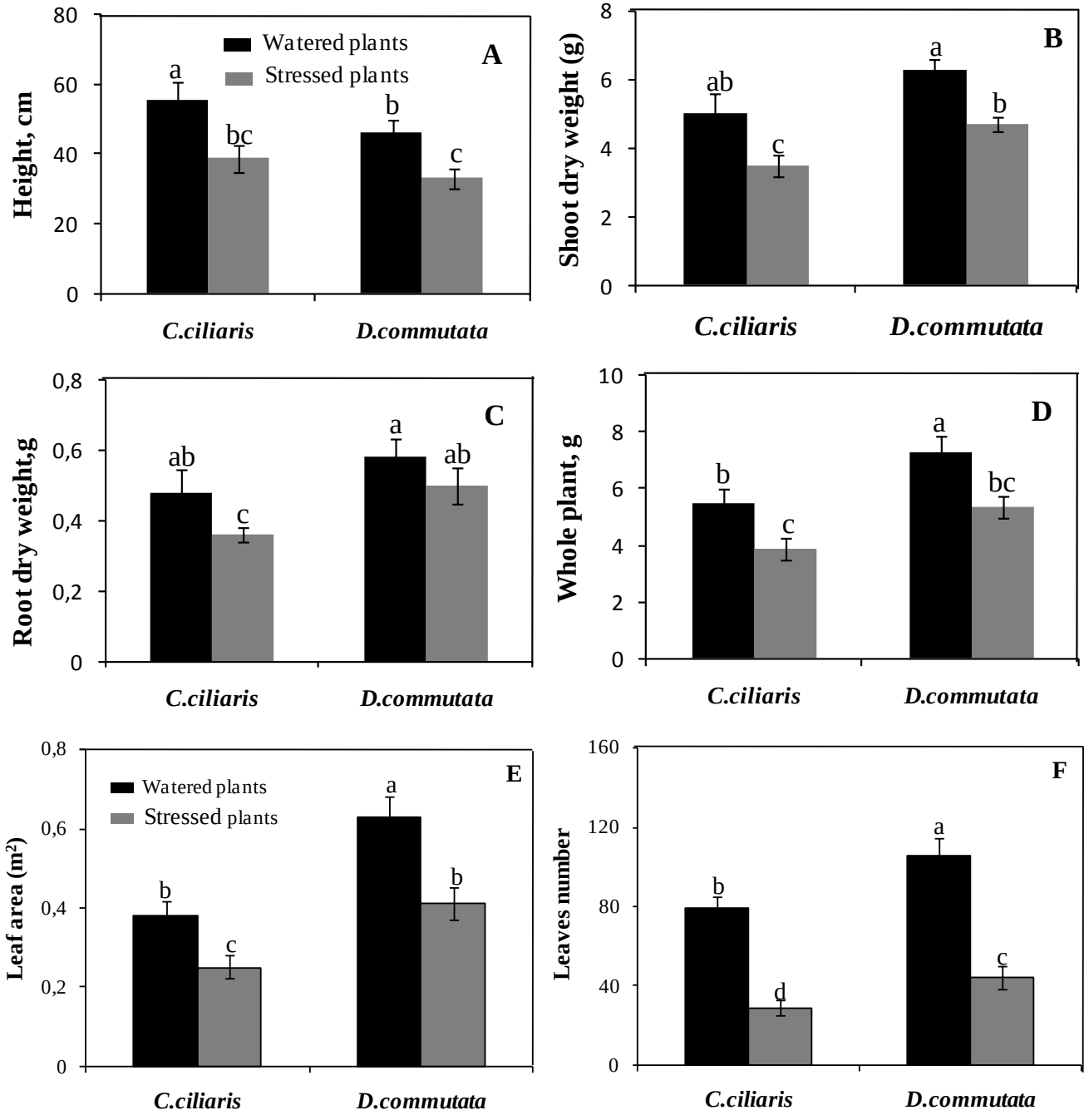

Figure 5. Effect of water stress on the height (A), shoot biomass (B), root biomass (C) the whole plant dry weight (D), Leaf area (E) and leaves number (F) of *Cenchrus ciliaris* and *Digitaria commutata*. Means (n = 6 per treatment ± SE) with at least one same letter are not significantly different at $P \leq 0.05$

4. Discussion

Cenchrus ciliaris and *Digitaria commutata* has been described as being adapted to arid climates and dry-land agricultural ecosystems [11]. It is incontestable that under adequate soil moisture conditions, plants species will show higher productivity. Nevertheless, plants developed a wide range of strategies, at morphological, anatomical and cellular levels to cope with water stress [17]. Among mechanisms that allow the plant to avoid the stress or to increase its tolerance, the reduction of the leaf water potential is a common way maintaining cell function through elevated relative water content and stomatal closure [17]. In the present study, there is a slight decrease in both Ψw and the relative water content (Fig. 1 and 2). A similar result has been reported by Fini et al. (2013) [18] in *Jatropha curcas* exposed to water deficit. The conservation of the water content in *C. ciliaris* and *D. commutata* can be considered a strategy to avoid dryness in plant tissues. It was assumed that osmotic adjustment is responsible for maintaining an adequate RWC in plants [19]. The water use efficiency was increased in *C. ciliaris* and *D. commutata* plants (Fig. 4). This is consistent with other studies showing that maintain adequate water use efficiency under drought with several physiological traits could be linked to water and carbon balances [20].

Photosynthesis is vital metabolic process for plant development and productivity, which is known to be severely impacted by biotic and abiotic constraints. Gaining a better understanding of how environmental stresses affect the plant physiological status, either in field studies or under controlled conditions especially imposes a reliable diagnosis of the photosynthetic functioning. Continuous technical improvements allowed the utilization of simple, quick and non-destructive portable devices like Chlorophyll fluorescence and gas exchange [16]. Water stress is one of the most factors inhibiting photosynthesis [8, 10]. In our experiment, the decline of foliar photosynthetic rate (Fig. 3A-B) in *C. ciliaris* and *D. commutata* was associated with a parallel decrease in stomatal conductance (Fig. 3C-D). The transpiration rate decreased also significantly in both species (Fig. 3E-F). According to the literature, the simplest explanation for the reduction of photosynthetic activity during water stress would be that the stomatal conductance and the internal CO_2 concentration decrease, following closure of stomata [21]. These authors have reported that stomatal conductance is more reduced when a plant is stressed. Photosynthesis decline under drought through metabolic impairment is a complex phenomenon than stomatal limitation [22]. It could be due to the biochemical processes perturbations [22].

Chlorophyll fluorescence data showed changes of photochemical activity in water-stressed plant of *C. ciliaris* and *D. commutata* (table 1). The maximal quantum yield of PSII photochemistry (F_v/F_m) remained almost constant during the time course of water stress treatment since F_v/F_m was close to 0.80, a value typical of healthy plants [16]. The quantum yield of PSII electron transport (ΦPSII) declined significantly. The decreased of ΦPSII could be due to the reduction in the efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m') because of no change in photochemical quenching (q_P) [15] (Table 1). According to Baker (1991) [23] the decrease in F_v'/F_m' may reflect light-induced non photochemical quenching. Results show that water stress induced an increase in q_N. This increased q_N would dissipate some excitation energy at the photochemical utilization expense [24]. Therefore, high q_N in water-stressed conditions was described in some plants as a regulatory mechanism to down-regulate photosynthetic electron transport so that production of ATP and NADPH would match with the decreased CO_2 assimilation due to the closure of stomata [7].

Photosynthetic pigments are important to plants mainly for collecting light and may be used as indicators of water stress damage. They may predict subsequent events at the organism level [21]. In this study, water stress produced changes in chlorophyll content (Chl a, b and Ch_T) (Table 2). The chlorophyll tissue concentrations were decreased in stressed plants of of *C. ciliaris* and *D. commutata*. Similar results have been reported in drought stressed *Catharanthus roseus* seedlings [5]. The water stressed plants of both species showed significantly higher carotenoids concentration as compared to the control (table 2). The increase of carotenoids content in plant was considered an important process to alleviate water stress [25].

It has been established that drought stress is a very important limiting constraint in plant growth and establishment. It greatly suppresses both elongation and expansion growth [4]. In the present study, water deficit negatively affected the plant growth in both *C. ciliaris* and *D. commutata* (Fig.5). Biomass productivity of shoots and roots, were significantly reduced in response to water stress, with shoot biomass being more impacted than root biomass (Fig. 5B-C). Significant reduction of the height plant was also observed (Fig. 5A). This result was similar to previous studies showing the decrease of the stem length and the plant biomass production under drought stress [26].

In our experiment, water deficit reduced numbers of leaves and in turn the leaf area (Fig. 5E-F). A similar trend was found by Akıncı and Lösel (2010) [6] in cotton plants and some *Cucurbitaceae* members. Although, the development of optimal leaf area is essential to photosynthesis and dry matter yield. Reduction in leaf area by rolling may also be important in controlling water loss and reflects changes in leaf turgor [27]. Leaf adaptations were considered a tolerance mechanism favoring the success of plants and allowing the resistance to drought [28]. Some authors pointed out that, in some species any reduction in cell size, due to loss of turgor during expansion, will lead to a higher stomatal frequency [29]. It is also worth mentioning that plants, under severe drought conditions, tend to develop morphological characters namely increases in proportion of leaf vein tissue compared to leaf area, increased stomatal numbers per unit of leaf surface, smaller sizes of stomata,

epidermal and mesophyll cells, greater density of leaf hairs but smaller hairs, thicker outer epidermal walls and cuticle [30]. In addition, several physiological and biochemical mechanisms could govern drought tolerance in plants.

5. Conclusions

Our finding indicated that water stress negatively affect the gas exchange and the growth activity of both *C. ciliaris* and *D. commutata*. Both species showed a better aptitude to preserve the PSII functional integrity when challenged with water deficit. The maintenance of an adequate relative water content and water potential suggests that both species are water savers. The morphological change, especially reduction of leaves numbers and leaf area, under water stress, can be also considered as an eventual strategy to control water loss.

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