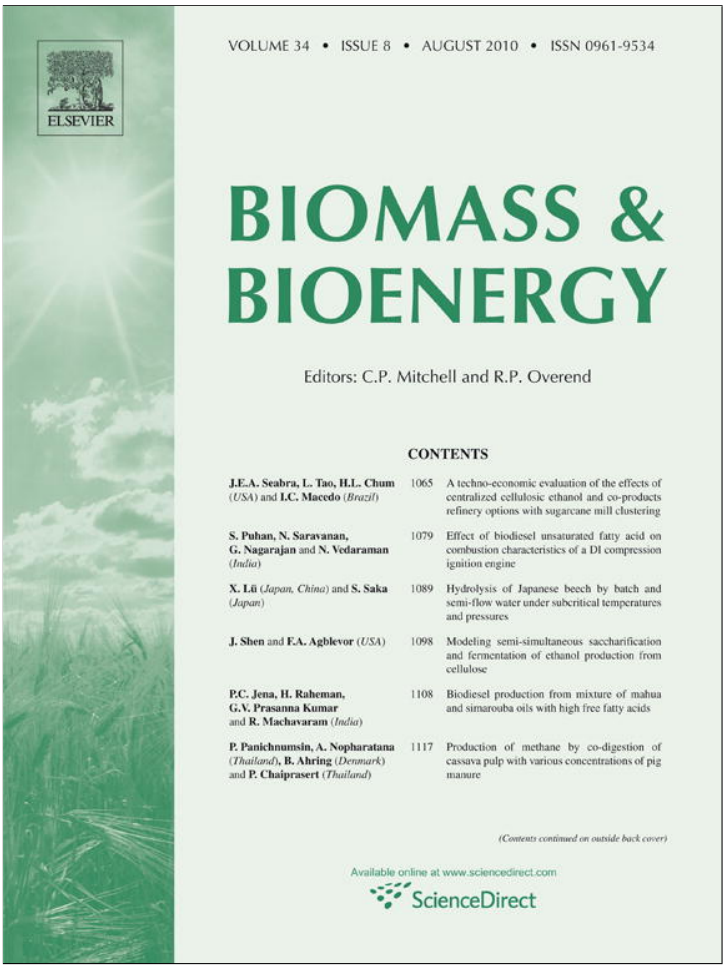

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Photosynthesis, photoprotection and antioxidant activity of purging nut under drought deficit and recovery

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ABSTRACT

Biodiesel is an alternative to petroleum diesel fuel. It is a renewable, a biodegradable, and also a non-toxic fuel. The general interest to produce biodiesel from *Jatropha* (*Jatropha curcas* L.) seeds oil has increased but its ability to grow on drought-prone areas has barely been investigated. The objective of this work was to identify some physiological processes that allow the Jatropha to produce in severe arid conditions by studying its leaf gas exchange and antioxidant systems under drought stress and recovering. It measured the activity of antioxidant enzymes involved in the scavenge of reactive oxygen species (ROS), as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutamine synthetase (GS), as well as malondialdehyde (MDA) content. It was also analyzed the chlorophyll (CHL), carotenoids, amino acids and soluble proteins contents. Net photosynthesis (*A*) and stomata conductance (g_s) decreased associate with drought stress and dropped to zero in soil water beneath 5%. Drought induced decrease in stomatal and non-stomatal photosynthetic activity. The activities of SOD, CAT, APX and GS and MDA content in leaves were significantly higher in the water-stressed plants compared to well-watered plants and decreased when the plants were rewatered. These observations suggest that oxidative stress resulting from drought deficit in Jatropha could result in the production of antioxidative enzymes to counteract the oxidative damage, and the enzymes may contribute to its ability to survive in the adverse arid environment.

1. Introduction

Jatropha curcas is until now an undomesticated plant, which can grow without irrigation in a broad spectrum of rainfall regimes, from 250 up to 3000 mm per year [1,2]. This species grows well on poor stony soils and therefore, it is recommended for cultivation on poor degraded soils [3] as a multipurpose tree for cultivation in tropical and subtropical regions of the world [4].

Biofuel, an alternative to petroleum diesel fuel, has attracted considerable attention during the past decade as a renewable, biodegradable, and non-toxic fuel. Perennial plants, as Jatropha, have been attracting increasing attention

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as alternative source of biodiesel. Jatropha seeds contain viscous, non-edible oil, used as a substitute for diesel (hence called biodiesel). Biodiesel production from oil of Jatropha seeds has become a booming business. The oil produced by this crop can be easily converted to liquid bio-fuel, which meets the American and European standards [5]. Additionally, the press cake can be used as a fertilizer and the organic waste products can be digested to produce biogas (CH_4) [6].

Soil water shortage is the most limiting environmental factor limiting for plant growth and yield in most parts of the world [7]. Drought-related reduction of plant growth and yield is largely owed to stomatal closure in response to low soil water content, which decreases the intake of CO_2 and, as a result, decreases photosynthesis. A consequence of the drought-induced limitation of photosynthesis is the exposure of plants to excess energy, which, if not safely dissipated, may be harmful to photosystem (PS) II because of over reduction of the reaction centers [8] and increased production of reactive oxygen species (ROS) in the chloroplasts [9]. In plants, lipids present in the thylakoids contain a high percentage of poly-unsaturated fatty acid residues and are thus susceptible to peroxidation [10]. The content of MDA, a product of lipid peroxidation, has been considered an indicator of oxidative damage [11]. In fact, MDA is one of the ultimate products as a result of lipid peroxidation damage by free radicals.

Stomatal regulation of photosynthesis during drought stress conditions has been well documented [12,13]. Levels of light which are optimal for photosynthesis in well-watered plants become excessive in plants suffering water deprivation [14]. Photosynthesis is particularly sensitive to water deficit because the stomata close to conserve water as available soil water declines. Stomatal closure deprives the leaves of carbon dioxide and photosynthetic carbon assimilation is decreased in favor of photorespiration. Moreover, oxygen uptake loading on the tissues as both processes generate reactive oxygen species, particularly H_2O_2 that produced at very high rates by the glycollate oxidase reaction in the peroxisomes in photorespiration [15].

This study had the general objective to evaluate the effects of water deficit on the photosynthesis and the photo-protection system. The relative importance of stomatal and non-stomatal limitations to photosynthesis was determined on well-watered, severely drought-stressed and rewatered plants. The specific objectives of this study were: (i) to identify physiological processes that allows Jatropha to resist a severe drought conditions, (ii) to investigate the capacity of Jatropha from recover after a severe drought, and (iii) to investigate the effect of drought stress on the plant antioxidant systems thus determining the importance of these antioxidant systems in conferring tolerance on drought stress.

2. Material and methods

2.1. Plant materials and growth conditions

Pot experiments were carried out in a glasshouse at the experimental station of the Federal University of Alagoas, located at Rio Largo city (9°27′55″ S, 35°49′31″ W, 130 m a.s.l) during the months of May and June of 2008. Jatropha seeds were planted and grown in 10 L of soil and filter cake from sugar mill industries (3:1, w w^{-1}) under natural photoperiod. The daily maximum and minimum air temperatures were registered throughout the experiment and are shown in Fig. 1.

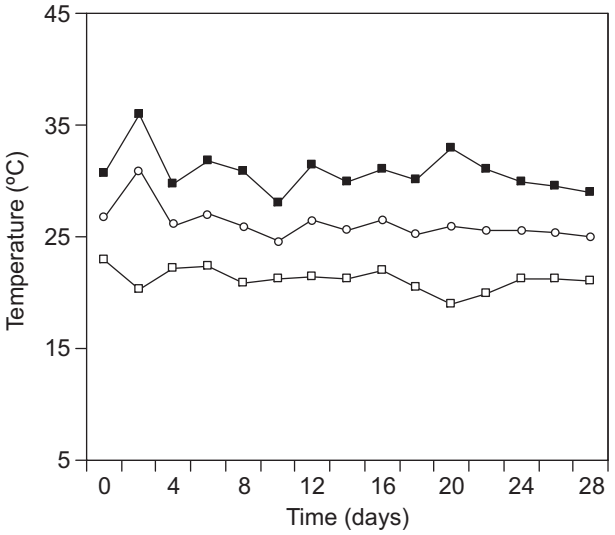

Fig. 1 – Maximum (-■-), minimum (-□-) and mean (-○-) temperature during the experiment.

Eleven-month-old plants of uniform height were selected for all experiments. Plants were subjected to water deficit by withholding irrigation to 4, 8 and 18 days and rewatering afterward for recovering from the stress. Control plants were maintained well watered. It was used at least five plants for each treatment. All experiments were performed using completely expanded leaves from the third or fourth leaf from the top of the orthotropic axis of the plants. Water potential of soil was monitored every 2 days by digital thermo-higrometer (Soil Moisture Sensor – SM200, Delta-T Devices, Cambridge, UK).

2.2. Measurements of photosynthesis

Every other day, during the drought stress period and after soil rewatering, net photosynthesis (A), stomatal conductance (g_s), transpiration (E), and internal CO_2 concentration (C_i) of leaves were measured using the LCi portable gas exchange system (ADC BioScientific Ltd, Hoddesdon, UK) under 1080 μmol (photons) m^{-2} s^{-1} light intensity at 8:00–10:00 am. Photosynthesis measurements were taken on five fully expanded main-stem leaves. Intrinsic water use efficiency (WUE_i) was calculated as the A/E ratio.

2.3. Biochemical measurements

Leaves were sampled at 0, 4, 8 and 18 days after initiation of drought stress, and after its recovery (i.e., at least 6 days), between 9:30 and 10:30 am. A fully expanded leaf from each plant was detached and immediately immersed in liquid nitrogen and stored in freezer (−20 °C) until used. The extraction of enzyme from the tissues was thoroughly ground with a cold mortar and pestle in an ice bath, until no fibrous residue could be seen. The extraction of superoxide dismutase (SOD) was done by the grinding medium consisted of 0.1 mol

K-phosphate (pH 7.8), 0.0001 mol EDTA, 0.001 mol dithiothreitol, 0.01 mol β-mercaptoethanol, 0.1% (w w^{-1}) triton X100 and 1% (w w^{-1}) polyvinylpyrrolidone. To extraction of catalase (CAT) and ascorbate peroxidase (APX) the grinding medium consisted of 0.05 mol K-phosphate (pH 7.5), 0.0001 mol H_2O_2 and 0.0005 mol sodium ascorbate. To extraction of glutamine synthetase (GS) the grinding medium consisted of 0.05 mol Tris–HCl buffer (pH 7.9), 0.02 mol $MgSO_4$ and 0.07 mol β-mercaptoethanol. The homogenate was centrifuged at 15000×*g* for 15 min in a refrigerated centrifuge at 4 °C. The supernatant was used for determination of the activity of these enzymes.

The samples were homogenized in 2 mL of 0.1% (w w^{-1}) trichloroacetic acid and centrifuged at 10000×*g* for 15 min to determine malondialdehyde (MDA). MDA of extracts was determined by the thiobarbituric acid reaction [10].

2.4. *Enzyme activity assay*

A total activity of SOD (EC 1.1.5.1.1) was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The 3 mL reaction mixture contained 75 μmol NBT, 2 μmol riboflavin, 0.013 mol methionine, 0.1 μmol EDTA, 0.05 mol K-phosphate buffer (pH 7.8), and 5 mm^{-3} enzyme extract. The test tubes containing the mixture were placed in a box 3.5 cm from two fluorescent lamps at 40 W, receiving 130 μmol (photons) m^{-2} s^{-1} of photosynthetic active radiation (PAR) at reaction tube surface. The reaction was started by switching on the light and run for 10 min. The reaction was stopped by switching off the light and then the absorbance at 560 nm was recorded. A non-irradiated reaction mixture that did not develop color served as the control, and its absorbance was subtracted from sample measurements absorbance at A_{560}. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction.

Activities of CAT (EC 1.11.1.6) and APX (EC 1.11.1.11) were measured to decomposition of H_2O_2 or ascorbate respectively. The decomposition of H_2O_2 to CAT was measured by the decline in absorbance at 240 nm for 1 min. The 1 mL reaction mixture contained 0.05 mol K-phosphate buffer (pH 7.5), 0.0125 mol H_2O_2, and 30 mm^{-3} enzyme extract. The decomposition of H_2O_2 to APX was measured by the decline in absorbance at 290 nm for 1 min. The 1 mL reaction mixture contained 0.05 mol K-phosphate buffer (pH 7.5), 0.0001 mol H_2O_2, 0.0005 mol sodium ascorbate and 25 mm^{-3} enzyme extract. The reactions were initiated with enzyme extract. One unit of CAT or APX was defined as the amount of enzyme required to oxidize 1 μmol of H_2O_2 or ascorbate per minute.

Activities of GS (EC 6.3.1.2) were measured by production of γ-glutamylhydroxamate detected in absorbance at 530 nm. The 1.2 mL reaction mixture contained 240 μmol L-glutamate, 18 μmol hydroxylamine, 24 μmol ATP, 600 μmol tricine buffer (pH 7.8), 12 μmol $MgSO_4$, 45 μmol 2-mercaptoethanol. The reaction was started by adding 60 mm^{-3} enzyme extract and after 20 min at 30 °C the reaction was stopped by $FeCl_3$ addition. One unit of GS was defined as the amount of enzyme required to produce 0.001 mol of γ-glutamylhydroxamate per hour, by the use of extinction coefficient 0.532 μmol L^{-1} cm^{-1}.

Amino acids and protein contents of crude extracts were determined according to Moore and Stein [16] and Bradford [17] methods respectively. Chlorophyll and carotenoid content in extracts were determined spectrophotometrically according to Lichtenthaler [18].

2.5. *Statistical analysis*

Results were analyzed by a mixed-model ANOVA and means were compared by Newman–Keuls test using Statgraphics Plus Version 5.1 (StatPoint, Inc., Herndon, Virginia, USA). The correlation coefficients were calculated by Statistica 7.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

3. Results

The stomatal conductance (g_s) was well related to soil water content (Fig. 2). The soil lost most of its water in the first four days, after that soil dryness rate was reduced. The stomatal conductance reduced to almost zero in four days after withholding the irrigation, remaining so until rewatering. Even recovering was faster when a shorter stress was given (Fig. 2A), no more than three days were necessary for stomatal conductance recovery when plants were expose to long period of drought (Fig. 2C). When considered all data, the stomatal conductance had a high correlation to soil water content ($R^2 = 0.700$, $p < 0.001$, $n = 480$) (data not shown). This correlation increased when the plants were submitted to a long period of drought ($R^2 = 0.89$, $p < 0.001$, $n = 120$) (Fig. 2C).

Leaf transpiration (*E*) was very efficiently regulated by g_s under drought stress conditions (Fig. 2). Under stress conditions, the correlation coefficients between g_s and *E* were 0.69, 0.81 and 0.95 ($p < 0.001$, $n = 480$) in seedlings under 4, 8 and 18 days of stressed periods, respectively, while leaf-to-air pressure vapour deficit had only a slight effect over well water seedlings with weak correlation between the two parameters in this situation (data not shown).

In stress period, *A* decreased significantly, however *A* values below 5 μmol CO_2 m^{-2} s^{-1} were observed only when the soil content decreased to below 5% (Figs. 2 and 3). When withdraw the pot irrigation, g_s and *A* had a strong correlation to soil water content. Once rewatered, g_s, and *A* recovered fully at least in 4 days (Fig. 3) and overall remain higher than unstressed plants. At all soil moisture levels, changes in C_i were apparently attributable to both changes in stomatal conductance and chloroplast activity. Although a large decrease in g_s under drought-stressed conditions was observed (Fig. 2), C_i remained the same as that observed in well-watered and rewatered seedlings (data not shown), indicating that the stomatal and mesophyll components of photosynthesis were equally affected.

Severe stress inhibited g_s at least 99%, which was probably attributable at least in part to CO_2 withdrawal in mesophyll; consequently *A* was lowering until 98.0% (Fig. 3). There was no significant correlation between *A* and g_s in well-watered plants but when plants were drought stressed it was a strong correlation emerged with coefficient correlation of 0.86, 0.90, and 0.93 when irrigation was interrupted for 4, 8 and 18 days, respectively. The stomata closure cause a lowering of

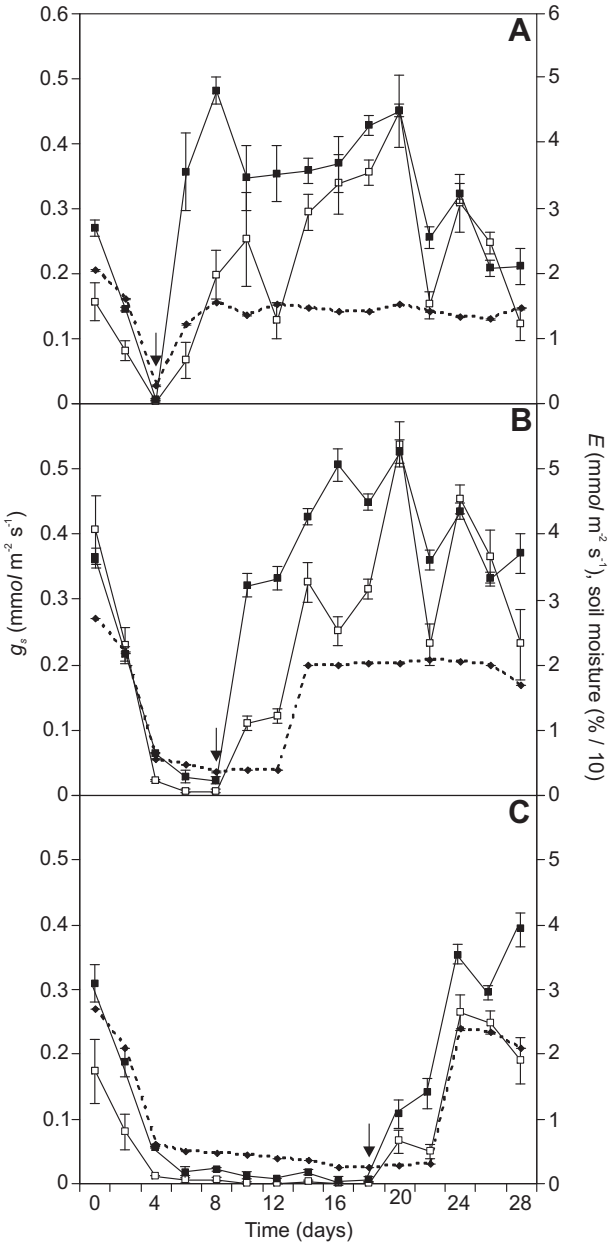

Fig. 2 – Effect of drought stress on soil moisture (-◆-), stomatal conductance (-□-) and transpiration (-■-) in *Jatropha curcas* L. subjected to drought stress by withholding irrigation to 4 (A), 8 (B), and 18 (C) days and rewatered subsequently and watered every 2 days of the recovery period. Arrows indicate rewatering of soil $n = 10$.

WUE_i, respectively, in 77%, 95%, and 67% in 4, 8, and 18 days of drought stress (Fig. 4). A/C_i rate reveal the same pattern of response. This seemed to indicate that the reductions in A were due to both decrease in g_s and direct drought stress effect on photosynthetic apparatus.

Senescence-induced loss of CHL, amino acids, and protein was more distinct in 8 and 18 days than in 4 days (Table 1). Drought stress treatment aggravated these losses and the highest decline of amino acids was observed in plants experiencing the drought stress of 8 days to amino acids, while the proteins losses were more significant in plants submitted to 18 days. By contrast, carotenoids pool increased in plants submitted to 4 and 8 days of drought stress while in plants submitted to 18 days their content has been reduced.

The drought stress induced the antioxidant enzymes SOD, CAT, and APX in leaves of Jatropha (Table 2). The activities of SOD, CAT, and APX in plants submitted to 8 and 18 days were more evident than plants submitted to 4 days. The SOD activity also rose in recovery period; however only in 8-day plants the activity were significantly different of control plants. The CAT activity, in this time, was elevated in plants submitted to 4 and 18 days of stress, while no alteration was verified in 8-day stressed plants. In the drought stress period, the APX activity was not affected in 4-and 8-day plants while in 18-day plants its activity has decreased. Compared to full water supply, the activity of SOD, CAT, and APX in plants under stress has increased significantly. The increased of SOD was 49.5%, 192.5%, and 72.7% at 4, 8, and 18 days of drought stress, respectively. The activities of CAT and APX followed the same tendency: 40.0%, and 20.0% to CAT and 64.0%, and 45.0% to APX at 8 and 18 days of drought stress, respectively. In plants submitted to 4 days of drought stress the CAT and APX activities was not significantly affected. The GS activity rose significantly only in 18-day stressed plants.

Under drought stress, the MDA content increased as drought stress intensity progresses, i.e., 18%, 26%, and 57% at 4, 8, and 18 days of stress, respectively (Table 2). The MDA content reached 195.98 μmol kg^{-1} DW in plants submitted to 18 days of stress. The higher content of MDA in stressed plants during recovery period indicates that stress was not properly dissipated.

4. Discussion

The A, g_s, and E were negligible under stress of low soil water potential. It is common that A and gs approached zero when pre-dawn leaf water potential decrease, as described in *Copaifera langsdorffii* [19], *Dalbergia miscolobium* Benth [20], *Coffea canephora* [21], and *Euterpe oleracea* Mart [22]. Although in this work the leaf water potential was not measured, the values of A, and g_s suggest high sensitivity of the gas exchange variables to soil drought in agreement to these authors. The high control over stomata conductance by Jatropha can also be seen by the low correlation between leaf-to-air vapour pressure deficit and transpiration when the seedlings were placed under drought stress. In such conditions, the low g_s was responsible for reducing to zero the transpiration even with high leaf-to-air vapour pressure deficit.

In the current study, C_i was not reduced even when g_s was zero. These results confirm the tolerance of Jatropha to water stress. On the other hand, they suggest that it is possible to manipulate the stomatal conductance of this species. Theoretically, manipulation of water deficit responses, through techniques such as 'partial rootzone drying' [23] and 'regulated deficit irrigation' [24] allows the exploration of the plant's long distance signaling system, preventing the development of severe water deficit [25]. All leaves showed constant values of C_i/C_a throughout the period of observation

(data not shown), implying that the reduction of A cannot be attributed to lower intercellular CO_2 concentration caused by lower g_s, but to direct effect of drought over photosynthetic apparatus. Other limiting factors, such as shortage in Rubisco and/or other relevant metabolites may be responsible for the reduction of A/C_i under drought conditions in Jatropha. We did not examine the biochemical reactions of photosynthesis in the present work but various researchers have observed a decline in Calvin cycle enzymes activity and reduced rates of CO_2 fixation during drought stress treatment of plants or plant parts [26] which may be a factor for overproduction of ROS resulting in lipid peroxidation. Recovery of photosynthesis seemed to have the same pattern of the stomatal conductance; however the g_s recovery was slower than A. In accord of Mansfield and Davies [27] the g_s recover only when the concentration of leaf ABA decreases, and the time needed for this recovery varies with plant species and the degree of stress. For example, in plants of *Euterpe oleraceae* submitted to 61 days without irrigation and rehydrated, the recovery of stomatal conductance occurred only 14 days after to rehydrate the soil [22]. Whether ABA levels affected the stomata aperture of the Jatropha leaves or not yet to confirmed as, in this work, ABA was not measured. Even so, we could observe a slower recuperation of g_s in seedling under longer drought periods in relation to seedlings under short drought periods.

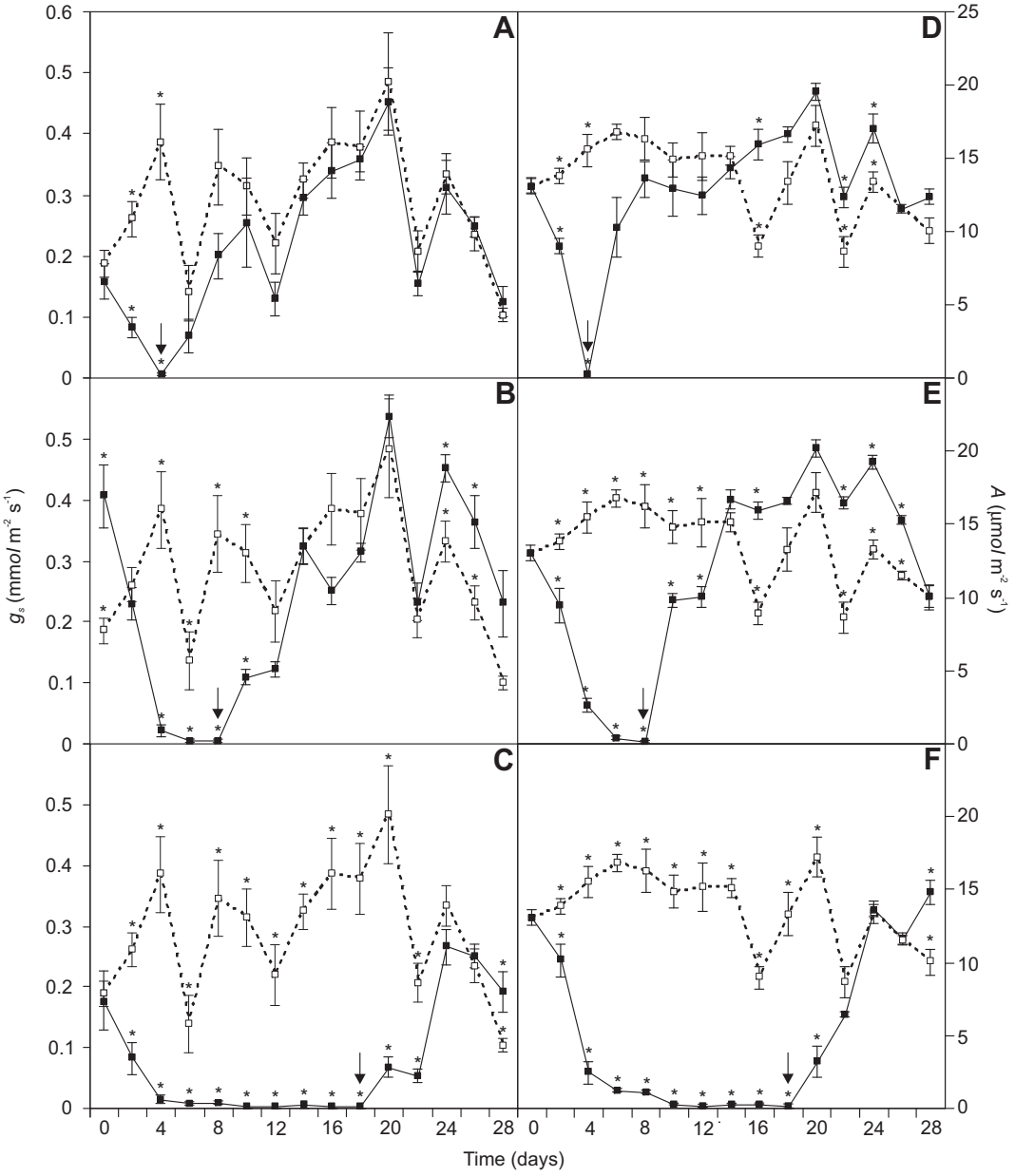

Fig. 3 – Effect of drought stress on stomatal conductance (A, B, and C) and net photosynthesis (D, E, and F) in *Jatropha curcas* L. subjected to drought stress by withholding irrigation to 4 (A, D), 8 (B, E), and 18 (C, F) days and rewatered subsequently and watered every 2 days of the recovery period. Arrows indicate rewatering of soil. * Denote statistical significance (SNK $p \leq 0.05$). □ Control plants, ■ Stressed plants $n = 5$.

A drought-induced reduction in pigment contents was previously reported in several species, including pea [28], *Nerium oleander* [29] and wheat [30]. Photoinhibition and photoprotection of pigments may contribute to such changes. The loss of chlorophyll was attributable to photoprotection of PS since the chlorophyll decrease helped to reduce the pressure on the PS under drought stress and high irradiance [31]. Among biochemical parameters analyzed, free amino acid was the best indicator of drought stress as its concentration decreased under stress and increased when rewatering in relation to unstressed plants, accompanying similar behavior of photosynthesis (Table 1). The losses of chlorophyll, amino acids and proteins, are used as markers of senescence in green tissues. As reported previously [32–34], they were indicative that these processes were caused by drought stress. In this work, the leaf abscission was verified from the 11th day of drought stress imposition. The chlorophyll *a*/*b* ratio decreased significantly during drought, but in re-hydration the ratio was compared to the control. Carotenoids are responsible for energy excess dissipation and scavenging of singlet oxygen and hence their comparative levels in a genotype will determine its relative tolerance. Therefore, the raise of carotenoids content will be result a reduction in the O_2^- levels and/or superoxide dismutase activity. However, in our work,

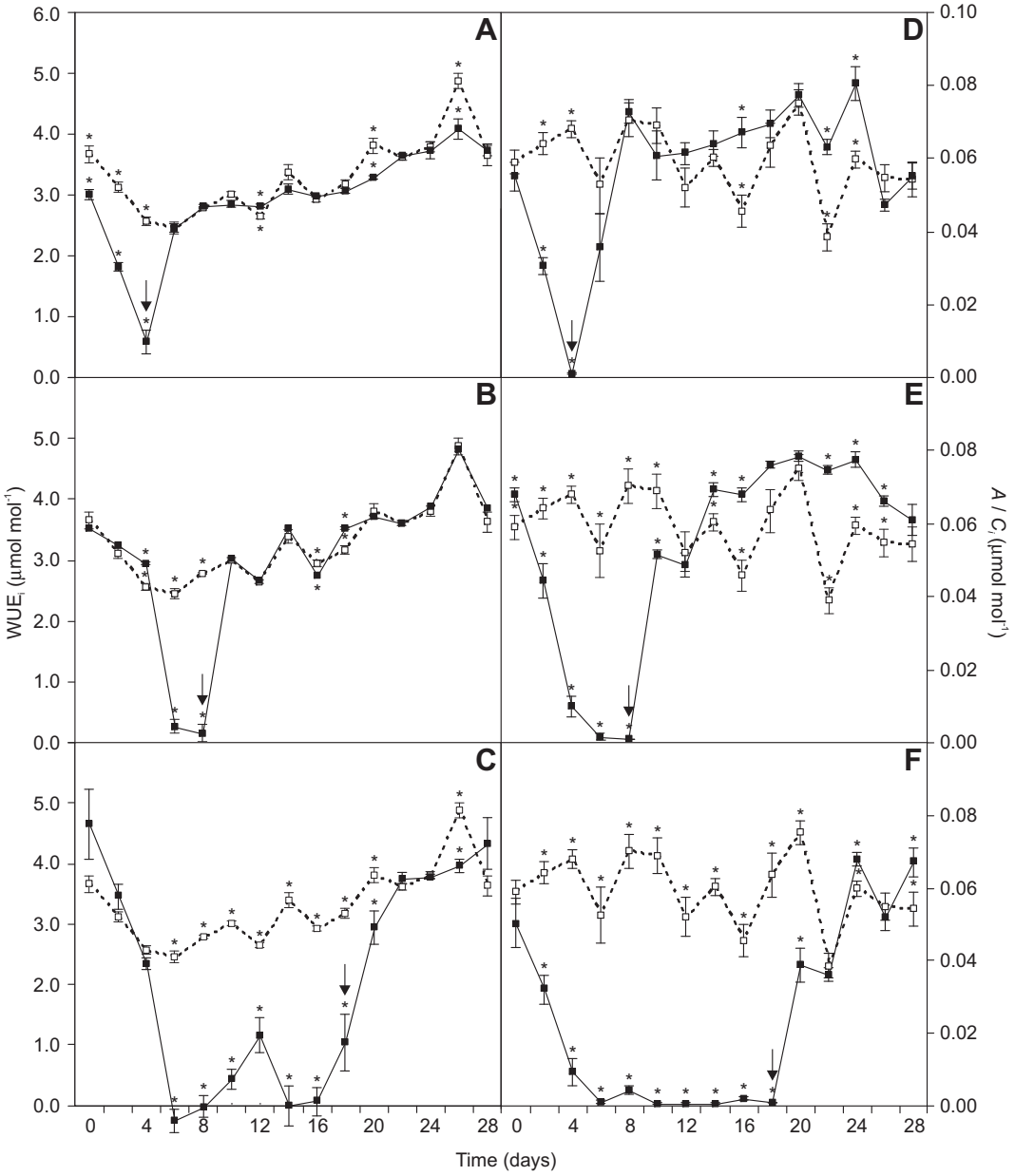

Fig. 4 – Effect of drought stress on WUE$_i$ (A, B, and C) and A/C$_i$ rate (D, E, and F) in *Jatropha curcas* L. subjected to drought stress by withholding irrigation to 4 (A, D), 8 (B, E), and 18 (C, F) days and rewatered subsequently and watered every 2 days of the recovery period. Arrows indicate rewatering of soil. * Denote statistical significance (SNK $p \leq 0.05$). □ Control plants, ■ Stressed plants $n = 5$.

Table 1 – Effect of drought stress on chlorophyll, carotenoids, amino acids, and protein content in leaves of *Jatropha curcas* L. subjected to drought stress by withholding irrigation to 4, 8, and 18 days and recovery period after rewatered of the soil. Differences means followed by *, **, and * were significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ level, respectively at $n = 5$.**

Stress days	Before stress	Stress			Recovery		
		Control	Stress	Difference	Control	Stress	Difference
Chlorophyll "a" (g kg^{-1} DW)							
4	6.74 ± 0.43	6.41 ± 0.43	5.76 ± 0.21	−0.65	4.94 ± 0.70	7.88 ± 0.42	2.94 **
8	6.74 ± 0.43	7.92 ± 0.51	5.98 ± 0.48	−1.93*	5.02 ± 0.59	7.83 ± 0.32	2.80 **
18	6.74 ± 0.43	5.68 ± 0.40	2.76 ± 0.31	−2.92***	7.95 ± 0.34	3.63 ± 0.44	−4.32***
Chlorophyll "b" (g kg^{-1} DW)							
4	2.15 ± 0.19	1.86 ± 0.17	2.09 ± 0.12	0.24	1.45 ± 0.18	2.12 ± 0.11	0.67*
8	2.15 ± 0.19	2.30 ± 0.06	1.87 ± 0.16	−0.43*	1.66 ± 0.15	1.46 ± 0.28	−0.21
18	2.15 ± 0.19	1.44 ± 0.07	0.88 ± 0.13	−0.56**	2.06 ± 0.08	2.49 ± 0.40	0.43
Chlorophyll "a/b"							
4	3.33 ± 0.08	3.30 ± 0.16	2.83 ± 0.10	−0.47*	3.82 ± 0.09	3.72 ± 0.06	−0.10
8	3.33 ± 0.08	3.87 ± 0.03	3.34 ± 0.06	0.53 ***	3.60 ± 0.09	3.79 ± 0.06	0.19
18	3.33 ± 0.08	3.71 ± 0.10	3.02 ± 0.06	−0.70***	3.49 ± 0.13	4.01 ± 0.09	0.51 *
Carotenoids (g kg^{-1} DW)							
4	1.05 ± 0.10	1.07 ± 0.07	1.53 ± 0.05	0.45 **	0.71 ± 0.09	1.22 ± 0.18	0.50 *
8	1.05 ± 0.10	0.46 ± 0.03	1.44 ± 0.13	0.98 ***	1.04 ± 0.06	0.99 ± 0.17	−0.05
18	1.05 ± 0.10	1.03 ± 0.06	0.57 ± 0.04	−0.46***	0.30 ± 0.04	0.51 ± 0.02	0.21 **
Chlorophyll/Carotenoids							
4	7.63 ± 0.41	7.01 ± 0.23	6.07 ± 0.93	−0.94	5.68 ± 0.23	6.32 ± 0.41	0.64
8	7.63 ± 0.41	5.86 ± 0.37	5.77 ± 0.23	−0.09	6.43 ± 0.146	7.23 ± 0.20	0.81
18	7.63 ± 0.41	6.62 ± 0.49	6.48 ± 0.38	−0.14	6.90 ± 0.55	6.99 ± 0.51	0.09
Amino acids free (mmol kg^{-1} DW)							
4	222.24 ± 5.78	211.38 ± 5.81	169.93 ± 5.02	−41.45***	260.22 ± 13.02	261.31 ± 10.09	1.09
8	222.24 ± 5.78	239.98 ± 3.60	157.04 ± 11.42	−82.94***	215.64 ± 6.97	271.12 ± 9.17	55.48 ***
18	222.24 ± 5.78	200.03 ± 4.19	170.04 ± 3.04	−29.99***	189.97 ± 7.17	288.34 ± 24.59	98.38 **
Soluble proteins (g kg^{-1} DW)							
4	135.11 ± 6.58	144.47 ± 14.52	104.78 ± 8.14	−39.69*	104.45 ± 4.97	101.61 ± 5.07	−2.84
8	135.11 ± 6.58	116.24 ± 12.49	47.22 ± 6.48	−69.03***	103.92 ± 8.94	95.09 ± 9.89	−8.83
18	135.11 ± 6.58	118.28 ± 8.52	95.46 ± 6.62	−22.83*	84.23 ± 9.53	149.52 ± 15.82	65.29 **

Table 2 – Effects of drought stress on antioxidative enzyme activities and malondialdehyde in leaves of *Jatropha curcas* L. subjected to drought stress by withholding irrigation to 4, 8, and 18 days and recovery period after rewatered of the soil. Differences means followed by *, **, and * were significant at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ level, respectively at $n = 5$.**

Stress days	Before stress	Stress			Recovery		
		Control	Stress	Difference	Control	Stress	Difference
Superoxide dismutase (U mg protein^{-1})							
4	24.21 ± 3.29	15.70 ± 2.13	23.45 ± 5.36	7.74	19.80 ± 5.92	12.20 ± 2.39	−7.61
8	24.21 ± 3.29	17.03 ± 2.65	49.82 ± 4.16	32.78***	23.96 ± 4.27	49.18 ± 13.79	25.22*
18	24.21 ± 3.29	13.75 ± 1.28	23.74 ± 3.12	9.99**	12.41 ± 2.05	21.39 ± 7.27	8.98
Catalase (U mg protein^{-1})							
4	0.09 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.03	0.10 ± 0.01	0.14 ± 0.01	0.05**
8	0.09 ± 0.01	0.10 ± 0.01	0.14 ± 0.01	0.04**	0.15 ± 0.01	0.16 ± 0.01	0.01
18	0.09 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.03*	0.24 ± 0.01	0.29 ± 0.02	0.06*
Ascorbate peroxidase (U mg protein^{-1})							
4	0.42 ± 0.04	0.86 ± 0.08	0.65 ± 0.06	−0.21	0.41 ± 0.08	0.49 ± 0.10	0.08
8	0.42 ± 0.04	0.69 ± 0.02	1.13 ± 0.11	0.45***	0.81 ± 0.07	0.84 ± 0.07	0.03
18	0.42 ± 0.04	0.98 ± 0.17	1.42 ± 0.13	0.43*	0.93 ± 0.08	0.63 ± 0.08	−0.30*
Glutamine synthetase (U mg protein^{-1})							
4	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.01	0.14 ± 0.01	0.11 ± 0.01	−0.03
8	0.11 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.03	0.10 ± 0.01	0.11 ± 0.01	0.01
18	0.11 ± 0.01	0.08 ± 0.02	0.14 ± 0.02	0.06*	0.11 ± 0.01	0.11 ± 0.01	0
Malonic aldehyde (μmol kg^{-1} DW)							
4	100.17 ± 1.76	108.40 ± 3.19	128.05 ± 2.66	19.65***	85.51 ± 2.92	101.18 ± 4.46	15.67**
8	100.17 ± 1.76	109.78 ± 3.29	138.14 ± 2.86	28.35***	84.77 ± 2.67	107.15 ± 5.76	22.37**
18	100.17 ± 1.76	124.61 ± 6.58	195.98 ± 4.22	71.37***	124.45 ± 7.03	114.71 ± 5.39	−9.74

although the levels of carotenoids were higher than in control, the photoprotective effect of the carotenoids was not verified, since chlorophyll/carotenoids ratio not changed in all treatments. It is clear that the response of antioxidants to a water deficit depends on the severity of stress and on the species and age of plants [32,33].

Drought stress, like many other abiotic stresses, may also lead to oxidative stress through the increase of ROS. The ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins, and nucleic acids [35]. Lipids have vital roles in the tolerance to several physiological stressors in a variety of organisms. In the present work, the elevated levels of MDA (Table 2) in stressed plants point to oxidative stress, which might cause cellular damage, suggesting that oxidative stress is a consequence of the deterioration of lipid peroxidation (indicated by MDA) brought about by ROS. Smirnoff [9] suggested that ROS may directly damage some cellular components as the PS II complex and membrane lipids. Besides drought stress, some other stress conditions may also cause oxidative damage in plants. For example, it has been reported that salt stress, air pollution, elevated temperatures, high irradiance can induce increased levels of ROS and MDA in the plants [36].

Adaptation to water deficit may depend on different mechanisms, including the capacity to maintain high levels of antioxidants, such as antioxidative enzymes as SOD, CAT, and APX [10,29]. Modulation of antioxidative enzymes activities may be an important mechanism to understand the responses of plants to environmental stresses [29]. In the present study, the changes in SOD activity of stressed plants were consistent with the changes in MDA and ROS, especially in 8-day and 18-day plants. This suggests that increase ROS levels not only result in lipid peroxidation but also stimulate production of the antioxidative enzyme SOD. In the stressed plants, levels of both SOD and CAT remained high, although the extent of variation in SOD content was less than that in CAT, probably due to effects of APX on H_2O_2 dismutase produced by SOD. However, SOD offers a more sensitive and effective intrinsic defense than CAT or APX, which serves as a potential antioxidant. Similar patterns of matching changes in CAT, APX and SOD were also observed in other plant tissues under water deficit [36,37].

The content of MDA, a product of lipid peroxidation, has been considered an indicator of oxidative damage [11]. In the present work, the elevated levels of MDA (Table 2) in stressed plants point to oxidative stress, which might cause cellular damage, that suggests that oxidative stress is a consequence of the deterioration of lipid peroxidation (indicated by MDA) brought about by ROS. Smirnoff [9] suggested that ROS may directly damage such cellular components as the PS II complex and membrane lipids. Besides water stress, some other stress conditions may also cause oxidative damage in plants. Water stress raised MDA in all treatments, especially in 18-day plants, suggesting that prolonged drought caused membrane lipid peroxidation, and this occurred even with an increase in the antioxidant enzymes levels. The increases observed in leaf MDA content of fully dried plants after a prolonged period (18 days) were in agreement with results of other studies [38,39].

An increase of GS activity, especially in 18 days of drought, beside the increase of CAT activity, and amino acids and proteins content decrease reveal a photorespiration activity in drought-stressed plants in accord of Guo et al. [40] and Oliveira et al. [41].

5. Conclusion

We showed that drought induce decrease in stomatal and non-stomatal photosynthetic activity in Jatropha. This decrease may result in lower biomass and oil production per area. On the other hand, the increase of antioxidant enzymes activity in plants under drought may contribute to its ability to survive in the adverse arid environment.

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