Water relations and some aspects of leaf metabolism of *Jatropha curcas* young plants under two water deficit levels and recovery

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ABSTRACT

Stomatal conductance (g_s), transpiration (E), relative water content (RWC), superoxide dismutase activity (SOD), malondialdehyde (MDA), leaf carbohydrate soluble (LCS), free amino acids (FAA) and total protein (TP) content were measured under mild and severe drought stress in young potted *Jatropha curcas* L. in the greenhouse. The plants were kept for 12 days under three different water regimes, control (well hydrated), 50% irrigation control (mild stress) and 25% (severe stress), after rehydration were measured on the second and fourth day. Both mild and severe stress decreased g_s and E; however, only severe stress reduced LCS, FAA and TP content and increased SOD activity (70%) and MDA content (60%) compared with the control. Moreover, under these conditions, the plants showed severe leaf senescence. These results show that only severe drought stress decrease foliar metabolism in potted plants. These results show that severe drought decreased metabolism and leaf RWC, in potted plants. However, 48 h under a moderate stress is enough to drive the stomatal control is the main barrier against water loss. Finally, in the fourth day after rehydration the plants under a moderate and severe drought even had a g_s and E reduced in both evaluation periods.

Key words: biodiesel, drought tolerance, MDA, oxidative stress, ROS, water deficit.

RESUMO

Relações hídricas e alguns aspectos do metabolismo foliar de plantas jovens de *Jatropha curcas* sob dois níveis de déficit hídrico e recuperação. A condutância estomática (g_s), transpiração (E), conteúdo relativo de água (RWC), a atividade da superóxido dismutase (SOD), conteúdo foliar de malondialdeído (MDA), carboidratos solúveis (LCS), aminoácidos livres (FAA) e proteína total (TP) foram mensurados sob estresse hídrico moderado, severo e na reidratação em plantas jovens envasadas de *Jatropha curcas* L. em casa de vegetação. As plantas foram mantidas por 12 dias, sob três diferentes regimes hídricos: controle (bem hidratada), 50% da irrigação do controle (estresse moderado) e 25% (estresse severo), após a reidratação foram mensuradas no segundo e no quarto dia. Tanto o déficit moderado quanto o severo reduziram g_s e E, no entanto, apenas o estresse severo reduziu o conteúdo de LCS, FAA e TP e aumentou a atividade da SOD (70%) e conteúdo de MDA (60%) em comparação com o controle (plantas bem hidratadas). Além disso, sob seca severa, as plantas apresentaram senescência foliar acentuada. Estes resultados mostram que apenas a seca severa diminuiu o metabolismo foliar e RWC, em plantas envasadas. No entanto, 48 h sob um estresse moderado é o suficiente

para que o rígido controle estomático seja a principal barreira contra a perda de água. O quarto dia após a reidratação, as plantas sob uma seca moderada e severa ainda apresentavam uma g_s e E reduzidas em ambas as épocas de avaliação.

Palavras chave: biodiesel, déficit hídrico, MDA, estresse oxidativo, ERRO, tolerância à seca

INTRODUCTION

Jatropha curcas L. has great economic potential as an alternative to oil biofuel (Maes et al., 2009; Kumar et al., 2010). This species is widely distributed in tropical areas, both wild and cultivated, in Central and South America, Africa, India, Southeast Asia and Australia (King et al., 2009). Worldwide, water deficit is considered the environmental factor that most significantly influences plant growth and yield (Kramer and Boyer, 1995). In addition, global climate change is expected to strong drought periods in semi-arid ecosystems like the Brazil northeastern (IPCC, 2007). Thus, plants with seed oil, with drought tolerance, but without food useful may be a great option for semi-arid areas.

There are few studies concerning the ecophysiological aspects of *J. curcas* (Maes et al., 2009; Pompelli et al., 2010; Silva et al., 2010). As a Euphorbiaceae, drought tolerance is an expected result (El-Sharkawy, 2007). The majority of crops used today to produce oil from the seeds present annual cycle. Among the non-traditional oilseeds great interest in *J. curcas* is justified by being tolerant to marginal soil and climatic conditions for traditional cultures. Nowadays more than 2.5 million hectares in China and India (Fairless, 2007), on the other hand, South America begins its cultivation only.

The ecophysiological responses in plants adapted to semi-arid regions is of interest (Ribeiro et al., 2009; Rodrigues et al., 2010; Souza et al., 2010), particularly when the species has numerous economic applications, like *J. curcas* (Maes et al., 2009; Pompelli et al., 2010). Strict stomatal control is common to species of Euphorbiaceae in order to prevent water loss (El-sharkawy, 2007), though such behavior leads to a lack of CO_2, which in turn changes the electron transport chain (Chaves et al., 2009), leading to oxidative stress that can cause damage to membranes due to reactive oxygen species (ROS) (Pompelli et al., 2010). Thus, the main objective of this study is to evaluate the behavior of young plants of *J. curcas* potted under greenhouse conditions, as to stomatal control, leaf relative water content and some aspects of leaf metabolism under two levels of water deficit.

MATERIALS AND METHODS

Plant material and growth conditions: Seeds of *Jatropha curcas* L. were sown in 10 L pots with 9.0 kg of substrate: a mixture of organic horizon (black earth) + B horizon clay (red clay) + washed sand in the proportion of 3:1:1, in the state of Pernambuco, Recife city. The pots received no chemical fertilizer, but had an adequate level of nutrients and pH (data not shown). The pot size was large enough for the plant to age without the roots winding. After 90 days of emergence, the plants were divided into three water regimes, 100% (control, 600 mL three times a week), 50% of the control (mild stress) and 25% of the control (severe stress). Water control was previously determined before the start of the experiment and was set to maintain the substrate fully hydrated without leaching. The maximum temperature ranged between 28 and 32°C and the minimum between 20 and 25.5°C. The relative humidity of air ranged between 50 and 95% during the experiment. The different water regimes were maintained for 12 days at a time of high temperatures and median radiation, due to cloudy days in January (Figure 1). The maximum stress was established when the plants began to lose leaves. The plants were kept under different water regime for 12 days and evaluated by the fourth day after rehydration.
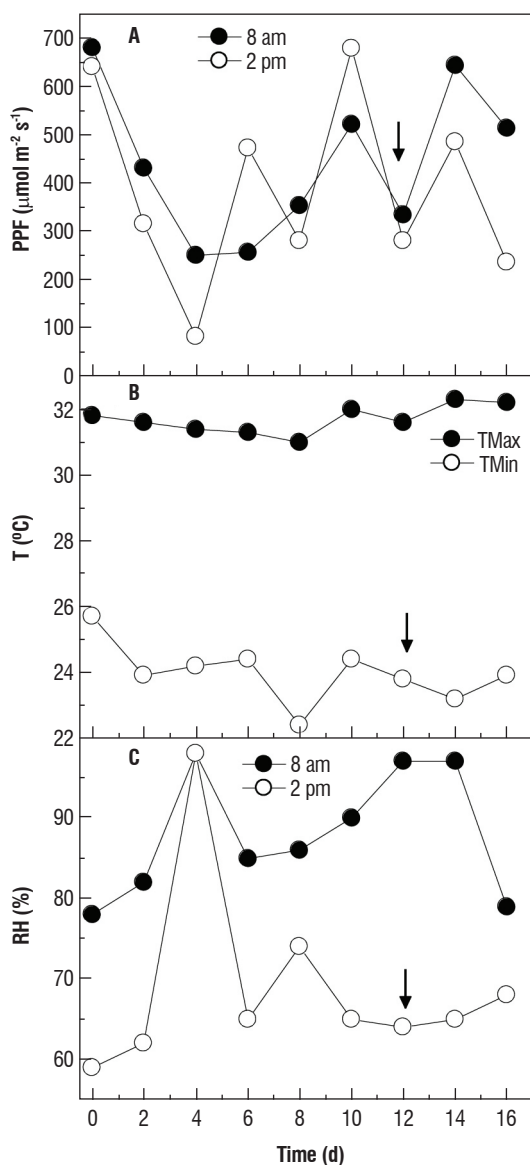
Figure 1. Data of Photosynthetic Photon Flux (PPF) (A), maximum and minimum temperature (B) and relative humidity(C) in a greenhouse during the differentiation of the water regime and rehydration. The arrow indicates the day of rehydration.

Relative water content (RWC): The water status of each plant was determined by the RWC. Thus, leaf discs were collected at 6 am from four plants per treatment and processed according to Barrs and Weatherley (1962).

Leaf stomatal conductance and transpiration: Stomatal conductance (g_s) and transpiration (E) were measured in five plants, with a porometer model Li-1600 (Licor, NE, USA) between 8-9 am and 2-3 pm, in a mature leaf exposed to sunlight.

Biochemical analysis: Leaves were always collected at 3 pm, the period of greatest accumulation of photoassimilates (Santos and Pimentel, 2009), the plant tissue was immediately wrapped with aluminum foil, then frozen in liquid nitrogen and stored at -80ºC freezer. Total soluble carbohydrates, total proteins, amino acids, malondialdehyde (MDA) and superoxide dismutase (SOD) activity were all analyzed according to the methodologies of Dubois et al. (1956), Bradford (1976), Moore and Stein (1948), Cakmak and Horst (1991) and Giannopolitis and Reis (1997), respectively. The samples were homogenized in 2 mL of 0.1% (w w^{-1}) trichloroacetic acid and centrifuged at 10000xg for 15 min to determine malondialdehyde (MDA). MDA of extracts was determined by the thiobarbituric acid reaction (Imlay, 2003).

Enzyme activity assay: Total activity of SOD (EC: 1.15.1.1) 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 m^{-2} s^{-1} of photosynthetic Photon Flux (PPF) at reaction tube surface. The reaction was started by switching on the light and was allowed to run for 10 min. The reaction was stopped by switching off the light, then the absorbance at 560 nm was recorded. A nonirradiated reaction mixture that did not develop color served as control and its absorbance was subtracted from sample measurements of absorbance at A560. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction.

Statistical analysis: The experimental design was completely randomized with three water levels and five replicates. The data were subjected to analysis of variance (ANOVA) and means were compared and segregated by the Student-Newman-Keuls test ($p < 0.05$), when significance was detected (program MSTATC).

RESULTS

J. curcas plants showed continuous vegetative growth, since they were maintained at the maximum capacity of the substrate contained in pots of 10 L and a favorable average temperature, with minimal variation. This growth was sustained by maintaining a high relative water

content (RWC) (Figure 2), maintained under control and moderate stress conditions, when compared with severely stressed plants ($P < 0.05$). However, plants under severe stress, with 25% of control irrigation, showed a reduction

in growth during the period under different water regimes, in the parameters height, stem diameter and leaf number (data not shown).
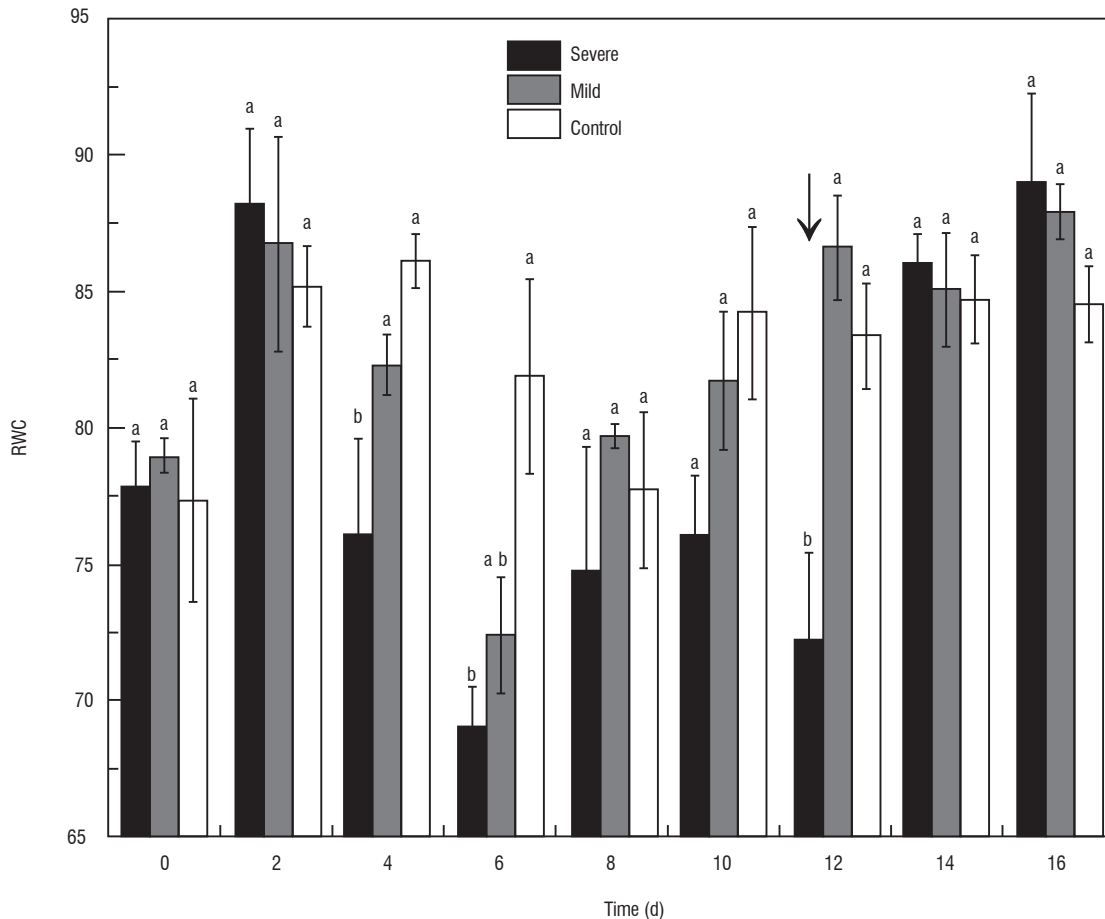

Figure 2. Relative water content at 6 am, in *Jatropha curcas* grown under greenhouse conditions: control (irrigation 100% - 600 mL), mild stress (50%) and severe stress (25%). Two and four days after rehydration (arrow). Each bar represents the mean of four replicates (±SE). Means followed by the same letter between treatments per day did not differ by test of Student Newman Keuls ($P < 0.05$).

The control plants showed the highest values of both g_s and E from day 2 of differentiation efficiency ($P < 0.05$) compared with plants under moderate and severe stress (Figure 3A-D). Among the stress treatments, a reduction in g_s and E ($P < 0.05$) was observed from day 8 of different water regimes in the morning (Figure 3A-B). In the afternoon,

only minimal differences were observed in plants of both treatments under stress (Figure 3C-D). The effect of water stress can be considered severe, since even after rehydration of the substrate for 4 days, the values of g_s and E were lower ($P < 0.05$) in plants exposed to moderate and severe stress compared to the values verified for control plants (Figure 3).
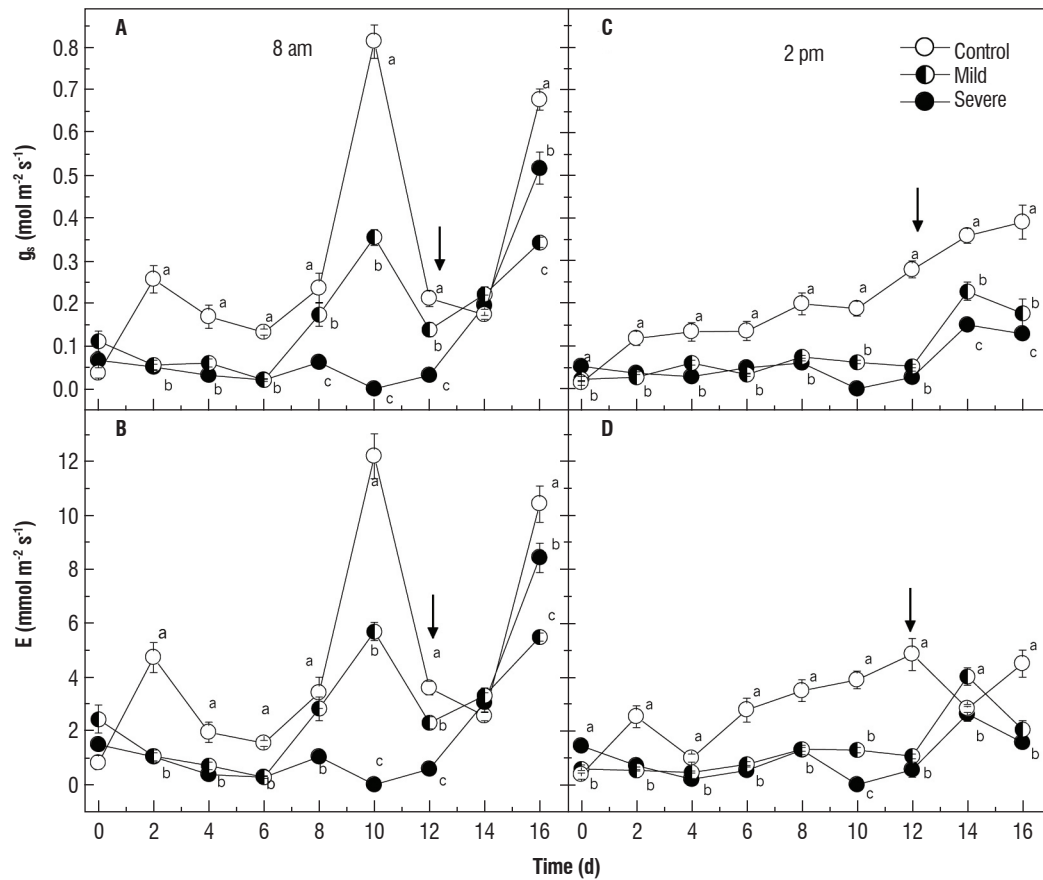
Figure 3. Stomatal conductance (g_s) and transpiration (E) at 8 am (A-B) and 2 pm (C-D), *Jatropha curcas* grown under greenhouse conditions: control (irrigation 100% - 600 mL), mild stress (50%) and severe stress (25%). The arrow indicates two and four days after rehydration. Each point represents the mean of five replicates (±SE). Means followed by the same letter between treatments per day did not differ by test of Student Newman Keuls ($P < 0.05$).

The values of g_s and E presented by the young plants under greenhouse conditions showed strong stomatal control (Figure 3A,C). Such control appears to be the first barrier against drought in this species, since soon after day 2 of water restriction, the plants under moderate and severe stress showed a reduction in g_s and E ($P < 0.05$) (Figure 3A-D). Stomatal conductance was reduced even more sharply after day 8 of water deficit in plants under severe stress ($P < 0.05$) (Figure 3A), which led to a lower rate of E (Figure 3B,D). During rehydration, four days were not sufficient for the stressed plants to exhibit the same rate of g_s and E as the control plants ($P < 0.05$), which further highlights the efficiency of stomatal opening control, particularly during the period of greatest water demand by the atmosphere (2 pm) (Figure 3A,C).

The different water regimes led to a reduction in LSC only at maximum stress (12 days) and during day 2 of rehydration ($P < 0.05$) (Table 1). In contrast, the contents of FAA and TP were not different at maximum stress ($P > 0.05$), difference only occurred between treatments following rehydration ($P < 0.05$) and the control plants showed the highest values (Table 1).

The strict stomatal control in plants under stress, compared to control plants, could be the main cause of the reduction in total soluble carbohydrate content (LSC) ($P < 0.05$) only in plants under severe stress after 12 days (Table 1). However, four days after rehydration, the plants showed the same LSC as well watered plants ($P > 0.05$), while g_s and E remained under tight control in plants that had suffered stress (Table 1).

Superoxide dismutase (SOD) activity was measured during stress, for which the highest peak activity was registered 10 days after stress in plants under severe stress ($P < 0.05$). Although less intense, SOD activity at maximum stress in plants under severe drought was higher than in control plants ($P < 0.05$); however, after substrate rehydration no differences were observed between treatments (Figure 4A). On day 10 of stress, greater malondialdehyde (MDA) content was observed in plants under moderate and severe stress ($P < 0.05$) (Figure 4B), nevertheless, after rehydration no differences were observed.

Table 1. Leaf carbohydrate soluble content (LCS – mmol kg^{-1} DW), total free amino acids (FAA – mmol kg^{-1} DW) e total proteins (TP – g kg^{-1} DW) in plants of Jatropha curcas under greenhouse conditions and different water regimes control (irrigation 100% - 600 mL), mild stress (50% - 300 mL) and severe stress (25% - 150 mL). Ten and twelve (maximum stress) days after begin different water regimes. In the recovery, two and four days after rehidration.

Variable	Water status	Time (d)			
		Stress		Recovery	
		10	12	14	16
LCS	Control	1470.1 ± 58.9^{ns}	1216.2 ± 48.9^{a}	1408.8 ± 33.3^{a}	835.6 ± 26.9^{ns}
	Mild	1247.5 ± 74.1^{ns}	1113.5 ± 26.4^{a}	565.0 ± 8.9^{b}	755.6 ± 20.4^{ns}
	Severe	1387.1 ± 66.4^{ns}	843.1 ± 28.2^{b}	475.6 ± 62.1^{b}	827.3 ± 39.7^{ns}
FAA	Control	22.4 ± 3.6^{b}	71.9 ± 4.2^{ns}	45.9 ± 2.1^{b}	22.2 ± 6.3^{b}
	Mild	40.5 ± 4.7^{a}	86.4 ± 4.3^{ns}	91.6 ± 10.2^{a}	40.6 ± 5.1^{a}
	Severe	24.3 ± 1.5^{b}	81.9 ± 3.3^{ns}	109.1 ± 3.3^{a}	45.5 ± 1.7^{a}
TP	Control	236.5 ± 8.9^{a}	160.2 ± 6.8^{ns}	120.2 ± 8.1^{ns}	148.0 ± 2.1^{c}
	Mild	246.3 ± 5.2^{a}	165.8 ± 5.3^{ns}	95.9 ± 7.6^{ns}	202.6 ± 1.7^{a}
	Severe	203.0 ± 4.5^{b}	158.5 ± 6.1^{ns}	89.1 ± 5.6^{ns}	171.9 ± 1.3^{b}

Values represent the average of four replicates. In the column, means followed by same letter do not differ by the test of Student Newman Keuls ($P < 0.05$).
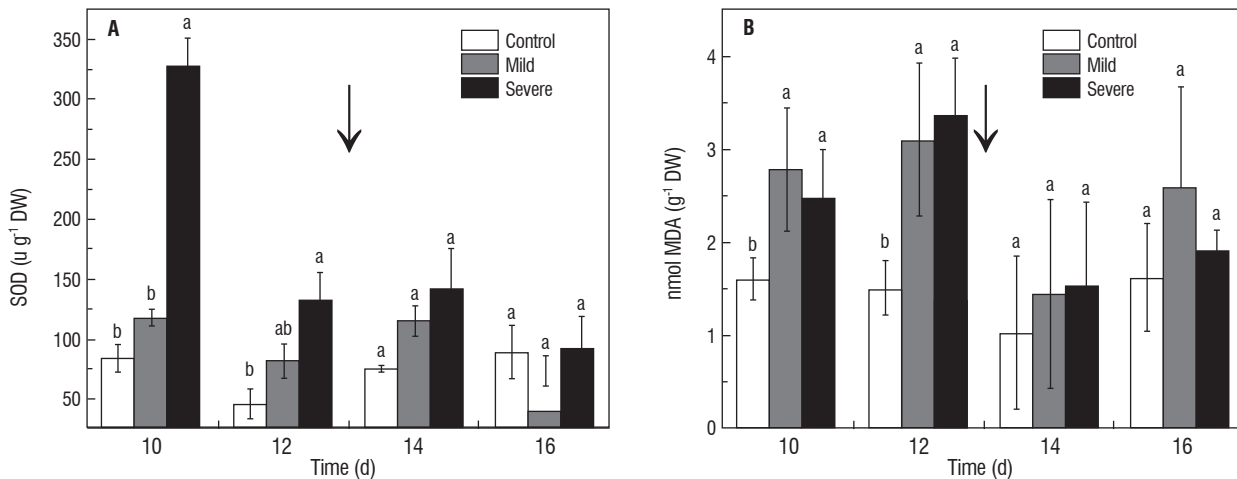

Figure 4. Superoxide dismutase (SOD) (A) activity and malondialdehyde content (MDA) (B) in *Jatropha curcas* plants grown under greenhouse conditions for 90 days when it was differentiated irrigation: control (100% - 600 mL), moderate (50%) and severe stress (25%). The arrow indicates two and four days after rehydration. Each bar represents the mean of three replicates ($\pm$SE). Means followed by the same letter between treatments per day did not differ by Student Newman Keuls test ($P > 0.05$).

DISCUSSION

Some results are beginning to be published on *J. curcas* on crop science aspects (Achten et al., 2010; Behera et al., 2010), ecophysiological and phytochemicals (Maes et al., 2009; Pompelli et al., 2010; Silva et al., 2010) but very little are the results for such pretense about this species not yet tamed. As more results are published will further support the decision on the best use of culture.

The fine control of stomatal aperture in *J. curcas* plants under water restriction in the greenhouse, seems to be the immediate response to low soil water availability (Pompelli et al., 2010; Silva et al., 2010). Even before the leaf relative water content show some reduction. The present results did not show correlations between RWC and g_s behavior, except under severe stress from fourth day after starter withholding water. Moreover under mild stress, plants showed reduced g_s despite high RWC in the same period (Figure 2 and 3A, C). Thus, young plants of *J. curcas* in pots were able to maintain a high RWC, controlling stomatal aperture when water deficit was moderate, with the onset of stomatal regulation even under a high RWC from the second day of treatment (Figure 3A, C), in both time of measurement.

These physiological characteristics of *J. curcas* contribute to its adaptation in a semi-arid region. Stomatal conductance control seem to be the main aspect of drought tolerance in the Euphorbiaceae, similar to cassava (*Manihot* sp.), which also has a strict stomatal control strategy, the plant is deciduous, to tolerate long dry periods, which increases the efficiency of water use, and has a highly efficient root system for soil water uptake (El-Sharkawy, 2007). Stomatal control is the first step to achieve high water use efficient, one of the major characteristics for pretending to cultivate this species on marginal land without irrigation technology.

Plants that exhibit tolerance to stress tend to minimize damage to their metabolism, for example, by regulating enzymatic activity, including enzymes that inactivate ROS, which show increased activity following stress. The increase can be attributed to a favorable adjustment of enzyme activity or new synthesis of these proteins (Jin et al., 2009), which may be an indicator of drought tolerance. In *J. curcas*, water deficit (Pompelli et al., 2010), like heat stress (Silva et al., 2010), may increase ROS. ROS production causes damage to lipids, proteins and nucleic acids (Mitter and Zilinskas, 1994).

In the present study, plants under severe water stress showed greater superoxide dismutase (SOD) activity after 10 and 12 days of stress ($P < 0.05$) compared to moderate stress and controls, but two days after rehydration, SOD activity was similar to the control (Figure 4A). On day 10 of stress, higher SOD activity was observed, which may have occurred due to high solar radiation on this day (Figure 1A), compared with maximal stress.

Water deficit may cause the degradation of lipids in the plasma membrane, causing changes in their fluidity (Chaves et al., 2009; Posch and Bennett, 2009). Degradation of fatty acids due to peroxidation produces not only peroxide ions, but also MDA. The content of MDA, a product of lipid peroxidation, has been considered an indicator of oxidative damage (Pompelli et al., 2010; Silva et al., 2010). In this study, the high level of MDA suggests an oxidative stress, which can cause cell damage, both under moderate drought as severe (Figure 4B). However, after rehydration the level of damage was reduced. Silva et al. (2010) working with young plants of *J. curcas* potted under water deficit in the same region of this study, also reported damage caused by ROS, which the authors are accentuated by the presence of other abiotic stress common in tropical environment, such as heat. Damage would affect the leaf photosynthetic metabolism, particularly photosystem II. However, water deficit was more stressful, in *J. curcas* young plants, in terms of oxidative stress damage than heat stress.

The results showed that only severe drought stress induced low metabolism, beginning with stomata closure, which leads to increased oxidative stress in young plants. In addition, the results suggested that plants of *J. curcas* have different mechanisms of tolerance to water deficit and the loss of leaves. *J. curcas* is a Euphorbiaceae, which already qualifies it as belonging to the group of species with high drought tolerance. However, multiple studies, at same time, under different environmental conditions are required, including soil, water availability, light, nutrients, temperature and other conditions of biotic and abiotic stress. The results corroborate with others finds about *Jatropha curcas* young plants which concluded that plants of this species have mechanisms to tolerate different water deficit intensity; with the stomatal control is the primary mechanism to maintain high leaf water content. The antioxidant mechanism in *J. curcas* leaf was effective increasing their activity from moderate stress.

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