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Morphophysiological changes in young plants of *Jatropha curcas* L. (Euphorbiaceae) subjected to water stress and recovery

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To investigate drought-induced changes in morphophysiological characteristics, seedlings of two genotypes of *Jatropha curcas* (CNPAE 183 and CNPAE 191) were grown under two watering regimes: irrigated (-33.1 to 13.5 to kPa) and water deficit (-409.5 to 49.5 to kPa) for 55 days, followed by six days of rehydration (*DAR*). Withholding water led to a significant reduction ($p<0.05$) of leaf water potential (Ψ_W) and an increase in relative water content (*RWC*). The values of net photosynthetic rate (P_N), stomatal conductance to water vapor (g_S) and transpiration (*E*) were significantly ($p<0.05$) reduced 21 and 34 days after starting treatment (*DAST*) in plants of genotypes CNPAE 183 and CNPAE 191, respectively. After 6 DAR, only CNPAE 191 achieved a recovery of P_N and *E*. Moreover, significantly ($p<0.05$) lower g_S was measured in recovering plants of both genotypes, as compared to the controls. Drought stress led to reductions of 57 and 65% in whole-plant hydraulic conductance (K_L) in genotypes CNPAE 183 and CNPAE 191, respectively. Full recovery of K_L was observed after 6 DAR. The average water consumption was 18% lower in plants subjected to water shortage, as compared to irrigated plants. However, drought-induced reduction in growth led to lower biomass water use efficiency ($WUE_{biomass}$) in plants subjected to water deficit. The effect of water stress was more intense in CNPAE 183 than in CNPAE 191, regarding the growth variables (leaf area, height and diameter), dry mass and root volume. Moreover, a delay in the effect of water stress in genotype CNPAE 191 was also observed, which suggests a higher tolerance of this genotype as compared to CNPAE 183. Altogether, the results showed strong drought-induced stomatal limitation of carbon assimilation and growth in *J. curcas*. Slight genotypic differences were detected, CNPAE 191 being less sensitive to the imposed experimental conditions than CNPAE 183.

Key words: Hydraulic conductivity, root volume, tolerance, leaf gas exchange, water relations, water status.

INTRODUCTION

Jatropha curcas L. (Euphorbiaceae) is an oilseed species, which, despite showing a strong capacity for survival and recovery from water stressed conditions (King et al., 2009; Wang et al., 2011; Verma et al., 2012), has shown negative responses to water deficit in reduced growth and biomass production (Fini et al., 2013; Sapeta

et al., 2013). The attractive characteristics of *J. curcas* include its expected lifespan of 50 years and its broad climatic tolerance, covering zones with annual precipitation between 250 and 1200 mm (Achten et al., 2008). Moreover, production of *J. curcas* oil does not entail competition with food crops, because its oil is non-edible (Tiwari et al., 2007; Islam et al., 2014) and this is one of the effective ways to overcome the problems associated with energy crisis and environmental issues (Ong et al., 2013). Its seeds may contain 11.7 to 42.1% oil depending on soil type and environmental conditions (Kaushik and Bahrdway, 2013), which makes it very promising for the production of biodiesel (Kheira et al., 2009).

Water is one of the main limiting factors for plant production worldwide. Due to the high economic and ecological costs of irrigation and the need for plant production in increasingly arid environments, the production and use of cultivars adapted to drought is of great importance. One of the more studied mechanisms through which plants can increase water use efficiency (WUE) is the stomatal regulation of water loss by transpiration. However, drought tolerant species with such characteristics tend to exhibit reduced growth rates, due to stomatal limitations to the uptake of CO_2 for photosynthesis (Verma et al., 2012).

The use of deficit irrigation has achieved promising results for *Cocos nucifera* (Arecaceae) (Azevedo et al., 2006) and *Citrus latifolia* (Rutaceae) (Sampaio et al., 2010) as a tool for increasing WUE. Through deficit irrigation regimes, three strategies could be used to increase WUE: (1) increase the capacity of water absorption; (2) increase the transpiration efficiency by drought-induced signaling and (3) modify the pattern of allocation of assimilates in favor of the economically viable structure (Condon et al., 2004), that is, augmenting the harvest index.

Regardless of some results showing a delay in the growth of *J. curcas* subjected to water deficit, Fini et al. (2013) concluded that the species can survive dry periods (20% of field capacity) of moderate duration (18 days). However, the effects on the economic profitability of these crops are still unknown. Thus, studies dealing with drought effects on growth and development of different genotypes are likely to be essential components in the success of breeding programs.

This experiment aimed to evaluate the initial growth and estimate the effects of water stress and post-drought recovery on the water relations of two genotypes of *J. curcas* (CNPAE 183 and CNPAE 191). The main hypothesis is that the effects of water stress on the morphophysiological characteristics of *J. curcas* vary with time and intensity of stress and are genotype dependent.

MATERIALS AND METHODS

Plant material and growth conditions

The experiment was conducted under greenhouse conditions from June to September 2012, in the campus of the State University of Santa Cruz, Ilhéus, BA (14°47'00"S, 39°02'00" W). According to the Köppen climate classification, the local climate is type Af, with annual average temperatures of 22to 25°C (Koppen, 1900).

J. curcas seeds of two genotypes were used, the CNPAE 183 and the CNPAE 191, from Jaíba/MG and São Francisco of Assis/RS, respectively. The selection of these genotypes was based on the differences obtained from preliminary data from EMBRAPA Agroenergia. The CNPAE 183 is a non-toxic genotype with a low yield (500 g of seed plant^{1}), average height of 2.4 m, and is from a tropical region. The CNPAE 191 has a high productivity (1030 g seed plant^{-1}), 3 m of height, is toxic to animals and is from a region of subtropical climate. The *J. curcas* seeds were germinated in pots containing 65 kg of substrate soil : sand (2:1), to match the loamy sand textural class. Forty days 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, 22 plants were subjected to a controlled irrigation treatment (60% of field capacity) for 55 days, which led to substrate water deficit (-49.5 to -409.2 kPa) and the other 22 plants to field capacity (-13 1 to -33.1 kPa), followed by rehydration for 6 days.

Water consumption in the two treatments was measured by means of periodic weighing of pots, using load cells CSA/ZL - 100 (MK Control Instruments, Brazil) coupled with an automatic data collector. The determination of the soil water content was carried out weekly by the gravimetric method, and simultaneously the soil matric potential for each treatment was determined using a soil water retention curve. The photosynthetically active radiation (PAR) was monitored using quantum sensors S-LIA-M003. The temperature and relative humidity were recorded using Hobo H8 Pro Series data loggers (Onset, USA). A summary of the microclimatic conditions during the experiment is shown in Table 1.

Water relations

Leaf water potential (Ψ_W) was measured in three randomly selected leaves per genotype per treatment, using a pressure chamber model 1000 (PMS Instrument Company, USA). The measurements were made between 2 and 4 am ($\Psi_{Wpredawn}$) on 35, 50 and 55 DAST and 6 DAR.

Whole plant hydraulic conductivity (K_L) was estimated on the peak of water stress (55 DAST) using the formula $K_L = g_S$ VPD/($\Psi_{Wpredawn}$) – $\Psi_{Wmidday}$, where g_S is the stomatal conductance to water vapor (see below), VPD is the vapor pressure deficit between the atmosphere and the leaf and $\Psi_{Wmidday}$ is the water potential measured at midday (Hubbard et al., 1999).

Leaf relative water content (RWC) was measured at 55 DAST and 6 DAR. Measurements were performed between 6 to 7 am. 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

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Table 1. Photosynthetically active radiation (PAR - mol photons m^{-2} day^{-1}), air temperature (Tair - ° C) and relative humidity (RH-%) over the trial period.

Variable	Mean	Maximum	Minimum
PAR	14.22 (0.44)	20.35	8.8
Tair	25.20 (0.20)	28.40	21.9
RH	76.80 (1.13)	97.90	57.0

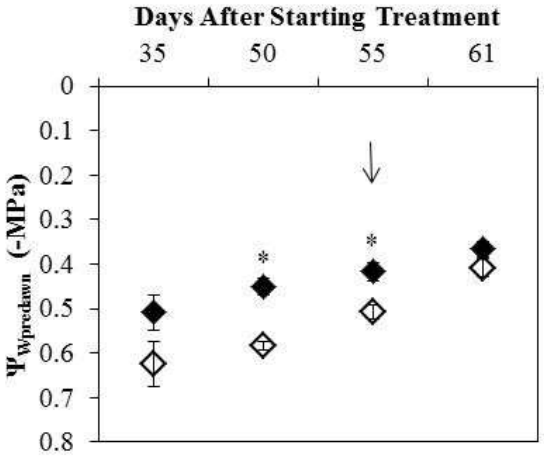

Figure 1. Leaf water potential (Ψ_w) of two genotypes of *J. curcas* plants exposed to water stress and after recovery. Control plants (filled symbols) and plants under water stress (open symbols). The arrow indicates the start of water replacement. Data refer to mean values (n = 6). * Significant by F test (p <0.05).

weighed again to obtain the turgid mass (Mt) and placed in a forced ventilation oven at 75°C until constant dry mass (Md). From these variables, the RWC was calculated as [RWC = ((Mf-Md) / (Mt-Md)) x100] (Nauš et al., 2016).

Leaf gas exchange

Leaf gas exchange variables (net photosynthesis (P_N), transpiration rate (E), stomatal conductance to water vapor (g_S) and the internal CO_2 concentration (C_i)) were measured at 21, 28, 34, 41, 49, 55 DAST and 6 DAR in fully mature leaves positioned on the stem opposite to those sampled for leaf water potential, between 7 and 10 h in all individuals of each treatment (De Santana et al., 2015). A portable gas exchange system (LI-6400, LI-Cor®, Nebraska / USA) was used. During the measurements, the LI-6400 was set to hold constant photosynthetically active radiation (PAR) at 1000 µmol photons m^{-2} s^{-1} and reference CO_2 concentration at 380 µmol mol^{-1}.

Water use efficiencies and water consumption of the plant

Instantaneous and intrinsic water use efficiencies were estimated as the ratios of P_N and E and of P_N and g_S, respectively. Water use efficiency of biomass was calculated as the ratio of the total biomass of the last harvest and water consumed during the experiment, which was measured by sequentially weighting the pots using load cells placed beneath each pot.

Growth

The height, stem diameter, 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). The results were summed to obtain the total leaf area.

Root volume of all plants was measured by displacement of water equivalent units (1 mL = 1 cm^3). The length of root systems was also quantified at the end of the experiment by taking the average of the three largest roots. The plants were then collected for the determination of total dry matter of root, stem and leaves, after complete dehydration in a forced ventilation oven (65 ± 5°C).

Statistical analysis and experimental design

The experiment was arranged in a completely randomized factorial (2x2) design, formed by two watering regimes and two genotypes of *J. curcas*, with six replicates. The data were subjected to a factorial analysis of variance and means were compared by F test (comparison between genotypes and water regimes) with a significance criterion of 0.05.

RESULTS

Water relations

Significant reduction ($p<0.05$) of $\Psi_{Wpredawn}$ was observed from 50 DAST in plants of both genotypes, growing under water stress (Figure 1). Full recovery of water status was observed 6 DAR. Hydraulic conductivity (K_L) was significantly ($P<0.05$) lower in stressed plants of CNPAE 183 and CNPAE 191. However, after rehydration there was no significant ($p<0.05$) difference of K_L between the control and stressed plants of either genotype (Figure 2).

Significant differences ($p<0.05$) between treatments (but not between genotypes) were observed 55 DAST for RWC. RWC was shown to increase in water stressed plants of both genotypes (Figure 3A). After rehydration, RWC decreased in water stressed plants of both genotypes as compared to their control but this decrease was only significant ($p<0.05$) in CNPAE 191 (Figure 3B).

Leaf gas exchange

Net photosynthetic rate (P_N), stomatal conductance to water vapor (g_S) and transpiration rate (E) were significantly ($p<0.05$) reduced in both genotypes by the water stress treatment. Moreover, this reduction was more delayed but greater in magnitude in CNPAE 191 than in CNPAE 183 (Figure 4). Water deficit led to P_N, g_S and E reductions of 34, 67 and 48% from 21 DAST on for genotype CNPAE 183 and of 49, 74 and 67% from 34 DAST on for CNPAE 191, respectively. After the six-day

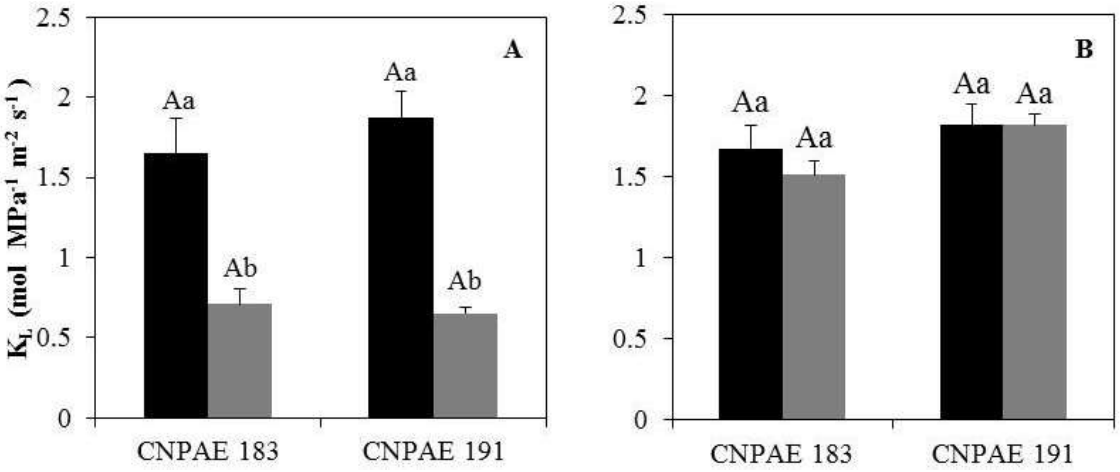

Figure 2. Hydraulic conductivity (K_L - mol H_2O MPa $m^{-1}s^{-1}$) of two genotypes of *J. curcas* (CNPAE 183 and 191) under irrigation (black bars) and water stress (gray bars) after 55 days of starting treatment (A) and after six days rehydration (B). Capital letters indicate comparison between genotypes and lowercase letters between water regimes, by the F test ($p<0.05$). Data refer to mean values of 3 repetitions and the bars indicate the standard error of the mean.

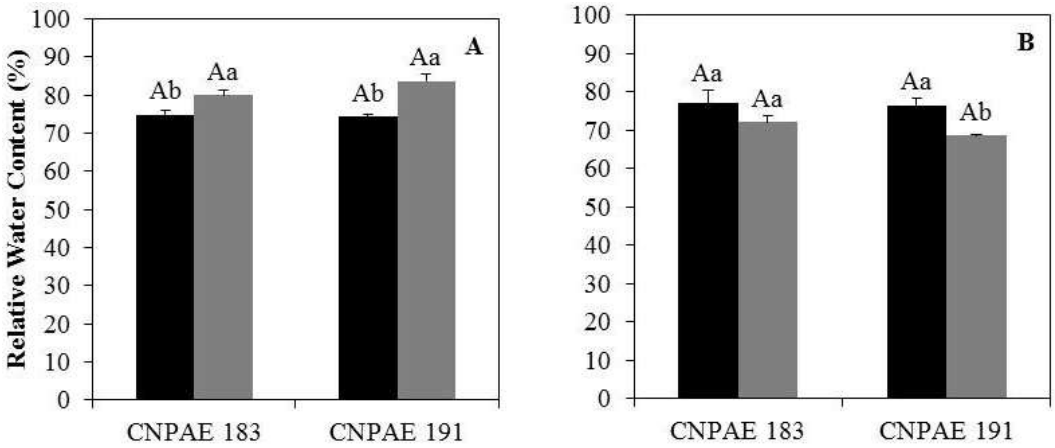

Figure 3. Relative water content (RWC) measured at peak stress to 55 days after starting treatment (A) and six days after rehydration (B) in two genotypes of *J. curcas*. Control plants (black bars) and plants under water stress (gray bars). Capital letters indicate comparison of genotypes and lowercase letters between water regimes, by the F test ($p<0.05$). The points represent the mean values of 3 replicates and the bars indicate the standard error of the mean.

rehydration period, significant differences ($p<0.05$) between treatments for g_S were still observed in both genotypes (Figure 4C and D).

There were no significant differences ($P<0.05$) between genotypes for the intrinsic (P_N/g_S) and instantaneous (P_N/E) water use efficiencies. While no significant differences were observed for P_N/E in CNPAE 183 throughout the experimental period (Figure 5A), a significant ($p<0.05$) increase (30%) of P_N/E was observed from 34 DAST in water stressed plants of CNPAE 191 (Figure 5B). The trend of P_N/g_S was similar for both genotypes, with a significant increase ($p<0.05$) from 34 DAST, which peaked at 85 and 96% increases relative to their controls at 55 DAST in CNPAE 183 and 191, respectively (Figure 5C and D). However, both measures of WUE dropped upon rewatering, but reached values comparable to control only in CNPAE 191 at 6 DAR (Figure 5).

Growth

There were significant differences between genotypes and water regimes for the height, number of leaves and

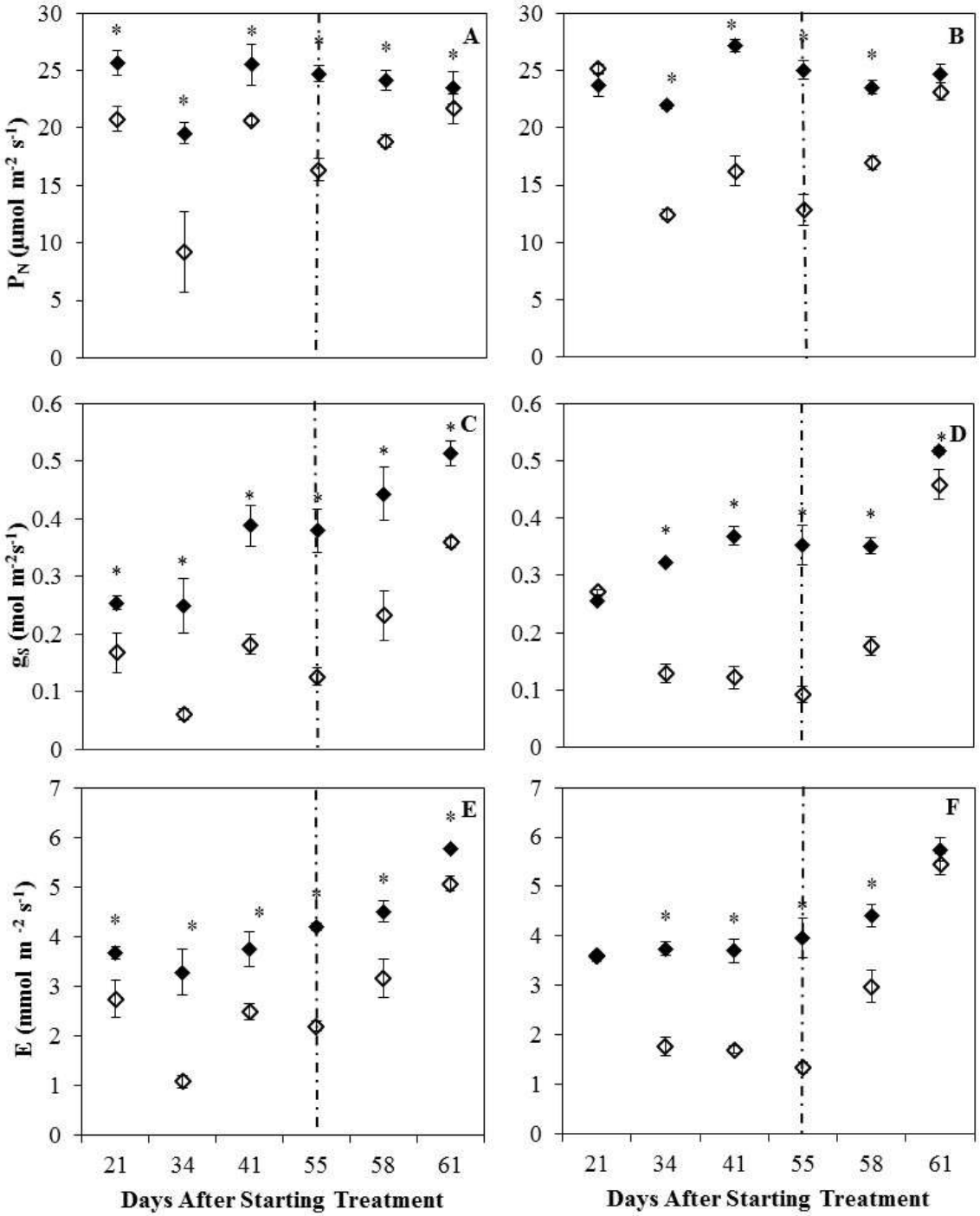

Figure 4. (A and B) Net photosynthetic rate (P_N), (C and D) stomatal conductance (g_S), (Eand F) transpiration (E) of two genotypes of *J. curcas*, CNPAE 183 (A, C and E) and CNPAE 191 (B, D and F), during 55 days of water stress and six days after rehydration. Control plants (filled diamonds) and plants under water stress (open diamonds). Asterisks (*) indicate significant differences between water regimes (F test, $p < 0.05$). Pointed-line indicate the beginning of rehydration. Points represent mean values of 3 to 5 replicates and bars indicate standard error of the mean.

leaf area. However, the diameter was affected significantly only for the treatment of water stress (Figure 6). Although, smaller than CNPAE 183 (69 vs. 85 cm in height), CNPAE 191 exhibited a delay in the effects of water stress as indicated by measurements of growth variables. Significant reductions ($p < 0.05$) in height were

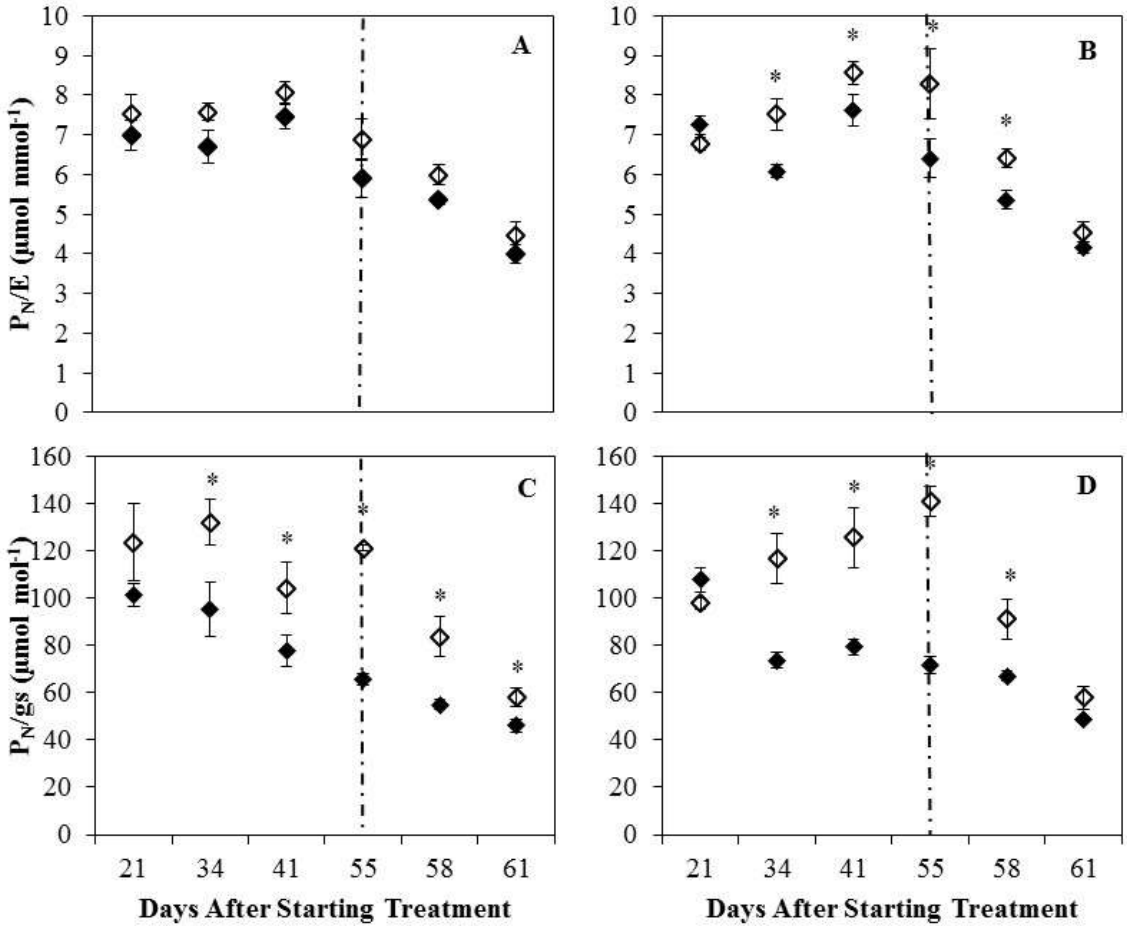

Figure 5. Instantaneous (P_N/E) and intrinsic (P_N/g_S) water use efficiency in two genotypes of *J. curcas,* CNPAE 183 (A and C) and CNPAE 191 (B and D) under control treatment (filled diamonds) and water stress (open diamonds). Asterisks (*) indicate significant differences between water regimes (F test, P<0.05). Pointed-line indicates the beginning of rehydration. Points represent mean values of 5 to 6 replicates and bars indicate standard error of the mean.

observed at 61 and 54 DAST in CNPAE 191 (10%) and CNPAE 183 (30%), respectively (Figure 6A and B). The reduction in diameter was 10% for both genotypes. However, this effect was observed from 41 DAST in CNPAE 183 and from 47 DAST in CNPAE 191 (Figure 6C and D). The number of leaves decreased (24%) significantly ($p < 0.05$) only for CNPAE 183 from 54 DAST (Figure 6E and F).

Significant differences ($p<0.05$) between genotypes under irrigated conditions were also observed for mean leaf area (0.67 m² in CNPAE 183 and 0.45 m² in CNPAE 191). The effect of water deficit occurred earlier (41 DAST) and was more pronounced (45% reduction in relation to control) in CNPAE 183. In CNPAE 191, a significant difference ($p<0.05$) between the water regimes for leaf area was observed from 47 DAST, with a reduction of 25% relative to the control (Figure 7).

Water stress led to reductions of leaf (LDM) and total (TDM) dry mass in both genotypes, and of root (RDM) and shoot (SDM) dry mass only in CNPAE 183 (Figure 8). The total biomass yield at the end of the experiment was affected by water stress in plants of CNPAE 183, with observed reductions of 29, 50, 78 and 55% of RDM SDM, LDM and TDM, respectively. In CNPAE 191, the observed reductions in dry weights water stressed plants relative to control plants were 70% for LDM and 49% for TDM (Figure 8).

There were no significant differences between genotypes for water consumption. However, water deficit did lead to a reduction in water consumption by the plants (Figure 9A) and, consequently, a reduction in the production of biomass. There was a 20 and 15% reduction in water consumption for genotypes CNPAE 183 and 191, respectively. Nevertheless, water deficit led to a significant ($p<0.05$) reduction in the biomass water use efficiency in both genotypes (Figure 9B).

The root volume in well-watered plants of CNPAE 191 was significantly ($p<0.05$) lower than in CNPAE 183.

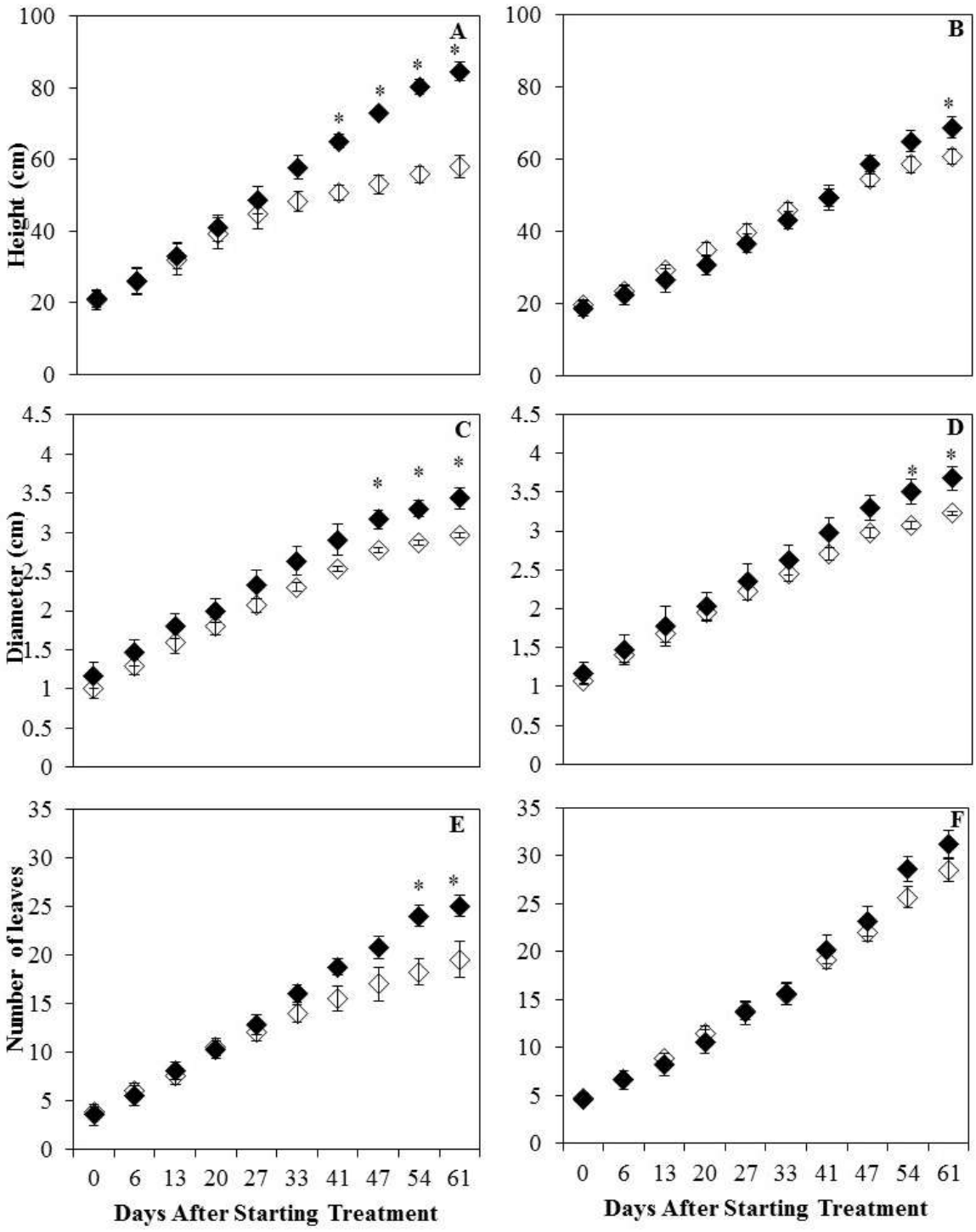

Figure 6. Height, diameter and number of leaves of seedlings of *J. curcas,* genotypes CNPAE 183 (A, C e E) and CNPAE 191(B, D e F), submitted to 55 days of water stress and six days of rehydration. Control plants (filled diamonds) and plants under water stress (open diamonds). Asterisks (*) indicate significant differences between water regimes (F test, $p<0.05$). Pointed-line indicate the beginning of rehydration. Points represent mean values of 4 to 5 replicates and bars indicate standard error of the mean.

However, water stress induced significant reduction (25% lower than control) of root volume in CNPAE183 and significant increase of root length in CNPAE 191 (Figure 10). For CNPAE 183, there was no difference between

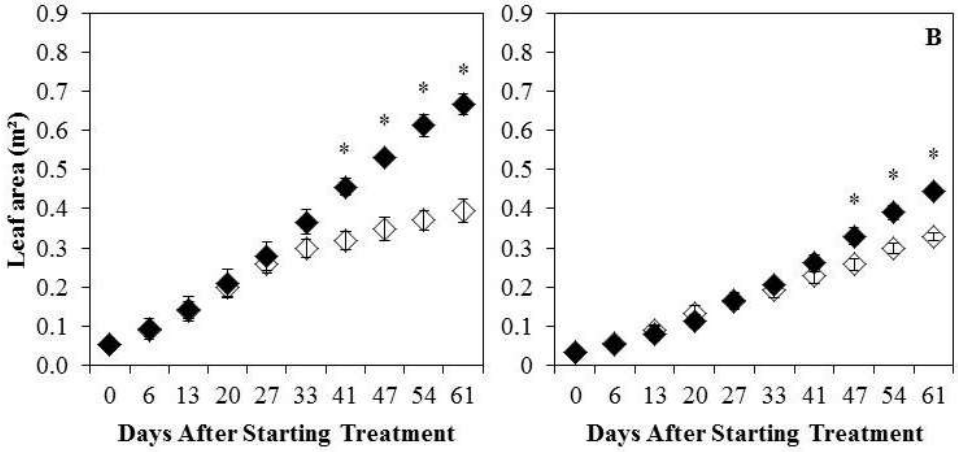

Figure 7. Leaf area (m^2) of seedlings of *J. curcas* genotypes CNPAE 183 (A) and CNPAE 191(B) submitted to 55 days of water stress and six days of rehydration. Control plants (filled diamonds) and plants under water stress (open diamonds). Asterisks (*) indicate significant differences between water regimes (F test, P<0.05). Points represent mean values of 4 to 5 replicates and bars indicate standard error of the mean.

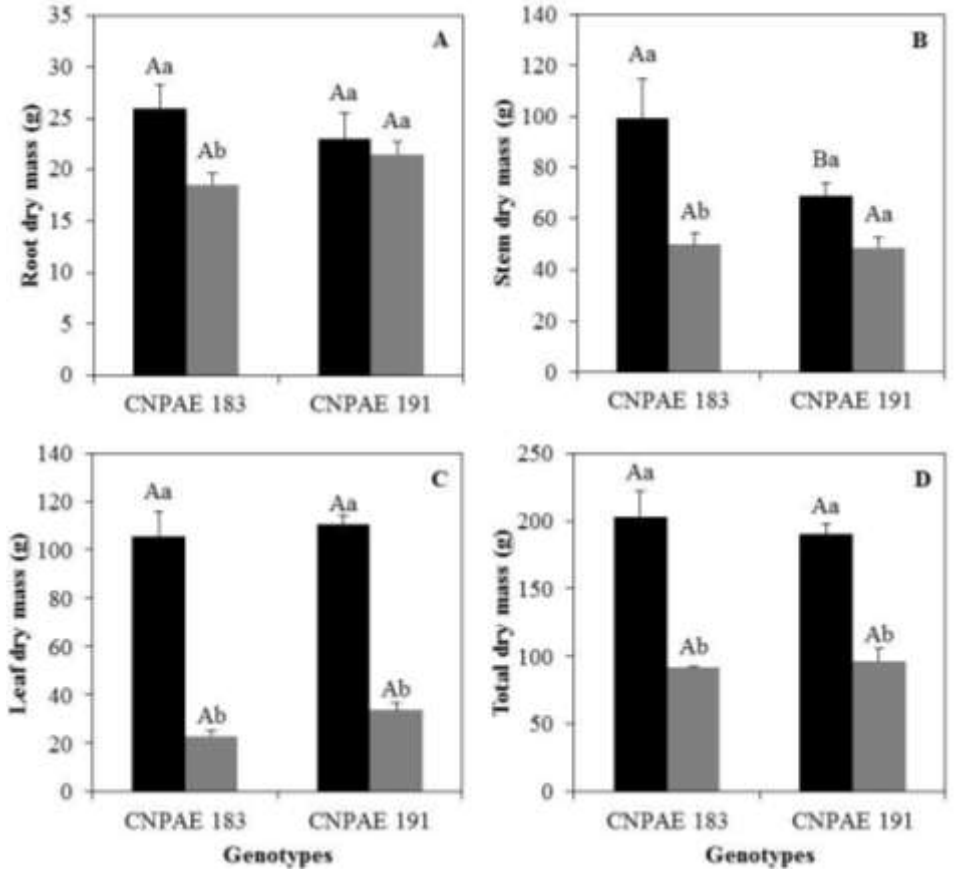

Figura 8. Root dry mass (A), stem dry mass (B), leaf dry mass (C) and total dry mass (D) of two genotypes of *J. curcas* (102 days). Control plants (black bars) and plants under water stress (gray bars). Capital letters indicate comparison of genotypes and lowercase letters between water regimes (F test, p<0.05). Points represent mean values of 4 to 5 replicates and bars indicate the standard error of the mean.

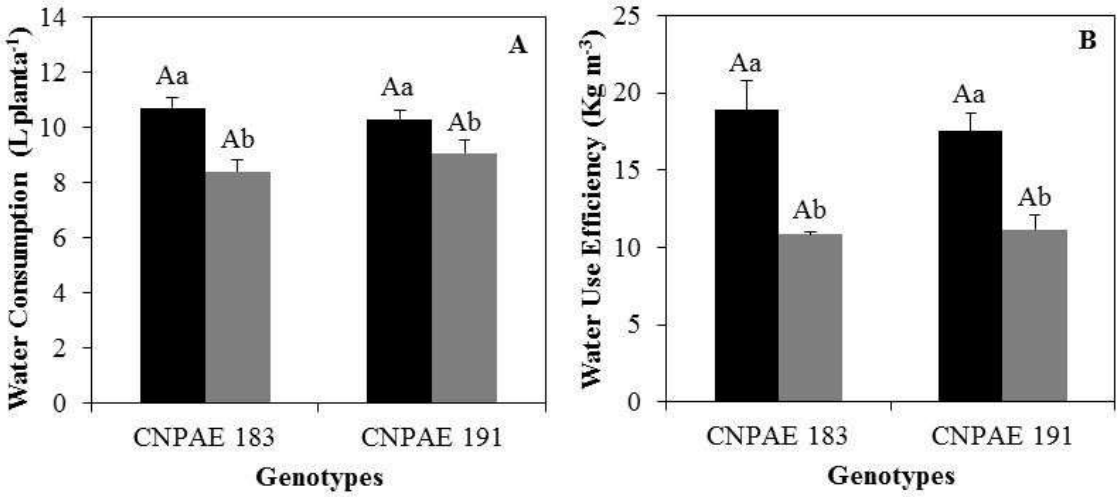

Figure 9. Total water consumption (A) and the biomass water use efficiency (B) calculated at the end of the experimental period. Control plants (black bars) and plants under water stress (gray bars). Capital letters indicate comparison between genotypes and lowercase letters indicate comparison between water regimes (F test, $p<0.05$). Columns represent mean values of 3 replicates and bars indicate standard error of the mean.

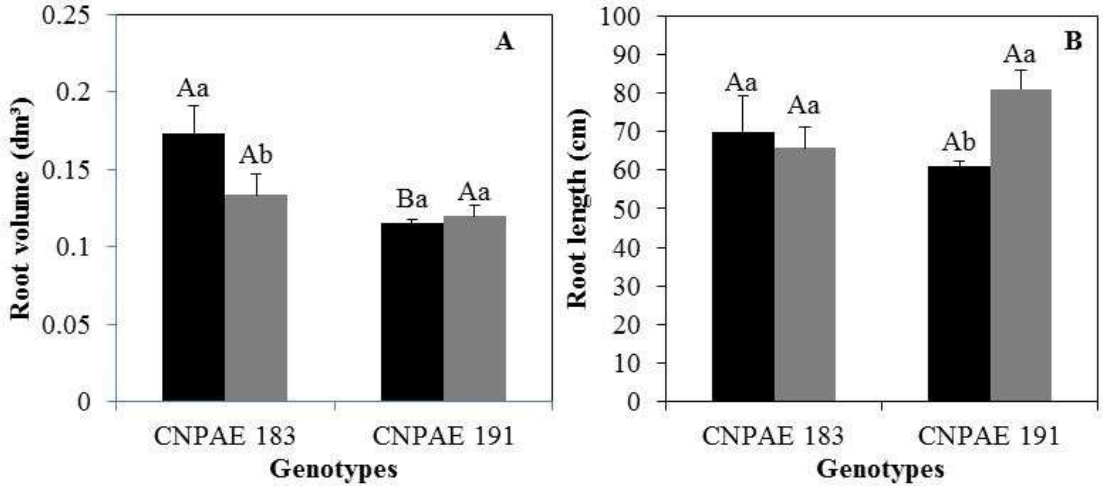

Figure 10. Root volume and root length of genotypes CNPAE 183 and CNPAE 191 of *J. curcas*. Control plants (black bars) and plants under water stress (gray bars). Capital letters indicate comparison of genotypes and lowercase letters between water regimes (F test, $p<0.05$). Points represent mean values of 4-5 replicates and bars indicate standard error of the mean.

roots lengths of water-stressed and control plants.

DISCUSSION

Although, *J. curcas* has been described as being adapted to arid conditions (King et al., 2009; Verma et al., 2012), it is indisputable that under adequate water availability, this species will show higher productivity. However, plants use various strategies to survive the water restriction periods, such as roots and leaves morphological changes, osmotic adjustment, increased abscisic acid content (ABA). The capacity Ψ_W reduction (osmotic adjustment) is also a common mechanism to avoid dryness in *J. curcas*, maintaining cell function through high RWC and stomatal closure (Fini et al., 2013; Tiwari et al., 2013; Fang and Xiong, 2014; Silva et al., 2015).

The continuation of drought stress caused no major decrease in $\Psi_{W,}$ because as stomata close, there is also a decrease in leaf water loss (Figures 1 and 4),

confirming the findings of Fini et al. (2013). These authors also noted that Ψ_W from plants that were drought stressed matched that of control plants after rehydration, thus demonstrating the species' ability to fully recover after a period of water stress. Silva et al. (2010), by imposing water stress on plants of *J. curcas* observed a reduced Ψ_W in stressed plants as compared to the control, but the relative water content (RWC) was only affected by water stress when the substrate water content was lowered to 10% of field capacity. This led to an increase of RWC as compared to the control. The increase in RWC in this work shows that conservation of the water content in plant tissues can also be considered a strategy of this species to survive periods of water deficit in soil. Results obtained by Sapeta et al. (2013) are similar to those found in this work, with an increase in RWC at the peak of water stress and recovery after 7 days of rehydration. According to Silva et al. (2012) osmotic adjustment is responsible for maintaining a high RWC in *J. curcas* tissues.

The coordination of hydraulic conductance with several physiological traits has been demonstrated to be linked to water and carbon balances (Brodribb and Jordan, 2008; Brodribb et al., 2010; Martinez-Vilalta et al., 2014; Pivovaroff et al., 2014). Water stress strongly affects the leaf hydraulic system, causing a decline in water potential (Brodribb and Holbrook, 2006). Genotypic differences in *J. curcas* have been detected recently regarding the trade-offs between K_L and growth under moderate water deficit (Santana et al., 2015). The authors demonstrated that three genotypes showed similar reductions in biomass accumulation (about 37%), although one of them (CNPAE 126) showed lower reduction in K_L (62% as compared to 88% in the other two genotypes). In the present work, a similar effect of water deficit on biomass accumulation (50% of reduction as compared to their controls) was observed for both genotypes (Figure 8D), although a greater reduction of K_L (down to 67% of the control value) was observed in CNPAE 191 than in CNPAE 183 (58%) (Figure 2A).

In a study by Díaz-Lopez et al. (2012), *J. curcas* showed higher P_N/g_S after 27 days under deficit irrigation (75% field capacity). This effect was corroborated in our study by 34 DAST for both genotypes. After six days of rehydration, the stressed plants of genotype CNPAE 191 matched the control, which shows once again that these plants decreased g_S and E to prevent dehydration under water deficit. Similar results were found by Fini et al. (2013), where the decrease of irrigation resulted in a gain of P_N/g_S and P_N/E, and after 12 days of rehydration plants fully recovered. This shows that *J. curcas* is able to use physiological mechanisms to survive periods of water deficit, resuming normal physiological function upon return to well-watered conditions.

Water stress reduces photosynthetic rate due to decreased stomatal conductance (Figure 4), through stomatal closure. However, with closed stomata, gas exchange and CO_2 assimilation by C3 photosynthesis is negligible (Chaves et al., 2009). This may account for a delay in the growth of the plant.

Verma et al. (2012) found similar patterns in gas exchange when they subjected plants to water stress at 50% of field capacity (FC). Sousa et al. (2012), also using a drought treatment of 50% of FC, found reductions of around 70% of carbon assimilation as compared to the control. Variation in the results of such studies can be attributed to the soil and climatic conditions of each region, pot size, the vapor pressure deficit and temperature, as well as nutritional status and genetic factors that may influence the physiological characteristics of the plant.

After six days of rehydration P_N and E recovered to the control values in genotype CNPAE 191. However, g_S shown by rehydrated plants remained significantly different from that of control plants. Similar results show that the recovery of g_S is slower than P_N, because this recovery is linked to the gradual decrease in the concentration of abscisic acid (ABA), and the time required for this to occur depends on the plant species and degree of stress (Pompelli et al., 2010). On the other hand, Silva et al. (2015) recently showed a more rapid restoration (5 days) of g_S than of to P_N (10 days), suggesting the need for recovery of g_S to facilitate the restoration of P_N.

Pompelli et al. (2010) reported values of P_N lower than 5 µmol CO_2 m^{-2} s^{-1}, when soil water content had reached 5%. However, plants recovered in at least four days, reaching values of P_N and g_S higher than the control plants, confirming the data presented here, which allows us to infer that these plants controlled dehydration by reducing g_S. However, the decrease of g_S caused by the closure of the stomata for water conservation entails an unavoidable decrease in CO_2 up take, thereby limiting plant productivity.

Water stress causes negative effects on cell expansion and photosynthesis, which causes a reduction in plant growth (Zhu, 2002), as observed in this study. Drought-induced reduction of growth in *J. curcas* has been considered a bottleneck concerning the potential use of this species as a bioenergy crop in arid and semiarid environments worldwide, where soil water potential may become very low during long time of drought (Fini et al., 2013). Reductions of 50% in the number of leaves for genotypes *J. curcas* from Brazil and Tanzania and a 90% for genotypes from Suriname, after 18 days without irrigation have been demonstrated (Fini et al., 2013). In this study, drought led to reductions of 22 and 9% in the number of leaves for genotypes CNPAE 183 and CNPAE 191, respectively. However there was no shedding leaf, but a reduction in the emission of new leaves.

Sapeta et al. (2013) demonstrated significant reductions in height and number of leaves of *J. curcas* from the 7th day of severe water stress imposition. In this study, the lowest number of leaves on the water stressed plants of

genotype CNPAE 183 was due to reduced initiation of new leaves, as a mechanism to decrease the surface area for transpiration. However, this mechanism may cause losses in crop yield, because with a reduced leaf area, there is a decrease in light interception, thus decreasing overall photosynthetic capacity. Maes et al. (2009), in subjecting *J. curcas* to water deficit (40% FC), found reductions in leaf area of approximately 57% as compared to control. Verma et al. (2012), after 50 days of imposing water stress at 75 and 25% of field capacity reported 11 and 55% reductions in leaf area of *J. curcas*.

The similarity between the RDW of control and stressed plants for CNPAE 191 explains the smaller effect of water stress on the biometric characteristics (height, diameter, number of leaves and leaf area), making a good indicator of drought tolerance (Figure 8). The stressed plants of genotype CNPAE 191, however, as a mechanism to prevent dehydration, increased the length of their roots, to explore a larger volume of soil (Figure 10), which according to Hammer et al. (2009) are characteristics associated with drought resistance. With the reduction in leaf area and maintenance of RDW, there is greater hydration of plant tissue (measured as RWC), favoring the continued growth and development of the plant (Silva et al., 2010). As experimental water stress was not imposed for a long time, the recovery of gs was faster, favoring CO_2 entry into the cell, which is an interesting quality for genetic improvement, as it allows to increase the effective use of water (EUW), influencing the plant stress tolerance and avoiding reduced productivity (Blum, 2009).

Conclusions

Moderate and rapidly-imposed water deficit, as imposed here, negatively affect the gas exchange and biomass water use efficiency of *J. curcas*, despite reductions in water consumption and increased photosynthetic water use efficiency. Maintenance of high RWC and Ψw under water deficit, as well as a lack of genotypic variation in that characteristic indicate that both *J. curcas* genotypes are water savers. The genotype CNPAE 191 should be considered for further investigation concerning the tradeoffs between RWC and root traits, in the search for genetic material suitable for cultivation in areas subject to short periods of soil water deficit.

Abbreviations

CI, Internal **CO2** concentration; **DAR**, days after rehydration; **DAST**, days after starting treatment; **DW**, dry weight; **E**, transpiration rate; **FC**, field capacity; **gS**, stomatal conductance to water vapour; **KL**, hydraulic conductivity; **LA**, leaf area; **PAR**, photosynthetically active radiation; **PN**, net photosynthesis; **PN/gS**, intrinsic water use efficiency; **PN/E**, instantaneous water use efficiency; **RWC**, relative water content; **Ψw**, leaf water potential; **WS**, water stress conditions; **WUE**, water use efficiency.

Conflict of Interests

The authors have not declared any conflict of interests.

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