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Abscisic acid-mediated stomatal closure and antioxidant defenses in *Jatropha curcas* L. seedlings submitted to moderate water deficit

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The objective of this research was to evaluate the biochemical changes in leaves from different genotypes of *J. curcas* in order to extend knowledge regarding the mechanisms of tolerance to water deficit and its variation in different genotypes. Potted plants of three genotypes (CNPAE 126, 137 and 139) were cultivated under water deficit conditions for 66 days. Two watering regimes, as measured by the percentage of field capacity (FC), were imposed: Control plants (100% FC) and plants submitted to water deficit (70% FC). After 66 days, no significant effects of treatment and of genotype on leaf water potential (Ψw) were observed. While the water deficit treatment led to significant increments in foliar concentrations of proline and soluble sugars in all genotypes, no significant effects of genotype or treatment on K^+ concentration were detected. In addition, significant differences among treatments and genotypes in the activity of antioxidant enzymes (SOD, CAT and POD) and [ABA] in leaf and root were demonstrated. The genotypes exhibited an effective mechanism of response against the effects of water deficit, involving accumulation of compatible osmolytes, and increased antioxidants enzymes and ABA. Taken together, that results configure a strategy for maintenance of leaf hydration under moderate water deficit.

Key words: Abiotic stress, antioxidant enzymes, compatible osmolytes, Euphorbiaceae, gas exchange.

INTRODUCTION

The development of human civilization and industrialization has long been based on fossil fuels utilization. However, oil reserves are running out at an ever-increasing speed, this being accompanied by a sharp increase of CO_2 in the atmosphere, one of the main greenhouse gases. In the quest for sustainable systems,

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coupled with gradual reduction of oil reserves, productive alternatives are being studied, and *Jatropha curcas* L. stands out due to its high oil productivity per hectare (Dias et al., 2007) and development in areas of low rainfall (Maes et al., 2009).

J. curcas, belonging to the Euphorbiaceae family, is native to the Americas and is widespread in tropical and subtropical regions of the globe, such as Asia, Africa and India (Divakara et al., 2010). Although it is considered to be a plant with the potential to provide raw material for fuel, this oilseed species is still in its domestication phase (Laviola et al., 2011). In recent years, a subject of special attention because of high oil content in seeds, which can easily be converted into biodiesel. Thus, *J. curcas* is being considered to be a source of energy and has attracted scientific and economic interest (Kumar and Sharma, 2008).

Water deficit is considered to be the environmental factor that most significantly influences plant growth and yield (Kramer and Boyer, 1995). Therefore, understanding the physiological and biochemical mechanisms that are involved with any level of plant response to water deficit is of uppermost interest (Slama et al., 2007). In order to increase their tolerance to dry conditions, some species develop some mechanisms such as, for example, accumulating osmotically active solutes in the cell cytoplasm, such as proline, soluble sugars and potassium (Silva et al., 2010a) as well as antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7) and catalase (CAT; EC 1.11.1.6) (Pompelli et al., 2010a).

The mechanism of osmotic adjustment in plants has been considered as an important physiological strategy associated with drought tolerance (Hessine et al., 2009). It leads to increased water uptake and to increased cell growth of plants during drought stress associated with the partial opening of the stomata allowing CO_2 assimilation at low water potential (Alves and Setter, 2004). According to Silva et al. (2010b), an efficient mechanism of osmotic adjustment was demonstrated in *J. curcas* in response to drought stress, which involved inorganic and organic osmolytes.

The activity of antioxidant enzymes such as SOD, POD and CAT may be an important mechanism in response to environmental stresses such as drought. Arcoverde et al. (2011), working with young plants of *J. curcas* in 10 L pots, found that the antioxidant mechanism has been effective thus increasing their activity from moderate stress.

The objective of the present work was to evaluate the biochemical responses in genotypes of *J. curcas* in order to generate additional information for the studies on physiological processes involved in plant tolerance to water deficit. We hypothesized that (1) accumulation of solutes osmotically active (osmotic adjustment) and stomatal control mediated by ABA are strategies used by *J. curcas* aimed at maintaining the hydration status under water stress conditions and (2) different genotypes show distinct responses to water deficit in terms of concentration of abscisic acid and antioxidant enzymes.

MATERIALS AND METHODS

Plant material and growing conditions

The experiment was performed in a greenhouse, at the State University of Santa Cruz (UESC) campus, located in Ilhéus, Bahia (14°47'00" S, 39°02'00" W), between July and September 2011. The maximum and minimum values of air temperature measured during the experimental period were 27 and 22°C, respectively. The mean relative air humidity was recorded at 77%. The mean daily value of photosynthetically active radiation was 11.7 mol photons m^{-2} day^{-1}.

J. curcas seeds from the genotypes CNPAE 126, 137 and 139 were provided by the germplasm bank of EMBRAPA Agroenergia - Distrito Federal, Brazil. Seeds were germinated in pots containing 50 dm^3 of a soil:sand (2:1) mixture, which was previously prepared according to chemical analysis of the substrate. After a germination period of 15 days, thinning was performed, leaving only one plant per pot. The pots were immediately covered with aluminum foil to prevent evaporation and heating of the soil and the water deficit treatment was set. Water deficit treatment was maintained for a period of 66 days.

The treatments were made up of two watering regimes, which were adjusted as a percentage of field capacity (FC): control plants (100% FC) and plants submitted to water deficit (70% FC). Throughout the experimental period the control plants were irrigated close to field capacity (matric potential of -7.4 to -9.8 kPa). Water deficit plants were kept in the range between -99.0 and -33.5 kPa. The water content and matric potential in the substrate were determined by use of the gravimetric method and of a characteristic water retention curve, respectively.

Water relations parameters

Pre-dawn (Ψpd) and midday (Ψmd) leaf water potential were measured weekly at 04:00 and 12:00, respectively. A PMS-1000 pressure Chamber (PMS Instrument Company, USA) was used in accordance with the methodology proposed by Scholander et al. (1956) with some modifications. After placing the sheet on the cylinder, we used absorbent paper pressing lightly on incision to extract the excess latex.

The leaf relative water content (RWC) was calculated using the formula: RWC = [(FW-DW)/(TW-DW)] × 100, where FW is the fresh weight, TW is the turgid weight measured after 24 h of saturation in deionized water in the dark, and DW is the dry weight determined after 48 h in an oven at 75°C.

Leaf gas exchanges

The leaf gas exchanges were evaluated 66 days after imposition of treatment (DAT) in fully mature leaves, between 8 and 11 h with a Li-6400 portable photosynthesis measurement system (Li-Cor, Inc., Lincoln, NE, USA) under artificial light saturating 1000 µmol photons m^{-2} s^{-1} and atmospheric CO_2 concentration (C_A) of 380 µmol mol^{-1}. The photosynthetic rate (P_N, µmol m^{-2} s^{-1}), the stomatal conductance to water vapor (g_S, mol m^{-2} s^{-1}), the transpiration rate (E, mmol m^{-2} s^{-1}) and the ratio between the internal and atmospheric concentrations of CO_2 (C_I/C_A) were calculated using the values of the variations of the concentrations of CO_2 and H_2O vapor inside the chamber. Through the gas exchange data were

estimated photosynthetic efficiency of water use, intrinsic (P_N /g_S, μmol mol^{-1}) and instant (P_N /E, μmol $mmol^{-1}$).

Osmotic potential and osmotic adjustment

Five leaf disks (5 mm in diameter) were collected at 66 DAT from the leaves of the middle third of the shoot of four randomly chosen plants from each treatment. The discs were frozen in liquid nitrogen, and after thawing and temperature stabilization were placed in the chamber to obtain the osmotic potential (Ψ_s) readings. Osmotic potential was measured using a thermocouple psychrometer (C-52 Chamber model, Wescor Inc., Logan, Utah, USA), connected to a dew point microvoltimeter (PsyPRO, Wescor Inc., Logan, USA) operating in a psychrometric method. The values Ψs were corrected to eliminate the effect of passive concentration of solutes caused by foliar dehydration, in accordance with Wilson et al. (1979). Osmotic adjustment was calculated as the difference in corrected Ψs of control and stressed plants.

Antioxidant enzymes activities

Antioxidant enzymes were determined at the end of the experiment (66 DAT). Leaf samples were collected from the third fully expanded mature leaf, immediately frozen in liquid nitrogen, lyophilized and stored in a freezer (-20°C) until the moment of analyses.

Peroxidase activity (POD; EC 1.11.1.7) was determined in accordance with the method by Rehem et al. (2011). Microplates with 96 wells and a 300 µL capacity were used, containing 140 µl of POD reaction buffer 2× [40 mmol L^{-1} of guaiacol, H_2O_2 at 0.06% and sodium phosphate (20 mmol L^{-1}, pH 6.0)], 139 µL of phosphate buffer (50 mmol L^{-1}, pH 6.0) and 1 µL of enzyme extract which was previously diluted. The absorbance variation at 470 ηm was monitored for 60 s of reaction at 25°C, in a microplate spectrophotometer (*VERSAmax*).

The superoxide dismutase (SOD; EC 1.15.1.1) was determined by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). 100 µL aliquots of the enzyme extraction were then transferred to test tubes and protected from light; these contained 50 mM of potassium phosphate buffer, pH 7.8, 0.1 mM of EDTA, 13 mM of L-methionine and 75 µM of NBT. The reaction was initiated through the addition of 2 µM of riboflavin and the concomitant transfer of the tubes to a chamber lit by a 30 Watt circular fluorescent lamp, for a 15 min period. Following this, the absorbance readings at 560 ηm were performed in a spectrophotometer. The activity was determined by calculating the amount of extract that inhibited 50% of NBT reduction (Beauchamp and Fridovich, 1971) and expressed in U kg^{-1} DM.

The enzyme catalase activity (CAT; EC 1.11.1.6) was determined according to the methodology as described by Madhusudhan et al. (2003), whose activity is defined by the amount of enzyme required to catalyze the decomposition of H_2O_2. For the test, 20 µL of the enzymatic extract was added to 0.98 mL of sodium phosphate buffer 0.05 M pH 7.0, H_2O_2 0.0125 M which was supplemented with hydrogen peroxide to a final concentration of 12.5 mM. CAT activity was determined through measuring the reduction in absorbance of samples at 240 ηm, this being a consequence of H_2O_2 consumption, using the molar extinction coefficient of 36 M^{-1} cm^{-1}.

Determination of organic and inorganic solutes

At 66 DAT, concentrations of compatible osmolytes were determined. Concentrations of proline were analyzed using the acidic Ninhydrin method (Bates et al., 1973). The total soluble sugar content was determined using the phenol-sulfuric method according to Clegg (1956) and potassium (K^+), was analyzed by flame photometry, according to the methodology as described by Viégas et al. (2001).

Abscisic acid concentration (ABA)

ABA extraction was carried out at 66 DAT according to the method as described by Kato et al. (2006). ABA quantification in the extracts was performed by means of the ELISA test, using a commercial kit (Phytodetek® ABA Test Kit), in accordance with the manufacturers recommendations. Following this, optical density readings were taken by means of a microplate spectrophotometer (Biotek, ELX 800 Instruments, Inc, Winooski, VE, USA) using a wavelength of 405 ηm.

Experimental design and statistical analysis

The experimental design was completely randomized with a 2×3 factorial scheme, made up of two levels of water availability and three genotypes of *J. curcas*, with four repetitions per treatment. The results were submitted to an F-test at a 5% significance level, by factorial ANOVA and, when indicated, the mean comparisons were performed by means of a Tukey test at the same significance level.

RESULTS

Substrate water content was 9.8 and 6.3%, on average, in control (well-watered) and water deficit (WD) treatments, respectively. The substrate matric potential (Ψm) calculated using the characteristic curve of water retention, varied between -9.8 and -7.4 kPa and -98.6 and -33.5 kPa in control and WD, respectively.

There were the significant differences ($P<0.05$) in pre-dawn (Ψpd) and midday leaf water potential (Ψmd) and in RWC among genotypes after 66 DAT (Table 1). Despite non significant, plants of CNPAE-126 presented a smaller amplitude (-0.25 MPa) between the Ψpd and Ψmd when compared with other genotypes. On the other hand, a significant difference ($P<0.05$) between the genotypes for osmotic adjustment (OA) was detected. The genotypes CNPAE-126 and 139 showed no OA, as the values of Ψs were -0.80 and 0.02, respectively. However, the genotype CNPAE-137 showed a value 0.30, which may indicate some degree of OA, despite no significant effect of treatments has been detected (Table 1).

No significant differences were observed ($P<0.05$) among the genotypes of *J. curcas* for the foliar concentration of proline (Figure 1A). Water deficit led to a significant ($P<0.05$) increase (58%) in foliar concentration of proline when compared to control plants. Genotype CNPAE-126 was the only in which significant differences ($P<0.05$) between treatments for total soluble sugars (TSS) were observed. Interestingly, high concentration (160.0 mg g^{-1}) was measured in control, as compared to water deficit plants (94.5 mg g^{-1}) (Figure 1B). There was no significant effect of genotype for TSS. In addition, no

Table 1. Pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water pontential, corrected osmotic potential (Ψ_{sc}) and osmotic adjustment (OA) in plants of *J.curcas* under different water conditions, control (C) and water defict (WD) at 66 days after the imposition of treatment (DAT). Values are means (s.e.) of five replicates.

Genotypes	CNPAE-126	CNPAE-137	CNPAE-139
		Ψ_{pd} (- MPA)	
C	0.47±0.04^{Aa}	0.45±0.05^{Aa}	0.46±0.03^{Aa}
WD	0.50±0.08^{Aa}	0.46±0.13^{Aa}	0.55±0.05^{Aa}
		Ψ_{md}(-MPa)	
C	0.72±0.06^{Aa}	0.81±0.04^{Aa}	0.75±0.08^{Aa}
WD	0.75±0.09^{Aa}	0.87±0.06^{Aa}	0.95±0.01^{Aa}
		Ψ_{sc}(-MPa)	
C	2.40±1.26^{Aa}	1.34±0.15^{Ba}	1.41±0.14^{Ba}
WD	1.60±0.31^{Ab}	1.64±0.12^{Aa}	1.43±0.10^{Aa}
		OA	
	-0.80±0.10^{b}	0.30±0.03^{a}	0.02±0.07^{a}

Lower case letters indicate significant differences between treatments within each genotype and capital letters indicate significant differences by genotypes within each treatment by Tukey test (P<0.05).

significant (P<0.05) differences were observed neither among the genotypes, nor between the treatments for the concentration of K^+ in *J. curcas* leaves (Figure 1C).

The values of P_N, g_S, E, and C_I/C_A at 66 DAT were significantly (P<0.05) reduced in stressed as compared to control plants. Such reduction reached 81, 90, 86 and 37%, respectively (Figure 2). Moreover, the values of P_N/g_S and P_N/E in WD plants increase of 52 and 74%, respectively, as compared to control plants (Figure 2).

Water deficit led to a significant increase in the SOD, POD and CAT enzymes in *J. curcas* leaves 66 DAT. Regarding SOD, the genotype CNPAE-137 differed from the others, showing a lower activity of this enzyme. The values of SOD activity in genotype CNPAE-137 were, on average, 0.06 and 0.04 U kg^{-1} DM in WD and control plants, respectively (Figure 3A). Significant (P<0.05) effects of genotype and of treatments in POD activity were detected. Water stress led to increases of 25, 35 and 2% in POD activity measured in WD plants of CNPAE-126, 137 and 139, respectively, as compared to their control plants (Figure 3B). Moreover, POD activity in WD plants was significantly (P<0.05) higher in CNPAE-137 (0.690 mmol h^{-1} kg^{-1} DM) than in CNPAE-126 (0.598 mmol h^{-1} kg^{-1} DM) and CNPAE-139 (0.604 mmol h^{-1} kg^{-1} DM). There were significant differences (P<0.05) among genotypes, as well as between treatments in CAT activity (Figure 3C). Water stress led to 32% increase, on average, in CAT activity, when compared to control plants. CAT activity was significantly higher in genotype CNPAE-126 of the two treatments, than in the other two genotypes. The values of CAT activity measured in WD plants were 39% higher than in control plants.

During the 66 DAT, there were significant differences (P<0.05) in ABA foliar concentration ([ABA]) among the water regimes, as well as among the genotypes. Water stress led to an increase in [ABA], of 136.4, 21.8 and 15.7 for the genotypes CNPAE-126, 137 and 139, respectively. The higher [ABA] leaf was found in the genotype CNPAE-126 which showed 100.42 ng g^{-1} DM for WD leaves, when compared to their controls (Figure 4A). Upon comparison, the *J. curcas* roots presented a lower [ABA] in relation to the leaves, presenting average values of 11.14, 13.19 and 10.09 ng g^{-1} DM for the genotypes CNPAE-126, 137 and 139, respectively (Figure 4B).

DISCUSSION

The lack of variation on the Ψw in *J. curcas* plants is probable explained by the internal redistribution of the water stored in the succulent stems. Therefore, water supply and conservation in order to foster a water deficit tolerance could be suggested as an important role for the succulent stem, as quoted by Maes et al. (2009).

Water conservative behavior through an efficient stomatal regulation, as indicated by measurements of leaf RWC and Ψw, has been commonly observed in young and adult plants (Díaz-López et al., 2012; Fini et al., 2013; Sapeta et al., 2013). The water content in tissues in *J. curcas* reveals a conservation strategy for this species to tolerate dry periods. According to Silva et al. (2011), osmotic adjustment mechanism is responsible for maintaining a high RWC in *J. curcas* of tissues.

In the present study, even though a tissue water maintenance strategy has been demonstrated, no significant osmotic adjustment was found. The genotypes avoided the loss of water in leaves through an efficient stomatal control of transpiration (Silva et al., 2010b). Rather than acting as an osmoregulator, in the present

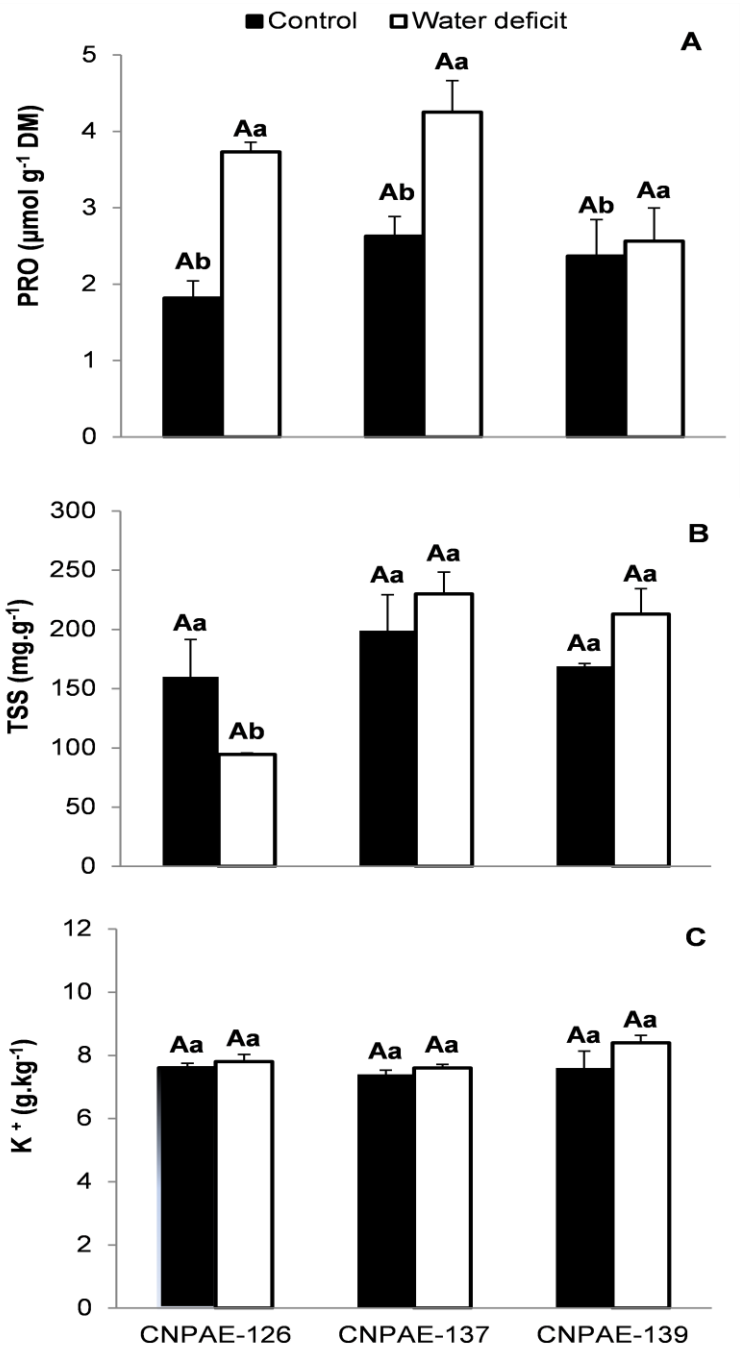

Figure 1. Foliar concentrations of proline (PRO, A), total soluble sugars (SST, B) and potassium (K^+, C) in young plants of *J. curcas* subjected to water deficit for 66 days. The columns are mean values (n = 5) and bars represent the standard error of the mean. Capital letters indicate comparison between genotypes and lower case between water regimes by Tukey test (P<0.05).

experiment, proline is suggested to be related to an osmoprotective role and can also serve as a reserve of carbon and nitrogen for growth (Silveira et al., 2003). It is worth noting that an increase in antioxidant activity, as well as lower growth (data not shown) were demonstrated when the plants were subjected to stress. However, there are other osmolytes which can accumulate in the cells and which were not quantified in this study, thus leading to the suggestion of OA in CNPAE-137 as an adaptation mechanism to tolerate the moderate water deficit.

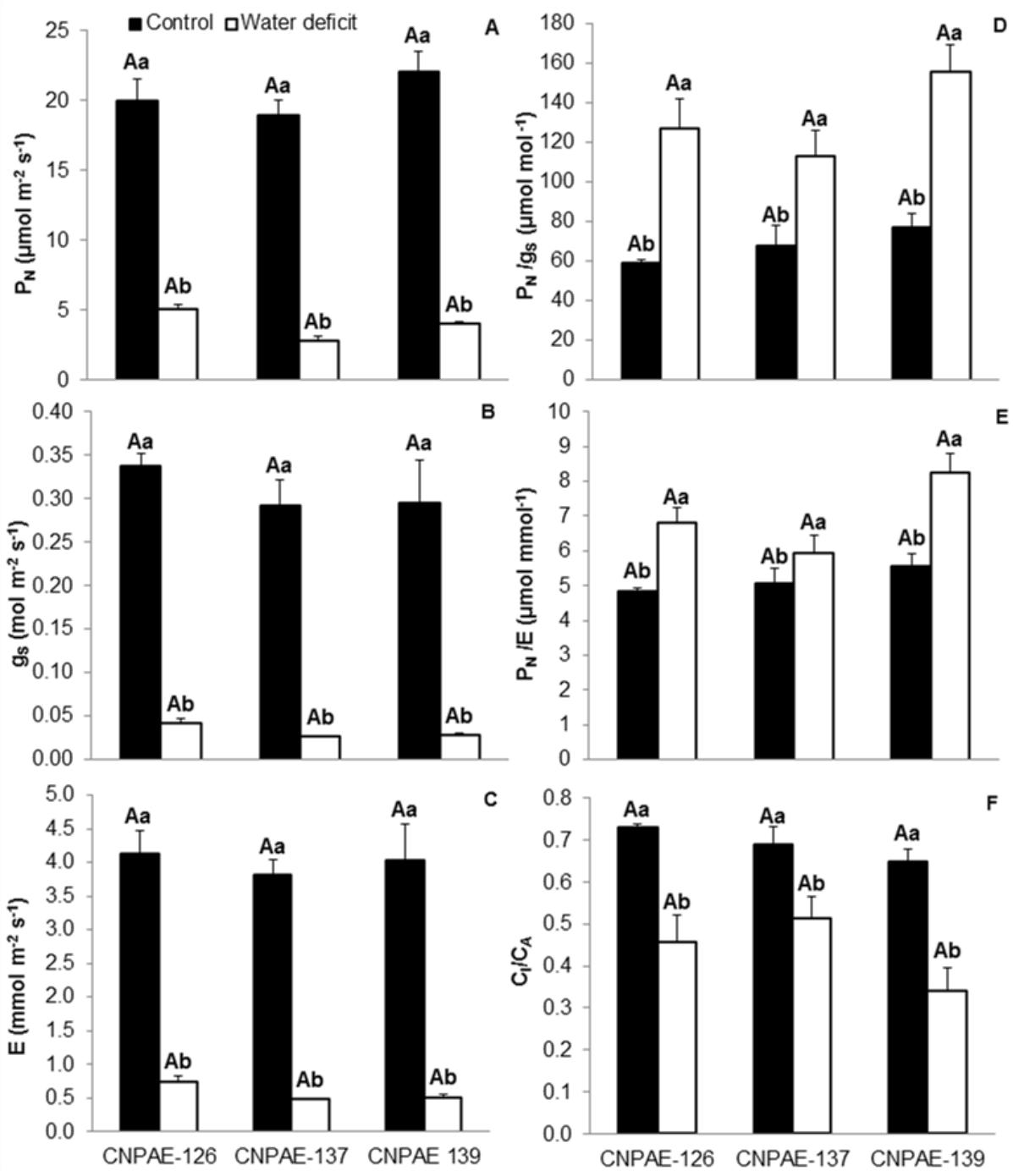

Figure 2. Net photosynthetic rate (P_N, A), stomatal conductance (g_S, B), transpiration rate (E, C), intrinsic efficiency (P_N / g_S, D), instantaneous efficiency (P_N/ E, E) e ratio of intercellular and atmospheric concentrations of CO_2 (C_I/C_A, F) in young plants of *J. curcas* subjected to water deficit for 66 days. The columns are mean values (n = 4) and bars represent the standard error of the mean. Capital letters indicate comparison between genotypes and lower case between water regimes by Tukey test (P<0.05).

The accumulation of compatible osmolytes (TSS and proline) in *J. curcas* plants subjected to WD may be related to the mechanism that prevents the water loss. In another survey, Babita et al. (2010) detected increased concentration of osmolytes such as proline and soluble sugars in a drought tolerant genotype *Ricinus communis* L. under water deficit. Although our data do not show significant differences in the concentration of TSS,

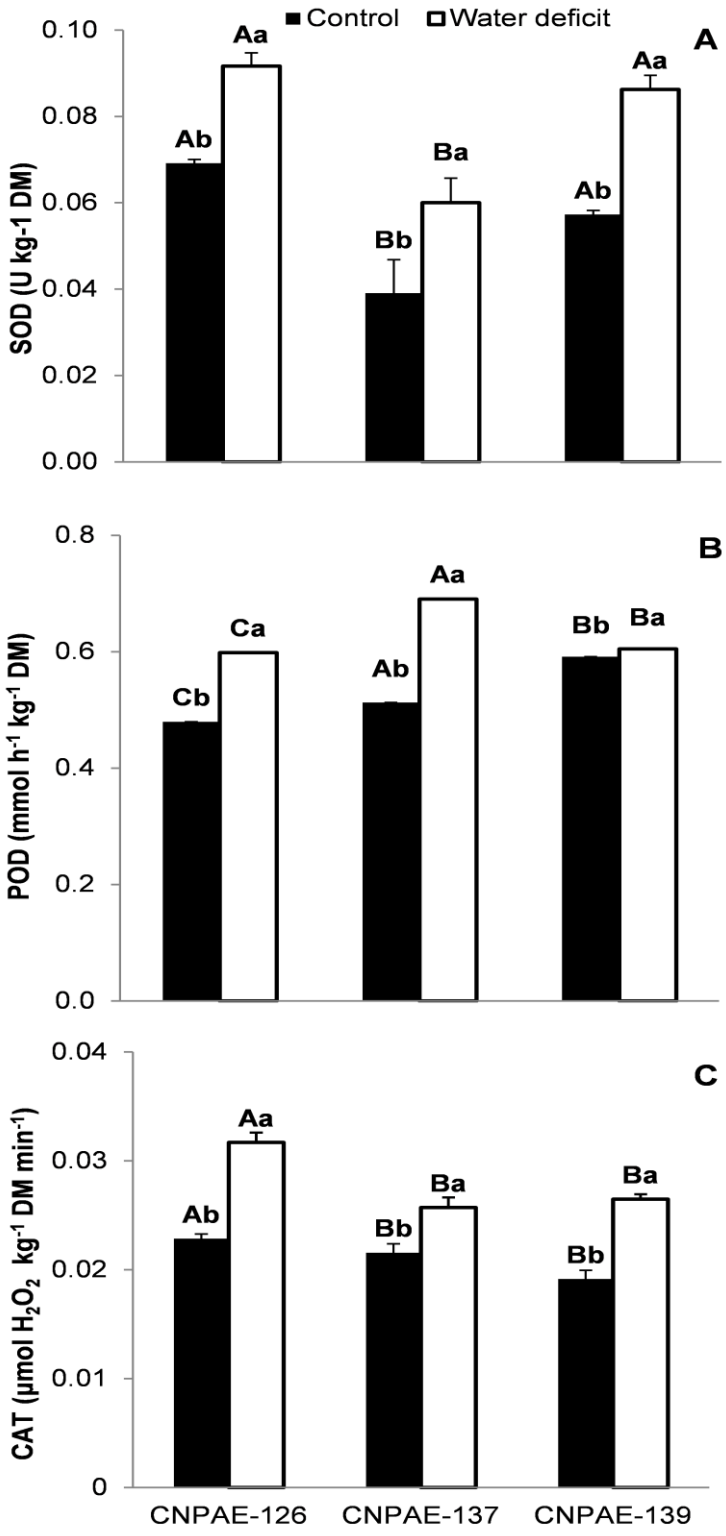

Figure 3. Activities of the enzymes superoxide dismutase (SOD, A), peroxidase (POD, B) and catalase (CAT, C) in leaves of three genotypes of *J. curcas* subjected to water deficit for 66 days. The columns are mean values (n = 5) and bars represent the standard error of the mean. Capital letters indicate comparison between genotypes and lower case between water regimes by Tukey test ($P<0.05$).

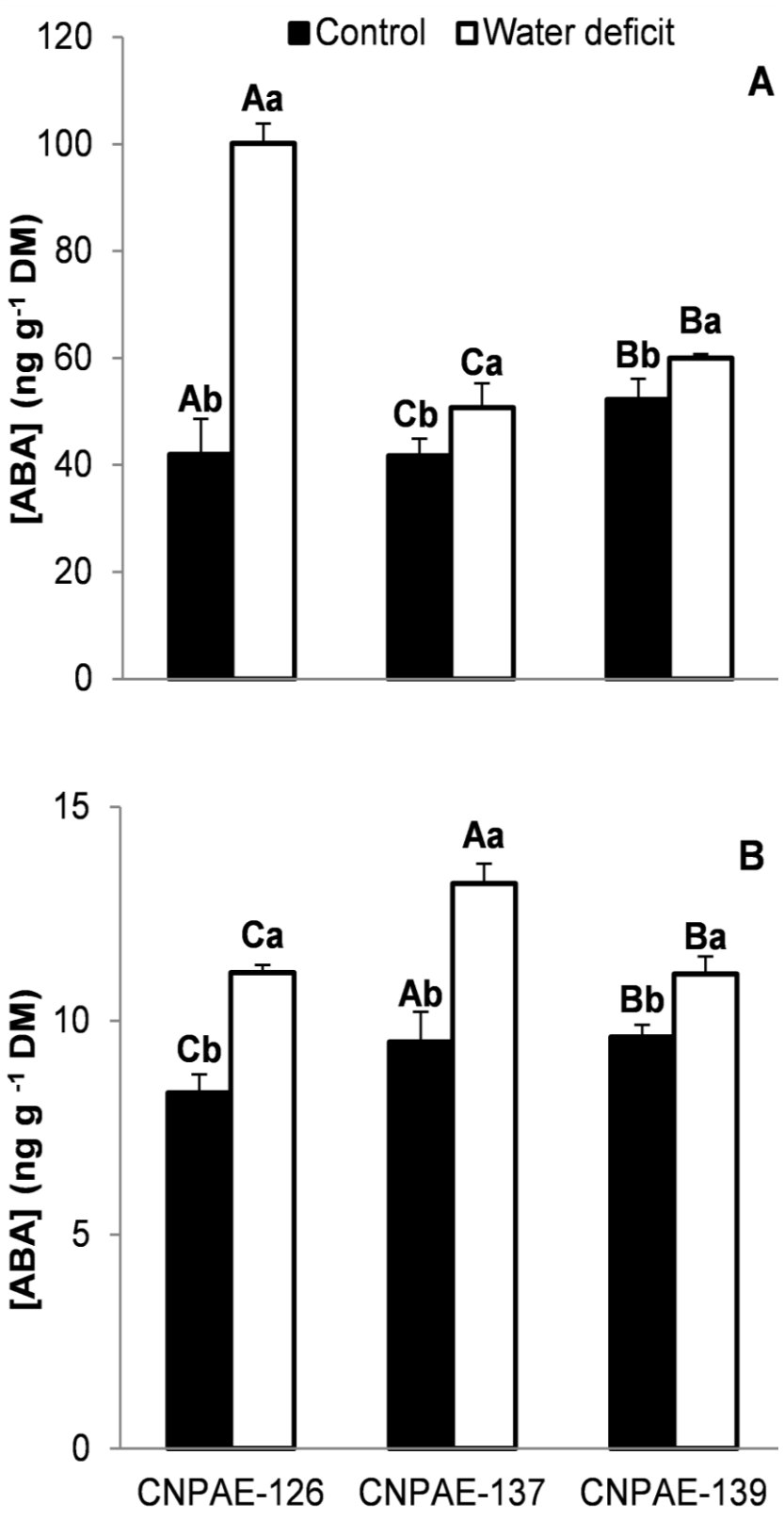

Figure 4. Concentration of ABA in leaves (A) and roots (B) in three genotypes of *Jatropha curcas* subjected to water deficit for 66 days. The columns are means (n = 5) and bars represent the standard error of the mean. Capital letters indicate comparison between genotypes and lower case between water regimes by Tukey test ($P<0.05$).

possibly by the stress applied, Silva et al. (2010b) report that the increase of this osmolyte is the largest contributor to OA.

Even though there were no changes in foliar concentration of K^+ in this research, this inorganic ion is involved in osmotic regulation for both WD as well as control plants. It has been demonstrated that, despite non-significantly changed in leaves, the content of K^+ contributed to 25% of the osmotic potential in stressed as compared to control plants (Silva et al., 2010b).

Water deficit-induced stomatal closure, as observed in the present investigation, is a strong mechanism of response in *J. curcas*, being an important component of drought tolerance in this species. Similar studies were reported by Pompelli et al. (2010), Silva et al. (2012), Sapeta et al. (2013) and De Santana et al. (2015). Pompelli et al. (2010a) demonstrated a reduction of P_N to values lower than 5 CO_2 μmol m^{-2} s^{-1} when the water content of the soil has reached the level of 5%. With the significant effect on P_N, g_S and E the intrinsic water use efficiency (P_N/g_S) and instant (P_N/E) had increases in stressed plants, demonstrating that the limitation of P_N was mainly stomatal. Higher P_N/gs and P_N/E in plants under WD have been demonstrated elsewhere, under several levels of water deficit (Diaz-Lopez et al., 2012; Fini et al., 2013).

Studies have shown a decline in the carbon cycle and increase of antioxidant enzymes when plants are water stressed (Da Matta et al., 2008). Among the several mechanisms of response to water deficit, the ability to maintain high levels of antioxidant enzymes such as SOD, POD and CAT, has been demonstrated (Zimmerman et al., 2006). The activity of one or more antioxidant enzymes generally increases in plants that are exposed to stress conditions, and this activity correlates with an increased tolerance to stress (Pilon et al., 2006). In this study, moderate water stress led to significant an increase in antioxidant enzymes.

Some studies have reported that water shortage produces oxidative stress as a result of an increase in ROS and also that many plants are able to cope with this by activating their antioxidant enzymes (Simova-Stoilova et al., 2009). Lower SOD activity was measured in leaves from the genotype CNPAE-137, thus the genotypes CNPAE-126 and 139 can be considered as more efficient at removing $O_2^{\bullet-}$ toxic radicals. Various abiotic stresses often led to an increase in ROS generation in which SOD has been an important enzyme for the plant stress tolerance, providing the first line of defense against the toxic effects of high levels of ROS (Gill and Tuteja, 2010).

It has been reported that the activity of POD in *J. curcas* is sensitive to water deficit (Kumar and Sharma, 2008). This means that the genotypes that were studied maintain a higher POD activity in plant leaves under WD, thereby giving rise to water deficit tolerance. The CAT antioxidant enzyme works to remove the H_2O_2 that is generated in peroxisomes by oxidases involved in the β-oxidation of fatty acids, photorespiration, purine catabolism and during oxidative stress (Vellosillo et al., 2010). Our data show that CAT activity increased in plants subjected to WD, which suggest a more efficient system in the elimination of ROS and thus maintaining a balance between ROS production and antioxidant enzymes for preventing oxidative damage under water deficit. Pompelli et al. (2010a), studying *J. curcas* under two water regimes (control and water deficit) for 4, 8 and 18 days, found that the WD induced the increase of antioxidant enzymes such as SOD, CAT and POD in leaves. The authors also report that the activity of these enzymes were more significant at 8 and 18 days of stress.

In the present study, elevated activity of SOD (43.7%) have been demonstrated, which can be related to the regulation of the expression of isoforms of this enzyme. The POD increased by 19.5% in stressed plants, showing an efficient mechanism against the accumulation of ROS, especially H_2O_2 present in chloroplasts. CAT had already increased 33.3% in plants subjected to WD compared to control plants, support in the removal of H_2O_2 present in peroxisomes. Thus, our data show that the antioxidant enzymes are effective in the removal of ROS in leaves of *J. curcas*. Similar results were found by Pompelli et al. (2010a), studying *J. curcas* subjected to water deficit for 18 days was found to increase SOD activity by 72.7%, POD by 45.0% and CAT by 20.0%, thus indicating that antioxidant enzymes are involved in the defense mechanism against oxidative stress during water deficit.

ABA has a central role in plant responses to water deficit by means of a large number of processes, with signaling pathways that have not yet been completely understood, despite the discovery of putative ABA receptors (Pandey et al., 2009). At the cellular level it controls the enzyme synthesis that acts to protect cells under high stress, such as dehydration (Li et al., 2002), stomatal closure (Christmann et al., 2007), hydraulic conductivity (Parent et al., 2009) and also to protect the growth of roots and shoots (Sharp, 2002). Throughout this research, there was a buildup in leaf [ABA] in all *J. curcas* genotypes, thereby corroborating those results found in cassava (Alves and Setter, 2004) and castor beans (Jokhan et al., 1996) two other species of Euphorbiaceae.

The concentration of ABA increased substantially roots of WD plants of all genotypes. However, only in CNPAE-137 such increment corresponded to an increase of organic solutes such as sugars and proline. However, Wilkinson and Davies (2002) reported that once inside the root, ABA may be translocated by the symplast and then stored or degraded, or can be transferred from one cell to another through the vessels of xylem, or may be carried by the apoplast to the transpiration stream to the xylem. In this research, the [ABA] the roots may have contributed to the increase in hydraulic conductivity, occurring thus, increased water absorption in *J. curcas* of

tissues under conditions of moderate water deficit.

Conclusion

The results revealed an efficient system for protection against drought-induced oxidative stress, through increased activity of antioxidant enzymes. Such strategy, observed in all the three genotypes, is suggested to be an important component of drought tolerance in *J. curcas*. Osmotic adjustment was not observed despite the increase in proline which apparently acts as an osmoprotector. Drought-induced increased foliar [ABA] was demonstrated and may have influenced stomatal control leading to the maintenance of foliar water status.

Abbreviations

ABA, abscisic acid; **[ABA],** abscisic acid foliar concentration; **CAT,** catalase; **DAT,** days after treatment initiation; D**M,** dry matter; **FC,** field capacity; **K^+,** potassium; **NBT,** nitroblue tetrazolium; **OA,** osmotic adjustment; **POD,** peroxidase; **PRO,** proline; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase; **TSS,** total soluble sugar; **WD,** water deficit; **Ψ_{am},** leaf water potential pre-dawn; **Ψ_{md},** leaf water potential midday; **Ψ_m,** substrate matric potential; **Ψ_s,** osmotic potential.

Conflict of Interests

The authors have not declared any conflict of interests.

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