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Antioxidative and osmorregulatory responses of *Jatropha curcas* to water stress are genotype dependent

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Abstract

Jatropha curcas L., Euphorbiaceae, is an undomesticated species noted for its high oil content of the seed (17.5 - 41.6%), which can be used to produce biodiesel, among other products. The objective of this study was to evaluate the behavior of nine Brazilian genotypes of *J. curcas* under the effect of water deficit. Seedlings of these genotypes (CNPAE 121, 124, 148, 168, 222, 215, 226, 298 and 299) were submitted to two irrigation regimes for 42 days: controlled irrigation (100% of tank capacity, TC) and water stress (WS, 50% TC). The foliar content of proline increased 56% on average in water stressed plants compared to the control plants. Water deficit-induced decreases of 62, 112, 78 and 23% in photosynthetic rate, stomatal conductance, transpiration rate and internal to atmospheric CO_2 concentration ratio (*Ci/Ca)*, respectively, were observed. Foliar activities of dismutase superoxide, guaiacol peroxidase and catalase increased in the stressed in comparison with the control plants. The genotypes CNPAE-148 and 222 were considered tolerant, as indicated by strong water conservation and significant increase in the activity of antioxidant enzymes when subjected to water stress.

Keywords: antioxidant enzymes; abiotic stress; Euphorbiaceae; gas exchange; osmoregulation; physic nut.
Abbreviations: CAT_catalase; DAT_days after treatment; FC_field capacity; GPX_guaiacol peroxidase; POD_peroxidase; ROS_ reactive oxygen species; SOD_superoxide dismutase; WUE_water use efficiency; WS_water stress.

Introduction

The species *Jatropha curcas* L., commonly known as physic nut, is a small tree widely distributed in cultivated tropical regions of Africa, India, Latin America and Southeast Asia. Due to its multiple products and sub-products, *J. curcas* has a highly diversified productive chain. In particular, it has been reported as an alternative source of sustainable energy (Pandey et al., 2012; Contran et al., 2013).

The oil content of *J. curcas* seeds can reach 38.3%, and the oil can be easily extracted and used to produce biodiesel, soap, biopesticides and fuel for lighting, as well as for medicinal purposes. The seed presscake, a sub-product of oil extraction, is used as organic fertilizer, fuel or for biogas production (Pandey et al., 2012; Contran et al., 2013). Moreover, *J. curcas* is a perennial and has a succulent stem. It is also resistant to drought in arid and semiarid conditions, meaning it does not directly compete with food crops, thus helping to mitigate soil degradation and recover marginal land or abandoned agricultural land (Pandey et al., 2012).

Studies indicate that *J. curcas* is drought tolerant because it develops morphophysiological strategies in reaction to water scarcity in the soil. For example, increased stem succulence (Maes et al., 2009), osmotic adjustments (Silva et al., 2010a) and redistribution of photo-assimilated compounds (Díaz-López et al., 2012) have been reported. Water conservation through efficient stomatal control of transpiration, as indicated by measures of leaf relative water content (RWC) and leaf water potential (Ψw), have been commonly observed in young and adult *J. curcas* plants (Díaz-López et al., 2012; Fini et al., 2013; Santos et al., 2013; Sapeta et al., 2013; Santana et al., 2015; Silva et al., 2019).

The osmotic adjustment mechanism of plants is an important physiological strategy associated with tolerance to drought (Hessine et al., 2009). This leads to increased water capture and increased cell growth of plants during water stress, associated with the partial opening of stomata, enabling assimilation of CO_2 under low water potential (Alves and Setter, 2004). Silva et al. (2016) observed an efficient mechanism of osmotic adjustment, involving organic and inorganic osmolytes, in *J. curcas* in response to drought stress.

Stomatal control to avoid water loss is common in species of Euphorbiaceae (El-Sharkawy, 2007). Such behavior, however, results in lower CO_2 input to the leaves, with negative effects on the electron transport chain of chloroplasts (Chaves et al., 2009) and oxidative stress, which may cause lesions to membranes due to reactive oxygen

species (ROS) (Pompelli et al., 2010). The overproduction of ROS in plants under stress may damage cell components, including DNA, proteins, and lipid membranes (Mittler, 2002). Some genotypes develop defense mechanisms, such as those based on the action of antioxidant enzymes, including superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC1.11.1.1) and catalase (CAT, EC 1.11.1.6) (Pompelli et al., 2010; Silva et al., 2016).

The aim of this study was to evaluate the differences in the responses of nine Brazilian genotypes of *J. curcas* to water stress by measuring leaf gas exchange variables and activities of antioxidative enzymes. We tested the following hypotheses: (1) the responses to water stress in terms of oxidative stress are variable among different genotypes, related to the region of origin; and (2) *J. curcas* has an efficient mechanism under water stress conditions to fight oxidative stress, which helps protect the photosynthetic system.

Results

Water relations

No significant differences were found among genotypes or between irrigation treatments (WS and control) for pre-dawn water potential (Ψpd). Regarding the midday leaf water potential (Ψmd), only in genotype CNPAE-222 was a significant effect observed (decrease). No significant differences were observed among genotypes for Ψmd (Table 1) and among genotypes and between WS treatments for RWC were observed.

Significant differences were observed among genotypes and WS treatments for osmotic potential (Ψs). A decrease of 52.5% in Ψs was found in the WS plants in comparison with the control plants (Fig. 1A). Significantly lower values of Ψs were measured in leaves of the genotypes 215 (-2.06) and 222 (-2.1 MPa) in comparison with the others.

With the decrease of Ψs in the WS plants in comparison with the control plants, there were significant differences among the genotypes for osmotic adjustment (OA) (Fig. 1B). There was a variation of OA in genotypes CNPAE 121 and 124, with values of -1.86 and -1.97 MPa, respectively, which showed higher osmotic adjustments in relation to the others. However, genotype CNPAE-126 had the lowest AO (33%).

Proline and potassium content

A significant difference was found among the *J. curcas* genotypes and between WS treatments at 42 DAT for proline content (Fig. 2A). Water stress led to an increase of 56% in proline content compared with the irrigated plants. On the other hand, no significant variation of potassium content (K^+) was found (Fig. 2B).

Leaf gas exchange, pigment content and Fv/Fm

All the measured leaf gas exchange variables decreased significantly in the WS plants compared to control plants. Such decreases reached 62, 112, 78 and 23% for P_N, *gs*, *E*, and *Ci/Ca*, respectively (Fig. 3). Moreover, significant effects of genotype and WS treatment on P_N/gs were observed (Fig. 4). Higher P_N/gs values were observed in most of the genotypes subjected to WS in relation to the control plants. In control plants, P_N/gs varied between 40 and 133 µmol CO_2 mol^{-1} H_2O, while in the WS plants, P_N/gs varied from 48 to 181 µmol CO_2 mol^{-1} H_2O, with an average value of approximately 47% in relation to control plants.

Only non-significant differences between the WS treatments for *Chl a* content were observed, with the exception of genotype CNPAE 121, where an increase of 24% in relation to the control was observed in WS plants (Fig. 5A). Moreover, WS led to a significant increase in *Chl b* content in the leaves of genotypes 124 and 168. (Fig. 5B). Regarding the *Chl a/Chl b* ratio, no significant differences were found either among genotypes or between WS treatments (Fig. 5C). No significant effects were observed of WS treatment on *Car* content (Fig. 5D). The maximum quantum yield of photosystem II (*Fv/Fm*) was not affected by the WS treatment in any of the studied genotypes.

Antioxidant enzymes and TBARS

Significant differences among genotypes and between treatments were observed for superoxide dismutase (SOD) activity (Fig. 6A). The highest mean value was recorded in WS plants of genotypes CNPAE-299 (0.465 U/kg DM), followed by CNPAE 121 (0.341 U/kg DM), both of them differing significantly from the others.

Differences among genotypes and between treatments were also found for guaiacol peroxidase (GPX). When comparing the treatments, increases in GPX activity of 260, 54, 15 and 3395% were observed for WS plants of genotypes CNPAE 121, 148, 168 and 226, respectively, in comparison with the control plants (Fig. 6B).

For catalase (CAT), significant differences were found among genotypes and between treatments (Fig. 6C), with the exception of genotypes CNPAE 121, 148 and 168. Lower activity was observed in control plants of genotypes CNPAE-215, 222 and 226 when compared to the others. Greater CAT activity was found in genotypes CNPAE-298 and 299 compared to treatment with WS, where there were increases of 50 and 43%, respectively, in relation to the others. Regarding thiobarbituric acid reactive substances (TBARS), significant differences among genotypes and between WS treatments were found. Genotypes CNPAE-168 and 215 differed from the others, and overall, WS treatment led to an increase of 26.4% in relation to the control plants (Fig. 6D).

Discussion

Maintenance of plant water status under low soil water content, as indicated by Ψw (Table 1) and RWC, confirms that *J. curcas* can be considered tolerant to drought. In non-stressed (control) plants, similar values of Ψw were found, especially at the hottest times of the day. This was mostly the result of stomata closure to avoid excessive water loss through transpiration. Indeed, many studies have found leaf water potential to be less affected by moderate water deficit in the soil in young plants of *J. curcas* (Santana et al., 2015; Silva et al., 2016; Oliveira et al., 2016). Moreover, no changes in RWC were observed for the genotypes, even at minimum soil water potential (-207.0 kPa), suggesting the plants quickly adapt to prevent water loss, as demonstrated elsewhere (Díaz-López et al., 2012; Silva et al., 2012; Fini et al., 2013; Meng et al., 2013; Sapeta et al., 2013). The lack of genotype effect on Ψw and RWC suggests low variability of these traits. This result indicates the introduction of genetic variability in future studies is needed to ascertain good physiological markers for breeding programs with *J. curcas*.

The water conservation strategy demonstrated here can be partially explained by the capacity to accumulate organic acids during dark period (Gomes et al., 2021) and compatible osmolytes, thus contributing to osmotic adjustment (Fig. 1A,

B), as also demonstrated by Silva et al. (2010) and Silva et al. (2016). Active OA can occur when concentrations of compatible solutes (Silveira et al., 2009), such as proline (Silva et al., 2015a; Silva et al., 2016) or total soluble sugars (TSS) (Silva et al., 2016), increase significantly in stressed plants. Our results show that the increase in proline is of utmost importance for WS plants of *J. curcas*, since the low similarity found for this variable demonstrated the high genetic diversity among genotypes of different origins (Silva et al., 2020). Therefore, foliar proline content can be suggested as a reliable marker for genotype classification under different soil water contents. On the other hand, the increase of foliar proline content did not lead to high osmotic adjustment among all genotypes (Fig. 1B). For example, a huge increase of foliar proline and low OA were observed in leaves of genotypes CNPAE-222 and 226, suggesting that more than acting as an osmolyte, proline has a protective role in *J. curcas* under water stress. Indeed, such inferences partially explain the lack of drought effect on *Fv/Fm* for all genotypes and TBARS for most of them (Fig. 6D).

The water stress conditions to which the *J. curcas* plants were subjected led to decreases of leaf gas exchange variables (Fig. 3A, B, C, D). Santana et al. (2015) found similar results in a study of three *J. curcas* genotypes from the state of Maranhão, Brazil. Decreasing P_N is related to low CO_2 uptake due to stomatal closure, as observed here. Moreover, as discussed above, Ψw remained unchanged as *gs* decreased. Thus, the drought-induced decrease of *gs* is probably connected to other factors, such as hormonal signaling through abscisic acid, as demonstrated by Silva et al. (2016). The genetic variation in gas exchanges showed that the genotypes analyzed here have strong potential to adapt to water shortage situations. Some genotypes (CNPAE-148 and 222) exhibited little change in relation to the conditions to which they were subjected in the present study. Therefore, genotypes with greater flexibility to adjust to photosynthetic rates under environmental conditions can be especially important to stabilize biomass production. Sunil et al. (2013) investigated the parameters of photosynthetic activity to determine the environmental adaptation of *J. curcas* genotypes. The authors found strong correlations between these parameters and proposed their use in breeding programs of the species. These parameters directly reflect the response of the plants to environmental conditions and simplify identification of stress-tolerant genotypes.

Water use efficiency increased in the plants subjected to water stress. Values of P_N/g_S were similar between the WS treatments in relation to control plants, with the exception of genotype CNPAE-226. Similar findings were reported by Díaz-López et al. (2012), who observed that after 27 days of deficient irrigation (75% TC) the P_N/g_S was greater than in irrigated plants. The high water-use efficiency is a direct consequence of the reduction in g_S prior to that of P_N, a response observed in species cultivated under moderate WS (Medrano et al., 2010). On the other hand, a decrease of P_N/g_S under conditions of severe water stress may indicate that at low g_S, P_N decreases due to stomatal limitations and/or mesophilic limitations, or that cuticular transpiration may significantly contribute to water loss (Fini et al., 2013).

The lack of drought-induced effect on chloroplastidic pigments observed in the present study has been reported by other authors, such as Sapeta et al. (2013), who observed *Chl a* values of around 3.5 and 2.2 mg g^{-1} of FW for dry and control treatments, respectively. However, damage to photosynthetic apparatus during photosynthesis due to decreased chlorophyll content is more frequent under more severe stress, as reported by Kiani et al. (2008) in sunflower, Massacci et al. (2008) in cotton, and Pompelli et al. (2010) in *J. curcas* plants maintained without irrigation for 18 days.

The lack of effect of WS on maximum quantum yield of PS II (*Fv/Fm*) in the present study reveals that the water stress imposed in this study did not cause damage to the photosynthetic system, indicating the absence of photoinhibition. Furthermore, other protective mechanisms associated with proline and TSS together with the role of antioxidative enzymes may explain the maintenance of membranes. When studying the photochemical activity of *J. curcas* under water stress conditions, Silva et al. (2010b) observed that *Fv/Fm* was not altered and maintained mean values of 0.85 during the stress period.

Some researchers have reported that water stress produces oxidative stress due to increased ROS and that many plants deal with this situation by activating antioxidant enzymes (Pompelli et al., 2010; Silva et al., 2015b; Silva et al., 2016). ROS production is controlled by various enzymatic defense systems, including SOD, GPX, and CAT (Gill and Tuteja, 2010; Karuppanapandian et al., 2011), as well as non-enzymatic systems, among which ascorbic acid (vitamin C), glutathione, proline, α-tocopherol (vitamin E) and flavonoids stand out (Mittler et al., 2004).

In the present experiment, higher SOD activity was found in the leaves of plants subjected to WS in comparison with the control (Fig. 6A). However, lower values in the activity of this enzyme were found for the CNPAE-148, 215 and 298 genotypes, reinforcing that the WS applied in this study was not sufficient to cause damage to the photosynthetic apparatus. In this respect, Silva et al. (2012), studying *J. curcas* plants subjected to different watering regimes, reported a slight increase (20%) in SOD activity in leaves. This may indicate that SOD performs an important role in regulating the removal of the superoxide radical (O_2^-) when the plants are subjected to water stress. However, Gill and Tuteja (2010) reported that several abiotic stresses frequently lead to increased ROS generation, in which SOD is an important enzyme for stress tolerance of plants, being the first enzyme involved in the process of eliminating ROS.

The GPX activity increased 44% on average in plants subjected to WS compared with the control plants (Fig. 6B). This increase in GPX in *J. curcas*, especially in genotypes CNPAE 121, 148, 168, and 226, may be enough to protect the proteins, chlorophyll and lipids against ROS attack. This means that these genotypes maintain greater GPX activity in leaves under WS, thus causing greater water retention and tolerance to water stress, at least under the moderate stress applied by us. Nevertheless, previous studies have reported drought-induced increased activity of peroxidases in *J. curcas* (Silva et al., 2015b; Silva et al., 2016).

Data from the present study reveal there was a mean increase of 35% in CAT activity in the WS plants in comparison with the control plants (Fig. 6C). Various authors have described increased CAT activity under WS conditions (Pompelli et al., 2010; Santos et al., 2013; Silva et al., 2015b; Silva et al., 2016). Furthermore, CAT is an essential enzyme for oxidative protection from the harmful effects caused by ROS in the cells. Therefore, SOD, GPX, and CAT may be considered effective indicators of physiological stress, involved in the defense mechanisms of *J. curcas* under water stress conditions.

Table 1. Leaf water potential measured at predawn (Ψam) and at midday (Ψmd), in *J. curcas* seedlings irrigated (control) or subjected to water deficit for 42 days (WS).

Genotypes	Ψpd (MPa)		Ψmd (MPa)	
	Control	WS	Control	WS
CNPAE-121	-0.47±0.08 Aa	-0.57±0.08 Aa	-1.00±0.05 Aa	-1.33±0.16 Aa
CNPAE-124	-0.43±0.06 Aa	-0.48±0.07 Aa	-1.03±0.03 Aa	-1.13±0.20 Aa
CNPAE-148	-0.32±0.04 Aa	-0.33±0.03 Aa	-1.07±0.06 Aa	-1.17±0.28 Aa
CNPAE-168	-0.32±0.04 Aa	-0.43±0.03 Aa	-0.87±0.06 Aa	-1.27±0.14 Aa
CNPAE-215	-0.33±0.06 Aa	-0.42±0.07 Aa	-1.27±0.03 Aa	-1.27±0.13 Aa
CNPAE-222	-0.33±0.06 Aa	-0.40±0.02 Aa	-0.83±0.12 Aa	-1.37±0.15 Ab
CNPAE-226	-0.27±0.04 Aa	-0.42±0.01 Aa	-0.75±0.10 Aa	-0.97±0.20 Aa
CNPAE-298	-0.35±0.07 Aa	-0.42±0.06 Aa	-0.78±0.20 Aa	-1.03±0.14 Aa
CNPAE-299	-0.35±0.08 Aa	-0.52±0.04 Aa	-0.87±0.06 Aa	-1.00±0.17 Aa

Values are mean (± standard error, n = 3). Lower case letters indicate significant differences between treatments within each genotype by the F-test and upper-case letters indicate significant differences among genotypes within each treatment by the Scott-Knott test (p< 0.05)

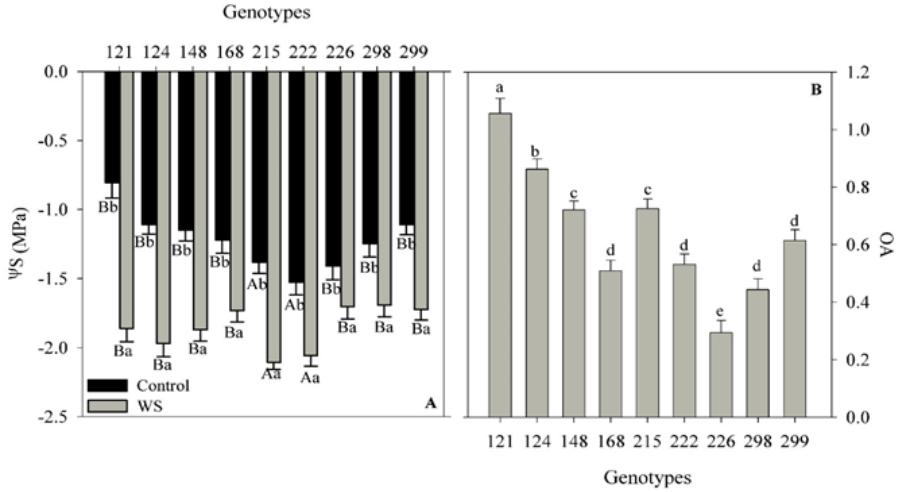

Fig 1. Leaf osmotic potential (Ψs, A) and osmotic adjustment (OA, B) in *J. curcas* seedlings irrigated (control) or subjected to water deficit for 42 days (WS). The columns are means of 5 replicates and the error bars represent the standard error of the mean. Upper case letters indicate comparison among genotypes within each treatment (control or WS) by the Scott-Knott test and lower case letters indicate comparison between watering regimes by F test (p<0.05)

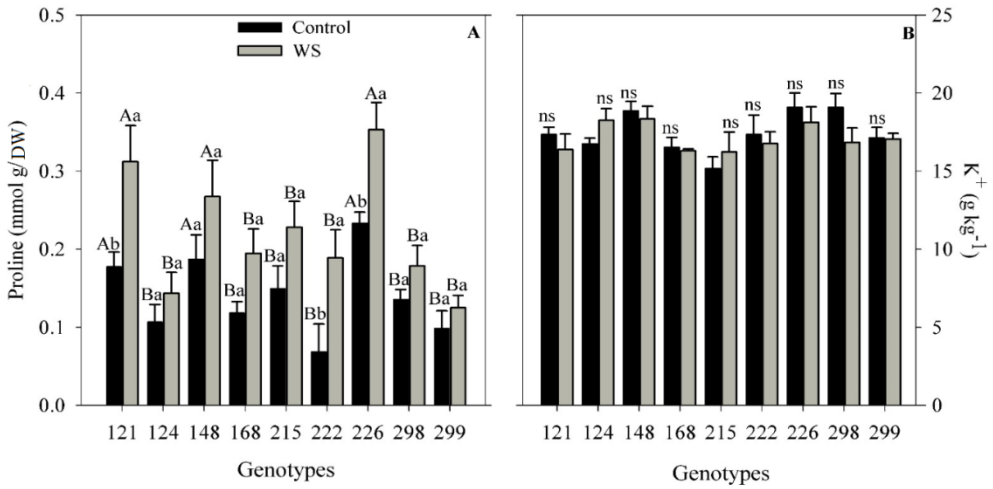

Fig 2. Proline (A) and potassium K^+ (B) content in leaves of *J. curcas* seedlings irrigated (control) or subjected to water deficit for 42 days (WS). The columns are means of 5 replicates and the error bars represent the standard error of the mean. Upper case letters indicate comparison among genotypes within each treatment (control or WS) by the Scott-Knott test and lower case letters indicate comparison between watering regimes by F test (p<0.05)

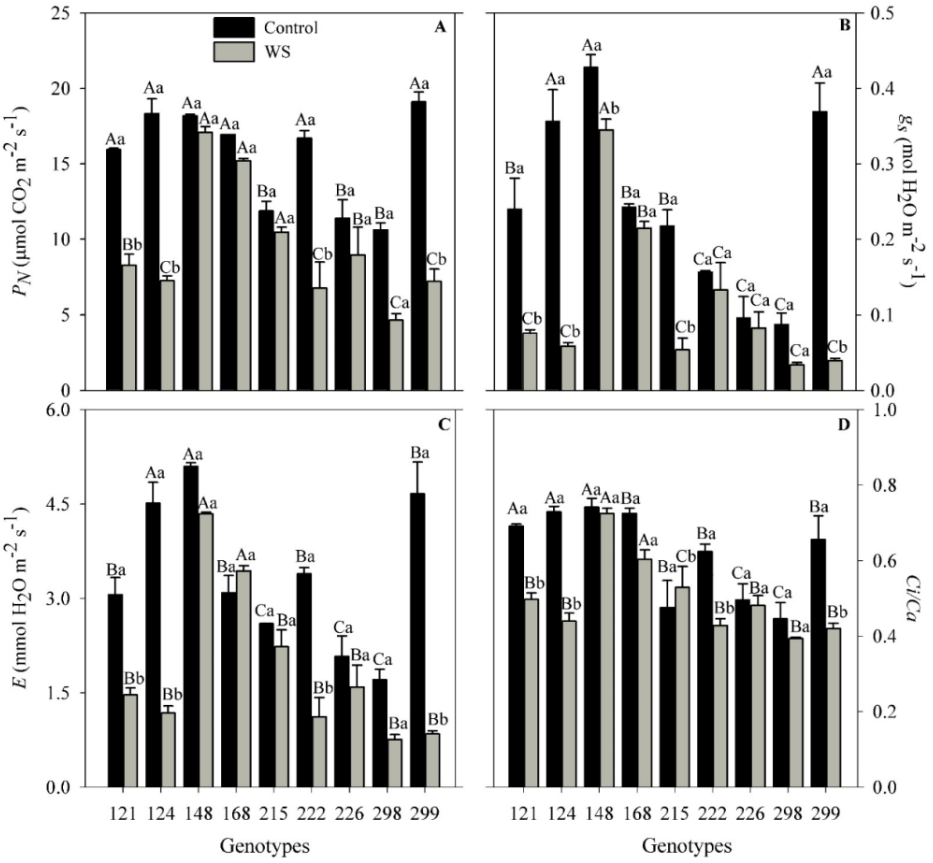

Fig 3. (A) Net photosynthetic rate (P_N), (B) stomatal conductance (g_s), (C) transpiration rate (E) and (D) *Ci/Ca* ratio measured in leaves of *J. curcas* seedlings irrigated (control) or subjected to water deficit for 42 days (WS). Columns are means of 3 replicates and the error bars represent the standard error of the mean. Upper case letters indicate comparison among genotypes within each treatment (control or WS) by the Scott-Knott test and lower case letters indicate comparison between watering regimes by F test (p<0.05)

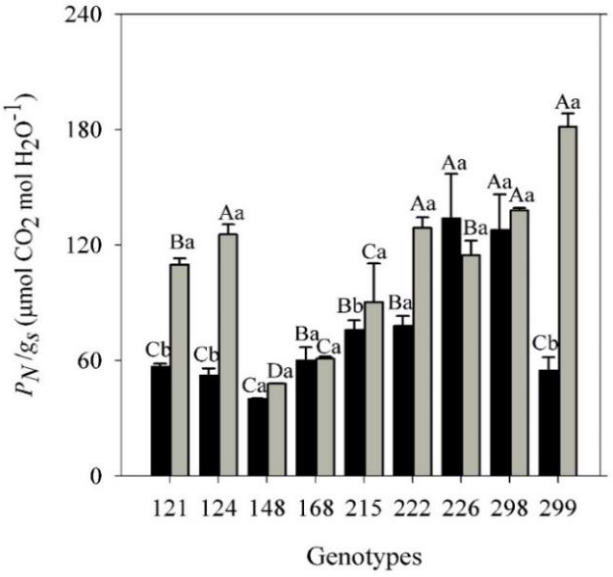

Fig 4. Intrinsic water use efficiency (P_N/g_s) in leaves of *J. curcas* seedlings irrigated (black) or subjected to water deficit for 42 days (gray). The columns are means of 3 replicates and the error bars represent the standard error of the mean. Upper case letters indicate comparison among genotypes within each treatment (control or WS) by the Scott-Knott test and lower case letters indicate comparison between watering regimes by F test (p<0.05)

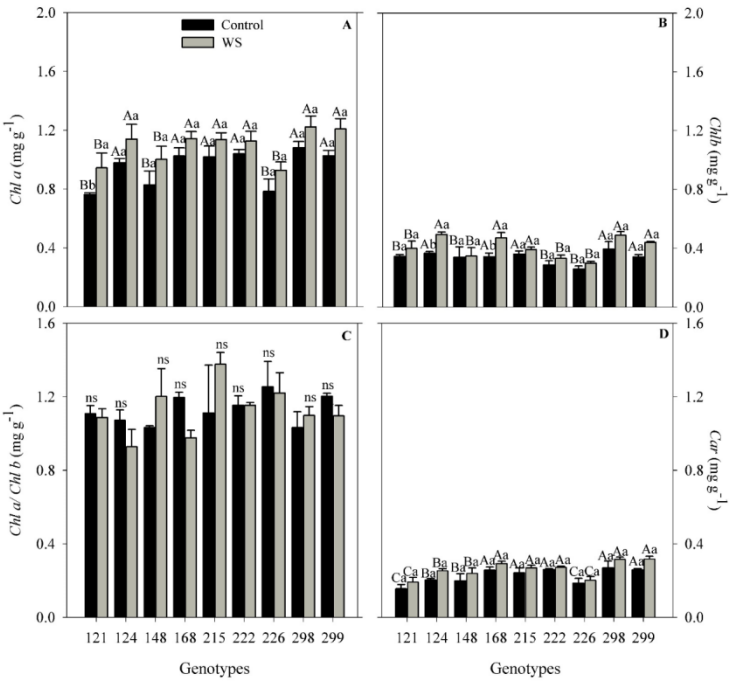

Fig 5. Chlorophyll a (*Chl* a, A), chlorophyll b (*Chl* b, B), *Chl* a/*Chl* b ratio (C) and carotenoids (*Car*, D) contents in leaves of *J. curcas* seedlings irrigated (control) or subjected to water deficit for 42 days (gray). The columns are means of 4 replicates and the error bars represent the standard error of the mean. Upper case letters indicate comparison among genotypes within each treatment (control or WS) by the Scott-Knott test and lower case letters indicate comparison between watering regimes by F test (p<0.05). ns - not significant

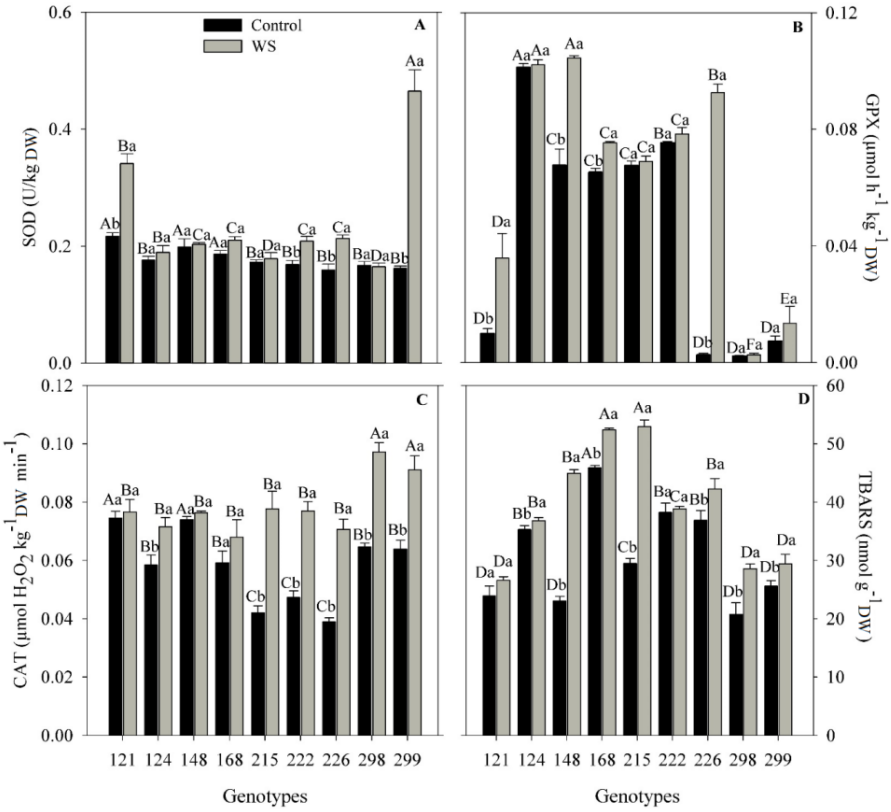

Fig 6. Activity of superoxide dismutase (SOD, A), guaiacol peroxidase (GPX, B), catalase (CAT, C) and thiobarbituric acid substances contents (TBARS, D) in leaves of *J. curcas* seedlings irrigated (control) or subjected to water deficit for 42 days (WS). The columns are means of 4 replicates and the error bars represent the standard error of the mean. Upper case letters indicate comparison among genotypes within each treatment (control or WS) by the Scott-Knott test and lower case letters indicate comparison between watering regimes by F test (p<0.05).

When analyzing lipid peroxidation in *J. curcas* leaves, we found an increase of 26% in plants subjected to WS compared to the control plants (Fig. 6D). In addition, genotypes CNPAE 124, 148, 168, 215, 226, 298 and 299 showed increases of 4, 95, 14, 97, 15, 99 and 15%, respectively, when submitted to WS in relation to the control plants. However, no differences were found for genotypes CNPAE 121 and 222. The content of TBARS, which is a product of lipid peroxidation, has been considered an indicator of oxidative damage (Moller et al., 2007). *J. curcas* plants suffered oxidative stress, indicated by increased lipid peroxidation and damage to the membranes induced by drought. This oxidative stress may also have been aggravated by increased SOD activity, especially in plants stressed and inhibited by CAT, since these enzymes catalyze H_2O_2 production reactions (Silva et al., 2019).

Therefore, TBARS is one of the final products resulting from lipid peroxidation damage caused by free radicals. In the present study, the elevated TBARS levels in plants subjected to WS revealed there was little oxidative stress, insufficient to cause damage to membranes or cells, suggesting this stress is a consequence of lipid degradation. Therefore, *J. curcas* has an efficient enzymatic and oxidative protection system, since the plants were able to attenuate the effects of water stress conditions, thus avoiding damage to the photosynthetic apparatus (Yang et al., 2015).

Material and methods

Plant growth conditions

A greenhouse experiment was conducted at the campus of State University of Santa Cruz (UESC), located in Ilhéus, Bahia, Brazil (14°47'00" S, 39°02'00" W). The photosynthetically active radiation (PAR) was monitored using S-LIA-M003 quantum sensors, while temperature and relative air humidity were monitored using Hobo H8 Pro Series micro-processed sensors (Onset, USA). These variables were systematically measured, and the data were stored using a Hobo Micro Station Data Logger (Onset, USA).

Seeds of nine genotypes of *J. curcas* (CNPAE 121, 124, 148, 168, 222, 215, 226, 298 and 299) from the germplasm bank of Embrapa Agroenergia, Brasília, were placed in pots (5 per pot), containing 12 dm³ of dystrophic yellow latosol (sandy clay textural class) and fertilization was carried out according to the chemical analysis of the soil. After the emission of the first pair of leaves (20 days after germination), thinning was carried out, leaving one plant per pot, and the water stress treatment began, which lasted 42 days

During the experimental period, the average value of photosynthetically active radiation integrated along the day was 12.1 mol of photons m^{-2} day^{-1}. The minimum and maximum air temperatures were 20 and 29 °C, respectively, and the average relative air humidity was 75%.

Treatments

Half of the plants ~received controlled irrigation of 100% of tank capacity (TC) and the other half were subjected to water stress (WS, 50% TC). The pots were watered on a daily basis, at 24 h intervals, in accordance to each watering regime. Irrigation was maintained close to tank capacity (with soil matric potential of the substrate varying from -33 to -15 kPa) in control pots for the entire experimental period, while for the other plants, water stress was maintained between -207 and -90 kPa. We determined soil moisture for both plant groups using the gravimetric method and estimated soil matric potential using a previously determined characteristic soil water retention curve.

Leaf water potential

Leaf water potential (Ψpd) was evaluated before sunrise (4:00 h), and at midday (Ψmd) using a PMS1000 pressure chamber (PMS Instrument Company, USA), according to Scholander et al. (1965).

Relative water content

Leaf relative water content (RWC) was measured in leaf disks collected between 6:00 h and 7:00 h. Five discs were removed from mature leaves and immediately weighed to obtain fresh weight (FW), after which they were placed in the dark to hydrate for 12 h, before being weighed again to obtain the turgid weight (TW). Subsequently, they were placed in a forced-air oven at 75 °C for 48 h to obtain dry weight (DW). RWC was then calculated from these variables based on the following equation:

$$\mathrm{RWC} = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})} \mathrm{x}\ 100$$

Osmotic potential and osmotic adjustment

To determine osmotic potential (Ψs), we collected five leaf discs (5 mm in diameter) from the middle third of the aerial part of the plant and used a thermocouple psychrometer (C-52 sample chamber, Wescor) connected to a dew point microvoltimeter (Psy-PRO, Wescor, Logan, USA). The leaf discs were frozen in liquid nitrogen, and after thawing and temperature stabilization, placed in a C-52 chamber to obtain the Ψs readings. The values of Ψs were corrected to eliminate the effect of passive concentration of solutes caused by foliar dehydration (Wilson et al., 1979). Osmotic adjustment was calculated as the difference between corrected Ψs of control and stressed plants.

Biochemical determinations

Completely expanded leaves were removed from the third pair at the end of the experiment and dried in a forced-air oven (65 ° C ± 5 °). They were then macerated and stored for subsequent measurements.

Proline and potassium content

Foliar content of proline was analyzed using the ninhydrin acid method (Bates et al., 1973) and potassium ion (K^+) level was determined according to Viégas et al. (2001).

Photosynthetic metabolism

Leaf gas exchange

Leaf gas exchange measurements were performed in completely expanded and mature leaves using a Li-6400 XT portable photosynthesis system (LI-COR Biosciences Inc., Nebraska, USA). Net photosynthetic rate (P_N), stomatal conductance to water vapor (g_S), transpiration rate (E), and the intercellular to atmospheric CO_2 concentration ratio (Ci/Ca) were obtained, always from 08:30 to 11:30 h, under saturating irradicance of 1,000 µmol photons m^{-2} s^{-1} and atmospheric concentration of CO_2 (Ca) of ±390 µmol mol^{-1}. The intrinsic water use efficiency (P_N /g_S) was calculated from the values obtained.

Chloroplastidic pigments

Leaf discs were collected (5 mm diameter) and immediately incubated in glass tubes covered with aluminum foil containing 4 mL of dimethyl sulfoxide (DMSO) saturated with $CaCO_3$ (Hiscox and Israelstam, 1979), for 24 h at room temperature. The absorbance of the extracts was then determined using a spectrophotometer at wavelengths of 480, 649, and 665 ηm. The concentrations of chloroplastidic pigments were estimated using the equations proposed by Wellburn (1994).

Maximum quantum yield of photosystem II (Fv/Fm)

Chlorophyll fluorescence emission was measured using a portable continuous-excitation fluorometer (Pocket PEA, Hansatech Instruments, Norfolk, UK), between 08:00 and 12:00, in the same leaves used for gas exchange and pigment measurements. After adaptation to the dark, the leaves were exposed to a saturating pulse of light (3,500 µmol m^{-2} s^{-1}, wavelength of 650 ηm, per 1 s). The saturating light pulse lasted 0.3 s, at a frequency of 0.8 KHz. The maximum quantum yield of photosystem II (*Fv/Fm*) was then calculated using these data.

Enzymatic determinations

Antioxidant enzyme activity

Leaf samples were collected from the third pair of completely expanded, mature leaves and immediately frozen in liquid nitrogen, freeze-dried, and stored in a freezer (-20 °C). The activity of the enzymes guaiacol peroxidase (GPX, EC 1.11.1.7), superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) was determined according to the methods proposed by Pirovani et al. (2008), Beauchamp and Fridovich (1971) and Madhusudhan et al. (2003), respectively.

Thiobarbituric acid reactive substances (TBARS)

Extraction of thiobarbituric acid reactive substances (TBARS) was carried out using the method of Heath and Paker (1968).

Statistical analysis

The experiment was conducted in a completely randomized design, in a 2 x 9 factorial arrangement, corresponding to two water regimes (100 and 50% TC) and nine genotypes of *J. curcas* (CNPAE 121, 124, 148, 168, 222, 215, 226, 298 and 299), with five replications. Analysis of variance (ANOVA) was performed, and the treatment means were compared using the Scott-Knott test (p<0.05).

Conclusion

Although the capacity of the genotypes to store water in the plant tissue was similar, they had different physiological and biochemical characteristics. Genotypes 148 and 222 were the most tolerant to drought due to the greater activity of the antioxidant enzyme and maintenance of the rates of leaf gas exchange. The results reveal an efficient system that protects the genotypes against oxidative stress induced by drought through increased activity of antioxidant enzymes and non-enzymatic mechanisms such as proline and osmotic adjustment. Such strategy, observed in some genotypes, is considered an important component in the tolerance of *J. curcas* to drought.

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