DETERMINATIONS OF ORGANIC OSMOLITES IN *Jatropha curcas* LEAVES UNDER DIFFERENT WATER REGIMES

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ABSTRACT: In order to quantify total soluble sugars and proline in leaves of *J. curcas* under different water regimes, a greenhouse experiment was carried out using genotype 557 from Embrapa Agroenergia-DF germplasm bank. After 20 days of germination, thinning was performed and the plants were submitted, for a period of 48 days, to three water regimes measured as percentage of tank capacity (TC): control plants (90% of TC), 70% of TC and 50% of TC. Harvesting of leaf material for the determinations of total soluble sugars (TSS) and proline was carried out in two seasons, at 8 days after treatment (DAT) and 48 DAT. It is observed that the TSS content for the control treatment differed significantly ($p<0.05$) for both collection periods. When analyzing the proline content, there were differences between the collection periods where 48 DAT revealed higher values when compared to 8 DAT. However, at 48 DAT, plants submitted to 50% of TC, showed an increase of 53.3% and 190% for the control plants and 70% of TC, respectively. The increase of organic osmolytes (TSS and proline) in *Jatropha curcas* demonstrates that the species can tolerate water deficiency in the soil, in addition, these osmolytes reveal the role of osmoregulators against stress.

KEYWORDS: proline, total soluble sugars, water deficiency

DETERMINAÇÕES DE OSMOLITOS ORGÂNICOS EM FOLHAS DE *Jatropha curcas* SOB DIFERENTES REGIMES HÍDRICOS

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RESUMO: Com o objetivo de quantificar açúcares solúveis totais e prolina em folhas de *J. curcas* sob diferentes regimes hídricos, realizou-se um experimento em casa de vegetação utilizando o genótipo 557 oriundo do Banco de Germoplasma da Embrapa Agroenergia-DF. Após 20 dias da germinação realizou-se o desbaste e as plantas foram submetidas, por um período de 48 dias, a três regimes hídricos medidos em percentagem da capacidade de campo (CC): plantas controle (90% da CC), 70% da CC e 50% da CC. Foram realizadas em dois períodos, aos 8 dias após tratamento (DAT) e 48 DAT, coletas de material foliar para as determinações dos açúcares solúveis totais (AST) e prolina. Observa-se que o teor de AST para o tratamento controle diferiu significativamente ($p<0,05$) para ambos os períodos de coleta. Ao analisar o teor de prolina, verificou-se diferenças entre os períodos de coletas onde aos 48 DAT revelou maiores valores, quando comparados aos 8 DAT. Entretanto, aos 48 DAT, as plantas submetidas a 50% da CC, revelou um aumento de 53.3% e 190% para as plantas controle e com 70% da CC, respectivamente. O aumento dos osmolitos orgânicos (AST e prolina) em *Jatropha curcas* demonstram que a espécie consegue tolerar a deficiência hídrica no solo, além disso, esse osmolitos revelam a função de osmorreguladores frente ao estresse.

PALAVRAS-CHAVE: prolina, açúcares solúveis totais, deficiência hídrica

INTRODUCTION

Jatropha curcas L., belonging to the family Euphorbiaceae, 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 a plant with potential to supply raw material for fuel, this species of oleaginosa is still in the stage of domestication (Laviola et al., 2011). The species has been characterized as, as tolerant to drought, even demonstrating negative responses to water deficit, it is able to survive and show good recovery capacity (Fini et al., 2013). Although *J. curcas* has been widely reported as a drought tolerant species, recent results have demonstrated negative effects of water deficit on several physiological and morphological characteristics (Oliveira et al., 2016; Santana et al., 2015; Silva et al., 2016).

The active accumulation of compatible osmolytes in response to drought has been reported as an important mechanism of adaptation to drought in several plants (Silva et al., 2010). The active occurrence of osmotic adjustment can be established if there is a significant increase in the concentrations of compatible solutes (Silveira et al., 2009), such as the increase

of total soluble sugars and proline (Silva et al., 2016). Thus, the objective of this work was to verify the osmotic adjustment by determinations of total soluble sugars and proline in *Jatropha curcas* plants under different water regimes.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the State University of the Southwest of Bahia (UESB), with coordinates 14° 53' 17" and 40° 48' 9" and altitude of 875m. According to Köppen, the municipality presents a tropical climate of altitude with summer rains and winter drought, with average annual temperature of 20 ºC, being classified as *Cwb*. Seeds of *Jatropha curcas* of genotype 557, from the germplasm bank of Embrapa Agroenergia-DF, were placed to germinate in pots (five per pot) containing 15 dm^3 of soil, classified as dystrophic yellow latosol previously prepared according to soil chemical analysis.

After 20 days the thinning was performed, leaving only one plant per pot, thus initiating the water deficiency treatments, being maintained for a period of 48 days of experiment The treatments consisted of three water regimes measured in percentage of tank capacity (TC): control plants (90% of TC), 70% of TC and 50% of TC and two sampling times (8 days after treatment (DAT) and 48 DAT).

Leaf tissues were analyzed for total soluble sugar (SST) content according to the methodology of Clegg et al. (1956), where they were extracted by homogenizing 100 mg of the powdered material and dried with 5 mL of boiling 80% ethanol, followed by stirring and subsequent rest for 5 minutes. Another 5 mL of ethanol was added, centrifuging for 10 minutes at 5.000 g. The supernatant was transferred to a 50 mL volumetric flask and the residue was washed with ethanolic solutions and centrifuged three more times. The supernatants were combined and the volume was made up to 25 mL with 80% ethanolic solution. Subsequently, the extracts were depigmented with chloroform and evaporated on a heating plate at 60 ºC. The resulting residue was dissolved in 5 mL of distilled water. From these extract aliquots were separated to quantify SST and absorbance readings at 490 nm were taken by spectrophotometer.

Proline was determined by the acid ninhydrin method (Bates et al., 1973), approximately 50 mg of powdered and dried leaf tissue was homogenized in 6 mL of 3% (p/v) sulfosalicylic acid. After centrifugation (8.000 g) for 10 min, 2 mL of the extract were collected, in which an additional 2 mL of ninhydrin solution (1.25 g of ninhydrin; 30 mL of

glacial acetic acid; 20 mL of phosphoric acid 6 M) and 2 mL glacial acetic acid. The samples were incubated at 100 °C for 1 h and then placed on ice to complete the reaction. Toluene (2 mL) was added to the solution, followed by stirring for 20 s for complete proline extraction. After resting, the less dense part (chromophore) was aspirated with a Pasteur pipette to read the spectrophotometer at 520 nm.

The results were subjected to F test at 5% significance, by factorial ANOVA and, when indicated, Tukey test at the same significance level. The experiment was conducted in a completely randomized design in a 3 x 2 factorial scheme, consisting of three levels of water availability and two collection times, with four replications per treatment.

RESULTS AND DISCUSSION

The total soluble sugars content (TSS) for the control treatment differed significantly ($p<0.05$) for both periods (Figure 1). It is observed that for the other water regimes, independently of the time of collection, they would not differ between them. Silva et al. (2016) when evaluating SST in three genotypes of *J. curcas*, showed that only genotype CNPAE-126 showed increase in control plants, in relation to the treatment of water deficit (70% of TC) at 66 days of treatment.

The increase of TSS in plants under drought may have been triggered by increased synthesis and reduced starch hydrolysis in its use due to translocation restriction or reduction as a carbon source (Silva et al., 2010). In addition, this increase in TSS levels was also verified by Silva et al. (2019), who studied *Jatropha* genotypes under moderate water stress with 42 days of stress, showed an increase of 62% when compared to control plants. In addition, Sousa et al. (2012) studying *J. curcas* plants submitted to different treatments of water deficit with wastewater and salinity found results of increase in TSS content.

McCormick et al. (2009) reported that the accumulation of TSS is a consequence of metabolic dysfunction affecting leaf sugar composition and translocation. They also suggested that this response may contribute to inhibition of photosynthesis during water deficit.

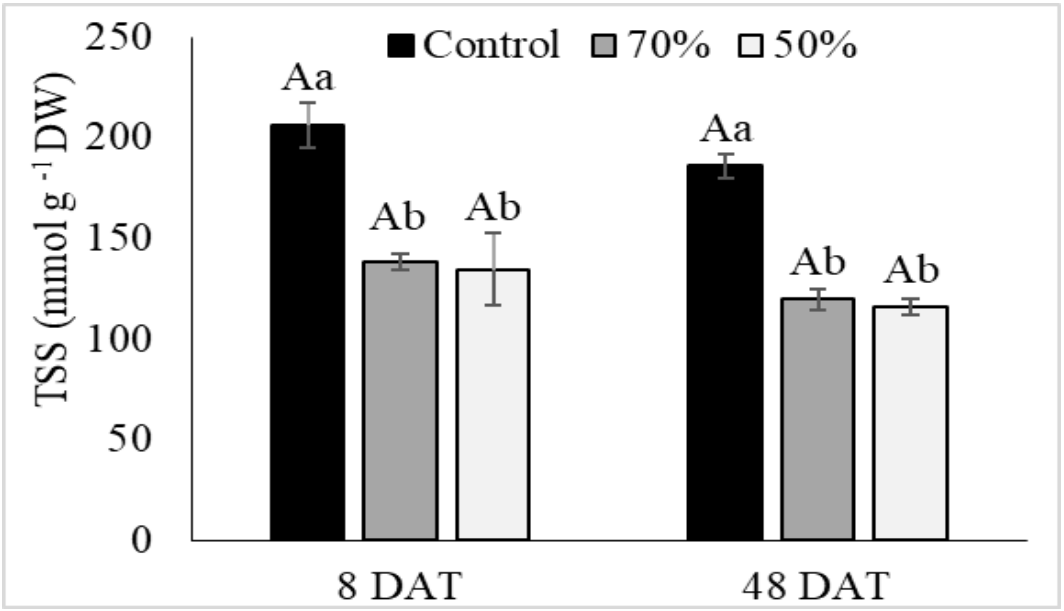

Figure 1: Total soluble sugar content (TSS) in leaves of *Jatropha curcas* under drought in two seasons. Columns are means of 4 replicates and the bars represent the standard error of mean. Upper case letters indicate comparison between two seasons and lower case letters comparing water regimes (control, 70% and 50%) by the F-test ($p < 0.05$).

In figure 2, it was observed that at the peak of stress (48 DAT), the proline content differed significantly ($p<0.05$) from the first week of treatment (8 DAT). The treatment of 50% of TC, differed in relation to the control plants at 8 DAT. However, at 48 DAT, plants submitted to 50% of TC, showed an increase of 53.3% and 190% for the control plants and 70% of TC, respectively. Silva et al. (2016) when studying three genotypes of *Jatropha curcas* (CNPAE-126, 137 and 139) submitted to two water conditions (control and 70% of TC). The authors showed increases of 111%, 60% and 10% for CNPAE-126, 137 and 139 genotypes, respectively, corroborating, with the values found in this research. The TSS and proline accumulation in *J. curcas* plants submitted to water deficit may be related to the mechanism that prevents water loss.

Studies with different hybrids of *Ricinnus communis* showed that this species accumulates high levels of TSS and proline after 33 days under water deficiency, and that TSS are the main osmolites in the osmotic adjustment in castor leaves (Babita et al., 2010). In addition, a positive correlation between proline accumulation and water deficit tolerance has been demonstrated in different species Guo et al., 2010; Valliyodan & Nguyen, 2006).

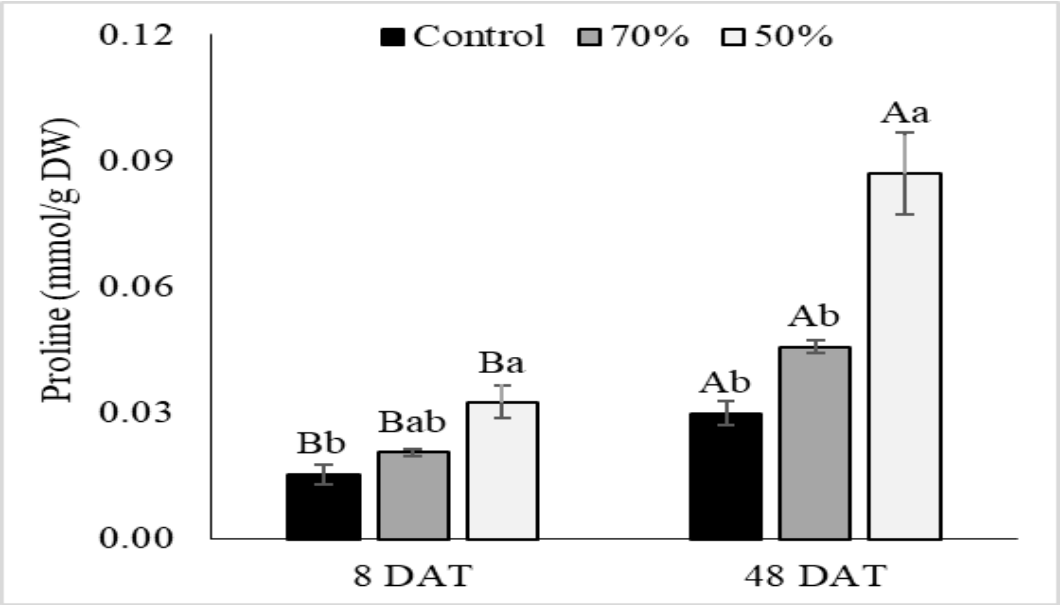

Figure 2: Proline content in leaves of *Jatropha curcas* under drought in two seasons. Columns are means of 4 replicates and the bars represent the standard error of mean. Upper case letters indicate comparison between two seasons and lower case letters comparing water regimes (control, 70% and 50%) by the F-test ($p < 0.05$).

CONCLUSIONS

The increase of organic osmolytes (TSS and proline) in *Jatropha curcas* demonstrates that the species can tolerate water deficiency in the soil, in addition, these osmolytes reveal the role of osmoregulators against stress.

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