Water relations and carbon isotope composition in young plants of two provenances of *Jatropha curcas* L. subjected to water deficit

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Palavras Chave: Drought tolerance, Physic nut, Stomatal conductance, Whole plant transpiration.

1 - Introduction

Jatropha curcas L. is a perennial slightly stem-succulent Euphorbiaceae, a family with other economically important species, such as castor bean (*Ricinus communis* L.), cassava (*Manihot esculenta* Crantz) and rubber tree (*Hevea brasiliensis* Müll. Arg.). It has been globally quoted as a promising natural feedstock for biodiesel production in tropical and subtropical countries (1).

A drought-induced water saving strategy, in which an early stomatal closure is the first line of defence against desiccation, has been thoroughly demonstrated in *J. curcas* (2, 3). Even though such strategy may lead to high photosynthetic water use efficiency, low carbon assimilation rates, growth and biomass production have also been reported as "side effects" of such water saving strategy. The maintenance of open stomata (and the leaf gas exchange) depends on a well-hydrated leaf mesophyll sustained by the whole plant hydraulic conductance. Thus, the aim of this study was to investigate water deficit-induced changes on whole plant transpiration and carbon isotope composition ($\delta^{13}C$) in different tissues of young plants of *J. curcas*. We tested the hypothesis of the existence of genetic variability for water use efficiency in *J. curcas.*

2 - Material and Methods

A glasshouse experiment was conducted at the Sainsbury Laboratory, University of Cambridge, from April to July 2015. Air temperature, relative humidity and a photoperiod of 12h inside the glasshouse were controlled to keep the values around, respectively, 28ºC, 65% and 400 W m^{-2}. Seed-born plants of two provenances of *J. curcas* (CNPAE183 and CNPAE222) were cultivated for 30 days under full irrigation in 18.0 L pots filled with substrate. The plants were submitted to water deficit by cessation of watering for 15 days. Physiological variables and substrate water availability were measured 0, 7 and 15 days after starting treatment (DAST). Substrate water content (SWC) was measured using a ThetaProbe Soil Moisture Sensor ML3 (Delta-T devices Ltd., Cambridge, UK). Leaf relative water content (RWC) was measured between 10:00 and 12:00 h a.m. in leaf discs collected from mature leaves. Leaf gas exchange variables were measured using a photosynthesis system LI-6400XT (LICOR, Lincoln, NE, USA) from 8:00 to 10:00 h a.m. in one completely expanded and physiologically mature leaf per plant. Whole plant transpiration by leaf area unit (TLA) was measured by sequentially weighting covered pots. Total leaf area per plant was computed as the sum of individual areas of each leaf, estimated from its linear dimensions (4). At the end of experiment (15 DAST), leaf and stem bark samples were collected from basal third (mature) and apical (young) regions of the plant for analysis of $\delta^{13}C$ following the protocols of the Godwin Laboratory, University of Cambridge, using a Costech Elemental Analyzer attached to a Thermo DELTA V mass spectrometer in continuous flow mode (Thermo Fisher Scientific, Waltham, MA, USA).

A completely randomized factorial design with two treatments (water deficit and irrigated) and two provenances with eight replicates was set. The data were submitted to factorial ANOVA and the means were compared using the *F* or Tukey's tests at 5% of probability.

3 - Results and Discussion

Cessation of watering led to decrease of SWC from 28%, on average, in irrigated pots to 15 and 9%, at 7 and 15 DAST, respectively. Significant effects of water deficit were detected in both provenances, as measured by decrease of RWC, TLA, net photosynthetic rate (P_N) and stomatal conductance (gs) with decreasing SWC (Figure 1).

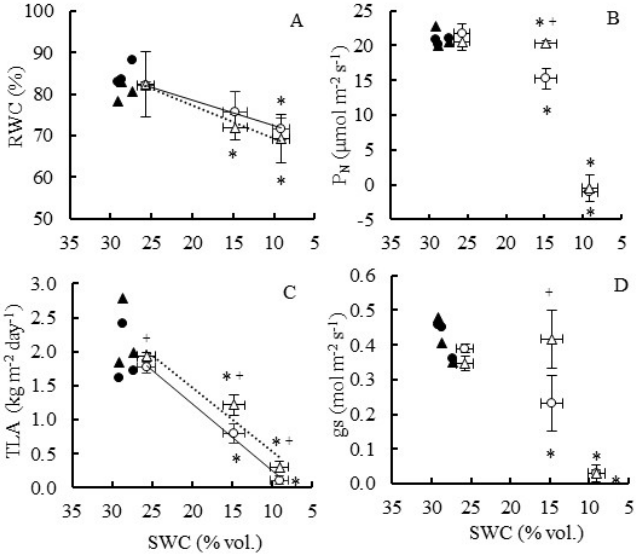

Figure 1. Mean (s.d., n=4-8) values of RWC (A), P_N (B), TLA (C) and gs (D) in young plants of *J. curcas* cultivated under irrigated (dark) or water deficit (open). CNPAE183, circles; CNPAE222, triangles. Differences between treatments (*) between provenances (+) (*F* test, P<0.05)

Significantly higher TLA was measured in CNPAE222 than in CNPAE183 during all the experiment.

Despite non-significant, RWC at the end of experiment was lower in CNPAE222 than in CNPAE183. A slow response of gs (and of P_N) to decreasing SWC was observed in CNPAE222 as compared to CNPAE183. The values of gs and PN of water deficit plants at 7 DAST had decreased, respectively, 49 and 27% in CNPAE183 and 13 and 11% in CNPAE222. Notwithstanding, both variables were significantly different between the two provenances. Intrinsic water use efficiency (P_N/gs), did not vary in CNPAE222, but increased significantly in CNPAE183 at 7 DAST (Figure 2). At this point, a clear picture can be drawn from that results, in which CNPAE222 can be categorized as "water spender" and CNPAE183 as "water saver". Such inference is supported by the fact that the two provenances were collected from contrasting regions of Brazil with respect to climate. While CNPAE183 is from an semi-arid region in the Northeast, CNPAE222 is from a region with mild weather in the South.

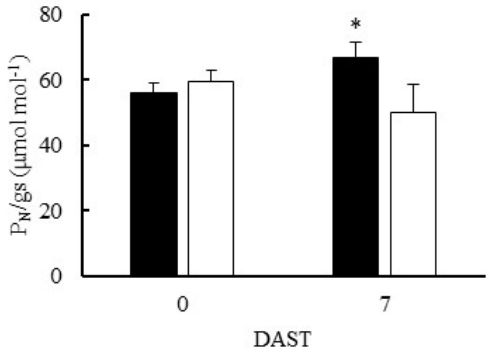

Figura 2. Mean (n=4, s.d.) values of P_N/gs in leaves of young plants of *J. curcas* under water deficit. CNPAE183, dark; CNPAE222, open. * (*F* test, P<0.05)

Significant effects of water deficit, provenance and sample were observed for $\delta^{13}C$ (Table 1). Higher values were measured in young as compared to mature tissue of both treatments (irrigated and water deficit) and provenances (CNPAE183 and 222). Moreover, in all cases, significantly higher values were found in leaves as compared to stem bark samples. Water deficit-induced increase of $\delta^{13}C$ was more pronounced in CNPAE222 than in CNPAE183. In four provenances of *J. curcas*, $\delta^{13}C$ increased with soil water deficit, probably, as suggested by the authors, due to decrease in stomatal conductance (gs) and transpiration (E) under water deficit. (5). While the increase in $\delta^{13}C$ may be due to decrease in gs and Ci/Ca under low water supply, values close to -20 ‰, as observed here, mainly in young leaves of water deficit plants, may suggest a 'cryptic' low-level of Crassulacean Acid Metabolism (CAM) (6). Less negative $\delta^{13}C$ found in young tissues of unwatered plants can be due to the source of carbon, i.e., carbohydrates produced by young leaves performing CAM (7). Within C_3 species, plants which are more enriched in ^{13}C will show greater water use efficiency at the leaf level (8), which was not observed here for *J. curcas*. Such result may suggest that the drought-induced metabolic change may be linked to carbon rather than water conservation under stressful conditions (6).

More negative values of $\delta^{13}C$ in stem bark, as observed here, can be due to differences in the relative proportions of the structural constituents, with a higher proportion of lignin (more ^{13}C depleted) in stem bark than in leaf tissue (9). Moreover, foliar depletion in ^{13}C during leaf development combined with export of relatively ^{13}C-enriched C by mature source leaves may help to explain the higher $\delta^{13}C$ in young than in mature leaves (10).

Table 1. Mean (±SE, n=4) values of $\delta^{13}C$ (‰) in young and mature leaves (YL, ML) and stem bark (YS, MS) of two provenances of *J. curcas* subjected to water deficit for 15 days.

sample	Irrigated	Water deficit	%
	CNPAE183		
YL	-26.26 ± 0.03 ^{Ba}	-23.41 ± 0.06 ^{Aa*}	11
YS	-26.42 ± 0.03 ^{Bb}	-24.37 ± 0.04 ^{Ab*}	8
ML	-26.79 ± 0.07 ^{Bc}	-25.86 ± 0.05 ^{Ac*}	3
MS	-28.16 ± 0.04 ^{Bd}	-26.85 ± 0.02 ^{Ad*}	5
	CNPAE222		
YL	-26.34 ± 0.06 ^{Ba}	-22.80 ± 0.03 ^{Aa}	13
YS	-26.47 ± 0.03 ^{Ba}	-23.63 ± 0.02 ^{Ab}	11
ML	-26.83 ± 0.04 ^{Bb}	-25.39 ± 0.02 ^{Ac}	5
MS	-27.98 ± 0.19 ^{Bc}	-26.40 ± 0.05 ^{Ad}	6

Significant (P<0.05) differences between treatments within each sample (lower case) or among samples within each treatment for each provenance (capital letters) were obtained by the *F* and Tukey's tests, respectively. *, indicates significant differences between provenances for each sample (F test, P<0.05). % is the effect of water deficit in percentage

4 – Conclusions

Water deficit led to physiological and biochemical changes, which allowed to discriminate the provenances as "water saver" (CNPAE183) and "water spender" (CNPAE222). Our results suggest the existence of genetic diversity for water relation and metabolic traits linked to drought tolerance in *J. curcas*. Also, water deficit and provenance effects on $\delta^{13}C$ may suggest different pattern of C allocation among the several tissues, drought-induced metabolic change (C_3-CAM), or a combination of them. Temporal variation of such drought-induced metabolic changes and its role in *J. curcas* is now under investigation.

5 – Acknowledgement

We thank CAPES for the scholarship provided, UESC and the Physiological Ecology Group at the University of Cambridge for financial support.

6 - Bibliografia

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