**Growth and carbon assimilation limitations in *Ricinus communis* (Euphorbiaceae) under soil water stress conditions**

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**ABSTRACT** – (Growth and limitations to carbon assimilation in *Ricinus communis* (Euphorbiaceae) under soil water stress conditions). Water availability may influence plant carbon gain and growth, with large impacts on plant yield. *Ricinus communis* (L.), a drought resistant species, is a crop with increasing economic importance in Brazil, due to its use in chemical industry and for the production of biofuels. Some of the mechanisms involved in this drought resistance were analyzed in this study by imposing progressive water stress to pot-grown plants under glasshouse conditions. Water withholding for 53 days decreased soil water gravimetric content and the leaf water potential. Plant growth was negatively and significantly reduced by increasing soil water deficits. With irrigation suspension, carbon assimilation and transpiration were reduced and remained mostly constant throughout the day. Analysis of A/Ci curves showed increased stomatal limitation, indicating that limitation imposed by stomatal closure is the main factor responsible for photosynthesis reduction. Carboxylation efficiency and electron transport rate were not affected by water stress up to 15 days after withholding water. Drought resistance of castor bean seems to be related to a pronounced, early growth response, an efficient stomatal control and the capacity to keep high net CO₂ fixation rates under water stress conditions.

**Key words:** A/Ci curves, diurnal changes, photosynthesis, stomatal limitations, water stress

**Introduction**

Drought is the major factor limiting crop productivity worldwide (Jones & Corlett 1992), and crops with increased resistance to this stress appear to be crucial for maintaining yields in areas where dry seasons are common. *Ricinus communis* (L.), also known as castor bean, is a crop with increasing economic importance in Brazil, due to its use in pharmaceutical and chemical industry and, mainly, as a substitute for diesel in the production of biofuels. Castor bean is considered a drought resistant species, and, for this reason, it has been grown mostly in arid zones in Brazil. However, little is known about the physiological mechanisms underlying its resistance to drought. Dai et al. (1992) observed that, under increased vapor pressure deficits, the inhibitory effect of drought upon photosynthesis in castor bean plants is caused by limited leaf CO₂ concentration, due to stomatal closure. Castor bean is a species in which stomata remain partially open at night and it was observed that drought reduced stomatal conductance over the day and also at night (Barbour & Buckley 2007).

Despite significant advances in the understanding of mechanisms by which photosynthesis responds to environmental factors, there is still controversy about the actual importance of diffusional and metabolic limitations to photosynthesis imposed by drought. Generally, the initial decrease of photosynthesis is due to stomatal closure, which protects the plant against excessive water loss, but simultaneously limits CO₂ availability to the leaf mesophyll (Chaves 1991; Flexas et al. 2002; Flexas & Medrano 2002). Besides stomatal limitations, metabolic limitations to photosynthesis, related to reduced RuBP regeneration (Gimenez et al. 1992; Gunasekera & Berkowitz 1993), Rubisco activity (Medrano et al. 1997), ATP production (Tezara et al. 1999; Lawlor & Cornic 2002), photosystem II (PSII) activity and electron transport rate (Long et al. 1994) have also been proposed. The extension of stomatal and metabolic limitations imposed by water stress on photosynthesis will affect diurnal patterns of gas exchange. An efficient regulation of photosynthesis throughout the day reflects plant capacity to maintain an internal balance among several processes as it responds to varying environmental conditions (Geiger & Servaites 1994). The general objective of this study is to better understand the mechanisms used by castor bean to tolerate drought. More specifically this paper aims to study the effects of soil water-deficits on plant growth and biomass allocation and on the daily

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pattern of gas exchange in castor bean plants, quantifying and comparing the diffusional and metabolic limitations to the photosynthetic process under limited water availability.

**Material and methods**

Plant material and growth conditions - Seeds of *Ricinus communis* (L.), cultivar BRS 149 Nordestina, were germinated on a mixture of commercial growth medium (TerraPreta®) and sand (2:1, v:v) in plastic cups (150 mL), under constant temperature (25 ± 2 °C) and 12 hour light/night period provided by a germination chamber (BOD-NT708). Upon opening of the cotyledonary leaves, seedlings were transferred to 3 L plastic pots (1 plant per pot) filled with the same growth medium and acclimated for 53 days, so that all plants would have, at least, 3 fully developed leaves, under natural environmental conditions, in a glasshouse at the School of Agronomy of the Federal University of Rio Grande do Sul, Brazil. Temperature and humidity inside the glasshouse recorded on a data logger (Pinguin, Novus Automation) installed at plant height, revealed temperature varying between 20 and 40°C and relative humidity between 24 and 80% during the growth period.

After the acclimation period, plants were assigned to one of two treatments, on a completely randomized design: well-watered (control) and water-stressed plants. Twenty and twenty-seven experimental units (plants) were assigned to the control and water stress treatments, respectively. The control plants continued to receive daily irrigation, with enough water to sustain all plants would have, at least, 3 fully developed leaves, under natural environmental conditions, in a glasshouse at the School of Agronomy of the Federal University of Rio Grande do Sul, Brazil. Temperature and humidity inside the glasshouse recorded on a data logger (Pinguin, Novus Automation) installed at plant height, revealed temperature varying between 20 and 40°C and relative humidity between 24 and 80% during the growth period.

Gas exchange measurements - The daily gas exchange pattern was evaluated every 15 days (with a total of 4 evaluations) by measuring carbon assimilation (A) and transpiration rate (E) in 10 plants from each treatment, every two hours, from 8:00 am to 6:00 pm, using a portable infrared gas analyzer (Licor 6400; LiCor Inc., Lincoln, NE, USA). The youngest fully expanded leaves of the main branch of each plant were used for these measurements. Photosynthetically active radiation (PAR) inside the leaf chamber was fixed in 1500 μmol m⁻² s⁻¹. The CO₂ concentration inside the chamber was controlled using CO₂ bottles and fixed on 400 ppm. Temperature and relative humidity fluctuated naturally, according to the environmental conditions inside the glasshouse. In order to analyze the diurnal variations of gas exchange, all four evaluations taken on control plants were grouped together and measurements were averaged for each time of the day. In the water-stressed plants, two groups were established according to the time elapsed from the moment of water withholding. The first group includes measurements taken up to 15 days after withholding water (water stressed, WS). The second group includes measurements taken from 30 days after withholding water up to the last day of measurements (progressive water stress, progressive WS).

The CO₂ response curves of photosynthesis (A-C) were analyzed at the 14th day after irrigation suspension (leaf water potential between –1.6 MPa and –2.1 MPa). The A-C curves were made in the laboratory, in four plants from each treatment (control and water stressed). Chamber temperature was kept between 25° and 26°C. Photosynthetically active radiation was 1500 μmol m⁻² s⁻¹, and relative humidity between 50 and 60%. The A-C curves were obtained with short shot measurements (approximately 2 minutes in each point), starting with a CO₂ concentration of 400 μmol mol⁻¹ and progressively reducing it to 50 μmol mol⁻¹. At this point, CO₂ concentration was progressively raised until 800 μmol mol⁻¹. A set of parameters were then calculated, according to the mathematical model for carbon assimilation proposed by von Caemmerer & Farquhar (1981): Vcmax (maximum carboxylation rate), Jmmax (maximum electron transport rate), Amax (maximum carbon assimilation rate upon light and CO₂ saturation conditions). Stomatal limitation (Ls) was also calculated, according to the model of Farquhar & Sharkey (1982). Another curve was performed on the 40th day after water withholding, when plants reached pre-dawn leaf water potentials between –1.6 MPa and –2.1 MPa. However, due to the pronounced stomatal closure of these plants, the response curves were not meaningful and are not presented.

**Results and discussion**

Gas exchange and plant water deficits - Irrigation suspension resulted in reduction of gravimetric soil water content (θ) from 0.30 to 0.18 g·g⁻¹. Pre-dawn values of leaf water potential (Ψᵞᵥ) recorded at the beginning of each day of measurement varied between –0.7 and –0.9 MPa in well-watered plants (WW) and showed a progressive reduction to –2.1 MPa in water–stressed plants (WS) (Fig. 1A and B). Maximum photosynthetic and transpiration rates reached each day were significantly reduced by water stress throughout the experiment (Fig. 1C and D). At the end, photosynthesis and transpiration had reduced by 59 and 96 %, respectively. The fact that transpiration was more greatly reduced than photosynthesis on stressed plants indicates an optimization on carbon gain versus water loss (Dai et al. 1992; Schurr et al. 2000).

Diurnal variations in photosynthesis and transpiration - Well-watered plants displayed increased photosynthetic (A) and transpiration (E) rates along the day, reaching maximum values at 3:00 pm (34 and 13.4 μmol m⁻² s⁻¹, for A and E, respectively), followed by a progressive decline later in the day (Fig. 2A). Plants under water stress had 45 % and 59 % average reduction in carbon assimilation along the day, respectively, when compared to control plants. Also, their rates of photosynthesis remained mostly constant along the day, with maximum values of 19 μmol m⁻² s⁻¹ on plants under WS and 14 μmol m⁻² s⁻¹ under progressive WS. Transpiration rates in plants under WS and progressive WS showed diurnal patterns which were similar to A, but plants under severe water stress had transpiration values close to zero (Fig. 2B).
Under well-watered conditions, leaf transpiration and carbon assimilation were high during most of the day. According to Dai et al. (1992), the very high photosynthetic rates exhibited by the well-watered plants can be explained by their high area-based concentration of soluble proteins, chlorophylls and Rubisco. However, under water stress conditions, castor bean showed a strong stomatal regulation of gas exchange, associated with reductions in transpiration and CO₂ assimilation. Nevertheless, even with reductions between 30% (WS) to 50% (progressive WS), photosynthesis values were still high. The maintenance of the high values of photosynthesis along the day was also observed in water-stressed plants of *Carica papaya* (L.) by Marler & Mickelbart (1998), and seems to be associated with the vigorous growth habitat of this species. However previous works had shown that daily gas exchange pattern of water stressed plants usually depends on the time of day (Romero & Botía 2006; Osório et al. 2006). This pattern was not observed in this study, since the rates of A and E showed little variation along the day on the water stressed plants. This behavior might be the result of strong stomatal regulation on castor bean plants, caused by the reduction of soil water potential. The maintenance of constant values of gas exchange, on the water stressed plants, all day long may be an important characteristic of castor bean plants, as reported in previous studies (Dai et al. 1992, Schurr et al. 2000), and it may help explain the vigorous growth of the species commonly observed under high-light conditions.

Stomatal limitations of photosynthesis under water deficit - The validity of the internal CO₂ concentration (*C₅*) calculated from A/C₅ curves has been questioned (Lawlor & Cornic 2002) because of: the possibility of heterogeneous stomatal closure and increase of cuticular transpiration under conditions of water stress (Boyer et al. 1997; Buckley et al. 1997). Besides, performing these curves can become extremely difficult when low photosynthesis values are observed due to reduced levels of stomatal conductance under high CO₂ concentrations (Lawlor 2002). In this study, uniform stomatal closure in castor bean in response to water deficit was assumed when analysing the stomatal vs. biochemical limitations to photosynthesis.

The A/C₅ curves obtained for control and water-stressed plants (14 days after water withholding, leaf water potential between -1.0 MPa to -1.5 MPa) were different (Fig. 3). In

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**Figure 1.** Effect of water withholding on (A) soil water content, (B) leaf water potential (Ψₜ), (C) maximum photosynthetic and (D) maximum transpiration rate. Each point is the mean of ten measurements. Vertical bars indicate the standard deviation of the mean. Asterisks indicate difference at *P* ≤ 0.05. WW= well-watered; WS= water-stressed.
The stressed plants, Rubisco saturation was not observed. The analysis of biochemical parameters derived from these curves shows that the limitation imposed by stomatal closure (Fig. 4) was higher under drought (0.49) than under well-watered (0.17) conditions. The parameters $V_{\text{cmax}}$ and $J_{\text{max}}$ and $A_{\text{max}}$ did not differ between stressed and control plants.

These results indicate that reduction of photosynthesis on WS plants was caused by the increase on CO$_2$ diffusion resistance, because of stomatal closure, and that Rubisco activity, carboxylation efficiency and electron transport rate are not affected. Similar results have been observed in previous studies (Chaves 1991; Gimen$_{\text{e}}$z$_{\text{e}}$ et al. 1992; Tezara et al. 1999). This suggests that biochemistry of photosynthesis is preserved on WS plants and that the photosynthetic limitations observed, under these conditions, are mostly due to diffusive resistance.

Figure 2. Diurnal courses of photosynthetic (A) and transpiration (B) rates of castor bean leaves. Well-watered plants ($\psi_w = -0.6$ a $-0.9$ MPa), water stress plants ($\psi_w = -1.0$ MPa $a -1.5$ MPa) and progressive water stress plants ($\psi_w = -1.6$ MPa $a -2.1$ MPa). Vertical bars indicate the standard deviation of the mean. Mean values followed by same letters are not different at $P \leq 0.05$ using the Tukey test.
Growth and chlorophyll responses to water deficit - As expected, at the end of the drought period, the water-stressed plants had reached predawn values of water potential below –2.0 MPa, indicating severe reduction in water availability to their roots. Plant growth was negatively and significantly affected by this severe water deficit, expressed by reduction in shoot length, root and shoot biomasses and total leaf area (Tab. 1).

Shoot elongation was affected very early after irrigation was suspended, indicating great sensitivity of shoot growth to incipient reduction in water availability. This early growth response and the pronounced cumulative growth reduction may have contributed to plant survival after 53 days of irrigation suspension. The very small size attained by the water-stressed plants reduced plant demand for water enhancing chances of survival. Growth reduction is regarded as a characteristic associated to water stress resistance (Lecoeur et al. 1995). An early response at shoot growth to reduced water availability is related to a hormonal signaling (ABA) coming from roots (Qaderi et al. 2006). Interestingly, water deficit did not result in increased mass allocation between root and shoot and in reduced leaf area ratio (Table 1), as reported by many other studies (Poorter & Nagel 2000; Anya & Herzog 2004). Also, no reduction in LAR was observed. It is possible that within-organ allocation patterns (eg., lateral vs. tip root), not evaluated in this study, might have responded to imposed drought.

Leaf chlorophyll concentration expressed on an area basis was significantly reduced under drought conditions (Tab. 1). Although many species increase LMA when under drought stress, such increases are more commonly associated to nutrient stress (Aerts & Chapin 2000). Considering the major reductions in leaf area and the restriction to water loss imposed by stomatal closure, it is very likely that the reduction in LMA exhibited by castor bean plants affects the leaf allocation pattern that prioritizes leaf expansion at the express thickening in order to add not an extra limitation to photosynthesis.

Castor bean plants resisted water stress by avoidance mechanisms, based on early stomatal closure, thus minimizing water loss by transpiration and demand for soil water. The results also indicate that castor bean plants combine an efficient stomatal control with high levels of net CO₂ fixation. Field experiments should be conducted in order to validate the results obtained in this glasshouse study. It should be noted that if castor bean plants also exhibit such avoidance responses under natural conditions, its vigorous growth will only be expressed when soil water availability is maintained at adequate levels.
Figure 4. Effects of withholding water on (A) stomatal limitation to photosynthesis ($L_s$) (B) maximum carboxylation rate ($V_{cmax}$) (C) electron transport driving regeneration of RuBP ($J_{max}$) and (D) maximum carbon assimilation rate upon light and CO$_2$ saturation conditions ($A_{max}$) of castor bean plants, estimated from A/C$_i$ response curves. Each point is the mean of four measurements. Vertical bars indicate the standard deviation of the mean. Asterisks indicate difference by Tukey test at $P \leq 0.05$. WW= well-watered; WS= water-stressed.

Table 1. Effects of water withholding on shoot length, root and shoot dry mass, total chlorophyll concentration, leaf mass per area, total leaf area, root: shoot mass ratio and the leaf area ratio of castor bean plants. WW= well-watered plants; WS= water-stressed plants.

<table>
<thead>
<tr>
<th>Water status</th>
<th>Shoot length (cm)</th>
<th>Root biomass (g)</th>
<th>Shoot biomass (g)</th>
<th>Chl g/m$^2$</th>
<th>LMA (g.m$^{-2}$)</th>
<th>TLA (m$^2$)</th>
<th>R-S (g.g$^{-1}$)</th>
<th>LAR (m$^2$.g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>16.1 ± 5.1</td>
<td>9.33 ± 6.3</td>
<td>8.13 ± 4.5</td>
<td>462 ± 58.5</td>
<td>45.0 ± 4.82</td>
<td>768.8 ± 514</td>
<td>1.69 ± 2.1</td>
<td>41.4 ± 19.5</td>
</tr>
<tr>
<td>WS</td>
<td>3.99 ± 0.88</td>
<td>1.12 ± 0.36</td>
<td>1.02 ± 0.24</td>
<td>555 ± 84.5</td>
<td>39.1 ± 4.48</td>
<td>78.59 ± 24.6</td>
<td>1.10 ± 0.32</td>
<td>37.9 ± 12.2</td>
</tr>
</tbody>
</table>

Tukey test * * * * * * ns ns

* Values are mean ± S.D (n=20 for WW and 27 for WS). Asterisks (*) indicate difference at $P \leq 0.05$

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