



Gas exchange and antioxidant activity in seedlings of *Copaifera langsdorffii* Desf. under different water conditions

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ABSTRACT

The aim of this study was to evaluate gas exchange, efficiency of the photosynthetic apparatus, and antioxidant activity in *Copaifera langsdorffii* Desf. The seedlings were cultivated under different conditions of water availability, in order to improve the utilization efficiency of available water resources. The seedlings were cultivated in four different water retention capacities (WRC- 25%, 50%, 75%, and 100%), and evaluated at four different time (T- 30, 60, 90, and 120 days). During the experimental period, seedlings presented the highest values for carboxylation efficiency of Rubisco (A/Ci), intrinsic water use efficiency (IWUE = A/gs), chlorophyll index, and stomatal opening, when grown in the substrate with 75% WRC, but the stomatal index (SI) was less the 25% WRC. The efficiency of photosystem II was not significantly altered by the treatments. Comparison between the extreme treatments in terms of water availability, represented by 25% and 100% WRC, represent stress conditions for the species. Water availability causes a high activity of antioxidant enzymes (superoxide dismutase, peroxidase, and catalase) in the plant.

Key words: antioxidant enzymes, chlorophyll a fluorescence, photosynthesis, water stress.

INTRODUCTION

Plant species are continuously exposed to a variety of stress factors, which limit their productivity and/or maximum expression of their genetic potential. Among these factors, water availability, the shortage of which affects leaf water potential, nutritional status, and leaf gas exchanges, might alter several metabolic and physiological processes; this, in turn, compromises the growth of plant species (Gonçalves et al. 2009, Cunha et al. 2013,

Campelo et al. 2015), and affects their occurrence and distribution (Sakamoto and Murata 2002).

Among the processes that could be affected by water stress, stomatal closure and reduction in the mesophyll conductance are particularly important, as they lead to a reduction in photosynthetic rate, and synthesis of ATP and antioxidant enzymes, in addition to damage to tissue membranes and impairing the activity of enzymes responsible for carbon fixation and assimilation processes (Loreto et al. 2003, Flexas et al. 2012, Campelo et al. 2015, Shao et al. 2007, Pompelli et al. 2010).

Copaifera langsdorffii Desf., popularly known as the diesel tree or “copaiba”, is a species native

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to the Cerrado and semi-deciduous forests, but has a wide geographic distribution in the Brazilian territory. Due to the medicinal properties of its oil, this species faces intense over exploitation. Thus, it is crucial to investigate its agronomic and acclimation features to facilitate its *ex situ* cultivation. Moreover, with the increase in climatic variations, there has been an increasing demand for data on species that could be potentially deployed in degraded environments, wherein the plant-water relations get significantly affected (Nascimento et al. 2014, Santana et al. 2016).

Soil water availability is considered the factor with the greatest impact on the productivity of agricultural or forest species, which might influence their spatial distribution as well. Sustainable management of water resources, and the communication of research results to different countries and regions, as well as to different species and forest management regimes, continues to be difficult (Portes et al. 2006, Caldato and Schumacher 2013). Therefore, knowledge of the responses of species and their responses under the influence of different water conditions is fundamental, starting right from the first few months of growth, when the seedlings are highly susceptible to environmental variations.

In order to improve the use of available water resources, the aim of this study was to evaluate gas exchange, efficiency of the photosynthetic apparatus, and antioxidant activity of *Copaifera langsdorffii* Desf. seedlings cultivated under different conditions of water availability.

MATERIALS AND METHODS

The study was carried out at the Plant Nursery of the Faculty of Agrarian Sciences of the Federal University of Grande Dourados (UFGD), in the municipality of Dourados - MS, from June to October 2015. We used 60-day-old seedlings of *Copaifera langsdorffii* Desf.

The duration of the experiment was 120 days. A complete randomized design was used, with four replicates per treatment. The treatments were arranged in a 4×4 factorial scheme, with four values of water-availability (water retention capacity -WRC- 25%, 50%, 75%, and 100%) and four different evaluation times (T- 30, 60, 90, and 120 days). An experimental unit consisted of a vessel with two seedlings each.

WRC was calculated based on the methodology proposed by Souza et al. (2000). The 100% WRC treatment was determined based on the amount of water retained after the drainage of excess water, while 25%, 50%, and 75% WRC were obtained using the rule of three from the mass. Irrigation was provided on alternate days, and had individualized control, using the gravimetric method, with enough water to reach the pre-established mass for each WRC.

The substrate used to fill the vessels (6-L volume) was a mixture, comprising equal volumes of Bioplant[®], vermiculite, and dystroferric Red Latosol. Upon filling of substrate, the experimental units were transferred to the designated growth area in the nursery, under 30% shading, and covered with transparent plastic to protect them from precipitation.

At 30, 60, 90, and 120 days, leaf water potential (Ψ_w) of the seedlings was evaluated, using individual leaves belonging to the second pair of leaves fully expanded from the apex to the base, and the assay was performed between 10:00 and 11:00 h in the morning, immediately after the collection of the leaves, using a Scholander pressure chamber (Portable Plant Water Status Console - model 3115) (Scholander et al. 1964). The chlorophyll index (SPAD), the potential quantum efficiency of photosystem II (F_v/F_m), and the maximum efficiency of the photochemical processes in photosystem II, obtained from chlorophyll a fluorescence data, were measured using the portable fluorometer model OS-30p (Opti-Sciences Chlorophyll Fluorometer,

Hudson, USA). Photosynthetic and transpiration rates, stomatal conductance (gs), internal carbon dioxide concentration (Ci), water use efficiency (WUE), carboxylation efficiency of Rubisco (A/Ci), and intrinsic water use efficiency (IWUE) were determined using the infrared gas analyzer (IRGA), ADC, model LCi PRO. The evaluation was performed using four seedlings per treatment, in the morning, between 08:00 and 11:00 h, in fully expanded leaves that were previously marked, and all measurements were performed on the same leaves, and only the data measured under a photosynthetic photon flux (PPF) higher than 700 $\text{mmol m}^{-2} \text{s}^{-1}$ was considered.

Stomatal opening was determined with the aid of digital camera Moticam 2000 coupled to the optical microscope by means of the program Motic Image 2000 and adjusted scales in the optical conditions. The stomatal index (SI %) were calculated using the formula proposed by Salisbury (1928): $SI = [NS / (EC + NS)] \times 100$, where NS is the number of stomata and EC the number of epidermal

cells. The activity of the antioxidant enzymes, i.e., superoxide dismutase, peroxidase, and catalase, was measured in leaf and root tissues, following the methodology compiled by Broetto (2014).

Data for temperature and relative humidity during the experimental period (Figure 1) were obtained from the Embrapa Agropecuária Oeste Meteorological Station, in Dourados - MS.

The results were analyzed using the statistical program SISVAR 5.3 (Ferreira 2010). The data were subjected to analysis of variance, at the 5% significance level by the F test, and modeled using regression equations.

RESULTS

There was a significant interaction ($p < 0.05$) between the WRC and the evaluation time for photosynthetic rate (A), transpiration (E), stomatal conductance (gs), internal carbon dioxide concentration (Ci), WUE, IWUE, A/Ci, chlorophyll, stomatal opening and stomatal index, and enzymatic activity of peroxidase in the leaves, and for catalase in the

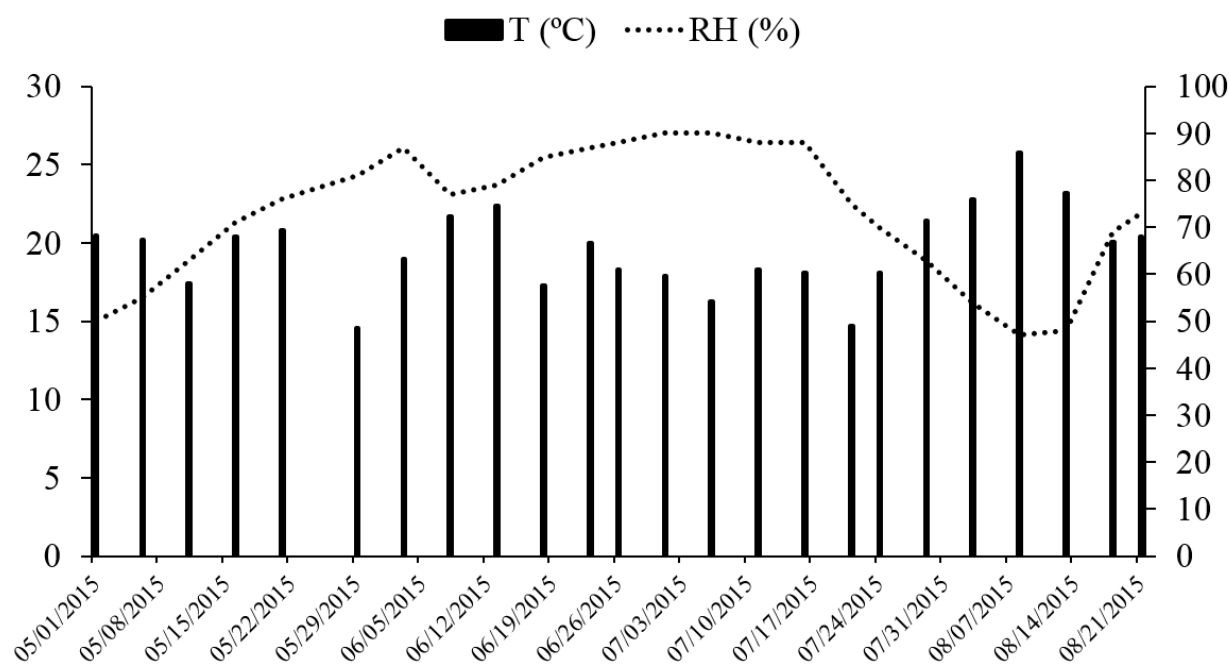


Figure 1 - Temperature and relative humidity during the experimental conduction period.

roots. A significant isolated effect of the WRC ($p < 0.01$) was observed for the enzymatic activity of superoxide dismutase in both leaves and roots, and for peroxidase in the roots. The isolated effect of time was significant ($p < 0.01$) only for the leaf water potential (Ψ_w). Neither F_v/F_M nor the maximum efficiency of the photochemical process in photosystem II (F_v/F_o) were influenced ($p > 0.05$) by the studied factors, presenting mean values of 0.70 and 3.90, respectively.

The maximum leaf water potential (Figure 2a) was observed at 86 days (-1.23 MPa), however, there were no significant differences among the different WRC treatments, which mean value of -1.4 MPa.

Photosynthetic rate (Figure 2b) was low for the seedlings cultivated under 100%, 25%, and 50% WRC. Although for seedlings cultivated under 75% WRC, the minimum observed value was $3.28 \mu\text{mol m}^{-2}\text{s}^{-1}$ (at 96 days), but on average, it is observed tendency of seedlings cultivated under this 75% WRC showed high photosynthetic rate.

Transpiration (Figure 2c), regardless of the evaluation time, had the lowest value under 25% WRC and the highest value under 100% WRC; for the other treatments, intermediate values were observed.

WUE under 25% WRC presented a maximum value of $5.26 \mu\text{mol CO}_2\text{mmol}^{-1}\text{H}_2\text{O}$, at 69 days (Figure 2d). For the other treatments, the WUE was inferior, and it differed between seedlings cultivated under 100% and 75% WRC, with lower values under 100% WRC. Data for seedlings cultivated under 50% WRC did not fit well to the regression equations.

The minimum value of C_i was observed under 75% WRC, at 60 days ($218.26 \mu\text{molmol}^{-1}$), and from this point on, the values increased. For seedlings under 100% WRC, the performance decreased over time, with values of 296.95 and $252.55 \mu\text{molmol}^{-1}$, at 30 and 120 days, respectively (Figure 3a). For the remaining WRC treatments, the data did not present any pattern, as it did not

show a good fit to the tested equations; however, at the end of the evaluation, the data were found to between the values obtained for 75% and 100% WRC.

A/C_i values decreased over time in all the evaluated water resource conditions, while remaining high in the seedlings cultivated under 75%, and low in the ones under 25% WRC. There was a greater temporal variation in the A/C_i under 100% WRC (difference of $0.014 \mu\text{molm}^{-1}\text{s}^{-1}\mu\text{mol mol}^{-1}$) in comparison with the other treatments evaluated (Figure 3b).

The values for stomatal conductance of the seedlings cultivated under 25% WRC decreased throughout the evaluation period. For the 50% WRC treatment, the minimum value was $0.046 \text{mol m}^{-2}\text{s}^{-1}$, at 88 days. For the 75% and 100% WRC treatments, the minimum values (0.037 and $0.041 \text{mol m}^{-2}\text{s}^{-1}$, respectively) were observed at 100 days (Figure 3c). The IWUE presented increasing values under 75% WRC during the evaluation period, with the highest value ($110.99 \mu\text{molCO}_2 \text{mmol}^{-1}\text{H}_2\text{O}$) at 120 days. For the 100% WRC treatment, the maximum value ($63.51 \text{CO}_2 \text{mmol}^{-1}\text{H}_2\text{O}$) was observed at 78 days (Figure 3d).

In general, the SPAD index was the highest throughout the evaluation period for seedlings cultivated under 75% WRC. Maximum chlorophyll index of 45.62, 51.33, and 7.12 was observed at 67, 77, and 57 days, under the 25%, 75%, and 100% WRC treatment, respectively. For seedlings cultivated under 50% WRC, the minimum value was 43.62, at 88 days. At the end of the evaluation period, the chlorophyll index exceeded the values for the seedlings cultivated under 100 and 25% WRC treatments, reaching values close to those of seedlings cultivated under 75% WRC (Figure 3e) capacity (WRC) and time.

Stomatal opening (Figure 4a) under 75% WRC, was, in general, higher than in the other treatments, with the minimum value ($0.54 \mu\text{m}$) observed at 54 days. The stomatal index (Figure

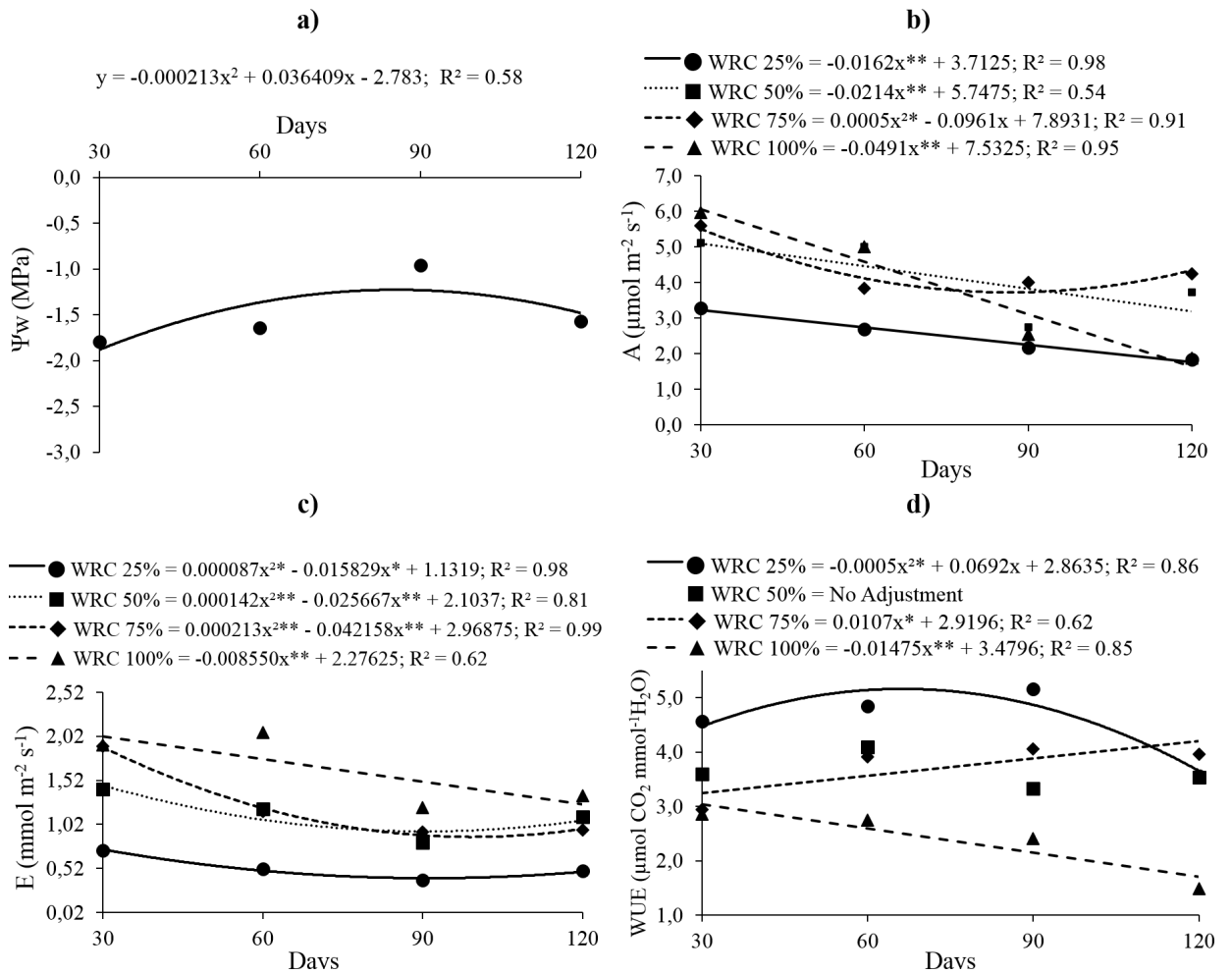


Figure 2 - Water potential of the leaves – Ψ_w (a) liquid photosynthesis - A (b); transpiration - E (c) and water use efficiency - WUE (d) in *Copaifera langsdorffii* Desf. depending on different water retention capacity (WRC) and time.

4b) values increased over time under the 25% and 75% WRC treatments, and the average value was higher for seedlings cultivated under 25% WRC. For seedlings cultivated under 100% WRC, the stomatal index presented a opposite behavior, decrease over time, with the lowest value (9.96%) at 120 days.

Superoxide dismutase activity in the leaves (Figure 5a) was highest in seedlings cultivated under 25% and 100% WRC, and in roots under 25% WRC (Figure 5b). The peroxidase content in the leaves (Figure 5c) remained high under the 25% WRC treatment throughout the evaluation; however, it tended to decrease, reaching a minimum

value of $0.65 \mu\text{Kat } \mu\text{g Prot}^{-1}$, at 86.78 days. For seedlings cultivated under 75% WRC, the values increased and reached the highest value at the end of the evaluation, with values similar to those for seedlings cultivated under 25% WRC. Peroxidase activity in the roots (Figure 5d) increased as water availability increased, with the highest values ($1.85 \mu\text{Kat } \mu\text{g Prot}^{-1}$) under the 100% WRC treatment. As for catalase activity in the roots (Figure 5e), the highest value was observed in the seedlings after 30 days of cultivation under 100% WRC, and the minimum value ($1.20 \mu\text{Kat } \mu\text{g Prot}^{-1}$) was observed for seedlings cultivated under 50% WRC at 55.26 days.

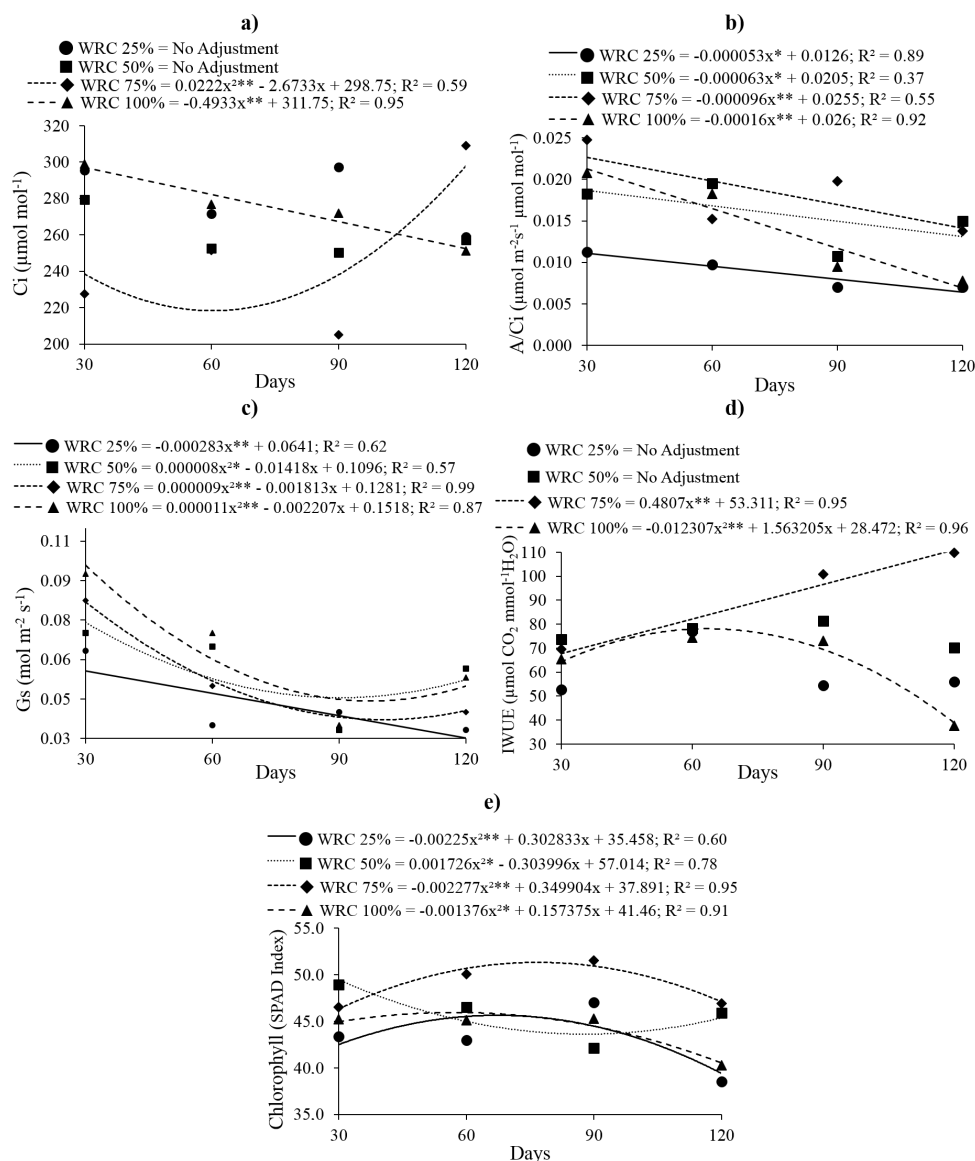


Figure 3 - Internal carbon dioxide gas - C_i (a), Carboxylation efficiency of rubisco - A/C_i (b); Stomatal conductance - g_s (c), intrinsic water use efficiency - IWUE (d) and Chlorophyll (e) in *Copaifera langsdorffii* Desf. depending on different water retention capacity (WRC) and time.

DISCUSSION

It is interesting to note that, under different conditions of water availability, *Copaifera langsdorffii* leaves did not present a significant difference in their water potential (Ψ_w). A potential reason is that, the species presents resources to adapt to the changes of the environment (represented in this study by different WRC values). Trovão et al. (2007) evaluated the

water potential of 11 tree species from Caatinga, in the dry and rainy seasons, and observed that seven species of the total did not present significant differences in the water potential across the two seasons. The most interesting explanation utilizes the fact that plants, in order to minimize water loss and maintain turgidity for a period of time, present physiological changes.

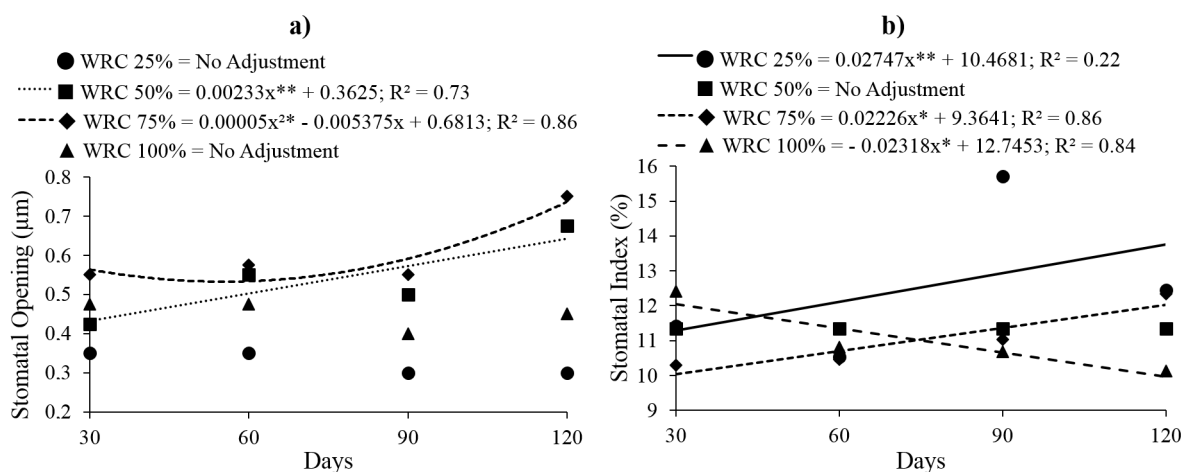


Figure 4 - Stomatal opening (a) and stomatal index (b) in *Copaifera langsdorffii* Desf. depending on different water retention capacity (WRC) and time.

The Ψ_w response pattern in *C. langsdorffii* seedlings might also be a response to the environmental conditions during the study period. In the days prior to the 30 and 120-day evaluations, the relative humidity of the environment was low, which could have contributed to low water availability in the seedling tissues, compared with the other evaluation periods. However, not all physiological relationships evaluated under different WRC treatments were negatively affected by this environmental condition.

Although the observed average value of Ψ_w for *C. langsdorffii* plants has been considered in the literature as a limiting factor for the development of several species, the value obtained for F_v/F_M , regardless of the evaluated treatments, was 0.70. This result suggests that the plants showed very little reduction towards the critical limit, suggesting that they have some evolutionary characteristic to tolerate water shortage. According to the literature, F_v/F_M decreases under conditions of low water availability, leading to an increase in the photoinhibition processes (Lage-Pinto et al. 2012).

Results similar to those obtained for *C. langsdorffii* seedlings were also observed by Trovão et al. (2007) in their study on Caatinga trees; four of the seven species that did not present significant

alterations in Ψ_w (*Myracrodruon urundeuva* Allem., *Amburana cearenses* Allem., *Commiphora leptophloeos* Mart., and *Maytenus rigida* Mart.) did not present changes in F_v/F_M either. Campelo et al. (2015) observed a reduction in this trait in only three of the evaluated tree species (*Calophyllum brasiliense* Cambess., *Swietenia macrophylla* King, and *Handroanthus serratifolius* Vahl), and only when the seedlings were subjected to a severe water stress, with F_v/F_M values varying from 0.555 to 0.619.

Photosynthetic rate decreased for most WRC values. The highest decrease occurred under the 100% WRC treatment (Figure 3a), which suggests that the 100% constant maintenance of WRC led to an effective decrease in the amount of air around the roots. This would impair the water absorption, which could consequently affect the photosynthetic process to a greater extent than under the 25% WRC treatment, wherein the values remained low throughout the experimental period. These results suggested a water stress in *C. langsdorffii* seedlings under the 100% WRC treatment.

After 120 days of evaluation, it was possible to observe that the seedlings cultivated under 75% WRC presented the highest photosynthetic rates and WUE. This was the only treatment where these characteristics continuously increased with time,

which might be justified by the higher observed stomatal opening that allows better internal carbon input and water vapor release. Very low values of transpiration and/or very high photosynthetic rate contribute to the elevation of the WUE. In this experiment, under the 25% WRC treatment, low transpiration led to the highest WUE values. On the other hand, the high photosynthetic rate observed under the 75% WRC treatment resulted in increasing values of WUE over time.

Higher values of WUE are characteristic of plants tolerant to lower availability of water resources (Ma et al. 2004). Moreover, they serve as an indicative parameter for the physiological plasticity of the plants with respect to the abiotic factors. In other words, the values of WUE represent the ability of the species to adapt to environmental adversities. In the case of *C. langsdorffii* seedlings, this fact is evident between 30 and 90 days of the experimental period, wherein the highest value of WUE was recorded under the 25% WRC treatment. However, this value decreased over time, which indicates that *C. langsdorffii* is vulnerable to extended periods of low water availability (Figure 3c).

The IWUE was highest in the 75% WRC treatment, demonstrating that this WRC value was the most favorable for gas exchange in *C. langsdorffii* seedlings, in comparison with the other treatments. High values of IWUE and WUE are usually the characteristics of plants tolerant to low water availability in the soil (Ferreira et al. 2012), which is the most favorable condition for the cultivation of *C. langsdorffii* seedlings.

As observed in Figure 4c, the stomatal conductance (g_s) up to the 60 days of evaluation presented a trend consistent with the different WRC values, because the lower the water availability, the lower the g_s values, which was an immediate and strategic response of the plants to reduce the water loss by transpiration, avoiding dehydration of the tissues (Albuquerque et al. 2013). This fact is evident from the similarity between the transpiration

curves (Figure 3b) and the stomatal conductance curves, for the 50% and 75% WRC treatments, which confirms the existing relationship.

In the 75% WRC treatment, a low C_i and a decrease in its values, up to 60 days of cultivation, were observed. This was followed by an increase in these values, which would explain the pattern of decreasing response during the experimental period, for both the photosynthetic rate and the A/C_i .

The decreasing values observed for the A/C_i under the 25% and 100% WRC treatments during the experimental period might be a consequence of water deficit and an effective decrease in the amount of air in the substrate, respectively. Both of these factors could cause damage to the photosynthetic apparatus, which might lead to the impairment and reduction of Rubisco activity (Flexas et al. 2006, Xu et al. 2009).

A similar trend was observed for the chlorophyll index. The highest values were observed under 75% WRC, and the lowest values were observed under 25% and 100% WRC treatments. This result indicates that the water stress accelerated the degradation of chlorophyll contents (Mafakheri et al. 2010), which might have affected the conditions for photosynthesis in the plants, via a reduction in the amount of energy absorbed by the light-harvesting complex (Baker 2008). However, during the experimental period, no typical chlorosis, resulting from low amounts of chlorophyll, was observed.

Although the number of stomatal openings was high under 75% and 50% WRC, the same trend was not observed for the stomatal conductance, which had the highest value under 100% WRC. These results confirm that these characteristics are not directly related or dependent, and the maintenance of stomatal openings does not represent a high diffusion of CO_2 and water, as observed in plants cultivated under 75% WRC, where in a reduction in the transpiration was noted before the decrease in the photosynthetic rates, resulting in an influx of CO_2 and low water loss due to transpiration.

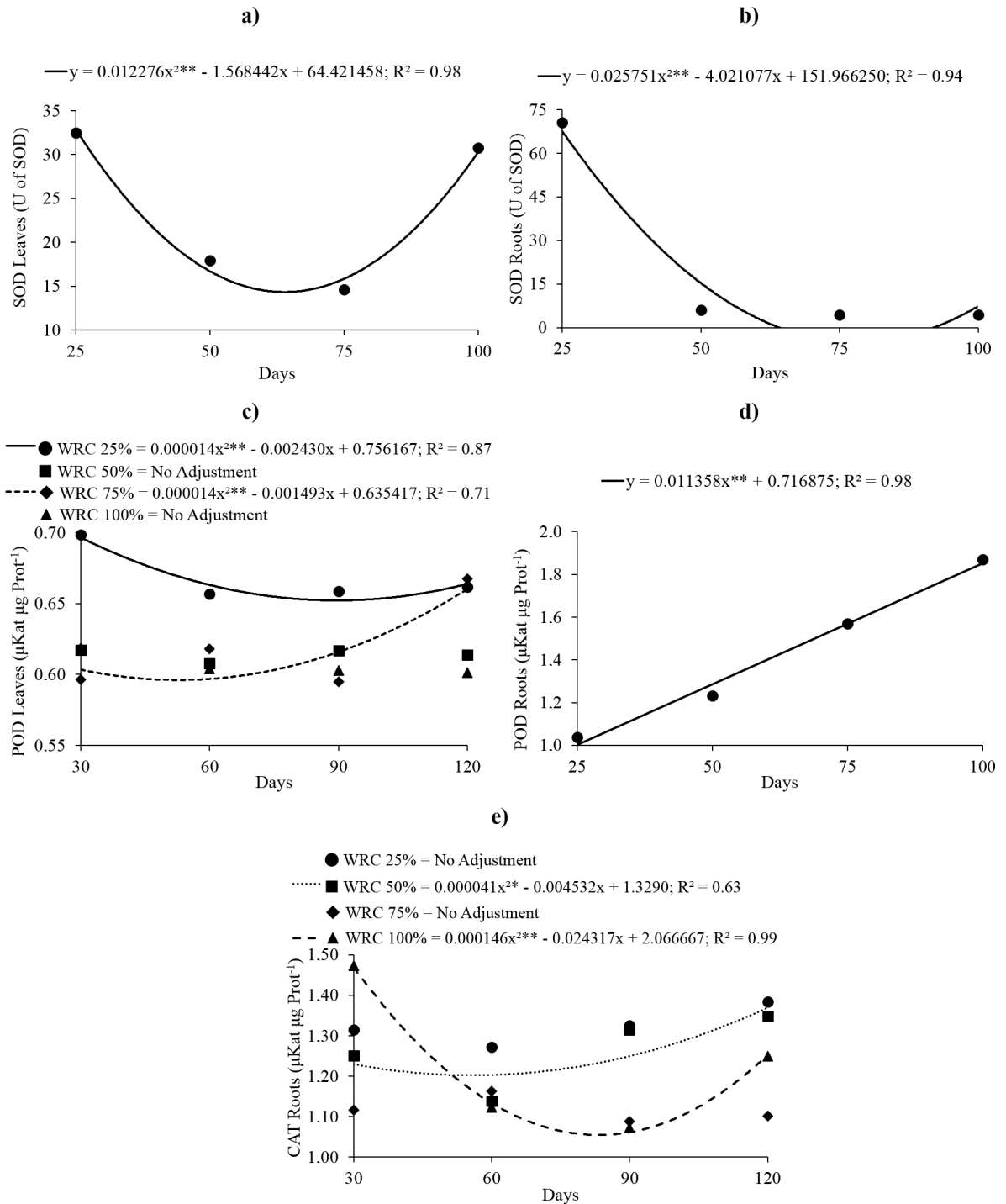


Figure 5 - Enzymatic activity of Superoxide dismutase in leaves - SOD (a) and roots (b); of Peroxidase in leaves - POD (c) and roots (d) and Catalase in leaves - CAT (e) in *Copaifera langsdorffii* Desf. depending on different water retention capacity (WRC) and time.

However, the water deficit caused several changes, as represented by the reduction in the number of stomatal openings. In the literature, stomatal changes (density, index, opening, size, diameters) are related to the regulation of gas exchange under stress conditions. Therefore, leaves with fewer stomatal openings show higher efficiency under water-deficit conditions, because they present a smaller size of the stomatal pores, which reduces water loss through transpiration (Boeger and Wisniewski 2003, Souza et al. 2010, Taiz and Zaiger 2013). In the case of *C. langsdorffii* seedlings, the reduction in the stomatal opening was a quick response to the water deficit, a strategy adopted to avoid water loss under a stress condition. However, it led to a reduction in the transpiration and photosynthetic rates.

The activity of superoxide dismutase, in both leaves and roots, was higher at the extremes of WRC, suggesting that such conditions (25% and 100% WRC) represent a growth-limiting condition for *C. langsdorffii* seedlings. Regarding peroxidase, its activity in the roots was more intense under 25% WRC than in the other treatments. However, an inverse trend was observed in the leaves; with increasing water availability, its activity increased. This fact suggests that different parts of the plant respond differently to the mechanisms of stress protection, and in the specific case of peroxidase, this could be due to its polymorphism. Therefore, this enzyme has different functions in each plant part (organ or cell compartment), which contributes to a better adaptation of the plant in the environment (Oliveira et al. 2008, Taiz and Zeiger 2013).

Regarding the catalase activity in the roots, although the data did not fit well to the tested equations, it can be seen that the highest values were generally recorded in the 25% WRC treatment. Seedlings cultivated under 75% and 100% WRC presented a low CAT activity during the experimental period, except at 30 days of cultivation under 100% WRC, which could be attributed to the fact that the

seedlings were still too young to support a large amount of water in the substrate, and consequently, increased the catalase production in response to a momentary oxidative stress.

Abiotic stress conditions might be related to the production of reactive oxygen species, which alter the cellular metabolism. It is known, however, that adaptation to water stress might be related to the ability of plants to maintain high levels of antioxidants in their tissues, such as the enzymes superoxide dismutase, peroxidase, and catalase, thus, activity of such enzymes constitutes an important parameter for quantifying plant responses to environmental stresses (Shao et al. 2007, Oliveira et al. 2008, Pompelli et al. 2010, Taiz and Zeiger 2013).

Previous studies have shown that water stress can cause reduction in the contents of photosynthetic pigments due to oxidative damage in plants; thus, the plants protect themselves by synthesizing carotenoids and increasing the content of enzymes such as peroxidases (Egert and Tevini 2002). It is noteworthy that this behavior was observed in the *C. langsdorffii* seedlings in the present study.

It is interesting to note that, among the enzymes quantified in *C. langsdorffii* seedlings, SOD was the most sensitive parameter for the detection and protection to stress. Similar results were also observed in *Jatropha curcas* L. subjected to water deficit (Pompelli et al. 2010). However, in the 11 different genotypes of *Triticum aestivum* L. evaluated by Shao et al. (2007), the differences in the expression of antioxidant enzymes under stress conditions might have been due to several factors, such as gene expression, cultivation site, atmospheric pressure, and natural and artificial selection.

CONCLUSION

Copaifera langsdorffii Desf. seedlings presented the highest gas exchange, photoassimilate production, WUE, and maintenance of the photosynthetic

apparatus, when cultivated on a substrate with 75% WRC. During the evaluation period, the efficiency of photosystem II was not significantly altered by any of the treatments. Extreme treatments, in terms of water availability, represented by 25% and 100% WRC, resulted in water stress for this species, leading to a high activity of antioxidant enzymes.

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