Gas Exchange in Caryocar Brasiliense Cambess Seedlings in Water Deficit Conditions

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Abstract

In this study, we assessed the gas exchange in Caryocar brasiliense seedlings in water deficit conditions and their capacity to recover after rehydration. We assessed plant photosynthetic rates, internal carbon concentrations, stomatal conductance, transpiration rates, water-use efficiency, instantaneous carboxylation efficiency, chlorophyll index, and photosystem II quantum efficiency. All gas exchange parameters were reduced after the suspension of irrigation. Seedling photosynthetic rates approached zero after 12 days. The equilibrium was reestablished in all variables 11 days after re-initiating irrigation, with the exception of internal CO\(_2\) concentration and stomatal conductance. Exposure of seedlings to water stress resulted in drastically reduced photosynthetic processes. This reduction occurred due to the limitation of stomatal conductance and low rubisco carboxylation efficiency but not to irreversible damage photosystem II. All of these parameters demonstrated recuperation with rehydration, but only the photosynthetic rates reached initial control values.

Keywords: hydric stress, native fruit, Pequi, Caryocaceae.

1. INTRODUCTION

Cerrado (Neotropical Savanna) is the second largest Brazilian biome and, according to a review by Klink & Machado (2005), it demonstrates great biodiversity with more vascular plants than most regions of the world. However, these authors also noted that rapid destruction of Cerrado ecosystems is occurring. By 2004, approximately 55% of the total area of this biome had been deforested or transformed by anthropogenic actions. Therefore, it is relevant to better understand the abiotic factors that can affect natural regeneration and thus improve reforestation programs of native Cerrado species to further restore and sustain the Cerrado ecosystem.

Water deficit alters plant physiological processes, mainly the gas exchange, thus limiting photosynthetic processes and the synthesis of organic solutes (and consequently the growth and primary production of plants) as carbon absorption is restricted (Chartzoulakis et al., 2002). During the dry season, seedlings of woody Cerrado species have been observed to present photosynthetic rates near to zero, probably because of severe water loss related to transpiration (Franco, 2006; Mariano et al., 2009).

Although measurements of gas exchange, as regulated by stomatal movements, are important to determine photosynthetic rates, they are not efficient in evaluating the deleterious effects of stress on chloroplasts (Durães et al., 2005). However, reductions in photosynthetic activities due to environmental stress are the result of the photosystem II activity inhibition and they can be evaluated as diminishing chlorophyll fluorescence (Camejo et al., 2005).

A number of researchers have examined the effects of water deficits on different plant species to determine their tolerance mechanisms (Bento et al., 2016; Franco, 2006; Gonçalves et al., 2009; Junglos et al., 2016; Mariano et al., 2009; Medrano et al., 2002; Pacheco et al., 2011; Parry, 2002). The results of these investigations have been useful in arboreal
species plantation projects and they could be used to enhance silvicultural techniques for species such as Caryocar brasiliense Cambess, a tropical tree of the Caryocaraceae family. This tree ("pequi") has significant economic relevance in the Cerrado biome because of its food and wood uses, as well as the oil extracted from its seeds (used in cosmetic and pharmaceutical industries). This species received the protected status due to its many uses, and these trees cannot be legally cut for lumber in Brazil (Almeida & Silva, 1994; Silva Neto & Costa, 2010).

Considering the wide distribution of this species in the Cerrado environment with irregular distribution of rainfall, characteristic of "Cerradão" (Ribeiro & Walter, 1998), it is believed that C. brasiliense seedlings can be cultivated for a few days under water restriction. The aim of this study was to investigate the characteristics of the photosynthetic metabolism of C. brasiliense seedlings to determine their tolerance to water deficits and subsequent metabolic recuperation after rehydration.

2. MATERIALS AND METHODS

2.1. Place of conduction of the experiment and production of seedlings

The following experiments were carried out under greenhouse conditions located in the municipality of Dourados, Mato Grosso do Sul State, Brazil (22°13′16″S, 54°17′01″W; 430 ma.s.l.). The regional climate is classified as Savanna climate (Aw) with an average annual rainfall of 1500 mm and of 22 °C (Kottek & Grieser, 2006; Pinto et al., 2016).

Caryocar brasiliense Cambess ("pequi") seedlings were produced from seeds harvested in a native population in the Cerrado region of southern Mato Grosso do Sul State. Seedlings were cultivated in polyethylene planters (capacity 5 kg) in a substrate composed of 50% distroferric Red Latosols and 50% sand (v:v), under 30% shading. The experiment started 20 months after sowing. The seedlings presented 6 to 9 expanded leaves and were between 16.3 and 18.5 cm tall, with stem diameters varying between 3.22 and 4.52 mm and primary roots between 24.0 and 31.0 cm long.

The cultivation planters were hydrated to 70% of their soil water retention capacity (WRC) (Souza et al., 2000).

2.2. Water regimes

The seedlings were divided in two groups:

1 – The first group (Control) was irrigated daily during the entire experiment period, and the soil was maintained at 70% of its water retention capacity.

2 – The second group (Stress) had irrigation suspended until the photosynthetic rates of the seedlings approached zero (12 days after the suspended irrigation). Starting at photosynthetic zero, all pots were watered daily. The plants were maintained at 70% of the water retention capacity until recovery, when the seedlings under water deficit treatment reached similar levels of photosynthesis as those of the Control group (11 days after the rehydration, 23 days after the start of the experiment). During the experiments, the seedlings were maintained in an environment protected from natural rainfall.

2.3. Characteristics evaluated

Gas exchange: Internal carbon concentrations (Ci - μmol CO₂ mol⁻¹); stomatal conductance (gs - mol m⁻² s⁻¹); transpiration rate (E - mmol m⁻² s⁻¹); and photosynthetic rate (A - μmol m⁻² s⁻¹) were determined using an infrared gas analyzer (IRGA) (ADC, model LCI PRO; Analytical Development Co. Ltd, Hoddesdon, UK). From the gas exchange data, two ratios were calculated – instantaneous water-use efficiency (A/E μmol CO₂ / mmol⁻¹ H₂O) and instantaneous carboxylation efficiency (A/Ci μmol m⁻² s⁻¹/ μmol mol⁻¹).

The chlorophyll index: was obtained using a Konica Minolta, SPAD 502 chlorophyll analyzer.

Efficiency of photosystem II: The potential quantum efficiency of photosystem II (Fv/Fm) was determined using a portable fluorometer (model OS-30p) (Opti-Sciences Chlorophyll Fluorometer, Hudson, USA). Fluorescence determination was performed between 8 a.m. and 11 a.m., on the same leaves used to assess gas exchanges. They were subject to a period of 30-minute dark adaptation, using leaf-clip holders. This enabled the reaction centers in the foliar region to acquire the "open" configuration, indicating the complete oxidation of the photosynthetic electron transport system.

All measurements were performed between 8 a.m. and 11 a.m. starting the seventh day after suspending irrigation. All measurements were performed on the same expanded leaf.

2.4. Evaluations periods

Five seedlings were used in each treatment and the evaluations occurred 7, 10, 12, 14, 16, 18, 20, 23, and 27 days after suspending the water supply. The irregular intervals between evaluations reflect the necessity of performing the measurements under appropriate climatic conditions, i.e. sunny days without clouds. The evaluations were finished when the photosynthetic rate of the stressed seedlings approached that of the control seedlings.
2.5. Statistical delineation and data analysis

For gas exchanges, the design was completely randomized with two water regimes — periodic irrigation, (Control) and no irrigation (Stress) — and five repetitions — each repetition representing a different seedling. After normality (Lilliefors test) and homogeneity (Bartlett test) tests, the results were subjected to analysis of variance. The means of each treatment were subjected to a t-test at 5 % probability in the Sisvar statistical program when statistical significance occurred.

3. RESULTS

The mean photosynthetic rate (A) of the control seedlings of *C. brasiliense* was 7.485 µmol m⁻² s⁻¹ (Figure 1a). When seedlings were subjected to water stress conditions a pronounced decrease in their photosynthetic rates until the seventh day occurred. By the 12th day their photosynthetic rates approached zero (0.280 µmol m⁻² s⁻¹), after which irrigation was reinitiated.

This rehydration of the seedlings exposed to conditions of water stress enabled them to recuperate their photosynthetic metabolism and regain high photosynthetic levels. The *C. brasiliense* seedlings required only 11 days to recuperate their initial photosynthetic rates.

The transpiration rates (E) of the water stressed seedlings reduced similarly to the photosynthetic rate. The transpiration values of the stressed seedlings were lower than those of the control during the entire evaluation period (Figure 1b). The transpiration rates of the stressed seedlings was higher after rehydration, although the final values never reached those for the control (6.26 and 8.55 mmol m⁻² s⁻¹ respectively).

The water-use efficiency (A/E) of the seedlings exposed to water deficit conditions was lower than that of the controls until the 14th day of evaluation (Figure 1c); however, after rehydration the A/E values were higher than those of the control, reaching their peak on the 20th day (average 1.96 µmol CO₂ / mmol H₂O). The control seedlings had an average A/E of 0.99 µmol CO₂ / mmol H₂O, below the value observed in stressed seedlings (average 1.27).

![Figure 1](image_url). Mean values of photosynthetic rate – A (a), transpiration rate – E (b), water use efficiency – A/E (c) of *Caryocar brasiliense* Cambess seedlings subjected to intermittent water deficit. Means followed by different letters differ by t-test at 5% probability.
The internal CO$_2$ concentrations ($C_i$) of the seedlings did not show the same response to the water deficit as the photosynthetic rate did (Figure 2a). Starting at the 14th day of evaluation the seedlings presented a decrease in their internal CO$_2$ concentrations, with a small recovery by the 18th day, although they did not reach the same values as the control.

The instantaneous carboxylation efficiency ($A/C_i$) of the stressed seedlings was greater than that observed in the control, demonstrating an accentuated decrease between the 7th and 10th day of experiment, with rapid recuperation after rehydration, surpassing the control again by the 18th day (Figure 2b).

Figure 2c presents the stomatal conductance (gs) of near-zero values by the 12th day (0.03 mol m$^{-2}$ s$^{-1}$). Conductance values recovered slowly after rehydration, but they did not achieve initial values of the control in the end of the experimental period.

The chlorophyll index of the $C. brasiliense$ seedlings varied little with the seedlings exposed to water deficit conditions, showing greater mean values than did the controls between the 14th and 18th days of evaluation (Figure 3a).

The PS II quantum efficiency values ($F_v/F_m$) of the stressed seedlings strongly decreased. After rehydration, this efficiency increased after the 18th day, showing an average of 0.78 during subsequent evaluations (Figure 3b).

**Figure 2.** Mean values of internal CO$_2$ concentration – $C_i$ (a), instantaneous carboxylation efficiency of CO$_2$ – $A/C_i$ (b) and stomatal conductance – gs (c) of *Caryocar brasiliense* Cambess subjected to intermittent water deficit. Means followed by different letters differ by t-test at 5% probability.
4. DISCUSSION

The observed decreases in the photosynthetic rates \( (A) \) of \textit{C. brasiliense} seedlings could be attributed to reductions in their stomatal conductance, as it has been observed in other plant species by Dias & Marenco (2007). This closing mechanism reduces transpiration and diminishes internal CO\(_2\) concentrations in the mesophyll, thus gradually halting the photosynthetic processes of water stressed plants.

According to Pacheco et al. (2011), plants exposed to water stress conditions normally present higher concentrations of abscisic acid (ABA)—a hormone that affects many physiological and developmental characteristics in plants. ABA has also been identified as a messenger for water stress perception (and other environmental stress factors).

A reduction in stomatal conductance \( (gs) \) in \textit{C. brasiliense} seedlings was observed on the seventh day after suspending irrigation; transpiration remained stable and low until approximately the 14th day, although photosynthesis was drastically reduced on the seventh day of evaluation. Transpiration in control plants increased throughout the experiment, which can be attributed to the high number of days without rain and with high temperature, factors that also contributed to raising the vapor pressure deficit (personal observation).

After rehydration, \( gs \) values slowly recovered but did not attain the mean values of the control, suggesting that the stomata of this species are slow to recover after rehydration. The decrease in \( A/E \) was observed in both control and stressed plants, suggesting a rapid stress response, even in the control seedlings which received water through soil. Furthermore, the increased \( A/E \) above control values is related to the incomplete recovery of \( gs \), leading to reduction of \( E \).

These results were caused by low water availability in the plants that may decrease leaf water potential, resulting in the loss of turgor, which consequently reduced stomatal conductance and photosynthetic rate (Medrano et al., 2002). These results suggest the existence of adaptive mechanisms in \textit{C. brasiliense} that can diminish water losses under water stress conditions.

The stomatal conductance of \textit{C. brasiliense} seedlings decreased during the first 12 days after interrupting irrigation, indicating that the \( gs \) response reflected decreased soil moisture. This result suggests the existence of connection between the plants roots and the aerial portion intermediated by growth substances, probably ABA (produced in roots), that enabled maintenance of water levels in leaves for longer periods, even though the soil is progressively drying (Gomes et al., 2004; Magalhães Filho et al., 2008).

The fact that \( gs \) was reduced and \( Ci \) remained elevated in the seedlings until re-initiating irrigation suggests that other factors act in controlling photosynthesis. According to Medrano et al. (2002) and Parry et al. (2002), drought conditions reduce metabolism in the mesophyll by diminishing the activation
and activity of rubisco carboxylase. The lower efficiency of rubisco may also be the result of greater mesophyll resistance due to stomatal closure, restricting the absorption of CO₂ by the chloroplasts and increasing the action of rubisco oxygenase and (consequently) photorespiration. This result can be visualized by the rapid reduction in A/CI, which represents the instantaneous carboxylation efficiency of CO₂ by rubisco. The recuperation of gs in C. brasiliense was proportionally slower than that of A, which would explain the decrease in CI after the rehydration of the leaf tissues.

There were notable continuations of various metabolic processes after rehydration. This led to an increase in water-use efficiency due to a significant increase in photosynthetic processes and the maintenance of transpiration levels, reaching maximum values (1.96) by the 20th day (8 days after rehydration). These stressed plants attained values slightly higher than those of the control seedlings.

Responses to water deficits vary among plant species. Mariano et al. (2009) evaluated the effects of water stress conditions on the physiological characteristics of Myracrodruon urundeuva Allemão and observed similar tendencies to those seen in C. brasiliense, with transpiration showing a direct relationship with stomatal conductance, resulting in less diffusion of CO₂ into the leaves and consequently reductions in photosynthetic levels. The photosynthetic indicators in young Carapa guianensis Aubl. plants decreased in water deficit conditions, reaching a near-zero photosynthetic rate at 21 days; the recovery of these indicators was observed approximately 8 days after rehydration, reestablishing all of the photosynthetic characteristics of normally irrigated plants (Gonçalves et al., 2009).

Campomanesia adamantium (Cambes.) O. Berg plants subjected to water deficit conditions demonstrated markedly decreased stomatal conductance and transpiration between the 23rd and the 31st day. At the 31st day, the photosynthetic rate, water-use efficiency, and Rubisco carboxylation activity reached levels of almost zero, but when the plants were rehydrated, the metabolism recovered rapidly (Junglos et al., 2016).

Water deficit decreases the water potential in the leaves and all the traits of the photosynthetic metabolism in Campomanesia xanthocarpa Mart. ex O. Berg seedlings in 20 days of suspension of irrigation. These seedlings later recovered with the re-establishment of the water supply. However, the exposure of the seedlings to a second cycle of water deficit during the evaluation period demonstrated that the metabolism traits do not re-establish equilibrium (Bento et al., 2016).

In relation to PS II photochemical quantum efficiency, it was observed that water deficits did not cause any permanent damage to the photosynthetic apparatus. Subsequent means of 0.82 indicated recuperation of the photosynthetic rate and Fv/Fm values. Plants with intact photosynthetic apparatus will have maximum chlorophyll fluorescence values (Fv/Fm) between 0.75 and 0.85 (Bolhar-Nordenkampf et al., 1989), with any decrease in this ratio reflecting photo inhibitory damage to the PSI reaction centers (Björkman & Demming 1987; Thach et al., 2007).

The results obtained in this work indicate that the water stress conditions experienced by C. brasiliense seedlings were not severe enough to provoke photo-inhibition, and that the light collector complex of photosystem II was not damaged. These results are similar to those reported by Queiroz et al. (2002) in M. urundeuva Allemão seedlings, as the control seedlings had average values of 0.804 while seedlings after 14 days without irrigation demonstrated values of 0.798.

The maintenance of high chlorophyll levels in seedlings cultivated in water deficit regimes can be attributed to the fact that less hydration resulted in apparent higher concentrations of these pigments in the cells. Although the chlorophyll index increased under water deficit conditions, a yellowing of the leaves was observed, indicating chlorosis. Water deficits represent one of the environmental stress factors responsible for pigment losses in leaves, due to ABA-induced relocation of metabolites to other parts. This relocation within the plant causes alterations in photosynthetic rates and alterations of the growth cycle. Coscolin et al. (2011) noted in their review that reductions in chlorophyll and protein levels can be characteristic symptoms of oxidative stress in plants exposed to low soil water availability, representing the beginning of leaf senescence.

The data obtained in this study indicate that C. brasiliense seedlings exposed to water stress conditions show reduced photosynthetic activities (reaching near-zero values 12 days after suspending irrigation). This reduction occurred because of limitations in the stomatal conductance and low rubisco carboxylation efficiency rather than irreversible damage to photosystem II. All of these parameters initiated their recuperation after rehydration, but only the photosynthetic rate fully attained initial values (11 days after rehydration).

These results suggest that C. brasiliense seedlings can be cultivated with periods of water restriction in a greenhouse or in areas of reforestation, where the periods of water deficit do not exceed 12 days. Further studies should be carried out under field conditions to assess more accurately how these plants behave when planted directly in the soil in full sun.
5. CONCLUSIONS

Exposure to water deficit conditions reduced the values all of the parameters examined in *C. brasiliense* with the exception of the chlorophyll index. The stressed seedlings required only 12 days to approach zero for their photosynthetic rate, but they were able to reestablish photosynthetic equilibrium 11 days after rehydration. The water deficit did not cause any deleterious effects on the photosynthetic apparatus of the studied seedlings.

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