Full Length Research Paper

Potential for recovery of *Campomanesia xanthocarpa* Mart. ex O. berg seedlings from water deficit

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The objective of this study is to evaluate the effect of water deficit and the recovery potential following rehydration, on the metabolism of *Campomanesia xanthocarpa* seedlings. The seedlings were distributed in two groups: the first group was the control, in which, plants were hydrated periodically in order to maintain 70% water holding capacity and the second was the treatment group characterized by stress, in which irrigation was suspended until the photosynthetic rate showed levels close to zero, at which point the plants were once again rehydrated with subsequent daily irrigation for one week, maintaining the water holding capacity at 70%. *C. xanthocarpa* shows a reduction in stomatal conductance and photosystem PSII efficiency. Water deficit decreases the water potential in the leaves and all the traits of the photosynthetic metabolism in *C. xanthocarpa* seedlings in twenty days of suspension of irrigation. These are later recovered with the re-establishment of the water supply. However, 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.

Key words: Cerrado, guabiroba, photosynthesis.

INTRODUCTON

Plants are frequently exposed to multiple stress conditions, which limit their growth and development. Among the environmental factors, water deficiency is one of the stress factors that cause more damage to the physiological and metabolic processes of plants (Larcher, 2006; Taiz and Zeiger, 2013), thus determining their distribution (Sakamoto and Murata, 2002).

Several physiological and biochemical responses are observed as a result of water deficit. CO_2 assimilation by the leaves is reduced mainly because of stomatal

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closure, damage to the membranes, and impaired enzymatic activity, especially that of enzymes involved in CO_2 fixation and ATP synthesis. Meanwhile, it has also been reported that under severe water stress, in addition to stomatal restrictions to CO_2 uptake, limitations can occur in non-stomatal components, such as reduction in RuBisCO activity, CO_2 availability in chloroplasts, and damage to the reaction centers of photosystem II, however, the extent of the effects of water deficiency will depend, among other factors, on its intensity and duration and on their recovery potential, as it requires adaptive changes and/or deleterious effects (Flexas et al., 2006; Xu et al., 2009).

Brazil has a rich and diversified flora regarding fruit species especially those producing edible and commercially useful fruits. *Campomanesia xanthocarpa* Mart. ex O. Berg, commonly known as 'gabiroba', which belongs to the family Myrtaceae, is native to Brazil and found in almost all forest formations from the state of Minas Gerais to the southernmost edge of Rio Grande do Sul (Lorenzi, 1992). The fruit presents an abundant and succulent pulp, and is appreciated regionally. It is used in the production of refreshments, ice cream, liqueurs, and homemade sweets, owing to the presence of significant levels of pectic substances. Gabiroba fruits have good nutritional value owing to the high level of vitamin C, minerals, and phenolic compounds, which allows them to be considered a functional food (Santos et al., 2009). The species is also important for the production of coal and good-quality firewood.

Few studies have focused on the effect of water deficit on the physiological processes in native plants. This is an important area for research, since this abiotic stress has effects on various plant processes, many of which reflect mechanisms of adaptation to different habitats. Such knowledge of *C. xanthocarpa* metabolism will allow the production of quality seedlings. This will be useful since there is a need to replant owing to high mortality rates when the plants are exposed to environmental stresses to which they are not adapted (Carvalho et al., 2003), in addition to permitting sustainable exploration, and the possibility of regeneration of degraded areas.

Considering the natural habitat of *C. xanthocarpa*, it is believed that their occurrence is related to increased photosynthetic efficiency and the plant's capacity for recuperation, following periods of water stress sustained in their natural environment. In light of this, the present work aimed to evaluate the effect of water deficit and the recovery potential following rehydration, on the metabolism of *C. xanthocarpa* seedlings.

MATERIALS AND METHODS

C. xanthocarpa fruits were collected at the beginning of the month of December 2013, using matrices distributed in the Cerrado areas, located near the municipality of Dourados/Mato Grosso do Sul State. After collection, the fruits were manually processed and the seeds extracted and selected according to their integrity and uniformity. The selected seeds were washed in running water to eliminate pulp remains and then dried using Germitest® paper tissues.

In order to obtain the seedlings, the seeds were sown in tubes of 50 × 190 mm at a depth of one centimeter, which contained distroferric red latosol, sand, and Bioplant® commercial substrate, at the proportion of 1:1:1. The seedlings, measuring about 15 cm, were transplanted into 5 kg capacity pots in the same soil, where they were acclimatized for 30 days, with irrigation at 70% water holding capacity and kept in a 40% shade (Sombrite®) greenhouse. During the course of the experiment, the plants were protected from pluviometric precipitation by a plastic cover.

The pots were distributed in two groups. The first group was the control, in which, plants were hydrated periodically in order to maintain 70% water holding capacity. The second was the treatment group characterized by stress, in which irrigation was suspended until the photosynthetic rate showed levels close to zero, at which point the plants were once again rehydrated with subsequent daily irrigation for one week, maintaining the water holding capacity at 70%. This treatment was applied twice. The seedlings were evaluated up to 172 days since the start of the treatments.

To evaluate the effect of intermittent water deficit, the plants were monitored every two days, until the photosynthetic rate was at levels close to zero, and following rehydration with daily hydration for one week, at which point the following characteristics were evaluated:

1. Chlorophyll index: Obtained with the help of a chlorophyll meter (Konica Minolta, SPAD 502).
2. Gas exchanges: The photosynthetic rate - A (μmol m^{-2} s^{-1}), foliar transpiration - E (mmol m^{-2} s^{-1}), stomatal conductance 3. Gs (mol m^{-2} s^{-1}) and internal CO_2 concentration - Ci (μmol mol^{-1}) were measured using an infrared gas analyzer (IRGA) (Model LCi PRO; Analytical Development Co. Ltd., Hoddesdon, UK). The measurements were performed on three seedlings per treatment in the morning, between 08:00 h and 11:00 h, and were recorded from two fully extended previously marked leaves. All measurements were taken from these marked leaves. Only the data measured under a photosynthetic photon flux (PPF) above 700 mmol m^{-2} s^{-1} were considered. From the gas exchange data, the following ratios were calculated: instantaneous water-use efficiency (WUE μmol CO_2/ mmol^{-1} H_2O), intrinsic water-use efficiency (IWUE μmol CO_2/ mmol^{-1} H_2O), and instantaneous carboxylation efficiency (A/Ci μmol m^{-2} s^{-1}/ μmol mol^{-1}).
4. Chlorophyll a fluorescence: A portable fluorometer (model OS-30p) (Opti-Sciences Chlorophyll Fluorometer, Hudson, USA), was used to measure the initial fluorescence (F_0), the maximum fluorescence (F_m), and the potential quantum efficiency of photosystem II (F_v/F_m). From these fluorescence data, the following parameters were calculated: variable fluorescence ($F_v = F_m - F_0$) and effective absorbed energy conversion efficiency of photosystem II (F_v/F_0). Fluorescence determinations were performed between 08:00 h and 11:00 h, on the same leaves used to evaluate gas exchanges, and they were submitted to a 30-minute dark adaptation period using leaf-clip holders, so that all the reaction centers in that foliar region acquired the 'open' configuration, indicating the complete oxidation of the photosynthetic electron transport system.
5. Leaf water potential ($\Psi\omega$): Obtained from readings performed on the second pair of fully extended leaves from the apex to the base, between 10:00 h and 11:00 h, using pressure chamber Scholander (Portable Plant water status console - modelo 3115) (Scholander et al., 1964), immediately after leaf collection. The values were obtained in bar and later converted to MPa.

For gas exchanges, the design was completely randomized with two water regimes (control - irrigated periodically and stress) and four repetitions, in which each one corresponded to one seedling.
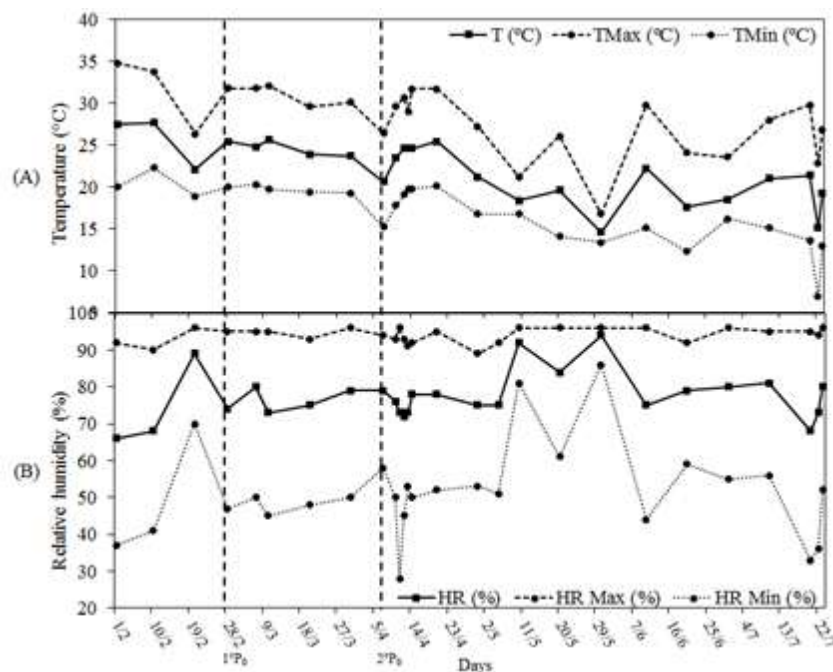
Figure 1. Average temperature data (°C) (A) and relative humidity (RH) (B) during the months of February to July 2015, in Dourados/Mato Grosso do Sul State, Brazil.

The results were submitted to analysis of variance and where there was a statistical significance, the means of each treatment were submitted to a *t* test at 5 % probability.

For the other reviews, the design was completely randomized design in factorial scheme 2 water regimes (control - irrigated periodically and stress) x 6 evaluation periods (Time zero - T_0, first and second photosynthesis - 1° P_0 and 2° P_0, first and second cycle of drought/rehydration - 1°D/R and 2°D/R, and 90 days after rehydration - 90 d/R) and four repetitions, in which each one corresponded to one seedling. The results were submitted to analysis of variance and statistical effect to water regimes; the averages were compared by *t* test and interaction between treatments by Scott Knott, both 5% probability.

During the experimental period, relative humidity (RH) and temperature (°C) data were recorded and collected from the database of the Embrapa Western Region Agriculture, located in Dourados/Mato Grosso do Sul State (Figure 1).

RESULTS

The water potential (Ψw) of *C. xanthocarpa* seedlings was influenced by the treatment (Figure 2). Seedlings subjected to water deficit showed significant reduction in Ψw in the periods referring to the first and second cycles of null photosynthesis (first and second P_0) with mean values of −2.6 and −2.3 MPa respectively. These were 26 times lower than those for control plants, which permitted us to infer that the leaf wilting was caused by a reduction in water availability. After 90 days of rehydration, the stressed seedlings did not differ from the controls.

During the experimental period, the control seedlings presented mean photosynthesis rates (A) of 6.81 µmol

m^{-2} s^{-1} (Figure 2B). The photosynthesis rates of seedlings under water deficit showed significant variation, with reductions from the 23rd day that intensified until the 28th day, averaging 0.79 µmol m^{-2} s^{-1} during the first cycle of drought/rehydration (D/R). This was on an average 0.60 µmol m^{-2} s^{-1} during the second cycle, from the 58th until the 64th day, when the rate reached values close to zero and irrigation was resumed.

After rehydration, the seedlings exposed to water deficit rapidly recovered their photosynthetic metabolism in such a way that the values reached control levels by the 38th day (8.42 µmol m^{-2} s^{-1}) necessitating only 7 days for the photosynthesis rate to recover. However, this recovery reached control seedling values only in the first D/R cycle, and in the remaining days, including the second D/R cycle, the rate remained lower until the end of the experimental period (Figure 3A).

In the first D/R cycle, the transpiration rate (E) values of the seedlings without irrigation remained close to the values of control seedlings until the 13th day (Figure 3B). From that day, significant reductions in the E rate of stressed seedlings were observed up to the 27th day, averaging 0.4 mmol m^{-2} s^{-1} when they were rehydrated. In the second D/R cycle however, these reductions were observed from the 35th to the 65th day, averaging 0.38 mmol m^{-2} s^{-1}. Similar to the photosynthesis rate, the transpiration rate recovered after irrigation was re-established, but the values remained lower to those observed for control seedlings, averaging 2.98 mmol m^{-2} s^{-1} during the whole evaluation period.
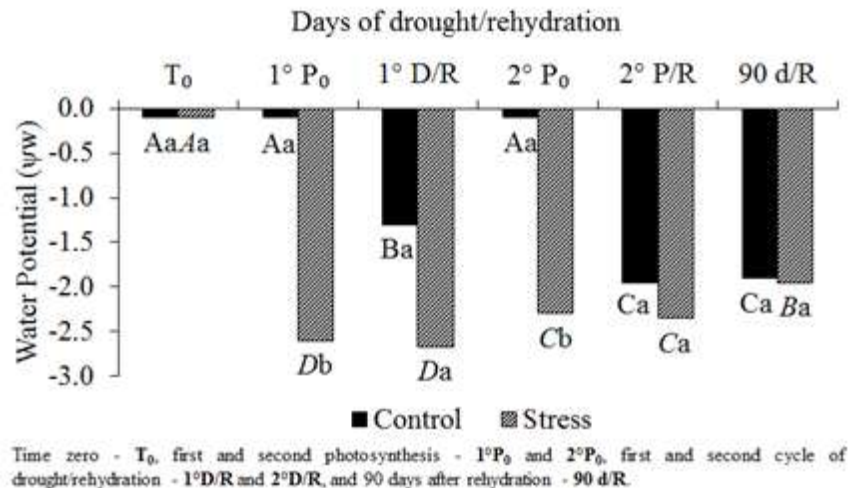
Figure 2. Water potential - Ψw of seedlings *Campomanesia xanthocarpa* in function water regimes. Small letters compare treatments (stressed and control) and capital letters compare control and capital letters in italics compare the stressed seedlings within the evaluation periods.

Regarding the water use efficiency (A/E) ratio, no significant differences were observed in seedlings from both treatments until the 23rd day of evaluation (Figure 3C). After the 35th day, fluctuations in the average A/E of the seedlings under water deficit were observed, indicating values lower than those observed in the controls until the end of the evaluation period, with the exception of day 65 (2.9 µmol CO_2/mmol H_2O) when a higher A/E was observed in stressed seedlings than in control seedlings.

The mean internal CO_2 concentration (Ci) fluctuated during the whole experiment (Figure 4A). The stressed seedlings showed a significant increase in concentration of this gas at the 9th day of suspension of irrigation (322 µmol mol^{-1}), a behavior that was also observed on the 38th and 65th days, averaging 326 µmol mol^{-1}, until the end of the experiment. It is worthwhile to note that the 28th and 65th days were when the seedlings under stress showed photosynthesis rates close to zero, in addition to showing the biggest reductions in transpiration and stomatal conductance.

The mean values of instantaneous carboxylation efficiency (A/Ci) were similar in both treatments until the 23rd day (Figure 4B). However, the plants under water stress showed a marked decline until the 28th day (0.014 µmol m^{-2} s^{-1}/µmol mol^{-1}) during the first DR cycle, and in the second cycle, this reduction was most pronounced on the 65th day (0.001 µmol m^{-2} s^{-1}/µmol mol^{-1}) regardless of the re-establishment of irrigation, and this condition was maintained until the end of the experiment.

The stressed seedlings showed significant reductions in stomatal conductance (Gs) from the 23rd to the 27th day in the first D/R cycle, when rehydration then occurred and a rapid recovery was observed (Figure 4C).

However, in the second cycle this reduction was noticeable from the 38th until the 65th day. The observed behavior denotes slow recovery following rehydration and, in both cycles, the levels detected remained below control levels until the 137th day of evaluation, suggesting that the stomata of this species need time to recover their hydration and/or the leaves their water potential.

With respect to the intrinsic water-use efficiency (A/Gs), the means observed showed oscillations during the whole evaluation period, but a trend was observed in which the values were higher in stressed seedling, except on days 9 and 38 (Figure 4D), when they were significantly reduced, averaging 16.03 and 26.95 µmol CO_2/mol H_2O, respectively.

With respect to the chlorophyll index (SPAD), a significant interaction was observed between the irrigation strategies and the evaluation periods (Figure 5A). The seedlings under water deficit showed reductions in chlorophyll index in the periods referring to the first D/R cycle, second P_0 cycle, and second D/R cycle. After 90 days of rehydration, no difference was observed in the stressed seedlings compared to the control.

Generally, chlorophyll a fluorescence parameters were influenced by the interaction between the treatments (Figure 5), with the exception of the initial fluorescence (F_0), which did not significantly differ between the periods evaluated, with an average of 0.274 electrons quantum^{-1}, but was higher in the seedlings under stress when compared to control seedlings (Figure 5B).

Maximum fluorescence (F_m) and variable fluorescence (F_v) were negatively influenced by water deficit (Figures 5C and D), which led to significant reductions during the first P_0 and second P_0, with the Fm averaging 0.597 and
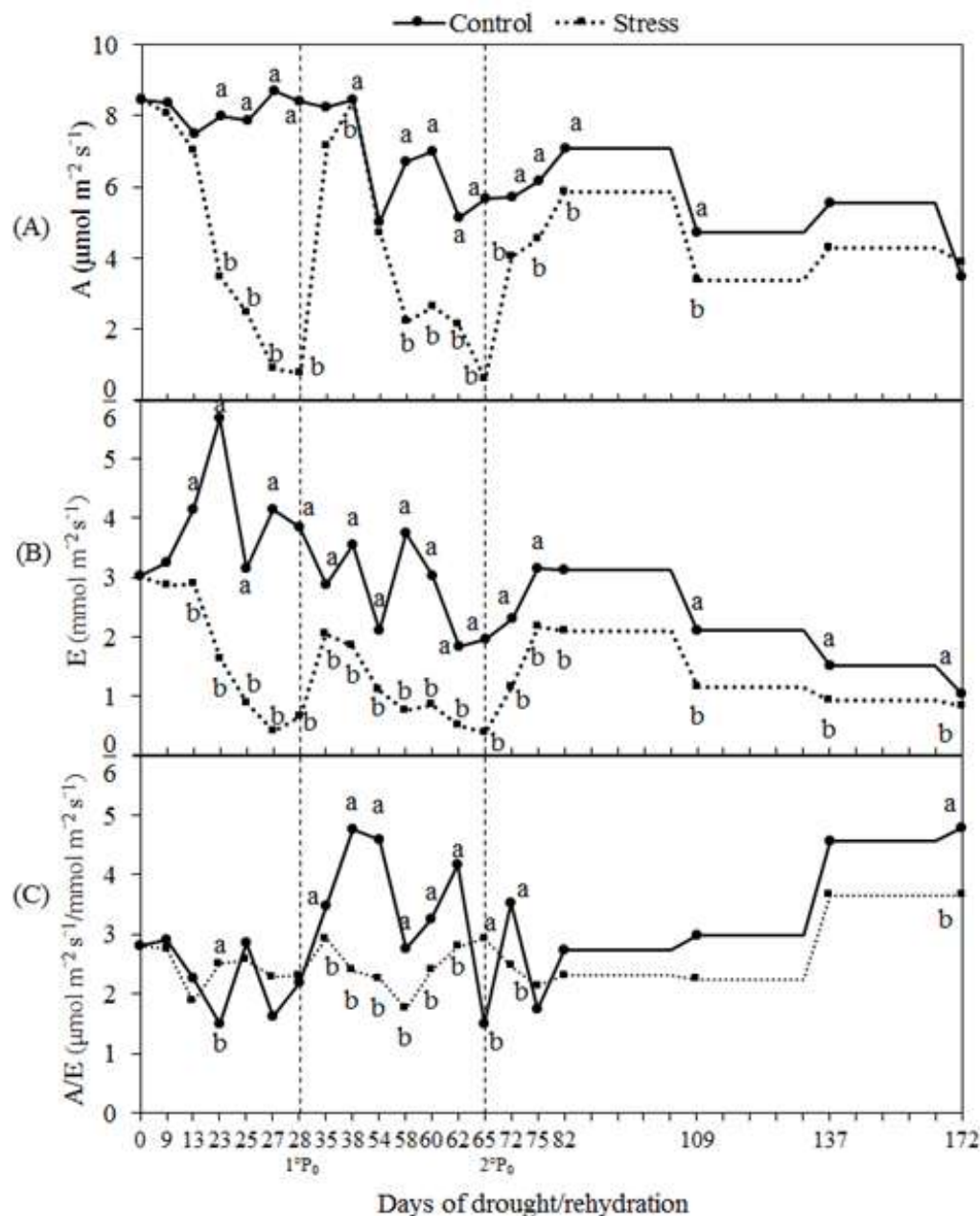
Figure 3. Photosynthetic rate - A (A), transpiration rate - E (B), water use efficiency - A/E (C) depending on the evaluation days between irrigated seedlings *Campomanesia xanthocarpa* and subject to conditions water stress.

0.590 electrons quantum^{-1} and the F_v 0.276 and 0.287 electrons quantum^{-1}. After 90 days rehydration, stressed seedlings differed from the control, showing an increase in F_m and F_v (Figures 5C and D).

The quantum efficiency of photosystem II (F_v/F_m) was reduced as a consequence of the stress caused by water deficiency. The lowest values (0.431 and 0.435 electrons quantum^{-1}) occurred in the periods during which the seedlings reached null photosynthesis (Figure 5E),

however in the second rehydration (R) the seedlings previously under stress showed significant recovery for this trait (0.431 and 0.435 electrons quantum^{-1}), which was maintained until the end of the evaluation.

The same response behavior was observed for the effective absorbed energy conversion efficiency of the photosystem II (F_v/F_0) as for F_v/F_m (Figure 5E), in which this variable recovered in the second R (3993.16 electrons quantum^{-1}) (Figure 5F).
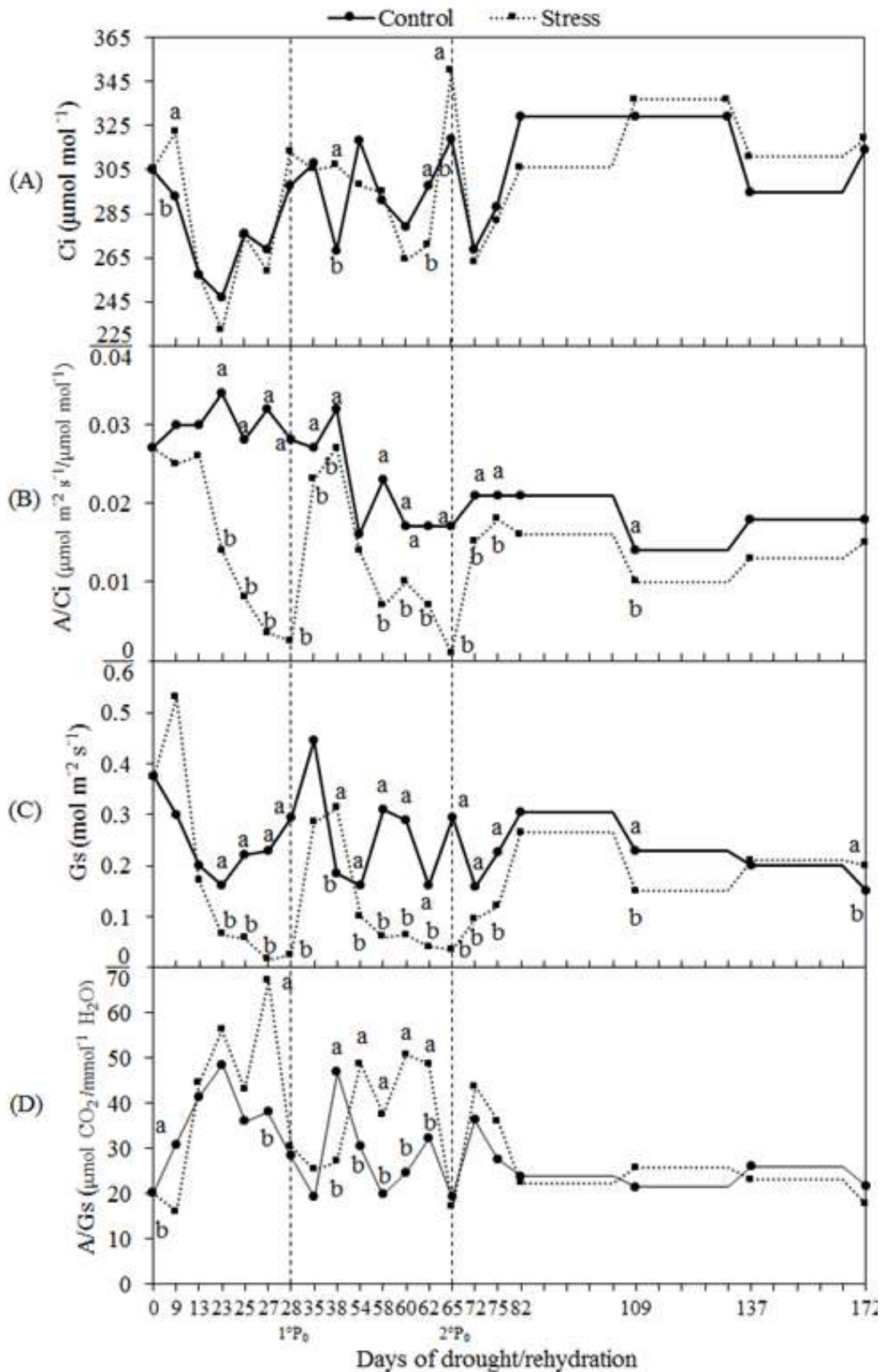
Figure 4. Mean values of internal CO_2 concentration - Ci (A), instantaneous carboxylation efficiency of CO_2 -A/Ci (B), stomatal conductance - Gs (C) and intrinsic efficiency of water use - A/Gs (D) in the light of day evaluation of seedlings irrigated *Campomanesia xanthocarpa* and under water stress conditions
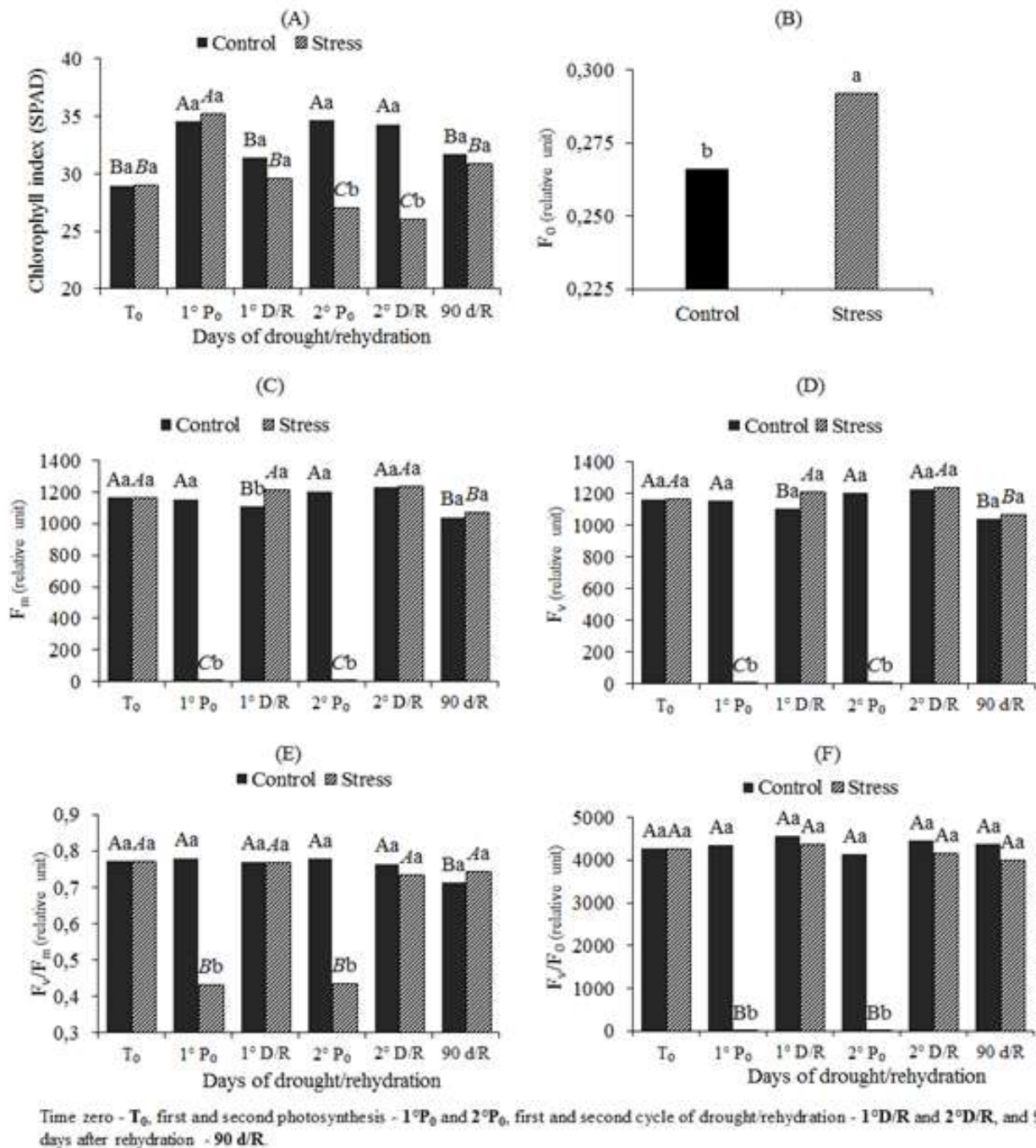
Figure 5. Chlorophyll index (A), F_0 - minimum fluorescence (B); F_m - maximum fluorescence (C); F_v - variable fluorescence (D); F_v/F_m - quantum efficiency of photosystem II (E) and F_v/F_0 - efficiency of photosystem effective the absorbed energy conversion (F) of seedlings *Campomanesia xanthocarpa* in function water regimes. Small letters compare treatments (stressed and control) and capital letters compare control and capital letters in italics compare the stressed seedlings within the evaluation periods.

DISCUSSION

C. xanthocarpa seedlings under water restriction showed a reduction in water potential (Ψw), reaching a mean value of −2.4 MPa when the photosynthesis rate was lowered to values close to zero, along with the loss of turgidity in the leaves (Figure 2). These Ψw values are considered critical for ligneous plants of the Cerrado

(Franco et al., 2005), and are sufficient to promote alterations in content and in the free energy of the water in the soil and in the plants. This reduction subsequently affects physiological processes, initially interrupting cell expansion, introducing stomatal closure, lowering photosynthesis, in addition to interfering in various other basic metabolic processes such as synthesis and degradation of carbohydrates and proteins, and the accumulation of solutes, which will have an impact on plant growth and productivity (Kumar and Sing, 1998).

Following rehydration, the Ψw of the seedlings previously kept under water restriction, recovered and reached values close to the control. However, the values observed both for the irrigated seedlings as well as for those maintained under water restriction remained above levels considered critical (−1.5 MPa) until the end of the experiment, which could have affected photosynthesis in field conditions (Da Matta et al., 2007). This fact is probably related to the lower relative air humidity (Figure 1) observed in the days around the evaluation periods, promoting water loss by the leaves. Environmental factors are known to not only act directly on water loss, but they can also act indirectly by controlling stomatal behavior, as is the case of air humidity (Seixas, 2009).

The initial decrease in photosynthesis, accompanied by stomatal conductance (Gs) and increase in internal CO_2 concentration (Ci) after the suspension of irrigation on day 23, suggests that stomatal restriction is initially responsible for the reduction in CO_2 uptake in the seedlings during the first days of water deficiency (Figure 3 and 4). This occurs because of the partial closure of the stomata, controlled either by dehydration of the guard cells or by hormonal response, which restricts water loss in the leaves due to transpiration. Our experiment showed that transpiration had already reduced significantly by the 13th day. At the same time, the partial closure of the stomata leads to a drop in CO_2 entry and assimilation, thus compromising the photosynthetic process (Magalhães et al., 2008; Araújo and Deminicis, 2009).

However, even under low Gs values, it was observed that during the longest stress period, when the photosynthesis rate was near zero, the stressed seedlings presented levels of internal CO_2 concentration similar to those of irrigated seedlings in the first D/R cycle, and an increase in the concentration of this gas in the second cycle (Figure 3). This behavior indicated that other factors influence CO_2 uptake. Further, it suggests the presence of chemical signals in the plants, such as abscisic acid, which control the stomatal opening during drought periods (Hirayama and Shinozaki, 2010; Oliveira et al., 2011). It constitutes a strategy used by the plants to reduce excessive water loss due to transpiration (Albuquerque et al., 2013) and therefore avoiding tissue dehydration, in addition to permitting the maintenance of the integrity of the water transport system and the development of water potential, when the soil is

undergoing progressive drought (Magalhães et al., 2008).

Similarly, the reduction in transpiration rate (E), an anticipated effect to the low Gs and the action of intrinsic factors (abscisic acid) was also demonstrated through the instantaneous water-use efficiency, which remained similar to the control during the first days of suspension of irrigation, until the 23rd day (Figura 3A, B). This occurs because instantaneous water-use efficiency (A/E) can increase when water deficiency is first established, since the partial closure of the stomata has a stronger effect on the exit of water molecules than on the quantity of CO_2 fixed (Pompelli et al., 2010; Silva et al., 2010).

It has been proposed that both stomatal (resistance to CO_2 entry) and non-stomatal factors (low enzymatic activity for CO_2 assimilation) may be the main causes for the reduction in carbon assimilation, reflecting changes in stomatal conductance patterns and internal carbon (Farquhar and Sharkey, 1982; Mielke et al., 2003; Herrera et al., 2008). Additionally, non-stomatal limitations gradually progress with the intensity and duration of the water stress, such as the decrease in RuBisCo activity, CO_2 availability in the chloroplast and photochemical efficiency of PSII (Flexas et al., 2006; Xu et al., 2009).

As such, the drop in photosynthesis in *C. xanthocarpa* seedlings can be attributed to the initial reduction in stomatal conductance associated with the reduction in CO_2 carboxylation efficiency (A/Ci) by RuBisCo (Figure 4B) as the duration and intensity of the stress increased. Values below 0.05 mol m^{-2} s^{-1}, observed in this study, are indicative of severe water deficit and are accompanied by non-stomatal limitations (Medrano et al., 2002; Sircelj et al., 2007) such as damage to the photochemical apparatus, through thylakoid membrane de-structing (Dias and Bruggermann, 2010), and reduction in the activity of enzymes involved in photosynthetic reactions, such as RuBisCo, leading to the interruption of photosynthetic processes (Tang et al., 2002; Ghannoum et al., 2003; Liu et al., 2014).

The capacity of the plants to recover their photosynthetic rate (A) following rehydration is of fundamental importance. This, along with their capacity to avoid and/or withstand water stress represent the resistance of the plants to drought, in addition to its ability to prevent decrease in productivity of plant cultivations (Chaves et al., 2009; Pinheiro and Chaves, 2011).

Following rehydration, total recovery of carbon assimilation by the seedlings was observed in the first D/R cycle; however, this recovery was partial in the second cycle, with 70% recovery in relation to the seedlings maintained under irrigation (Figure 3B). Usually, plants submitted to water stress present a maximum photosynthetic rate recovery of 40–60% after rehydration, whereby the recovery continues in the following days, yet the maximum photosynthesis rate is not always reached (Flexas et al., 2004; Sofo et al., 2004; Souza et al., 2004). This occurs because the carbon gain

obtained during the period of water stress and rehydration may depend on both the speed and degree of photosynthetic recovery, as well as on the degree and speed of the decline in photosynthesis during low water availability (Flexas et al., 2006; Xu et al., 2009).

In the case of the *C. xanthocarpa* seedlings, a total recovery of A in seven days is considered to be fast. This was similar to observations recorded for *Hancornia speciosa* Gomes (Scalon et al., 2015), *Myracrodruon urundeuva* Allemão (Costa et al., 2015), *Khaya ivorensis* A. Chev (Albuquerque et al., 2013), *Eucalyptus* and *Acacia* spp. (Warren et al., 2011), *Tabebuia aurea* S. Manso (Oliveria et al., 2011), and *Carapa guianensis* Fusée-Aublet (Gonçalves et al., 2009) seedlings. For other species, the recovery of photosynthetic traits occurred only after fourteen days after rehydration (Calbo and Moraes, 2000).

As the stress prolonged, *C. xanthocarpa* seedlings showed a marked decrease in Gs (from the 23rd day of suspension of irrigation) (Figure 4C), leading to reduction in A due to the low intracellular CO_2 concentrations (Ci) and the inhibition of foliar metabolism (non-stomatal factors) (Ben et al., 2009; Ashraf, 2010). Such reductions in A were reflected in lower A/E for the stressed seedlings, in which they remained at levels below those of the control, even after rehydration, throughout the experimental period.

In some species, the maintenance of lower stomatal conductance following rehydration imposes a substantial limitation for photosynthesis (Gallé and Feller, 2007; Galmés et al., 2007) and transpiration recovery. In the present work, it was possible to observe such behavior, since even after seven days of rehydration the recovery of stomatal conductance was partial and lower in both D/R cycles when compared to the irrigated seedlings, reaching a maximum of 63% (Figure 3C).

Similar results to those obtained for *C. xanthocarpa* were observed in other species. In *H. speciosa*, stomatal conductance recovery (63%) occurred six days after rehydration, yet at lower levels when compared to irrigated plants, suggesting that the stomata in those species are slow in recuperating hydration (Scalon et al., 2015). Likewise, 60% recovery of stomatal conductance was observed in *Populus euphratica* after six days of rehydration (Bogeat-Triboulot et al., 2007), while in *Bactris gasipaes* Kunth seedlings, 50% recovery of stomatal conductance occurred three days after water was added back to the soil (Oliveira et al., 2002).

In the case of *C. xanthocarpa* seedlings, considering that the recovery of stomatal conductance was partial and lower when compared to the control, it is noteworthy that, such recovery was much slower in the second D/R cycle, suggesting that prolonged stress on the seedlings may have created water tension in the xylem, which affected the water transport system. According to Manzoni et al. (2012), both stomatal as well as xylem conductance are reduced when water is limiting, in comparison to well-hydrated plants. This is because, the regulation of foliar water conductance depends on cavitations and on the recovery of xylem vessels, and these vessels are more sensitive in leaf veins (Cochard et al., 2002). Under water stress, chlorophyll levels in the leaves may be reduced, which will affect photosynthesis (Din et al., 2011; Asharaf and Harris, 2013).

In the present study, maintenance of chlorophyll levels in the seedlings grown under suspension of irrigation during one of the periods of higher stress (1° F_0) may have been due to the lower hydration state of the cells in the leaves. This could have caused the pigment to concentrate and contribute to a higher chlorophyll quantification, thus masking the effect of the stress, while explaining the observed reduction in photosynthetic rate in the same period.

However, in the first D/R, the stressed seedlings showed reduction in chlorophyll levels, which was also observed in the second P_0 and second D/R, indicating that the water deficit led to a reduction in the efficiency of the photosynthetic apparatus, which was maintained even after irrigation was re-established. These data usually result from the degradation of chlorophyll molecules or from impaired chlorophyll synthesis (Dalmolin, 2013). The reduction in chlorophyll index in *C. xanthocarpa* seedlings seems to have occurred in a more pronounced way as the result of reduced pigment synthesis, since yellowing of the leaves, a typical indication of chlorosis resulting from chlorophyll degradation, was not observed. Meanwhile, in these seedlings, the chlorophyll levels were recovered 90 days after rehydration.

The increase in initial photosynthesis (F_0) in the seedlings cultivated under suspension of irrigation indicates that the functionality of the photosynthetic apparatus is compromised, as a consequence of damage to the reaction center of photosystem II (PSII) or the reduction of the capacity of excitation energy transfer from the antenna to the reaction center (Maxwell and Johnson, 2000; Baker, 2008). With the decrease in chlorophyll content in the leaves, it is believed that a lower amount of energy was absorbed by the antenna complex, meaning it was not transmitted, causing increased alterations in the photosynthetic capacity because of the stress caused by water deficiency.

In addition, the photochemical quantum efficiency of PSII (F_v/F_m) also decreased as a function of the water deficit, averaging 0.433 electrons quantum^{-1}, suggesting the occurrence of photo-inhibitory damage to the reaction centers of PSII (Figure 5). When intact, the observed values of F_v/F_m remained between 0.75 and 0.85 electrons quantum^{-1} (Baker and Rosenqvst, 2004). Under severe water stress, plants frequently present a marked photo-inhibitory effect characterized by a significant decrease in quantum yield (Araújo and Deminicis, 2009). The damage to the photosynthetic apparatus occurs due to photo-oxidation of D1 proteins in the PSII, which are

localized in the thylakoid membranes and show high sensitivity to oxygen reactive species, produced during the photochemical stage of photosynthesis (Biswall et al., 2011) which increase due to water deficit. This is proved by the reduction in Fm and the reduction in electron flux between the photosystems (Tatagiba et al., 2014) and confirmed by the decrease in Fv during the periods of increased water stress ($1° P_0$ and $2° P_0$).

Similarly, the F_v/F_0 ratio was reduced, with the mean value observed (0.901 electrons quantum^{-1}) being much lower than 4 to 6 electrons quantum^{-1}, the levels at which the functionality of the reaction centers of PSII are preserved when facing water stress (Rohácek, 2002). As such, the values verified for the ratio F_v/F_0 confirm the damage to the photosynthetic apparatus, since this ratio has been widely used to amplify small variations detected in F_v/F_m (Reis and Campostrini, 2011), being an indicator more sensitive to perturbations in PSII functionality.

At the end of the 90 days after re-establishment of irrigation, the plants previously submitted to stress presented normal PSII functionality, evidenced by the recovery in F_v/F_m and F_v/F_0 ratios, demonstrating that the damage caused by water stress was reversible. However, the time spent in this recovery indicates that the type of photo-inhibition developed by the stressed seedlings was chronic, in which the lower water content in the leaves, damages the photosynthetic system by intensifying the damage from light exposure. This in turn reduces the quantum efficiency and the maximum photosynthetic rate, exhibiting effects with relatively long duration, persisting for weeks or months (Taiz and Zeiger, 2013).

Conclusion

C. xanthocarpa shows a reduction in stomatal conductance as the first line of defense against water deficit; however, as the intensity and duration of the stress increase, non-stomatal factors are observed, presenting a reduction in photosystem PSII efficiency, which is repaired after irrigation is resumed. Water deficit decreases the water potential in the leaves and all the traits of the photosynthetic metabolism in *C. xanthocarpa* seedlings in twenty days of suspension of irrigation. These are later recovered with the re-establishment of the water supply.

However, 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.

Conflict of Interests

The authors have not declared any conflict of interests.

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