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HYDROGEL AND WATER REGIMES IN THE CHLOROPHYLL-*A* FLUORESCENCE AND GROWTH OF *Campomanesia xanthocarpa* SEEDLINGS

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KEYWORDS

Dickson quality, physiological indexes, photosystem II, water-retaining polymer, water retention capacity.

ABSTRACT

Water availability is one of the most important factors for the growth of tree seedlings in forestry-related regions. We hypothesized that under different water regimes, a water-retaining polymer (hydrogel) can positively contribute to chlorophyll-*a* fluorescence and growth in *Campomanesia xanthocarpa* (Mart.) O. Berg. Four water retention capacities (WRC) were evaluated: 25%, 50%, 75%, and 100%, depending on the presence or absence of hydrogel at the substrate. The lowest WRCs, particularly those under 25% without hydrogel, reduced chlorophyll index and negatively affected the photochemical activities of photosystem II. However, under low water availability the hydrogel mitigated the damage inflicted on the reaction centers and chlorophyll synthesis. The greatest growth effects occurred at 100% WRC in the presence of the hydrogel. Physiological indices were higher under 100% WRC without hydrogel and 50% with hydrogel. The increase in biomass and Dickson quality were more pronounced in the seedlings produced under 50% WRC and hydrogel, and the addition of these parameters to the substrate contributed to more viable morphophysiological indicators for the production of *C. xanthocarpa* seedlings.

INTRODUCTION

In areas of forest recomposition/degradation or regions involved in the formation of integrated sustainable base production systems, water availability in the soil is a determining factor for the growth and initial establishment of tree species seedlings. The difficulty in implementation of irrigation systems and/or the irregularity of precipitation in these areas at certain times of the year are the main limiting factors for water accessibility (Mátyás & Sun, 2014).

With the reduction of water availability in the soil, the growth characteristics of seedlings are reduced due to instability of the leaf metabolism and damage to the reaction centers of photosystem II (PS II) (Khatri & Rathore, 2019; Reis et al., 2020), impeding the initial formation. Environmental stress mitigation agents are required in these situations in order to maintain the quality of tree seedlings.

Water-retaining polymers (WRP), known as hydrogels, have the capacity to absorb water and retain it for use during dry periods (Kalhapure et al., 2016; Silva et al., 2019). The composition base of the hydrogel contains polyacrylamide, which when incorporated into the soil, promotes aeration and drainage improvements and reduces nutrient leaching (Azevedo et al., 2002; Freitas et al., 2019), contributing to root and other vegetative organ growth through the stability of metabolic processes and the prevention of plant dehydration.

Therefore, the use of hydrogels may be an alternative for seedlings planted in recovery areas of previously degraded forests, aiding in the success of silvicultural activities (Fonseca et al., 2017). Seedlings of some tree species have shown strong growth responses with hydrogel addition to the soil, including *Handroanthus ochraceus* (Cham.) Mattos (Mews et al., 2015), *Mimosa scabrella* Benth. (Konzen et al., 2017), *Eucalyptus urophylla* × *Eucalyptus grandis* (Teixeira et al., 2019), and *Enterolobium contortisiliquum* (Vell.) Morong. (Turchetto et al., 2020).

Among the species with the potential to recover degraded areas in the Cerrado, Brazil, or to be included in agroforestry systems, *Campomanesia xanthocarpa* (Mart.) O. Berg. (Myrtaceae) is a native, fruitful tree species,

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popularly known as “guabiroba,” “guavirova,” or “gabiroba,” with fruits that can be consumed *in natura* and are attractive to avifauna. This species is located in different forest configurations, and develops preferentially in moist soils from alluvial formations, capons, and more open areas of secondary forest, as well as in regions of the Cerrado (Gogosz et al., 2010; Teleginski et al., 2018).

Considering that *C. xanthocarpa* generally grows in areas with moist and well-drained soils, we hypothesized that, since the photochemical metabolism and growth of seedlings are reduced under low water availability, the incorporation of WRP into the substrate may mitigate the damage to PS II and other morphometric aspects. Thus, the aim of this study was to evaluate the effect of water-retaining polymers on chlorophyll-*a* fluorescence and the quality of *C. xanthocarpa* seedlings subjected to different water regimes.

MATERIAL AND METHODS

The experiment was conducted at the Faculty of Agricultural Sciences (22°11′43.7″ S and 54°56′08.5″ W, 452 m), at the Federal University of Grande Dourados (UFGD), Dourados, Mato Grosso do Sul (MS), Brazil, using the species *C. xanthocarpa*, whose exsiccate is deposited at the Herbarium of UFGD (DDMS) under No. 4644. Ripe fruits of this species were collected from the matrices of natural populations (Access Register No. A9CDAAE-CGEN-MMA) in the Itamarati Settlement, city of Ponta Porã (23º32'30″ S and 55º37′30″ W), MS, Brazil.

The fruits were manually pulped, and the seeds were immersed in sodium hypochlorite solution (2%) for 5 min. Sowing was performed in 72 cell polystyrene trays filled with Tropstrato®. When the seedlings reached 3.0 cm in height, 60 days after sowing, they were moved to 290 cm^3 polyethylene tubes, and remained in the nursery with 50% shading and daily irrigation.

When the seedlings grew to an average height of 6.0 cm, they were transferred to plastic pots filled with 1.5 kg of Dystrophic Red Latosol + sand (3:1, v/v), with water and hydrogel in the corresponding portions. The seedlings were placed in a nursery with 30% shading, with additional protection from rainfall by 150 µm thick top and side plastic covers. The soil presented the following chemical attributes: pH $CaCl_2$= 5.67; P= 27.92 mg dm^{-3}; K= 0.63 $cmol_c$ dm^{-3}; Ca= 8.55 $cmol_c$ dm^{-3}; Mg= 2.04 $cmol_c$ dm^{-3}; Al= 0.00 $cmol_c$ dm^{-3}; H+Al= 2.37 $cmol_c$ dm^{-3}; sum of bases= 11.22 $cmol_c$ dm^{-3}; cationic exchange capacity= 13.59 $cmol_c$ dm^{-3}; S= 4.22 mg dm^{-3}; B= 0.48 mg dm^{-3}; Fe= 52.13 mg dm^{-3}; Cu= 3.90 mg dm^{-3}; Mn= 78.60 mg dm^{-3}; Zn= 1.75 mg dm^{-3}; organic matter= 20.82 g dm^{-3}; and base saturation (V%)= 82.6.

The seedlings were grown according to four water regimes, based on the water retention capacity (WRC) of the substrates: 25%, 50%, 75%, and 100%, and whether water-retaining polymers (WRP) were added to the substrate. The treatments were arranged in a 4 × 2 factorial scheme, in a randomized block design with four replications, with each experimental unit consisting of four pots containing one plant each.

The water retention capacity was determined on alternate days using the gravimetric method (Souza et al., 2000). Forth Gel® was the hydrogel used as the water-retaining polymer, consisting of polyacrylic potassium polyacrylamide (soil conditioner – class E; CEC= 53.22 $cmol_c$ dm^3) at a dose of 4 g L^{-1} substrate, added in an incorporated granule form and homogenized to the substrate. Three hours before transplanting the seedlings the substrate was moistened.

Sixty days after transplanting, the seedlings were evaluated for chlorophyll-*a* fluorescence and growth characteristics as follows:

a) Chlorophyll index (SPAD): obtained using a portable chlorophyll meter SPAD 502 (Soil Plant Analyzer Development), with the evaluation carried out between 8:00 to 10:00 a.m.;

b) Chlorophyll-*a* fluorescence: fully expanded leaves were subjected to dark conditions using leaf clips for 30 min, and subsequently, using a flash of 1,500 µmol m^{-2} s^{-1}, with a portable fluorometer (OS-30p; Opti-Sciences Chlorophyll Fluorometer, Hudson, NY, USA), the emission of the initial (F_0) and maximum (F_m) chlorophyll-*a* fluorescence and the potential photochemical efficiency of photosystem II (F_v/F_m) were evaluated. The variable fluorescence ($F_v = F_m - F_0$), efficiency of conversion of absorbed energy (F_v/F_0), and the maximum basal yield of non-photochemical processes (F_0/F_m) were determined, and the results were expressed as electrons per quantum;

c) Growth: the height of plants was determined using a ruler graduated in millimeters, considering the evaluation standard, distance between the collar to the inflection of the highest leaf (cm), stem diameter (mm, with digital calipers 1.0 above the substrate level), and the number of leaves (NL). The seedlings were then harvested, separated into leaves, stems, and roots, and the leaf area (LA, cm^2) was determined using an area integrator (LI-COR, 3100 C – Area Meter, NE, USA), and the length of the largest root was measured with a ruler graduated in centimeters;

d) Production, physiological indexes, and quality of seedlings: The different organs were placed in an oven with forced air circulation at 60 ± 5 °C until the mass was stabilized, followed by weight assessment on a precision scale (0.0001 g). From the leaf area and dry biomass data, the leaf area ratio (LAR, cm^2 g^{-1}), specific leaf area (SLA, cm^2 g^{-1}), and specific leaf mass (SLM, g cm^{-2}) were calculated (Benincasa, 2003). The seedling quality index (DQI) was determined according to the proposal of Dickson et al. (1960).

The data were subjected to analysis of variance (ANOVA), and when significant differences were detected (F test, $p < 0.05$), the means were compared using the Bonferroni *t* test for the water-retaining polymer, and Tukey’s test for water retention capacity ($p \leq 0.05$), using the SISVAR software (Ferreira, 2019).

RESULTS AND DISCUSSION

The water regimes affected the photochemical characteristics of photosynthesis and initial growth of *C. xanthocarpa* seedlings, and the lower water retention capacities (WRC) of 25% and 50% in the substrate produced lower values, indicating a water deficit; however, the addition of the water-retaining polymer (WRP) contributed to mitigating the damage caused to the various indicators evaluated, particularly under low water availability, which corroborates our hypothesis.

Seedlings produced under 25% and 50% WRC without WRP showed a lower chlorophyll index (Figure 1a), while those under 25% WRC with WRP showed an increase in this measure, with values close to those of seedlings with 75% or 100% WRC without WRP. However, the addition of WRP to the substrate and maintenance of 100% WRC caused a reduction in the SPAD index.

The indicators of chlorophyll-*a* fluorescence and activities on photosystem II were influenced by the interaction between the factors under study, apart from the F_m ($p < 0.05$). The lower water availability (25% WRC) in the substrate without WRP resulted in an increase in F_0 (0.280 electrons per quantum) (Figure 1b) and a decrease in F_v (0.098 electrons per quantum) (Figure 1c), indicating oxidative damage to the chloroplast. In general, with 25% and 50% of the WRC and the absence of WRP, the seedlings reduced the efficiency of PS II (F_v/F_m) (Figure 1d) and absorbed energy conversion (F_v/F_0) (Figure 1e), causing an increase in the yield of non-photochemical processes (F_0/F_m) (Figure 1f).

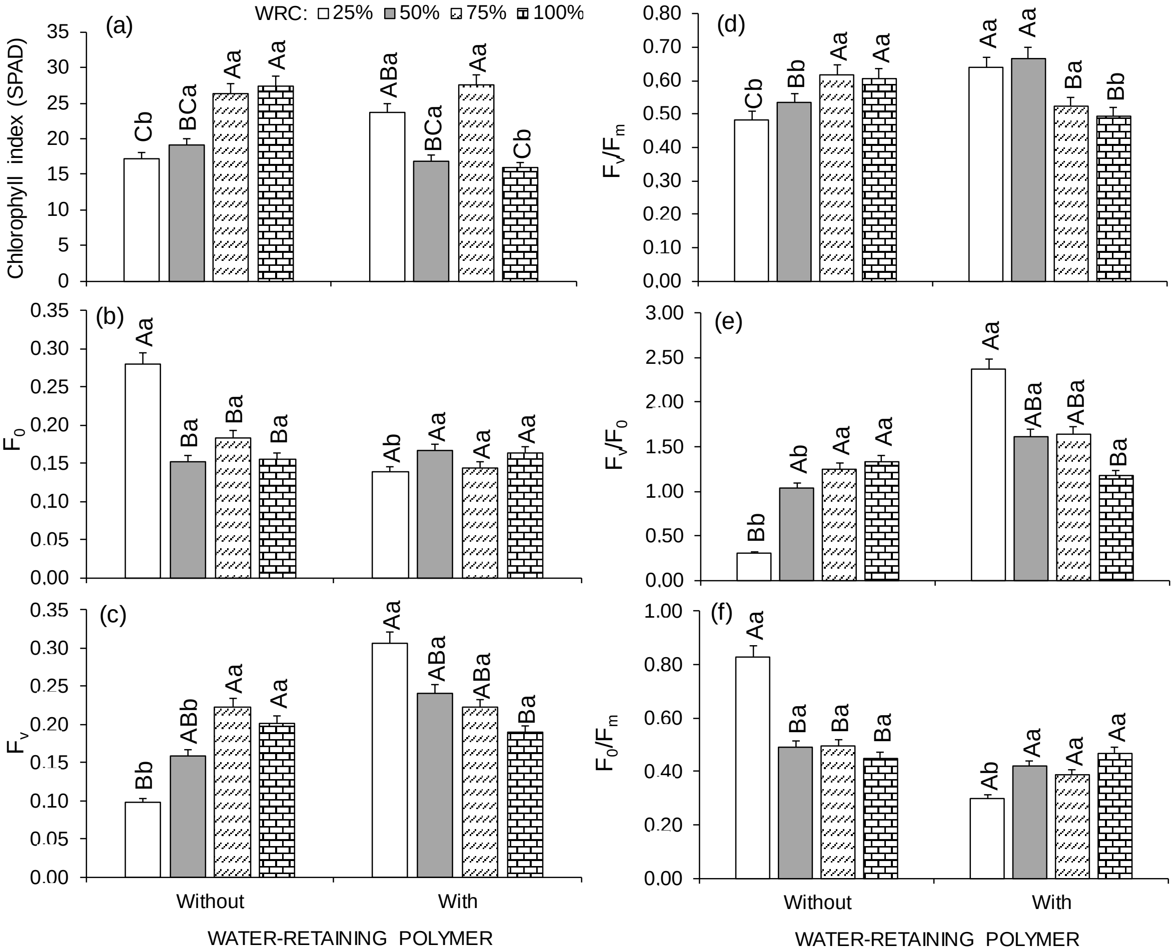

FIGURE 1. Chlorophyll index (a), initial – F_0 (b) and variable – F_v (c) fluorescence, potential photochemical efficiency of photosystem II – F_v/F_m (d), efficiency of conversion of absorbed energy – F_v/F_0 (e) and maximum basal yield of non-photochemical processes – F_0/F_m (f) in leaves of *C. xanthocarpa* seedlings in function of association water-retaining polymer and water retention capacity – WRC on substrate. Uppercase letters compare the different WRC within the use of WRP (Tukey, $p < 0.05$) and lowercase letters compare the use of WRP within each WRC (Bonferroni *t* test, $p < 0.05$).

A partial inactivation in the P_{680} reaction centers results from a reduction in the potential collector of light energy in the antenna complex and transfer to the acceptors, indicated by the increase in F_0 and F_0/F_m and decrease in the other photochemical indicators under water deficit (Meng et al., 2016; Khatri & Rathore, 2019; Faseela et al., 2020). Thus, a decline in the flow of electrons occurs in the transport chain, particularly those of plastoquinone A, and the reduced chlorophyll synthesis and tissue dehydration causes the conversion of absorbed energy (Zheng et al., 2019; Badr & Brüggemann, 2020), promoting energy dissipation and photochemical apparatus alterations.

When WRP was incorporated into the substrate, the photochemical processes were stable, especially under 25% and 50% WRC, reinforcing our hypothesis that the hydrogel attenuates the damage in the reaction centers even under low water availability, returning values similar to those of seedlings with the largest WRC, without WRP. The polymer is formed by a hydrophilic group able to absorb up to 400 times its weight in water. Therefore, when water potential begins to decrease in the substrate, the gel uniformly supplies up to 95% of the water that has been stored in the soil, thus mitigating the water deficit (Kalhapure et al., 2016) and regulating electron transfer, energy balance, and leaf metabolism.

However, we found that seedlings with 75% and 100% WRC and the addition of WRP presented lower values of F_v/F_m and F_v/F_0, indicating stress, since there was sufficient irrigation with the WRC, along with the water stored in the WRP. These reductions may be associated with water saturation, supra-optimal conditions beyond the required levels; thus, seedlings show symptoms of stress likely in relation to the production of reactive oxygen species (ROS); reduction of CO_2 fixation, causing lipid peroxidation; and oxidative damage (Hasanuzzaman et al., 2020).

The height of the seedlings was greater (14.85 cm) with WRP, independent of the WRC (Figure 2a). The stem diameter was larger under 50% and 100% WRC with WRP when compared to the same water regimes in the absence of WRP (Figure 2b). As the WRC increased in the substrates, the number of leaves also increased, especially under 100% without and with WRP (22 and 27 leaves, respectively), and the seedlings grown with WRP presented more expressive values that differed statistically from those without WRP within the regimes of 25%, 75%, and 100% WRC (Figure 2c).

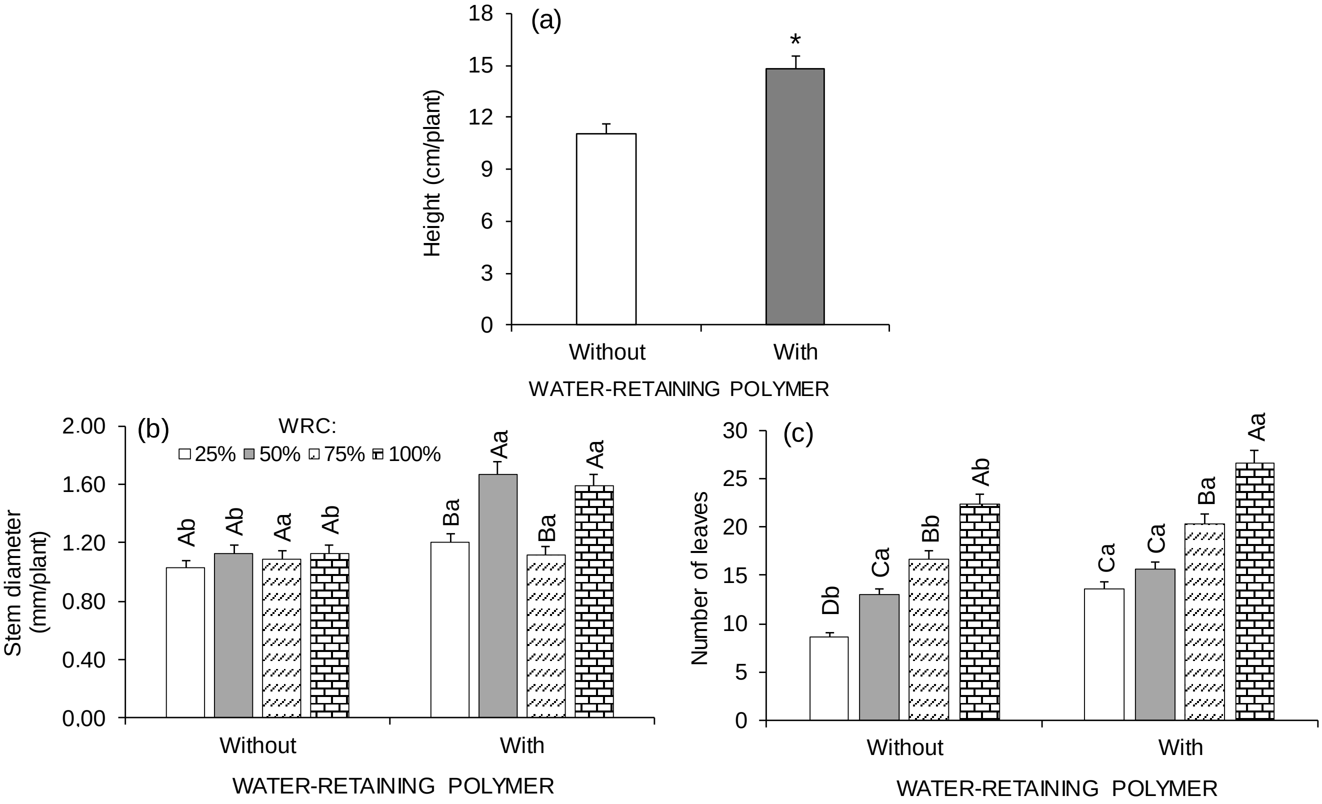

FIGURE 2. Height (a), stem diameter (b) and number of leaves (c) in *C. xanthocarpa* seedlings in function of association water-retaining polymer and water retention capacity – WRC on substrate. Uppercase letters compare the different WRC within the use of WRP (Tukey, $p < 0.05$) and lowercase letters compare the use of WRP within each WRC (*Bonferroni *t* test, $p < 0.05$).

Our results demonstrate that WRP contributed to the growth of *C. xanthocarpa* through a longer supply of water from the storage of hydrophilic polymer granules. This response was also observed in two cultivars of *Olea europapeae* L., under two water regimes (without and with water restriction), and addition or absence of hydrogel, where the growth parameters of this species were improved by the hydrogel because of its ability to prevent dehydration damage to young plants (M'Barki et al., 2019).

Regarding the leaf morphological characteristics, hormonal signaling occurs under lower WRC in the substrate, in terms of ABA transport from the roots to the cytosol, reducing leaf expansion both in number and in area, mitigating the transpiratory rate, as well as regulating the conductance in the sub-stomatal and mesophilic chambers for CO_2 assimilation (Sorrentino et al., 2016). In contrast, the mechanisms presented by the seedlings under adequate water status keep their metabolic processes stabilized.

The leaf area of the *C. xanthocarpa* seedlings were influenced by the factors alone, with greater values in the seedlings produced with WRP (28.49 cm^2) (Figure 3a) and under 100% WRC (32.96 cm^2) (Figure 3b). The addition of WRP increases the water potential gradient in the substrate (Kalhapure et al., 2016), and water translocates more efficiently through the xylemic vessels to the leaves, expanding their per unit area (Lauri et al., 2014; Hernandez-Santana et al., 2016), thereby maximizing photosynthetic capacity. Similarly, *Tabebuia roseoalba* (Ridl.) seedlings had a larger leaf area under 100% WRC that reduced with lower water availability (Scalon & Mussury, 2020).

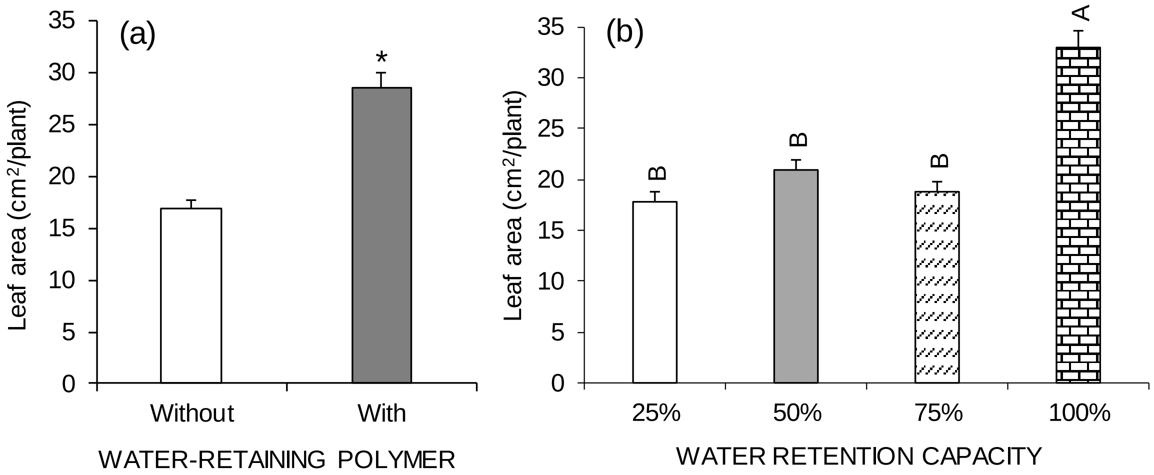

FIGURE 3. Leaf area in *C. xanthocarpa* seedlings in function of use water-retaining polymer (a) or water retention capacity – WRC (b) on substrate. * (Bonferroni *t* test, $p < 0.05$); Equal letters between columns in WRC not differ from each other (Tukey, $p > 0.05$).

The length of the largest root averaged 22.0 cm and was not influenced by the factors under study ($p > 0.05$). Conversely, the physiological indexes were influenced by factor interactions, with the highest LAR values (266.81 and 233.73 cm^2 g^{-1}) occurring in the seedlings with 100% and 50% WRC, without and with WRP, respectively (Figure 4a). The values of SLA and SLM showed the same trend, higher values in seedlings under 100% WRC without WRP, while only the 50% WRC with WRP group presented lower values compared to the other water regimes (Figure 4b and 4c).

The responses of the physiological indexes to seedlings in these water conditions, especially 50% WRC with WRP, demonstrate an increase in structural tissues for dehydration protection, allowing less stomatal limitation under adverse environmental conditions (Ricote et al., 2019), and greater photoassimilate production per unit area.

The greatest dry mass of leaves (0.234 g/plant) occurred in seedlings produced under 100% WRC with WRP (Figure 4d), whereas the water regimes without WRP, did not differ statistically. According to Milani et al. (2017), the presence of polyacrylamide in the WRP can increase the hydraulic cohesion and conductivity in the soil, even under deficit, thereby reducing the required frequency or depth of irrigation, as well as maintaining the cell membrane integrity, mitigating the ROS production, and enabling greater biomass allocation of in the seedlings. The stem and root dry masses were not influenced by the factors under study ($p > 0.05$), with averages of 0.131 and 0.188 g/plant, respectively.

The aerial part/root ratio (APRR) was higher under 75% and 50% WRC, without and with WRP, respectively, and it should be noted that when WRP was added to the substrate, different water regimes, except 50% WRC, returned statistically higher values when compared to the seedlings without hydrogel (Figure 4e). This increase relates to the benefits of WRP for water retention in the substrate (Freitas et al., 2019), demonstrating investment in root biomass due to greater water absorption, contributing to translocation to the aerial part, promoting better physiological indexes and growth characteristics.

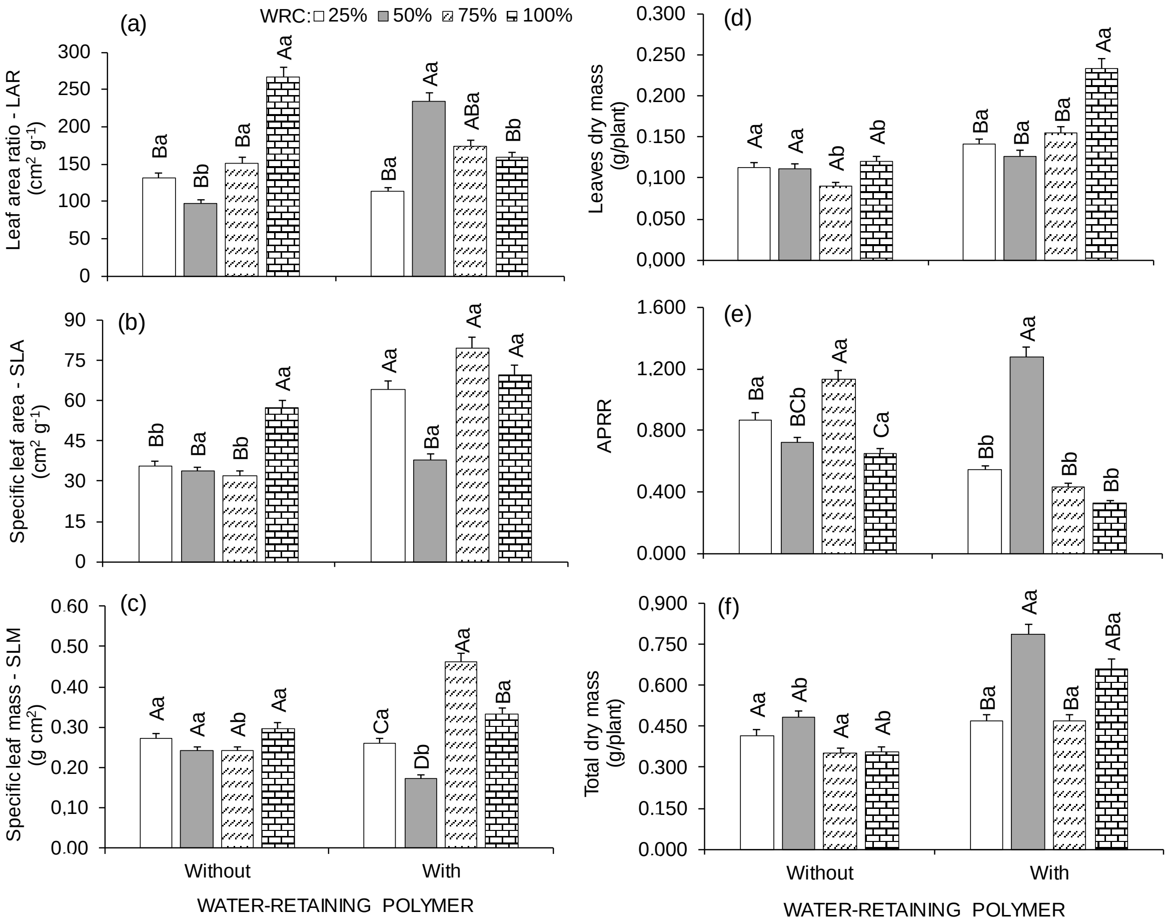

FIGURE 4. Leaf area ratio – LAR (a), specific leaf area – SLA (b), specific leaf mass – SLM (c), leaves (d) aerial part/root ratio – APRR (e) and total dry mass (f) in *C. xanthocarpa* seedlings in function of association water-retaining polymer and water retention capacity – WRC on substrate. Uppercase letters compare the different WRC within the use of WRP (Tukey, $p < 0.05$) and lowercase letters compare the use of WRP within each WRC (Bonferroni *t* test, $p < 0.05$).

The seedlings showed higher total dry mass yields under 50% and 100% WRC, both with WRP (Figure 4f), demonstrating the beneficial effect of the hydrogel on the photoassimilate production. The largest biomasses were associated with the highest vegetative characteristic values of the aerial part, such as the number of leaves (Figure 1c) and leaf area (Figure 3) under the same growing conditions.

The Dickson quality index (DQI) was influenced by the factors separately, with the highest values occurring in seedlings produced with WRP (Figure 5a) and under 50% WRC (Figure 5b). The increase in DQI in these conditions is associated with the benefits of WRP in terms of growth, APRR, physiological indexes, and biomass. This indicator has been used as a quality parameter for forest seedlings because it makes inferences based on the morphometric characters and photoassimilates partitioning in the different organs.

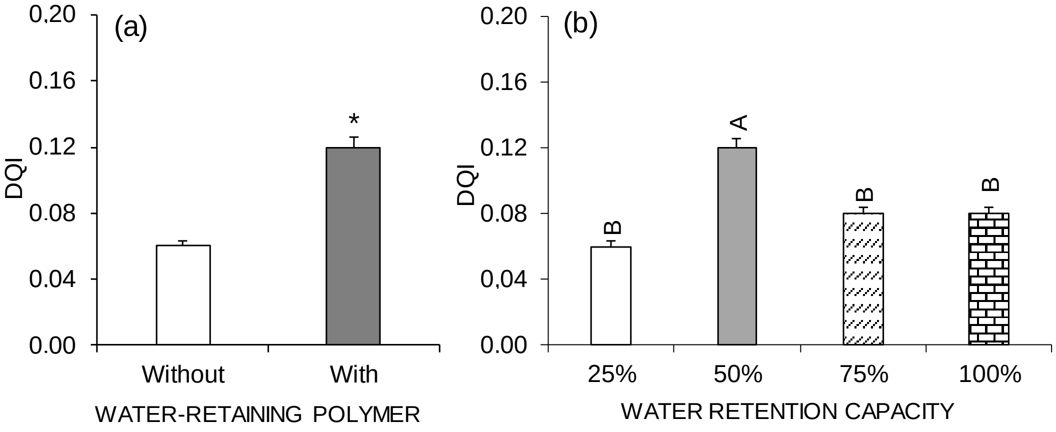

FIGURE 5. Dickson quality index – DQI in *C. xanthocarpa* seedlings in function of use water-retaining polymer (a) or water retention capacity – WRC (b) on substrate. * (Bonferroni *t* test, $p < 0.05$); Equal letters between columns in WRC not differ from each other (Tukey, $p > 0.05$).

Although the efficiencies of the photochemical processes of *C. xanthocarpa* seedlings was reduced without WRP, the damage in the reaction centers was reversible as no harm to the biochemical processes of photosynthesis occurred, since there was still an increase in biomass independent of the WRC. However, the addition of WRP maintained the photochemical apparatus integrity by attenuating disturbances in chlorophyll-*a* fluorescence emissions, thereby enhancing the production of photoassimilates.

Therefore, in regions where irrigation management is challenging or rainfall is irregular, such as the Cerrado, the addition of a water-retaining polymer has a promising mitigating effect, contributing to the quality of seedlings, and reducing costs with its replacement after transplanting, ensuring sound silvicultural practices. However, further studies should be conducted to verify the duration of action of the WRP in the soil and thus determine the ideal time for transplanting the seedlings with the addition of the polymer to the substrate.

CONCLUSIONS

Water deficiencies destabilize the activities in the photochemical apparatus, compromising the growth of *Campomanesia xanthocarpa* (Mart.) O. Berg, seedlings; however, a water-retaining polymer can attenuate these deleterious effects. The addition of a hydrogel and attaining a water retention capacity of 50% promotes greater morphophysiological indicators and quality of seedlings.

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