Floresta e Ambiente 2020; 27(2): e20180263
https://doi.org/10.1590/2179-8087.026318
ISSN 2179-8087 (online)

Floresta e Ambiente

ORIGINAL ARTICLE – Silviculture

Irrigation Regimes on Growth and Metabolic Processes of *Enterolobium contortisiliquum* Seedlings

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Abstract

In forest nurseries, one of the factors that change the growth and quality of seedlings is water supply, which has a direct influence on metabolic processes such as stomatal conductance and photochemical efficiency of photosystem II. This study aims to identify the influence of different watering regimes as well as the use of a water-retaining polymer on the initial growth and metabolic processes of Enterolobium contortisiliquum seedlings. The experimental design was in random blocks with a factorial scheme. Morphological attributes, as well as physiological and biochemical attributes were investigated. We verified that the performance of E. contortisiliquum seedlings depends on the watering regime provided to the plants; furthermore, up to 60 days after the application of the treatments, both the height and diameter of the collection and the leaf water potential were similar in the water regimes of 8 mm day-1 and 12 mm day-1.

Keywords: abiotic stress, water potential, oxidative stress, antioxidant enzymes.

1. 1. INTRODUCTION

Enterolobium contortisiliquum (Vell.) Morong belongs to the Fabaceae family. It is a tree native to South America found in Brazil, Argentina, Paraguay, and Uruguay (Burkart, 1967). This species can occur in several forest formations; however, it is more commonly found in subtropical forests (Moreira et al., 2015).

This species is important for different sectors of the economy, such as timber, shipbuilding, and civil construction (Zuchiwschi et al., 2010). It also has medicinal value in view of the anti-inflammatory and antibiotic properties of substances present in its bark and fruit, respectively (Nakahata et al., 2011). In addition, *E. contortisiliquum* is recommended for reforestation of degraded areas, permanent preservation, and mixed plantings, mainly due to its rapid initial growth (Araújo & Paiva Sobrinho, 2011).

In forest nurseries, one of the factors that influence the development and quality of seedlings is the management of irrigation (Silva et al., 2012). This aspect of forestry, together with the worldwide concern for sustainable use of water, requires studies that optimize irrigation in several productive sectors, such as forestry.

Water is an indispensable component for the maintenance of metabolic homeostasis and structural integrity of cell macromolecules. Leaf watering regime has a direct influence on metabolic processes, such as stomatal conductance, cell

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elongation and expansion, ψ, and photochemical efficiency of photosystem II (Silva et al., 2007). In addition, water acts as a solvent for electrolytes and is involved in the assembly and reactivity of native biomolecules (Vyumvuhore et al., 2015).

The effects of environmental stresses, such as water restriction, can be measured by the increase in production of reactive oxygen species (ROS) (Møller et al., 2007). ROS, such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide (O_2•-), and hydroxyl radical (HO•) are capable of causing oxidative damage to lipids, proteins, and DNA within the plant (Møller et al., 2007; Sharma et al., 2012), which ultimately causes reduced growth and death. Thus, to minimize the cytotoxic effects of ROS, plants have a complex antioxidative system, where specific enzymes act to neutralize the action of these radicals, starting with superoxide dismutase (SOD) that dismutes the superoxide anion (O_2•-) to H_2O_2 (Pandey et al., 2016). In sequence, hydrogen peroxide is detoxified by peroxidases such as ascorbate peroxidases (APXs), glutathione peroxidase (GPX) and catalase (Asada, 2006; Choudhury et al., 2016; Waszczak et al., 2018).

In addition, the use of synthetic conditioners is reported to contribute toward an increase in the water retention capacity of the substrate, which reduces the frequency of irrigation, and thus, allows more effective use of resources from the culture medium and water, which ultimately optimizes crop yield (Navroski et al., 2015). Moreover, the use of water-retaining polymers improves the chemical and physical characteristics of the substrate, allowing better utilization of the nutrients incorporated into the culture medium (Felippe et al., 2016; Navroski et al., 2016)

This investigation sought to identify the influence of different watering regimes and the addition of water-retaining polymer to the substrate on the initial growth and metabolic processes of *E. contortisiliquum* seedlings. The main questions that we aimed to answer are: a) Is the growth of *E. contortisiliquum* seedlings dependent on the watering regime adopted?; b) Does the use of water-retaining polymer allow a reduction in the amount of water available to the plants?; c) Under conditions of low water availability, does *E. contortisiliquum* keep the metabolic processes unchanged?

2. MATERIAL AND METHODS

2.1. Plant material and experimental design

The experiment was conducted in the forest nursery of the Universidade de Santa Maria (29°43'S and 53°43'W), located in the central region of the Rio Grande do Sul State, Brazil. The approximate altitude here is 90 meters. The local climate, according to the Köppen classification, is subtropical, type Cfa, with annual average precipitation between 1,900 and 2,200 mm. The average temperature is 19.1 °C, with maximum and minimum values of 32 and 9 °C, respectively (Alvares et al., 2013), which sets four distinct seasons in the area under study. The research was conducted from February to June 2016, corresponding to the period between summer and fall.

Mature fruits of *E. contortisiliquum* were collected from eight trees located in a fragment of Deciduous Seasonal Forest (29°38'S and 53°40'W), in Santa Maria, Rio Grande do Sul State, Brazil. After the collection, seeds were extracted by pruning shears and manual processing. Only completely formed seeds were selected for further analysis. Following seed collection, the tegument was manually scarified with sandpaper in the region opposite to the micropyle without accessing the cotyledons, as recommended by Brasil (2013).

Seedlings were germinated with the use of cylindrical-conical polypropylene tubes with a volume of 110 cm^3 that were placed in plastic trays with a capacity for 96 tubes. They were suspended at a depth of 16 cm from the soil surface. We used a commercial substrate composed of sphagnum peat and expanded vermiculite with charcoal rice husk in a 4:1 (v:v) ratio. For base fertilization, 8 g per liter substrate of the controlled-release fertilizer with an NPK formulation of 18-05-09 were employed. Substrates were prepared in a concrete mixer by adding the fertilizer and the water-retaining polymer for the treatment that required its presence.

Two seeds were sowed per container. After about 15 days, thinning was performed to leave only one seedling per container, which was central and the strongest one. The seedlings were kept for 30 days in a greenhouse, where they received micro-sprinkler irrigation (5 mm day^{-1}). After this period, the trays were placed in an open and sunny environment, where the seedlings were subjected to different watering regimes. In this environment, there were low tunnels and clear polyethylene tarpaulins that were manually operated to cover the seedlings whenever necessary in order to avoid contact with precipitation.

The experimental design was random blocks in a factorial scheme. Factor A was constituted by three randomized irrigation regimes (4, 8, and 12 mm day^{-1}). Factor D was prepared on the basis of the absence (P0 – 0 g L^{-1}) or presence (P4 – 4 g L^{-1}) of the water-retaining polymer. The assay was performed using four replicates per treatment, totaling 24 experimental units, each unit composed of 24 seedlings.

Before installing the experiment, physical and chemical analyses of the tested substrates with or without the water-retaining polymer tested (Table 1) were carried out in the Laboratory of Analysis of Substrates for Plants from the Departamento de Diagnóstico e Pesquisa Agropecuária. The analyses were conducted according to Brasil (2008) and Verdonck et al. (1984).

Table 1. Physical and chemical attributes of the substrates used in the production of *E. contortisiliquum* seedlings.

Substrate	MD (kg/m³)	AS %	WEA %*	EC (dS/m)	Condition EC*	pH	Condition pH*
P0	413	33.0	22.0	0.51	Normal	4.43	Low
P4	329	30.0	25.0	0.47	Normal	4.53	Low

P0: absence of water-retaining polymer; P4: presence of water-retaining polymer; MD: moist density; AS: aeration space; WEA: water easily available; EC: electrical conductivity. *Regan (2014).

Previously, the uniformity of the irrigation system was determined using the Christiansen Uniformity Coefficient (CUC) as described by Bernardo et al. (2006). The CUC obtained was 83.2%, which was considered adequate by these authors. Irrigation regimes (RR) were programmed at different frequencies and schedules by means of timers linked to the irrigation system: RR4 – 4 mm day^{-1} (2 mm at 8 a.m. and 1 p.m.); RR8 – 8 mm day^{-1} (2 mm at 8 a.m. and 1 p.m. and 4 mm at 3 p.m.); and RR12 – 12 mm day^{-1} (4 mm at 8 a.m., 1 p.m. and 3 p.m.).

2.2. Morphological attributes

The morphological attributes were determined at four-time intervals (30, 60, 90, and 120 days after sowing), corresponding to 0, 30, 60, and 90 days after applying the irrigation regimes, in 8 seedlings per experimental unit. Morphological attributes included height, and stem diameter. The relationship between height and stem diameter of the collected samples from the eight central plants of each replicate was also evaluated. The height was measured with a millimeter ruler from the apical bud of the plant to the surface of the substrate. The diameter of each specimen was measured with a digital caliper (accuracy of 0.01 mm). Shoot dry mass, root dry mass, and total dry mass were obtained from four individuals of each experimental unit. Dry mass was measured by weighing samples after drying them for a period of approximately 72 hours in a forced circulation oven at 70 °C.

2.3. Water potential

The water potential (ψ) was measured at four-time intervals (30, 60, 90, and 120 days after sowing). The measurements were performed with the aid of a Scholander Pressure Chamber (model 600) in four plants per replicate and the evaluations carried out between 1 p.m. and 2 p.m. To perform this evaluation, about 8 cm of plant were sectioned from the apex of the molt and inserted into the chamber, followed by the application of pressure until the first droplets of exudation were observed. At this time the reading was performed in MPa.

2.4. Biochemical attributes

At the end of the experiment (90 days after application of irrigation regimes), the photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids content) and attributes of oxidative stress (lipid peroxidation, hydrogen peroxide concentration, and activity of the enzyme superoxide dismutase) were measured. Samples of expanded leaves were collected and immediately frozen in liquid N_2 and then stored in an ultrafreezer (−80 °C) until the moment of determination. The analyses were carried out at the Plant Biotechnology Laboratory, Department of Biology (Universidade Federal de Santa Maria).

In the quantification of the photosynthetic pigments, the concentrations of chlorophyll *a*, chlorophyll *b*, and carotenoids were determined according to the methodology described by Hiscox & Israelstam (1979) and were then estimated using the Lichtenthaler formula (Lichtenthaler, 1987). Fresh leaf samples (0.05 g) were incubated at 65 °C with dimethyl sulfoxide for 1.5 hours. The absorbances of the solutions were measured in a spectrophotometer (Celm E-205D) at 663, 645, and 470 nm for chlorophyll *a*, chlorophyll *b,* and carotenoids, respectively.

Lipid peroxidation was estimated following the method of El-moshaty et al. (1993). Samples of leaves (0.5 g) previously macerated in liquid N_2 were homogenized and then centrifuged, with 1 ml of the supernatant added to 1 ml of 20% (w/v) of trichloroacetic acid containing 0.5% (w/v) of thiobarbituric acid. The mixture was heated at 95 °C for 40 min, cooled in an ice bath for 15 min, and then centrifuged at 10,000 × g for 15 min. The absorbance of the supernatant was read in a spectrophotometer at 532 and 600 nm (to correct non-specific turbidity). Lipid peroxidation was expressed as nmol MDA mg^{-1} protein.

The concentration of H_2O_2 was determined according to the method described by Loreto & Velikova (2001). Approximately 0.1 g of leaf samples were homogenized in 3 mL of 0.1% (w/v) TCA. The homogenate was centrifuged at 12,000 × g for 10 min at 4 °C. Following this, 0.5 mL of the supernatant were added to 0.5 mL of 10 mM K-phosphate buffer (pH 7.0) and 1 mL of 1M KI. H_2O_2 concentration of the supernatant was evaluated by comparing the absorbance obtained in a spectrophotometer at 390 nm with a standard calibration curve. The concentration of hydrogen peroxide was expressed as μmol g^{-1} of fresh mass.

SOD activity was determined according to the spectrophotometric method described by Giannopolitis & Ries (1977). Samples (0.5 g) were homogenized in 3 mL of 0.05 M sodium phosphate buffer (pH 7.8) containing 1 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVP). Afterwards, the homogenate was centrifuged at 13,000 × g for 15 min at 4 °C (Zhu et al., 2004). To determine the activity, 50 μl of sample was mixed with 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 2 μM riboflavin, 75 μM *Nitroblue tetrazolium* (NBT), and 0.1 mM EDTA. The tubes containing the solution were packed in 15-watt bulbs for 15 min followed by reading the absorbances at 560 nm (Beauchamp & Fridovich, 1971). The activity of the enzyme was expressed as U mg^{-1} protein. Total proteins were determined following the method of Bradford (1976) using bovine serum albumin as standard.

2.5. Statistical analysis

The data were subjected to analysis of normality and homogeneity assumptions, followed by analysis of variance (ANOVA), Tukey HSD test ($p < 0.05$), and/or linear regression to observe the significance coefficient of determination (R^2). For statistical analysis, we used the software SISVAR v. 5.3 (Ferreira, 2014). When considering the quantitative factor, time intervals of 0, 30, 60, and 90 days after the application of irrigation regimes were performed.

3. RESULTS

The analysis of variance indicated an interaction between irrigation regimes (IR) and the time of measurements, for height, stem diameter, height/stem diameter ratio, root dry mass, and water potential ($p < 0.05$) variables. Individually, the factors affected the other morphological and physiological variables (Table 2).

We verified the highest averages of height, stem diameter, and height/stem diameter ratio, when the *E. contortisiliquum* seedlings were grown under irrigation regime RR12 (12 mm day^{-1}). In addition, after 90 days in the nursery, seedlings under the RR12 regime showed an increase in height and stem diameter, which was about 50% higher than of those grown under RR4 (4 mm day^{-1}) (Figure 1).

Table 2. Summary analysis of variance of morphological and physiological variables of *Enterolobium contortisiliquum* seedlings, according to the tested treatments.

Source of variation	DF	Mean square – Non-destructive measurement					
		H	SD	H/SD	SDM	RDM	ψ
Irrigation regime (IR)	2	54.90**	1.15**	1.08**	0.04^{ns}	0.08*	0.85**
Water-retaining polymer (WRP)	1	15.53*	1.00**	0.02^{ns}	0.14*	0.00^{ns}	0.04^{ns}
Time	3	153.13**	9.58**	4.86**	0.02^{ns}	2.62**	0.67**
IR*WRP	2	4.11^{ns}	$0,17^{ns}$	0.11^{ns}	0.02^{ns}	0.03^{ns}	0.07^{ns}
IR*time	6	14.65**	15.97**	0.84**	0.00^{ns}	0.09**	0.34*
WRP*time	3	1.19^{ns}	0.03^{ns}	0.11^{ns}	0.05^{ns}	0.00^{ns}	0.14^{ns}
IR*WRP*time	6	0.22^{ns}	0.03^{ns}	0.04^{ns}	0.01^{ns}	0.01^{ns}	0.02^{ns}
Residue	72	2.54	0.09	0.10	0.02	0.02	0.09
CV (%)		11.54	8.77	7.82	20.78	22.50	14.45
		Mean square – Destructive measurement					
		Chl *a*	Chl *b*	MDA	H_2O_2	SOD	
Irrigation regime (IR)	2	0.06^{ns}	0.002^{ns}	0.0002**	0.32*	11102**	
water-retaining polymer (WRP)	1	0.11**	0.008*	0.000005^{ns}	0.03^{ns}	1120.1^{ns}	
IR*WRP	2	0.01^{ns}	0.0004^{ns}	0.00004^{ns}	0.04^{ns}	$910,67^{ns}$	
Residue	14	0.01	0.001	0.00002^{ns}	0.08	542.41	
CV (%)		13.94	16.90	35.41	15.04	15.49	

CV%: coefficient of variation; DF: degree of freedom; ns: not significant; H: height; SD: stem diameter; H/SD: height/stem diameter ratio; SDM: shoot dry mass; RDM: root dry mass; ψ: Water potential; Chl *a*: chlorophyll *a*; Chl *b*: chlorophyll *b*; MDA: malondialdehyde content of shoot; H2O2: hydrogen peroxide; SOD: superoxide dismutase. * significant at 5% probability by the F test; ** significant at 1% probability by the F test.

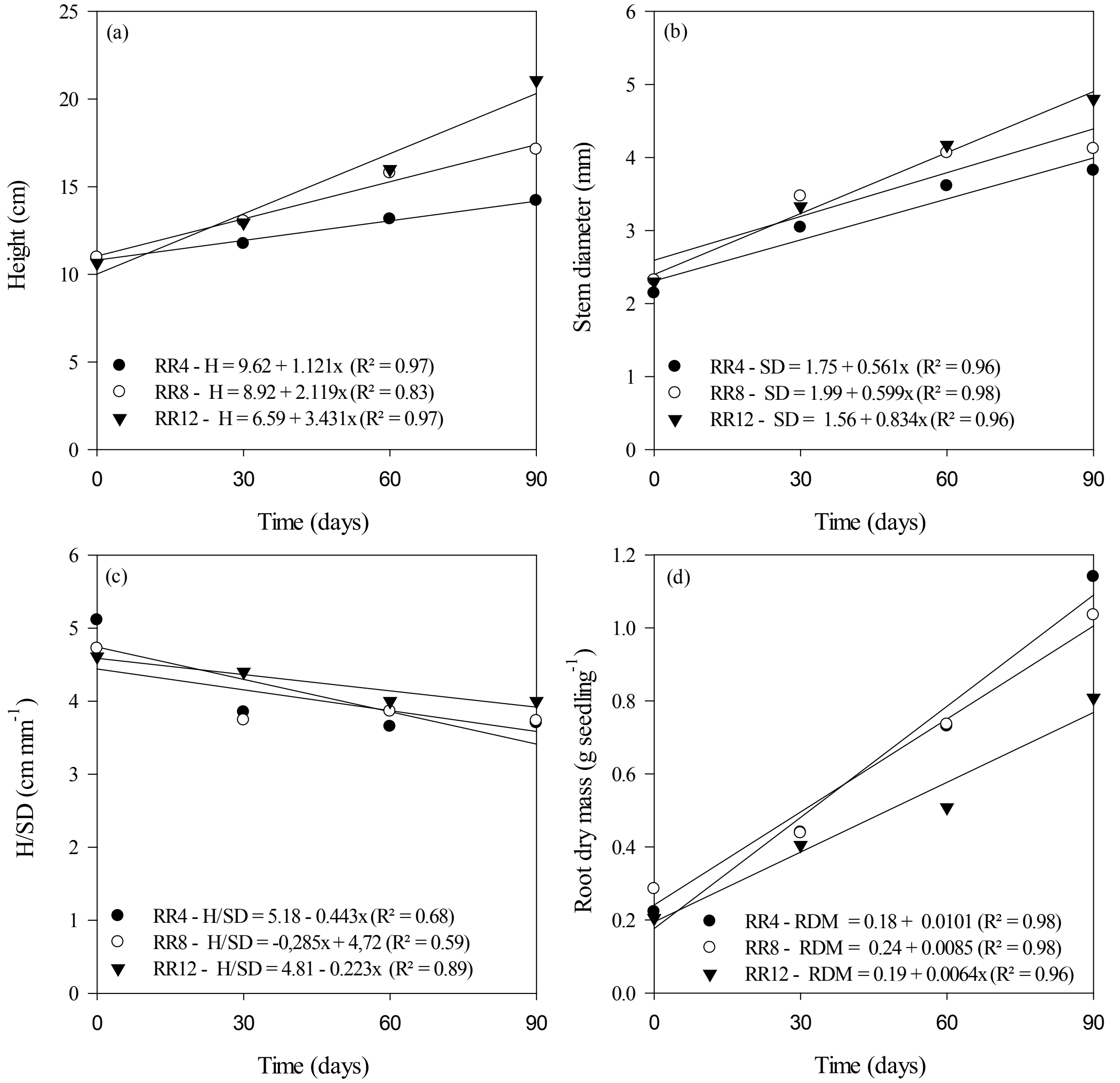

Figure 1. Height (a), stem diameter (b), height/stem diameter ratio (c) and root dry mass (d) of *Enterolobium contortisiliquum*, according to the irrigation regimes of 4 (RR4), 8 (RR8) and 12 (RR12) mm day^{-1} at 0, 30, 60 and 90 days after irrigation.

On the other hand, we found that under the irrigation regime RR12 (12 mm day^{-1}), root dry mass had the lowest mean. For this attribute, the regimes that provided the lowest water supply (RR4 and RR8) showed the highest values of dry mass of roots (Figure 1).

The attributes of height, stem diameter, and shoot dry mass presented higher values ($p < 0.05$), when the *E. contortisiliquum* seedlings were raised in the presence of the water-retaining polymer (Figure 2).

We observed that the ψ of the plants was influenced by the watering regimes used. Water supply restriction (RR4 – 4 mm day^{-1}) reduced the hydration state of the cells (ψ −2.04 MPa) 90 days after the application of the treatments (Figure 3). With other regimens, RR8 and RR12, 60 days after the application of the treatments, similar results of −0.56 and −0.69 MPa, respectively, were obtained.

The analysis of the photosynthetic pigments revealed that *E. contortisiliquum* seedlings had the same behavior regarding the use of water-retaining polymer. We observed that seedlings grown in the presence of the polymer (P4 – 4 g L^{-1}) had the highest levels of chlorophyll *a* (0.94 mg g MF^{-1}) and *b* (0.22 mg g MF^{-1}) (Figure 4).

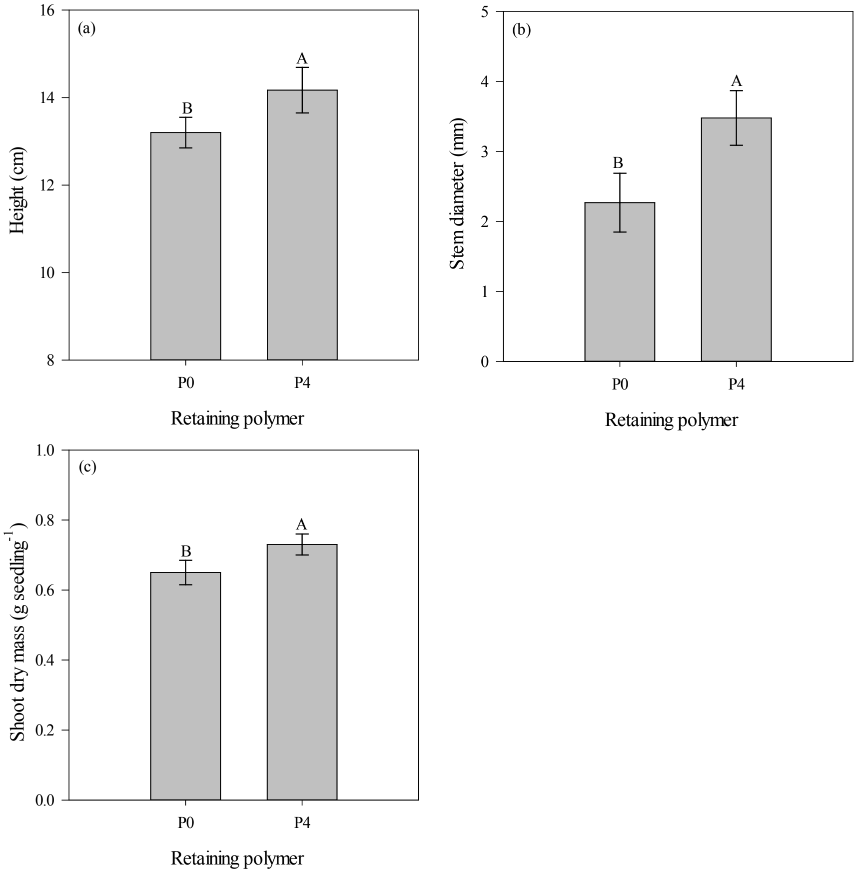

Figure 2. Height (a), stem diameter (b) shoot dry mass (c) of *Enterolobium contortisiliquum* seedlings, conducted in the absence (P0 – 0 g L^{-1}) or in the presence (P4 – 4 g L^{-1}) of the water-retaining polymer in the substrate formulation. Data are mean ± SE.

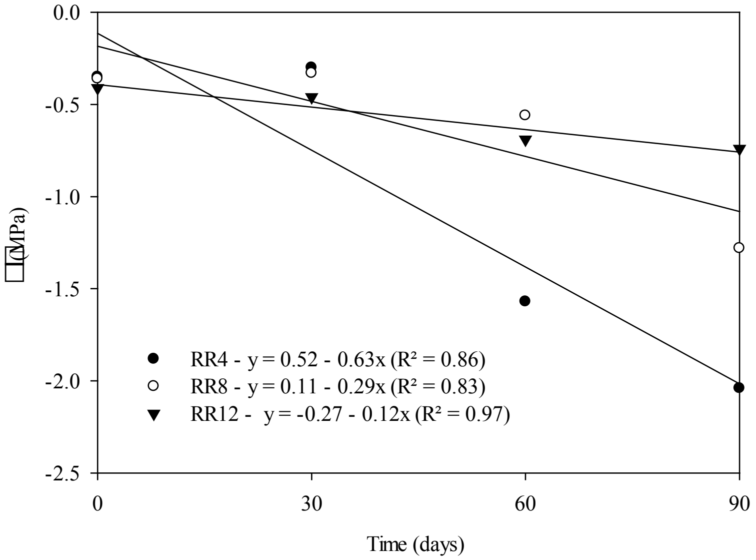

Figure 3. Water potential (ψ) of *Enterolobium contortisiliquum* seedlings, according to irrigation regimes of 4 (RR4), 8 (RR8) and 12 (RR12) mm day^{-1} at 0, 30, 60 and 90 days after application of irrigation.

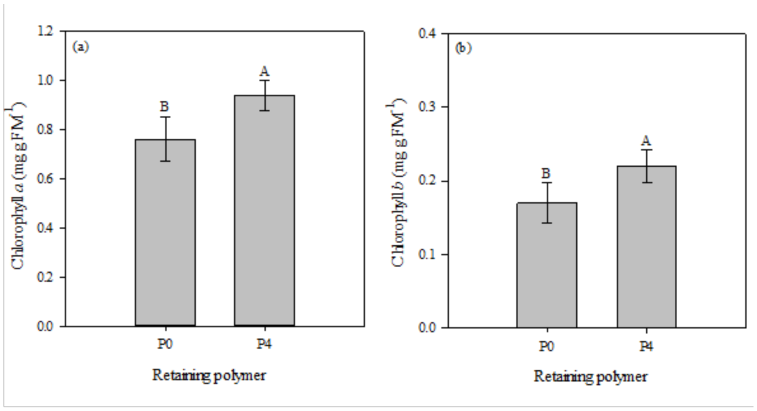

Figure 4. Chlorophyll a (a) and chlorophyll b (b) of Enterolobium contortisiliquum seedlings, conducted in the absence (P0 – 0 g L-1) or in the presence (P4 – 4 g L-1) of water-retaining polymer in the substrate formulation. Data are mean ± SE

For other biochemical attributes, we observed a significant effect of the irrigation regimes, when analyzing lipid peroxidation, hydrogen peroxide concentration, and the activity of the enzyme superoxide dismutase (Figure 5). The level of lipid peroxidation that was measured by the accumulation of malondialdehyde (MDA) and H_2O_2 content increased as the water availability decreased. The highest values of 0.13 µmol MDA mg^{-1} protein and 2.11 µmol g^{-1} of fresh mass, respectively, were observed in the seedlings subjected to the RR4 irrigation regime (4 mm day^{-1}) as compared to the RR12 irrigation regime (12 mm day^{-1}) (Figure 5).

The plant's defense system investigated in this study by the activity of the SOD increased significantly with the reduction of their water supply (Figure 5). In general, plants subjected to RR4 irrigation regimen (4 mm day^{-1}) showed a 46% increase in SOD activity as compared to RR12 (12 mm day^{-1}) regimen.

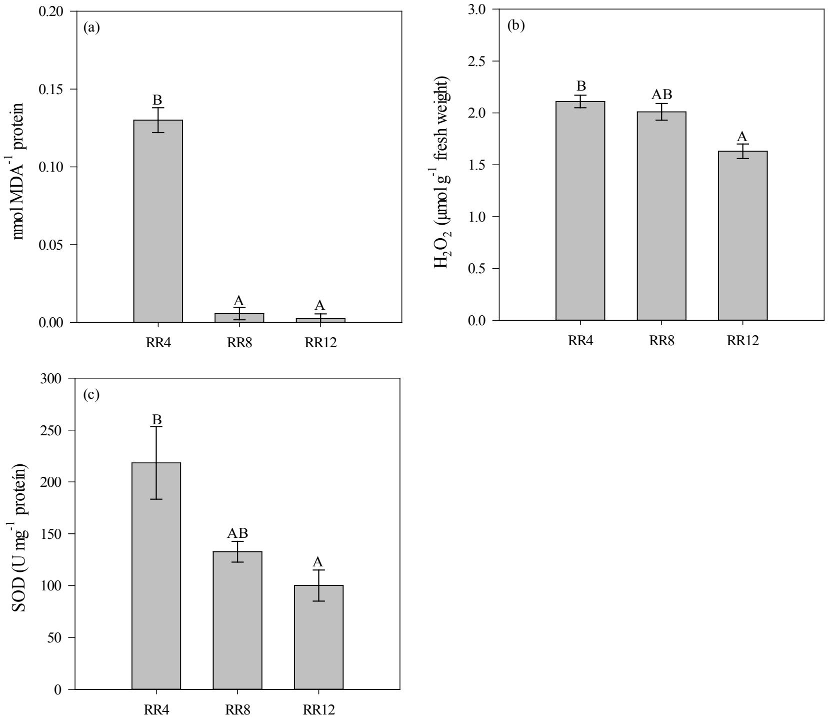

Figure 5. Malondialdehyde (MDA) content of shoot (a), hydrogen peroxide (H_2O_2) concentration (b) and superoxide dismutase (SOD) enzyme activity (c) of *Enterolobium contortisiliquum* seedlings, according to the irrigation regimens of 4 (RR4), 8 (RR8) and 12 (RR12) mm day^{-1} at 90 days after the application of irrigation. Data are mean ± SE.

4. DISCUSSION

Morphologically, *E. contortisiliquum* seedlings presented positive results when the RR12 irrigation regime (12 mm day^{-1}) was applied. Silva et al. (2016) studied the responses of water availability in sixteen species of the *Eucalyptus* genus to verify the negative effect of water restriction during seedling production. However, Dutra et al. (2016) evaluated the effect of different substrates and irrigation regimes to report that the use of 4 mm day^{-1} was enough to produce healthy seedlings of *Parapiptadenia rigida* (Benth.) Brenan. Similarly, we also verified it is fundamental to characterize the specificities of each species in relation to the factor of watering regimes. This will allow the optimization of the productive activity and the rational use of water in nurseries because even species from the same family, such as *E. contortisiliquum* and *P. rigida,* have different water requirements.

Enterolobium contortisiliquum expressed the opposite behavior under limited water conditions in the substrate concerning root dry mass. The results show that water deficiency in the substrate has a significant influence on root growth, where this deficit increases the root biomass under stress. The contrasting response in the production of root biomass reflects a strategy used by different forest species (Dutra et al., 2016; Silva et al., 2016) in relation to carbon allocation. In general, water restriction induces carbon partitioning for root synthesis in order to increase the surface of fine roots, and thus, to increase the area of contact with the soil, which consequently enhances the capacity of water and nutrients absorption (Litton et al., 2007; Ryan et al., 2010).

One of the main results obtained in our study refers to the fact that the watering regimes had a significant interaction ($p < 0.05$) with the time of permanence of the seedlings in the nursery. Up to 60 days after the application of irrigation regimes, there was no difference observed between seedlings grown at RR12 and RR8. Therefore, both irrigation regimes can be applied during the growth phase. However, this should be done in combination, where in the first 60 days, 8 mm day^{-1} may be used and then changed to 12 mm^{-1} after this period. In this manner, 77.8% of water was saved during the production period of the *E. contortisiliquum* seedlings.

The positive effects of the combined use of irrigation regimes, RR8 and RR12, are explicitly observable, when analyzing data on water potential in plants. We observed here that up to 60 days after the application of the treatments, water potential presented values close to RR8 and RR12: −0.56 MPa and −0.69 MPa, respectively. However, when analyzing the irrigation regime, RR4 (4 mm day^{-1}), we identified severe water stress (−1.56 MPa). According to Taiz et al. (2017), plants with water potential below −1.00 MPa are under severe water stress, and adverse symptoms can be seen in the processes of expansion and cell expansion.

Additionally, according to Bergonci et al. (2000), the potential of water in a plant indicates the state of turgidity of its cells. In a situation of low availability of water in the soil, plants reduce the loss of water by restricting stomatal conductance. Water stress substantially changes the metabolism and gas exchange in plants (Taiz et al., 2017), which can, in turn, directly hinder the growth if water stress inhibits photosynthesis by stalling the dehydration of mesophyll cells (Silva et al., 2007). Thus, the lower water potential confirms the lower growth rates and aerial biomass allocation in plants grown under the 4 mm day^{-1} regime (RR4).

We observed that the addition of water-retaining polymer to the substrate contributes to the growth and biomass increase in the *E. contortisiliquum* seedlings, regardless of the watering regime adopted. These results corroborate those obtained by Navroski et al. (2015) and Felippe et al. (2016) on the production of the genus Eucalyptus seedlings. The increase in biomass with the addition of water-retaining polymer occurs due to the reduction of percolation of irrigation water and improvement in the aeration and drainage in soil (Bernardi et al., 2012) as well as the reduction in nutrient leaching (Navroski et al., 2015), which contributes directly to the improvement of the nutritional status of the plant, reducing the consumption of fertilizers from 25% to 50% (Navroski et al., 2016).

The results obtained for the photosynthetic pigment content demonstrate the beneficial effect of the use of water-retaining polymers in reducing the leaching of nutrients from the substrate, especially nitrogen (N). Chlorophyll *a* and *b* contents were higher in plants grown with the addition of the polymer to the substrate. Photosynthetic pigments are used to estimate the nutritional status of N in plants because the amount of these pigments correlates positively with N content in the plant (Smeal & Zhang, 1994). This relationship is attributed mainly to the fact that, in general, 50% to 70% of the total N of leaves is incorporated in enzymes that are associated to the chloroplasts (Chapman & Barreto, 1997), other than being a part of the chlorophyll molecule (Taiz et al., 2017).

However, stressful environmental conditions, such as water restriction, provoke a common response involving the overproduction of reactive oxygen species (ROS), including $^1O_2$, H_2O_2, and O_2•- in plant cells (Pandey et al., 2016). These ROS are unstable and highly reactive molecules that cause serious damage to vital organelles, such as chloroplasts, mitochondria, and DNA on reaction with other molecules, thus causing peroxidation of membrane lipids, protein oxidation, and nucleic acid fragmentation (Gill & Tuteja, 2010). We demonstrated higher lipid peroxidation in plants cultivated under water restriction (4 mm day^{-1}) as compared to plants grown in irrigation regimes with higher water supply (8 and 12 mm day^{-1}). This indicates that the low water supply to *E. contortisiliquum* seedlings causes oxidative stress, causing damage to membrane lipids. In addition, there was a higher content of ROS represented by H_2O_2 in plants under water restriction (4 mm day^{-1}), as compared to the plants in the irrigation regime of 12 mm day^{-1}.

The aforementioned responses obtained are attributable to the fact that under conditions of water stress the photosynthetic activity is inhibited in plant tissues due to an imbalance between light capture and its use (Foyer & Noctor, 2000). The dissipation of excess light energy in the PSII core and the antenna complex leads to the generation of ROS that are potentially hazardous under water restriction conditions (Sharma et al., 2012).

In an attempt to neutralize the ROS and achieve a balance between the synthesis and degradation of these molecules, the plant defense system, which is represented by the antioxidant enzyme superoxide dismutase (SOD), showed 105% higher activity in plants under water restriction (4 mm day^{-1}) as compared to the watering regime that provided 12 mm day^{-1}. Activation of antioxidative enzyme activity has been reported in several plant species under water stress (Sgherri et al., 2004; Sharma & Dubey, 2005). This higher activity of the SOD enzyme under water restriction observed in our study coincides with the higher content of H_2O_2. Overproduction of ROS is opposed enzymatically by means of a complex coordinate system of antioxidant enzymes (Shapiguzov et al., 2012). Among these enzymes, SOD is considered the first line of defense of the plant against $O_2^{\bullet-}$ (Favaretto et al., 2011); it plays a primordial role in the protection of cells against oxidative damage by dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 (Wang et al., 2005). The H_2O_2 has a deleterious action because it participates, together with the $O_2^{\bullet-}$, in the formation reaction of $OH^{\bullet}$, the most reactive oxidant in the ROS family (Sharma et al., 2012). Thus, after the dismutation of the $O_2^{\bullet-}$, the produced H_2O_2 requires the action of other enzymes such as catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidases (POX), which catalyzes the dismutation of H_2O_2 into water and oxygen (Barbosa et al., 2014).

According to results of MDA, H_2O_2 and SOD activity, the irrigation condition of 4 mm day^{-1} presented oxidative damages due to the high levels of peroxidation of lipids and H_2O_2. The accumulation of H_2O_2 favors the peroxidation of lipids, causing oxidative damages (Sharma et al., 2012). As there was a high production of H_2O_2, SOD probably performed the dismutation of the superoxide anion in H_2O_2. However, the enzymes responsible for H_2O_2 elimination, such as catalase and peroxidases, were not efficient in the control of the production of H_2O_2. This probably favored oxidative stress. Thus, in the 4 mm day^{-1} condition, the SOD activity was elevated, but, together with the other antioxidant enzymes, they were not able to neutralize the oxidative damages to the more severe condition of water restriction, observed by the high levels of MDA and H_2O_2, resulting in seedlings with lower morphological quality. Thus, we noticed that water restriction in the 4 mm day^{-1} irrigation regime triggered an abiotic stress situation.

For conditions of 8 and 12 mm day^{-1} even with high levels of H_2O_2 production, there was no oxidative damage, indicated by the reduced levels of lipid peroxidation. ROS under low levels participate in various biological processes in plants such as cell signaling to regulate development, cellular proliferation and differentiation, redox levels, stress signaling, immune response, interactions with other organisms, systemic responses, circadian rhythms, and cell death regulation (Mittler, 2017). That is, ROS participate in various biochemical and physiological processes even under normal growth conditions.

We emphasize the importance of research involving concomitant morphophysiological analysis to allow technical recommendations based on morphological and metabolic analyses.

5. CONCLUSIONS

The development of *E. contortisiliquum* seedlings is dependent on the water regime adopted by the nursery. In this sense, it can be used 8 mm day^{-1} (RR8) in the first 60 days and for the remainder of the period the daily 12 mm day^{-1} (RR12).

The use of water-retaining polymer does not reduce the need for irrigation in *E. contortisiliquum* seedlings. However, it provides greater growth and biomass allocation, regardless of the daily water regime.

E. contortisiliquum seedlings tend to reduce their metabolism when submitted under conditions of low water availability, independent of the addition of the water-retaining polymer. Under these conditions, the seedlings present a smaller increase in biomass due to the overproduction of ROS and oxidative damages, not effectively neutralized by the action of the antioxidant system.

ACKNOWLEDGEMENTS

We would like to thank the Universidade Federal de Santa Maria for the necessary infrastructure for this study.

SUBMISSION STATUS

Received: 10 June 2018
Accepted: 7 July 2019
Associate editor: Marcel Carvalho Abreu
0000-0002-6457-421X

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