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Does the origin of *Eucalyptus* species influence their response to water regime changes?

¿El origen de las especies de Eucalyptus influye en su respuesta a los cambios en el régimen hídrico?

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ABSTRACT

Eucalyptus has been cultivated worldwide because of its good adaptability to different climatic conditions and ease of use in plant breeding programs. However, environmental stress such as drought is unavoidable, affecting the yield of this crop and making the search for genotypes more adapted to these situations, extremely important. The aim of this study was to characterize the physiological responses of young plants in six *Eucalyptus* species that originated from contrasting environments and then subject them to three water regimes consisting of 40, 60 and 80% field capacity (FC). For this, 180-day-old seedlings were transplanted to pots, where they were kept for 85 days in a greenhouse. After the adoption of water regimes, the net photosynthesis (A), transpiration (E), stomatal conductance (g_s) and leaf water potential (Ψ_w) were analyzed. Significant differences were found in all the physiological characteristics evaluated. There were reductions in the A, E, g_s and Ψ_w for all species as the water stress intensified. At 40% FC, *E. tereticornis* was the only one for which plant death occurred. Among the six *Eucalyptus* species studied, *E. saligna* was the most tolerant to water stress followed by *E. urophylla* and *E. camaldulensis*, while *E. tereticornis* was the most sensitive, and it was preceded by *E. brassiana* and *E. grandis*. The interspecific comparative study enabled the identification of the most contrasting species in terms of water stress tolerance, providing support to studies aimed at obtaining drought-tolerant clones.

Keywords: seedlings, eucalypt, water stress, drought tolerance, plant physiology.

RESUMEN

El eucalipto se ha cultivado en todo el mundo debido a su buena adaptabilidad a diferentes condiciones climáticas y su facilidad de uso en programas de fitomejoramiento. Sin embargo, el estrés ambiental como la sequía es inevitable, afectando el rendimiento de este cultivo y haciendo que la búsqueda de genotipos más adaptados a estas situaciones sea de suma importancia. El objetivo de este estudio fue caracterizar las respuestas fisiológicas de plantas jóvenes en seis especies de *Eucalyptus* que se originaron en ambientes contrastantes y luego las sometieron a diferentes regímenes hídricos. Para ello, se trasplantaron plántulas de 180 días a macetas, donde se mantuvieron durante 85 días en un invernadero. Después de la adopción de regímenes hídricos que constan de 40, 60 y 80% de capacidad de campo (FC), se analizaron: fotosíntesis neta (A), transpiración (E), conductancia estomática (gs) y potencial hídrico (Ψw). Hubo reducciones en A, E, gs y Ψw para todas las especies a medida que se intensificó el estrés hídrico. Con 40% FC, *E. tereticornis* fue la única en la que se produjo la muerte de la planta. Entre las seis especies de *Eucalyptus*, *E. saligna* fue la más tolerante al estrés hídrico seguida por *E. urophylla* y *E. camaldulensis*, mientras que *E. tereticornis* fue la más sensible, precedida por *E. brassiana* y *E. grandis*. El estudio comparativo interespecífico permitió identificar las especies más contrastantes en términos de tolerancia al estrés hídrico, apoyando los estudios dirigidos a la obtención de clones tolerantes a la sequía.

Palabras clave: plántulas, eucalipto, estrés hídrico, tolerancia a la sequía, fisiología vegetal.

INTRODUCTION

Eucalyptus is one of the most important genera for the pulp and paper industry, comprising 747 species (Beech et al., 2017). These species are widely cultivated due to their rapid growth, versatile wood properties, and adaptability to diverse soils and climates (Grattapaglia et al., 2012; Torre et al., 2014).

However, environmental stress–particularly drought–is a major abiotic factor limiting plant growth (Galmés et al., 2007), and its frequency is expected to increase with global climate change (Blunden & Arndt, 2012; IPCC, 2014). Increased aridity is expected to reduce crop yields, highlighting the need to expand planting areas and improve drought tolerance in forest species (Kröger, 2012). Drought is considered one of the three most concerning global risks (World Economic Forum, 2016) and causes physiological damage such as reduced turgor, CO_2 assimilation, and photosynthetic efficiency (Taiz et al., 2017).

The first plant response to water deficit is stomatal closure, which limits water loss but also reduces CO_2 diffusion and photosynthesis. Under prolonged stress, osmotic adjustment may temporarily maintain cell turgor, but growth and yield eventually decline (Taiz et al., 2017; Utkhao & Yingjajaval, 2015). Projected global temperature increases may intensify these effects (Leonardi et al., 2015; Pagter & Williams, 2011). Although many *Eucalyptus* species are adapted to different climates, their responses to water deficit vary widely depending on species, developmental stage, and drought intensity (Shulaev, 2008).

Plants have evolved mechanisms to regulate water status and maintain turgor under stress (Chai et al., 2016), and understanding these mechanisms is essential for identifying tolerant species for breeding programs. In regions where water scarcity limits plant productivity, comparative interspecific studies are crucial for identifying traits associated with drought tolerance. Therefore, this study tested the hypothesis that *Eucalyptus* species from contrasting environments differ in their physiological responses to water deficit. Specifically, we evaluated six species—*E. brassiana*, *E. camaldulensis*, *E. urophylla*, *E. tereticornis*, *E. saligna*, and *E. grandis*—under three water regimes to identify the most contrasting species in terms of drought tolerance.

METHODS

Study species. Eucalyptus species were selected from contrasting environments, particularly differing in water availability (Table 1).

Production of Eucalyptus seedlings. This experiment was initially conducted in a greenhouse and later under full sunlight between February and July 2017, using seeds of six *Eucalyptus* species (*E. brassiana, E. camaldulensis, E. urophylla*, *E. tereticornis*, *E. saligna* and *E. grandis*) obtained from the Forestry Science and Research Institute (IPEF). Seeds were sown in 55 cm^3 polyethylene tubes filled with a substrate mixture of coconut fiber, carbonized rice husk and commercial substrate at a 4:3:3 ratio (v/v/v) supplemented with slow-release NPK fertilizer (19:06:10) at 4.0 kg m^{-3}. The tubes were kept in a greenhouse for 35 days after sowing with automatic sprinkler irrigation applied every three hours each day from 8:00 a.m. to 5:00 p.m., each event lasting two minutes. Subsequently, the tubes were transferred to a full-sun area and irrigated by spray four times a day, with each irrigation lasting five minutes. At 45 days after sowing, topdressing fertilization was applied using a solution of monoammonium phosphate (MAP) and potassium chloride (KCl) (1,000 g MAP + 150 g KCl per 100 L of water), at a rate of 10 mL per tube.

Eucalyptus growth under greenhouse conditions. At 180 days after sowing, plants were selected for uniformity, and their root systems were immersed in a MAP solution (1,000 g of MAP / 100 liters of water) to accelerate root development after planting. They were then transplanted into polyethylene pots containing eight liters of a substrate composed of sand and Red Latosol in a 1:1

Table 1. Characteristics of the regions where the studied eucalyptus species naturally occur.

Características de las regiones donde se encuentran naturalmente las especies de eucaliptos estudiadas.

Species	Regions of natural occurrence^{1,2}	Latitudinal range (S)^{2}	Altitudinal range (m)	Annual rainfall (mm)	Dry season (months)*
E. brassiana	Australia, Papua New Guinea	6°-18°	not known	1,000^{2}-1,500^{2}	2-3^{2}
E. camaldulensis	Australia	15°-38°	near sea level to 700^{1}	150^{1}-2,500^{3}	4-8^{2}
E. grandis	Australia	17°-32°	near sea level to 1,100^{1}	1,000^{2,3}-3,500^{1}	3^{2}
E. saligna	Australia	28°-35°	near sea level to 1,100^{1}	800^{2,3}-1,800^{13}	up to 4^{2}
E. tereticornis	Australia, Papua New Guinea	6°-38°	near sea level to 1,000^{1,2}	500^{2,3}-2,500^{1}	up to 7^{2}
E. urophylla	Timor, Indonesia	8°-10°	near sea level to 3,000^{2}	600^{4,5}-1,500^{4,5}	3-8^{4,5}

Source: [1]Boland et al. (2006); [2]FAO (1979); [3]Orwa (2009); [4]PROSEA (1993); [5]Sein & Mitlöhner (2011). *Dry season duration (number of consecutive months with less than 30 mm of rainfall per month).

[1]Boland et al. (2006); [2]FAO (1979); [3]Orwa (2009); [4]PROSEA (1993); [5]Sein & Mitlöhner (2011). *Duración de la estación seca (número de meses consecutivos con menos de 30 mm de lluvia por mes).

ratio (v/v) supplemented with slow-release NPK fertilizer at (19:06:10) at 1.5 kg m^{-3} and single superphosphate at 1.0 kg m^{-3}.

Soil chemical analysis revealed: pH = 6.5; P = 0.87 mg dm^{-3}; K = 79.8 mg dm^{-3}; OM (soil organic matter) = 0.61 dag kg^{-1}; V (%) = 39.47 and the following concentrations (cmol dm^{-3} soil): H+Al = 1.07; Ca = 0.38; Mg = 0.11; SB (sum of bases) = 0.69; and CEC (effective cation exchange capacity) = 1.76. Physical analysis indicated that the soil consisted of 24, 5 and 71% sand, silt and clay, respectively. After transplanting, the plants were maintained in a non-climate-controlled greenhouse with a clear plastic roof and shade cloth sidewalls for 85 days, with soil water status maintained at 80% field capacity (FC).

Mean air temperature and relative humidity inside the greenhouse were monitored using a DT-17 hygrothermograph, programmed to record readings every three hours between August and October 2017 (Figure 1).

Experimental design. The experiment was conducted in a completely randomized design with a 6 x 3 factorial arrangement consisting of six *Eucalyptus* species (*E. brassiana, E. camaldulensis, E. urophylla, E. tereticornis*, *E. saligna* and *E. grandis*) and three water regimes (80, 60 and 40%) using seven replicates with one plant per pot.

Water regimes applied to the plants. At 85 days after transplanting, the plants were subjected to water stress for eight days, during which they were maintained at 40, 60 and 80% FC, where 80% FC was considered the control (without stress) and 40% represented severe water stress based on preliminary tests.

The FC was determined based on soil water potential (water tension) in a known volume of soil irrigated at 100% FC and also for completely dry soil (105 °C 24 h^{-1}). The values were determined using an ML2x ThetaProbe soil moisture sensor (Delta-T Devices Ltd) with DeltaLINK 3.6 software. The volume of water required to reach 40, 60 and 80% FC in each pot was determined based on the soil water tension values and the soil volume used in the pot (8 dm^3). Soil moisture loss was monitored three times per day using the soil moisture sensor to determine the volume of water needed in each pot to maintain the pre-established moisture levels throughout the experiment.

Morphophysiological variables. On the eighth day of water stress (one day after the onset of the first signs of wilting in some plants at 40% FC), the net photosynthesis *(A),* stomatal conductance *(g_s),* transpiration (E) and maximum ($\Psi_{w\,max}$) and minimum leaf water potentials ($\Psi_{w\,min}$) were evaluated.

The A, g_s and E values were obtained by measuring gas exchange using a portable infrared gas analyzer (IRGA LI-6400 model, LI-COR®, Nebraska/USA). In the greenhouse, the plants received irradiance of approximately 1,000 mmol of photons m^{-2} s^{-1} during the measurements. The readings were performed between 8:00 a.m. and 10:30 a.m. on five fully expanded leaves from the middle third of the plants, one leaf per plant of the experimental unit.

The Ψw was measured using a pressure chamber (Scholander et al., 1965) in four fully expanded leaves from the middle third of the plants, one leaf per plant in the experimental unit, which were collected at 5:00 a.m. ($\Psi_{w\,max}$) and 12:00 a.m. ($\Psi_{w\,min}$).

At the end of the eight-day water stress period, plant mortality was recorded for each water regime. Plants subjected to 40% and 80% FC were then harvested and separated into leaves, stems, and roots. These parts were dried in a forced-air oven at 65 °C until constant weight to determine the dry mass of each component. Total dry mass (sum of root and shoot) was calculated for each

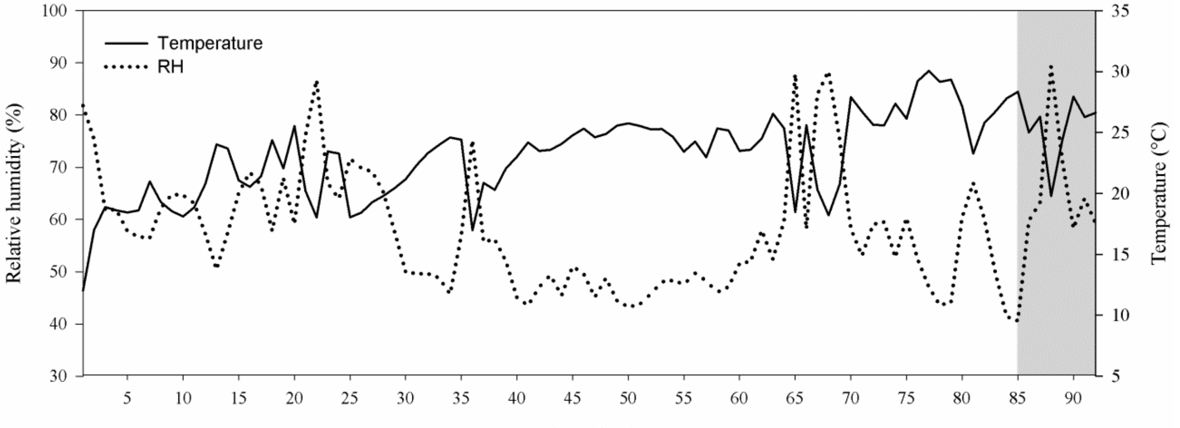

Figure 1. Mean daily air temperature and relative humidity during the experiment in a greenhouse. The gray color represents the period in which the plants were subjected to water regimes of 40, 60 and 80% field capacity.

Temperatura media diaria del aire y humedad relativa durante el experimento en invernadero. El color gris representa el periodo en el que las plantas fueron sometidas a regímenes hídricos de 40, 60 y 80% de la capacidad de campo.

plant to evaluate the effect of water stress on biomass accumulation.

Statistical analysis. The A, g_s, E, Ψ_w and biomass variables (shoot, root, and total dry mass) were subjected to an analysis of variance (ANOVA), and the means were compared by Scott-Knott test ($P < 0.05$). All the analyses were performed using SISVAR software (Ferreira 2014).

RESULTS

The plant mortality rate was calculated at the end of the experiment (on the eighth day of water stress treatment). The only species that exhibited mortality was *E. tereticornis*, with a 57% mortality rate.

Regarding dry mass, although *E. grandis* exhibited the highest total dry mass (TDM) under well-watered conditions (80% FC), the evaluation based on the percentage reduction in biomass between 80% and 40% FC revealed that *E. saligna* was the species that maintained its biomass most effectively under water deficit, with only a 0.36% reduction in TDM, followed by *E. urophylla* (0.67%). In contrast, *E. tereticornis* showed the greatest reduction (19.1%), consistent with its higher mortality rate at 40% FC. These results demonstrate that while absolute TDM values under optimal moisture conditions are informative, the relative reduction in biomass under stress is a more sensitive indicator of drought tolerance (Table 2).

In the species under study, significant simultaneous effects ($P < 0.05$) were observed for the pecies × water regime interaction across all analyzed physiological variables (A, E and g_s), with a decrease in these variables for all the species as the water stress intensified (Table 3).

With regard to photosynthesis *(A),* at 80% FC, the species *E. camaldulensis* (22.67 µmol m^{-2} s^{-1}), *E. saligna* (21.16 µmol m^{-2} s^{-1}), *E. tereticornis* (21.56 µmol m^{-2} s^{-1}) and *E. brassiana* (22.96 µmol m^{-2} s^{-1}) showed the highest A rates. However, a marked reduction in this variable was observed for all the species when they were kept at 40% FC, except for *E. saligna*, which, despite exhibiting reduced photosynthesis with increased water stress, maintained higher rates than the other species. These reductions were on the orders of 15, 42, 46, 55, 74 and 81% for *E. saligna*, *E. urophylla*, *E. tereticornis*, *E. grandis*, *E. brassiana* and *E. camaldulensis*, respectively. At 60% FC, the A remained similar across species, not differing from the 80% FC condition.

Unlike A, the stomatal conductance (g_s) was higher in *E. brassiana* (0.52 mol m^{-2} s^{-1}) at 80% FC. However, when the plants were kept at 40% FC, the reduction in this variable was more pronounced (88%). When kept in pots at 60% FC, the g_s did not show significant differences between species, while under severe water stress (40% FC), the species *E. saligna* and *E. tereticornis* showed the highest g_s values and reductions of 48 and 57%, respectively, when compared to the control (80% FC).

It was also observed that at 80% FC, the transpiration (E) was higher in *E. saligna* (6.91 mmol m^{-2} s^{-1}) and *E. brassiana* (7.32 mmol m^{-2} s^{-1}). However, under severe water stress conditions (40% FC), these species experienced reductions of 44% (3.88 mmol m^{-2} s^{-1}) and 81% (1.41 mmol m^{-2} s^{-1}), respectively.

Significant differences ($P < 0.05$) were found for the species and water regime sources in terms of the maximum ($\Psi_{w\,max}$) and minimum leaf water potentials ($\Psi_{w\,min}$), and an interaction effect between species and water regime was observed.

Table 4 shows the mean water potential for the six *Eucalyptus* species that were kept in pots at three FC levels. At 80% FC, there was no significant difference

Table 2. Root, shoot, and total plant dry mass (g) of six *Eucalyptus* species under 40 and 80% field capacity (FC).

Masa seca de raíz, parte aérea y masa seca total deseis especies de *Eucalyptus* bajo diferentes regímenes hídricos.

Species	FC (%)											
	80		40		80		40		80		40	
	Root (g)				Shoot (g)				Total plant (g)			
E. grandis	16.75	Aa	16.00	Aa	65.00	Aa	62.00	Aa	81.75	Aa	78.00	Aa
E. camaldulensis	18.75	Aa	17.75	Aa	56.75	Aa	56.00	Aa	75.50	Aa	73.75	Aa
E. saligna	15.25	Aa	16.75	Aa	54.00	Aa	52.25	Aa	69.25	Aa	69.00	Aa
E. urophylla	19.25	Aa	18.50	Aa	56.00	Aa	55.25	Aa	74.25	Aa	73.75	Aa
E. tereticornis	13.75	Aa	11.75	Ba	53.00	Aa	42.25	Bb	66.75	Aa	54.00	Aa
E. brassiana	17.00	Aa	15.25	Aa	51.75	Aa	47.50	Ba	68.75	Aa	62.75	Aa

Means followed by the same letters for the same physiological variable, with uppercase letters in the columns and lowercase letters in the rows, do not differ statistically from one another according to the Scott-Knott test ($P < 0.05$).

Las medias seguidas de las mismas letras para una misma variable fisiológica, con letras mayúsculas en las columnas y minúsculas en las filas, no difieren estadísticamente entre sí según la prueba de Scott-Knott ($P < 0,05$).

between the species for $\Psi_{w\,max}$. However, the $\Psi_{w\,min}$ exhibited a significant difference, ranging from -1.14 MPa (*E. tereticornis*) to -1.86 MPa (*E. camaldulensis*). At 60% FC, there was no significant difference between the species for either potential. Under low soil water availability conditions (40%), a significant difference was observed between the species for both $\Psi_{w\,max}$ and $\Psi_{w\,min}$, with the greatest difference in $\Psi_{w\,max}$ being 0.50 MPa, observed in *E. saligna* (-0.33 MPa) and *E. brassiana* (-0.83 MPa). For $\Psi_{w\,min}$, the greatest difference was 0.86 MPa between the species *E. saligna* (-1.55 MPa) and *E. brassiana* (-2.41 MPa).

There was a decreasing trend in the potentials (maximum and minimum) as a function of the water regime adopted for all the studied species. In general, this trend was more pronounced for Ψ_{wmin} at 40% FC, where *E. saligna* reached a value of -1.55 MPa, while the other species maintained values close to -2.0 MPa. In addition, *E. saligna* showed the smallest difference between $\Psi_{w\,max}$ and $\Psi_{w\,min}$ (1.22 MPa), followed by *E. urophylla* (1.24 MPa), *E. camaldulensis* (1.29 MPa), *E. grandis* (1.50 MPa) and *E. brassiana* (1.59 MPa). In turn, *E. tereticornis* showed the greatest difference between $\Psi_{w\,max}$ and $\Psi_{w\,min}$ (1.73 MPa), and during the period of the day corresponding to the highest water

Table 3. Net photosynthesis (*A*), transpiration (*E*) and stomatal conductance (g_s) of six *Eucalyptus* species under 40, 60 and 80% field capacity (FC).

Fotosíntesis (A), transpiración (E) y conductancia estomática (gs) de seis especies de Eucalyptus bajo diferentes regímenes hídricos.

Species	FC (%)								
	80	60	40	80	60	40	80	60	40
	A (µmol $m^{-2}\,s^{-1}$)			*E* (mmol $m^{-2}\,s^{-1}$)			g_s (mol $m^{-2}\,s^{-1}$)		
E. grandis	17.80 Ba	19.39 Aa	8.02 Cb	4.52 Ca	4.11 Ca	1.39 Bb	0.42 Ba	0.35 Aa	0.06 Bb
E. camaldulensis	22.67 Aa	20.23 Aa	4.26 Cb	5.47 Ba	3.80 Cb	1.19 Bc	0.40 Ba	0.33 Aa	0.05 Bb
E. saligna	21.16 Aa	22.45 Aa	17.98 Ab	6.91 Aa	6.45 Aa	3.88 Ab	0.37 Ba	0.37 Aa	0.19 Ab
E. urophylla	18.60 Ba	19.37 Aa	10.72 Bb	4.12 Ca	3.82 Ca	0.93 Bb	0.40 Ba	0.33 Aa	0.06 Bb
E. tereticornis	21.56 Aa	22.98 Aa	11.74 Bb	5.43 Ba	5.10 Ba	1.79 Bb	0.39 Ba	0.42 Aa	0.17 Ab
E. brassiana	22.96 Aa	21.41 Aa	5.98 Cb	7.32 Aa	4.98 Bb	1.41 Bc	0.52 Aa	0.33 Ab	0.06 Bc

Means followed by the same letters for the same physiological variable, with uppercase letters in the columns and lowercase letters in the rows, do not differ statistically from one another according to the Scott-Knott test ($P < 0.05$).
Las medias seguidas de las mismas letras para una misma variable fisiológica, con letras mayúsculas en las columnas y minúsculas en las filas, no difieren estadísticamente entre sí según la prueba de Scott-Knott ($P < 0,05$).

Table 4. Maximum ($\Psi_{w\,max}$) and minimum leaf water potential ($\Psi_{w\,min}$) values of six *Eucalyptus* species under 40, 60 and 80% field capacity (FC).

Valores máximos (Ψw max) y mínimos del potencial hídrico foliar (Ψw min) de seis especies de Eucalyptus bajo diferentes regímenes hídricos.

Species	FC (%)					
	80	60	40	80	60	40
	$\Psi_{w\,max}$ (MPa)			$\Psi_{w\,min}$ (MPa)		
E. grandis	-0.37 Aa	-0.31 Aa	-0.45 Aa	-1.26 Aa	-1.47 Aa	-1.95 Bb
E. camaldulensis	-0.15 Aa	-0.16 Aa	-0.71 Bb	-1.86 Ba	-1.88 Aa	-2.00 Ba
E. saligna	-0.30 Aa	-0.30 Aa	-0.33 Aa	-1.45 Aa	-1.54 Aa	-1.55 Aa
E. urophylla	-0.28 Aa	-0.38 Aa	-0.69 Ba	-1.36 Aa	-1.55 Aa	-1.93 Bb
E. tereticornis	-0.23 Aa	-0.28 Aa	-0.34 Aa	-1.14 Aa	-1.62 Ab	-2.07 Bc
E. brassiana	-0.23 Aa	-0.23 Aa	-0.83 Bb	-1.57 Ba	-1.37 Aa	-2.41 Bb

Means followed by the same letters for the same leaf water potential, with uppercase letters in the columns and lowercase letters in the rows, do not differ statistically from one another according to the Scott-Knott test ($P < 0.05$).
Las medias seguidas de las mismas letras para el mismo potencial hídrico foliar, con letras mayúsculas en las columnas y minúsculas en las filas, no difieren estadísticamente entre sí según la prueba de Scott-Knott ($P < 0,05$).

stress, $\Psi_{w\ min}$ was less than -2.0 MPa. In addition, this was the only species for which the $\Psi_{w\ min}$ decreased at 60% FC.

DISCUSSION

Plant growth and biomass accumulation are integrative indicators of plant performance under contrasting water conditions. As observed in the results, differences in total dry mass (TDM) among *Eucalyptus* species under 80% and 40% FC revealed contrasting responses to water deficit. Species such as *E. saligna* and *E. urophylla* were able to maintain their biomass production with minimal reductions in TDM, while *E. tereticornis* showed a marked decrease under limited soil moisture conditions.

These patterns suggest that the higher drought tolerance of *E. saligna* and *E. urophylla* is associated with physiological mechanisms that support carbon assimilation and water-use efficiency under stress (Ngugi et al., 2003). In contrast, the reduction in biomass observed for *E. tereticornis* and *E. brassiana* indicates a lower capacity to sustain growth under reduced water availability (Merchant et al., 2007; Tatagiba et al., 2007).

In general, the water regime promoted a dramatic reduction in all the physiological variables (*A*, *E* and g_s) analyzed here, especially when contrasting extreme conditions (80 and 40% FC). This result demonstrates the strong effect of water availability on the physiological performance of the studied species. The species *E. saligna* displayed the highest values for these variables, showing that it is the most adapted to soil water stress conditions, which can positively affect its growth and establishment in the field when compared to the other species.

Regarding net photosynthesis, a 15% reduction was observed (from 21.16 to 17.98 μmol m^{-2} s^{-1}) for *E. saligna*, while the other species exhibited reductions greater than 42%, with *E. brassiana* and *E. camaldulensis* showing the highest decreases (74 and 81%, respectively). The marked reduction in photosynthesis is commonly associated with water stress and is a consequence of several physiological and biochemical processes, such as stomatal limitation of CO_2 influx due to abscisic acid (ABA) accumulation, damage to the photochemical apparatus, and decreased Rubisco activity (Bertolli et al*.*, 2015; Pinheiro et al*.*, 2011; Taiz et al*.*, 2017). Even under low soil water availability, *E. saligna* maintained a high photosynthetic rate, highlighting its photosynthetic efficiency and ability to sustain growth under drought stress.

At 80% FC, *E. saligna* and *E. brassiana* presented the highest transpiration values, suggesting similar water loss under optimal conditions. However, under severe water stress (40% FC), *E. saligna* reduced transpiration by 44%, compared to 81% in *E. brassiana*, indicating a more effective regulation of water loss. To avoid tissue dehydration, plants reduce transpiration to maintain high leaf water potential values, which is one of the primary mechanisms of plant adaptation to water stress (Taiz et al*.*, 2017). In the present study, there was a decreasing trend in the transpiration rates across all species when the water regimes of 80 and 40% FC were compared, coinciding with the reductions observed in the stomatal conductance. This direct relationship between *E* and g_s is expected because as the stomata close, there is a decrease in water vapor flow from the plant to the atmosphere, and consequently in transpiration (Gonçalves et al*.*, 2010).

The ability to restrict water loss through stomatal closure and to enhance water uptake by the root system are important mechanisms for maintaining cell turgor and physiological activity under water deficit (Ngugi et al., 2004). In *Eucalyptus* species, transpiration is typically high when water is readily available, but it can be substantially reduced as soil water declines through partial or total stomatal closure (Lane et al., 2004). In the present study, this mechanism is reflected in the observed decreases in stomatal conductance and transpiration at lower soil water regimes, which help to explain the variations in leaf water potential and photosynthetic performance among the species.

At 80% FC, stomatal conductance was highest in *E. brassiana*, and at 60% FC, this was the only species in which a reduction in this variable was observed. At 40% FC, the reduction in the g_s was more pronounced, suggesting that young plants of this species do not have a high adaptive capacity to environments with low soil water availability. Moreover, under severe water stress, there was a reduction of 48% for *E. saligna* and 57% for *E. tereticornis* when compared to the control (80% FC), whereas in the other species, this value was higher than 85%. These results demonstrate the greater capacity of *E. saligna* and *E. tereticornis* for regulating stomatal opening and closure. The greater opening of the stomata observed in these species may have contributed to their greater carbon assimilation because the influx of CO_2 occurs through the stomata, which is necessary for the photosynthetic process and the growth of the plants (Taiz et al*.* 2017).

Plants in the species *E. tereticornis* presented a high g_s value when kept at 40% FC. However, during the water stress period, this was the only species that exhibited plant death, with a mortality rate of 57% at the end of the experiment. This mechanism of stomatal closure and transpiration limitation that plants exhibit to minimize water loss can vary considerably between species, and it may be correlated with water stress tolerance (Inman-Bamber & Smith, 2005). According to Hopkins & Huner (2012), the plant hardly transpires at night, and the water uptake rate, although low, remains higher than the transpiration rate, promoting tissue rehydration. Thus, this result suggests that *E. tereticornis* is not able to adapt to low soil water availability conditions because the plants probably did not rehydrate during the night due to the lack of an efficient water transport process in the soil-plant-atmosphere system. This interpretation is based on the potential difference observed for the maximum and minimum leaf water potentials under severe drought stress (40% FC), which contributed to the death of the plants.

The decreasing trend for the maximum ($\Psi_{w\,max}$) and minimum water potentials ($\Psi_{w\,min}$) observed in *Eucalyptus* species subjected to three water regimes was expected, considering that the reduction in soil moisture affects the cell turgor through several physiological, biochemical and genetic mechanisms (Bartels & Sunkar, 2005; Bedon et al*.*, 2012).

Ψ_w indicates the degree of hydration of a plant; it is the primary component responsible for the water flow and may represent the water stress to which the plant is subjected (Ferreira et al., 1999). Chaves et al*.* (2004) observed a reduction in Ψ_w at the end of the drought cycles in eucalypt clones under water stress, and it ranged from -0.80 to -1.98 MPa. The leaf water potential of fully irrigated plants ranged from -0.25 to -0.35 MPa. Fernandes et al. (2015) also observed a decrease in Ψ_w in eucalyptus plants under water stress. These results were similar to those found in the present study since there was a reduction in Ψ_w according to the adopted water regime. Stomatal conductance is dependent on the cell volume, which decreases under water stress conditions, ultimately reducing the leaf water potential. The decrease in g_s and *E,* and ultimately in the photosynthesis rate, are the first changes that occur in plants in response to water stress (Chaves et al*.*, 2003; Pita et al*.*, 2005).

In the present study, *E. saligna* exhibited the highest values for $\Psi_{w\,max}$ (-0.33 MPa) and $\Psi_{w\,min}$ (-1.55 MPa) at 40% FC. The leaf water potentials between -1.0 and -2.0 MPa indicate moderate water stress and relative drought tolerance, according to Taiz et al*.* (2017). In addition, this species showed the smallest difference between $\Psi_{w\,max}$ and $\Psi_{w\,min}$ (1.22 MPa) under low soil water availability conditions, indicating a greater ability to regulate water loss through transpiration and, therefore, more efficient stomatal control. For all the physiological variables analyzed here, *E. saligna* demonstrated the best performance under water stress conditions, which is a strong indicator of the greater tolerance of this species compared to the other study species. These results are consistent with those reported by Orwa (2009), who stated that *E. saligna* tolerates short periods of drought, although this species occurs preferentially in regions with high precipitation that is distributed evenly throughout the year.

Although the region of natural occurrence for *E. urophylla* is restricted in terms of latitude (8-10º S), the altitudinal range is high (0-3,000 m), covering areas with annual precipitation ranging from 600 to 1,500 mm (Table 1). Similar to *E. saligna*, *E. urophylla* exhibited a small difference between the $\Psi_{w\,max}$ and $\Psi_{w\,min}$ (1.24 MPa) under severe water stress, indicating that this species also has efficient mechanisms to regulate water loss.

For the species *E. camaldulensis* and *E. grandis*, the differences between the $\Psi_{w\,max}$ and $\Psi_{w\,min}$ at 40% FC were 1.29 and 1.50 MPa, respectively. In addition, at 80% FC, both *A* and *E* were higher in *E. camaldulensis*. This species has the widest geographic distribution within the natural area of occurrence for the *Eucalyptus* genus, having been found throughout the entire Australian territory. Its natural occurrence region has a broad annual precipitation range (150 to 2,500 mm), and the dry season in some of these regions can last up to eight months (Table 1). For this reason, *E. camaldulensis* is able to grow under a wide range of climatic conditions, from mild to hot and from humid to arid areas (Orwa, 2009). Thus, based on the results of the present study, it is possible that the material used here comes from a region with a more pronounced dry season because *E. camaldulensis* exhibited a notable degree of tolerance to water stress.

In turn, *E. grandis* demonstratedsensitivity to water stress. In fact, this species is known to occur in humid and hot subtropical regions (FAO, 1979) where the annual precipitation is high (1,000 - 3,500) and the dry season is rarely severe, not exceeding three months (Table 1). This information may explain the greater sensitivity of the species.

Similarly, *E. brassiana* showed a large difference between $\Psi_{w\,max}$ and $\Psi_{w\,min}$ (1.58 MPa), which adversely affected the physiological variables under study, in addition to the lowest values for $\Psi_{w\,max}$ (-0.83 MPa) and $\Psi_{w\,min}$ (-2.41 MPa). According to Taiz et al. (2017), leaf water potentials below -2.0 MPa indicate severe water stress. Although the stress period was only eight days, *E. brassiana* showed sharp reductions in photosynthesis (−74%), transpiration (−81%), and stomatal conductance (−88%) at 40% FC compared to well-watered plants. These results indicate rapid stomatal closure and strong limitation to gas exchange, which restricted CO_2 assimilation and led to severe dehydration. This rapid decline under short-term stress suggests that *E. brassiana* has low physiological plasticity to cope with sudden reductions in soil moisture, consistent with its natural occurrence in regions where the dry season is relatively short (Table 1).

The species *E. tereticornis* exhibited the largest difference between the $\Psi_{w\,max}$ and $\Psi_{w\,min}$ (1.73 MPa) at 40% FC, and during the day with the highest transpiration demand, the $\Psi_{w\,min}$ was -2.07 MPa, which is considered severe water stress. According to Taiz et al*.* (2017), when critical Ψ_w values (below -2.0 MPa) are reached, the stomata begin to close substantially, thus affecting the physiological characteristics of the plant, such as $g_{s,}$ *E* and *A.* These results suggest that *E. tereticornis* may be the most sensitive to water stress among all the studied species. Within the genus *Eucalyptus, E. tereticornis* is the one with the widest latitudinal distribution and the one that is subject to one of the largest annual precipitation ranges (Table 1). Therefore, it is also possible that the genetic material used in the present study comes from a region with high precipitation because the species behaved as the most sensitive among those tested based on the evaluation of the leaf water potential, which is the most effective parameter to indicate the tolerance/sensitivity of a species (Merchant et al., 2007; Tatagiba et al., 2007).

Variations in the leaf water potential may affect carbon assimilation, leading to stomatal closure and decreased photosynthesis, so that under drought conditions, plants reduce their water loss by reducing stomatal conductance. Therefore, to promote cell turgor in water stress situations, adjustments to cell metabolism occur, such as the synthesis and/or accumulation of osmolytes, which contribute to plant osmoregulation (Silva et al., 2004).

CONCLUSIONS

Among the six species studied here, *E. saligna* demonstrated the greatest tolerance to water stress, followed by *E. urophylla* and *E. camaldulensis*. Regarding sensitivity, *E. tereticornis* was identified as the most sensitive, and *E. brassiana* and *E. grandis* also exhibited sensitivity to water stress. At the end of the experiment, *E. tereticornis* was the only species that exhibited plant death, with a mortality rate of 57%. For the studied species, we confirmed the hypothesis that the physiological characteristics of eucalyptus species originating from contrasting environments in terms of water availability can differ, allowing the identification of species with distinct levels of water stress tolerance.

AUTHORS CONTRIBUTIONS

All authors contributed to the study conception and design; R.S.M., G.P.C., B.S.B. and B.T.F collected the data; R.S.M. and G.P.C performed the data analyses; all authors interpreted the results; R.S.M., A.C.J., L.A.M and J.M.R.F wrote the first draft of the manuscript; all authors reviewed and edited the manuscript; all authors read and approved the final manuscript.

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REFERENCES

Bartels, D., & Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences 24*(1): 23-58. DOI: https://doi.org/10.1080/07352680590910410

Beech, E., Rivers, M., Oldfield, S., & Smith, P. P. (2017). Global Tree Search: The first complete global database of tree species and country distributions. *Journal of Sustainable Forestry 36*(5): 454-489. DOI: https://doi.org/10.1080/10549811.2017.1310049

Bedon, F. E., Villar, D., Vicent, J-W., Dupuy, A-M., Lomenech, A., Mabialangoma, P., Chaumeil, A., Barré, C., Plomion, & J., Gion. (2012). Proteomic plasticity of two *Eucalyptus* genotypes under contrasted water regimes in the field. *Plant, Cell & Environment 35*(4): 790-805. DOI: https://doi.org/10.1111/j.1365-3040.2011.02452.x

Bertolli, S. C., Souza, J. D., & Souza, G. M. (2015). Caracterização fotossintética da espécie isohídrica pata-de-elefante em condições de deficiência hídrica. *Revista Caatinga 28*(3): 196-205. DOI: http://dx.doi.org/10.1590/1983-21252015v28n322rc

Blunden, J., & Arndt, D. S. (2012). State of the climate in 2011. Special Supplement. *Bulletin of the American Meteorological Society 93*(7): 1-264. DOI: https://doi.org/10.1175/2012BAMSStateoftheClimate.1

Boland, D. J., Brooker, M. I. H., Chippendale, G. M., Hall, N., Hyland, B.P.M., Johnston, R. D., Kleinig, D. A., McDonald, M. W., & Turner, J. D. (2006). *Forest trees of Australia*, CSIRO Publishing, Australia. 736 p.

Chai, Q., Gan, Y., Zhao, C., Xu, H. L., Waskom, R. M., Niu, Y., & Siddique, K. H. (2016). Regulated deficit irrigation for crop production under drought stress. A review. *Agronomy for Sustainable Development 36*(3): 1-21. DOI: https://doi.org/10.1007/s13593-015-0338-6

Chaves, J. H., Reis, G. G. D., Reis, M. D. G. F., Neves, J. C. L., Pezzopane, J. E. M., & Polli, H. Q. (2004). Seleção precoce de clones de eucalipto para ambientes com disponibilidade diferenciada de água no solo: relações hídricas de plantas em tubetes. *Revista Árvore 28*(3): 333-341. DOI: http://dx.doi.org/10.1590/S0100-67622004000300003

Chaves, M. M., Maroco, J. P., & Pereira, J. S. (2003). Understanding plant responses to drought: from genes to the whole plant. *Functional Plant Biology 30*(3): 239-264. DOI: http://dx.doi.org/10.1071/FP02076

FAO.(1979). Eucalypts for Planting. Food & Agriculture Organization of the United Nations, Italy.

Fernandes, E. T., Cairo, P. A. R., & Novaes, A. B. D. (2015). Respostas fisiológicas de clones de eucalipto cultivados em casa de vegetação sob deficiência hídrica. *Ciência Rural 45*(1): 29-34. DOI: http://dx.doi.org/10.1590/0103-8478cr20120152

Ferreira, C. A. G., Davide, A. C., & de Carvalho, L. R. (1999). Relações hídricas em mudas de *Eucalyptus citriodora* Hook., em tubetes, aclimatadas por tratamentos hídricos. *Cerne 5*(2): 95-104.

Ferreira, D. F. (2014). Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. *Ciência e Agrotecnologia* 38(2): 109-112. DOI: http://dx.doi.org/10.1590/S1413-70542014000200001

Galmés, J., Medrano, H., & Flexas, J. (2007). Photosynthetic limitations in response to water stress and recovery in mediterranean plants with different growth forms. *New Phytologist 175*(1): 81-93. DOI: http://dx.doi.org/10.1111/j.1469-8137.2007.02087.x

Gonçalves, E. R., Ferreira, V. M., Silva, J. V., Endres, L., Barbosa, T. P., & Duarte, W. D. G. (2010). Trocas gasosas e fluorescência da clorofila a em variedades de cana-de-açúcar submetidas à deficiência hídrica. *Revista Brasileira de Engenharia Agrícola e Ambiental 14*(4): 378-386. DOI: http://dx.doi.org/10.1590/S1415-43662010000400006

Grattapaglia, D., Vaillancourt, R. E., Shepherd, M., Thumma, B. R., Foley, W., Külheim, C., & Myburg, A. A. (2012). Progress in Myrtaceae genetics and genomics: *Eucalyptus* as the pivotal genus. *Tree Genetics & Genomes 8*,463-508. DOI: https://doi.org/10.1007/s11295-012-0491-x

Hopkins, W. G., & Huner, N. P. A. (2012). *Introduction to Plant Physiology*. India. Wiley. 576 p.

Inman-Bamber, N. G., & Smith, D. M. (2005). Water relations in sugarcane and response to water deficits. *Field Crops Research* 92(2-3): 185-202. DOI: https://doi.org/10.1016/j.fcr.2005.01.023

Intergovernmental Panel on Climate Change - IPCC. (2014).

Kröger, M. (2012). Global tree plantation expansion. ICAS review paper series

Lane, P. N., Morris, J., Ningnan, Z., Guangyi, Z., Guoyi, Z., & Daping, X. (2004). Water balance of tropical eucalypt plantations in southeastern China. ↱ *Agricultural and Forest Meteorology* (3): 253-267. DOI: https://doi.org/10.1016/j.agrformet.2004.01.015

Leonardi, G. D. A., Carlos, N. A., Mazzafera, P., & Balbuena, T. S. (2015). *Eucalyptus urograndis* stem proteome is responsive to short-term cold stress. *Genetics and Molecular Biology* (2): 191-198. DOI: https://doi.org/10.1590/S1415-475738220140235

Merchant, A., Callister, A., Arndt, S., Tausz, M., & Adams, M. (2007). Contrasting Physiological Responses of Six *Eucalyptus* Species to Water Deficit. *Annals of Botany 100*(7): 1507-1515. DOI: https://doi.org/10.1093/aob/mcm234

Ngugi, M. R., Doley, D., Hunt, M. A., Dart, P., & Ryan, P. (2003). Leaf water relations of *Eucalyptus cloeziana* and *Eucalyptus argophloia* in response to water deficit*.* Tree Physiology: 23(5): 335-343. DOI: https://doi.org/10.1093/treephys/23.5.335

Ngugi, M. R., Doley, D., Hunt, M. A., Ryan, P., & Dart, P. (2004). Physiological responses to water stress in *Eucalyptus cloeziana* and *E. argophloia* seedlings. *Trees 18*(4): 381-389. DOI: https://doi.org/10.1007/s00468-003-0316-5

Orwa, C. (2009). *Agroforestree Database: a tree reference and selection guide version 4.0*. World Agroforestry Centre, Kenya.

Pagter, M., & Williams, M. (2011). Frost dehardening and rehardening of *Hydrangea macrophylla* stems and buds. *HortScience 46*(8): 1121-1126. DOI: https://doi.org/10.21273/HORTSCI.46.8.1121

Pinheiro, C., & Chaves, M. M. (2011). Photosynthesis and drought: can we make metabolic connections from avaiable data? *Journal of Experimental Botany 62*(3): 869-882. DOI: https://doi.org/10.1093/jxb/erq340

Pita, P., Cañas, I., Soria, F., Ruiz, F., & Toval, G. (2005). Use of physiological traits in tree breeding for improved yield in drought-prone environments: the case of *Eucalyptus globulus*. *Investigación agrarian 14*(3): 383-393. DOI: https://doi.org/10.5424/srf/2005143-00931

PROSEA. 1993. Timber trees: Minor commercial timbers. In: Soerianegara I and Lemmens RHMJ (Eds) *Plant resources of South-East Asia*. Netherlands. Pudoc Scientific Publishers. 656 p.

Sein, C. C., & Mitlöhner, R. (2011). *Eucalyptus urophylla* S.T. Blake: ecology and silviculture. Indonesia. CIFOR. 16 p.

Scholander, P. F., Bradstreet, E. D., Hemmingsen, E. A., & Hammel, H. T. (1965). Sap pressure in vascular plants. *Science 148*(3668): 339-346. DOI: https://doi.org/10.1126/science.148.3668.339

Shulaev, V., Cortes, D., Miller, G., & Mittler, R. (2008). Metabolomics for plant stress response. *Physiologia Plantarum 132*(2): 199-208. DOI: https://doi.org/10.1111/j.1399-3054.2007.01025.x

da Silva, E. C., Nogueira, R. J. M. C., de Azevedo Neto, A. D., de Brito, J. Z., & Cabral, E. L. (2004). Aspectos ecofisiológicos de dez espécies em uma área de caatinga no município de Cabaceiras, Paraíba, Brasil. *Série Botânica 59*(2): 201-205.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2017). Fisiologia e Desenvolvimento Vegetal. Porto Alegre. Editora Artmed. 888 p.

Tatagiba, S. D., Pezzopane, J. E. M., & dos Reis, E. F. (2007). Avaliação do crescimento de clones de *Eucalyptus* submetidos a diferentes manejos de irrigação. *Cerne, 13*(1): 1-9

de la Torre, F., Rodríguez, R., Jorge, G., Villar, B., Alvarez-Otero, R., Grima-Pettenati, J., & Gallego, P. P. (2014). Genetic transformation of Eucalyptus globulus using the vasculars-pecific EgCCR as an alternative to the constitutive CaMV35S promoter. *Plant Cell, Tissue Organ Culture 117*(1): 77-84. DOI: https://doi.org/10.1007/s11240-013-0422-z

Utkhao, W., & Yingjajaval, S. (2015). Changes in leaf gas exchange and biomass of *Eucalyptus camaldulensis* in response to increasing drought stress induced by polyethylene glycol. Trees *29*(5): 1581-1592. DOI: https://doi.org/10.1007/s00468-015-1240-1

World Economic Forum. (2016). *The global risks report 2016* (11th ed.). Geneva.

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