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Original Article

Role of *Trichoderma* spp. in mitigating water deficiency and promoting *Eucalyptus urophylla* growth

Papel de *Trichoderma* spp. na mitigação da deficiência hídrica e promoção do crescimento de *Eucalyptus urophylla*

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Abstract

Trichoderma spp. induce physiological and biochemical changes in plants, potentially enhancing growth and mitigating water deficit effects. This study evaluated morphophysiological parameters, water status indicators, gas exchange, and biochemical and metabolic responses of *Eucalyptus urophylla* under different water regimes and inoculation with *T. asperellum* and *T. harzianum*, as well as their impact on early field growth. A greenhouse experiment followed a 3 × 2 factorial design: three inoculation treatments (*T. asperellum* [TA], *T. harzianum* [TH], and control [C]) and two water regimes—water deficit (30% pot capacity) and well-irrigated (90% pot capacity). Morphophysiological traits, water status, gas exchange, and metabolic parameters were assessed. Additionally, three independent field experiments tested inoculation effects, with 250 seedlings per treatment planted at 2.5 × 3.5 m spacing. The effective experimental area comprised 60 central plots per treatment. Plant height, leaf number, and stem diameter were measured at 90 days post-transplant, while photosynthetic pigments, sugars (total, reducing, and soluble), starch, and proline were analyzed at 270 days. Water deficit reduced plant growth, leaf water potential, relative water content, and gas exchange while increasing thiobarbituric acid-reactive substances and hydrogen peroxide levels. Inoculation with *T. asperellum* and *T. harzianum* did not alleviate water deficit-induced growth reduction. However, regardless of the water regime, inoculation enhanced photosynthetic activity, water-use efficiency, and soluble sugars and amino acid accumulation. Under field conditions, inoculated plants exhibited greater height, stem diameter, and leaf number but lower reducing sugars, soluble sugars, and starch levels compared to control plants.

Keywords: eucalyptus, water stress, physiological responses, *Trichoderma asperellum*, *Trichoderma harzianum*.

Resumo

Trichoderma spp. induz mudanças fisiológicas e bioquímicas em plantas, potencialmente aumentando o crescimento e mitigando os efeitos do deficit hídrico. Este estudo avaliou parâmetros morfofisiológicos, indicadores do estado hídrico, trocas gasosas e respostas bioquímicas e metabólicas de *Eucalyptus urophylla* sob diferentes regimes hídricos e inoculação com *T. asperellum* e *T. harzianum*, bem como seu impacto no crescimento inicial em campo. Um experimento em casa de vegetação seguiu um delineamento fatorial 3 × 2: três tratamentos de inoculação (*T. asperellum* [TA], *T. harzianum* [TH] e controle [C]) e dois regimes hídricos — deficit hídrico (30% da capacidade do vaso) e bem irrigado (90% da capacidade do vaso). Características morfofisiológicas, estado hídrico, trocas gasosas e parâmetros metabólicos foram avaliados. Além disso, três experimentos de campo independentes testaram os efeitos da inoculação, com 250 mudas por tratamento plantadas em espaçamento de 2,5 × 3,5 m. A área experimental efetiva compreendeu 60 parcelas centrais por tratamento. A altura da planta, o número de folhas e o diâmetro do caule foram medidos 90 dias após o transplante, enquanto pigmentos fotossintéticos, açúcares (total, redutor e solúvel), amido e prolina foram analisados em 270 dias. O deficit hídrico reduziu o crescimento da planta, o potencial hídrico foliar, o teor relativo de água e as trocas gasosas, enquanto aumentou os teores de substâncias reativas ao ácido tiobarbitúrico e de peróxido de hidrogênio. A inoculação com *T. asperellum* e *T. harzianum* não aliviou a redução do crescimento induzida pelo deficit hídrico. No entanto, independentemente do regime hídrico, a inoculação aumentou a atividade fotossintética, a eficiência do uso da água e os teores de açúcares solúveis e aminoácidos. Em condições de campo, as plantas inoculadas exibiram maior altura, diâmetro do caule e número de folhas, mas menores teores de açúcares redutores, açúcares solúveis e amido em comparação a plantas controle.

Palavras-chave: eucalipto, estresse hídrico, respostas fisiológicas, *Trichoderma asperellum*, *Trichoderma harzianum*.

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1. Introduction

Eucalyptus cultivation plays a crucial role in supplying forest products such as timber, charcoal, resins, plywood, cellulose, and paper while reducing pressure on native forests (Tupinambá-Simões et al., 2022). However, drought is a major constraint on eucalyptus growth and productivity. In *Eucalyptus urophylla*, water restriction reduces leaf water potential, relative water content, gas exchange, and sugar, starch, and photosynthetic pigment levels while increasing hydrogen peroxide, malondialdehyde, and electrolyte leakage (Barros Junior et al., 2021; Santos et al., 2021; Soares et al., 2022). These physiological and biochemical alterations hinder growth and may compromise plant survival, particularly in early forest establishment (Felippe et al., 2021).

Symbiotic relationships with *Trichoderma* spp. have been explored as a strategy to enhance plant growth and mitigate water deficiency effects in crops such as rice, cocoa, sugarcane, corn, tomato, and wheat (Bae et al., 2009; Mastouri et al., 2012; Shukla et al., 2012; Guler et al., 2016; Alwhibi et al., 2017; Scudeletti et al., 2021). Under water deficit conditions, inoculation with *T. asperellum* and *T. harzianum* enhances antioxidant enzyme activity, reduces oxidative damage from reactive oxygen species (ROS), improves photosynthetic pigment content and gas exchange regulation, and increases water-use efficiency and nutrient uptake, collectively promoting plant growth (Mastouri et al., 2012; Estévez-Geffriaud et al., 2020; Khoshmanzar et al., 2020; Scudeletti et al., 2021; Singh et al., 2023).

Some *Trichoderma* species promote the growth of forest species (Griebeler et al., 2021). In eucalyptus, their effects on growth and phytopathogen control are well documented (Maciel et al., 2012; Azevedo et al., 2017; Bandeira et al., 2023; Gomes et al., 2023), yet their role in water deficiency tolerance remains unclear. In *Pinus massoniana*, *T. longibrachiatum* inoculation under water stress reduced membrane damage, increased antioxidant enzyme activity, enhanced osmolyte accumulation, and improved photosynthesis (Yu et al., 2023). The ability of *Trichoderma* spp. to mitigate water stress effects is generally attributed to enhanced nutrient uptake (Bononi et al., 2020; Harman, 2006), which promotes root growth and water absorption (Bae et al., 2009; Shukla et al., 2012). Additionally, these fungi upregulate antioxidant enzyme expression (Mastouri et al., 2012) and may induce phytohormone synthesis (Illescas et al., 2021).

Given the hypothesis that *Trichoderma* spp. influence *E. urophylla* tolerance to water deficiency and promote growth, this study aimed to evaluate the morphophysiological parameters, water status indicators, gas exchange, and biochemical and metabolic responses of plants under different water regimes and inoculation treatments. The study also assessed the effects of *T. asperellum* and *T. harzianum* inoculation on early field growth.

2. Materials and Methods

2.1. Greenhouse experiment: cultivation conditions and experimental design

The experiment was conducted in a greenhouse covered with plastic and enclosed with an anti-aphid screen (12 × 6 m structure, 3 m arch height) at the State University of Southwest Bahia, Vitória da Conquista, Bahia, Brazil, from October to December 2021. The study site is located at 14°53'34" S, 40°48'15" W, at an altitude of 858 m. During the experiment, temperatures in the greenhouse ranged from 19°C (minimum) to 43°C (maximum), with an average of 31°C, while the mean relative humidity was 61%.

Clonal seedlings of *Eucalyptus urophylla* (clone AEC 144), 100 days old, approximately 25 cm in height with around 10 leaves, were used for the experiment. The seedlings were transplanted into 15 dm^3 plastic pots filled with a substrate composed of sand and ravine soil (2:1 ratio) and fertilized based on a prior substrate analysis.

The experiment followed a completely randomized design in a 3 × 2 factorial scheme. The first factor was inoculation treatment: *Trichoderma asperellum* (TA), *T. harzianum* (TH), and a non-inoculated control (C). The second factor was the water regime: water deficit (30% pot capacity) and well-irrigated (90% pot capacity) conditions (WD and WW, respectively). Each treatment had four replicates, with one plant per experimental plot.

For inoculation, seedlings were removed from their tubes, and roots were immersed for 30 minutes in a solution of commercial products containing *T. harzianum* (Trichodermil®, CEPA ESALQ 1306, 2×10^9 viable conidia mL^{-1}) or *T. asperellum* (TrichobiolMax®, 1×10^9 CFU mL^{-1}) at a concentration of 10 mL L^{-1}. After transplanting, 200 mL of the inoculum solution was applied to each pot. Water regimes were established 30 days after transplanting (DAT), with pots weighed daily and watered to maintain 30% or 90% pot capacity (Alves et al., 2010).

At 60 DAT, plants were evaluated. Leaf samples were collected from the middle third of each plant, frozen in liquid nitrogen, lyophilized, and stored in a freezer for subsequent determination of thiobarbituric acid-reactive substances and the activity of guaiacol peroxidase (GPX) and superoxide dismutase (SOD) enzymes.

2.1.1. Biometric characteristics

A graduated ruler and a digital caliper were used to measure plant height and collar diameter, respectively. The total leaf area was assessed using a leaf area meter (LICOR, LI-3100). Shoot and root dry masses were determined by drying samples in a forced-ventilation oven at 65°C for 72 hours, followed by weighing on a precision scale (Bel, S243).

2.1.2. Leaf water potential and relative water content

Leaf water potential was measured just before dawn using a pressure chamber (Model 1000, PMS) following Scholander et al. (1965). Fully expanded adult leaves from the middle part of the plant, specifically at the fourth to fifth node of the branch, were used as references.

Relative water content (RWC) was determined using ten 7-mm leaf discs, using the formula proposed by Weatherley (1950).

2.1.3. Thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide

Lipid peroxidation was assessed using the thiobarbituric acid (TBA) reaction (Heath and Packer, 1968), while

leaf hydrogen peroxide (H_2O_2) content was determined following the method described by Junglee et al. (2014).

2.1.4. Leaf gas exchange

Leaf gas exchange parameters—net photosynthesis (*A*), transpiration (*E*), and stomatal conductance (g_s)—were measured between 8:00 and 11:00 a.m. using an infrared gas analyzer (LI-COR, LI-6400XT). Four measurements were taken per treatment on fully expanded leaves from the middle portion of the plants. Intrinsic water use efficiency (iWUE) was calculated as the ratio of net photosynthesis rate to stomatal conductance (iWUE = Ag_s^{-1}).

2.1.5. Photosynthetic pigments

Photosynthetic pigments were extracted using four 7 mm leaf discs and 4 mL of dimethyl sulfoxide (DMSO) saturated with calcium carbonate, following the method of Hiscox and Israelstam (1979). In the pot experiment, four samples were analyzed per treatment, while in the field experiment, 10 samples per treatment were evaluated. Chlorophyll *a* and carotenoid contents were determined according to Wellburn (1994), using reference wavelengths of 664 nm and 480 nm, respectively.

2.1.6. Reducing sugars, soluble sugars, and starch

Reducing sugars content was quantified using the dinitrosalicylic acid (DNS) method (Miller, 1959), while soluble sugars content was determined following the method of Yemm and Willis (1954). For starch quantification, 0.25 g of dried, hexane-defatted leaf samples were treated with 5 mL of 0.5 M H_2SO_4 and heated at 100°C for 1 hour. Starch content was then determined according to Normative Instruction No. 20 (Brasil, 1999).

2.1.7. Total amino acids

Total amino acid levels were quantified using the same extracts as in sugars quantification, following the method of Yemm and Cocking (1955). Absorbance readings were taken at 570 nm using a spectrophotometer, and results were expressed as µmol of amino acids per gram of fresh weight.

2.1.8. Proline

Proline content was determined following the method of Bates et al. (1973). Extraction was performed using 10 mL of water and 0.02 g of dried, crushed leaves, which were heated in a water bath at 100 ºC for 1 hour.

2.1.9. Quantification of antioxidant enzymes guaiacol peroxidase (GPX) and superoxide dismutase (SOD)

Enzyme extracts were obtained from 0.05 g of lyophilized material, which was added to 1.5 mL of NaH_2PO_4 buffer (50 mM, pH 6.0) and centrifuged at 10,000 rpm for 10 minutes. Guaiacol peroxidase (GPX) activity was determined following the methodology of Burguieres et al. (2007) with modifications. Superoxide dismutase (SOD) activity was assessed by measuring the extract volume required to inhibit 50% of nitro blue tetrazolium (NBT) reduction (Beauchamp and Fridovich, 1971).

2.2. Field study

A field experiment was conducted in Montezuma, MG (15°13'36" S, 42°34'34" W) from August 11 to November 23, 2022. Three independent trials were established to evaluate seedling inoculation with *T. asperellum* (TA), *T. harzianum* (TH), and a control (C). Clonal *E. urophylla* (AEC 144) seedlings were planted at 2.5 × 3.5 m spacing, with 250 seedlings per treatment. The effective experimental area consisted of 60 centrally located plants per treatment.

Inoculation was performed by immersing roots in a *Trichoderma* solution (1 mL L^{-1}) for 30 minutes, followed by the application of 1 L of the same solution near the plant's base to ensure each plant received 1 mL of the commercial product. Control plants underwent the same procedure using only water. Since planting occurred during a dry period, irrigation was applied weekly using a hose and water truck, delivering approximately 4 L of water per plant.

2.2.1. Evaluations

The number of leaves was recorded at 90 days after transplanting (DAT). Plant height was measured at 90 and 150 DAT using a tape measure, while stem diameter was assessed at 90, 150, and 270 DAT with a digital caliper. These measurements were taken from the 60 plants in the effective experimental area of each treatment.

At 270 DAT, leaf discs were collected from fully expanded leaves located in the middle canopy (fourth to seventh leaf on the branch) from 10 plants per treatment to determine photosynthetic pigment content, as described in Section 2.1.6. Additionally, for each treatment, 40 leaves from 10 plants were collected from the same canopy position, dried in a forced-air circulation oven, crushed, and analyzed for total and reducing sugars, starch, and proline content, following the methods in Sections 2.1.7 and 2.1.9.

2.3. Statistical analysis

Data were subjected to the Shapiro-Wilk test for residual normality and Bartlett's test for homogeneity of variances using PAST 4.10 (Hammer et al., 2001). Subsequently, analysis of variance (ANOVA) and Tukey's mean comparison test were performed using the SISVAR statistical program (Ferreira, 2011).

3. Results

3.1. Greenhouse experiment

Eucalyptus growth and dry mass parameters were influenced solely by the water regime factor (Table 1). Plants under water deficiency (WD) exhibited reduced height, stem diameter, leaf area, and dry mass of both shoots and roots compared to well-watered plants (WW).

Although inoculation did not significantly affect growth parameters, plants inoculated with *Trichoderma asperellum* (TA) exhibited 9.05%, 14.87%, and 15.99% higher values for height, stem diameter, and leaf area, respectively, compared to control plants (Table 1).

Table 1. Plant height (PH), stem diameter (SD), leaf area (LA), and shoot (SDM) and root (RDM) dry masses of young *Eucalyptus urophylla* plants under water deficient (WD) and well-watered (WW) conditions and inoculated with *Trichoderma asperellum* (TA) and *Trichoderma harzianum* (TH), and non-inoculated ones (C).

Water regime	PH (cm)	SD (cm)	LA (cm²)	SDM (g)	RDM (g)
WD	54.08±[1.80] b	7.17 ±[0.41] b	2188.12 ± [159.53] b	23.66 ±[1.63] b	7.17 ±[0.50] b
WW	59.83 ±[1.69] a	9.10 ±[0.33] a	3951.24 ±[256.05] a	41.24 ± [2.62] a	12.86 ±[0.96] a
Inoculation[ns]	**PH (cm)**	**SD (cm)**	**LA (cm²)**	**SDM (g)**	**RDM (g)**
TA	60.25 ±[1.36]	9.19 ±[0.48]	3399.45 ±[355.06]	36.97 ±[4.34]	11.11 ±[1.51]
TH	55.37 ±[2.25]	7.24 ±[0.50]	2878.75 ±[438.86]	28.2 ±[3.68]	9.73 ±[1.2]
C	55.25 ±[2.95]	8.00 ±[0.56]	2930.83 ±[453.24]	32.22 ±[4.22]	9.22 ±[1.50]

Different lowercase letters between treatments indicate significant differences (Tukey's test, $p < 0.05$). [ns]: non-significant difference (F-test, $p < 0.05$).

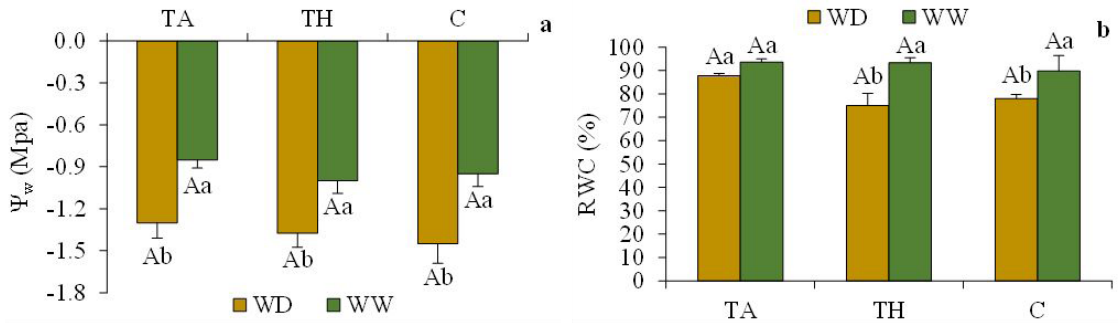

Figure 1. Leaf water potential (Ψw) [a] and relative water content (RWC) [b] of *Eucalyptus urophylla* plants inoculated with *Trichoderma asperellum* (TA) and *Trichoderma harzianum* (TH), as well as non-inoculated (C), under water deficient (WD) and well-watered (WW) conditions. Different uppercase letters indicate significant differences between inoculation treatments, and lowercase letters indicate significant differences between water regimes (Tukey's test, $p < 0.05$).

Leaf water potential was influenced by the water regime, with lower values observed under WD. For RWC, a significant interaction was found between the water regime and inoculation (Figure 1). Regardless of the water regime, no differences were observed between inoculated treatments. However, under TA inoculation, RWC remained similar in both WD and WW plants. In contrast, under TH inoculation and in the control treatment, RWC was lower in WD plants.

Chlorophyll *a* and carotenoid levels were influenced by inoculation, with lower concentrations observed in plants inoculated with TH compared to the control (Table 2). TA resulted in intermediate values.

Stomatal conductance (g_s) and transpiration rate (E) were influenced by the water regime, with lower values observed under WD. The photosynthetic rate (A) was affected by both factors individually, while intrinsic water-use efficiency (iWUE) was influenced solely by inoculation (Table 3). The WD regime also led to a reduction in *A*. In terms of inoculation effects, plants inoculated with TA and TH exhibited higher A and iWUE compared to the non-inoculated control (Table 3).

TA-inoculated and control plants showed lower reducing sugars contents under WD. In contrast, TH-inoculated plants exhibited no significant differences between water regimes. Regardless of water availability, TA-inoculated plants had the lowest reducing sugars contents (Figure 2a).

Under inoculation with TA, starch content remained similar between WD and WW regimes. In TH-inoculated plants, starch content was higher under WW, while in the control group, it was higher under WD. Under WD, inoculated plants (TA and TH) had lower starch content than control plants, whereas no differences were observed under WW (Figure 2b).

Soluble sugars and proline contents were influenced by the water regime and inoculation independently. TBARS content was affected only by the water regime, while total amino acid content was influenced solely by inoculation. Under WD, plants exhibited higher levels of soluble sugars, TBARS, and proline (Table 4).

TA and TH inoculations increased soluble sugars and total amino acid content compared to the control. Plants inoculated with TA had higher proline levels than those inoculated with TH, with values similar to the control.

H_2O_2 content was influenced solely by the water regime, with higher levels observed in plants under WD (Figure 3).

The interaction between inoculation and water regime influenced guaiacol peroxidase (GPX) and superoxide dismutase (SOD) activities (Figure 4). TA-inoculated and control plants exhibited higher GPX activity under WD, whereas those inoculated with TH showed no differences between water regimes. Regardless of the water regime, TA-inoculated plants had higher GPX activity than those inoculated with TH and the control (Figure 4a).

TH-inoculated and control plants exhibited higher SOD activity under WW. In contrast, under TA inoculation, SOD activity remained similar between WD and WW (Figure 4b).

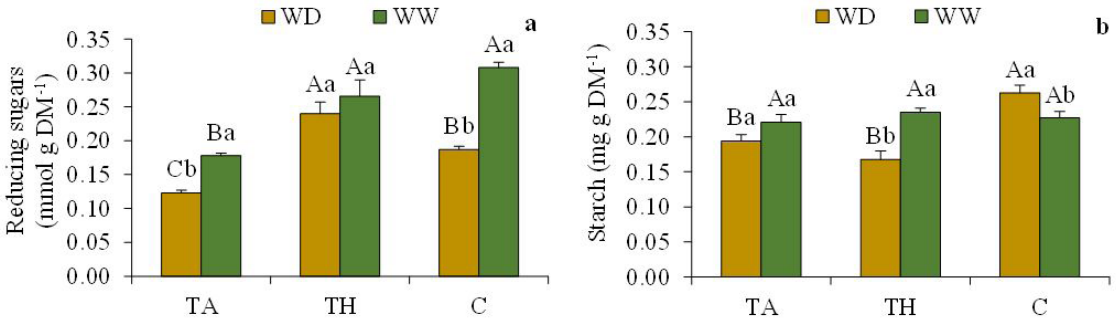

Figure 2. Contents of reducing sugars (a) and starch (b) in *Eucalyptus urophylla* plants inoculated with *Trichoderma asperellum* (TA) and *Trichoderma harzianum* (TH), as well as non-inoculated (C), under water deficient (WD) and well-watered (WW) conditions. Different uppercase letters indicate significant differences between inoculation treatments, and lowercase letters indicate significant differences between water regimes (Tukey's test, $p < 0.05$).

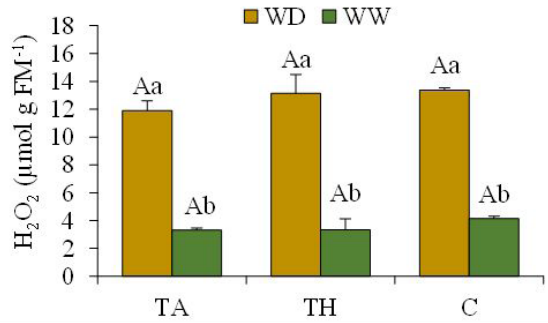

Figure 3. Hydrogen peroxide (H_2O_2) content in *Eucalyptus urophylla* plants inoculated with *Trichoderma asperellum* (TA), *Trichoderma harzianum* (TH), as well as non-inoculated (C), under water deficient (WD) and well-watered (WW) conditions. Different uppercase letters indicate significant differences between inoculation treatments, and lowercase letters indicate significant differences between water regimes (Tukey's test, $p < 0.01$).

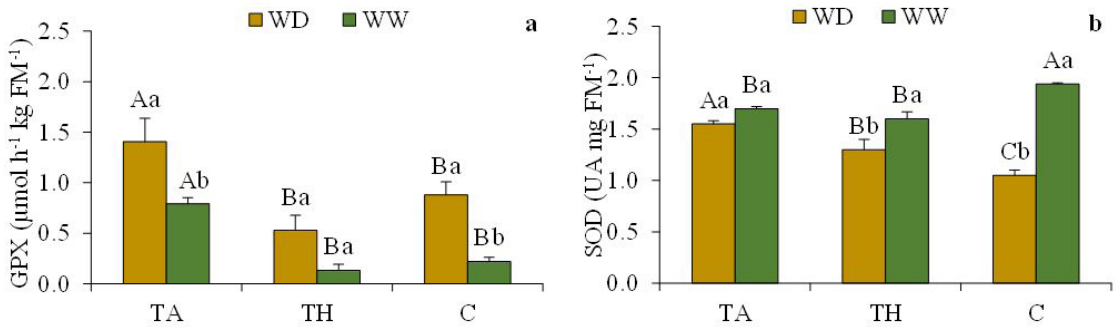

Figure 4. Activity of guaiacol peroxidase (GPX) [a] and superoxide dismutase (SOD) [b] in leaves of *Eucalyptus urophylla* plants inoculated with *Trichoderma asperellum* (TA), *Trichoderma harzianum* (TH), as well as non-inoculated (C), under water deficient (WD) and well-watered (WW) conditions. Different uppercase letters indicate significant differences between inoculation treatments, and lowercase letters indicate significant differences between water regimes (Tukey's test, $p < 0.05$).

Table 2. Contents of chlorophyll *a* and carotenoids in *Eucalyptus urophylla* plants inoculated with *Trichoderma asperellum* and *Trichoderma* and non-inoculated ones (control).

Inoculation	Chlorophyll *a* (µg cm^{-2})	Carotenoids (µg cm^{-2})
T. asperellum	0.00491 ± 0.00079 ab	0.000313 ± 0.00005 ab
T. harzianum	0.00389 ± 0.00079 b	0.000251 ± 0.00004 b
Control	0.00727 ± 0.00046 a	0.000460 ± 0.00006 a

Different lowercase letters between treatments indicate significant differences (Tukey's test, $p < 0.05$).

Table 3. Gas exchange of *Eucalyptus urophylla* plants as a function of water regimes and inoculation with *Trichoderma asperellum* and *Trichoderma harzianum*.

Water regime	g_s (mol $m^{-2}s^{-1}$)	E (mmol H_2O $m^{-2}s^{-1}$)	A (μmol CO_2 $m^{-2}s^{-1}$)
Water deficit	0.22 ± 0.02 b	4.93 ± 0.19 b	14.49 ± 0.61 b
Well-watered	0.33 ± 0.02 a	6.60 ± 0.14 a	19.07 ± 0.36 a
Inoculation	**A (μmol CO_2 $m^{-2}s^{-1}$)**		**iWUE (μmol mol^{-1})**
T. asperellum	17.45 ± 0.87 a		71.54 ± 6.76 a
T. harzianum	17.40 ± 1.03 a		74.58 ± 6.69 a
Control	15.48 ± 0.96 b		52.69 ± 5.47 b

Different lowercase letters between treatments indicate significant differences (Tukey's test, $p < 0.05$).

Table 4. Biochemical parameters of *Eucalyptus urophylla* plants under different water regimes and inoculation conditions.

Water regime	Soluble sugars (mmol g DM^{-1})	TBARS (nmol g FM^{-1})	Proline (mmol g DM^{-1})
Water deficit	1.15 ± 0.09 a	80.27 ± 5.59 a	0.0257 ± 0.003 a
Well-watered	0.92 ± 0.10 b	63.65 ± 4.17 b	0.0192 ± 0.002 b
Inoculation	**Soluble sugars (mmol g DM^{-1})**	**Total amino acids (μmol g DM^{-1})**	**Proline (mmol g DM^{-1})**
T. asperellum	1.22 ± 0.077 a	7.11 ± 0.364 a	0.0259 ± 0.003 a
T. harzianum	1.12 ± 0.125 a	6.65 ± 0.532 a	0.0162 ± 0.003 b
Control	0.73 ± 0.105 b	4.83 ± 0.207 b	0.0254 ± 0.003 ab

Different lowercase letters between treatments indicate significant differences (Tukey's test, $p < 0.05$).

Under WW, the highest SOD activity was observed in control plants, while under WD, it was highest in TA-inoculated plants, followed by those inoculated with TH.

3.2. Field study

At 90 DAT, the number of leaves was highest in plants inoculated with TH, followed by those inoculated with TA, both of which had more leaves than the control (non-inoculated plants) (Figure 5a). Plant height, measured at 90 and 150 DAT, was greater in TA- and TH-inoculated plants compared to the control, with no significant differences between the two inoculated treatments (Figure 5b). Similarly, stem diameter, assessed at 90, 150, and 270 DAT, was greater in inoculated plants, which did not differ from each other (Figure 5c).

Total chlorophyll and carotenoid contents were higher in TA-inoculated plants compared to the control. Plants inoculated with TH showed intermediate values, similar to both the control and TA (Figure 6).

At 270 DAT, total soluble sugar, reducing sugar, and starch contents were lower in TA- and TH-inoculated plants compared to the control (Figure 7a, b). Proline content showed no significant differences between treatments (Figure 7c).

4. Discussions

The adverse impact of water deficiency on *Eucalyptus urophylla* growth has been well documented (Barros Júnior et al., 2021; Santos et al., 2021; Soares et al., 2022). Plant height, stem diameter, leaf area, and dry mass reductions due to water deficit are linked to lower water potential, relative water content, and leaf gas exchange.

Compared to pot-grown plants, the positive effects of TA and TH inoculations on leaf number, height, and stem diameter in field-grown *E. urophylla* suggest that inoculation benefits increase over time, highlighting the importance of interaction duration (Segarra et al., 2007). Under field conditions, *Trichoderma* species can interact more effectively with other beneficial soil microorganisms, enhancing plant growth (Schuelter et al., 2024).

Although TA inoculation did not mitigate water deficit effects on potted plant growth, it enhanced shoot and root dry mass compared to the control. This increase may be linked to improved cell turgor maintenance, as evidenced by similar RWC values between WD and WW plants inoculated with TA, whereas other treatments showed RWC reductions under WD.

The ability of TA-inoculated plants to maintain similar RWC levels under WD and WW suggests improved water regulation. *Trichoderma*-inoculated plants may have an enhanced capacity to extract water from the rhizosphere (Guler et al., 2016) and regulate stomatal opening, thereby reducing water loss (Contreras-Cornejo et al., 2015). Increased leaf RWC in plants inoculated with *T. hamatum*, *T. atroviride*, and *T. asperellum* has also been reported in cocoa and maize (Bae et al., 2009; Guler et al., 2016; Estévez-Geffriaud et al., 2020).

The greater efficiency of iWUE in TA- and TH-inoculated plants, regardless of the water regime, aligns with findings in tomato and rice (Pandey et al., 2016; Khoshmanzar et al., 2020). This effect may be attributed to increased expression of genes regulating water influx

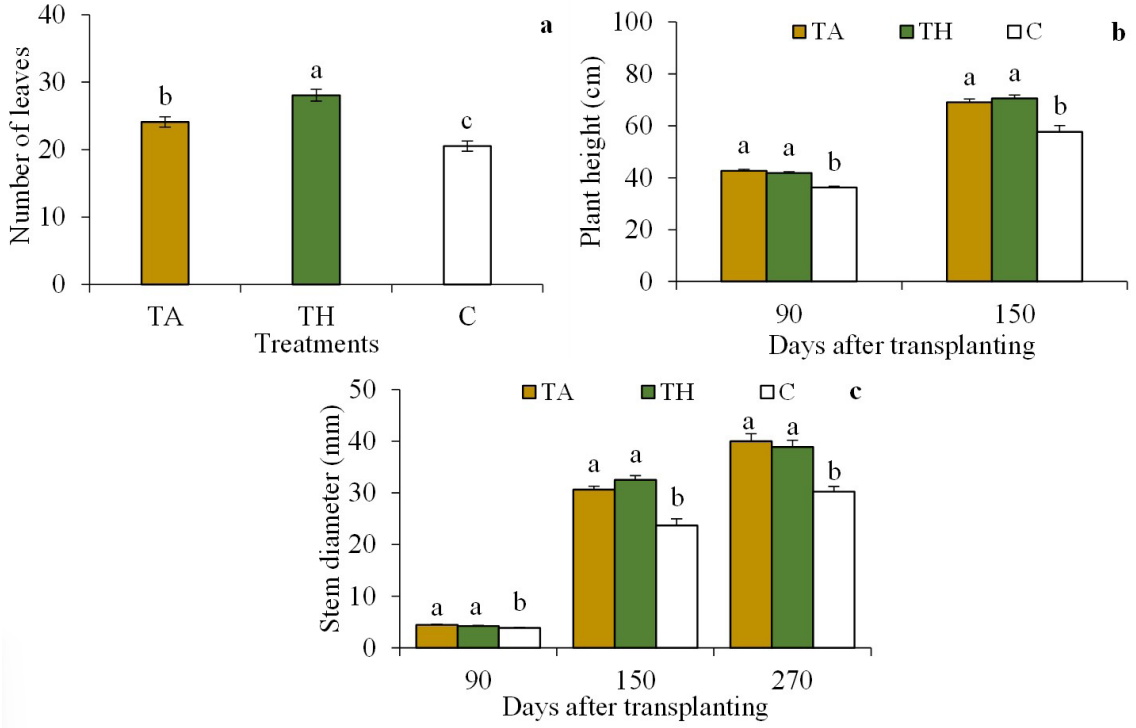

Figure 5. Number of leaves (a), plant height (b), and stem diameter (C) of eucalyptus plants 90 days after transplanting as a function of the control treatments (c) and inoculation of *Trichoderma asperellum* (TA) and *Trichoderma harzianum* (TH).

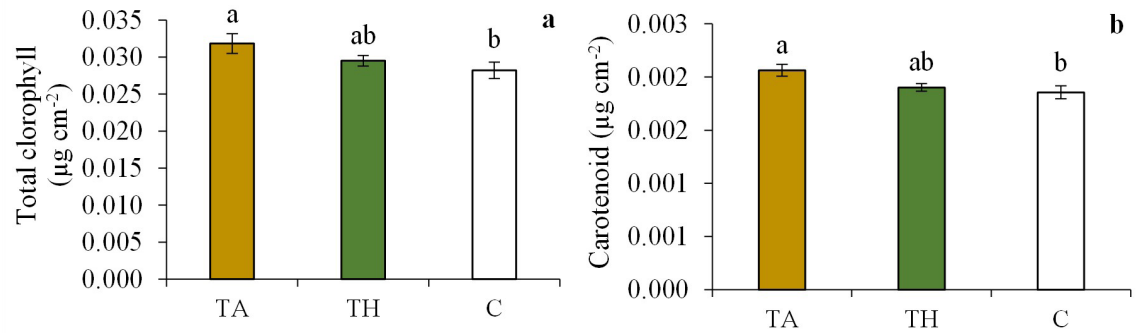

Figure 6. Contents of total chlorophyll (A) and carotenoids (B) in eucalyptus leaves 270 days after transplanting as a function of the control treatments (C) and inoculation of *Trichoderma asperellum* (TA) and *Trichoderma harzianum* (TH).

and efflux in cells (Pandey et al., 2016), contributing to improved water balance.

Under water restriction, *Eucalyptus* plants exhibit lower g_s, *E*, and *A* values (Fernandes et al., 2015; Chen et al., 2020), primarily due to partial stomatal closure (Sharma et al., 2020). In this study, TA and TH inoculation did not counteract the negative effects of water deficit on gas exchange, as g_s reductions led to decreased *E* and *A*. However, regardless of water regime, TA and TH inoculations promoted higher *A* and iWUE. Similar increases in photosynthetic rate following *Trichoderma* inoculation have been observed in wheat and sugarcane (Oljira et al., 2020; Scudeletti et al., 2021). This enhancement is generally associated with increased photosynthetic pigment content and antioxidant activity, which help maintain favorable physiological conditions for photosynthesis. Furthermore, *Trichoderma* strains have been shown to upregulate multiple photosynthesis-related genes and components (Harman et al., 2021).

Although some studies have reported increased photosynthetic pigment content in stressed plants inoculated with *Trichoderma* (Alwhibi et al., 2017; Zhang et al., 2019), this was not observed in potted plants inoculated with TA and TH in the present study. However, in the field, total chlorophyll and carotenoid contents were higher in TA-inoculated plants. This increase in pigment content may be attributed to molecular changes, as *Trichoderma* colonization upregulates genes, proteins, and pigments involved in photosynthesis across various crops (Harman et al., 2021; Smiderle et al., 2024).

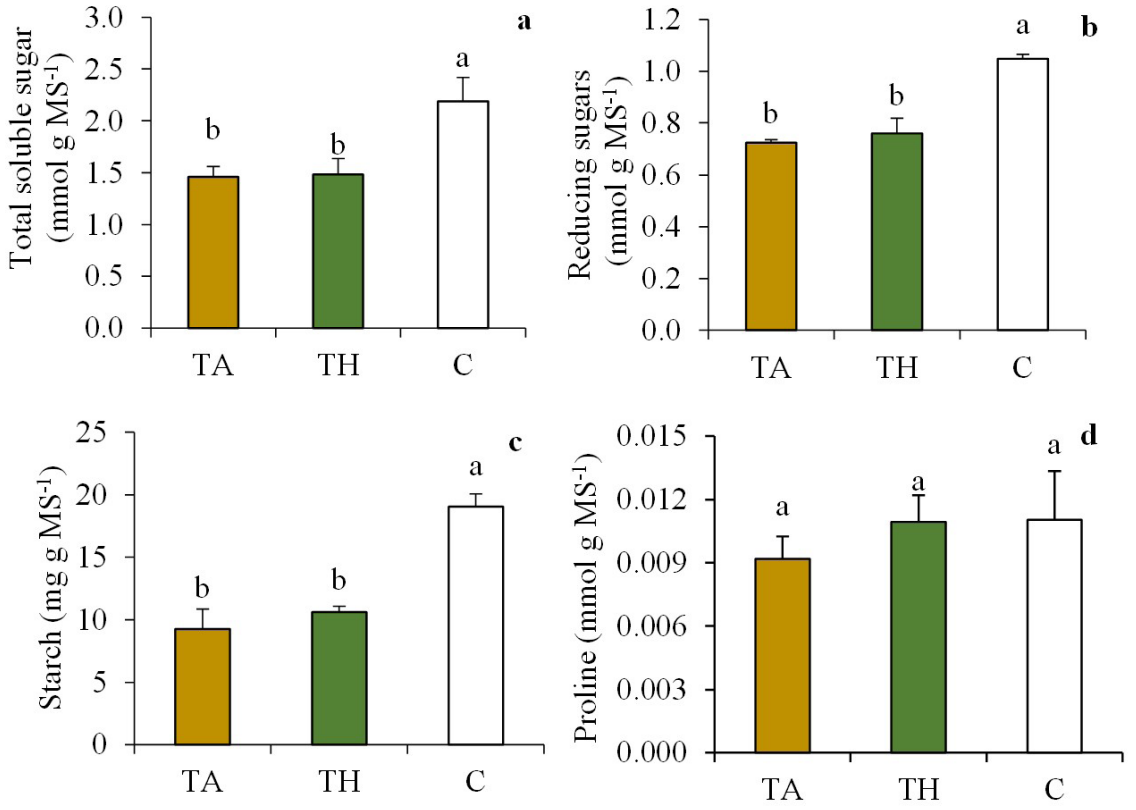

Figure 7. Contents of total soluble sugars (a), reducing sugars (b), starch (c), and proline (d) in eucalyptus leaves 270 days after transplanting as a function of the control treatments (C) and inoculation of *Trichoderma asperellum* (TA) and *Trichoderma harzianum* (TH).

The higher reducing sugars content in WD plants inoculated with TH may contribute to maintaining cell membrane and protein integrity, enhancing dehydration tolerance (Shvaleva et al., 2006). *Trichoderma* inoculation has also been associated with increased soluble sugars protein, and proline content in tomato and cucumber plants (Ghorbanpour et al., 2018; Alwhibi et al., 2017; Zhang et al., 2019). Under field conditions, the lower total soluble sugar, reducing sugar, and starch contents in inoculated plants may be due to higher carbohydrate demand to support increased plant growth.

Starch content reduction, along with increased soluble sugars levels in WD plants inoculated with TA and TH, may facilitate osmotic adjustment while providing energy and carbon when photosynthesis is limited (Thalmann and Santelia, 2017). The higher total amino acid content in TA- and TH-inoculated plants, regardless of water regime, aligns with findings in cocoa plants inoculated with *T. asperellum* (Tchameni et al., 2011) and maize plants inoculated with *T. asperelloides* strain T02 (Meneses et al., 2022). Amino acid accumulation not only aids in osmotic adjustment but also plays a role in ion transport regulation, protein synthesis, gene expression, and redox homeostasis (Rai, 2002).

Water deficiency restricts plant growth primarily by disrupting cellular physiology and biochemistry through oxidative stress (Gill et al., 2015). The higher lipid peroxidation (measured as TBARS content) and increased H_2O_2 levels in WD plants compared to WW plants indicate an imbalance between reactive oxygen species (ROS) production and the antioxidant system's quenching activity. However, under water deficiency, TA and TH inoculation enhanced antioxidant activity, with TA specifically promoting higher SOD and GPX activity. SOD protects cells from oxidative damage by catalyzing the conversion of singlet oxygen into O_2 and H_2O_2 (Gill et al., 2015), while GPX facilitates H_2O_2 detoxification by converting it into H_2O (Das and Roychoudhury, 2014).

Overall, SOD and GPX activity under WD was highest in TA-inoculated plants. Activation of the antioxidant system is a well-documented mechanism employed by *T. asperelloides* and *T. harzianum* strains to enhance tolerance to salinity and water deficit in *Arabidopsis*, cucumber, and tomato plants (Mastouri et al., 2012; Brotman et al., 2013).

The distinct responses induced by TA and TH in eucalyptus plants grown in pots versus those in the field may be attributed to the specific and variable mechanisms of action of these microorganisms, which depend on environmental conditions and the time elapsed between inoculation and evaluation (Hoyos-Carvajal et al., 2009; Stewart and Hill, 2014). Additionally, environmental factors affect the ability of the *Trichoderma* species tested to thrive in the rhizosphere and effectively colonize the plant.

5. Conclusions

Water restriction in the greenhouse reduced *Eucalyptus urophylla* growth by lowering leaf water potential, relative water content, and gas exchange. Moreover, inoculation with *Trichoderma asperellum* and *T. harzianum* boosted photosynthetic activity, water-use efficiency, and soluble sugars and amino acid levels, regardless of the water regime. However, these improvements were insufficient to counteract the negative effects of water deficiency on plant growth.

Under field conditions, inoculated plants (*T. asperellum* and *T. harzianum*) exhibited greater leaf number, height, and stem diameter, while reducing and soluble sugars and starch levels were lower compared to non-inoculated plants.

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