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Boron as mitigator of drought damage in *Eucalyptus*: a genotype-dependent mechanism?

Boro como mitigador dos danos causados pela seca em eucalipto: um processo genótipo-dependente?

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Resumo

A identificação de caracteres morfofisiológicos associados à resistência à seca é de grande importância para a seleção de genótipos de eucalipto mais tolerantes. Além de características intrínsecas da espécie, fatores externos, como a suplementação de boro (B), podem potencializar essa tolerância. Este trabalho objetivou avaliar os efeitos da deficência hídrica em dois clones de eucalipto com tolerância diferencial à seca, bem como o potencial do B na mitigação dos danos. Mudas dos clones i144 (tolerante) e gg100 (sensível) foram cultivadas em solo, em casa de vegetação, com e sem restrição hídrica e de B. Foram realizadas avaliações fotossintéticas, do potencial hídrico foliar (Ψw_{leaf}) e de crescimento. Ambos os clones sofreram redução drástica do Ψw_{leaf} quando a umidade do solo atingiu 65 % da capacidade de campo, independentemente da suplementação de B. O principal efeito do B como mitigador dos efeitos da seca no clone i144, tolerante, foi estimular o aumento da produção de raízes e redução na produção de biomassa foliar, otimizando a absorção de água e as perdas por transpiração. Em ambos os clones, as trocas gasosas foram afetadas pela seca, mas respostas diferenciais não foram observadas em função da suplementação de B. Verificou-se redução significativa da taxa de assimilação de CO_2 e na eficiência instantânea de uso da água no clone gg100, e aumento considerável da eficiência intrínseca de uso da água no clone i144. Ambos os clones apresentaram queda das taxas transpiratórias, a qual foi mais evidente no clone i144, como resultado da redução da condutância estomática. Verificou-se redução da taxa de transporte de elétrons no clone gg100 e do rendimento quântico efetivo do fotossistema II em ambos os clones, quando submetidos à seca. No clone i144, o B atenuou a dissipação de energia térmica nas plantas controle e submetidas à seca, fazendo com que, nestas últimas, os valores se igualassem aos do controle. Conclui-se que os efeitos da seca, bem como o potencial do B em mitigar seus danos, variam entre os genótipos, sendo o clone tolerante mais beneficiado pela suplementação de B.

Palavras-chave: estresse hídrico, clone gg100, clone i144, fotossíntese, crescimento.

Abstract

The identification of morphological-physiological traits associated with drought resistance is extremely important for the selection of the most drought-tolerant *Eucalyptus* genotypes. Aside from the intrinsic characteristics of the species, external factors such as boron (B) supplementation can raise tolerance. This work aimed to evaluate the effects of water deficit on two *Eucalyptus* clones with differential drought tolerance, and the potential of B to mitigate the damage. Plants of clones i144 (tolerant) and gg100 (sensitive) were grown in soil, in a greenhouse, with and without water and B restriction. We evaluated the photosynthetic rates, the leaf water potential (Ψw_{leaf}), and the growth. In both clones, Ψw_{leaf} decreased drastically when soil moisture reached 65 % of the field capacity, regardless of B supply. The main mitigating effect of drought impacts of B on the tolerant clone i144 was to stimulate root and reduce leaf biomass production, optimizing water uptake and reducing transpiration losses. In both clones, gas exchange was affected by drought, but no differential responses were observed after B supplementation. In clone gg100, the CO_2 assimilation rate and instantaneous water use efficiency were significantly reduced, whereas in clone i144 the intrinsic water use efficiency increased considerably. The transpiration rates dropped in both clones, which it was more evident in clone i144, as a result of the reduced stomata conductance. The electron transport rate was reduced and the effective quantum yield of photosystem II decreased in both clones when exposed to drought. In clone i144, boron attenuated the dissipation of thermal energy in the control and drought-stressed plants, which caused the values in the latter to become equal to those of the control plants. We concluded that the drought effects as well as the damage mitigation potential of B vary among genotypes, and that the tolerant clone i144 benefitted the most from B supplementation.

Keywords: water stress, clone gg100, clone i144, photosynthesis, growth.

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INTRODUCTION

The increased demand for forest products has promoted an expansion of *Eucalyptus* plantations into the regions of Brazil with long drought periods and low-fertility soils. During the dry season, the water and nutrient uptake by eucalypts roots is greatly compromised, limiting tree growth (GRANDA et al. 2014); and in some cases widespread death occurs in forest stands. The main strategy to mitigate these stress effects is the selection of genotypes more tolerant to these restrictions.

The traditional process of selecting forest genotypes is both time and labor demanding and in most cases, prioritizes productivity over drought-tolerance (NAVARRETE-CAMPOS et al., 2012). The selection of drought-tolerant genotypes requires a thorough knowledge of molecular, biochemical, physiological, and anatomical aspects of different genotypes. This process is fundamental for the success of eucalyptus cultivation in areas with water deficit (GRANDA et al. 2014). Additionally, understanding the contribution of nutrients to mitigate drought damage is extremely necessary for this purpose and is also important for decision-making of forestry companies on specific silvicultural treatments.

Stomata conductance is reduced during water stress, later resulting in a drop of the photosynthetic rate, due to CO_2 limitation at the carboxylation sites of chloroplasts (LAWLOR; CORNIC, 2002), and in a reduction in plant growth. The stomata control of transpiration is considered to be the determinant process of short-term responses of a plant to drought, and is directly related to the rate of soil water depletion, the water potential of the plant (CALLISTER et al., 2008), and to the water and solute transport in the xylem (WILLIGEN; PAMMENTER, 1997). If CO_2 assimilation decreases, but the radiation intensity remains high, the electron transfer reactions along the thylakoid membrane may be inactivated, this results in excessive reducing power. A part of this excess can be used to reduce molecular oxygen, leading to the formation of reactive oxygen species (ROS), which are potentially capable of causing oxidative damage to cells (TARDIEU; SIMONNEAU, 1998; FU; HUANG, 2001; OZKUR et al., 2009; BEN-AHMED et al., 2009).

During drought periods, boron (B) uptake by *Eucalyptus* is severely reduced due to the low transpiration flow of the plant (MATTIELLO et al., 2009a). B-deficient *Eucalyptus* plants may have necrotic apical meristems (shoot die-back), aside from the overdevelopment of the lateral meristems (ALTHOFF et al., 1991), since most B in plants is concentrated in rhamnogalacturonan II molecules (O'NEIL et al., 2004) of pectin, a major component of the primary cell wall (HU; BROWN, 1994; MATOH et al., 1996). Furthermore, there is evidence that B deficiency can lead to the loss of stomata functions (WIMMER; EICHERT, 2013) and reduce the uptake of some nutrients, such as K (POLLARD, 1977; ROTH-BEJERANO; ITAI, 1981; SCHON et al., 1990; HAJIBOLAND; FARHANGI, 2011), which aggravates drought-induced stress.

Recent studies indicate that B fertilization can be effective in mitigating drought effects in some species, as observed in *Picea abies* (MÖTTÖNEN et al., 2001; MÖTTÖNEN et al., 2005), *Helianthus annuus* (HASSAN et al., 2011), *Brassica rapa* (HAJIBOLAND; FARHANGHI, 2011) and more recently, in *E. urophylla* (HODECKER et al., 2014). In addition, this nutrient has been suggested to increase the water use efficiency in *Eucalyptus urophylla* (HODECKER et al., 2014). However, information related to the affected photosynthetic processes and the influence of the nutrient on clones with differential drought tolerance is still scarce, requiring further studies on the subject.

Given the above, we hypothesize that the drought tolerant genotype has higher root growth, water use efficiency and photosynthetic rate and will be more positive influenced by B supply under low water availability compared to the sensitive genotype. The objective of this study was to evaluate the effects of water stress on morphological-physiological adaptation in two *Eucalyptus* clones with differential drought tolerance, and the potential of B in mitigating drought damage.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse, using rhizotrons (diameter 0.1 m diameter, length 1.0 m) to optimize vertical root growth and volume of 8.5 dm^3 soil. The soil had the following characteristics: 1.4 % organic matter; 42.0 % clay; 1.1 $mg.dm^{-3}$ P; 38.0 $mg.dm^{-3}$ K; 70 % CTC. To each m^3 of soil we added 0.2 kg of dolomitic lime (28 and 18 % of CaO and MgO, respectively),

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150 g N, 700 g P_2O_5, 100 g K_2O, and 90 g $MgSO_4^{-2}$. The micronutrients were added to the soil by drip irrigation (0.07 mg L^{-1} Cu; 3 mg L^{-1} Fe; 1.0 mg L^{-1} Mn; 0.02 mg L^{-1} Mo; 0.25 mg L^{-1} Zn) on alternate days. The supplementation of B (0.4 mg L^{-1}) was made together to the drip irrigation (MATTIELLO et al., 2009b). On the other days, irrigation with ultrapure water only was applied.

The treatments were arranged in a completely-randomized design with four replications in a 2 x 2 x 2 factorial scheme: two-hybrid clones *E. urophylla* (**i144** = drought-tolerant; **gg100** = drought-sensitive) x two levels of water availability (**C** = no water deficit, i.e., control; **D** = drought) x two doses of B (**-B** = absence of B application, **+B** = B application to the soil). In the treatments without B application, supplementation of this micronutrient was suspended 10 days after transplantation, i.e., 15 days before the beginning of water restriction. Each replication consisted of a rhizotron with one plant. In addition, four rhizotrons without plants were installed to determine soil water evaporation.

Seedlings were planted in the rhizotrons and the soil moisture maintained close to 100 % moisture content of field capacity (FC) until 25 days after transplanting, when the plants were approximately 30 cm high. Thereafter, the water supply was partially reduced to gradually decrease soil moisture in the treatments with water restriction. After this acclimation period, the values achieved were 90, 80 and 65 % FC after one, two and three weeks, respectively. During the entire experiment, the gravimetric transpiration was determined, by weighing the pots daily with and without plants, and the water replenished, always in the evening, in an amount sufficient to reach FC. The leaked amount was calculated by subtracting the values of the rhizotrons with plants from those without plants, considering that all had the same initial weight (rhizotron + soil). A moisture content of 65 % was maintained until the end of the experiment. The transpiration curve was determined when 100 %, 90 %, 80 % and 65 % of soil FC were reached.

The photosynthetic and leaf water potential analyses were performed when 65 % of soil FC was reached, from the youngest fully expanded leaves (one leaf per plant/repetition). An infrared gas analyzer coupled to a fluorometer (IRGA, 6400xt liquor, Lincoln, USA) was used for the determination of the following photosynthetic parameters: liquid carbon assimilation (A), stomata conductance (g_s), transpiration rate (E), and ratio between internal and external CO_2 concentrations (C_i/C_a), determined at around 9 AM, under saturating artificial light (1000 µmol m^{-2} s^{-1}) and environmental CO_2 concentration (around 400 µmol CO_2 mol^{-1} air). From these parameters, we calculated the instantaneous water use efficiency (WUE = A/E) and intrinsic water use efficiency ($WUE_i = A/g_s$). The parameters of chlorophyll fluorescence were estimated to determine the photochemical efficiency of PSII (F_v/F_m), the apparent electron transport rate (ETR) and non-photochemical energy quenching (NPQ). The leaf water potential was determined at predawn, with a Scholander pressure pump.

At the end of the experiment (60 days after transplanting, 50 days after terminating B supply and 35 days after initiating water stress), shoots and roots of the plants were harvested to determine dry matter production. The samples were dried to constant weight in a forced-air oven and the dry matter was weighed on a precision scale. The dry matter of the young leaves (YL), expanded leaves (EL), stem (St), and root system (Rt) were weighed separately.

The data were subjected to ANOVA and compared by the Scott-Knott test at 5 % probability, using software Assistat (version 7.7). All the analyses were conducted in four repetitions (plants) per treatment.

RESULTS AND DISCUSSION

Growth

The increase in dry matter production in the evaluated clones were severely affected by water restriction. Under this condition, only clone i144 (tolerant) responded positively to B nutrition (Table 1). The dry matter accumulation of this clone was higher under adequate supply of water and of B (C + B), resulting in increases of up to 23 % (young leaves dry matter) compared to plants grown without B (C-B). B application to drought-stressed plants had a positive effect on the dry matter production of young leaves, roots and total dry matter of the tolerant clone; however, it was not a determining factor in the production of expanded/expanding leaves and stems. Similar results were observed for *Picea abies, Helianthus annuus, Brassica rapa* and *E. urophylla*, in which

B favored plant growth in drought-stressed plants (MÖTTÖNEN et al. 2001; MÖTTÖNEN et al. 2005; HASSAN et al. 2011; HAJIBOLAND; FARHANGHI, 2011; HODECKER et al. 2014). No positive response to B supplementation was observed in the sensitive clone gg100.

The deficiency of B can lead to an accumulation of non-structural carbohydrates in leaves (ZHAO; OOSTERHUIS, 2002; HAN et al. 2008; HAN et al. 2009; LU et al. 2014), reducing translocation into root cells and, thereby affecting the growth of this organ. Hodecker et al. (2014) reported that foliar application of B to a drought-tolerant *E. urophylla* clone favored carbon allocation to the roots and intensified their growth, which was at the expense of the shoot, allowing better water uptake and plant hydration during water stress. Conversely to the tolerant genotype, no positive responses of B nutrition were observed in plant dry matter of the sensitive clone (gg100) under drought (Table 1).

Table 1. Dry mass of young (YL) and expanded leaves (EL), stem (St) and root (Rt) of eucalyptus clones i144 and gg100 under drought and boron supply.

Tabela 1. Massa de matéria seca de folhas jovens (YL) e expandidas (EL), caule (St) e raiz (Rt) dos clones de eucalipto i144 e gg100 submetidos à restrição hídrica e de boro.

Clone	Drought	B	Young leaves (g)	Expanded leaves (g)	Stem (g)	Root (g)	Total (g) (shoot + root)
i144	+	+	13.38 B	17.68 C	14.95 C	39.95 C	85.96 C
i144		-	9.81 C	19.25 C	12.71 C	31.71 D	73.48 D
i144	-	+	17.49 A	34.21 A	26.28 A	51.26 A	129.24 A
i144		-	13.10 B	29.93 B	22.53 B	48.82 B	114.38 B
gg100	+	+	6.76 B	21.34 C	12.35 C	31.78 C	72.23 D
gg100		-	9.92 B	22.46 C	17.01 B	33.82 C	83.21 C
gg100	-	+	18.62 A	26.38 B	23.79 A	46.24 B	115.03 B
gg100		-	16.98 A	29.00 A	22.95 A	51.74 A	120.67 A

Different letters indicate statistical differences between treatments for the same clone (n = 4).

Letras diferentes indicam diferenças estatísticas entre tratamentos, para um mesmo clone (n = 4).

Leaf water potential and gas exchange

When the soil contained 65 % FC, the leaf water potential decreased drastically in drought-stressed plants. No significant differences in the data of this parameter were detected in B-supplemented plants (Figure 1). Merchant et al. (2007) evaluated six *Eucalyptus* species and found that the osmotic adjustment, along with the reduction in leaf water potential, was a common physiological response in all species evaluated. In relation to B as stress mitigation factor, not only different species, and/or genotypes have different degrees of drought tolerance (MERCHANT et al., 2007; GRANDA et al., 2014), but the mitigating efficiency of B to dehydration can also vary between *Eucalyptus* genotypes or from different forms of fertilization. Hodecker et al. (2014) also found that plants of *E. urophylla* under long drought periods resulted in a leaf water potential that was important trait in the selector of draught-tolerant genotypes (LI et al. 2009).

Gas exchanges were adversely affected by drought, but were little responsive to B supplementation. A significant reduction of *A* (approximately 30 %), due to the low water availability in the soil, was only observed in drought-sensitive clone gg100 (Figure 2A). On the other hand, g_s decreased only in clone i144, indicating that the occurrence of stomata closure was in response to water deficit (Figure 2B). Stomata conductance generally regulates CO_2 assimilation, particularly when water is the limiting factor. A direct relationship between *A* and g_s was reported in a large number of species, including some of the genus *Eucalyptus* (FARQUHAR; SHARKEY, 1982; LEWIS et al., 2011). The growth and development of plants under water stress depends on the stomata ability to control water loss while growth is still maintained (DUNIN; ASTON, 1984), as was observed in the drought-tolerant clone in this experiment.

Despite the reduction in g_s in clone i144, no significant differences were observed in the C_i/C_a ratio between treatments (data not shown). This indicates that the biochemical stage of photosynthesis was not significantly affected. In both clones, the values of *E* decreased under water stress. This reduction was more marked in clone i144, reinforcing the indication of greater drought tolerance (Figure 2C). An efficient control of the transpiration rate, thus increasing the water use efficiency, is considered to be an important trait in the selection of drought-tolerant genotypes (LI et al. 2009).

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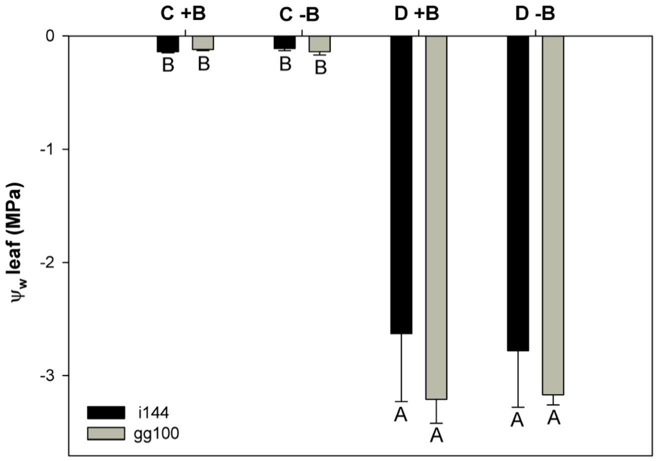

Figure 1. Water potential of *Eucalyptus* leaves in the predawn period of genotypes i144 and gg100 under different conditions of water availability and boron supplementation. Different letters indicate statistical differences between treatments. Means followed by the standard deviation (n = 4). Abbreviations: -B = no boron; + B = boron; C = no water stress (control); D = drought.

Figura 1. Potencial hídrico de folhas de *Eucalyptus*, no período de antemanhã, dos genótipos i144 e gg100 submetidos a diferentes condições de disponibilidade hídrica e suplementação de boro. Letras diferentes indicam diferenças estatísticas entre os tratamentos. Médias seguidas por desvio padrão (n = 4). Abreviaturas: -B = sem boro; +B = com boro; C = sem deficiência hídrica (controle); D = com deficiência hídrica.

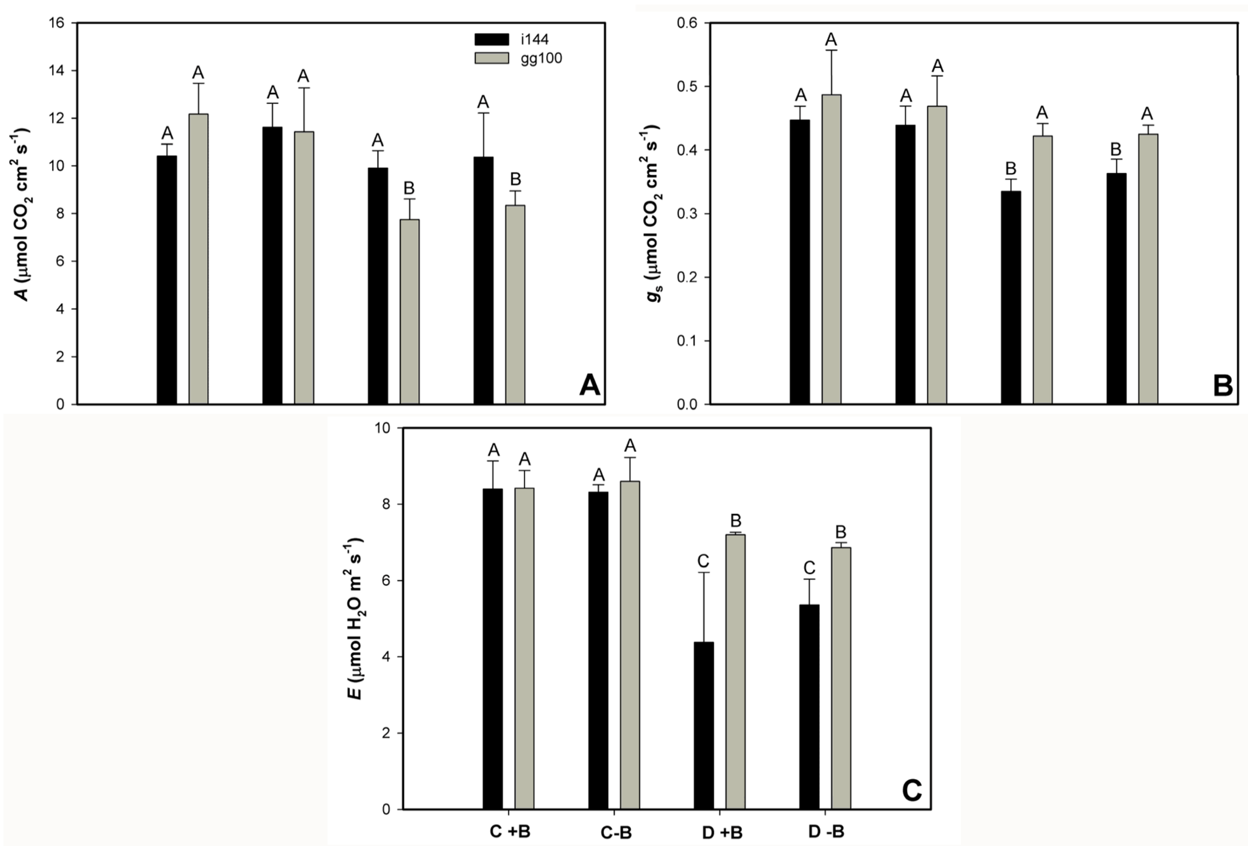

Figure 2. Gas exchange in *Eucalyptus* genotypes i144 and gg100 under different levels of water availability and boron supplementation. A. net CO2 assimilation. B. stomata conductance. C. transpiration. Different letters indicate statistical differences between treatments. Means followed by the standard deviation (n = 4). Abbreviations: -B = no boron; + B = boron; C = no water stress (control); D = drought.

Figura 2. Trocas gasosas em plantas de *Eucalyptus* dos genótipos i144 e gg100 submetidos a diferentes condições de disponibilidade hídrica e suplementação de boro. A. Assimilação líquida de CO2. B. Condutância estomática. C. Transpiração. Letras diferentes indicam diferenças estatísticas entre os tratamentos (n = 4). Médias seguidas por desvio padrão. Abreviaturas: -B = sem boro; +B = com boro; C = sem deficiência hídrica (controle); D = com deficiência hídrica.

Water Use Efficiency

Water stress promoted a reduction in instantaneous water use efficiency, WUE (A/E), in the sensitive clone gg100, unlike in the more drought-tolerant clone i144, regardless of B supplementation. On the other hand, the intrinsic water use efficiency, WUE_i (A/g_s), increased sharply in clone i144 under drought, mainly in B-supplemented plants (Figure 3). The reason for this significant increase in WUE_i was that after drought, the reduction in E (related to WUE) was greater than the reduction in g_s (related to WUE_i). It is possible that this asynchrony between E and g_s was related to morphological-anatomical changes that may have occurred during leaf development, e.g., the increase in thickness of the mesophyll tissues or even of the cuticle, increasing resistance to water loss by non-stomata transpiration.

A possible beneficial effect of the increase in WUE observed in clone i144 would be a reduction in the water consumption rate per unit of leaf area. However, it is noteworthy that this effect is only advantageous when the leaf biomass production remains the same or decreases in relation to the control (as occurred in both clones). Otherwise, the plant water consumption can increase, which can be masked depending on the way the analysis is performed (LIMA et al., 2003). For species with increased WUE under water deficit, like in the case with clone i144, drier environments can be advantageous (LI et al., 2009). However, there is a physiological cost for this, since the reduction in g_s promotes a proportionally greater reduction in transpiration than in CO_2 assimilation, increasing WUE (DE LUCIA; HECKATHORN, 1989), as observed in clone i144 in this study.

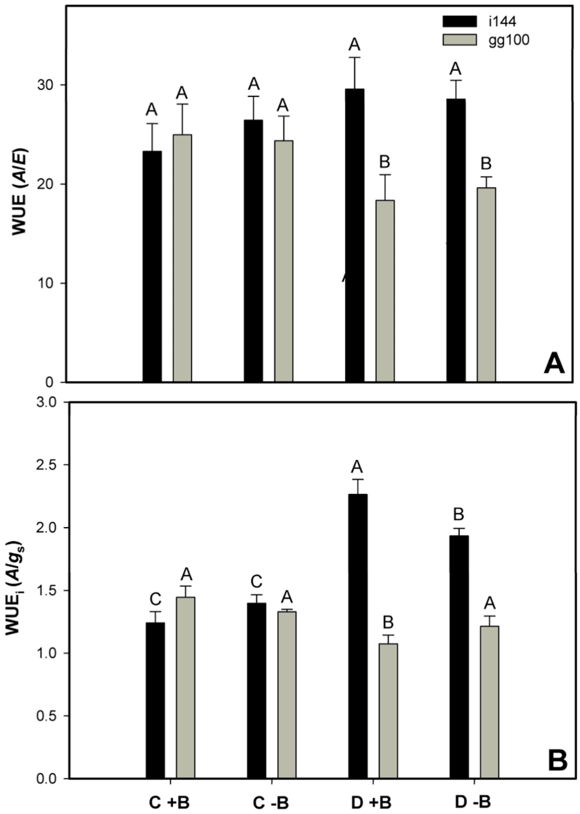

Figure 3. Water use efficiency of plants of *Eucalyptus* genotypes i144 and gg100 subjected to different water availability conditions and boron supplementation. A. instantaneous water use efficiency (WUE). B. intrinsic water use efficiency (WUEi). C. Transpiration. Different letters indicate statistical differences between treatments. Means followed by the standard deviation (n = 4). Abbreviations: -B = no boron; + B = boron; C = no water stress (control); D = drought.

Figura 3. Eficiência de uso da água de plantas de *Eucalyptus* dos genótipos i144 e gg100 submetidos a diferentes condições de disponibilidade hídrica e suplementação de boro. A. Eficiência instantânea de uso da água (WUE). B. Eficiência intrínseca de uso da água (WUEi). C. Transpiração. Letras diferentes indicam diferenças estatísticas entre os tratamentos. Médias seguidas por desvio padrão (n = 4). Abreviaturas: -B = sem boro; +B = com boro; C = sem deficiência hídrica (controle); D = com deficiência hídrica.

The analysis of transpiration curve throughout this experiment showed high water losses from drought-stressed plants and very similar transpirations patterns between clones and treatments with and without B (Figure 4). It was therefore concluded that B supplementation through roots were inefficient to mitigate drought-induced water losses in these plants.

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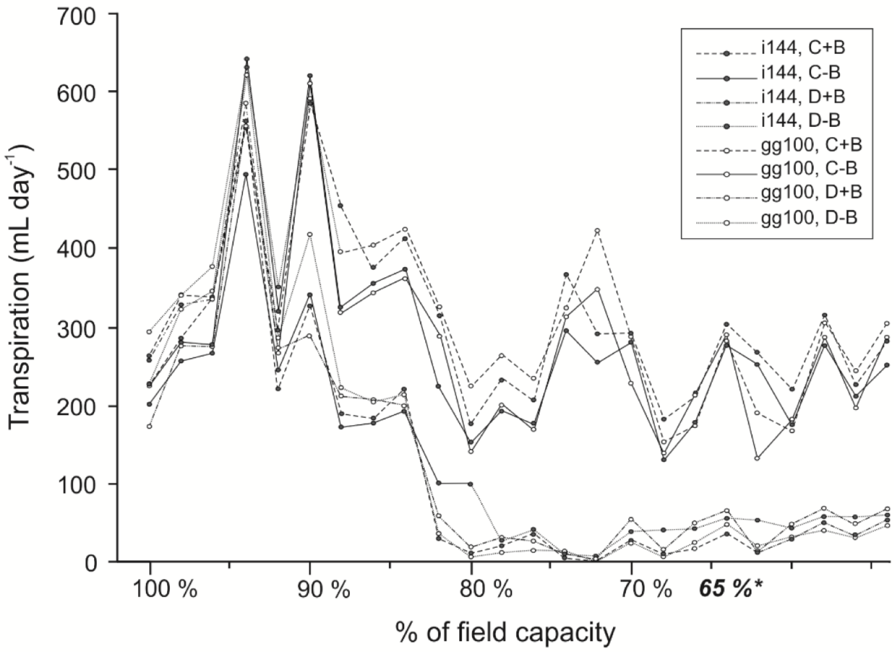

Figure 4. Transpiration curve of *Eucalyptus* plants (clones i144 and gg100) under different conditions of water availability and boron supplementation. Abbreviations: -B = no boron; + B = boron; C = no water stress (control); D = drought. * = Indicates sampling time.

Figura 4. Curva de transpiração de plantas de *Eucalyptus* (clones i144 e gg100) submetidos a diferentes condições de disponibilidade hídrica e suplementação de boro. Abreviaturas: -B = sem boro; +B = com boro; C = sem deficiência hídrica (controle); D = com deficiência hídrica.

Chlorophyll fluorescence

Water stress reduced the electron transport rate (ETR) through the thylakoid membrane only in the sensitive clone gg100, although the values did not differ in response to B supply (Figure 5A). Similarly, there was a reduction in the effective quantum efficiency of photosystem II in drought-stressed plants. This reduction was most drastic in clone gg100, and the effect was not mitigated by B supply in either clone (Figure 5B). The drop in ETR may lead to increased pairing of electrons along the thylakoid membranes, which reduces the molecular oxygen produced in the chloroplast. This can cause an increase in the production of reactive oxygen species (ROS), resulting in the oxidation of membrane lipids, proteins and nucleic acids if not removed effectively (RUBIO et al., 2002; ARORA et al., 2002).

In the drought treatments, the non-photochemical quenching energy dissipation (NPQ) increased in plants of clone gg100, with no differences between treatments with and without B. In treatments C and D without B supply, a significant increase in NPQ was detected in drought-stressed plants of clone i144, compared to the control. In these two treatments, B supply was effective in attenuating the dissipation of heat energy in chloroplasts, so that the NPQ value in treatment D +B was statistically equal to its control (C +B). Thermal dissipation was directly related to the xanthophyll cycle in the antenna complex of photosystem II and is an important mechanism apparatus of photosynthetic protection, which reduces the formation of reactive oxygen species and the risk of damage to protein D1 (NIYOGI, 1999). It is likely that oxidative stress occurred on a larger scale in clone gg100 than in i144, in view of the reduction in ETR and F_v/F_m and increase in NPQ in that genotype, which would explain its higher sensitivity to drought.

In summary, B supplementation in clone i144 was effective in optimizing photosynthetic rates and electron flow in the thylakoid membrane, mitigating heat energy loss after the reduction of water availability, so that the values of plants in treatment D +B were statistically similar to those in the water-saturated treatment (Figure 5C). This reinforces the greater drought tolerance of this clone as well as the importance of the nutrient studied in maintaining physiological processes during periods of water restriction, as observed in some regions of Brazil.

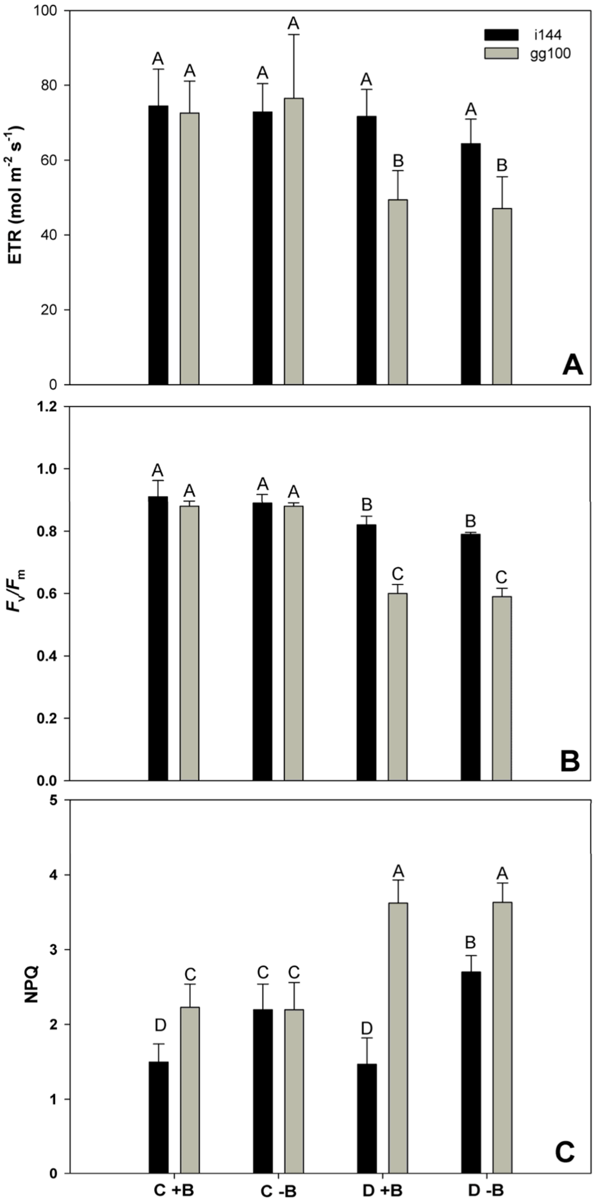

Figure 5. Parameters related to chlorophyll fluorescence in *Eucalyptus* genotypes i144 and gg100 under different conditions of water availability and boron supply. A. apparent electron transport rate. B. effective quantum yield of photosystem II. C. non-photochemical energy dissipation. Different letters indicate statistical differences between treatments. Means followed by standard deviation (n = 4). Abbreviations: -B = no boron; + B = boron; C = no water stress (control); D = drought.

Figura 5. Parâmetros relacionados à fluorescência da clorofila a em plantas de *Eucalyptus* dos genótipos i144 e gg100 submetidos a diferentes condições de disponibilidade hídrica e suplementação de boro. A. Taxa aparente de transporte de elétrons. B. Rendimento quântico efetivo do fotossistema II. C. Dissipação não fotoquímica. Letras diferentes indicam diferenças estatísticas entre os tratamentos. Médias seguidas por desvio padrão (n = 4). Abreviaturas: -B = sem boro; +B = com boro; C = sem deficiência hídrica (controle); D = com deficiência hídrica.

CONCLUSIONS

The effects of water deficit as well as the efficiency of B to mitigate negative drought effects in plants are genotype-dependent mechanisms, since clone i144 (tolerant) maintained some parameters of photosynthesis and growth when exposed to drought and supplemented with B, which was not observed in clone gg100 (sensitive). Thus, this result indicates that B is more effective in alleviating water stress symptoms in genotypes with effective mechanisms of drought tolerance. Low B levels in the soil can be sufficient to meet the demand of *Eucalyptus*. It is therefore believed that the control soil used in this experiment contained a sufficiently high B concentration to meet the plant nutrient demand and that the difference between treatments -B and + B was possibly not contrasting enough for clearer responses in plants under water stress. Thus, future research on complete B restriction is suggested to investigate the role of this nutrient in mitigating drought damages more effectively.

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