Científica

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Boron fertilization in Paricá seedlings mitigates negative effects of water deficit

Fertilização com boro em mudas de Paricá ameniza efeitos negativos do déficit hídrico

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Abstract

Boron (B) supply has been studied as an alternative to alleviate environmental stress conditions in forest essences due to the functions that B plays within the plant cell in the metabolism of compounds and polysaccharides connection. Thus, it was postulated the hypothesis that B may provide greater resistance from Paricá (*Schizolobium parahyba*) to water deficit. The aim was to evaluate growth parameters, photosynthetic rate, nutritional efficiencies, and B content of young *Schizolobium parahyba* plants as a function of the factors B levels and water deficit. Experiment was a completely randomized factorial, with two B concentrations (25 and 250 µmol L⁻¹, simulating sufficient and high B conditions, respectively) and two water conditions (deficit and no deficit). Factors analyzed influenced growth, accumulation, photosynthetic and nutritional efficiency variables. There was an increase in the growth of stem, root and in the Dickson quality index of plants of Paricá with high concentration of B, when they were under water deficit. Concentration and accumulation of B was higher in plants under water deficit with high concentration of B, as well as the efficiency of absorption and translocation, indicating that high B nutrition attenuates the effect of water deficit in young plants in Paricá.

Additional keywords: abiotic stress; gas exchange; micronutrient; seedling quality.

Resumo

O fornecimento de boro (B) tem sido estudado como alternativa para aliviar condições de estresse ambiental em essências florestais devido às funções que o B desempenha dentro da célula vegetal no metabolismo de compostos e conexão de polissacarídeos. Assim postulou-se a hipótese de que o B pode proporcionar maior resistência do Paricá (*Schizolobium parahyba*) ao déficit hídrico. O objetivo foi avaliar parâmetros de crescimento, taxa fotossintética, eficiências nutricionais, e conteúdo de B de plantas jovens de *Schizolobium parahyba* em função dos fatores níveis de B e déficit hídrico. O experimento foi um fatorial inteiramente casualizado, com duas concentrações de B (25 e 250 µmol L⁻¹, simulando condições de B suficiente e alto, respectivamente) e duas condições de água (déficit e sem déficit). Os fatores analisados influenciaram variáveis de crescimento, acumulação, fotossintética e eficiência nutricional. Houve incremento no crescimento de caule, de raiz e do índice de qualidade de Dickson (DQI) de plantas de Paricá com alta concentração de B, quando estavam sob déficit hídrico. A concentração e o acúmulo de B foi maior em plantas sob déficit hídrico com alta concentração de B, assim como a eficiência de absorção e translocação, indicando que a nutrição de B alta atenua o efeito do déficit hídrico em plantas jovens de Paricá.

Palavras-chave adicionais: estresse abiótico; micronutriente; qualidade de mudas; trocas gasosas.

Introduction

Paricá, *Schizolobium parahyba* var. *amazonicum* (Huber ex. Ducke) Barneby is a species that belongs to the Caesalpinaceae family, found in primary and secondary forests of the Amazon region (Ohashi et al., 2010). Agroforestry plantations of this species have been growing due to its rapid growth and wood quality for the manufacture of plywood, furniture, ceilings, pulp and paper (Rosa, 2006), besides being used for reforestation of anthropized areas (Locatelli et al., 2007). As a result, Paricá commercial plantations in Brazil reached about 87,901 ha in 2012, punctually concentrated in Amazon (Abraf, 2013).

Forest essences suffer from the deficiency of several nutrients absent in Brazilian Oxisols, among them boron (B). B is absorbed by the plants as (H_3BO_3) by simple diffusion, that is, diffusion of the non-dissociated boric acid without charge. This nutrient has structural function in vascular plants as it is concentrated in

cell wall performing activities such as cell division, joining of Ramnogalacturonan II (RGII) molecules, which provides physical strength to the cell wall, plasma membrane synthesis, and DNA synthesis and proteins (Acuña & Molina, 2007; Hansch & Mendel, 2009; Herrera-Rodríguez et al., 2010). Therefore, supply of B contributes to plant development, especially root growth and pollen tube, as well as providing tolerance to stresses caused by water deficit in forest essences (Gupta, 2007; Nunes, 2010).

B toxicity causes lower shoot and root growth in *Eucalyptus globulu* (Mattiello et al., 2009). Another study showed that B contents above 100 µmol L⁻¹ in solution caused a decrease in shoot and root dry matter growth, stem diameter, lower height, and quality index of Paricá seedlings (Souza et al., 2015). However, doses higher than normal B doses can also result in positive responses for plants, as described by Atique-ur-Rehman et al. (2018), in which boron supplementation improved performance in rice plants and by Oldoni et al. (2018), in which plants showed a better formation of plant tissue, benefiting its growth.

Water deficit is an abiotic phenomenon that causes stresses in the plant, such as stomatal closure and disturbances in photosynthesis, and respiration, which cause growth retardation in young plants (Kiani et al., 2008; Tahkokorpi et al., 2007). Research indicates that water deficit has limited early plant growth (Jaleel et al., 2009). In addition, it caused a reduction in height, leaf area and root dry mass in Popolus (Wullschleger et al., 2005), and fresh leaf and root mass of Petroselinum crispum [Mill.] Nym. Ex AW Hill (Petropoulos et al., 2007). On the other hand, little is known about the effects of a high supply of boron on plants with water deficit. However, in work developed by Nunes (2010) the adequate nutritional supply of forest essences in the early stages of growth can minimize the effects of stress caused by water deficiency.

Thus, the hypothesis of this work is that a high supply of B may cause resistance in *Schizolobium parahyba* with water deficit. Therefore, this research aims to evaluate growth parameters, photosynthetic rates and efficiencies nutritional B content of young plants *Schizolobium* parahyba.

Material and methods

Location and growth conditions

The experiment was carried out in an area located at the coordinates 2°55'S and 47°34'W in the municipality of Paragominas, Pará (PA) state, Brazil. Seedlings were grown in a greenhouse under environmental control to temperature and humidity. The minimum, maximum and median temperatures were 24 °C, 33 °C and 27.5 °C, respectively, and relative humidity of 65-85%.

Plants, containers and acclimation

Seeds of *S. parahyba* were sterilized for 3 min in a 1% of sodium hypochlorite, being scarified to increase speed of the germination. These seeds were placed into 1.2-L containers to germinate (0.15 m in height and 0.10 m in diameter) occupied with substrate mixture composed of sand and vermiculite in a 3:1 proportion. The solution contained 500 ml of nutritive solution adjusted to the nutritional exigencies of this species. The ionic force started at 25 %, and it was reformed to 50 % and 100 % at regular intervals over three days. Subsequently these periods, the nutritive solution persisted with the total ionic force (100%) from 22th until 37th day after sowing.

Experimental design

The experiment design was a factorial that was entirely randomised, with two boron concentrations (25 and 250 μ mol L⁻¹, simulating conditions of sufficient B and high B, respectively) and two water regimes (Deficit and no deficit), totalizing four treatments. B concentration was chosen in agreement with Hoagland & Arnon (1950). Five replicates were used and one plant per container corresponded to one unit experimental.

Treatments and nutrient solution

One young plant was preserved in each pot, during plant conduction. Fertilization was performed by supply of nutrient solution (Hoagland & Arnon, 1950) containing the following macronutrients and micronutrients: 5.71 mM KNO₃, 2.85 mM Ca(NO₃)²·4H₂O, 1.43 mM NH₄H₂PO₄, 3.21 mM MgSO₄·7 H₂O, 0.71 mM KCl, 1.42 mM KH₂PO₄, 1.42 μM MnSO₄·H₂O, 1.42 μM ZnSO₄·7H₂O, 0.35 µM CuSO₄·5H₂O, 0.35 µM NaMoO₄·5H₂O, 215 µM NaEDTAFe·3H₂O. To simulate the conditions of sufficient B and high B were used 25 and 250 µmol B L⁻¹, respectively, being applied by 23 days (37th until 60th days after experiment implementation), supplied via H₃BO₃. Water regimes (Deficit and no deficit) were applied by five days (60th until 65th days after sowing), and water deficit was obtained maintained plants without nutrient solution into container. All plants were physiologically measured on the 65th day, and leaf, stem and root were harvested for analysis.

Evaluation of photosynthetic rate

The net photosynthetic rate (P_N) was measured on the adaxial surface of fully expanded leaves that were collected from the middle region of the plant using an infrared gas analyser (model LCPro+; ADC BioScientific). The equipment chamber was adjusted to measure gas exchange under constant conditions to CO₂ concentration, photosynthetically active radiation, air-flow rate and temperature chamber in 360 µmol mol⁻¹ CO₂, 900 µmol photons m⁻² s⁻¹ 300 µmol s⁻¹ and 28 °C, respectively, during interval between 11:00 h and 12:00 h.

Evaluated characteristics

The height and stem diameter of the seedlings were measured 65 days after sowing. The plant material was separated in leaves, stem and roots, and washed with deionized water. Leaves, roots and stems were then dried in a forced circulation oven at 65 °C until constant weight to determine their dry matters. The Dickson quality index (DQI) was determined using the equation: DQI = TDM/[(H/SD) + (ShDM/RDM)]. (Dickson et al., 1960)

Where: TDM = total dry mass (g), SD= stem diameter (mm), H = height (cm), LDM = leaf dry mass (g), StDM=stem dry mass (g); ShDM = shoot dry mass (g), RDM = root dry mass (g); ShDM = StDM+LDM TDM = StMD+LDM+RDM.

Boron determinations

Leaf, stem and root dry mass were ground and analyzed for total B content, the samples were extracted by dry digestion (incineration) and determination was according to the colorimetric method described by Malavolta et al. (1997). Data from shoot and root dry mass and total B concentration were used to calculate the B accumulation, B uptake efficiency. From the dry matter content of nutrients in the plant indices were calculated: a) absorption efficiency (AE) = (total nutrient content of the plant)/(root dry matter) (Swiader et al., 1994); b) translocation efficiency (TE) = (nutrient content in the shoot)/(total content of nutrient in the plant)x100 (Li et al., 1991); c) use efficiency (EU) = (dry matter total produced)2/(total content of nutrient in plant) (Siddiqi & Glass, 1981).

Data analysis

The data were subjected to one-way analysis of variance, and significant differences between the means were determined using the Scott-Knott test at a probability level of 5%. Standard deviations were determined for each treatment. The statistical analyses were performed with Assistat software.

Results

There was a significant effect of the interaction of factors, water deficit and B levels for stem diameter (SD), root dry mass (RDM) and Dickson quality index (DQI) in young plants of Paricá (P < 0.05) (Table 1). Plants not subjected to deficit and with a high concentration of B present SD 15.77% higher when compared to plants under water deficit. Water deficit induced a significant reduction of SD of 16.8% and 32.7% for plants with sufficient and high B concentration, respectively (Table 1).

Stem dry mass (StDM) did not suffer significant effect of B concentration and water deficit, but the plants that did not suffer deficit and with high B concentration presented an increase of 15% in StDM if compared with plants under water deficit and high B concentration (Table 1). There was a reduction in the StDM of 25.8% and 14.4% in plants under sufficient and high B concentration, respectively. The production of leaf dry mass (LDM) had a reduction of 21.7% in plants under water deficit and high B concentration supply compared to plants without water restriction (Table 1). In plants under water deficit and sufficient B concentration there was a 19.6% reduction compared to plants not under deficit. However, these reductions were not significant.

Root dry mass (RDM) was significantly altered by interaction between them, however, for plants not subjected to water deficit there was no significant difference between the B concentrations (Table 1). For plants subjected to water deficit there was an increase of 13.3% in RDM at time of supplementation with high B. Deficit isolated effect promoted a 15% reduction in RDM compared to plants not submitted to deficit.

There was an increase in total dry mass (TDM) in plants influenced by high B concentration of 9% and 5.3% out and under water deficit, respectively, but not significant (Table 1). In addition, water deficit isolated effect caused decrease of 19.3% and 22.4% of the TDM for plants supplemented with sufficient B and high B concentration, respectively.

Dickson quality index (DQI) was significantly affected by interaction between the factors, because high B concentration provided an increase in seedling quality of 23.3% for plants without water restriction (Table 1). Plants under water deficit showed a decrease of 34.8% when supplemented with high B compared to plants under sufficient B.

B contents found in leaves and stem, as well as B accumulation in leaves, stem and roots were significantly influenced by B concentration and water deficit (P < 0.05) (Table 2). High B concentration provided significant increases in leaf B content, 28.2% and 51.4%, and stem, 7.1% and 40.2%, for young plants Paricá without and under water deficit, respectively. Analyzing water deficit isolated effect, an increase in B content in stem of 39.9% was observed with high B concentration.

Plants under influence of high B showed greater accumulations of B in leaves, stem and roots under water deficit or not. High B concentration provided an increase in B accumulation of 28.9% and 49.7% in leaves, 9.9% and 49.4% in stem, and 19.6% and 39.09% in roots of plants without and under water deficit, respectively (Table 2).

Net photosynthetic rate (*P*_N), absorption efficiency (AE) and translocation efficiency (TE) were significantly influenced by factors, B concentration and water deficit (P < 0.05) in young plants of Paricá (Table 3). High B concentration caused *P*_N to decrease by 21.1% and 26.5% in plants without and under water deficit, respectively (Table 3). Contrary behavior was observed in values of EA, which were higher in the plants supplemented with high B concentration, 28.4% and 38.8% in the treatments without and under water deficit, respectively. High B concentration provided an increase in ET of plants under water deficit of 5.3%. For use efficiency (EU) the factors were not significant, but sufficient B treatment increased 38.8% and 24.6% in EU for plants without and under water deficit, respectively.

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Table 1 - Stem diameter (SD), stem (StDM), leaf (LDM), root (RDM) and total (TDM) dry mass and Dickson quality index (DQI) of *S. parahyba* plants as function of B concentration and water regimes.

	SD		StDM		LDM		RDM		TDM		DQI	
Water regimes	er regimes		B concentration (µmol L ⁻¹)									
	25	250	25	250	25	250	25	250	25	250	25	250
Deficit	5.48 Ab	5.24 Ab	2.90 aA	3.44 aA	3.99 aA	3.87 aA	1.70 bB	1.96 aA	10.90 aA	10.65 aA	4.01 aA	3.70 bA
No deficit	6.57 Ba	7.80 Aa	3.91 aA	4.02 aA	4.96 aA	4.94 aA	2.00 aA	1.90 aA	12.50 aA	13.73 aA	4.35 aB	5.67 aA

Means followed by different uppercase and lowercase letters in the row and column, respectively, differ by Skott-Knott test (P < 0.05).

Table 2 - Content and accumulation of B in leaf, stem and root of S. parahyba plants as function of B concentration and water regimes.

	L	₋eaf	St	em	F	Root			
Water regimes	B concentration (μmol L ⁻¹)								
	25	250	25	250	25	250			
Deficit	134.10 aB	276.21 bA	32.49 aB	54.39 aA	36.52 aA	51.90 aA			
No deficit	127.89 aB	178.12 bA	30.36 aA	32.69 bA	36.69 aA	51.90 aA			
	B accumulation (μmol L ⁻¹)								
Deficit	537.82 aB	1070.42 aA	94.64 bB	187.34 aA	62.08 bB	101.91 aA			
No deficit	623.75 aB	877.45 bA	118.43 aA	131.53 bA	73.32 aB	91.23 bA			

Means followed by different uppercase and lowercase letters in the row and column, respectively, differ by Skott-Knott test (P < 0.05).

Table 3 - Net photosynthetic rate (*P*_N) absorption efficiency (AE) e translocation efficiency (TE) and use efficiency (UE) of *S. parahyba* plants as function of B concentration (25 and 250 (µmol L⁻¹) and water regimes.

	<i>Ρ</i> _N (μm	iol m ⁻²)	AE (mg g⁻¹)	TE	(%)	UE (g ² mg ⁻¹)		
Water regimes	B concentration (µmol L ⁻¹)								
	25	250	25	250	25	250	25	250	
Deficit	2.49 bA	1.83 bA	119.71 aB	195.47 aA	81.92 aB	86.43 aA	0.61 aA	0.46 aA	
No deficit	11.00 aA	8.68 aB	98.15 bB	137.01 bA	81.16 aA	81.42 bA	0.36 aA	0.22 aA	

Means followed by different uppercase and lowercase letters in the row and column, respectively, differ by Skott-Knott test (P < 0.05).

Discussion

Increase in B concentration in solution has provided growth of young plants of Paricá, evidenced by higher values of SD, RDM and DQI. This is related to structural function that B plays on plant cell wall, as it is known that this nutrient is essential for the growth of higher plants, such as forest essences. It is thought that B is responsible for interconnecting two polysaccharide molecules, Ramnogalacturonan II (RGII), which is a primary complex to control cell wall porosity. In addition, B performs activities in hydrated carbon metabolism. flow synthesis of nucleic acids, proteins, enzymes in cytoskeleton, accumulation of phenols and polyamines (Miwa & Fujiwara, 2010; Camacho-Cristóbal et al., 2008). Similar results were found by São José et al. (2009) who studied eucalyptus clones and found that omission of B decreased production of dry mass roots, stems and leaves.

Beneficial effect of B on plants is usually associated with adequate water supply in soil due to transport mechanism of this nutrient until root system is predominantly passive by mass flow, thus requiring moisture, according to Mattiello et al. (2009) 100% of B can be absorbed by mass flow in eucalyptus plants.

Decrease in LDM and RDM is linked to sensitivity of Paricá to toxicity caused by high B concentration in solution (Lima et al., 2003), presenting foliar symptoms. B toxicity decreases chlorophyll content as well as photosynthetic rate because it inhibits cell division, growth and expansion (Reid, 2007; Landi et al., 2012). In response to these changes, plants showed reduced growth, especially in aerial part with visible symptoms of toxicity, such as chlorosis and represented by dark brown spot lesions, especially on old leaves (Turan et al., 2009). Different results from this research were found by Silva et al. (2008), who investigated the toxicity of B in *Ricinus communis* L., observed that the toxicity imposed by concentration of 5.40 mg L⁻¹ did not affect dry mass production of roots, stems and leaves.

Due to higher availability of this nutrient, there were better responses of DQI, as this index depends on growth parameters. It is worth noting that Dickson quality index is measured from TDM, SD, height, shoot dry mass and RDM data, and seedlings with DQI > 0.2 are within ideal quality standard, according to Dickson et al. (1960). Different results were found by Santos et al. (2013), who studied effect of nutrient omission on development of *Bertholletia excelsa* and observed that omission of B limited plant growth, in addition to reducing the DQI.

Water deficit has reduced growth of plants in Paricá, as it is one of main factors limiting plant growth in agricultural production due to absence of cell division and elongation, in addition to absorption of nutrients (Raif, 2008). Water stress occurs when there is a lack of water or when rate of transpiration is higher than the water supply in the environment, with consequent osmotic stress (Moreno, 2009). Effect of water deficit on eucalyptus growth was negative, because when water

supply was reduced below 75%, there was a gradual decrease in total biomass production (Schwider et al., 2013).

B deficiency reduces root length and absorption of water and nutrients, since B can be absorbed by passive diffusion, active diffusion and/or through absorption channels as water vapor, in addition, this nutrient is transported mainly via xylem (Miwa & Fujiwara, 2010; Wimmer & Eichert, 2013). Nunes (2010) verified influence of availability of B on water deficit tolerance of eucalyptus clones and argued that B may contribute to activation of water deficit tolerance mechanisms, or under B deficiency conditions there will be worsening of water stress in plants. Results that support our research were found by Möttönen et al. (2005), who studied the B concentration in growth of Picea abies (L.) Karst. under water deficit, found that plants with B deficiency were affected by water deficit through reduction in height, dry mass of the aerial part and root mass.

Content and accumulation of B in leaves and stem are highly influenced by their greater supply in solution, even under water deficit conditions. This is related to fact that B contributes to development of water deficit tolerance mechanisms in forest essences (Nunes, 2010). This research is corroborated by Silva et al. (2014), in *Carica papaya* L., when they found that leaf B content increased linearly with supply of B, confirming idea that B is transported mainly by perspiration flow and deposited in leaves after water evaporation (Rees et al., 2011). Furthermore, Euba Neto et al. (2014) in *Helianthus annuus* L. plants observed that high availability of B provided greater accumulation in stem, in addition to greater dry mass production of leaves and total.

Higher accumulation of B in leaves may be related to fact that this nutrient has a direct effect on polysaccharide synthesis, since B is responsible for binding of two monomers of ramnogalacturonan II (RGII), a pectic component that confers resistance to the cell wall (Acuña & Molina, 2007). However, under stress conditions, vegetables accumulate sugar in leaves (Gupta, 2007), and this greater accumulation is related to high amount of dry leaf mass.

Accumulation of B in roots with a high B concentration is due to capacity of roots to absorb B in high or even toxic amounts by passive absorption of mass flow (Miwa & Fujiwara, 2010). In cotton plants fed 4.5 mmol L⁻¹ B, 15% to 34% of B accumulated in roots in relation to total accumulated in plant (Rosolem et al., 2012).

High supply of B under water deficit conditions provided higher absorption efficiency (AE) and translocation (ET), and these results are directly related to plant growth. This is because these parameters depend mainly on total nutrient content of plant, total dry weight and dry mass of roots (Swiader et al., 1994; Araújo & Silva, 2012). They corroborate with this research Tomaz et al. (2011), who evaluated the absorption efficiency of B in *Coffea arabica* L. and observed AE, 44.94 µg g¹. Another study, Prado et al. (2006), with application of B in *Passiflora edulis*, observed that increasing doses of B decreased translocation rate of this nutrient in the plant, that is, the highest dose of 1 mg dm⁻³ was the lowest ET, with higher accumulation of B in roots.

Conclusion

High B supply under water deficit conditions was positive to improve growth parameters, photosynthetic rate and nutritional efficiency of *Schizolobium parahyba* demonstrating that adequate supply of B influences the ability of this species to withstand water stress conditions.

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