BORON INFLUENCE ON CONCENTRATION OF POLYOLS AND OTHER SUGARS IN *Eucalyptus*[1]

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ABSTRACT – Although the functions of the element Boron (B) in plants have not been sufficiently clarified, several hypotheses have been raised. Some of the functions attributed to this element are synthesis and transport of carbohydrates. To evaluate the effect of B on the synthesis of some polyols and sugars in *Eucalyptus grandis* and hybrids "urograndis", these species were submitted to situations of supply and restriction of B. The experiment was carried out in a greenhouse, arranged in a randomized block design in a 2 x 2 factorial scheme, with 5 replicates of each genotype. The B levels were provided in the form of nutrient solution. No significant difference was observed in the content of mannitol, sorbitol, myo-inositol and scyllo-inositol and sugars α-glucose and β - glucose between *E. grandis* and hybrids. Arabinose was the only one to present a higher content in *E. grandis* on restriction of B. The effect of the presence of B was very expressive, but regarding B supply, the plants showed significant increase in the synthesis of the compounds evaluated.

Keywords: Mannitol, metabolism of sugars in plants and sorbitol.

INFLUÊNCIA DO BORO NA SÍNTESE DE POLIÓIS E OUTROS AÇÚCARES EM Eucalyptus

RESUMO – Embora a função do elemento Boro (B) nas plantas ainda não tenha sido bem esclarecida, várias hipóteses vêm sido levantadas. Uma das funções atribuídas a esse elemento é a síntese de carboidratos. Com o objetivo de avaliar o efeito do B sobre a síntese de alguns polióis e açúcares em ***Eucalyptus grandis*** *e híbridos "urograndis", estes foram submetidos à situação de suprimento e restrição de B. O experimento foi conduzido em casa de vegetação de acordo com o delineamento de blocos casualizados com cinco repetições, e os elementos nutricionais, assim como o B, foram fornecidos na forma de solução nutritiva. Não se observou diferença significativa nos teores de manitol, sorbitol, myo-inositol e scyllo-inositol e dos açúcares* α*-glicose e* β*-glicose entre* ***E. grandis*** *e híbridos. Arabinose foi o único a apresentar maior teor em* ***E. grandis*** *em restrição de B. O efeito da presença do B foi bastante expressivo, mas em suprimento do elemento as plantas apresentaram acréscimo significativo na síntese dos compostos avaliados.*

Palavras-chave: Manitol, metabolismo de açúcares em plantas e sorbitol.

1. INTRODUCTION

The so-called polyols or polyhydroxy alcohols are reduced forms of sugar that are probably present in all species of plants (LOESCHER et al., 1995). The most common polyols are derived from hexose sugars in which the aldose or ketose group is reduced in a hydroxyl group. Thus, mannitol, sorbitol (or glucitol) and dulcitol (or galactitol) are the equivalent polyols of the hexoses glucose, fructose, and galactose, respectively, and are the most frequent hexitols in angiosperms (NOIRAUD et al., 2001).

Mannitol, sorbitol and dulcitol, like other sugars,

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[1] Recebido em 02.02.2007 e aceito para publicação em 22.08.2008.
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play an important role in several organisms. Mannitol is the most abundant polyol in nature, participating in the translocation and storage of metabolites and providing the plants with resistance against salinity and osmotic stress (YAMAMOTO et al., 1997), and against invasions by pathogens as well (STOOP et al., 1996). Sorbitol is known to be the product of primary photosynthesis transport in many common fruit trees (LOESCHER et al., 1995). In some species, such as apple trees and plum trees, sorbitol occurs at approximately the same concentrations as sucrose (BOLLARD, 1970).Inositol, another important polyol, seems to be involved, together with auxin, in root initiation (JAVIS and BOOTH, 1981) and has an important function as a secondary messenger for signal transduction in metabolic pathways, participating in the transmembrane transport of calcium and other compounds (TAIZ and ZEIGER, 2004). Despite their importance, the occurrence, metabolism, and physiology of polyols are still largely unknown, and more research work is needed on these compounds (BROWN and SHELP, 1997). Finally, arabinose is involved in the regulation of transcription-promoting sites (SAVIOLA et al., 1998), while glucose is extremely important for starch synthesis, which is involved in the process of growth and maintenance of plants during the non-photosynthetic period (at night, for example) (MADORE, 1994).

The polyols mannitol and sorbitol also have a crucial role in the mobility of the element Boron (B) within plants. According to studies, the mobility of B allows this element to link the compounds which have a cis-diol configuration. These compounds are polyols (sugar alcohols) as: sorbitol (tree), mannitol (broccoli) and dulcitol (DORDAS et al., 2001). The polyols are considered compound of stock formed during the photosynthetic process and transported by phloem in higher plants, and used as osmorregulator (LOESCHER et al., 1995). B would move therefore linked to these polyols.

Boron is an essential element for the development of higher plants (WARINGTON, 1923; CALDEIRA et al., 2004). There is evidence that B plays various roles within the plants, from primary metabolite synthesis, (MADORE, 1994) to cell wall biogenesis and pollen germination (GARCÍA HERNÁNDEZ and CASSAB LOPÉZ, 2005).

The objective of this work was to evaluate the effect of B on the concentration of some sugars, both in *Eucalyptus grandis* and in *E. grandis* × *E. urophylla* (“urograndis”) hybrids, as well as to evaluate possible differences between these genetic materials.

2. MATERIAL AND METHODS

To assess the biosynthesis of carbohydrates in situations of supply and restriction of B in *Eucalyptus grandis* and hybrid between *E. grandis* and *E. urophylla* (urograndis), 5 different clones of the species *E.grandis* in 11 different clones urograndis were used each clone with 5 replicates (each experimental plot is made up of a plant). The experiment was conducted under a plastic tunnel in a randomized block design in a 2 x 2 factorial (species / hybrids x levels of B) scheme. Each plant was kept in a container with a capacity of 8 L in silica.

The plants were irrigated daily with 1 L Hogland and Arnon’s (1950) modified nutritive solution, with the following formulation: 223 mg L^{-1} Cl, 220 mg L^{-1} N, 180 mg L^{-1} Ca, 180 mg L^{-1} K, 62 mg L^{-1} S, 50 mg L^{-1} Mg, 25 mg L^{-1} P, 5 mg L^{-1} Fe, 0.67 mg L^{-1} Mn, 0.5 mg L^{-1} B (0.0 mg L^{-1} B in B restriction), 0.32 mg L^{-1} Cu, 0.091 mg L^{-1} Zn, 0.024 mg L^{-1} Mo and 0.011 mg L^{-1} Na. The nutrient solution was prepared in a plastic container and refreshed every 15 days.

During the initial four months, the plants received a complete solution so that the plants could develop sufficient leaf area. After that period, B was removed from the nutrient solution (restriction B treatment) for 60 days.In the final 30 days, B was again added to the solution.

Sugar Analysis: Leaves were sampled under two situations. The first collection was carried out 162 days after the experiment had started, when the plants had already been without B in the nutrient solution for 42 days. At this stage, the plants already showed marked deficiency symptoms of that element. The second collection was carried out 30 days after application of the complete solution (that is, a solution that supplied B) had been restored.

Sugar analysis was performed on leaves harvested from the first pair of mature leaves in the distal section of the upper branches of the plants. The methodology suggested by Bellaloui et al. (1999), with a few modifications described ahead, was used for sap extraction. Three grams of fresh leaves were ground in approximately 10 mL 80% ethanol. The extract was centrifuged and an internal standard in the form of 50 μL of a 1 μg μL^{-1} xylitol solution was added to the supernatant. The samples were dried in a double boiler at 50 ^{o}C until reduced to a very thick paste and stored in a freezer until analysis. The samples were prepared for analysis by adding 400 µL acetic anhydride and 60µìL 1- methylimidazole. After 10 minutes, the reaction was interrupted by adding 2 mL distilled water and 2 mL dichloromethane and agitated with a vortex mixer. The supernatant was removed and the operation was repeated twice with the remaining part of the samples. Again, the samples were dried in a double boiler at 50 ^{o}C with an air flow.The samples already containing acetylated sugars were dissolved in 100 µL acetone and analyzed by means of a Refraction Index in a Perkin-Elmer brand (model 8320) gas chromatograph (GC). Additional information on the compounds was obtained by using a Hewlett-Packard model 5890 gas chromatograph coupled with a Hewlett-Packard model 5970 mass spectrometer operated as described by Greve and Labavitch (1991). The analyses were performed at the Chromatography Laboratory of the Pomology Department of the University of California at Davis, CA, USA.

Statistical Analysis: The ANOVA model of the SANEST software program was used to run the analysis of variance for the concentrations of sugars, followed by the Tukey Test at 5% significance level.. Due to the fact that some samples presented values equal to 0 (zero), the data were transformed by the equation: y=$\sqrt{x + 0,5}$, as suggested by Banzato and Kronka, 1995, to perform the analysis.

3. RESULTS AND DISCUSSION

The influence of the nutritional condition of the plant on the organic compound synthesis has been reported (CARMO et al., 2004). This effect can also be observed in the present work.

Concentration of the sugars mannitol, sorbitol, arabinose, a-glucose and b-glucose, myo-inositol and scyllo-inositol, in mature leaves of *Eucalyptus*, proved to be significantly susceptible to B supplementation, except for arabinose in *E. grandis* (Table 1). The concentration of these sugars considerably increased with B supplementation, reaching a 693.68% increase in the case of sorbitol, and 878.29% in the case of Scyllo-inositol, in *E. grandis*.

Boron is an essential micronutrient in higher plants, and its deficiency results in growth inhibition (HU and BROWN, 1994) and in substantial changes at the cell level (CAKMAK and RÖMHELD, 1994). Parr and Loughman (1983) also attributed physiological damages to B deficiency, such as in the synthesis and structure of the cell wall, membrane integrity, RNA metabolism, respiration, IAA and phenol metabolism, and also in the metabolism and transport of sugars. Other functions have been attributed to B (LUKASZEWSKI and BLEVINS, 1996), even though it is still not clear whether some of the physiological changes mentioned above would represent a direct or an indirect function of this micronutrient.

Tabela 1 – Concentração média e desvio padrão da média de açúcares em folhas maduras de *E. grandis* e híbrido na presença e ausência de Boro. Valores seguidos pela mesma letra indicam não significância a 5% pelo teste de Tukey.

Table 1 – *Mean concentration and standard deviation of the sugar mean in mature leaves of E. grandis and hybrids in the absence and presence of Boron. Values followed by a common letter indicate that the treatments (E. grandis and hybrids) did not differ at 5% level by the Tukey test*

Sugars	Boron	*E. grandis*	Hybrids
Arabinose	Supply	178,63 ± 14,58 aA	157,22 ± 22,99 aB
	Restriction	125,77 ± 21,38 aA	57,47 ± 9,26 bA
Mannitol	Supply	422,46 ± 33,04 aB	325,61 ± 25,81 aB
	Restriction	132,49 ± 9,06 aA	128,57 ± 8,59 aA
α-Glucose	Supply	3631,73 ± 925,07 aB	2815,40 ± 538,36 aB
	Restriction	879,95 ± 166,93 aA	681,92 ± 126,39 aA
β-Glucose	Supply	1957,09 ± 370,56 aB	1519,93 ± B221,23 aB
	Restriction	540,14 ± 80,59 aA	477,63 ± 69,82 aA
Sorbitol	Supply	1664,18 ± 645,57 aB	1113,12 ± 148,66 aB
	Restriction	209,68 ± 32,65 aA	221,76 ± 33,52 aA
Myo-inositol	Supply	2055,19 ± 434,73 aB	1664,12 ± 289,76 aB
	Restriction	487,31 ± 88,20 aA	545,41 ± 96,26 aA
Scyllo-inositol	Supply	1318,24 ± 186,25 aB	668,16 ± 85,96 bB
	Restriction	134,75 ± 15,95 aA	92,41 ± 10,64 aA

Lower-case letter: comparison of supply and restriction Boron (column). Lower-case letter: comparison of genetic materials (line).

Reductions in the synthesis of sugars were also observed by Kastori et al. (1995) in B-deficient sunflower leaves, which were explained as a decrease in their ability to assimilate O_2 and CO_2. Due to damage caused to the photosynthetic process, starches and sugars were accumulated for a short period in leaves submitted to B deficiency, indicating a reduction in the translocation of photosynthates in sink organs. Marschner (1995) attributed the reduction in sucrose translocation from sink tissues to the loss of activity in those tissues in roots and apical buds. The increase in the concentrations of sugars when the B nutrition status becomes normal again was explained by Dugger (1983), who stated that when the plant's requirements for B have been taken care of, the translocation of photosynthesized ^{14}C is stimulated from its source tissues to other parts of the plant, and the synthesis of sugars and starch is resumed normally.

Even though it was not statistically significant, both under B restriction and a full supply, the sugar contents were higher on average in *E. grandis* than in the hybrids, and this situation was maintained when the element was normally supplied (Table 1), except for arabinose.

Arabinose was the only carbohydrate that showed a significant difference between *E. grandis* and the hybrids, as it presented 125.77 mMol content in *E. grandis* and 57.47 mMol in hybrids, on average. Again, no significant differences were detected between the 16 genotypes tested, which could indicate low genetic variability with respect to the expression of genes related to the synthesis of these sugars in the tested materials. Intra- and interspecific variations have been reported by some authors with regard to responses between plants submitted to both B deficiency and B toxicity conditions (BROWN and SHELP, 1997), and to variations in the contents of certain polyols in tissues from different plant species (SHELP et al., 1996), which implies differences between genotypes. Among the sugars here evaluated, sorbitol and mannitol have deserved great consideration in the mineral nutrition of plants due to their importance for B mobilization (BROWN and HU, 1996; BELLALOUI et al., 2003). Species considered rich in sorbitol or mannitol have shown mobility of this element within the plant, which does not occur with most species that do not present these polyols, or synthesize them in small amounts. The contents of these polyols even among species considered sorbitol-rich or mannitol-rich is also quite variable. Species such as *Pyrus*, *Malus*, and *Prunus*, which are considered rich in sorbitol, show content variations from 300 to 1000 mMol (BROWN and HU, 1996), while transgenic tobacco shows a content variation for sorbitol between 0.3 and 1.0 µMol g^{-1} fresh leaf of this polyol (BELLALOUI et al., 1999).

Even though the variation found between *E. grandis* and urograndis hybrids was not significant under the conditions adopted, the genetic variation between other species in the genus *Eucalyptus* is quite possible. As a consequence, the determination of polyols in *Eucalyptus* could be of relevant importance in the selection of plants with higher polyol contents for the different purposes they are suitable for, be it B mobility (sorbitol and mannitol) or improved rooting of cuttings (inositol). The selection of superior genotypes can increase the likelihood of success in activities such as cutting propagation and even reforesting with regard to greater tolerance to B deficiency in soils subject to water deficits.

4. ACKNOWLEDGEMENTS

The authors wish to thank Patrick Brown, Nacer Bellaloui and Hening Hu for their support..

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