The effects of magnesium deficiency on sugar partitioning do not restrict the root growth in Eucalyptus young plants

Os efeitos da deficiência de magnésio na partição de açúcares não restringem o crescimento de raízes em plantas jovens de eucalipto

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Abstract

The symptoms of magnesium (Mg) deficiency have been well documented in crop plants. The relationship between Mg deficiency as an important abiotic stress factor and the sugar partitioning may restrict the root growth and limit the success of planting in the field. Despite of this, the primary physiological effects of low Mg availability remain largely unknown in eucalyptus. This paper aimed to investigate how the Mg deficiency affects biochemical aspects of sugar partitioning associated to dry matter accumulation in roots of Eucalyptus urophylla young plants, clone AEC 144. Experimental work was carried out in a greenhouse, arranged by completely randomized design, consisted by split plot 5x4, using the following Mg levels: 0, 25, 50, 75 and 100% in the Mg concentration of Clark’s nutrient solution. Evaluations were performed at 15, 30, 50 and 120 days after seedlings planting (DAP) in pots. Soluble (SS) and reducing (RS) sugar contents, invertase and sucrose synthase (susy) activity and shoot and root dry matter were measured. Increased sugar concentrations, both SS and RS, were found in leaf tissues from 30 DAP. In root tissues, neither RS nor SS content showed differences between Mg deficiency treatments and control. Significant differences were also not found in both root dry matter accumulation and shoot/root dry matter ratio. The Mg deficiency did not affect sucrose cleaving in roots, which was predominantly catalyzed by acidic invertase, followed by susy and neutral invertase. We concluded that Eucalyptus urophylla is tolerant to Mg deficiency, because the sugar accumulation in the leaf tissues was not enough to constrain the dry matter accumulation in roots.

Keywords: Woody plants; Mineral nutrition; Assimilate partitioning; Source-sink

Resumo

Os sintomas da deficiência de magnésio (Mg) têm sido bem documentados em plantas cultivadas. A relação entre a deficiência de Mg, como um importante fator de estresse abiótico, e a partição de açúcares, pode restringir o crescimento das raízes e limitar o sucesso do plantio no campo. Apesar disso, os efeitos fisiológicos primários da baixa disponibilidade de Mg permanecem amplamente desconhecidos no eucalipto. Este trabalho buscou investigar como a deficiência de Mg afeta aspectos bioquímicos da partição de açúcares, associados ao acúmulo de matéria seca em raízes de plantas jovens de Eucalyptus urophylla. O experimento foi conduzido em casa de vegetação em delineamento inteiramente casualizado, com parcelas subdivididas 5 x 4, utilizando os seguintes níveis de Mg: 0, 25, 50, 75 e 100% na concentração da solução nutritiva de Clark. As avaliações foram realizadas aos 15, 30, 50 e 120 dias após o plantio (DAP) das mudas em vasos. Determinaram-se os teores de açúcares solúveis (SS) e redutores (RS), as atividades da invertase e da sacarose síntese (susy), e a matéria seca da parte aérea e da raiz. Aumentos nas concentrações de açúcares, tanto SS como RS, foram encontrados em tecidos foliares a partir de 30 DAP. Nos tecidos de raízes, os teores de RS e SS não apresentaram diferenças entre os tratamentos com deficiência de Mg e o controle. Diferenças significativas também não foram encontradas, tanto no acúmulo de matéria seca da raiz, como na razão matéria seca de parte aérea / raízes. A deficiência de Mg não afetou a clivagem de sacarose nas raízes, que foi predominantemente catalisada pela invertase ácida, seguida de susy e invertase neutra. Concluiu-se que Eucalyptus urophylla é tolerante à deficiência de Mg, uma vez que o acúmulo de açúcares nos tecidos foliares não foi suficiente para restringir o acúmulo de matéria seca nas raízes.

Palavras-chave: Plantas lenhosas; Nutrição mineral; Partição de assimilados; Fonte-dreno

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Introduction

Eucalyptus is the most important forestry species in Brazil, with cultivated areas over more than 5.56 million hectares (INDÚSTRIA BRASILEIRA DE ARVORES, 2015). Cultivations are usually carried out by intensive regime, based on high productivity clones, which performances reaches 60 m³ ha⁻¹ year⁻¹ (MORA; GARCIA, 2000), depending on the genotype, edaphoclimatic characteristics and cultivation conditions (ALFENAS; ZAUZA; MAFIA, 2009).

Some species of the *Eucalyptus* genus are tolerant to low levels of magnesium (Mg) in the soil, even at levels below those established for most crops (BARROS; NOVAIS, 1999). Due to a great Mg demand by eucalyptus, the exhaustion of this nutrient in soil solution is concerning, although it is often overlooked in fertilization programs (CAKMAK; YAZICI, 2010).

Mg²⁺ is less adsorbed by soil colloids than the other cations, because of its larger radius of hydration, which makes it highly susceptible to leaching (DENG et al., 2006). Mg²⁺ availability also becomes reduced in acid soils, due to its competition with H⁺, Al³⁺ and Mn²⁺; in alkaline soils, Mg²⁺ competes with Ca²⁺, NH₄⁺, Mn²⁺ and especially with K⁺ (WILKINSON et al., 1990; MARSCHNER, 2012) which is widely used in fertilization programs and increases the competitive effect on the Mg²⁺ absorption, decreasing its content on the leaves (OLIVEIRA; CARMELO; MASCARENHAS, 2001; MARQUES et al., 2011).

Mg is an essential element for the plant development, which is required in several metabolic processes and reactions, such as photophosphorylation, carbon dioxide fixation in photosynthesis, protein and chlorophyll synthesis, phloem loading, assimilate partitioning, reactive oxygen species formation and photo-oxidation in leaf tissues (CAKMAK; YAZICI, 2010). In addition, Mg deficiency affects the size, structure and function of chloroplasts, including the electron transfer in photosystem II (MCISWAIN; TSUJIMOTO; ARNON, 1976; MAATHUIS, 2009). It also acts as a cofactor and allosteric activator of CO₂ fixation enzymes (MARSCHNER, 2012), and participates in energy transfer via ATP (IGAMBERDIEV; KLECZKOWSKI, 2003; MAATHUIS, 2009; MAATHUIS, 2009; PASTERNAK; KOCOT; HORECKA, 2010) and pH control (WU; PETERS; BERKOWITZ, 1991).

The sucrose available for exporting from mesophyll cells is transported through the phloem to the sink organs, where it can be stored as starch in amyloplasts or converted to hexoses (LEMOINE et al., 2013) by invertase (acidic and neutral isoforms) and sucrose synthase (susy) enzymes (STURM; TANG, 1999; WINTER; HUBER, 2000; WELHAM et al., 2009). Roots and young leaves are the most important sinks during the initial plant growth (WARDLAW, 1990).

The literature shows controversial results regarding to root-shoot ratio as a plant response to Mg deficiency. It has been reported that the shoot growth is greater than the root growth in *Pinus* (SUN; PAYN, 1999) and coffee (SILVA et al., 2014). However, in *Arabidopsis thaliana* (HERMANS; VERBRUGGEN, 2005) and sugar beet (HERMANS et al., 2005) occurs the inverse: root growth is greater than shoot growth. Carbohydrate accumulation in leaves and lower biomass partitioning from shoot to roots have been reported as some plant responses to Mg deficiency (HERMANS et al., 2004; TEWARI; KUMAR; SHARMA, 2006; CAKMAK; KIRBY, 2008). Carbon allocation to root growth increases the root-shoot ratio, which ultimately would result in greater capacity to water and nutrient uptake by the roots, providing better conditions for the seedling survival after its planting in the field (PALLARDY, 2008).

Low Mg availability tends to difficult sucrose exportation, causing leaves to retain sucrose three to twelve times more than the leaves of plants that receive Mg in adequate supply, indicating that the deficiency of this nutrient causes severe inhibition of sucrose transport (CAKMAK; HENGELER; MARSCHNER, 1994a) and other solutes, like K⁺ and aminoacids (MARSCHNER; CAKMAK, 1989), in the source-sink system.

Despite the well-known role of Mg for various critical functions in several species, there is surprisingly little research activity on the role of Mg nutrition on biochemical aspects involved in the sucrose transport from source to sink in eucalyptus. This study aimed to investigate...
The effects of magnesium deficiency on sugar partitioning do not restrict the root deepening of young plants of *Eucalyptus urophylla*.

**Materials and methods**

**Plant cultivation and experimental design**

The experiment started in July 2015, in a greenhouse of the State University of Southwest Bahia, Vitoria da Conquista, Brazil. *Eucalyptus urophylla* (clone AEC 144) 90 days old seedlings were planted in pots (8 dm$^3$) containing washed sand. In order to adapt to the hydroponic system, the seedlings were irrigated for 20 days using a nutrient solution (CLARK, 1975) – first at 50% of its ionic strength and then at 100%, in the last 10 days. Thereafter, the sand of the pots was abundantly washed with deionized water until the electrical conductivity of the water drained from the pots have decreased to a level equal or very close to 0.0 mS cm$^{-1}$.

To implement the treatments, seedlings started to be irrigated daily using a Clark’s nutrient solution, which was adjusted to the following Mg levels: 0, 25, 50, 75 and 100% in the Mg concentration of Clark’s nutrient solution. The electrical conductivity of the solution was 1-4 mS cm$^{-1}$ and pH was 5.5-6.5. Evaluations were performed at 15, 30, 50 and 120 days after seedlings planting in pots. Treatments were arranged in a split plot 5 x 4 (five Mg concentrations and four periods of evaluations), with three replicates (one plant pot$^{-1}$), in a completely randomized design.

Data were used in analysis of variance, based on F test. According to homogenity and normality, when necessary, data were transformed. Periods of evaluations were compared by Skott-Knott test (p < 5%), while Mg concentrations were submitted to regression analysis. Mean were compared using SAEG program, 9.1 version.

**Soluble and reducing sugars content**

Water soluble sugars (SS) were extracted from leaves and roots using 15 mL of KH$_2$PO$_4$ buffer 0.1 M as extractor. For each plant tissue, the samples (200 mg of dry weight) were centrifuged three times at 1.225 g for 40 min. The supernatants of the three extractions were combined for analysis.

To SS content determination, an aliquot of 0.2 mL of the extract was added to reaction medium at 4°C, which consisted of 2 mL of anthrone solution (40 mg anthrone, 1 mL deionized water, 20 mL H$_2$SO$_4$) and 0.8 mL of deionized water. Then, the reaction medium was placed in a water bath at 100°C for three minutes. After cooling, readings were taken at 620 nm (YEMM; WILLIS, 1954).

Reducing sugars (RS) were determined by adding an aliquot of 0.8 mL of the extract to a reaction medium, which consisted of 0.5 mL of dinitrosalicylic acid solution and 0.2 mL of deionized water. Then, the reaction medium was placed in a water bath at 100°C for 15 minutes. After cooling, 3.5 mL of deionized water were added to reaction medium, and readings were taken at 540 nm (MILLER, 1959).

**Invertases and sucrose synthase (susy) activities**

To evaluate the magnitude of sucrose hydrolysis in young root tissues, reducing sugars’ production was considered as a result from the sum of invertases (acidic and neutral isoforms) and susy activities. Reaction medium for each enzyme assay was prepared *in vivo* from young root tissues (200 mg of fresh weight), similarly to that proposed by Cairo *et al.* (2015).

Reaction medium for acidic invertase assay (vacuole and cell wall) was composed by 200 µL of sodium acetate buffer 1.0 M pH 4.7, 100 µL of MgCl$_2$ 0.1 M and 400 µL of sucrose 1.0 M,
with added water up to 2.000 µL. For neutral invertase assay, reaction medium was maintained, except for the pH, which was adjusted to 7.5, and for the buffer, which was KH$_2$PO$_4$ 1.0 M. Both assays were kept in a water bath at 37°C for 60 minutes. Reaction medium for susy assay was 200 µL of HEPES-KOH buffer 1.0 M pH 6.0, 100 µL of MgCl$_2$ 0.1 M, 100 µL of UDP 5.0 mM and 400 µL of sucrose 1.0 M, with added water up to 2000 µL. The assay was kept in a water bath at 25°C for 60 minutes. RS contents were determined according to Miller (1959) and readings were performed 540 nm.

**Dry matter weight**

Plants were removed from the pots and shoots and roots were taken separately to an oven dryer at 70°C for 72 hours. After that, the dry matter weight was gauged.

**Results and discussion**

In leaf tissues, RS content showed a significant increase as a response to Mg deficiency at 30 and 120 days after planting (DAP). In these two periods, the highest RS contents (0.13 and 0.26 mmol.g.dw$^{-1}$, respectively) were obtained in plants under total Mg absence. However, no significant differences were found in RS contents at 15 and 50 DAP, in response to Mg concentrations (Figure 1). In root tissues, both RS contents and SS contents showed no differences between Mg deficiency treatments and control (data not shown).

**Figure 1** – Reducing sugar (RS) contents in leaves of *Eucalyptus urophylla* young plants, clone AEC 144, at four evaluation dates after planting (DAP), grown under different Mg concentrations.

Figura 1 – Teores de açúcares redutores (RS) em folhas de plantas jovens de *Eucalyptus urophylla*, clone AEC 144, em quatro datas de avaliação após o plantio (DAP), crescidas sob diferentes concentrações de Mg.

![Graph showing RS contents in leaves of Eucalyptus urophylla under different Mg concentrations](image_url)
SS content in leaf tissues also increased, from 30 DAP, in response to progressive lack of Mg (Figure 2). The highest SS contents (2.19; 1.87 and 2.64 mmol·g dw⁻¹) were obtained in plants submitted to 0% Mg, at 30, 50 and 120 DAP, respectively.

**Figure 2 – Soluble sugar (SS) contents in leaves of Eucalyptus urophylla young plants, clone AEC 144, at four evaluation dates after planting (DAP), grown under different Mg concentrations. Data presented were transformed using log (x).**

Figura 2 – Teores de açúcares solúveis (SS) em folhas de plantas jovens de Eucalyptus urophylla, clone AEC 144, em quarto datas de avaliação após o plantio (DAP), crescidas sob diferentes concentrações de Mg. Os dados apresentados foram transformados, usando log (x).

Increased sugar concentrations, both SS and RS, were found in leaf tissues only after 15 DAP. These observations suggest that the effects of Mg deficiency on sugar accumulation are tolerable in the early two weeks after planting.

Increased leaf sugar contents were also found in other Mg-deficient trees (WIKSTRÖM; ERICSSON, 1995). In spruce, elevated pools of carbohydrates occurred before (MEHNE-JAKOBS, 1995) or during the appearance of yellowing symptoms (SOLBERG; RINDAL; OGNER, 1998). Disturbance of phloem transport in Mg-deficient spruce needles could be also attributed to an increased sucrose gradient from the mesophyll toward the central cylinder in yellowing needles. This increased gradient occurred transiently, during the first few weeks of symptom development, until the cell disintegration of the phloem tissue could take place. The overall content of sucrose in these samples, however, changed only slightly probably due to a down-regulation of sucrose synthesis, as indicated by a reduced activation state of sucrose-P synthase (SPS) in these samples (MEHNE-JAKOBS, 1995).

Sugar accumulation in leaves have been reported in Mg-deficient crop plants, such as beans (FISCHER; BREMER, 1993; CAKMAK; HENGELER; MARSCHNER, 1994a; 1994b), spinach (FISCHER et al., 1998), sugar beet (HERMANS et al., 2004; 2005) and coffee (SILVA et al., 2014). The increase in soluble sugar concentration may occur prior to any noticeable change in photosynthetic activity, growth and chlorophyll content (CAKMAK; HENGELER; MARSCHNER, 1994a; HERMANS et al., 2004).
Different explanations for similar sugar accumulation in leaves have been proposed. Fischer and Bremer (1993) attributed to a source to sink sucrose transport interruption. For Cakmak, Hengeler and Marschner (1994a; 1994b), an increase in sugar content assimilate was due to a Mg-limited phloem sucrose loading. For Marschner (2012), a malfunctioning of the proton pumping ATPase involved in the sucrose phloem loading was proposed as the reason for phloem loading inhibition.

**Dry weight**

In shoot dry weight, the results showed significant effects of Mg concentration and time of evaluation, although the interaction between the factors was not significant (Table 1). A significant, but not so strong, decrease in dry weight was found only at 120 DAP, as a response to Mg deficiency, mainly in the total absence of this nutrient (Figure 3). In respect of root dry weight, there was no significant difference between the parameters. No differences were also found in the shoot/root dry weight ratio.

**Table 1 – Analysis of variance and coefficients of variation (CV) of shoot, root and shoot / root ratio dry weight in Eucalyptus urophylla young plants, clone AEC 144, submitted to different Mg concentrations, in four evaluation dates after planting. Data were transformed using log (x).**

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>Degrees of Freedom</th>
<th>Mean Squares</th>
<th>Shoot</th>
<th>Root</th>
<th>Shoot / Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg concentrations (C)</td>
<td>4</td>
<td>0.020881*</td>
<td>0.012047NS</td>
<td>0.059NS</td>
<td></td>
</tr>
<tr>
<td>Error 1</td>
<td>8</td>
<td>0.001953*</td>
<td>0.006959*</td>
<td>0.034**</td>
<td></td>
</tr>
<tr>
<td>Times of evaluation (T)</td>
<td>3</td>
<td>6.856207*</td>
<td>3.687135*</td>
<td>6.114**</td>
<td></td>
</tr>
<tr>
<td>(C) x (T)</td>
<td>12</td>
<td>0.006644NS</td>
<td>0.004220NS</td>
<td>0.054 NS</td>
<td></td>
</tr>
<tr>
<td>Error 2</td>
<td>32</td>
<td>0.009868*</td>
<td>0.006194*</td>
<td>0.050**</td>
<td></td>
</tr>
<tr>
<td>CV 1 (%)</td>
<td></td>
<td>3.89</td>
<td>8.48</td>
<td>11.99</td>
<td></td>
</tr>
<tr>
<td>CV 2 (%)</td>
<td></td>
<td>8.75</td>
<td>8.00</td>
<td>14.41</td>
<td></td>
</tr>
</tbody>
</table>

* F significant (p < 0.05); NS: non-significant

Mg deficiency has been reported to affect the plant growth and the biomass partitioning between root and shoot (CAKMAK; HENGELER; MARSCHNER, 1994b; FISCHER et al., 1998). Sucrose accumulation in leaves seems to be the major growth constraint especially for roots, under Mg-limiting conditions (ERICSSON; KAHR, 1995). Nevertheless, studies have been reported that diverge from this understanding. In Beta vulgaris, after prolonged Mg deficiency treatment, biomass allocation in roots was not markedly lower as that in control plants (HERMANS et al., 2005). Regardless of the intrinsic differences of plant species and growth stages between the present and other previous studies, results reported here also suggest that an adaptive mechanism would be responsible for the unaffected root growth found in Mg-deficient eucalyptus plants. Such mechanism would enhance the mineral uptake ability from the nutrient medium in the absence of Mg.
The effects of magnesium deficiency on sugar partitioning do not restrict the root...

Figure 3 – Shoot dry weight of *Eucalyptus urophylla* young plants, clone AEC 144, at 120 DAP, grown under nutrient solutions with different Mg concentrations. Data were transformed using log (x).

Figura 3 – Peso da matéria seca da parte aérea de plantas jovens de *Eucalyptus urophylla*, clone AEC 144, aos 120 DAP, crescidas em soluções nutritivas com diferentes concentrações de Mg. Os dados foram transformados usando log (x). Fonte: Barbosa, Cairo, Lacerda and Botelho

Sucrose cleaving and utilization enzymes activity in roots

Comparing the performance of these enzymes, sucrose cleavage was predominantly catalyzed by acidic invertase, followed by susy and neutral invertase (Figure 4).

Figure 4 – Relative contribution of invertase (acidic and neutral) and susy to sucrose cleaving at four evaluation dates after planting (DAP) in root tissues of *Eucalyptus urophylla* young plants, clone AEC 144, grown in nutrient solutions with different Mg concentrations.

Figura 4 – Contribuição relativa da invertase (ácida e neutra) e susy para a clivagem de sacarose em quatro datas de avaliação após o plantio (DAP) em tecidos da raiz de plantas jovens de *Eucalyptus urophylla*, clone AEC 144, cultivadas em soluções nutritivas com diferentes concentrações de Mg.

Source: Barbosa, Cairo, Lacerda and Botelho (2019)
The performance of invertase (acidic and neutral isoforms) and sucrose synthase (susy) enzymes in root tissues was not affected by the variation of Mg concentration in nutrient solution (data not shown). These results may be related to the RS and SS contents found in root tissues, which were also not affected by Mg deficiency.

The cleavage of sucrose by invertase is generally correlated with growth and cell expansion (RICARDO; AP REES, 1970), while susy is associated with anabolic processes, in which UDP-glucose is the precursor of numerous compounds (STURM et al., 1999). Acidic invertase is a key enzyme in phloem unloading and maintenance of hexoses supply to meristems (STURM; TANG, 1999), while neutral invertase controls internal hexose levels into the cell of mature tissues, where the susy activity is low (VAN DEN ENDE; VAN LAERE, 1995).

Conclusions

This experimental study has shown that in *Eucalyptus urophylla* young plants, clone AEC 144, Mg deficiency increases SS and RS contents in leaf tissues from 30 days after planting. In root tissues, both RS and SS contents were unaffected by Mg deficiency. For this reason, invertase (acidic and neutral) and susy activities were also not affected. Sucrose cleaving in roots appeared to be predominantly catalyzed by acidic invertase, followed by susy and neutral invertase.

The effects of Mg concentrations on sugar contents in leaves showed no effect on root dry matter accumulation and on the shoot/root dry matter ratio. We concluded that *Eucalyptus urophylla* young plants, clone AEC 144, can tolerate Mg deficiency up to 120 days after planting, since the accumulation of sugar in the leaf tissues is not sufficient to restrict the accumulation of dry matter in the roots.

References


