Growth, Chlorophyll Content and Photosynthetic Capacity of Eucalyptus Clones Under Nutrition Omission

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OBJECTIVE

Since the mineral nutrition is very important for the proper development of planted forests in Brazil, analyzing the behavior of different commercial genotypes under the failure of some nutrients may allow better optimization of fertilizer application on this crop and decrease costs production. Objective: It was aimed to evaluate the effect of the omission of macronutrients, B, Cu and Zn over the growth, total chlorophyll index and potential quantic efficiency of the photosystem II of hybrid clones of Eucalyptus grandis x Eucalyptus urophylla. Results: The default of nutrients negatively affect height growth and total chlorophyll content of the clone studied. It was found that Zn and Mg were the nutrients more limited height growth of seedlings, while N was the nutrient that more limited the total chlorophyll index. The treatment with omission of S considerably affected the potential quantum efficiency of photosystem II of the treated plants. Conclusion: The absence of some nutrients caused significant damage to the photosynthetic apparatus of the plants studied. Further investigation on the effects of macro and micronutrients in the photosynthetic efficiency of PSII are longed.

INTRODUCTION

Eucalyptus genus is native from Australia and adjacent islands, belongs to the division Angiospermae, family Myrtaceae and comprises more than 600 described species, with broad genotypic plasticity and global dispersion (Santos et al., 2001; Fonseca et al., 2010). This genus is distinguished as the most planted in the world (Fonseca et al., 2010) and has supplied significantly the worldwide demand for timber and non-timber products.

Because of the necessities of the environmental conservation and to ensure the best soils for the food production, the eucalypt is generally cultivated in low fertility soils. Considering that the nutrients availability to supply the needs of the plants in poor soils is small, when it’s not onerous to the production for its high cost, to understand e characterize the diverse ways through which the different genotypes of Eucalyptus unfold their nutritional state can provide a more proper use of the fertilizers according to the nutritional demands of different clones (Sgarbi et al., 1999; Silveira et al., 2001).

A quick and economic way of generating information related to the nutritional state of the vegetable is find the limiting element, using the leaf diagnosis, by means of visual aspects of plants. The mineral liability promote change in the vegetable metabolism which, frequently, may modify the morphological and physiological aspects of the plant (Silveira et al., 2002; Epstein and Bloom, 2004). Yet according to Malavolta et al. (1997) a regular plant is that one which presents in it tissue all the macro and micronutrients in no limiting amounts and proportions to the growth in all the stages of its life circle. So, the lack of essential nutrients will provide slow development of the plant, which restrain the production, and causes morphological and physiological changes to the tissues.

A variable that has been discarded in nutritional studies, but which may be of great importance to evaluate the deleterious effects of the absence of each nutrient to the level of chloroplasts, is the reason Fv/Fm, that indicates the potential quantic efficiency of the photosystem II (Maxwell and Johnson, 2000). This relation may vary in the range of 0.75 to 0.85 in plants that are in great environmental conditions. Reductions in this reason...
may be an indicator of the photoinhibitory effect to the photosynthetic apparatus (Bolhár-Nordenkampf et al., 1989).

Given the above, it was aimed to evaluate the effect of the omission of macronutrients, B, Cu and Zn over the growth, total chlorophyll index and potential quantic efficiency of the photosystem II of hybrid clones of *Eucalyptus grandis* × *Eucalyptus urophylla*.

**MATERIAL AND METHODS**

The experiment was developed in the period of August/November 2012, in the CIPEF’s (Integrated Center for Forest Species Spread) greenhouse, which is installed in the JK Campus of Universidade Federal dos Vales do Jequitinhonha and Mucuri (UFVJM), located in the city of Diamantina, Minas Gerais State, Brazil. The seedlings of commercial clone 244 (*Eucalyptus grandis* × *Eucalyptus urophylla*), provided by the Company Aperam Bioenergia LDTA, located in the city of Itamarandiba – Minas Gerais, were grown according to standards commercially adopted by the company, in propylene tubes containing vermiculite (40%), carbonized rice husk (30%) and coconut fiber (30%). At the 90 days of age, the roots were washed with distilled water in order to remove all the substrate. Afterward, they were transplanted to plastic pots, with capacity for 1 L, containing coarse washed sand. The treatments used were full (fertilized with N, P, K, Ca, Mg, S, B, Cl, Cu, Fe, Mn, Mo and Zn) and individual omission of the nutrients (N, P, K, Ca, Mg, S, Cu and Zn). The full fertilization consisted of 300mg of N, 200mg of P, 150 mg of K, 75 mg of Ca, 15.5 mg of Mg, 50 mg of S, 0.5 mg of B, 1.5 mg of Cu, 5mg of Fe, 0.1 mg of Mo, 5 mg of Zn and 4 mg of Mn. The chemical analysis of the washed sand showed that the amount Fe present in the substrate was sufficient for the plant development. The physical and chemical analysis was performed by the analysis soils lab of the Universidade Federal de Viçosa. The nutrition in the treatments of omission was identical to the full one, except for the omitted nutrient, which was not present. The nutrients were totally mixed to substrate volume corresponding to each treatment. The incorporation was done by using a concrete mixer, so as to guarantee a full homogenization.

The plants were daily watered, with distilled water, at 8:00, during all the experimental period, with a volume that was sufficient to keep the substrate with 12% of gravimetric humidity. The humidity control was performed through the gravimetric method, characterized by the daily weighing of the pot-substrate-plant set and subsequent re-placement of the water lost by evapotranspiration. Completely randomized design was used, with ten treatments and four repetitions each, totaling forty experimental units. At the end of the experiment (60 days), the height, total chlorophyll index and chlorophyll a fluorescence variables were measured.

The plants total height was measured with a centimeter graded ruler and the total chlorophyll index was indirectly quantified (non-destructive method) with a chlorophyll meter (brand: ClorofiLOG, model: CFL 1030), following the manufacturer’s instructions and expressed in a dimensionless unit, named Falkor Chlorophyll Index (Falkor Automação Agrícola, 2008). Measurements were made on the first leaf completely expanded (from the apex to the base of the plant). Three readings were made, avoiding nerve areas and the ones damaged by pests and pathogens, being used in the analysis the average value obtained from them. The chlorophyll a fluorescence variables were obtained using a portable Fluor meter (brand: JUNIOR-PAM). From the values of Fo (minimum fluorescence) and Fm (maximum fluorescence), the variable fluorescence was calculated (Fv=Fm-Fo), thus, obtaining the potential quantic efficiency of PSII (Fv/Fm) (Maxwell and Johnson, 2000). When obtaining the chlorophyll a fluorescence variables the seedlings were kept in the dark so that the leaves reaction centers could get the condition “open” (non-reduced photochemical extinguisher) (Maxwell and Johnson, 2000). The readings were made at 8p.m. and 10p.m., with the help of magnetic leaf clips attached to the Fluor meter, placed in median region, on the adaxial side of the leaf blade, avoiding the central nerve. The obtained fluorescence values were automatically stored in the Fluor meter and, afterward, transferred to a computer, using the Wincontrol software.

The obtained data were submitted to variance analysis (ANAVA) and, when meaningful, the averages were compared by the Tukey’s test at 5% significance. Statistical analysis were made in the R environment (R Core Team, 2013).

**RESULTS AND DISCUSSION**

The treatments presented different behavior in relation to the height growth, total chlorophyll index and Fv/Fm (Table 1).

Although not statistically different from most of the treatments (Figure 1), the omission of the nutrients Mg and Zn were the ones that mostly limited height growth of the referred clone. The absence of these nutrients decreased in 19.25% and 24.15%, respectively, the height when compared to the treatment without P, such treatment was the one the least restrained the height growth. This decrease in growth in plants under omission of Mg probably took place as a result of impaired export of assimilates via the phloem, which causes the accumulation of carbohydrates in leaves. This reduced accumulation and consequent transport, typical symptoms of lack of Mg, cause changes in the photosynthetic carbon metabolism and restrict CO2 fixation.
compromising thus plant growth (Cakmak and Kirkby, 2008; Ding et al., 2008). The depressive effect of the omission of Zn on the growth of plants is reported in literature and its function is related to the synthesis of growth hormone Auxin (Furlani et al., 2005; Zabini, et al., 2007; Leal and Prado, 2008; Carmo et al., 2010).

Table 1: Average results for the height variables, total chlorophyll index and potential quantic efficiency of the PSII of eucalyptus seedlings, 60 days from the application of the treatments, because of the individual omission of each nutrient (averages followed by the same letter do not differ by the Turkey test at 5% of significance).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Height (cm)</th>
<th>Total chlorophyll</th>
<th>Fv/Fm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omission of P</td>
<td>33.125 a</td>
<td>46.325 a</td>
<td>0.827 a</td>
</tr>
<tr>
<td>Omission of N</td>
<td>29.875 ab</td>
<td>30.510 b</td>
<td>0.742 a</td>
</tr>
<tr>
<td>Omission of Cu</td>
<td>29.500 ab</td>
<td>44.985 a</td>
<td>0.585 ab</td>
</tr>
<tr>
<td>Omission of K</td>
<td>29.375 ab</td>
<td>40.234 ab</td>
<td>0.687 ab</td>
</tr>
<tr>
<td>Omission of B</td>
<td>29.375 ab</td>
<td>39.982 ab</td>
<td>0.762 a</td>
</tr>
<tr>
<td>Full</td>
<td>28.687 ab</td>
<td>42.225 a</td>
<td>0.832 a</td>
</tr>
<tr>
<td>Omission of Ca</td>
<td>28.250 ab</td>
<td>47.235 a</td>
<td>0.835 a</td>
</tr>
<tr>
<td>Omission of S</td>
<td>27.875 ab</td>
<td>40.210 ab</td>
<td>0.247 b</td>
</tr>
<tr>
<td>Omission of Zn</td>
<td>26.750 b</td>
<td>41.225 ab</td>
<td>0.612 ab</td>
</tr>
<tr>
<td>Omission of Mg</td>
<td>25.125 b</td>
<td>40.257 ab</td>
<td>0.747 a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>8.27</td>
<td>13.32</td>
<td>26.01</td>
</tr>
</tbody>
</table>

Fig. 1: Average total height of *Eucalyptus grandis* x *Eucalyptus urophylla* hybrid clone by the treatments (averages followed by the same letter do not differ by Tukey’s test at 5% significance).

The effect on the height growth with absence of P was similar to the one obtained by Sgarbi et al. 1999, also with hybrid clones of *Eucalyptus grandis* x *Eucalyptus urophylla* e by Viégas et al. (2004) in *Myrciaria dubia*. In opposition Moretti et al. (2011) found reductions in the growth of *Toona ciliata* under deficit of P. To the full treatment, due to the optimal availability of nutrients, it was expected a major height growth, which was not noticed.

In relation to the total chlorophyll index, despite some treatments do not present statistical differences among themselves, the omission of some nutrients caused low content to that variable. The treatment with omission of N resulted in a considerable reduction in the total chlorophyll index (Figure 2). This can be explained by the fact that the N participates in the structural composition of the chlorophyll molecule in the porphyrin portion, in the tetra-pyrrolic rings (Malavolta et al., 1997; Taiz and Zeiger, 2009; Frazão et al., 2013). Besides this, the omission of N decreases the metabolism of assimilates by the plant, besides make larger quantities of carbon are diverted to the formation of starch (Rufty Jr. et al., 1998). When the accumulation of starch on the chloroplast is excessive, the photosynthesis can be seriously affected because it makes it difficult the incoming of CO₂ to the sites of carboxylation of the RubisCo, so affecting the total chlorophyll (Guidi et al., 1998). Similar effects were checked by Maffeis, et al. (2000) and Barroso et al. (2005), in seedlings of *Eucalyptus citriodora* and *Tectona grandis*, respectively. Lobo et al. (2012) also observed significant reductions in chlorophyll content in *Arachis hypogaea* plants subjected to deficit N.

Treatments with omission of P, Ca and Cu presented the greatest percentages of total chlorophyll. Some works, such as the one developed by Naiff (2007), have verified a more intense green coloring, almost bluey, in leaves with lack of P, associated, according to Malavolta (1980), to the rise of the relative concentration in the chlorophyll percentage. Similar coloring was noticed in this study when there was the omission of P. But to Ca and Cu, the obtaining of high levels of chlorophyll can be explained by a probable efficiency of the plant in the consumption of these nutrients, or by lack of direct functional relation of these ones with the chlorophyll molecule. Unlike these two, since 6% to 25% of the total Mg in the plant belong to the chlorophyll molecule (Vitti et al., 2006), it was expected that the absence of Mg would present less content of chlorophyll than it was found.
Fig. 2: Total chlorophyll index of *Eucalyptus urophylla* x *Eucalyptus grandis* hybrid clone by the treatments (Averages followed by the same letter do not differ by Tukey’s test at 5% significance).

In the same way, the chlorophyll a fluorescence because of the applied nutritional omission, in general, has showed little statistical difference between the means of the treatments adopted (Figure 3). The basic signal of the fluorescence has characteristic levels, which reflect the status of the plant in that moment, in relation to its own metabolism and this with the environment in which it is placed (Ribeiro *et al*., 2004; Baker, 2008).

Fig. 3: Potential quantum efficiency of PSII of *Eucalyptus grandis* x *Eucalyptus urophylla* clone hybrid by the treatments (averages followed by the same letter do not differ among themselves by Tukey’s test at 5% significance).

The potential quantic efficiency of the PSII, estimated by Fv/Fm, indicates the photochemical dissipation of energy and expresses the efficiency of capture of this energy of excitation through the open reaction centers of the PSII (Baker *et al*., 1991). This ratio can vary from 0.75 to 0.85 in plants with good environmental conditions. Reductions in these values may indicate a photo-inhibitory effect (Bolhàr-Nordenkampf *et al*., 1989).

Although, statistical differences have not been verified among the majority of the treatments, the N, K, Mg, Cu and Zn omission has caused a reduction in the Fv/Fm values below 0.75, what may be an indication either of reversible photo protector regulation, or an irreversible inactivation of the PSII (Figure 3). In the literature, there are a few reports that compare the effects of macro and micronutrients in the PSII photochemical efficiency, except for the N. Some studies have shown that the deficit of N causes decrease in the quantic efficiency of the electron transportation of the PSII, suggesting the occurrence of some damages to the PSII (Nunes *et al*., 1993; Verhoeven *et al*., 1997). On the other hand, other studies have shown that limiting amounts of N do not have any meaningful effect over the quantic efficiency of electrons transportation of the PSII and therefore, do not cause damages to the PSII (Henley *et al*., 1991; Lu and Zhang, 2000; Vieira *et al*., 2010).

The treatment that more limited photochemical efficiency of PSII was one in which S was omitted. The noticed values, considerably low to the relation Fv/Fm, can be indicative of the occurrence of a very severe damage to the photosynthetic apparatus. The influence of S in photochemical phase of the photosynthesis is not reported in the literature, but its assimilation depends on the reduced ferredoxin, what could result in changes in the fluorescence signal (Vieira *et al*., 2010).

For the other treatments reductions have not been verified in the ratio Fv/Fm, even for the full treatment. Considering that most of the nutrients studied here directly or indirectly influences in the operation of the photosynthetic apparatus of the plant, values below 0.75 were expected for the majority, otherwise to all the tested treatments, except for the full treatment.
Conclusions:
The nutrients omission have interfered negatively in the height and in total chlorophyll content in the studied clone, especially Zn and Mg, which significantly reduced height growth and the omission of N, which promoted noticeable drop in chlorophyll content.
The absence of some nutrients caused significant damage to the photosynthetic apparatus of the plants studied, with emphasis on the treatment without S. Further investigation on the effects of macro and micronutrients in the photochemical efficiency of PSII are longed.

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