Monitoring the end of the *in vitro* phase of *Anthurium andreanum* Lindl. plantlets

 Giulio Cesare Stancato* and Maria Luiza Sant’Anna Tucci

1 Centro de Horticultura, Instituto Agronômico (IAC), Caixa Postal 28, 13012-970, Campinas, SP, Brasil.

* Corresponding author: stancato@iac.sp.gov.br

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ABSTRACT

Estimulation of autotrophy in *in vitro* plantlets could be achieved through changes in the culture medium, or by changing the traditional hermetic caps by one that could allow gas exchanges between the culture and the environment. Besides that, the use of lamps with distinct emission spectrum irradiation has propitiated successful results. This work was carried out aiming to evaluate the either the combined or the single action of some factors that can induce autotrophy on *in vitro* *A. andraeanum* cv. Eidibel plantlets. 3 sucrose concentrations were used: 0, 15 and 60 mM and for each one, to kinds of flasks according to the cap ventilation: under (0.038 L.h⁻¹) and without ventilation. Flasks were kept under cold light fluorescent lamps or under gro-lux lamps. At the end of the experiment showing the highest shoot dry mass treatment was 60 mM, under ventilation and gro-lux, and the treatment which accumulate root dry mass to a lesser extent were 0 mM with ventilation and cold light and 15 mM without ventilation and cold light. In average, treatments with higher sucrose content in the culture medium, that is, 60 mM, under gro-lux lamps, presented the highest chlorophyll *a* and *b* and total contents, than those under cold lamp. Steps of carbohydrates metabolism could be associated with the total soluble sugars (sucrose and reducing sugars) levels, highlighting the steps where nutrient requirements were higher, showing the role of the plantlets sink.

Key words: acclimatization, Araceae, plantlets metabolism

RESUMO

Monitoramento do final da fase *in vitro* de plântulas de *Anthurium andraeanum*. O estímulo à autotrofia em plântulas *in vitro* pode ser alcançado através de mudanças no meio de cultura, ou pela troca de tampas herméticas por tampas que permitam a troca de gases entre a cultura e o ambiente. Além disso, o uso de lâmpadas com distintos espectros de irradição tem propiciado bons resultados. Este trabalho foi conduzido com o objetivo de avaliar a ação isolada ou em conjunto de fatores que podem induzir a autotrofia em plântulas de *A. andraeanum* cv. Eidibel *in vitro*. 3 concentrações de sacarose foram usadas: 0, 15 e 60 mM e para cada concentração foram empregados frascos com ventilação (0,038 L.h⁻¹) ou sem ventilação. Os frascos foram mantidos sob lâmpada fluorescente fria ou gro-lux. O final dos experimentos mostrou que o maior acúmulo de massa seca da parte aérea ocorreu a 60 mM, sob ventilação e gro-lux, e os tratamentos que acumularam menor massa seca de raízes foram 0 mM com ventilação e sob luz fria e 15 mM sem ventilação e luz fria. Em média, os tratamentos com o maior teor de sacarose no meio, 60 mM, sob lâmpada gro-lux, apresentaram as maiores concentrações de clorofila *a*, *b* e total, do que sob luz fria. Os níveis de açúcares solúveis totais (sacarose e açúcares redutores) mostraram passos do metabolismo de carboidratos nessas plantas, realçando os momentos em que a exigência por nutrientes foi maior, destacando-se o papel de dreno das plântulas.

Palavras-chave: aclimatização, Araceae, metabolismo de plântulas
INTRODUCTION

Anthurium andreanum has been of great significance in the Brazilian flowers production either for the cut flowers market or for the pot plants one, and the development of cultivars suitable to the tropical conditions (Tombolato, 2004) has contributed to the increasing growing area as well as to the high quality of flowers.

The Anthurium market has shown interest in plants uniformity, high floral quality as well as high yield, and the method of production through tissue culture in vitro, has been an important tool for the consecution of those purposes.

It is known that plantlets grown in vitro show feeble systems of protection against water loss, and don’t have yet developed photosynthetic machinery for CO$_2$ assimilation. Therefore is essential to provide suitable conditions to plantlets development from the beginning to transplantation. According to literature (Grattapaglia and Machado, 1998) besides low irradiance around and appropriate mineral nutrition (N:P:K), plantlets in vitro need high relative humidity, between 70 and 80% and air temperature never below 15ºC.

The success of a in vitro culture depends mainly on the physiological and anatomical adjustments that plantlets undergo at the last stage in vitro and throughout the ex vitro one, and the dry mass accumulation will be a consequence of the interaction of the environmental conditions and carbohydrates metabolism.

In general, the control of the environment and the medium culture changes are part of a serie of estrategies performed throughout the pre-acclimatization of plantlets, being of great importance in its growth, development, and proper morphological changes promotion, in vitro (Kozai et al., 1987; Kozai et al., 1991). Estimulation of autotrophy could be achieved through changes in the culture medium, or by changing the traditional hermetic caps by one that could allow gas exchanges between the culture and the environment (Kozai, 1991). Besides that, the use of lamps with distinct light emission spectrum has propitiated successful results, but mostly restrict to few plant species.

This work was carried out aiming to evaluate the either the combined or the single action of some factors that can induce autotrophy on in vitro A. andreanum cv. Eidibel plantlets.

MATERIAL AND METHODS

A. andreanum, cv. Eidibel plantlets kept in Muraschige and Skoog (1962) medium were selected, being subsequently submitted to the same medium but under half salt concentration, in 300 mL capacity flasks with 50 mL of culture medium, with 5 plantlets per flask, for forty five days. Three sucrose concentrations were used: 0, 15 and 60 mM and for each one, to kinds of flasks according to the cap ventilation: under (0.038 L.h$^{-1}$) and without ventilation, u/v and w/v, respectively. Flasks were kept under cold light fluorescent lamps or under gro-lux lamps.

The experimental design was a random one, with three sucrose concentrations, and the use or not of ventilation and 2 kinds of illumination, that is twelve treatments and 10 replicates. Analyses were performed at 0, fifteen, thirty and forty five days of growing in vitro, and for each treatment all the 10 replicates were analyzed.

Plantlets dry weight was performed by submitting them to 70ºC temperature, until constant weight. At the end of the experiment chlorophyll $a$, $b$ and total contents were evaluated, according to the Amon (1949) method and the results were expressed in mass units. Total soluble sugars contents were determined according to Dubois et al. (1956), reducing sugars contents were determined according to Somogy (1952), and sucrose contents, according to Handel (1968).

RESULTS

There was no significant difference in shoot dry weight among treatments at the 0 day experiment (Table 1). From the onset of dry weight accumulation on, high values have been observed for the treatments 15 mM u/v and cold lamps (CL) corresponding to 5.2 mg and the treatment 60mM, under the same conditions, corresponding to 5.3 mg. At the 30$^{th}$ days the higher dry mass accumulation was for the treatments 0 mM u/v and gro-lux (GL), corresponding to 5.3 mg; 15 mM u/v and CL, corresponding to 5.5 mg, and 60 mM u/v and GL, 5.6 mg. At the end of the experiment showing the highest shoot dry mass treatment was 60 mM u/v and GL, corresponding to 7.2 mg.

Treatments that accumulated shoot dry mass to a lesser extent were: at the 15$^{th}$ day, 0 mM w/v and CL, and 60 mM w/v and GL; at the 30$^{th}$ day treatments 0 mM w/v and CL, 15 mM w/v and GL, and 60 mM w/v and GL; at 45$^{th}$ day the 0 mM w/v and CL.

Regarding to the root dry weight accumulation (Table 2), at the 30$^{th}$ day better performance was observed on treatments 0 mM, under and without ventilation and GL, corresponding to...
Table 1. Average shoot dry mass accumulation (mg) in Anthurium andraeanum cv. Eidibel plantlets at the 0, 15, 30, and 45 days of acclimatization in vitro, in media culture with 0, 15 and 60 mM sucrose, under (u/v) or without (w/v) flasks ventilation and under cold lamps (CL) or gro-lux (GL).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot dry mass</th>
<th>0 days</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0mM w/v CL</td>
<td>3.16ns^1,2</td>
<td>3.44h</td>
<td>4.08f</td>
<td>4.6g</td>
<td></td>
</tr>
<tr>
<td>0mM u/v CL</td>
<td>3.16ns</td>
<td>4.06f</td>
<td>4.44e</td>
<td>4.98f</td>
<td></td>
</tr>
<tr>
<td>0mM w/v GL</td>
<td>3.16ns</td>
<td>4.70c</td>
<td>5.16c</td>
<td>5.51de</td>
<td></td>
</tr>
<tr>
<td>0mM u/v GL</td>
<td>3.16ns</td>
<td>5bc</td>
<td>5.3abc</td>
<td>5.72cd</td>
<td></td>
</tr>
<tr>
<td>15mM w/v CL</td>
<td>3.16ns</td>
<td>4.4e</td>
<td>4.78d</td>
<td>5.3e</td>
<td></td>
</tr>
<tr>
<td>15mM u/v CL</td>
<td>3.16ns</td>
<td>5.16ab</td>
<td>5.54ab</td>
<td>5.76c</td>
<td></td>
</tr>
<tr>
<td>15mM w/v GL</td>
<td>3.16ns</td>
<td>3.96fg</td>
<td>4.18ef</td>
<td>5.76c</td>
<td></td>
</tr>
<tr>
<td>15mM u/v GL</td>
<td>3.16ns</td>
<td>4.04fg</td>
<td>4.4e</td>
<td>5.94c</td>
<td></td>
</tr>
<tr>
<td>60mM w/v CL</td>
<td>3.16ns</td>
<td>4.54de</td>
<td>4.84d</td>
<td>5.36e</td>
<td></td>
</tr>
<tr>
<td>60mM u/v CL</td>
<td>3.16ns</td>
<td>5.34a</td>
<td>5.58a</td>
<td>6.94b</td>
<td></td>
</tr>
<tr>
<td>60mM w/v GL</td>
<td>3.16ns</td>
<td>3.72gh</td>
<td>4.08f</td>
<td>5.82c</td>
<td></td>
</tr>
<tr>
<td>60mM u/v GL</td>
<td>3.16ns</td>
<td>4.0cd</td>
<td>5.26bc</td>
<td>7.22a</td>
<td></td>
</tr>
</tbody>
</table>

1 According to Tukey test at 5%.
2 Values followed by same letters on columns do not differ significantly.

Table 2. Average root dry mass accumulation (mg) in Anthurium andraeanum cv. Eidibel plantlets at the 0, 15, 30, and 45 days of acclimatization in vitro, in media culture with 0, 15 and 60 mM sucrose, under (u/v) or without (w/v) flasks ventilation and under cold lamps (CL) or gro-lux (GL).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root dry mass</th>
<th>0 days</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0mM w/v CL</td>
<td>0.9ns^1,2</td>
<td>1.04ns</td>
<td>1.22c</td>
<td>1.56g</td>
<td></td>
</tr>
<tr>
<td>0mM u/v CL</td>
<td>0.9ns</td>
<td>1.2ns</td>
<td>1.38bc</td>
<td>1.52g</td>
<td></td>
</tr>
<tr>
<td>0mM w/v GL</td>
<td>0.9ns</td>
<td>1.08ns</td>
<td>1.4bc</td>
<td>1.86ef</td>
<td></td>
</tr>
<tr>
<td>0mM u/v GL</td>
<td>0.9ns</td>
<td>1.16ns</td>
<td>1.4ab</td>
<td>1.74fg</td>
<td></td>
</tr>
<tr>
<td>15mM w/v CL</td>
<td>0.9ns</td>
<td>1.3ns</td>
<td>1.46ab</td>
<td>1.52g</td>
<td></td>
</tr>
<tr>
<td>15mM u/v CL</td>
<td>0.9ns</td>
<td>1.34ns</td>
<td>1.62a</td>
<td>1.74fg</td>
<td></td>
</tr>
<tr>
<td>15mM w/v GL</td>
<td>0.9ns</td>
<td>1.12ns</td>
<td>1.32bc</td>
<td>2.06de</td>
<td></td>
</tr>
<tr>
<td>15mM u/v GL</td>
<td>0.9ns</td>
<td>1.14ns</td>
<td>1.42abc</td>
<td>2.34c</td>
<td></td>
</tr>
<tr>
<td>60mM w/v CL</td>
<td>0.9ns</td>
<td>1.12ns</td>
<td>1.2bc</td>
<td>1.8f</td>
<td></td>
</tr>
<tr>
<td>60mM u/v CL</td>
<td>0.9ns</td>
<td>1.28ns</td>
<td>1.36bc</td>
<td>2.98b</td>
<td></td>
</tr>
<tr>
<td>60mM w/v GL</td>
<td>0.9ns</td>
<td>1.12ns</td>
<td>1.24bc</td>
<td>2.2cd</td>
<td></td>
</tr>
<tr>
<td>60mM u/v GL</td>
<td>0.9ns</td>
<td>1.28ns</td>
<td>1.46ab</td>
<td>3.36a</td>
<td></td>
</tr>
</tbody>
</table>

1 According to Tukey test at 5%.
2 Values followed by same letters on columns do not differ significantly.

Table 3 shows the plantlets leaf area increase throughout the 45 days acclimatization. Significant differences among treatments could be seen after 15 days, and it is worth mentioning treatments 15 mM u/v and GL, 15 mM w/v and GL, 60 mM u/v and GL, and 60 mM w/v and GL, presenting significant larger leaf area until the end of the experiment. It is worth noting that treatments with 15 mM sucrose presented larger leaf areas independently if plantlets were under CL or GL or u/v or w/v, and 60 mM u/v or w/v and GL. Treatments 15 mM w/v and GL, 60 mM u/v and GL, 60 mM w/v and GL, and 15 mM u/v CL, showed leaf areas about 43.1, 41.9, 38.9 and 35.0%, respectively as high as the other ones.

In average, 60 mM sucrose treatments presented higher chlorophyll a, b and total content (Table 4), while plantlets grown in 15 mM sucrose showed intermediate values and those under in 0 mM sucrose had the lowest chlorophylls content. Chlorophyll a content was higher than chlorophyll b (Table 4), in every treatment, corresponding to 2.22 on treatments under 0 mM, 2.89 under 15 mM and 2.45 under 60 mM. Total chlorophyll averages were 1.44 and 1.17 higher under 60 and 15 mM sucrose, respectively, when compared with the content observed under 0 mM. The ratio chlorophyll a/chlorophyll b varied significant among treatments, with average values of 2.2 for the 0 mM, 2.3 for 15 mM and 2.45 for the 60 mM sucrose.

Table 3. Average leaf area (cm²) in Anthurium andraeanum cv. Eidibel plantlets at the 0, 15, 30, and 45 days of acclimatization in vitro, in media culture with 0, 15 and 60 mM sucrose, under (u/v) or without (w/v) flasks ventilation and under cold lamps (CL) or gro-lux (GL).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf area</th>
<th>0 days</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0mM w/v CL</td>
<td>0.94ns</td>
<td>1.62d</td>
<td>2.45e</td>
<td>2.94c</td>
<td></td>
</tr>
<tr>
<td>0mM u/v CL</td>
<td>0.94ns</td>
<td>2.15cd</td>
<td>2.71de</td>
<td>2.97c</td>
<td></td>
</tr>
<tr>
<td>0mM w/v GL</td>
<td>0.94ns</td>
<td>2.29cd</td>
<td>2.92d</td>
<td>3.29bc</td>
<td></td>
</tr>
<tr>
<td>0mM u/v GL</td>
<td>0.94ns</td>
<td>2.29cd</td>
<td>2.91d</td>
<td>3.29bc</td>
<td></td>
</tr>
<tr>
<td>15mM w/v CL</td>
<td>0.94ns</td>
<td>3.16ab</td>
<td>3.46bc</td>
<td>3.78b</td>
<td></td>
</tr>
<tr>
<td>15mM u/v CL</td>
<td>0.94ns</td>
<td>3.58a</td>
<td>4.15a</td>
<td>4.51a</td>
<td></td>
</tr>
<tr>
<td>15mM w/v GL</td>
<td>0.94ns</td>
<td>3.17ab</td>
<td>3.82ab</td>
<td>4.78a</td>
<td></td>
</tr>
<tr>
<td>15mM u/v GL</td>
<td>0.94ns</td>
<td>2.48bc</td>
<td>2.82de</td>
<td>3.31bc</td>
<td></td>
</tr>
<tr>
<td>60mM w/v CL</td>
<td>0.94ns</td>
<td>2.37cd</td>
<td>2.85de</td>
<td>3.27bc</td>
<td></td>
</tr>
<tr>
<td>60mM u/v CL</td>
<td>0.94ns</td>
<td>2.49bc</td>
<td>3.12cd</td>
<td>3.86b</td>
<td></td>
</tr>
<tr>
<td>60mM w/v GL</td>
<td>0.94ns</td>
<td>3.77a</td>
<td>3.95a</td>
<td>4.64a</td>
<td></td>
</tr>
<tr>
<td>60mM u/v GL</td>
<td>0.94ns</td>
<td>3.49a</td>
<td>3.87ab</td>
<td>4.74a</td>
<td></td>
</tr>
</tbody>
</table>

1 According to Tukey test at 5%.
2 Values followed by same letters on columns do not differ significantly.
Table 4. Chlorophyll content (μg.100 mg^(-1) fresh mass), after 45 days of acclimatization in vitro, in media culture with 0, 15 and 60 mM sucrose, under (u/v) or without (w/v) flasks ventilation and under cold lamps (CL) or gro-lux (GL).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorophyll content</th>
<th>Chlorophyll a/b ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mM w/v CL</td>
<td>2.91e 1.48de 4.39e 1.98c</td>
<td></td>
</tr>
<tr>
<td>0 mM u/v CL</td>
<td>3.19e 1.46e 4.66e 2.18bc</td>
<td></td>
</tr>
<tr>
<td>0 mM w/v GL</td>
<td>5.33bcd 2.19abc 7.51bcd 2.43ab</td>
<td></td>
</tr>
<tr>
<td>0 mM u/v GL</td>
<td>4.48cd 2.02bcd 6.5cd 2.22bc</td>
<td></td>
</tr>
<tr>
<td>15 mM w/v CL</td>
<td>5.1bcd 2.15abc 7.25bcd 2.37ab</td>
<td></td>
</tr>
<tr>
<td>15 mM u/v CL</td>
<td>4.09de 1.83cde 5.92de 2.23abc</td>
<td></td>
</tr>
<tr>
<td>15 mM w/v GL</td>
<td>5.41bc 2.24abc 7.65abcd 2.42ab</td>
<td></td>
</tr>
<tr>
<td>15 mM u/v GL</td>
<td>4.15de 1.84cde 6.09de 2.26abc</td>
<td></td>
</tr>
<tr>
<td>60 mM w/v CL</td>
<td>5.59abc 2.31abc 7.9abc 2.42ab</td>
<td></td>
</tr>
<tr>
<td>60 mM u/v CL</td>
<td>6.67a 2.64a 9.31a 2.53a</td>
<td></td>
</tr>
<tr>
<td>60 mM w/v GL</td>
<td>4.98cd 2.1abc 7.08cd 2.36ab</td>
<td></td>
</tr>
<tr>
<td>60 mM u/v GL</td>
<td>6.32ab 2.55ab 8.87ab 2.48ab</td>
<td></td>
</tr>
</tbody>
</table>

1 According to Tukey test at 5%.
2 Values followed by same letters on columns do not differ significantly.

Figures 1A, 1B, 1C e 1D show that there were no significant differences among the 0 mM sucrose treatment, independently of flasks being ventilated or not and of the type of illumination. At the 0 day it was observed that plantlets presented low sucrose content while the reducing sugars content was high. At the 15th day total sugars and reducing sugars content decreased, while sucrose content shows an increase. At the 30th day the lowest contents of sucrose, reducing sugars and total sugars were observed. Henceforward and then until the end of the experiment at the 45th day, sucrose content has kept the same or showed a decrease while reducing sugars content showed a sharp increase.
The results showed in Figures 2A, 2B, 2C and 2D, under 15 mM sucrose, show that the total soluble sugars, reducing sugars and sucrose content varied significantly throughout the experiment. In general the variations on carbohydrates content seemed to have been similar in all treatments. Under w/v and CL and u/v and GL, the total soluble sugars level showed an increase from the 0 to the 15\textsuperscript{th} day \textit{in vitro} (Figures 2A and 2C). At the same period, all treatments showed decreases in the reducing sugars, while sucrose content has increased. From the 15 to the 30\textsuperscript{th} day, a decrease in reducing sugars as well as in sucrose was observed, coupled with a decrease in total soluble sugars content. The lowest level of all the carbohydrates were observed at the 30\textsuperscript{th} experiment. Figures 2A and 2C, showed that from the 30 to the 45\textsuperscript{th} day an increase in reducing sugars as well as in sucrose content was observed. At the same period, Figures 2B and 2D, show an increase in reducing sugars level and a decrease in sucrose content, coupled with an increase in total soluble sugars much lower however, than that one observed at the 0 day experiment. It is worth noticing that from the 15 to the 30\textsuperscript{th} the reducing sugars and sucrose levels were similar.

Figure 2. Total soluble sugars, reducing sugars and sucrose content variation, in \textit{Anthurium andreanum} cv. Eidibel plantlets, throughout 45 days of acclimatization \textit{in vitro}, in media culture with 15 mM sucrose, under (u/v) or without (w/v) flasks ventilation and under cold lamps (CL) or gro-lux (GL).

Figures 3A, 3B, 3C and 3D show the variations in total soluble sugars, reducing sugars and sucrose contents under 60 mM sucrose. Figures 3A, 3B and 3C, showed that the variations in carbohydrates contents were similar, and that there was no sharp decrease of them after 30 days \textit{in vitro}, as it was observed under 0 and 15 mM. From the 0 to the 15 day, there was a decrease in reducing sugars and in sucrose levels, which were maintained steady from the 15 to the 30\textsuperscript{th} day. Henceforward until the 45\textsuperscript{th} day, the sucrose levels increased while those of reducing sugars either unchanged
or showed a slight decrease. Meanwhile, total soluble sugars contents showed an increase, mainly due to the increase in sucrose levels.

Figure 3D shows an atypical performance when compared either with those of Figures 3A, 3B and 3C, or those under the other sucrose concentration in the culture medium (Figures 1 and 2). From the 0 to the 15\textsuperscript{th} day, the reducing sugars and sucrose contents decreased coupled with the decrease in total soluble sugars content. Henceforth until the end of the experiment at the 45\textsuperscript{th} day, the reducing sugars as well as the sucrose levels showed a significant increase, coupled with an increase in total soluble sugars levels.

![Figure 3A: Sucrose eq. (mg.g\textsuperscript{-1} DW) Acclimatization (days)](image)

![Figure 3B: Sucrose eq. (mg.g\textsuperscript{-1} DW) Acclimatization (days)](image)

![Figure 3C: Sucrose eq. (mg.g\textsuperscript{-1} DW) Acclimatization (days)](image)

![Figure 3D: Sucrose eq. (mg.g\textsuperscript{-1} DW) Acclimatization (days)](image)

**Figure 3.** Total soluble sugars, reducing sugars and sucrose content variation, in Anthurium andraeanum cv. Eidibel plantlets, throughout 45 days of acclimatization in vitro, in media culture with 60 mM sucrose, under (u/v) or without (w/v) flasks ventilation and under cold lamps (CL) or gro-lux (GL).

**DISCUSSION**

The high total soluble sugars levels, mainly reducing sugars at the beginning of the experiment and the subsequent metabolic changes throughout the 45 days may indicate that previously to the treatments imposition, plantlets had absorbed sucrose from the culture media which enzymatically was broken down to glucose and fructose. Taking into account that the sucrose levels have kept low, the results indicate high catalytic enzymes activity but low sucrose demand, that is the reducing sugars were not consumed highly mainly at the 15\textsuperscript{th} day. On the 30\textsuperscript{th} day there was a decrease in the total soluble...
sugars, reducing sugars and sucrose level, probably due to a
highest demand for photosynthates at this period, probably
due to the maximum sink strength at that moment.

According to Hazarika (2006), growth and respiration in
vitro, require a steady sucrose exogenous supply as a carbon
source. However, high sucrose and salts concentration within
the culture medium seem to restrain photosynthetic efficiency.
Also, Kovtum and Daie (1995) observed that an exogenous
sucrose source speeds leaves growth and development and
its transition sink-source in Beta vulgaris L. plantlets grown in
vitro, and concluded that the question was not the source but
the sink limitation, until the plantlets themselves developed its
capacity of metabolizing carbohydrates. Ticha et al. (1998)
have considered that exogenous sucrose would prevent
photicinhibition to happen.

The increasing sucrose concentration within the culture
medium maximizes the role of the nutrients in persistent
leaves (Grout and Millam, 1985; Desjardins et al., 1987), and
it is known that high sucrose concentration has propitiate
increasing shoot dry weight. Hazarika et al. (2000) observed
that pre-conditionement of Citrus sp in vitro under 3%
sucrose has increased subsequent survival and growth ex
vitro. According to the same authors, a linear increase in the
biochemical components with sucrose addition to the culture
medium.

From the 45th day on, there was a sink strength decrease
that could be corroborated by the high reducing sugars
content, while sucrose content was kept at its previous levels.
The results indicate an increase in the sucrose metabolism
enzymes which could be due to a decrease of sinks strength
or to an imbalance in carbohydrates partitioning (Stancato et
al., 2001). Once plantlets from 60 mM treatment presented
higher dry weight (Tables 1, 2, and 3) it is possible that a
higher demand for reducing sugars has resulted in a higher
demand for sucrose.

When the gain of dry mass was related to the leaf
area, it seemed that plantlets under 15 and 60 mM sucrose
in the culture medium are among those which presented a
bigger leaf area, indicating a higher demand for sugars from
the culture medium to be allocated to the leaves, resulting a
increase in leaf area. By the way, the leaf area does not seem
to be a suitable variable for to be used to evaluate the plantlets
acclimatization, although Premkumar et al. (2001) have
pointed out that the success of acclimatization depends on the
sources inside the developing plantlets. According to Ticha
et al. (1998), the addition of sucrose to the culture medium
influenced positively the increase of biomass and leaf area
as well as accumulation of chlorophyll and photosynthetic
capacity in tobacco plantlets.

Chlorophyll contents are in agreement with those
observed in plantlets of other species in vitro (Carvalho et
al., 2005). The results show that chlorophyll levels are better
related with sucrose concentration in the culture medium than
the lamp type. GL lamps have the emission spectrum in the
range of blue and red and the CL lamps emit in blue, but the
emission spectrum did not interfere on chlorophyll levels,
although its capacity of capturing and transforming light
energy depends on the simultaneous absorption of blue and
red wave lengths. Light intensity is the same for both lamps.

According to Premkumar (2001), while low light inhibits
chloroplasts development, sugars content in the culture
medium may restrain the photosynthetic enzymes activity,
as for instance the Rubisco (EC 4.1.1.39). Watanabe et al.
(1990); Tanaka et al. (1991); Rival et al. (1999), observed that
Chrysanthemum morifolium L., Spathiphyllum wallisi Regel and
coconut plantlets grown in vitro with 3% sucrose, presented
low Rubisco levels, what is characteristic for in vitro plantlets.
According to Carvalho et al., (2005) low light intensity inhibit
the proper development of chloroplasts, which exhibit low
content and activity of Rubisco in vitro.

In average, plantlets grown under gro-lux lamps grew
faster than those under cold light grown under the same
sucrose concentration, that is 60 mM. This may imply that
although the lamps emission spectrum has not been involved
in the chlorophyll level, there could have been higher Rubisco
activity associated to sucrose level in the culture medium,
resulting in a better utilization of the light energy. Studies
by Kozai et al. (1987) with species of ornamental plantlets
showed that the CO₂ level in the flasks, decreased from
3000-9000 ppm, in the dark period to 90 ppm in the light
period indicating that plantlets were able to photosynthesize
throughout the light period.

Total soluble sugars (sucrose and reducing sugars)
showed steps of the carbohydrates metabolism mainly
the higher the demand for nutrients by plantlets sinks.
Although the carbohydrates level in the culture medium
may have contributed to plantlets heterotrophy, the fact that high sugars concentrations have decreased significantly the medium osmotic potential besides the possible gas exchanges propitiated by the flasks with the holed cap, allowed a degree of autotrophy expressed by the dry weight accumulation in plantlets. Even under low light conditions it is possible to speculate that plantlets grown in 15 and 60 mM sucrose and holed caps have grown not only as a function of nutrients availability in the culture medium, but also via photosynthesis.

CONCLUSIONS
- The plantlets dry mass accumulation were positively correlated with the higher sucrose content in the culture medium;
- The ventilation in the flasks contributed significantly to the plantlets dry mass accumulation and to its acclimatization and consequently to its vigor;
- The emission spectrum of the GL lamps showed a positive influence on the plantlets development.

REFERENCES