

***In vitro Dendrobium nobile* plant growth and rooting in different sucrose concentrations**

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ABSTRACT

Sucrose is a very important component in *in vitro* culture media, serving as a source of carbon and energy. In this paper, the rooting and *in vitro* growth of *Dendrobium nobile* Lindl (Orchidaceae) were studied using different sucrose concentrations (0 g L⁻¹; 5 g L⁻¹; 10 g L⁻¹; 20 g L⁻¹; 30 g L⁻¹ and 60 g L⁻¹), in a modified MS medium containing half the regular concentration of macronutrients at pH 5.8. Greater increases in plant height (4.21±0.6 cm) and high seedling multiplication (1:4) were observed in the 60 g L⁻¹ sucrose treatment, even without the addition of plant hormones. Sucrose concentration in the culture medium did not influence *in vitro* plant rooting.

Keywords: *Dendrobium nobile*, *Orchidaceae*, carbohydrate, tissue culture.

RESUMO

Crescimento e enraizamento *in vitro* de plântulas de *Dendrobium nobile* sob diversas concentrações de sacarose

A sacarose é um componente muito importante no meio de cultura servindo como fonte de carbono e energia. Neste trabalho, avaliou-se o crescimento e enraizamento *in vitro* de plântulas de *Dendrobium nobile* Lindl. (Orchidaceae) cultivadas em diferentes concentrações de sacarose (0 g L⁻¹; 5 g L⁻¹; 10 g L⁻¹; 20 g L⁻¹; 30 g L⁻¹ e 60 g L⁻¹), utilizando como base o meio MS com metade da concentração de macronutrientes e pH 5,8. No tratamento com 60 g L⁻¹ de sacarose observou-se maior crescimento em altura (4,21 ± 0,9 cm) assim como uma alta taxa de multiplicação de mudas (1:4) mesmo sem adição de fitorreguladores. O acréscimo de sacarose no meio de cultura não influenciou o enraizamento *in vitro* das plantas.

Palavras-chave: *Dendrobium nobile*, *orchidaceae*, carboidrato, cultura de tecidos.

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The high heterozygosity of *Dendrobium nobile* hybrids has attracted the attention of biotechnology companies for large scale clonal propagation of elite genotypes belonging to this genus (Faria & Illg, 1995). The orchid species used in this experiment, *Dendrobium nobile*, is an epiphyte of Asian origin, found throughout tropical and subtropical Asia, as far east as the Fiji Islands and Southern Australia (Huber, 1994).

The source of carbon (sucrose, glucose or fructose) is a very important component in *in vitro* culture media. Carbon sources are added to the culture medium because of the light energy deficiency and low CO₂ concentration present in *in vitro* conditions (Tomblato & Costa, 1998). Sucrose concentrations of 20 and 30g L⁻¹ are the most commonly used in orchid tissue culture studies (Arditti, 1974). Reports from several laboratories emphasize the importance of the source and concentration of sugars used to promote *in vitro* orchid seed germination, as well as plant growth (Arditti, 1967; Ernest, 1967).

Plants cultured *in vitro* often show a low photosynthesis rate and incomplete autotrophy (mixotrophic or heterotrophic plants). This accounts for the low survival rates of plants during the acclimation stage (Grout & Price, 1987; Kozai, 1988). The most common procedure is micropropagation of sprouts and plants in culture media that mimic greenhouse conditions, aiming to achieve a shorter adaptation period (Debergh, 1991).

According to Debergh (1991), the absence of sugar reduces contamination problems in the culture medium and allows plants to grow autotrophically *in vitro*, when sufficient CO₂ is supplied and light intensity is increased. However, the presence of sugar in the culture medium is one of the factors that contribute to a significant increase in the production cost of micropropagated plantlets (Kozai, 1991).

Some researchers have been trying to solve the problem of *in vitro* autotrophic plant production adaptation. To achieve these objectives, the amount of sugar is reduced or omitted (Grout & Price, 1987; Kozai, 1988) and light intensity and CO₂

concentration are increased (Desjardins & Laforge, 1988; Kozai *et al.*, 1988).

Micropropagation requires, in many cases, the cultivation of explants under heterotrophic conditions, mainly in the sprout induction stage. At this stages, the explant generally consists of an agglomeration of sprouts, whose photosynthesis system is not able to supply energy for tissue growth and development (Kozai, 1992). Bearing in mind that each species has a different behavior, careful testing of partial decreases in sugar is recommended in the subsequent stages of the process, through assessment of the multiplication rate, growth, and quality of plants produced (Kozai, 1991).

Working with strawberry (cv. Campinas), Calvete *et al.* (2002) observed that the concentration of sucrose (45 g L⁻¹) is ideal for the formation of roots; however, in the absence of this sugar, roots are not formed under *in vitro* cultivation.

This study was carried out to assess *in vitro* plant growth and rooting of *Dendrobium nobile* seedlings in culture media containing different sucrose concentrations.

MATERIAL AND METHODS

Dendrobium nobile plantlets derived from seeds germinated *in vitro* were used with a mean initial height of 1.92 ± 0.5 cm and a mean fresh weight of 0.07 ± 0.02 mg.

The MS medium (Murashige and Skoog, 1962), containing half its regular concentration of macronutrients and at pH 5.8 was used to assess plantlet growth and rooting. The sucrose concentrations added to the MS medium were: (0 g L⁻¹) T₁; (5 g L⁻¹) T₂; (10 g L⁻¹) T₃; (20 g L⁻¹) T₄; (30 g L⁻¹) T₅, and (60 g L⁻¹) T₆.

Flasks (250 ml capacity) were used, containing 50 ml of autoclaved culture medium each. Five plants were inoculated in each flask, closed and sealed with PVC film, and kept in a culture chamber adjusted to a 16-hour photoperiod (fluorescent bulbs); temperature was set around 25°C. The study was carried out in a completely randomized design with ten replicates per treatment. Plantlet growth in height was assessed monthly for a period of four months. After that period, fresh weight, root length, and number of shoots were also assessed. The plantlets were transplanted to ceramic pots containing xaxim coir and transferred to a greenhouse with 50% of shading provided by a black polypropylene screen, 60% relative humidity, and temperature ranging from 25 to 28°C. The data obtained were submitted to analysis of variance, Tukey test, and regression analysis.

RESULTS AND DISCUSSION

There were no significant differences regarding the plant height variable among the six different treatments in the period from 30 to 60 days (Table 1).

The treatments containing different sucrose concentrations presented significant mean plant height differences after ninety days from the beginning of the experiment. In T6 (60 g L⁻¹ of sucrose), plantlets showed greater growth in height when compared to the other treatments. After 120 days of

Table 1. Mean values for plant height (PH), assessed at different periods (30, 60, 90 and 120 days of culture), fresh weight (FW), root length (RL) and number of shoots (NS), in the different sucrose concentrations after 120 cultivation. Londrina, UEL, 2003.

Treatments	Means							
	PH (cm) 30 days	FW (cm) 60 days	PH (cm) 90 days	PH (cm) 120 days	FW (mg) 120 days	RL (cm) 120 days	NS 120 days ¹	
T1 (0 g L ⁻¹)	2.04 a ²	2.15 a	2.29 c	2.75 d	85.50 bc	1.94 b	2.0 b	
T2 (5 g L ⁻¹)	1.98 a	2.11 a	2.32 c	2.89 cd	75.80 bc	2.96 ab	3.4 ab	
T3 (10 g L ⁻¹)	2.12 a	2.34 a	2.58 bc	3.09 cd	70.90 c	4.00 ab	4.0 ab	
T4 (20 g L ⁻¹)	2.05 a	2.24 a	2.64 bc	3.32 bc	93.50 bc	3.34 ab	3.6 ab	
T5 (30 g L ⁻¹)	2.10 a	2.27 a	2.95 b	3.56 b	122.40 b	4.79 a	3.2 ab	
T6 (60 g L ⁻¹)	1.95 a	2.09 a	3.54 a	4.21 a	171.70 a	4.75 a	4.4 a	
C.V.%	11.32	16.06	16.29	10.18	15.63	25.41	14.79	
DMS	0.62	0.45	0.42	0.56	30.0	1.62	1.04	

¹Means followed by the same letter in the column do not differ according to the Tukey test at 5% probability.

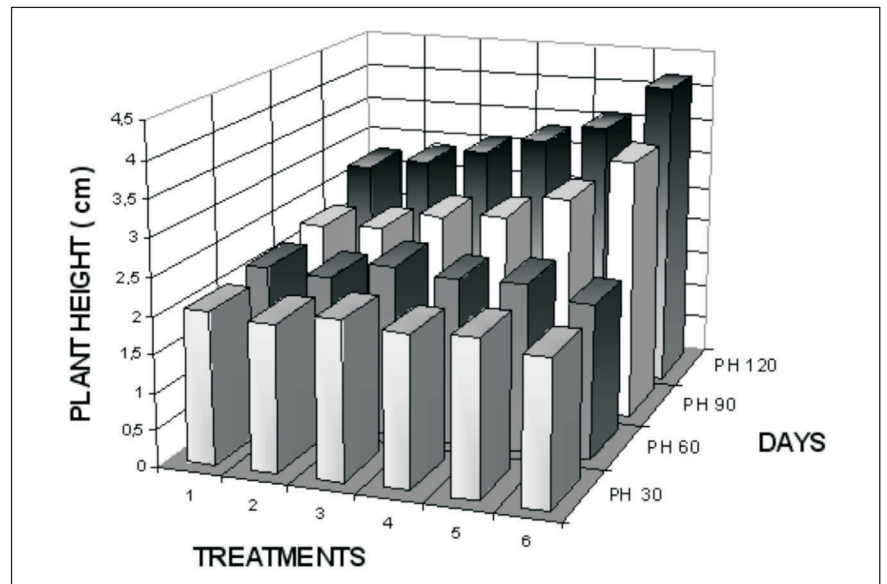


Figure 1. Plant height mean values (PH), according to sucrose concentration in the culture medium and the time in culture (30, 60, 90 e 120 days). Treatments:T1 (0 g L⁻¹); T2 (5 g L⁻¹); T3 (10 g L⁻¹); T4 (20 g L⁻¹); T5 (30 g L⁻¹) e T6 (60 g L⁻¹). Londrina, UEL, 2003.

culturing, the superiority of use of a higher sucrose concentration (60 g L⁻¹) became evident based on explant growth in height. Similar results were observed considering plant fresh weight (FW) (Table 1).

Plantlets grown in the six different treatments began to sprout after the second month, even in the absence of plant hormones.

The assessment of mean root length (RL) values and number of shoots (NS) in plantlets from each treatment indicated that there were no significant differences between treatments, except when sucrose was not added to the

culture medium, in which case root growth and shoot production were inhibited (Table 1). According to Mc Cown (1998), *in vitro* root formation does not occur when photosynthesis products are supplied in insufficient quantities.

In Figure 1 we can observe that *in vitro* growth of *Dendrobium nobile* plantlets was directly proportional to the sucrose concentration in the culture medium, after culturing for 120 days. The highest sucrose concentration in the culture medium (60 g L⁻¹) presented the best result, with the greatest mean plant height value (4.21 ± 0.9cm).

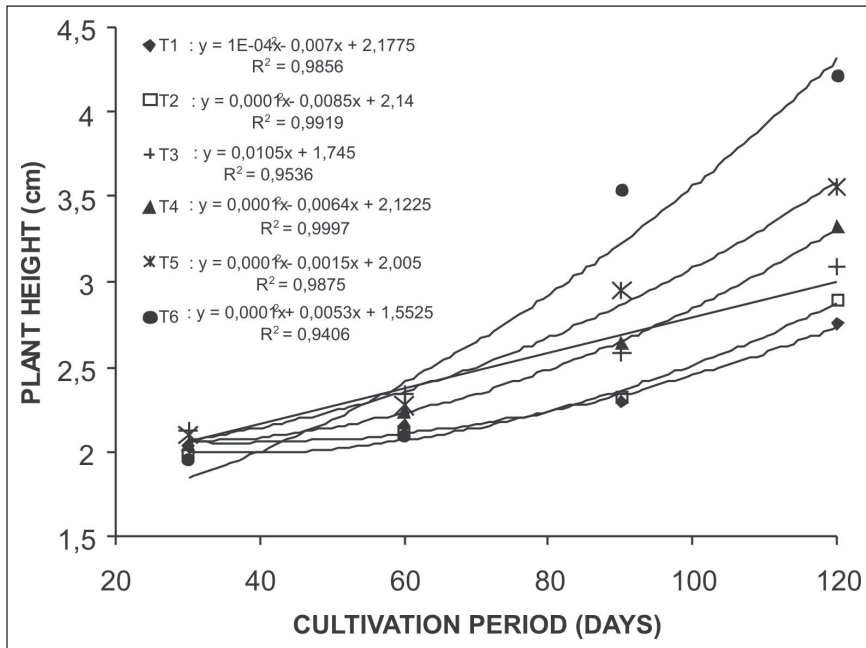


Figure 2. Curves of growth *in vitro* of *Dendrobium nobile* in agreement with the different sucrose dose: T1 (0 g L⁻¹); T2 (5 g L⁻¹); T3 (10 g L⁻¹); T4 (20 g L⁻¹); T5 (30 g L⁻¹) e T6 (60 g L⁻¹) assessed in different periods. Londrina, UEL, 2003.

Collins & Dixon (1992) studied different sucrose concentrations in *in vitro* culturing and observed that for the Australian terrestrial orchid *Diuris longifolia*, 20g L⁻¹ sucrose plus charcoal had a similar rooting effect as 40 g L⁻¹ sucrose without charcoal. According to Kerbauy (1993), high agar levels, together with sucrose, promote great longitudinal root growth and lateral aerial growth, while low levels of these components favor the formation of protocorms in *in vitro* culture of *Oncidium varicosum* (Orchidaceae). Ishii *et al.* (1998) observed that in *Phalaenopsis* the presence of sucrose in the culture medium caused protocorm formation and its absence caused callus proliferation.

The mean root length (RL) values and mean number of shoots (NS) were not influenced by the increase in sucrose concentration in the culture medium, as 5 g L⁻¹ would be sufficient. Without sucrose, smaller plant height increase, shorter root length, and a lower rate of shoot multiplication were observed.

Studies on micropropagation show that the presence of sugar in the culture medium is important for root development and shoot multiplication, as well as for plant height increase

(Kozai, 1991). Song *et al.* (1999) reported that a culture medium containing 3 g L⁻¹ of Peters 10-30-20 commercial rate, sucrose (20 g L⁻¹), banana pulp (60 g L⁻¹), and agar (4 g L⁻¹) was efficient to promote the vertical growth and rooting of *Dendrobium nobile* plants derived from *in vitro*-germinated seeds. Mitra *et al.* (1998) observed that *Dendrobium Sw.* roots in culture medium without sucrose did not develop, even when cultured within an atmosphere enriched with CO₂. The best culture medium combination for the micropropagation of *Dendrobium joannie* Ostenhault was 3% sucrose supplemented with 1 ppm of IAA, IBA, or NAA (Madhuri *et al.*, 1990).

It is important to verify whether plants cultured in no sucrose medium (0 g L⁻¹) would be more successful during the acclimation stage, as they were submitted to conditions that were quite similar to normal greenhouse conditions. These effects are still under observation. According to Vij *et al.* (1996) *Vanda cristata* Lindl. plantlets obtained from protocorms cultivated in culture medium with a reduced sucrose concentration (from 0.5 to 2 g L⁻¹) showed a success rate of 80% when transferred to greenhouse conditions.

Finally, a positive relationship between increase in sucrose concentration and increase in plant height was verified, as both treatments showed an increase in plant height as sucrose concentration increased. Treatment T6 presented a higher curve in relation to the treatment period, with an R₂ of 0.9406, while treatment T1 presented a lower curve in relation to the treatment period, with an R₂ of 0.9856 (Figure 2).

This increase in the amount of sucrose in the culture should be taken with caution and should not be progressive, because, according to Cappellades *et al.* (1991) and Hdidier & Desjardins (1994), high sucrose concentrations in *in vitro* cultures favor carbohydrate accumulation and hinder photosynthesis.

Sucrose concentration influenced growth and accumulation of biomass (fresh weight) of *Dendrobium* plantlets propagated *in vitro*. The presence of 60 g L⁻¹ sucrose in the culture medium was the most efficient treatment for increasing height and fresh weight of plants cultured *in vitro*.

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