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STIMULATION OF *IN VITRO* DEVELOPMENT OF *CATTLEYA GRANULOSA* BY SUCROSE

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Summary. *Cattleya granulosa*, a native orchid of Northeastern Brazil is under great risk of genetic erosion due to its location in areas threatened by massive urbanization as well as collection of plants in their natural habitat for commercialization. Because many papers report contrasting responses depending on sucrose concentration used, in this work we evaluated the effect of sucrose on *in vitro* development of *C. granulosa* with the objective to contribute to the development of an efficient seedling production method, thus discouraging the collection of plants and facilitating future reforestation programs. Four sucrose concentrations were tested (0, 15, 30 and 45 g L^{-1}) on seedlings obtained from seeds germinated *in vitro*. Sucrose enhanced strongly plant development, increasing significantly the height of seedlings and the production of new shoots, leaves and roots. In general, the higher the sucrose concentration was, the better the plant development was. Plant development was not limited by sucrose or other nutrients in the culture medium until 180 days of *in vitro* culture. Our results are important for reduction of cost of production of seedlings of this species.

Key words: Orchidaceae; seedling production; sucrose.

Abbreviations: DAS – days after start of the experiment.

INTRODUCTION

Cattleya granulosa Lindl. (Orchidaceae) is a native species of Brazilian Northeastern region with great commercial potential due the beauty of its flowers. However, nowadays it is under great risk of extinction due to its location in areas threatened by massive urbanization as well as collection of plants in their natural habitat.

Tissue culture techniques may be used for production of many seedlings by *in vitro* germination of seeds (Pierik,

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1990), thus maintaining the natural genetic variability, which is important for ecological purposes. In the natural habitat of *C. granulosa* a great diversity in the color and shape of flowers as well as in plant height is established, suggesting great genetic variation. Thus, propagation of this species from germination of seeds *in vitro*, will significantly contribute for its preservation. However, yet a problem in propagating plants by tissue culture is that this is a very expensive technique as compared to other propagation procedures. The high costs are well related to the manipulation and long time of maintaining the plants in laboratory, particularly orchids, which have low growth rates.

The external supply of carbohydrates is very important for the growth and development of plants *in vitro* (Kozai et al., 2005; Thorpe et. al., 2008; Peyvandi et al., 2009), sucrose being the most used source due to better responses in general and low costs (Thorpe et al., 2008). For orchids, there are different recommendations of concentrations of sucrose to be used according to species (Arditti and Ernst, 1992), and some results with other species showed also different responses (Langford and Wainwright, 1987; Kumar et al., 1999; Cui et al., 2000; Takayama and Misawa, 2006). However, this subject is in general overlooked in literature. It should be stressed that the establishment of an optimal concentration of sucrose would be important for the growth of plants, which will in turn reduce the manipulation and the time of maintaining them in laboratory. Therefore, the objective of this work was to evaluate the sucrose effect on *in vitro* development of *C. granulosa*, with the purpose to contribute to a more efficient seedling production, thus discouraging its collection from the natural habitat and facilitating future reforestation programs.

MATERIAL AND METHODS

Four sucrose concentrations were tested (0, 15, 30 and 45 g L^{-1}) in media containing the salts of Murashige and Skoog (1962), 0.55 mM *myo*-inositol, 0.0003 mM thiamine-HCl, 0.004 mM nicotinic acid, 0.0024 mM pyridoxine-HCl and 0.027 mM glycine. The pH of media was adjusted to 5.7 before gelling them with 7 g L^{-1} agar. Glass test tubes (25 x 150 mm) containing 10 ml of culture medium were used as flasks. Each flask was capped with aluminum foil before sterilization at 121 ºC for 15 min. Seedlings with an average height of 19.8 mm and fresh mass of 23.7 mg, obtained from seeds collected at Natal-RN, Brazil, and germinated *in vitro*, were used as plant material. The flasks were incubated at 26±2°C under cool white fluorescent light of 50 µmol m^{-2} s^{-1} photons flux density and a 16-h photoperiod.

The experiment was in a completely randomized design with 8 replicates. Plant height and the number of leaves, new shoots and roots, were evaluated initially and at 30, 60, 120 and 180 days after start of the experiment (DAS). The shoots and roots fresh mass were evaluated at 180 DAS. After checking the homogeneity of variances and normality according to Fligner-Killeen and Shapiro-Wilk tests, respectively, the data were submitted to analysis of variance and the least significant difference at 5% was calculated for each variable (Crawley, 2007). Data analysis and graphs generation were made using the software R (R Development

Core Team, 2010), version 2.10.1, in a Linux platform.

RESULTS

Seedlings which had not received sucrose, showed no formation of new roots and a poor increase in height and production of leaves and new shoots (Fig. 1). Sucrose enhanced strongly plant development, increasing significantly ($p \leq 0.05$) the height of seedlings and the production of new shoots, since 120 and 60 DAS, respectively (Fig. 1A, C). The number of leaves was significantly higher in the seedlings which received 45 g L^{-1} since 120 DAS onwards and in those which received 15 g L^{-1} at 180 DAS (Fig. 1B). The number of roots was significantly higher in the 30 g L^{-1} treatment since 120 DAS onwards (Fig. 1D). The only differences between sucrose treatments concerned plant height (Fig. 1A) in 45 g L^{-1} concentration compared to 15 g L^{-1}, number of leaves (Fig. 1B) in relation to 30 g L^{-1}, and both at 180 DAS.

The fresh mass of seedlings was also enhanced by sucrose. There was a trend of increase in shoot fresh mass in all sucrose treatments compared to control, though the difference was significant ($p \leq 0.05$) only in the 15 g L^{-1} treatment (Fig. 2A).

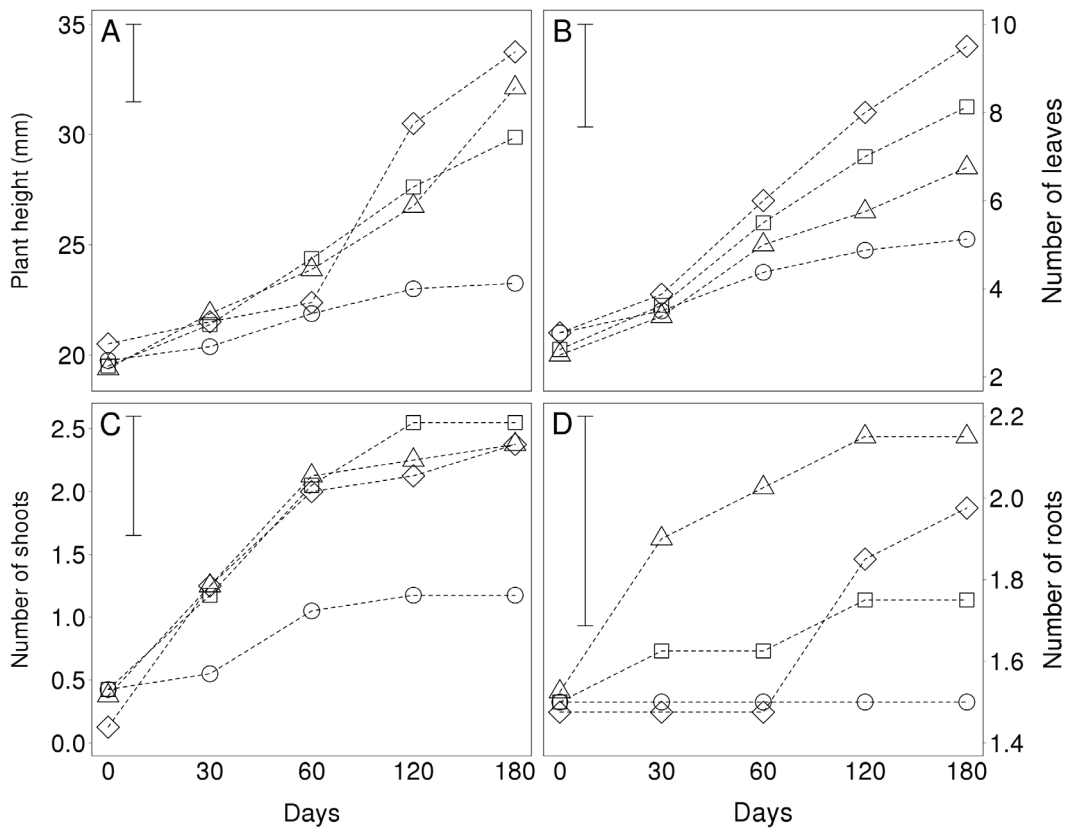

Fig. 1. Plant height (A), number of leaves (B), number of new shoots (C), and number of roots (D) of *Cattleya granulosa* grown for 180 days *in vitro* under different sucrose concentrations (0 g L^{-1} – circles; 15 g L^{-1} – squares; 30 g L^{-1} – triangles and 45 g L^{-1} – diamonds). The bars represent the least significant difference at 5%.

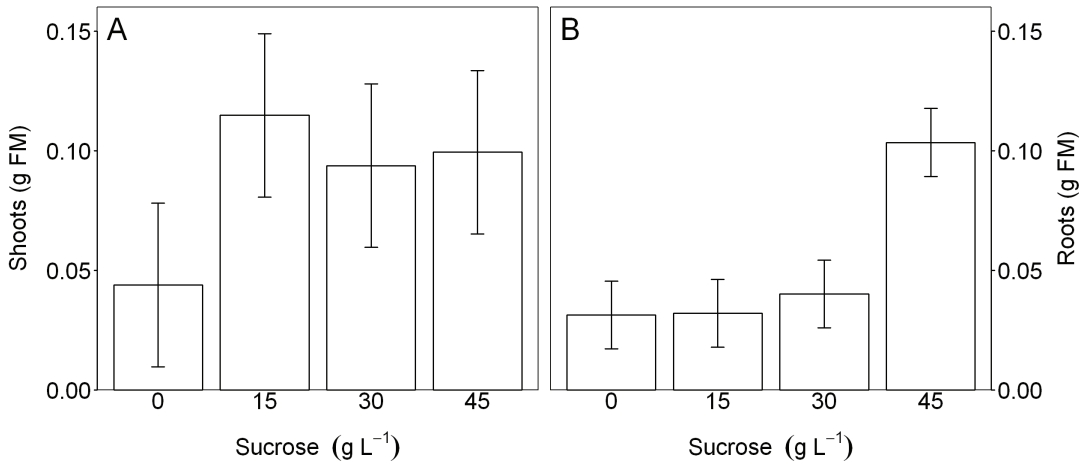

Figure 2: Fresh mass of shoots (A) and roots (B) of *Cattleya granulosa* grown for 180 days *in vitro* under different sucrose concentrations. The bars represent the least significant difference at 5%.

Concerning fresh mass of roots, the only significant increase was in the 45 g L^{-1} treatment (Fig. 2B).

DISCUSSION

We showed that in our experimental conditions *C. granulosa* is able for autotrophic growth *in vitro* since some increase in height and number of leaves and new shoots occurred during 180 days without external carbon source in the culture medium. In most cases, efficient autotrophic growth *in vitro* is possible only by enriching the CO_2 concentrations in the vessels during the photoperiod as well as optimizing the environment, e.g. by reducing the relative humidity by ventilation (Kozai et al., 2005). Our results indicated, however, that the higher the sucrose concentration in the medium was, the higher the plant development was, thus showing a strong dependency of adequate *in vitro* development of *C. granulosa* on the external carbon source. For some other orchids, the stimulatory effect of sucrose on *in vitro* development is observed at relatively low concentrations, 20 g L^{-1}, for *Cymbidium* (Kusumoto, 1980), *Diuris longifolia* (Collins and Dixon, 1992), which is a terrestrial species, and for hybrids of *Cattleya labiata* x *Laelia itambana* (Fráguas et al., 2003) and *Laelia purpurata* x *Cattleya warneri* (Moreira et al., 2007). However, there are also papers demonstrating the increase in plant development at higher sucrose concentrations in the culture medium as shown in the present work. In *Oncidium baueri* (Sorace et al., 2008) and *Cattleya walkeriana* (Dignart et al., 2009), the best results were obtained at 40 and 30 g L^{-1}, respectively. In *Dendrobium nobile* grown in liquid medium the best result was achieved at 60 g L^{-1}, which was the highest concentration tested (Faria et al., 2004). Furthermore, our results and the data of other researches with orchids propagation *in vitro* indicate that before commercial production of some species it is necessary to test which concentration of sucrose or other external carbon sources will give the best results.

The most intriguing result in this

work, though not statistically significant, was the tendency of 15 g L^{-1} sucrose to induce a higher increase in the number of leaves compared to 30 g L^{-1}. However, the latter concentration showed the highest tendency of increase in the number of roots. These results suggest a change in biomass allocation induced by sucrose concentration in the culture medium. Regulation of plant gene expression in relation to carbohydrates is well known (Thorpe et. al., 2008) and it could affect the biomass allocation in plants. Therefore, this hypothesis must be further investigated.

The shape of the response curves indicated that production of new shoots and roots occurred early, preferentially during the first two months after the onset of the experiment. However, the plants continued increasing in height and producing leaves during 180 days after incubation. The above results indicated that carbon source and other nutrients in the media were not limiting for the growth of plants. This is important for reduction of cost of seedlings production in *C. granulosa*, since it will reduce the manipulation of plant material in laboratory besides increasing their development. Furthermore, our results were obtained on plant material of seeds, thus being important for ecological and commercial purposes.

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