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PRODUÇÃO VEGETAL

Mauren Sorace1, Ricardo Tadeu de Faria2, Inês Cristina de Batista Fonseca3, Nathália Padovanni4, Arney Eduardo do Amaral Ecker5

ACCLIMATIZATION OF *CATTLEYA* (ORCHIDACEAE) IN PLASTIC, STYROFOAM AND CERAMIC CONTAINERS

ABSTRACT: Orchids are among the most cultivated ornamental plants in the world. Root formation and vegetal development of orchid *Cattleya loddigesiii* and hybrid *Cattleya intermedia* x *Hadrolaelia purpurata* (Orchidaceae) were evaluated using different recipients during the acclimatization phase. Seedlings, 3±0.5 cm mean height, were obtained from *in vitro* grown seeds in Murashige & Skoog (1962) culture medium and with half the macronutrient concentration. They were cultivated in different types of containers with sphagnum as substratum. Recipients consisted of a plastic tray with 120 cells, polystyrene tray; earthenware tray and a transparent plastic tray with lid. Seedlings were maintained under 60% luminosity at greenhouse. A randomized design with four treatments and four replications was prepared with twenty plants per container as a statistical model. Seven months after starting the experiment, the following factors were evaluated: frequency, length of the biggest root; number of roots; height; length of the largest leaf; number of leaves; number of sprouts and total fresh mass. The plastic trays with 120 cells produced the highest vegetal growth and roots of *Cattleya loddigesii* seedlings and *Cattleya intermedia* x *Hadrolaelia purpurata* hybrid during the acclimatization stage.

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1 Bióloga, Dra. Pós Doc em Engenharia Agrícola - UNIOESTE. Bolsista CNPq E-mail: mauren_band@hotmail.com; 2 Eng. Agr. Prof. Dr. Associado, bolsista de produtividade em pesquisa do CNPq - Nivel -2; Universidade Estadual de Londrina, Centro de Ciências Agrárias, Departamento de Agronomia, Caixa Postal 6001, Cep: 86051-990, Londrina - Pr. E-mail: faria@uel.br; 3 Eng. Agr. Profa. Dra. Bolsista de Produtividade em Pesquisa da Fundação Araucária; Universidade Estadual de Londrina, Centro de Ciências Agrárias, Departamento de Agronomia, Caixa Postal 6001, Cep: 86051-990, Londrina - Pr. E-mail: inescbf@uel.br; 4 Bióloga, Mestranda em Ecologia na Universidade Estadual de Campinas. Bolsista CNPq; 5 Eng. Agr. Dr. Prof Assistente Universidade Estadual de Maringá- UEM. E-mail:

KEYWORDS: Orchids, seedling production, *in vitro*, *ex vitro*.

ACLIMATIZAÇÃO DE *CATTLEYA* (ORCHIDACEAE) EM RECIPIENTES PLÁSTICOS, DE ISOPOR E CERÂMICA

RESUMO: As orquídeas estão entre as plantas ornamentais mais cultivadas no mundo. O presente trabalho objetivou avaliar o crescimento vegetativo e enraizamento de mudas das orquídeas *Cattleya loddigesii* e do híbrido *Cattleya intermedia* x *Hadrolaelia purpurata* em diferentes tipos de recipientes durante a fase de aclimatização. As plântulas, com altura média 3,0 ± 0,5 cm, foram obtidas a partir de sementes germinadas *in vitro* em meio de cultura Murashige & Skoog, 1962 e com metade da concentração dos macronutrientes e cultivadas nos diferentes tipos de recipientes contendo o esfagno como substrato. Os recipientes testados foram: bandeja plástica com 120 células, bandeja de isopor, vaso de cerâmica e bandeja transparente de plástico com tampa. As plântulas foram mantidas em casa de vegetação com 60% de luminosidade. O delineamento experimental foi inteiramente casualizado com quatro repetições, contendo vinte plântulas por recipiente. Após sete meses do início do experimento foram realizadas as seguintes avaliações: freqüência de pegamento, comprimento da maior raiz, número de raízes, altura da planta, comprimento da maior folha, número de folhas, número de brotos e massa fresca total. O tratamento com o recipiente bandeja plástica com 120 células proporcionou maior crescimento vegetativo e enraizamento de mudas de *Cattleya loddigesii* e do híbrido *Cattleya intermedia* x *Hadrolaelia purpurata*, durante a fase de aclimatização.

PALAVRA CHAVE: Orquídeas, produção de mudas, *in vitro*, *ex vitro*.

INTRODUCTION

The Orchidaceae family is the most developed group of the order *Liliales*. It comprises approximately 35.000 species (CRONQUIST, 1981) with highly specialized features that provide them with great adaptability in different environments (BENZING et al., 1982). Orchid species of the genus *Cattleya* are natives to regions in Mexico and Central and South America. Some 70 species have been identified including 20 species native to Brazil (PABST; DUNGS, 1975).

The culture of orchids by micro-propagation is due to the plants' commercial production and features fast manipulation, least time possible, increased productivity, production uniformity and seedling quality. Drastic changes occur when seedlings are removed from their flasks in which light and gas exchanges are limited and high sugar availability is extant. Since the transition transforms the plants from a heterotrophic to an autotrophic status (DÍAZ-PÉREZ et al., 1995),

acclimatization is a highly critical stage for plants due to stress in environmental change but especially to possible infections by fungi and bacteria which may develop during this phase (TOMBOLATO; COSTA, 1998).

Factors, such as water stress, temperature, luminosity, substrate, nutrients and phytosanity, which limit the seedlings' development, should be controlled during the seedling acclimatization process (MORAES et al., 2002).

Sorace et al., (2007) obtained results with naphthalenoacethic acid spraying (NAA) with a 200 mg L^{-1} dose in the substrate during the acclimatization period and had positive results in root induction of the orchid *Oncidium baueri*.

According to OLIVEIRA et al. (2002), the container size and substrate type are the first factors that must be investigated so that the good quality production of seedlings and plants' adequate growth may be warranted. The former affects directly the available volume with regard to the space for root development; the latter influences the root system and the plants' nutritional situation. It deeply affects seedling quality since plants retrieve most nutrients, needed for development, through the substrates.

Containers used during this phase greatly affect the development of the seedlings, with special emphasis to drainage and space for root growth (KÄMPF, 2000).

In vitro-propagated orchid seedlings are normally planted in a single container, called a collective container. In spite of the fact that orchid producers employ different types of containers during the acclimatization phase, the literature fails to indicate the most efficient ones for vegetal development and seedling rooting.

The current assay evaluates the vegetal growth and seedling rooting of the orchid *Cattleya loddigesii* and hybrid *Cattleya intermedia* x *Hadrolaelia purpurata* in different types of containers during the acclimatization phase.

MATERIAL AND METHODS

The experiment was carried out from May 2007 to November 2007 at the Universidade Estadual de Londrina, Paraná, Brazil (23°23'S and 51°11'W, at 566-m altitude). The climate is classified as Cfa, humid subtropical, according to Köppen.

In the experiment, seedlings from the orchid *Cattleya loddigesii*

and from the hybrid *Cattleya intermedia* x *Hadrolaelia purpurata,* propagated *in vitro* with approximately 3 cm ± 0,5 cm high were used. They were washed in running water for removal of any trace of medium culture (MURASHIGE; SKOOG, 1962), immersed in a diluted solution of sodium hypochlorite (5%) during two minutes and transplanted to different containers with sphagnum as substrate and were acclimatized in arched-roof greenhouse with 60% shade given by black polypropylene screens.

Containers comprised: T1 – plastic tray with 120 cells (33.5 cm wide; 5 cm high; 50 cm long); T2 – polystyrene tray (14.5 cm wide; 4 cm high; 21 cm long); T3 – transparent plastic tray with lid (16.5 cm wide; 10 cm high; 23 cm long); T4 – earthenware pot (8 cm wide; 17 cm diameter).

Three weekly applications of naphthalenoacethic acid solution (200 mg L^{-1}) were undertaken in the substrate so that seedling rooting may be triggered. Seedlings were watered daily and fertilized monthly with leaf manure featuring formula NPK: 6-6-8 liquid, in a concentration of 2 ml L^{-1} (SORACE et. al., 2007).

After seven months from the start of the experiment, the following parameters were evaluated: frequency of grounding in soil, length of greatest root, number of roots, plant height, length of largest leaf, number of leaves, number of sprouts and total fresh mass. Determination of the survival of plants was performed by counting the number of dead plants after the acclimatization time considered. The determination of the height of the air was made by measuring the region between the neck and the insertion of the last leaf of the plants, with digital caliper. For roots length was measured from the root tip to the stem beginning and length of the largest sheet was measured from base to tip of the leaf (ASSIS et al., 2011).

Randomized experimental design comprised four repetitions with 20 seedlings each. Results were evaluated by variance analysis and Tukey test at 5% probability. In the case of the variables number of roots, leaves and sprouts, data were transformed into the square root $\sqrt{x+1}$, and means are those which were not transformed.

RESULTS AND DISCUSSION

Grounding frequency was higher than 98% with no statistical difference among the containers. Whereas in the case of plant rooting, the plastic trays with 120 cells, polystyrene trays and the earthenware

pots gave better results for greatest root length than those for plastic trays with lid, in the case of the variable number of roots, results for the plastic trays with 120 cells statistically differed from those for transparent plastic trays with lid. Results for polystyrene tray and for earthenware pot did not differ from those of both (Table 1).

Table 1 Means of grounding rates, length of biggest root (CMR), number of roots (NR), plant height (AP), length of the largest leaf (CMF), number of sprouts (NB), number of leaves (NF), total fresh mass (MFT) of *Cattleya loddigesii* seedlings after seven months from the start of the experiment

Parâmetro		Profundidade do sulco	Profundidade da semente	Índice de velocidade de emergência	Espaçamento entre plantas	Estande inicial de plantas	Estande final de plantas
"F"	Semeadoras	0,12^{ns}	3,00^{ns}	0,11^{ns}	75,0*	1,67^{ns}	0,77^{ns}
	Velocidades	20,51*	44,16*	0,23^{ns}	0,75^{ns}	0,32^{ns}	0,33^{ns}
	Semeadoras x Velocidades	0,99^{ns}	0,57^{ns}	2,09^{ns}	0,75^{ns}	0,71^{ns}	0,95^{ns}
Nº de amostras		24	24	24	22	22	22
Mínimo		0,084 m	0,039 m	4,3	0,16 m	51390	41680
Máximo		0,119 m	0,059 m	10,8	0,25 m	77780	69440
Média		0,97 cm	0,048 m	7,4	0,207 m	63760	58780
Desvio Padrão		0,0092 m	0,054 m	1,25	0,024 m	6610	6050
Coeficiente de variação (CV)		9,5%	11,3%	17,0%	11,6%	10,4%	10,3%
Normalidade		sim	Sim	sim	sim	sim	sim

*Data transformed by square root ($\sqrt{x+1}$).

**Means followed by the same letter in the column do not differ among themselves by the Tukey test at 5% significance.
Legend: T1 – polystyrene tray; T2 – plastic tray with 120 cells; T3 – earthenware pot; T4 – transparent plastic tray with lid.

In vitro propagated plants have adaptation to difficulties with regard to the ex vitro environment. This is due to their small and fragile roots during the acclimatization phase. According to DEBERGH and MAENE (1981), roots produced in culture are not functional after the plants transference to an ex vitro condition. Only roots produced after transplantation are functional. During the acclimatization phase of the orchid *Cattleya chocolate* Drop x *Cattleya guttata* x *Laelia tenebrosa* COLOMBO et al. (2005) found excellent results with coconut substrate in polypropylene pots, whereas MORAES et al. (2002), when cultivating *Dendrobium nobile*, had satisfactory results in earthenware pots containing the substrates vermiculite + plantmax + ground coal +

ground polystyrene.

When plant height, length of the greatest leaf, number of leaves, number of sprouts and total fresh mass were analyzed with reference to the growth of the plant's aerial section, the results for the plastic trays with 120 cells statistically differed from those involving the transparent plastic tray with lid.

In the case of the variable number of leaves, results for plastic trays with 120 cells were higher than those for all treatments. Procedures for seedling planting in trays with cells are different from those with other types of trays. Seedlings are planted singly in this type of container, whereas they are planted within a collective system in the other containers.

According to OLIVEIRA et al. (2002), container size and substrate type are the first factors that must be investigated so that the production of good quality seedlings and the plants adequate growth may be warranted. The former affects directly the volume available with regard to the space for root development; the latter influences the root system and the plants' nutritional situation. It deeply affects seedling quality since plants retrieve most nutrients, needed for development, through the substrates.

No statistical difference exists in grounding rates, which ranged between 94 and 97% , among the different containers. Polystyrene trays (7.14 cm), plastic tray with 120 cells (8.37 cm) and earthenware pot (7.51 cm) had the best results with regard to the length of the greatest root. They differed statistically from those obtained for the transparent plastic tray with lid (5.97 cm). In the case of number of roots, the plastic tray with 120 cells had the largest number of roots (8.69) and differed statistically from the other treatments (Table 2).

Table 2 Mean grounding rates, root length (CMR), number of roots (NR), height of plant (AP), length of the largest leaf (CMF), number of sprouts (NB), number of leaves (NF), total fresh mass (MFT) of hybrid *Cattleya intermedia* x *Hadrolaelia purpurata* seedlings after seven months from the start of the experiment

TR	FP %	CMR (cm)	NR (*)	APA (cm)	CMF (cm)	NF (*)	NB (*)	MFT (mg)
T1	95.33 a**	7.14 ab	7.18 b	4.81 ab	3.95 ab	4.82 ab	1.33 a	2.74 ab
T2	97.66 a	8.37 a	8.69 a	5.47 a	4.23 a	5.35 a	1.47 a	3.47 a
T3	95.33 a	7.51 a	7.18 b	4.83 ab	3.79 ab	4.89 ab	1.46 a	2.25 bc
T4	94.66 a	5.97 b	6.22 b	3.91 b	3.58 b	4.20 b	1.14 b	1.66 c
CV%	**3.10**	**16.32**	**11.27**	**11.09**	**8.34**	**10.27**	**18.15**	**15.88**

*Data transformed by square root ().
**Means followed by the same letter in the column do not differ among themselves by the Tukey test at 5% significance.
Legend: T1 – polystyrene tray; T2 – plastic tray with 120 cells; T3 – earthenware pot; T4 – transparent plastic tray with lid.

In their experiments with in vitro rooting and kaki acclimatization in greenhouses, TELLES and BIASI (2005) found that during the acclimatization phase the plants in plastic pots without any lid had better survival rate (48.9%) than that of seedlings acclimatized in containers with lids. The latter had low survival rate (9.5%). According to these authors, this fact may be due to a temperature rise within the pots with lid which caused the seedlings' death.

Seedlings of the hybrid *Cattleya intermedia* x *Hadrolaelia purpurata* had the biggest growth in plant height (5.47 cm), length of the largest leaf (4.23), number of sprouts (1.47) and total fresh mass (3.47 mg) in the plastic tray with 120 cells. The plastic tray with lid provided statistically lower results for vegetal development compared to those of the plastic tray with 120 cells.

In the case of the variable number of leaves, results for polystyrene tray (4.82), plastic tray with 120 cells (5.35) and earthenware pot (4.89) did not statistically differ among themselves. However, results for the plastic tray with 120 cells (5.35) were higher than those of plastic tray with lid (4.2).

With regard to total fresh mass, there were no statistical differences between results for the polystyrene tray treatment (2.74

mg) and those for the plastic tray with 120 cells treatment (3.47 mg). The earthenware pot (2.25 mg) and the plastic tray with lid (1.66 mg) treatments had the lowest total fresh mass. JÚNIOR et al. (2001) evaluated the comportment of passionflower seedlings planted in different containers and perceived that the best development of seedlings occurred when the small black polyethylene tubes were employed rather than when used the big black polyethylene tubes were used with the sand/fiber/organic compost substrate. Plastic tubes in the production of pineapple seedlings aim at improvements of the production system with better quality seedlings and costs decrease.

The container with plastic tray cover was not provided a better development for both orchids tested for the variables, because this show is closed and increased moisture in the substrate during the experiment.

CONCLUSION

Treatments featuring plastic trays with 120 cells had better results in vegetal growth and rooting of seedlings of the species *Cattleya loddigesii* and the hybrid *Cattleya intermedia* x *Hadrolaelia purpurata* during the acclimatization stage.

The capped plastic container should not be used in growing orchids for having lower values for both analyzed variables.

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