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A. INVESTIGACIONES

Plant growth of *Laelia tenebrosa* Rolfe treated with gibberellic acid and grown on different substrates

Crecimiento de plantas de Laelia tenebrosa *Rolfe tratadas con ácido giberélico y cultivadas en diferentes sustratos*

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ABSTRACT

The effect of gibberellic acid (GA_3) and different substrates on the growth of *Laelia tenebrosa* was evaluated. *In vitro* germinated plants were transplanted to trays, acclimatized, and after nine months used in two experiments: (a) effect of different substrates: coconut powder, *Pinus* sp. bark, coconut powder + *Pinus* sp. bark, coconut powder + *Pinus* sp. bark + charcoal and coconut powder + *Pinus* sp. bark + charcoal + crushed walnut shells, with five plants/pot and seven repetitions; (b) effect of pulverization with GA_3 (0, 25, 50, 100 and 200 mg L^{-1}) with four plants/pot and seven repetitions. After 360 days, the following variables were evaluated: survival rate, shoot height, number of leaves and buds, length and width of leaves and root length. Significant differences were found for number of buds, length of leaves and roots, with the best results for the plants grown in coconut powder + *Pinus* sp. bark and coconut powder + *Pinus* sp. bark + charcoal + walnut shells. Significant differences were found with pulverization with GA_3 for all variables except for leaf width and the best results were obtained with 25, 50 and 200 mg L^{-1}.

Key words: Growth regulator, gibberillin, Orchidaceae.

RESUMEN

Se evaluó el efecto del ácido giberélico (GA_3) y de diferentes sustratos en el crecimiento de plantas de Laelia tenebrosa *oriundas de la germinación* in vitro*, trasplantadas para bandejas colectivas, aclimatadas y tras nueve meses utilizadas en dos experimentos: a) evaluación del efecto de la pulverización con GA_3 (0, 25, 50, 100 e 200 mgL^{-1}) con cuatro plantas/florero y siete repeticiones; b) evaluación del efecto de los sustratos: polvo de coco, cáscara de* Pinus *sp., polvo de coco + cáscara de* Pinus *sp., polvo de coco + cáscara de* Pinus *sp. + carbón y polvo de coco + cáscara de* Pinus *sp.+ carbón + cáscara de nueces, con cinco plantas/florero y siete repeticiones. Tras 360 días se evaluó: supervivencia, altura de la parte aérea, número de hojas y brotes, largura y anchura de las hojas y largura de la raíz. Diferencias significativas fueron observadas para todas las variables excepto anchura de las hojas con pulverización con GA_3 y mejores resultados con 25, 50 y 200 mgL^{-1}. Diferencias significativas fueron observadas para número de brotes, largura de las hojas y mayor raíz, con mejores resultados cuando las plantas fueron cultivadas con polvo de coco + cáscara de* Pinus *sp. y polvo de coco + cáscara de* Pinus *sp.+ carbón + cáscara de nueces.*

Palabras clave: *Regulador de crecimiento, giberelina, Orchidaceae.*

Introduction

The flower and ornamental plant market is expanding rapidly with gains in quality and competitiveness, requiring the use of advanced technologies, technical knowledge and efficient distribution and marketing systems. Brazil is a center of diversity with exceptionally beautiful plants, orchids among them. In Brazil orchids have been the target of several studies, because of the indiscriminate collection and commercialization leading to the destruction of their natural habitat.

Plants of the family Orchidaceae have slow development, which makes multiplication difficult and affects marketing. Of the thousands of seeds existing in a capsule, only a small percentage germinates (Pinheiro *et al.,* 2004). For this reason, the use of techniques to germinate these seeds *in vitro* with greater efficiency, ensuring a larger number of plants, is crucial for the spread of these species.

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After germination, *in vitro* plants are transferred to *ex vitro* conditions, known as the acclimatization stage. *In vitro* propagation mainly aims to increase the production of seedlings, reducing costs and helping to save many orchid species from extinction (Stancato *et al*., 2001).

Studies have been conducted recently with some species to evaluate the effect of growth regulators, including gibberellic acid (GA_3), on the stimulation of shoot growth and reproductive development (Chen *et al*., 1994; Vichiato *et al*., 2007; Cardoso *et al*., 2010; Cardoso *et al*., 2012); in general good results have been obtained. However, there are no reports of these studies on *Laelia tenebrosa*, a species native to Brazil and endangered.

The substrate used in the production of orchid seedlings strongly influences the growth and development of plants (Kämpf, 2000; Silva, 2000; Souza, 2003). The ideal substrate should be available in large quantities, be easy to use and of low cost (Villa *et al*., 2007). Among the alternative substrates to fern tree fiber, coconut based-substrates, pine bark, Styrofoam, charcoal, vermiculite and rice hulls produced satisfactory results for different species (Rego *et al*., 2000; Assis *et al*., 2005; Araujo *et al*., 2007).

According to Colombo *et al.* (2005) concern with the preservation of the fern tree has been expressed by several authors. Coconut fiber and coconut powder have been evaluated as alternative substrates for both agronomic crops such as tomato (Silveira *et al*., 2002) and for ornamentals such as chrysanthemum (Bezerra *et al*., 2001).

This study aimed to evaluate the effect of GA_3 concentrations and different substrates on the growth of *L. tenebrosa*.

Materials and Methods

Growing conditions and plant material

Two experiments were conducted in a greenhouse at the Universidade Federal do Paraná (UFPR) - Sector Palotina, Palotina, Paraná, Brazil, from October 2010 to September 2011.

Laelia tenebrosa plants derived from *in vitro* germination in MS medium (Murashige and Skoog, 1962) with 1.5 g L^{-1} of activated charcoal were transplanted into trays and acclimated in a greenhouse. After nine months, when plants were between 2.5 and 3 cm long and bearing two leaves, they were transferred to polyethylene pots (No. 1) and used in the experiments.

Gibberellic acid

Plants were sprayed with four concentrations of gibberellic acid (25, 50, 100 and 200 mg L^{-1}) every two weeks. The control plants were sprayed with water (0 mg L^{-1}). Sprayings were carried out in the morning and the plants were wet with the treatment until dripping. In alternate weeks to the GA_3 spraying, the fertilizer Biofert® (5 mL L^{-1}) was applied to the plants.

The substrate used for cultivation was based on the mixture of coconut powder, *Pinus* sp. bark, charcoal and walnut shells (1:1:1:1). The plants were kept under 70% shade. Irrigation was performed manually and according to the substrate moisture assessed by visual evaluation, on average three times a week.

The experiment was arranged in a completely randomized design, with four plants per pot and seven replicates, totaling 28 plants per treatment. After 360 days, the following variables were evaluated: survival rate, shoot height, number of leaves, length and width of leaves and length of roots. Length measures were taken with a ruler and data expressed in cm.

Substrates

Plants were grown in pots containing the following substrates: coconut powder (CP), *Pinus* sp. bark (PB), coconut powder + *Pinus* sp. bark (PB + CP), coconut powder + *Pinus* sp. bark + charcoal (CP + PB + C) and coconut powder + *Pinus* sp. bark + charcoal + walnut shells (CP + PB + C + WS). The experiment was arranged in a completely randomized design, with five plants per pot and seven replicates, totaling 35 plants per treatment.

Plants were kept under 70% shade and supplied with the fertilizer Biofert® (5 mL L^{-1}). Irrigation was performed manually and according to the substrate moisture assessed by visual evaluation, on average three times a week.

After 360 days, the following variables were evaluated: survival rate, shoot height, number of leaves, length and width of leaves and length of roots. Length measures were taken with a ruler and data expressed in cm.

Statistical analysis

Data from both experiments were subjected to analysis of variance (ANOVA) and means were compared by the Fisher's Least Significant Difference (LSD) Test at 5% significance level using the software Statistic 7.0.

Results and Discussion

Effect of gibberellic acid

Significant differences were found for the variables survival rate, shoot height, number of leaves, length of leaves and length of roots of *L. tenebrosa* plants subjected to different concentrations of gibberellic acid (Table 1). *L. tenebrosa* plants that showed the highest survival rate (96.4%) were treated with 25 mg L^{-1} GA_3, differing from the other treatments. The greatest shoot height (5.1 cm) was observed in plants treated with 50 mg L^{-1} GA_3. The plants sprayed with 50 mg L^{-1} GA_3 also formed more leaves, but not significantly different from the treatment with 25 mg L^{-1} GA_3 (3.9 and 3.7 cm, respectively). The length of leaves and of the longest root were favored by the treatment with 200 mg L^{-1} GA_3 (Table 1).

The cell division induced by gibberellins through the activation of hydrolytic enzymes increases the length of the cells compared to their diameters, making tissues and organs, such as leaves, stems and fruit longer and thinner (Taiz & Zeiger, 2009). Unlike the results found in this study, Vichiato *et al.* (2007) found no significant differences between *Dendrobium nobile* plants treated with different concentrations of GA_3 (50, 100, 200, 400 mg L^{-1}) for height, number of leaves, and length and width of leaves.

Spraying 12-month-old plants of Phalaenopsis (FSNT Dai-Itigo hybrid pink) with 125 mg L^{-1} gibberellic acid increased the length of leaves and promoted flowering and production of high quality flowers (Cardoso *et al*., 2012). However, higher concentrations of GA_3 (250, 500 and 1000 mg L^{-1}) caused a reduction in leaf width of these plants. Studying the effect of five concentrations of gibberellic acid (0, 125, 250, 500 and 1000 mg L^{-1}) and two water regimes (one and four irrigations per week) on flowering induction of two hybrids of *Cattleya* and *Brassocattleya,* Cardoso *et al*. (2010) found that the concentration of 250 mg L^{-1} associated with decrease in frequency of irrigation was the most effective treatment for *Brassocattleya* Marcella Koss, inducing flowering in 83% of the plants. GA_3 at the same concentration, but with more frequent irrigations induced only 17% of the plants to flower. The number and quality of flowers increased with increased concentrations of GA_3. The use of gibberellic acid did not induce flowering in the hybrid *Cattleya* Irene Holguin.

The positive effect of GA_3 added to the culture medium for the *in vitro* propagation of a number of orchid species has been reported. Rodrigues *et al*. (2007) tested different concentrations of gibberellic acid and number of seedlings per jar on the growth of *in vitro* plantlets of *Cattleya loddigesii* Lindl. Plantlets from seeds germinated *in vitro* approximately 1 cm long were inoculated into Knudson C medium plus GA_3 (0, 2.5, 5, 7.5 and 10 mg L^{-1}) at a density of 3, 6, 9 or 12 plants per jar. After 90 days, the best results for *in vitro* propagation of seedlings (plant length) were obtained with 3 seedlings per jar and 10 mg L^{-1} GA_3.

In other cases, however, the addition of GA_3 in the culture medium had a negative influence on the growth characteristics of orchids. According

Table 1. Means for survival rate (%), shoot height (SH), number of leaves (NL), width of leaves (WL), length of leaves (LL) and length of longest root (LLR) of *L. tenebrosa* plants treated with gibberellic acid (GA3) after 360 days of cultivation.

GA_3 (mg L^{-1})	Survival (%)	SH (cm)	NL	WL (cm)	LL (cm)	LLR (cm)
0	78.6 b	4.1 b	3. 05 b	1.0 a	4.1 b	8.2 b
25	96.4 a	3.9 b	3.7 a	0.8 a	3.6 b	6.5 b
50	82.1 b	5.1 a	3.9 a	1.0 a	3.2 b	8.6 b
100	89.3 b	3.7 b	2.3 b	0.7 a	3.5 b	7.7 b
200	78.6 b	4.2 b	2.2 b	0.9 a	4.9 a	9.8 a

* Means followed by different letters in the columns are significantly different by Fisher's LSD Test at 5% significance level.

Table 2. Means for survival rate, shoot height (SH), number of leaves (NL), number of buds (NB), length of leaves (LL), width of leaves (WL) and length of longest root (LLR) of *L. tenebrosa* plants in the substrates: coconut powder (CP); *Pinus* sp. bark (PB); coconut powder + *Pinus* sp. bark (CP + PB); coconut powder + *Pinus* sp. bark + charcoal (CP + PB + C); coconut powder + *Pinus* sp. bark + charcoal + walnut shells (CP + PB + C + WS) after 360 days of cultivation.

Substrate	Survival %	SH (cm)	NL	NB	LL (cm)	WL (cm)	LLR (cm)
CP	37.1	3.3 a	3.2a	0.00b	2.7b	0.7a	4.1 b
PB	17.1	3.1 a	3.2a	0.00b	3.9a	0.6a	4.8 b
CP+PB	45.7	3.5 a	3.9a	0.17[a]	2.8 b	0.7a	7.2a
CP+PB+C	31.4	3.0 a	3.2a	0.05b	2.2b	0.6a	4.2 b
CP+PB+C+WS	40.0	3.7 a	3.9a	0.25[a]	3.4a	0.7a	5.6 a

* Means followed by different letters in the columns are significantly different by Fisher's LSD Test at 5% significance level.

to Araujo *et al*. (2009), GA_3 (2.5, 5, 10 and 20 mg L^{-1}) added to WPM medium for *in vitro* propagation of *Cattleya loddigesii* did not favor shoot formation, but there was increase in the number of roots and the fresh weight of plants in the absence of gibberellin 90 days after inoculation. Soares *et al*. (2009) also found no change in shoot formation, shoot growth and fresh weight when gibberellic acid was added to Knudson C medium for *Hadrolaelia lobatta* x *Hadrolaelia purpurata* Aço and *Cattleya loddigesii*.

The response of *L. tenebrosa* plants to different substrates showed significant differences for the number of shoots, length of leaves and length of the longest root (Table 2). The number of shoots was larger when plants were cultivated in the mixtures coconut powder + *Pinus* sp. bark (0.17) and coconut powder + *Pinus* sp. bark + charcoal + walnut shells (0.25). The latter also promoted the formation of larger leaves (3.4 cm), but was not significantly different from the treatment with *Pinus* sp. bark used alone (3.9 cm). The mixture coconut powder + *Pinus* sp. bark + charcoal + walnut shells also promoted the formation of large roots (5.6 cm) in *L. tenebrosa* plants, however without significant difference from coconut powder + *Pinus* sp. bark (7.2).

Colombo *et al*. (2005) concluded that coconut powder was a suitable substrate for growing the orchid *Cattleya chocolate drop* x (*C. guttata x L. tenebrosa*) during acclimation. In this study, the mixture coconut powder with *Pinus* sp. bark, charcoal and walnut shells was efficient for most of the variables tested. This is likely because of the combination of a little permeable substrate such as coconut powder and others more permeable such as charcoal, *Pinus* sp. bark and walnut shells. The substrate for transplanting orchids should have good conditions to retain moisture and avoid compaction and lack of aeration and permeability, which would decrease gas exchange in the plant root (Kämpf, 2000).

Muller *et al*. (2007) also succeeded in replacing the fern tree fiber by coconut powder and pine bark during the acclimation of *Miltonia flavescens*. Demattê (2001) reported that the substitution of fern tree fiber by coconut fiber in substrate mixtures for *Tillandsia gardneri* (Bromeliaceae) promoted a higher number of leaves, induction of inflorescences, and later, buds in the plants.

Different orchid species may require different substrates to adapt to the environment when removed from *in vitro* conditions, but the mixture of substrates was effective for the *ex vitro* growth of *Laelia tenebrosa,* allowing good aeration and anchorage for the fixing of new roots during plant development.

Conclusions

Greater shoot height and larger number of leaves were recorded in *Laelia tenebrosa* plants treated with 50 mg L^{-1} GA_3, while the length of leaves and roots was favored by the treatment with 200 mg L^{-1} GA_3. The substrate coconut powder combined with *Pinus* sp. bark, charcoal and walnut shells gave the best results for plant growth.

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