PLANT GROWTH REGULATORS FOR *in vitro* MINIMAL GROWTH OF *Comanthera mucugensis*

REGULADORES VEGETAIS NO CRESCIMENTO MÍNIMO DE *Comanthera mucugensis*

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ABSTRACT

In vitro minimal growth storage is a viable strategy for *ex situ* conservation that can be obtained through alterations of the standard culture medium and environment. The present study evaluated the effects of growth regulators, on the slow growth storage of *Comanthera mucugensis* (Giul.) L. R. Parra & Giul. for future *in vitro* conservation. The shoots were inoculated in MS culture medium, containing half salt concentrations (MS ½) supplemented with different concentrations of either ancimidol or paclobutrazol (0; 0.85; 1.70; 3.40 and 6.80 µM). After 180 days of cultivation, the following characteristics were evaluated: percentage of plant survival, length of the aerial section, percentage of green leaves, leaf color, length of the longest root, number of roots, dry weight of the root and of the aerial portion, percentage of plants with shoots, number of shoots per plant, and the length of the shoots. The conservation of *C. mucugensis* may be done on MS ½ culture medium for up to 180 days, without subculturing.

Index terms: *Ex situ* conservation, floriculture, paclobutrazol, ancimidol.

RESUMO

O crescimento mínimo *in vitro* é uma estratégia de conservação *ex situ* que pode ser obtida por alterações no meio de cultura e no ambiente de cultivo. O objetivo deste estudo foi avaliar o efeito de reguladores vegetais na conservação in vitro de *Comanthera mucugensis* (Giul.) L. R. Parra & Giul. Os brotos foram inoculados em meio de cultura Murashige e Skoog com metade da concentração salina (MS ½) suplementado com diferentes concentrações de ancimidol ou paclobutrazol (0; 0,85; 1,70; 3,40 e 6,80 µM). Ao final de 180 dias foram analisados: porcentagem de sobrevivência das plantas, comprimento da parte aérea, porcentagem de folhas verdes, coloração das folhas, comprimento da maior raiz, número de raízes, matéria seca da raiz e da parte aérea, porcentagem de plantas com broto, número de brotos por planta e comprimento dos brotos. Os reguladores vegetais promoveram um decréscimo no crescimento das plantas, no entanto reduziram a sua viabilidade. A conservação de *C. mucugensis* pode ser feita em meio de cultura MS ½, por até 180 dias, sem subcultivo.

Termos para indexação: Conservação *ex situ*, floricultura, paclobutrazol, ancimidol.

INTRODUCTION

The Eriocaulaceae family is comprised of approximately 1,200 species, in 10 genera, and has a pantropical distribution (RAMOS *et al*., 2005). This species occurs mainly in the neotropics, especially in the mountains of Venezuela and in the rocky plateaus of the Espinhaço mountain range in Minas Gerais and Bahia, Brazil (GIULIETTI *et al*., 1988).

Among the species found only in Bahia, *Comanthera mucugensis* Giul. ("sempre viva" of Mucugê) stands out as the "sempre-viva" with the highest commercial value (GIULIETTI *et al*., 1996). Despite an extraction ban by Brazil's environmental protection agency IBAMA, harvesting of its flowers before seed

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production still persists, resulting in a significant reduction in natural population, making this a threatened species (CERQUEIRA *et al*., 2008).

The *ex situ* conservation of plants from plant tissue culture techniques can be made through the induction of minimal growth, and has been successfully applied to the conservation of other threatened ornamental plants, such *Ipsea malabarica* (Reichb.f.) J. D. Hook and *Dickia distachya* Hassler and *Vriesea inflata* (Wawra) Wawra (MARTIN & PRADEEP, 2003; POMPELI & GUERRA, 2004; PEDROSO *et al*., 2010); as well as other plant species, such grapevine (SILVA *et al*., 2012), sweet potato (ARRIGONI-BLANK *et al*., 2014), vetiver (SANTOS *et al*., 2012) e cassava (LONDE *et al*., 2012).

Lima-Brito *et al*. (2011) recommend the use of tissue culture for the in vitro culture of *C. mucugensis*. However, the authors found no reduction in the growth of plants with the use of osmotic agents. Thus, other strategies for reduction of in vitro growth must be tested with a view to the preservation of genetic diversity of this species.

In vitro growth restriction is obtained by reducing the plant metabolism through alterations in the growth environment or by modifying the culture medium as the addition of plant growth regulators and osmotic agents and the reduction of saline and organic components (ENGELMANN, 1991; VILLALOBOS *et al*., 1991; WITHERS & WILLIANS, 1998; LEMOS *et al*., 2002). This technique allows the increases the period between subcultures, which reduces the costs of maintaining an active germplasm bank and, above all, reduces the risks of genetic alteration and contamination (ENGELMANN, 1991; VILLALOBOS *et al*., 1991; LEMOS *et al*., 2002).

The plant growth regulators, that act as growth retardants, are synthetic compounds used in *in vitro* conservation to restrict the longitudinal growth of a plant without modifying its growth pattern or producing phytotoxic effects (RADEMACHER, 2000; THAKUR *et al*., 2006).

Ancimidol (pyrimidine) and paclobutrazol (triazole) are N-heterocyclic plant growth regulators that inhibit the action of P-450 mono-oxygenase enzymes, blocking the oxidation reaction in the conversion of ent-kaurene to kaurenoic acid in the gibberellin biosynthetic pathway, impeding the formation of GA_{12}-aldehyde which is oxidized to GA_{12}, the first gibberellin ring system in all plants and, as such, the precursor of all others (RADEMACHER, 2000; TAIZ & ZEIGER, 2013). The gibberellin promotes cellular division and elongation stimulating the growth of intact plants, especially in rosette plants (KERBAUY, 2004), such as sempre-vivas.

This study aimed to evaluate the effect of plant growth regulators ancimidol and paclobutrazol to reduce the *in vitro* growth of *C. mucugensis* shoots, allowing its further *in vitro* conservation.

MATERIAL AND METHODS

Plant matter and culture medium

Microplants of an *in vitro C. mucugensis* collection of LCTV-UEFS were used as source of explants. Shoots of approximately 10mm in length, obtained by direct organogenesis using stem segments inoculated on half strength MS medium – MS ½ (MURASHIGE & SKOOG, 1962) gelled with 7 g L^{-1} agar and supplemented with 15 g L^{-1} sucrose, were used as explants.

The explants were inoculated in test tubes (25x150 mm) containing 15 mL of the culture medium. The medium used is the one described above. The pH of the medium was adjusted to 5.7 before autoclaving, conducted at 121 ºC for 15 minutes.

Plant growth regulators

The culture medium was supplemented with plant growth regulators ancimidol or paclobutrazol in concentrations 0 (control); 0.85; 1.70; 3.40 and 6.80 µM, and maintained in growth rooms at a temperature of 25 ± 3 ºC and a 16h photoperiod.

After 180 days, the plants were analyzed according to the following variables: survival rate of plants (%S), length of aerial part (LAP), percentage of green leaves

(%GL), leaf color (LC), length of largest root (LR), number of roots (NR), root dry matter (RDM) aerial part dry matter (APDM), percentage of plants with shoots (%PS), number of shoots per plant (NS) and shoot length (SL). The RDM and APDM were calculated having dried the plants to a constant weight, at 65 ºC in a oven with forced air circulation. The following scale was used to evaluate the leaf color variable: 0 – dry leaves, 1 – yellow leaves, 2 – light green leaves and 3 – dark green leaves.

Statistical analysis

The experimental design was completely randomized, with eight repetitions and four tubes per repetition (one explant per tube).

The data were submitted to variance analyze and the averages tested by regression analyze and the Scott-Knott test at 5% of probability for quantitative and qualitative factors respectively. All analyses were performed using Sisvar 4.3 software (FERREIRA, 2011).

RESULTS AND DISCUSSION

According to the results of variance analysis, there were no significant differences ($p \leq 0.05$) observed between the plant growth regulators ancimidol (ANC) and paclobutrazol (PBZ), and no significant interaction between the types and concentrations of regulators for all the variables analyzed (Table 1). However, significant differences were obtained between regulator concentrations for the length of aerial part (LAP), survival rate (%S), percentage of green leaves (%GL), leaf color (LC), length of largest root (LR), number of roots (NR), root dry matter (RDM) and aerial part dry matter (APDM) variables (Table 1).

Increases in the concentration of ANC and PBZ significantly reduced the variables length of aerial part (LAP), %S, %GL and LC in comparison to the control group. The data obtained for these variables were fitted using a linear regression model (Figure 1). These results corroborate Lima-Brito *et al*. (2011) who found the highest rates of survival for the species *C. mucugensis* on MS ½ medium gelled with 7 g L^{-1} agar and supplemented with 15 g L^{-1} sucrose in a period of 180 days of storage *in vitro*.

The greatest average for LAP (22.6 mm) was obtained in medium without ANC or PBZ (control). A linear decline for this variable was observed as a result of an increase in the concentrations of these regulators (Figure 1A). In the treatments that used the greatest concentrations (6.80 µM) of ANC and PBZ, there was a LAP reduction of 70.80% and 73.00%, respectively, in comparison to the control.

The explanation for the reduction of LAP in *C. mucugensis*, in response to ANC and PBZ treatments, is based in the blocking action that these inhibitors provoke in the conversion of ent-kaureno to kaurenic acid in the gibberellin biosynthetic pathway, a hormone that principally effects cell elongation (RADEMACHER, 2000).

TABLE 1 – Summary of the variance analysis for length of aerial part (LAP), survival rate (%S), percentage of green leaves (%GL), leaf color (LC), length of largest root (LR), number of roots (NR), root dry matter (RDM) aerial part dry matter (APDM) of *Comanthera mucugensis* as a function of different concentrations of the plant growth regulators ancimidol and paclobutrazol.

Source	DF	Mean square							
		LAP	S (%)	GL (%)	LC	LR	NR	RDM	APDM
Regulator	1	4.28ns	195.31ns	0.45ns	0.01ns	55.28ns	20.00ns	0.02ns	197.19ns
Concentration	4	687.99*	8,328.12*	6,722.82*	2.82*	683.77*	70.18*	1.86*	5,303.33*
R x C	4	7.32ns	78.12ns	197.45ns	0.08ns	7.43ns	15.99ns	0.03ns	463.33ns
Residue	70	70.06	806.92	561.03	0.44	69.23	9.31	0.48	2,127.25
C.V. (%)		43.54	50.15	48.55	18.20	44.82	35.41	20.80	67.11

* significant by the T Test Pd - 0.05, ns: not significant.

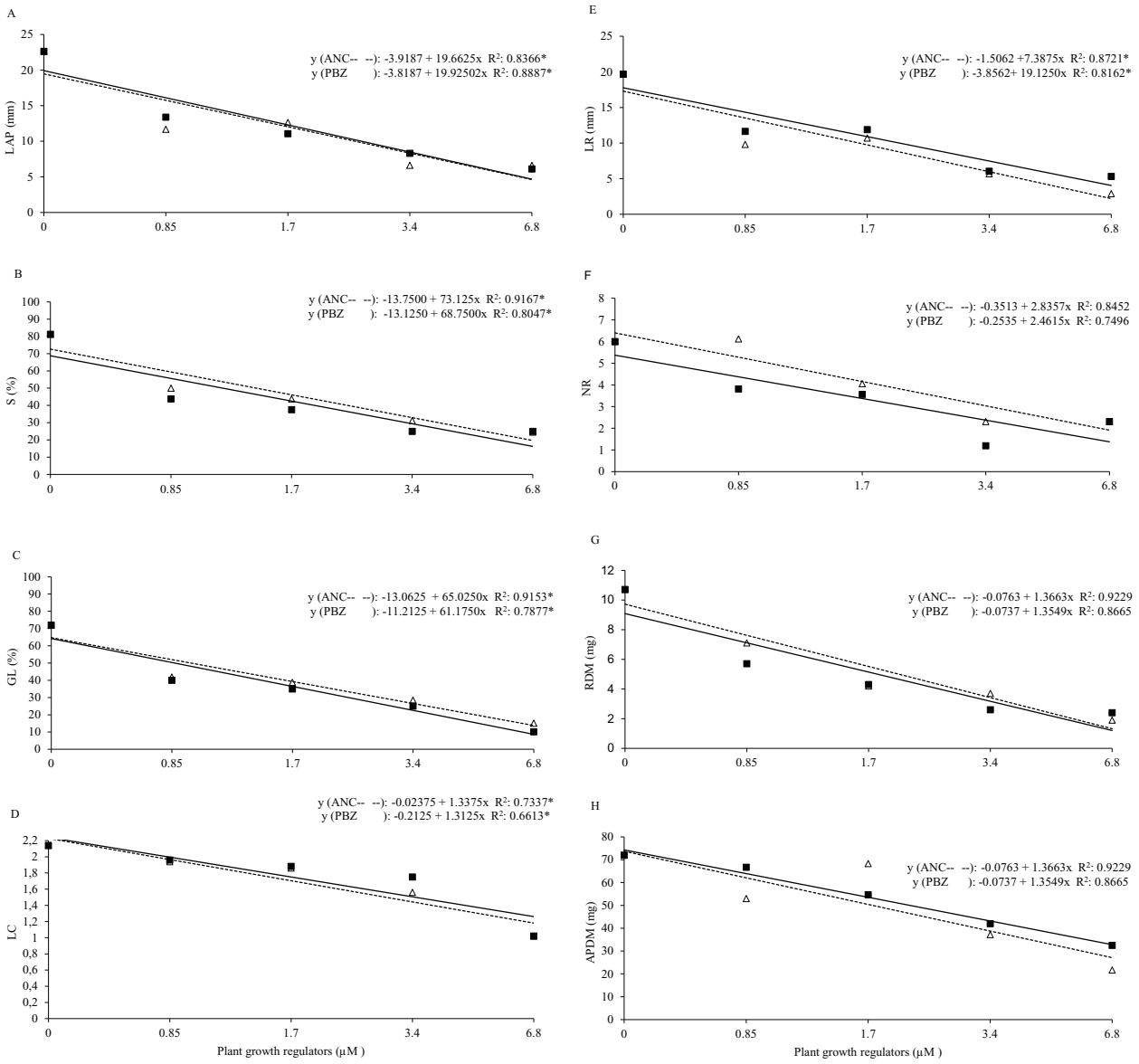

FIGURE 1 – Effect of different concentrations of plant growth regulators ancimidol (ANC) and paclobutrazol (PBZ) on length of aerial part – LAP (A); survival rate – %S (B); percentage of green leaves - %GL (C) and leaf color – LC (D); length of largest root – LR (E); number of root – NR (F); root dry matter – RDM (G); aerial part dry matter – ADPM (H) of *Comanthera mucugensis*.

Despite the inhibitory effects of ANC and PBZ on the *in vitro* growth of *C. mucugensis*, these compounds are not recommended for the conservation of this species since they reduce the viability of the plants. In contrast, Sarkar *et al*. (2005) and Canto *et al*. (2004) describe the positive effect that ANC and PBZ regulators had upon the *in vitro* conservation of *Solanum tuberosum* L. and *Ananas comosus* L., respectively.

At the end of the 180 day period the highest averages for %S (81.25%), %GL (72%), and LC (2.14) were obtained in the control group, with a linear decline in these variables as the concentration of plant growth regulators in the medium increased (Figure 1B - D). A reduction of 79% and 86% in %GL was observed in comparison to the control group when using 6.80 µM ANC or PBZ, respectively (Figure 1B).

For the variables %S and LC, there was a reduction in comparison to the control group of 69% and 85%, respectively, in the mediums supplemented with 6.80 µM of both plant growth regulators (Figure 1B and D). These results may be due to the phytotoxic effect of ANC and PBZ on the species studied.

In contrast to the results obtained for *C. mucugensis* Giul., other studies involving the application of PBZ, be it *in vitro* or *ex vitro*, have shown that plants treated with this regulator exhibit a more pronounced green color than the control group. This characteristic has been attributed to an increase in the synthesis of chlorophyll favored by PBZ, or to an increase in chlorophyll concentrations due to a reduction in cell size (FLETCHER *et al*., 2000).

The root system of *C. mucugensis* was negatively influenced by the use of ANC and PBZ. The data obtained for length of largest root (LR), number of roots (NR), root dry matter (RDM), as well as aerial part dry matter (APDM) fit the linear regression model (Figure 1).

The lowest averages for LR and NR were found when using the greatest concentration (6.80 µM) of regulators ANC (2,8 mm and 2.3, respectively) and PBZ (5,31 mm and 2.3, respectively) (Figure 1E and F). These results differ from those found in Thakur *et al*. (2006), that reported the formation of a large number of roots in *Lilium* sp. plants treated with 1.7 and 3.4 µM ANC or PBZ.

One of the secondary effects of the inhibition of gibberellin biosynthesis is an alteration in the plants' activity source-sink and, as a consequence, greater partitioning of assimilates, which can contribute to larger gains in the root system in detriment to the aerial part. However, this was not observed in the study of *C. mucugensis*, given that the dry matter in both the aerial part and root system decreased (Figure 1G e H).

The low average for %PS, NS and LS demonstrates that ANC and PBZ had little effect on the *in vitro* proliferation of *C. mucugensis* (Figure 2). Nonetheless, these plant growth regulators have induced high proliferation rates in the *in vitro* shoots of various ornamental plants, as related for genus species of *Lilium*, *Gladiolus* e *Philodendrum* (ZIV, 1989; ZIV & ARIEL, 1991; *THAKUR et al*., 2006). A result of the effect of these compounds on the endogenous levels of cytokines and auxins (DAVIS & CURRY, 1991; FLETCHER *et a*l., 2000).

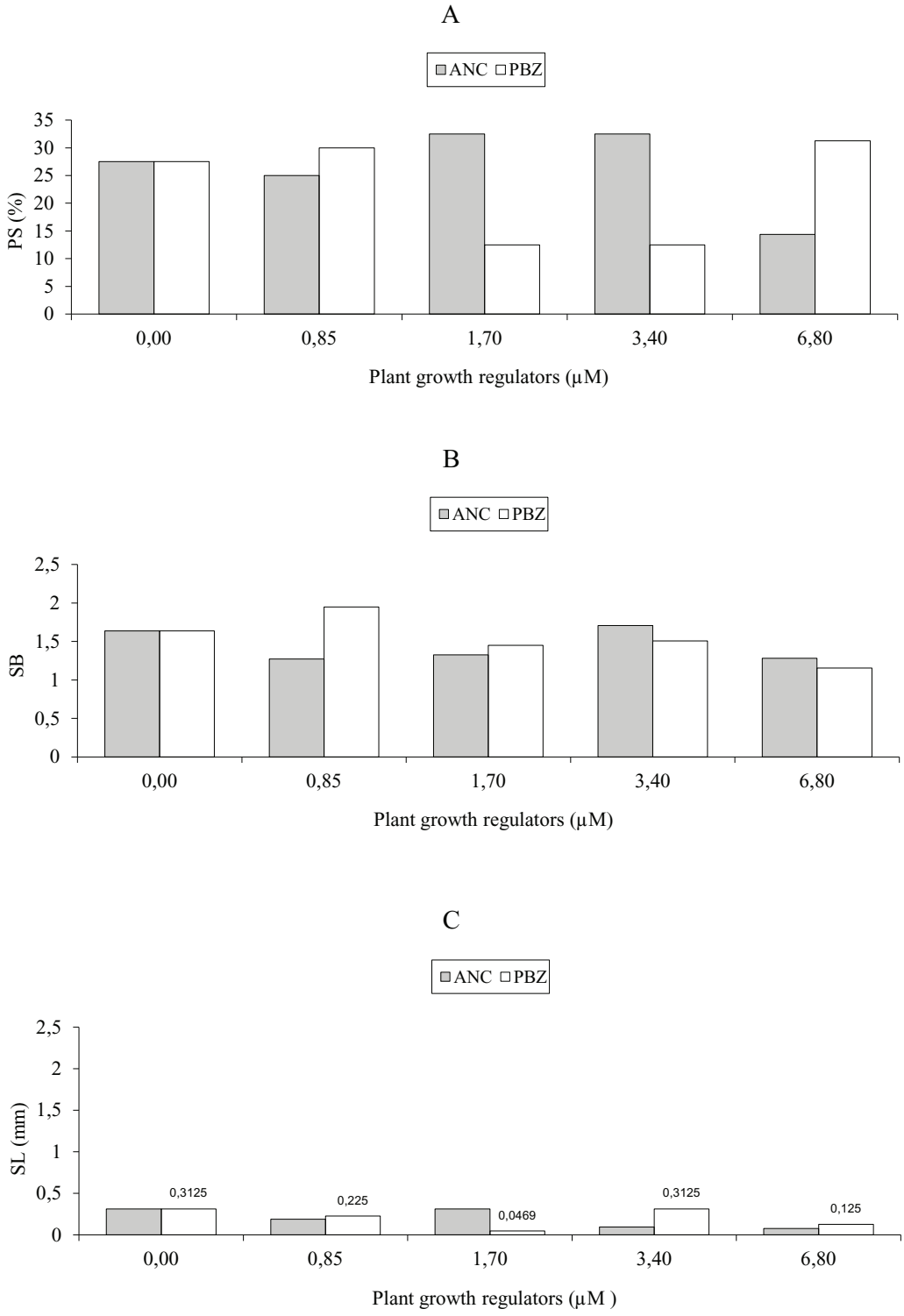

FIGURE 2 – Effect of different concentrations of plant growth regulators ancimidol and paclobutrazol on the percentage of plants with shoots – %PS (A); number of shoots per plant – SB (B) and shoot length – SL (C) of *Comanthera mucugensis* plants conserved *in vitro* for 180 days.

CONCLUSION

The *in vitro* culture allowed for the conservation of plants of *Comanthera mucugensis* for up to 180 days, without subculture, on medium MS ½ free of the growth regulators paclobutrazol and ancimidol.

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