In vitro multiplication and acclimatization of Heliconia hybrid (*Heliconia bihai* x *Heliconia caribaea* 'Jacquinii')

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ABSTRACT

For the implementation of commercial cultivation areas of heliconias, it is important to use pathogen-free seedlings and maintain the inherent characteristics of the hybrid plants to be cultivated. Thus, the objective of this work was to study a protocol for the *in vitro* propagation of the *Heliconia bihai x Heliconia caribaea* 'Jacquinii' hybrid and evaluate the plants survival rate during the acclimatization stage. Buds (explants) up to 5 mm long, originated from plantlets established *in vitro* were transferred to a ½ MS culture medium added by 0,5 mg L^{-1} of NAA combined with concentrations of 0.0; 0.5; 1.0; 1.5; 2.0 and 2.5 mg L^{-1} of 6-BA. Each treatment included 10 replications with one explant by replication, distributed at completely randomized design. After 100 days of growth under controlled conditions, rate of multiplication; plantlets length (cm) and cluster fresh and dry weight (g) were evaluated. Remaining plantlets were acclimatized in coconut fiber substrate to assess survival rate. In regards to the number of buds, the largest number of buds (four buds per explant) was verified in the absence of 6-BA, whereas in the presence of 6-BA, two to three buds per explant were observed. No significant differences were observed among 6-BA concentrations for plantlets length and cluster fresh weight. Plantlets survival rate during the acclimatization stage was 100%. Results from this study show that the MS culture medium added by 0.5 mg L^{-1} of NAA can be used for the in vitro propagation of the *H. bihai x H. caribaea* 'Jacquinii' hybrid.

Key words: Heliconiaceae, NAA, 6-BA, micropropagation.

INTRODUCTION

Among tropical cut flowers, the heliconias, undoubtedly, stand out for its intense colors and sculptural bracts forms that involve the flowers, attracting the attention and winning an increasing number of consumers.

The propagation of these plants by seeds becomes a limiting factor due to an increase in the plants juvenile period and lack of process uniformity caused by germination speed variation or genetic segregation. On the other hand, propagation by clumps is limited when there are a few mother plants or they are infected by some pathogenic organism (Nathan et al., 1992; Dias and Rodrigues 2001).

Thus, micropropagation becomes an indispensable tool for the massive propagation of species that present such limitations whenever conventional propagation methods are adopted. One of the obstacles found during the *in vitro* multiplication phase of the heliconias is the presence of endophytic bacteria, most frequently the *Pseudomonas solanacearum* (Atehortua 1997; Dias and Rodrigues 2001). After overcoming this initial obstacle, the culture medium must be adjusted to optimize the *in vitro* multiplication in order to obtain a large number of plantlets per explant. Yet, another phase of the process that deserves attention is the acclimatization, which consists in transferring the *in vitro* cultivated plantlets to an *ex vitro* cultivation condition where, sometimes, there is no total control of environmental conditions.

For some Heliconiaceae and related species, such as those from the Zingiberaceae and Musaceae families, there are some well-defined micropropagation protocols (Rodrigues et al., 2006; Colombo et al., 2010; Ulisses et al., 2010; Iqbal et al., 2013). However, there are no works on the propagation of *Heliconia bihai x Heliconia caribaea* 'Jacquinii' (Figure 1). Therefore, the aim of this work was to study a protocol for the in vitro propagation of the *H. bihai x H. caribaea* 'Jacquinii' hybrid and assess the survival of the plantlets during the acclimatization stage.

MATERIAL AND METHODS

The experiment was conducted at the Plant Tissue Culture Laboratory of Universidade Estadual de Londrina (UEL), Londrina, Paraná, between the months of November, 2014 and May, 2015. Heliconia (*Heliconia bihai x Heliconia caribaea* Jacquinii') plantlets eatablished *in vitro* through a shoot apex cultivation in a MS (Murashige and Skoog 1962) culture medium with half of the macronutrients concentration (½ MS) added by 0.5 mg L^{-1} of naphthalene acetic acid (NAA) and 2.0 mg L^{-1} of 6-benzylaminopurine (6-BA) were submitted to two sub-cultivations in the same culture medium without the NAA and 6-BA growth regulators.

Figure 1. Inflorescence of *Heliconoa bihai* x *Heliconia caribaea* 'Jacquinii' cultivation.

From these plantlets, buds (explants) up to 5 mm long were selected and transferred to a ½ MS culture mean added with 0.5 mg L^{-1} of NAA, combined with concentrations of 0.0; 0.5; 1.0; 1.5; 2.0 and 2.5 mg L^{-1} of 6-BA, to obtain six treatments with 10 replications with one explant per replication, distributed at completely randomized design.

Flasks with explants were kept in a growth chamber at 25 ± 2 ºC and photoperiod of 16 light hours. After 100 growing days under these conditions, multiplication rate based on the number of shoots per explant; main plantlet length (cm) and cluster fresh weight (g) were assessed.

Plantlets obtained from each *in vitro* treatment were marked and acclimatized. For this phase, coconut fiber substrate was used as substrate (Amafibra® - standard 47) and 180 mL translucent plastic pots as recipients. Plants were kept in a greenhouse covered with transparent polyethylene film and black plastic screen (Sombrite®), with 50% of light retention. Irrigation was realized by micro-spinklers, once day, until the substrate got saturated.

After 90 days of acclimatization, plantlets were evaluated in regards to survival rate, pseudo stalk diameter (mm) and aerial part height (cm), measured from the pseudo stalk to the insertion of the last expanded leaf.

Data collected during the *in vitro* multiplication and acclimatization phases were submitted to an ANOVA and regression analysis, to test the polynomial regression models of first, second and third degree.

RESULTS AND DISCUSSION

Based on the regression analysis, the cubic adjustment for the number of shoots per explant variable is shown in Figure 2. Thus, in the absence of the cytokinins (6-BA), the average number of shoots per explant was four and as the concentrations of 6-BA increased, the number of shoots remained between two to three per explant (Figure 3A).

Under these conditions, it can be inferred that the cytokinin inhibited the shoots multiplication and its addition is unnecessary to the culture medium for the multiplication of *Heliconia bihai x Heliconia caribaea* 'Jacquinii'. However, the presence of the auxin, alone, brought an increase in the number of shoots. Similar results were described by Al-Amin et al. (2010) for the *in vitro* multiplication of the 'BARI Banana-I' banana. The authors observed 2.5 shoots per explant for the 0.5 mg L^{-1} of NAA concentrations and 1.75 shoots per explant for the 0.5 mg L^{-1} of NAA + 2.5 mg L-1 de 6-BA combination. Colombo et al. (2010), assessing on *in vitro* multiplication of Etlingera elatior (Zingiberaceae), also obtained from three to four shoots per explant during the multiplication phase, regardless of the concentrations and growth regulators.

Although these results are not usually expected in micropropagation, they are interesting since they reveal that, for some species, the multiplication may occur in the absence of growth regulators. Yet, it is possible to cut costs during the multiplication process and reduce somaclonal and epigenetic variation risks.

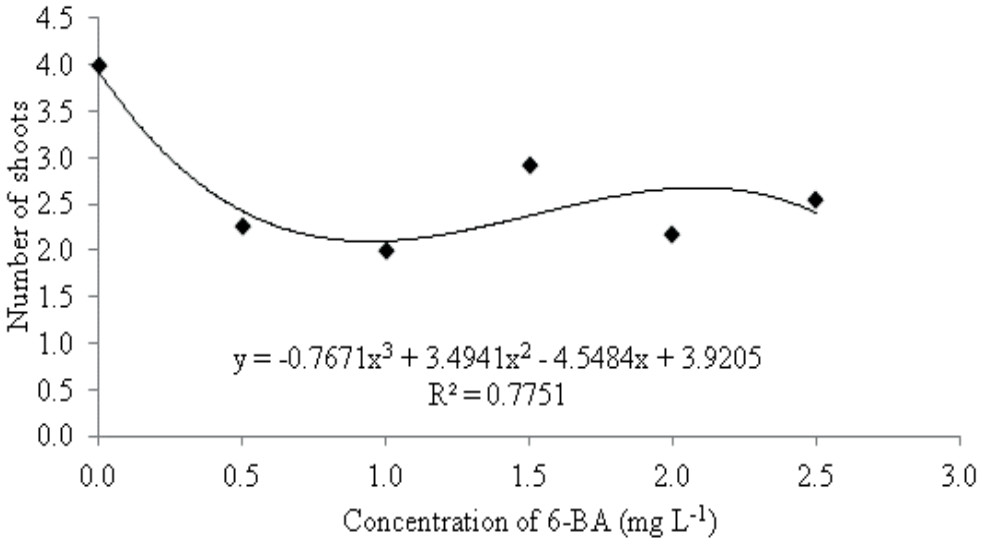

Figure 2. Number of shoots in micropropagated plantlets of *Heliconia bihai* x *Heliconia caribaea* 'Jacquinii' submitted to concentrations of 0; 0.5; 1.0; 1.5; 2.0 and 2.5 mg L^{-1} of 6-BA.

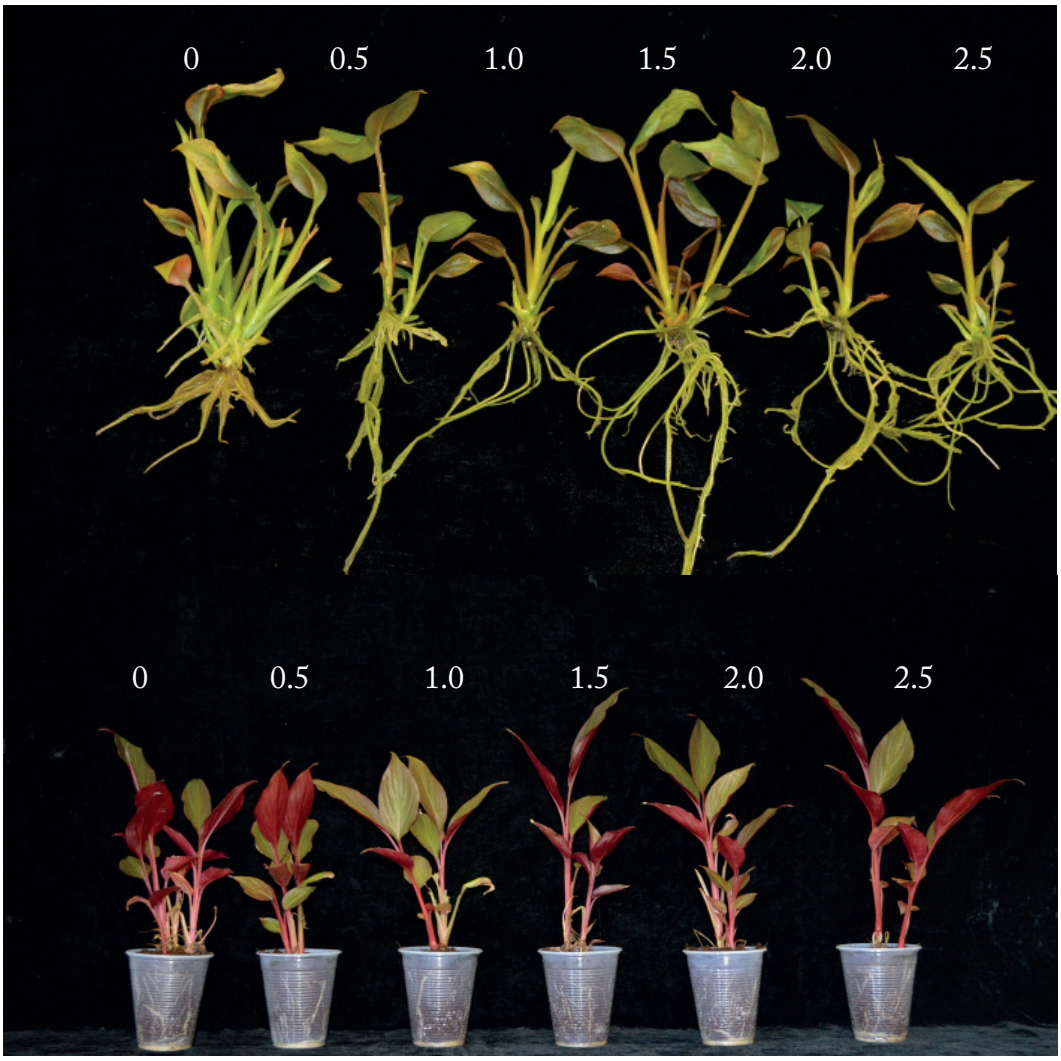

Figure 3. Micropropagated plantlets of *Heliconia bihai* x *Heliconia caribaea* 'Jacquinii' at 100 days of in vitro cultivation (A) and seedlings of the same species, after 90 days of acclimatization (B), submitted to concentrations of 0; 0.5; 1.0; 1.5; 2.0 and 2.5 mg L^{-1} of 6-BA.

On the other hand, Marulanda-Ángel et al. (2011) were recorded multiplication rate for Heliconia bihai of three shoots per explant, in a MS medium with 0.5 mg L^{-1} of IAA and 2.0 mg L^{-1} of 6-BA. However, the same authors observed that, in the absence of auxin (IAA) and regardless of the 6-BA concentrations used, the multiplication

rate was of one shoot per explant. Similar results are described by Sosa-Rodríguez et al. (2009) for the *in vitro* multiplication of *Heliconia standleyi* in a MS medium with 0.65 mg L^{-1} of IAA and 2.0 mg L^{-1} of 6-BA, with the production of up to 4.6 shoots per explant.

In regards to the aerial part height and clusters fresh weight , no significant differences were observed due to the 6-BA concentrations studied; the mean for these variables being 7.59 cm and 3.60 g, respectively. It is probable that this lack of significant difference in regards to the aerial part height was due to the fact that the concentration of auxin was the same for all treatments.

Working with different concentrations of 6-BA for the *in vitro* propagation of *Etlingera elatior,* Rescarolli and Zaffari (2009) observed that their application did not induce the development of lateral buds, which can be justified by the plantlets high apical dominance and/or inefficiency of the of the cytokinin levels present in culture medium in altering the auxins/cytokinin relation in the tissues.

During the plantlets acclimatization phase, the survival rate was 100% and the developed seedlings presented similar growth (Figure 3B). Quisen et al. (2013) and Rodrigues et al. (2005) also showed high survival rates during the acclimatization phase of the *Heliconia chartacea* 'Sexy Pink' and *Heliconia bihai* plantlets, respectively.

The general mean for the pseudo stalk diameter variable was 5.49 mm, with no significant differences between the 6-BA concentrations. However, a cubic polynomial regression model was adjusted for the aerial part height with a tendency to increase seedlings height due to the increase in 6-BA concentrations (Figure 4). Ulisses et al. (2010) also obtained greater plants length with the 2.5 mg L^{-1} of BAP concentration, of during the acclimatization phase of *Heliconia bihai* plantlets, since, according to Taiz and Zeiger (2013) the cytokinins are capable of influence the mobilization of nutrients to the leaves from other parts of the plant, establishing a new source drain relation in order to stimulate aerial part growth in plants.

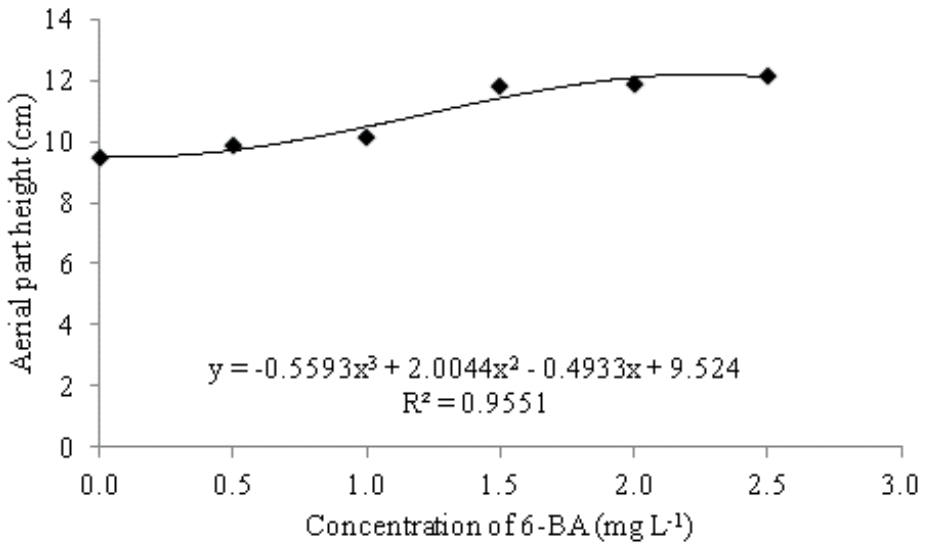

Figure 4. Aerial part height of plants micropropagated *Heliconia bihai* x *Heliconia caribaea* 'Jacquinii' plants submitted to concentrations of 0; 0.5; 1.0; 1.5; 2.0 and 2.5 mg L^{-1} of 6-BA after 90 days of acclimatization.

CONCLUSIONS

The MS culture medium with 0.5 mg L^{-1} of NAA can be used for the in vitro propagation of the *Heliconia bihai x Heliconia caribaea* 'Jacquinii' hybrid.

Heliconia bihai x Heliconia caribaea 'Jacquinii' plants propagated in vitro showed no adaptation and mortality problems during the acclimatization phase.

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