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Slow-grown *in vitro* conservation of *Heliconia champneiana* cv. Splash under different light spectra

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ABSTRACT: *In vitro* techniques, with the purpose of conserving the genetic resources of plants, are fundamental to the feasibility of establishing germplasm banks and enabling the commercial production of micropropagated plants. The aim of this study was to evaluate the use of different spectra of light in *in vitro* germplasm conservation by slow grown storage of *Heliconia Champneiana* cv. Splash. Explants of heliconia *in vitro* were submitted to the following light treatments: CW (control white), B100 (100 % blue), R100 (100 % red) and R70B30 (70 % red/30 % blue), all with PFD = 25 µmol m^2 s^{-1}, maintained *in vitro* for two time periods, namely P1, 6 weeks and P2, twelve weeks, and were statistically evaluated after each interval with respect to: height, fresh weight, number and length of roots, number of leaves, and pseudostem diameter, in addition to the rate of acclimatization in percentage terms. Treatment B100 presented the lowest level of development in the two periods, and 100 % survival in acclimatization.
Keywords: tissue culture, micropropagation, ornamental plants

Introduction

Flower growing in Brazil has been increasing constantly over the last few years, according to the Brazilian Institute of Floriculture (IBRAFLOR, 2015). One of the segments of floriculture that especially attracts attention is tropical flowers, with Heliconias presenting outstanding characteristics, such as rusticity, beauty, and various colors in their bracts. The production of *in vitro* plants is fundamental to commercial cultivation and the cuttings used in tissue culture (Rodrigues et al., 2013).

In vitro maintenance of genetic material, whether for conservation or plant production, requires frequent changes in the cultivation media that may vary according to the objective of the laboratory. This operational routine demands an adequate structure, consumption of cultivation medium and above all, qualified labor, which accounts for the major part of the cost of the operation (Tavazza et al., 2015). Other operational difficulties particular to *in vitro* propagation are as follows: *in vitro* plants are ready to be removed from the laboratory, but the greenhouse is not available to receive the plants, either because it is full of unsold plants, or occupied by plants that had previously been sold and the customer is not ready to remove the plants from the greenhouse. These operational difficulties may generate financial losses for both the laboratory and customer (Watt et al., 2000).

"Slow grown storage" refers to the techniques capable of reducing the *in vitro* development of a plant to a minimum; this involves reducing plant metabolism by increasing the *in vitro* period of the explant, without changing the genetic uniformity (or pattern) and the quality of the micropropagated plant (Kamińska et al., 2016). In effective reduction in the metabolism of plants *in vitro*, the cultivation conditions such as temperature, light period, and light intensity may be changed, and so may the components of the cultivation medium (organic and inorganic nutrients, osmotic and growth regulators) during the period of incubation (Thakur et al., 2015; Carvalho et al., 2014; Kaur et al., 2012).

Light sources with different wavelengths from light emitting diodes (LED) have been widely used in *in vitro* propagation in different crops such as sugarcane, anthurium and blueberry, with the purpose of increasing production and improving the quality of the plants (Maluta et al., 2013; Martinez-Estrada et al., 2016; Hung et al., 2016). Such light sources may have an effect on *in vitro* development with significant changes in the arrangements of the thylakoids in the chloroplasts, which reduces the size of the plants, in emission and length of the roots, number of stomata in the leaves and chlorophyl content (Maluta et al., 2013; Vieira et al., 2015; Macedo et al., 2011).

Thus, the aim of this study was to evaluate the use of different spectra of light at the *in vitro* stage of root growth and elongation of *Heliconia Champneiana* cv. Splash in slow grown storage.

Materials and Methods

Plants of *Heliconia Champneiana* cv. Splash were at the stage of the sixth subculture *in vitro*, in glass flasks (10 cm long by 10 cm in diameter) containing 40 mL of semi-solid MS (Murashige and Skoog, 1962) culture media, with the addition of MS vitamins; 30 g L^{-1} sucrose; 1.8 mg L^{-1} gellam gum and 3 mg L^{-1} BAP (benzilaminopurine), incubated at a temperature of 25 °C ± 2, subject to light period conditions of 16/8 h (day/night) at 50 µmol m^2 s^{-1} by using fluorescent lamps. Plants of the same size were selected and separated; the aerial parts were excised, so as to form structures 1.5 cm long by 0.5 cm in diameter, for a total of 120 explants.

The explants selected were transferred to PYREX test tubes (15 cm long by 2.5 cm in diameter) with Kimble polypropylene lids, 25 mm in diameter, each containing 7

mL of semi-solid culture media ½ MS, with the addition of MS vitamins; 30 g L^{-1} sucrose; 1.8 mg L^{-1} gellam gum; 0.1 mg L^{-1} IBA (idolbutiric acid) for the induction of root growth and elongation; pH was adjusted to 5.8 before sterilization and incubation was at a temperature of 25 °C ± 2; under light conditions of 16/8 h (day/night) consisting of light treatments with a LED lighting system in the colors red LPEL-06R3-B (620-630 nm) and blue LPEL-06B3-B (455-475 nm). Three combinations of LED treatments were evaluated: 100 % blue (B100), 100 % red (R100), 70 % red + 30 % blue (R70B30) and control white (CW). Spectra 380-780 nm, fluorescent lamps, with the photon flux density (PFD) adjusted to 25 µmols m^2 s^{-1} were used in all the treatments, and measurements were taken using a Light Meter (LI-250A). Each treatment group consisted of thirty explants (totaling 120); the growth and development of the explants were evaluated over two time periods, without any subcultures developing. For each treatment, the test tubes were centralized and arranged equidistant to uniformly receive the spectrum evaluated. In the first time period (P1), after 6 weeks, fifteen plants were removed and washed in running water to remove the culture media, and the plant length was evaluated, based on the pseudostem up to the tip of the largest leaf (mm); diameter of the pseudostem at 1.0 cm from the base (mm); number of leaves; fresh weight of the plant (g); number of roots and length of the largest root (mm). In the second time period (P2), the remaining explants were maintained in the proposed treatments until a period of 12 weeks had passed, when they were submitted to the same evaluation procedures as those previously mentioned. The experimental design used was entirely randomized in the split-plot arrangement, with the plots being represented by the treatments, and the sub-plots by time. The data obtained were submitted to an analysis of variance in the split-plot arrangement, and the mean values were compared by Tukey test at a 5 % level of significance (Statistical Analysis System) between the treatments proposed and between each time period evaluated. After the evaluations of each time period, the plants were acclimatized in 24-cell trays, using the commercial substrate "BASE Horticultura", and were maintained in the greenhouse with 80 % shade (Aluminet) and relative humidity of 90 % for seven days (Rodrigues et al., 2005), to evaluate the plant survival, expressed in percentage terms.

Results and Discussion

The characteristics measured in the trial, according to the Tukey test, were influenced by the use of the different light spectra. In the comparison, the characteristics evaluated showed a significant difference between treatments, except for the pseudostem diameter (Table 1). Specific wavelengths presented different effects when compared with CW, and these were repeated in the second time period evaluated. Blue light (B100) induced lower fresh mass, height of explants, number of roots/plant and root length, but induced a larger number of leaves/

Table 1– Fresh weight of the plant (g); plant length (mm); number of roots and length of the largest root (mm), number of leaves and pseudostem diameter (mm), in different evaluation times (P1) and (P2) from plants of *Heliconia champneiana* cv. Splash. (n = 15).

Treatments	Weight (g) P1	Weight (g) P2	Length (mm) P1	Length (mm) P2	nº roots P1	nº roots P2	Root Length (mm) P1	Root Length (mm) P2	nº of leaves P1	nº of leaves P2	Ø pseudostem P1	Ø pseudostem P2
CW	1.36 aB	1.84 aA	105.40 abB	121.00 bcA	3.46 abB	4.80 abA	86.0 aB	145.00 aA	3.06 aB	4.60 aA	4.26 aA	4.60 aA
B100	0.77 bA	1.08 bA	94.20 bB	112.80 cA	2.26 bB	3.60 bA	54.40 bB	85.40 bA	2.93 aB	4.40 aA	4.00 aA	4.40 aA
R100	1.26 abB	1.80 aA	105.46 abB	133.20 aA	2.46 abB	4.20 abA	82.60 aB	145.00 aA	2.06 bB	3.40 bA	4.33 aA	4.80 aA
R70B30	1.50 aB	2.19 aA	108.93 aB	125.40 abA	3.6 aB	5.40 aA	94.06 aB	115.40 aA	2.53 abB	4.00 abA	4.33 aA	4.80 aA
CV	38.97		11.09		41.37		25.71		23.98		15.86	

The means followed by distinct letters are statistically different from each other. The Tukey test was applied at the level of 5 % probability; Column rating lowercase; Line rating with a capital letter comparing times within the same variable; CW = control white; CV = coefficient variation.

plant, together with CW. Red light (R100) induced a lower number of leaves/plant, while the combination of red/blue light (R70B30) induced higher fresh weight, height of explants and number of roots. The majority of characteristics measured in (P1) were repeated in (P2), except for root length which in P2 was exceeded by CW and R100 compared with the combination R70B30. In the comparison between the two time periods, no significant differences were found for the characteristic fresh mass in B100, which in P1 presented 0.77 g and 1.08 g in P2 (Table 1). In the remaining characteristics evaluated, there were significant differences between the time periods evaluated. The effects and applications of different spectra of light from LEDS on the *in vitro* cultivation of plants have been extensively reported by different authors in diverse cultures (Gupta and Jatothu, 2013). The results of the present study indicated that the use of blue light in *in vitro* conservation in minimum growth was promising and cast a new perspective on this area. Inhibition of plant development related to the use of blue light during the *in vitro* process, has been reported in crops such as potato (Luz et al., 2015), chrysanthemum (Kurilčik et al., 2008), blueberry (Hung et al., 2016) and grape (Poudel et al., 2008). In general, the blue component inhibited elongation of the plantlets and root formation *in vitro*. In an *in vitro* study on chrysanthemums, Kim et al. (2004) related that active phytochromes are related to the stimulus of *in vitro* development and increase in mass. The activation of phytochromes occured at the red and far red wavelengths existent in the CW, R100 and R70B30 treatments evaluated. On the other hand, the blue light, in which the red and far red wavelengths do not occur, did not activate the phytochromes sufficiently, as they did in B100 (Nhut et al., 2003).

The reduced gain in fresh mass between the time periods, associated with the induction of a lower number of roots, root length and lower height of the plants, found in B100, were visually evident after 12 weeks (Figure 1). In the CW, R100 and R70B30 treatments, the authors noted that the high density of the roots formed at the base of the plants, was shown to be greater than that in B100. In addition to the lower height of the plant induced in B100, the absence of yellowing of the leaves was also visually evident, a characteristic that was visible in the other treatments evaluated. The occurrence of yellowing leaves is frequently associated with stress caused by the *in vitro* environment resulting from the scarcity of nutrients in the culture medium and/or difficulties in gaseous exchanges, which accelerates the processes of senescence. These characteristics are commonly observed in plants that exceed the maximum time spent *in vitro* (Pimenta et al., 2013; Kevers et al., 2004).

The final quality of plant material kept in slow growth storage *in vitro* conservation must be the best possible, given the conditions to which explants are submitted to *in vitro*. On many occasions, the process of *in vitro* propagation may cause morphological, physiological and anatomical changes that make the *ex vitro* acclimatization stage difficult (Chandra et al., 2010). In the acclimatization

Figure 1 – *Heliconia champneiana* cv. Splash plants, after 12 weeks *in vitro* under treatments with different light quality. Barr: 10 mm; CW = control white.

of *Heliconia bihai* cv. Lobster Claw, explants propagated *in vitro*, Rodrigues et al. (2005) reported greater efficiency in the process of acclimatization in a greenhouse with shade as beginning at 50 %, attaining a peak in efficiency of 80 %. The results obtained in the acclimatization trial from all the plants of all the treatments evaluated were 100 % of the plants acclimatized in the conditions proposed in P1. The same did not occur in P2, in which survival percentages of 87; 100; 87 and 73 %, were obtained for the CW, B100, R100 and R70B30 treatments, respectively. No effect of differentiated light treatments interfered in the acclimatization in P1, which could result in the loss of plants and consequent loss to the laboratory or to an *in vitro* conservation program. In P2, an advanced condition of senescence in the roots was observed in CW, R100 and in R70B30 this resulted in the loss of material during the acclimatization process. In B100, in spite of the reduced size, the plants were firm and without symptoms of senescence in the leaves and few roots emitted, which probably contributed to the better result in acclimatization after 12 weeks *in vitro*. In the surviving plants, no morphological changes such as leaf variegations or malformations of the leaf blade - common characteristics of possible somaclonal variants - were observed in any of the treatments after eight weeks of acclimatization. The authors' concern regarding this evaluation was justified, because a long period spent in *in vitro* conditions may favor the development of somaclonal variation (Bairu et al., 2011; Xin et al., 2015).

The results of the present study indicated that blue light used in cultivation *in vitro* was efficient in maintaining the explants at reduced growth, and did not alter the quality of the plants, even after 12 weeks *in vitro*. In the case of the culture under study, the subcultures proceeded regularly every 21 days. From the data that were reported, the culture with reduced vegetative development was maintained for 84 days. For a commercial laboratory, this result represents savings in labor and culture medium due to the increase in subculture periods, and the increased ease of cleaning explants resulting from reduced root formation.

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