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ORIGINAL ARTICLE

Acclimatization of micropropagated plantlets of Heliconia Sexy Pink

Aclimatização de mudas micropropagadas de Helicônia Sexy Pink

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ABSTRACT: Tropical ornamental plants are distinguished by their beauty and color, and *Heliconia chartacea* (Lane x Barreiros) cv. Sexy Pink is a species of great value in the flower market. Micropropagation can be used for the mass production of plantlets in this species, allowing for the multiplication of high-quality plant materials using less time and space. However, the various stages of this process, such as acclimatization, suffer from a lack of standardization, which limits the advancement of micropropagation culture. In light of this issue, the present study examined the influence of different substrates on the acclimatization of plantlets of Heliconia Sexy Pink. Plants obtained by micropropagation were planted in polypropylene tubes containing different substrates (Bioplant®; coconut fiber; coconut fiber + Bioplant®; coconut fiber + earthworm humus; coconut fiber + Bioplant® + earthworm humus; sand + vermiculite). The survival rate ranged from 83 to 92%, with no significant differences observed among the treatments. The combination of coconut fiber + Bioplant® allowed for the greater development of the root system than did the other treatments, and roots were significantly less developed in the Bioplant® and sand + vermiculite substrates. Dry matter accumulation in the shoots of plants was greatest in the substrate composed of coconut fiber, followed by the substrates containing Bioplant® or earthworm humus, yielding vigorous plantlets. The substrate prepared with coconut fiber was most favorable for the increase of shoot and root dry mass over the acclimatization of *Heliconia chartacea* cv. Sexy Pink.

RESUMO: *As plantas ornamentais tropicais diferenciam-se por sua beleza e coloração, sendo a Heliconia chartacea (Lane × Barreiros) cv. Sexy Pink uma espécie de grande valor neste segmento de mercado. Sua utilização na micropropagação para a produção massal de mudas permite a multiplicação de materiais de alta qualidade fitossanitária, em tempo e espaço reduzidos. Entretanto, a falta de definições de diferentes fases desta técnica, como a aclimatização, compromete o avanço da micropropagação nesta cultura. Neste sentido, o presente estudo teve como objetivo estudar a influência de diferentes substratos na aclimatização de plantas micropropagadas de Helicônia Sexy Pink. Para tal, as plantas foram transplantadas para tubetes contendo seis diferentes substratos: Bioplant®; fibra de coco; fibra de coco + Bioplant®; fibra de coco + húmus de minhoca; fibra de coco + Bioplant® + húmus de minhoca; areia + vermiculita. A taxa de sobrevivência variou de 83 a 92%, não havendo diferenças estatísticas entre os tratamentos avaliados. A combinação fibra de coco e Bioplant® proporcionou melhor desenvolvimento do sistema radicular do que os demais, sendo os substratos Bioplant® e areia + vermiculita menos eficientes. Houve um maior acúmulo de matéria seca na parte aérea das plantas aclimatizadas no substrato composto por fibra de coco, sendo seguido pelas suas variações com Bioplant® e com húmus de minhoca, propiciando mudas mais vigorosas. O substrato à base de fibra de coco seco foi o que proporcionou o maior incremento de massa seca da parte aérea e raiz na aclimatização de Heliconia chartacea cv. Sexy Pink.*

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1 Introduction

The production of flowers and ornamental plants is an economically promising activity. In Brazil, the flower market generates close to $4.4 billion Brazilian real (BRL) annually and has grown by 10 to 15% per year over the past ten years, with a planted area of approximately 11,800 hectares (IBRAFLOR, 2013).

In this economic segment, flowers from temperate climates have always dominated the market, but the commercialization of ornamental and landscape species has attracted increased interest in recent years. Tropical flowers are taking up increasingly more space in the flower market, and the demand for these products is growing, providing a viable alternative investment for agriculture in Brazil (IBRAFLOR, 2013). Among the many tropical species native to Brazil, heliconias offer particularly favorable prospects for markets such as cut flowers. *Heliconia chartacea* cv. Sexy Pink and the other species of this genus stand out due to their exotic-looking inflorescences, high durability after being cut and high acceptance in the domestic and international markets.

As with the other species of the genus, the success of the Sexy Pink heliconia is directly related to the quality of the propagation material used, and one of the bottlenecks in this industry is the demand by producers for stock plants of phytosanitary quality. The propagation of this species, which is usually performed using rhizomes, leads to the transmission of important diseases between successive plantings of contaminated rhizomes, which hinders the production potential of the heliconia (SANTOS et al., 2006). Seed propagation has a number of disadvantages compared to vegetative propagation, as the seeds have a dormancy period and the plantlets propagated using this method have a very long flowering period (TORRES et al., 2005).

The high demand by producers throughout the year for uniform plants with high genetic and phytosanitary quality has reinforced the commercial importance of *in vitro* propagation techniques for flowers and ornamental plants (CARVALHO; RODRIGUES; SANTOS, 2012). Within this context, the production of plantlets through micropropagation has become a viable alternative on a commercial scale. The cultivation of clones from selected plants on aseptic media produces high-quality genetic material that is free of fungi, bacteria and, most importantly, viruses and nematodes.

A fundamental step in plant production by tissue culture is acclimatization, as *in vitro* culture conditions modify the biochemical, anatomical and morphological characteristics of plants, thereby altering their normal physiological processes (LUCAS et al., 2002). In this step, plants are taken from the *in vitro* media and transferred to the greenhouse, representing a transfer from a low-transpiration condition to an environment in which the plants must control gas exchanges. This transfer can result in water stress if the new environment does not offer the ideal conditions for this transition phase.

According to Grattapaglia and Machado (1990), a number of factors should be considered in the transition from a heterotrophic state to an autotrophic one. Among the main factors is the change from aseptic conditions to conditions where the plantlet is subject to attack by saprophytic and eventually pathogenic microorganisms, as is the change from a substrate rich in mineral salts to one in which the plantlet must develop the ability to absorb nutrients.

The selection and adequate management of these substrates are therefore of the utmost importance for obtaining a good survival rate and high-quality plants. The substrates should have good porosity to facilitate root system aeration, good water retention capacity and a lack of excessive compaction, which compromise drainage and can lead to tissue death (GONÇALVES, 1995).

Given the relevance of this topic to tropical flower production and the lack of studies regarding the acclimatization of micropropagated plants of *Heliconia* species, the present study aimed to determine the influence of different substrates on the acclimatization of *Heliconia chartacea* (Lane x Barreiros) cv. Sexy Pink plantlets.

2 Materials and Methods

The experiment was conducted in a greenhouse belonging to Embrapa Western Amazon, municipality of Manaus, state of Amazonas, Brazil. The Sexy Pink heliconia plantlets were obtained by in vitro propagation in Murashige and Skoog (1962) medium, supplemented with cytokinin benzylaminopurine, in the Plant Tissue Culture Laboratory, Manaus, Amazonas. At the time of planting, the plantlets were completely rooted, with heights varying from 2.5 to 5.0 cm.

Before transplantation, the plants were preacclimated in uncovered flasks that were kept open for 16 h in a growth room at a temperature of 26 ± 1 °C, a 16 h photoperiod and a photon flow density of 38 μ mol $m^{-2}s^{-1}$. After the plantlets were taken from the flasks, their roots were washed to remove any medium residues and pruned to an approximate length of 3.0 cm to standardize the material.

The plants were then taken to the greenhouse and transferred to 250 cm^3 tubes containing the experimental substrates on a bench placed over rigid polypropylene supports 80 cm above the soil surface of a semicircular mini greenhouse with a height of 0.8 m and covered in a layer of clear plastic. A thermometer was placed inside the greenhouse to control the daily maximum and minimum temperatures (°C) and relative humidity, and the plants were watered daily by hand. The experimental design was a completely randomized block design with six treatments of four blocks each and six replicates (plants) per block, for a total of 144 experimental units. The treatments consisted of the following substrates: (1) Bioplant®; (2) coconut fiber; (3) Bioplant® + coconut fiber (1:1); (4) earthworm humus + coconut fiber (1:3); (5) Bioplant® + earthworm humus + coconut fiber (1:1:3); (6) sand + vermiculite (1:1).

Starting 15 days after transplantation, a fungicide composed of 0.05% Cercobin®, 0.05% Agrimicina® and 0.03% adhesive spreader was applied weekly until the end of the experiment. After 45 days of cultivation, the following variables were measured for each plants: survival; root and shoot biomass determined after drying in an oven at 65 °C until reaching constant weight (MALAVOLTA; VITTI; OLIVEIRA, 1997); and N, P, K, Ca, Mg, S, B, Zn, Fe, Mn and Cu levels.

The data were subjected to analysis of variance and Tukey's test at 5% significance to compare the means of the treatments.

3 Results and Discussion

No pests or diseases that could have hindered the acclimatization process were observed, a finding that may have resulted from the phytosanitary control performed and the use of the cultivation tunnel.

Plantlets survival did not significantly differ among the substrates ($p < 0.05$) (Figure 1). The survival rates were high even in the sand + vermiculite and coconut fiber substrates, which were considered inert by Carrijo, Liz and Makishima (2002). According to these authors, vermiculite and fiber should be used in combination with fertilizers or other substrates. Also in contrast to the results obtained in the present study, Rodrigues et al. (2005) found a low survival rate (32.5%) in plantlets of *Heliconia bihai* acclimated in vermiculite.

Maciel, Silva and Pasqual (2000) highlighted the importance of ambient temperature and humidity in the acclimatization period; these variables could have favored plantlets survival in the present study, as the mean ambient temperature and humidity were 30.3 °C and 74.5%, respectively.

Of the parameters analyzed, the only one that varied in response to the substrate type was the length of the longest root (LLR) (Table 1).

The values obtained for the treatments were similar for all of the parameters analyzed, which may be due to the short acclimatization period (45 days) evaluated in this study.

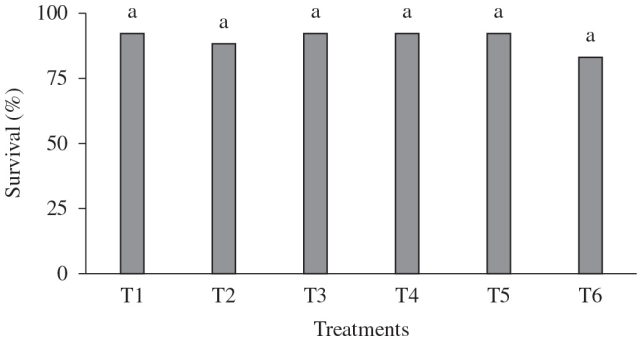

Figure 1. Percent survival of *Heliconia chartacea* cv. Sexy Pink, 45 days after transplantation to the greenhouse, on the following substrates: T1. Bioplant®; T2. Coconut fiber; T3. Bioplant® + Coconut fiber; T4. Earthworm humus + Coconut fiber; T5. Bioplant® + Coconut fiber + Earthworm humus and T6. Sand + Vermiculite.

According to Torres et al. (2001), under *in vitro* conditions, plants exhibit nonfunctional stomata and respond slowly to water stress, the epicuticular wax is thin or absent, and the vascular connection between the stem and the adventitious roots is inadequate for meeting the evapotranspiration demand. Thus, a longer acclimatization period could influence tissue differentiation and promote different growth and development patterns in the different substrates tested.

The lower means obtained for the development of the shortest root (LLR) in the treatments with Bioplant® (treatment 1) and sand + vermiculite (treatment 6) (Table 1) could be related to the density of these substrates, which may have negatively affected root system development during the evaluation period. Higher means were observed for those substrates containing coconut fiber, which have greater aeration and porosity and lower density; the fiber can also absorb excess nutrients, which would balance nutrient availability for the plants.

According to Lemle (2005), the good physical characteristics of coconut fiber include moisture retention, porosity and density, and when the fiber is used in mixtures such as commercial substrates, these characteristics are improved and the material becomes lighter. Similar results were found by Lacerda, Passos and Rodrigues (2006), who recommended substrates based on coir dust or mixed with Argisol for the acclimatization of *Mimosa caesalpiniifolia* plantlets. Bezerra et al. (2001) and Silveira, Rodrigues and Gomez (2002) also found that coconut fiber was an excellent substrate for growing tomatoes and for the acclimatization of chrysanthemums, respectively. Root proliferation depends on water and nutrient availability in the surrounding microenvironment. If the rhizosphere is poor in nutrients or very dry, root growth is slow and increases as conditions improve (TAIZ; ZEIGER, 2006).

Significant differences were observed between substrates for fresh root mass (FRM) and dry shoot mass (DSM), but the other variables did not significantly differ among the substrates tested (Table 2).

Dry shoot matter accumulation was greatest for the acclimatized plants in the coconut fiber–based substrate (treatment 2), followed by those grown in the variations of this material (treatments 3, 4 and 5); these results are in agreement with the positive effects observed for these substrates on plants vigor. In an evaluation of the effects of several substrates on

Table 1. Mean values of plant height (PH), pseudostem diameter (PD), number of leaves (NL), number of roots (NR) per plant, length of the longest root (LLR) and length of the secondary root (LSR) of acclimatized *Heliconia chartacea* cv. Sexy Pink plants 45 days after transplantation. Manaus, 2013.

Treatment	PH		PD		NL		NR		LLR		LSR	
	(cm)		(mm)		(Plant)		(Plant)		(mm)		(mm)	
1	4.6	a	0.6	a	5.0	a	3.9	a	4.3	b	0.2	a
2	4.2	a	0.5	a	4.4	a	4.5	a	7.7	ab	0.4	a
3	5.5	a	0.6	a	6.2	a	5.2	a	8.7	a	1.0	a
4	5.0	a	0.7	a	5.3	a	4.1	a	6.2	ab	0.6	a
5	5.6	a	0.7	a	6.3	a	3.4	a	7.7	ab	0.5	a
6	5.6	a	0.6	a	5.3	a	2.8	a	3.9	b	0.6	a

1: Bioplant®; 2: Coconut fiber; 3: Bioplant® + Coconut fiber; 4: Earthworm humus + Coconut fiber; 5: Bioplant® + Coconut fiber + Earthworm humus and 6: Sand + Vermiculite. Measurements followed by the same letter in each column did not differ according to Tukey's test at a 5% probability level.

Table 2. Mean values of the fresh root mass (FRM), fresh shoot mass (FSM), dry root mass (DRM) and dry shoot mass (DSM) of acclimatized *Heliconia chartacea* cv. Sexy Pink plants 45 days after transplantation.

Treatment	FRM		FSM		DRM		DSM	
	(g)		(g)		(g)		(g)	
1	1.4	b	15.1	a	0.08	a	0.9	b
2	2.8	a	18.1	a	0.10	a	1.6	a
3	3.2	a	24.8	a	0.11	a	1.4	ab
4	2.2	ab	23.8	a	0.07	a	1.4	ab
5	2.7	a	24.2	a	0.11	a	1.4	ab
6	2.4	ab	19.0	a	0.09	a	1.2	ab

1: Bioplant®; 2: Coconut fiber; 3: Bioplant® + Coconut fiber; 4: Earthworm humus + Coconut fiber; 5: Bioplant® + Coconut fiber + Earthworm humus and 6: Sand + Vermiculite. Means followed by the same letter in a column did not differ according to the Duncan test at a 5% probability level.

Table 3. Analysis of the nutrients in the shoot tissues for acclimatized *Heliconia chartacea* (Lane x Barreiros) cv. Sexy Pink plants.

Treatment	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	g kg^{-1}						mg kg^{-1}				
1	34.6	5.29	61	8.29	5.47	5.50	40	8.2	958	143.9	88
2	33.6	8.37	42	5.00	2.75	3.43	49	12.4	570	346.8	144
3	27.6	7.40	73	5.27	3.29	3.47	46	9.4	404	273.7	101
4	34.7	8.44	63	5.53	2.60	2.76	41	8.9	318	231.9	93
5	28.8	7.56	65	5.14	2.88	2.84	39	9.1	639	216.4	87
6	36.5	3.17	38	3.71	7.17	2.44	34	10.6	1724	211.7	82

1: Bioplant®; 2: Coconut fiber; 3: Bioplant® + Coconut fiber; 4: Earthworm humus + Coconut fiber; 5: Bioplant® + Coconut fiber + Earthworm humus and 6: Sand + Vermiculite.

the acclimatization of micropropagated anthurium plantlets, Silva et al. (2007a) found that after 90 days of cultivation, plants grown in dry coir dust were taller and had higher mean dry shoot mass. In a study of *Dyckia marítima* (Bromeliaceae) grown from tissue culture, Silva et al. (2007b) observed that the addition of coir dust to a hydroponic solution favored survival and reduced acclimatization time to a third of the period required for plants grown in substrates made of earthworm humus and coconut fiber + earthworm humus. In a study on the acclimatization of African violets, Cordão Terceiro Neto et al. (2004) found that the best results were obtained for plantlets acclimatized on commercial substrate and Bioplant®, followed by those acclimatized on dry coir dust and vermiculite, which is similar to the results found in the present study of heliconia acclimatization.

For N, only the levels in treatments 3 and 5 (Table 3) were outside the nutrient range recommended for *Heliconia* spp. by Atehortua et al. (1999), which are as follow: N = 31-38 g kg^{-1}; P = 20-40 g kg^{-1}; K = 35-45 g kg^{-1}; Ca = 12.6-17.5 g kg^{-1}; Mg = 2.5-8 g kg^{-1}; S = 2.5-8 g kg^{-1}; B = 10-75 mg kg^{-1}; Cu = 6-25 mg kg^{-1}; Fe = 76-300 mg kg^{-1}; Mn = 100-1000 mg kg^{-1}; Zn = 26-250 mg kg^{-1}.

According to the previously described values, the Ca levels in the present study were under the requirements of the plants, and this deficiency was characterized by the reduced growth of meristematic tissues. A more critical situation was observed for P, which in the sand + vermiculite treatment only showed a value of 15.8% of the minimum level (20 g kg^{-1}) (ATEHORTUA et al., 1999) required by the plants. This nutrient is related to rapid root formation and may have influenced the results, as no significant differences in the dry root mass were observed among the treatments analyzed.

Regarding the levels of micronutrients found in the plant tissues, B, Mn and Cu were all within the requirement ranges defined by Atehortua et al. (1999). Although the Fe levels were high, they did not reach the phytotoxic level of 1880 mg kg^{-1} defined by Kirkby and Römheld (2007). The microelements analyzed did not differ significantly among the treatments.

4 Conclusions

Under the conditions of the present study, the coconut fiber–based substrate was most favorable for the production of dry root and shoot mass in micropropagated plantlets of *Heliconia chartacea* (Lane x Barreiros) cv. Sexy Pink over 45 days of acclimatization.

To improve the acclimatization of this crop, complementary studies on the use of fertilizer and different volumes of coconut fiber–based substrate are recommended.

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