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MORPHOLOGICAL AND STOMATAL CHARACTERIZATION OF *Heliconia chartacea* VAR. SEXY PINK INDUCED POLYPLOIDY

CARACTERIZAÇÃO MORFOLÓGICA E ESTOMÁTICA DE *Heliconia chartacea* VAR. SEXY PINK INDUZIDAS A POLIPLOIDIA

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ABSTRACT: The growth of the tropical flower market has demanded a consistent search for new varieties, primarily those endowed with an exotic profile, but that are also beautiful and durable. The genus *Heliconia*, naturally found in the Amazon region, is among the most prominent of tropical flowers. Looking to augment the genetic variability available in *Heliconia chartacea* var. Sexy Pink, biotechnological research was conducted with the application of colchicine to induce polyploidy in plants from this species. With that in mind, this study was undertaken to evaluate the establishment of plants in the field drawn from *in vitro* polyploidy induction assay and to determine the morphological and physiological characteristics of 38 *H. chartacea* var. Sexy Pink clones. The characterization analyzes were performed through 49 morphological descriptors and a stomatal density evaluation using microscopy. The genotype 35 exhibited the greatest morphological variations, with alterations in the position and coloring of the inflorescence, in addition to having the edges of the entire limbus. Genotype 18 featured the lowest amounts for plant height and inflorescence size, showing promise for research geared towards use in reduced environments. Some genotypes did not have any flowering and are recommended exclusively for landscape composition such as foliage, since their exotic characteristics allow for this. The genotypes that were evaluated displayed stomata with tetracytic morphology and guard cells that had no significant changes. However, genotypes with greater equatorial diameter and stomatal density were obtained in relation to the mother-plant. Overall, the induction of polyploidy allowed for clones to be obtained with a high variability for the characteristics of the leaf, pseudostem and inflorescence, with various attributes that confer a more efficient post-harvest management to some genotypes, in addition to favorable aspects for commercialized purposes as a cut flower.

KEYWORDS: Floriculture. Heliconiaceae. Colchicine. Morphological descriptors. Stomatal density.

INTRODUCTION

The floriculture industry has one of the highest growth rates in national and international markets (JUNQUEIRA; PEETZ, 2014). The world floriculture production is growing at a rate of 10 per cent per year (HARISHA, 2017). Over the last few years, tropical flowers have been receiving particular attention due to the features that favor their scale production, such as beauty, exoticism, colors, shapes, resistance to transport and post-harvest durability (CARVALHO et al., 2012; PRIA, 2017). Yet, consumers seeking the latest innovations have forced plant producers and breeders to increasingly release new varieties to keep up with the demand in this market. In this regard, genetic breeding programs have been presented as a tool for diversifying and improving existing plant materials, particularly through using methods that increase the available genetic variability.

The artificial induction of polyploidy has been an important method for creating new varieties of plant species, and this area has been gaining ground in the floriculture industry with the increasingly efficient use of mutagenic agents. One of the most widely used chemical agents is colchicine. Many varieties boasting "sales-friendly" features have been developed through their use. For example, there are plants with characteristics that are resistant to environmental adversities, like in *Ziziphus mauritiana*, which is resistant to water stress (NTULI; ZOBOLO, 2008), as well as new flower phenotypes and ornamental plants, such as *Oncidium flexuosum* (VICHIATO et al., 2007), *Dendrobium nobile* (UNEMOTO et al., 2009) and *Heliconia bihail* (CAVALCANTE FILHO, 2011).

An increase in the chromosome number is usually illustrated through a higher cellular content, which can be reflected in an growth in the size of the plant organs and in a shift of its morphologies (WU et al., 2010). Thus, the association between the

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level of ploidy and morphological characteristics aids in identifying polyploid plants. The morphological characterization still allows the almost immediate adoption of plants with phenotypes that are pertinent to the producers and breeding programs, or even for differentiating and identifying potential genotypes that are to be designated as varieties. Because it is an easy and simple methodology, stomatal analysis has also been used for identifying supposed polyploids through the counting and comparative measurement of stomata (GŁOWACKA et al., 2010). Various polyploidy induction activities in vegetables used this characteristic to select polyploid candidates, like in *Centella asiática* (KAENSAKSIRI et al., 2011) and *Lagerstroemia indica* (WANG; LEI, 2012).

Taking into account that part of the technologies applied in Brazil's flower production comes from other countries, research for tropical ornamental plants is still scarce, particularly with native species like Amazonian heliconias. These plants have an enormous potential for growth in the floriculture market, but the characterization of exotic germplasms and information on crop and post-harvest management deserves greater attention, even for more valuable plants like *Heliconia chartacea* var. Sexy Pink.

Due to the need for ongoing research activities applied to the tropical flower and ornamental plants industry, Embrapa Amazônia Occidental coordinated a research project in 2009 titled "The development of technologies for heliconia production: a new niche market for the Amazon". The purpose of this project was to define criteria for the production of *Heliconia chartacea* var. Sexy Pink in the environmental conditions surrounding the city of Manaus in the State of Amazonas that involved agronomic and biotechnological studies. One of the main products it sought to generate was establishing a population of polyploid plants in a field that demonstrated early interesting and innovative characteristics for this species. Consequently, this project sought to characterize *Heliconia chartacea* var. Sexy Pink plants that are obtained through induced polyploidization for the morphological and physiological characteristics that are of commercial interest.

Therefore, this study was undertaken to evaluate the establishment of plants in the field drawn from *in vitro* polyploidy induction assay and to determine the morphological and physiological characteristics of 38 *H. chartacea* var. Sexy Pink clones.

MATERIAL AND METHODS

The genotypes used in the this study are linked to the collection of *Heliconia chartacea* var. Sexy Pink established in Embrapa Amazônia Ocidental's Experimental Field Headquarters in Manaus, Amazonas. These plants are derived from an induction of an in vitro polyploidy assay which consisted of the immersion of stem apices from a single mother-plant at different concentrations of colchicine (ranging from 0.01 to 0.10%).

After the mutation induction, the explants were cultured and multiplied in MS medium (MURASHIG; SKOOG, 1962) supplemented with 0.15 mg of AIA (indole-3-acetic acid) and 3 mg of BAB (6-benzylaminopurine) (RAIZER et al., 2017), in vitro rooted (QUISEN et al., 2013), followed by acclimatization in a greenhouse and planted in a field in March 2011. In May 2014, the collection with 38 genotypes was transferred to a new area divided by clumps and established with a spacing of 3.0m between rows and 1.5m between plants, arranged in random blocks with four replications per genotype. Fertilization and cultivation cleaning followed Embrapa's pre-established schedule and was based on recommendations for the cultivation of other tropical flowers (LUZ et al., 2005; SOUSA et al., 2009).

Monthly evaluations were conducted of morphological descriptors in the genotypes established in the field for two consecutive years, and the criteria used was based on forty seven qualitative descriptors and two quantitative descriptors (Table 1) (CASTRO et al., 2007; LOGES et al., 2007). In order to have a more accurate identification of hue-related descriptors, a Munsell color chart was used. Visual assessments were made of the pseudo stem, leaves and inflorescences and the measurements were done with the aid of a digital caliper and tape measure in four plants from each genotype.

To visualize the differences among *H. chartacea* genotypes based on the qualitative and quantitative descriptors, a dendrogram was designed through an analysis of clusters of the UPGMA type (Unweighted Pair Group Method using Arithmetical Averages) with the help of the statistical program R (R DEVELOPMENT CORE TEAM, 2017). The dissimilarity matrix was obtained using the Gower algorithm (1971) and the suitability of the cluster analysis to the original data was evaluated by the coefficient of co-optic correlation.

Table 1. Morphological descriptors used in the evaluation of induced polyploidy genotypes in *H. chartacea* var. Sexy Pink.

Descriptors	Identification of Phenotype
Pseudo stem	
Plant height ("ALP")	Small-sized (< 1.50 m); Medium-sized (1.51-2.50 m); Large-sized (> 2.51 m)
Diameter of pseudostem ("DPC")	Obtained a height of 30 cm from the soil
Color of pseudostem ("CPC")	Greenish-yellow (2.5 GY 8/12); Light green (2.5 GY (8/8); Medium green (5GY 6/6); Dark green (7.5 GY 4/4)
Waxiness of the pseudostem ("CER")	Absent; Small; Average; Much
Hairiness of pseudostem ("PIL")	Absent; Small; Average; Much
Intensity of anthocyanin (IAN)	Intense; Average; Weak; Absent
Leaf	
Length of third leaf ("CFO")	Measure from the base to the top of the Limbus
Width of third leaf ("LFO")	Narrow (up to 10 cm); Median (of 20 to 30 cm); Large (more than 31 cm)
Position of the leaves ("PFO")	Upright; Drooping; Arched
Limbus (LIM)	Torn; Not torn
Green color of the underside of the leaf ("CFI")	Greenish-yellow (2.5 GY 8/12); Light green (2.5 GY (8/8); Medium green (5GY 6/6); Dark green (7.5 GY 4/4)
Green color of the upper surface of the leaf ("CFS")	Greenish-yellow (2.5 GY 8/12); Light green (2.5 GY (8/8); Medium green (5GY 6/6); Dark green (7.5 GY 4/4)
Shade of color on the underside of the main vein("TCP")	Greenish-yellow (2.5 GY 8/12); Light green (2.5 GY (8/8); Medium green (5GY 6/6); Dark green (7.5 GY 4/4)
Color of the side of the petiole ("CMP")	Purple (5R 3/10); Pink (2.5 R 7/2); Green (5GY 6/6); Brown (5YR 3/4); Transparent
Waxiness of the limbus on the dorsal surface of the leaf ("CLD")	Absent; Small; Average; Much
Waxiness of the limbus on the ventral surface of the leaf (CLV)	Absent; Small; Average; Much
Presence of spots on the limbus of the sapling ("PML")	No spots; Few spots; Scarce spots
Shape of the limbus base ("FBL")	Both round; One round/ one tapered; Both tapered
Comparison of the sizes of the limbus base ("CBL")	Equal; Unequal; Variable in the same plant
Shape of the petiole base ("FBP")	Open with winged edges; Open with upright edges; Narrow with upright edges; Twisted edges on the inside part
Edge with scaly base ("MBE")	Absent; Small; Average; Much
Shape of the petiole edge ("FMP")	Closed; Semi-closed; Upright; Wide open
Internal color of the sheaths ("CIB")	Purple (5R 3/10); Reddish-Pink (2.5 R 7/2); Green (5GY 6/6); Brown (5YR 3/4); Transparent
Inflorescence	
Positioning of the inflorescence in relation to the stem ("PIH")	Upright; Drooping
Length of inflorescence ("CI")	Short (10 cm <); Average (between 11 to 40 cm); Large (> 41 cm)
Length of the 2nd bract ("CB")	Small (10 cm <); Average (between 11 to 40 cm); Large (> 41 cm)
Width of the second bract ("LB")	Narrow (up to 10 cm); Median (of 20 to 30 cm); Large (more than 31 cm)
Angle of the 1st bract in relation to the peduncle ("ABP")	> 90°; 75°-90°; 45°-60°; 15°-30°; 0°
Twisting the spine ("TR")	Present; Absent
Visualization of the spine ("VR")	Visible; Covered by bracts

Width of the inflorescence ("LI")	Narrow (10 cm <); Average (between 11 to 40 cm); Large (> 41 cm)
Leaf at the tip (1st bract)	Present ; Variable; Variable in the same plant
Shape of bract apex ("FAB")	Long (bulging); Narrow; Tappered
Waxiness of the bracts ("CEB")	Absent; Small; Average; Much
Hairiness of the bracts ("PB")	Absent; Small; Average; Much
Symmetry of the bract ("SB")	Actinomorphic; Zigomorphic; Asymmetric
Number of bracts in mature inflorescence ("NBI")	Small (5 <); Average (between 6 to 20); Much (> 21)
Color of the spine ("CRA")	Dark Pink (5R 4/6); Light Pink (5R 4/4); Pink Orange (10R 7/8); Baby pink (2.5R 8/4); Pink Baby Orange (2.5R 7/8); Dark Red (5R 3/10); Medium Red (5R 4/10).
Color of the external bract ("CBE")	Dark Pink (5R 4/6); Light Pink (5R 4/4); Pink Orange (10R 7/8); Baby pink (2.5R 8/4); Pink Baby Orange (2.5R 7/8); Dark Red (5R 3/10); Medium Red (5R 4/10).
Color of the internal bract ("CBI")	Dark Pink (5R 4/6); Light Pink (5R 4/4); Pink Orange (10R 7/8); Baby pink (2.5R 8/4); Pink Baby Orange (2.5R 7/8); Dark Red (5R 3/10); Medium Red (5R 4/10).
Color of the perigonium (CBP)	White; Cream; Yellow (5Y 8/12)
Color of the perigonium lobes (CLP)	White; Cream; Yellow (5Y 8/12)
Anthocyanin presence in the perigonium (PAP)	Absent; In the basal part; Presence of stripes; Uniform coloring
Presence of pollen ("PPO")	Absent; Small; Average; Much
Color of the sepal ("CSE")	Greenish-yellow (2.5 GY 8/12); Light green (2.5 GY (8/8); Medium green (5GY 6/6); Dark green (7.5 GY 4/4)
Petal Color ("CPE")	Greenish-yellow (2.5 GY 8/12); Light green (2.5 GY (8/8); Medium green (5GY 6/6); Dark green (7.5 GY 4/4)
Ovary: Color of the unripe fruit ("OCI")	White; Cream; Yellow (5Y 8/12)
Ovary: Color of the ripe fruit ("COM")	White; Cream; Purplish blue
Flowering period ("PF")	Short (< 5 months); Medium (between 5 to 8 months); Long (> 8 months)

To verify the existence of differences in the structures of a leaf blade, a clump of genotype with a good phytosanitary quality was selected, and three fully expanded leaves (third leaf) were collected. From the mid-part of each leaf, using a circular hole-puncher, a sample was taken of foliar tissue with 1 cm diameter. It was later placed in a petri dish and immersed in commercial hypochlorite solution until full depigmentation (24-48 hours). Next, using a soft-bristle brush, the abaxial and adaxial portions of all samples were separated, they were also stained with 1% toluidine blue and the blades were assembled with the addition of one drop of Canada balsam (Canada turpentine).

The size of the stomas and stomatal density (DE - number of stomatas per unit area) of both parts of the leaf, abaxial and adaxial, were analyzed under an optical microscope and measured under a 40X lens, observing fifteen fields of view for each sample. Based on a micrometer scale for the eyepiece used, the stomatal density was determined through the ratio between the number of stomata and the area of the real field visible from the 40X lens (0.14 mm^2).

Slides were prepared with toluidine blue in order to evaluate the polar diameter (PD) and equatorial diameter (QD) of the stomata. They were observed in a clear chamber, in accordance with the Labouriau technique (CASTRO et al., 2009). The stomatal functionality ("FUN") was calculated from this data, given by the ratio between the polar diameter and equatorial diameter (CASTRO et al., 2009). The averages obtained for all variables were compared through the Scott Knott test at 5% probability.

RESULTS AND DISCUSSION

The clustering analysis based on morphological descriptors and Gower algorithm allowed for three distinct clusters to form (Table 1). Group 2 gathered most of the genotypes, while genotype 35 was the only one remaining alone in group 1, thus demonstrating that it had morphological characters distinct from the others.

These differences could be observed in the coloring of the inflorescence in the genotypes present in distinct groups (Figure 2).

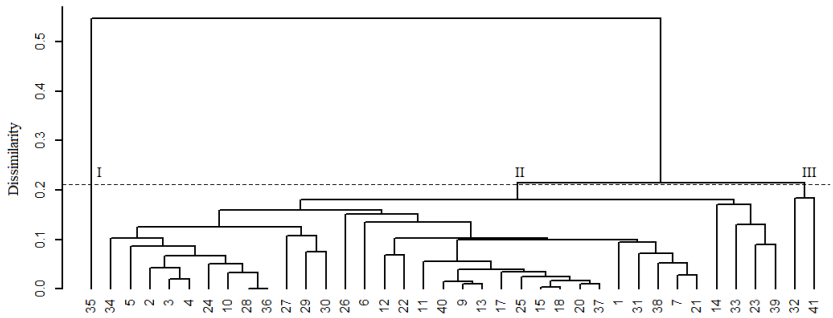

Figure 1. An analysis of the UPGMA cluster, based on qualitative and quantitative morphological characters and Gower algorithm, in *H. chartacea* var. Sexy Pink genotypes. Cophenetic correlation coefficient: r = 0.8552. The dotted horizontal line represents the cut estimated by the Mojema method.

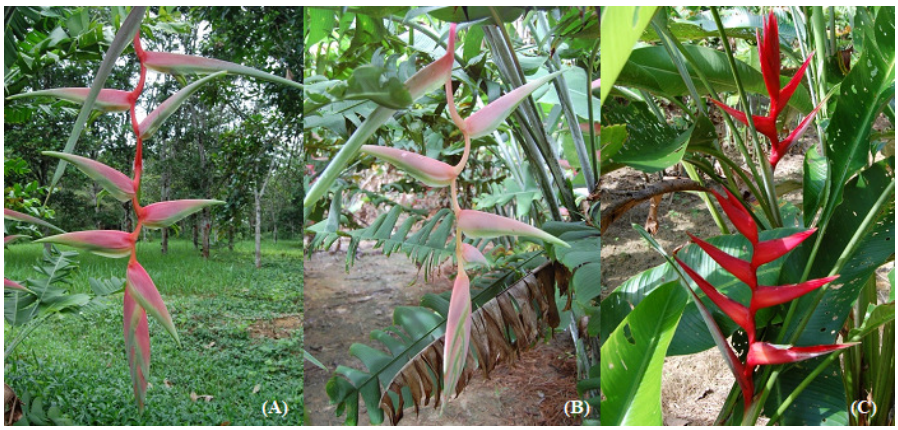

Figure 2. Inflorescences of *H. chartacea* var. Sexy Pink resulting from an in vitro polyploidy induction assay by somatic doubling using colchicine. (A) 29 Genotype; (B) 32 Genotype; (C) 35 Genotype.

There was no presence of hairiness in the pseudo stem in any of the genotypes. The presence of hairs is an important characteristic in the production of heliconia, especially an excess, because it has a negative effect on post-harvest handling and transport that requires additional cleaning, because the presence of hairs leads to the retention of debris (CASTRO et al., 2007).

The plant's height was over 1.5m in all genotypes (Table 2), which could hamper the handling and preparation of flower stems during post-harvest, because large stems are more prone to toppling or being ruptured. However, this is a characteristic that has already been described for this variety and the values obtained for the length of the floral stems are within what the market requires (ABBOTT et al., 2010). Clone 18 displayed the lowest plant height along with reduced inflorescences, which can be better used in landscaping projects that have a floristic composition in reduced environments.

For the diameter of the pseudo stem (DPS), there was a significant variation among the genotypes, ranging from 23.32 (genotype 5) to 58.22mm (genotype 26). According to Farias et al. (2013), the DPS warrants attention due to its influence on flower resistance, handling, selection, packaging and post-harvest durability. A study conducted by Castro et al. (2007) with 30 heliconia genotypes, classified *H. chartacea* flower stems as large, indicating a high degree of difficulty in handling, and requiring special care for packaging and transportation.

Both the diameter and stem length features are of highly important for the flower's resistance to wind, as stems that have larger diameters are also more rigid. According to Albuquerque et al. (2010), this is due to the carbon reserve contained in the stem that is used to prolong the potential longevity of the flowers. In this regard, Hermans et al. (2006) and Castro et al. (2007) claim that the longer the rod length and diameter, the greater the post-harvest durability. One explanation for the variation in the diameter of the stems is that it is due to the chromosome duplication in the evaluated genotypes.

Table 2. Characterization of *H. chartacea* genotypes when considering morphological descriptors of pseudo stem analysis.

Gen.	ALP[1]	DPC	CPC	CER	IAN	Gen.	ALP	DPC	CPC	CER	IAN
1	Medium	34.44	Dark Green	Much	Weak	23	Large	30.00	Dark Green	Much	Weak
2	Medium	36.25	Mid Green	Much	Aus.	24	Medium	28.50	Light green	Much	Aus.
3	Medium	36.00	Mid Green	Much	Aus.	25	Medium	26.08	Mid Green	Much	Average
4	Medium	35.00	Mid Green	Much	Aus.	26	Medium	58.22	Mid Green	Much	Average
5	Medium	23.32	Mid Green	Much	Aus.	27	Large	26.50	Mid Green	Much	Aus.
6	Medium	25.99	Dark Green	Much	Weak	28	Medium	27.25	Light green	Much	Aus.
7	Medium	27.25	Mid Green	Much	Weak	29	Large	33.00	Mid Green	Much	Weak
9	Medium	29.32	Mid Green	Much	Weak	30	Medium	30.00	Mid Green	Much	Aus.
10	Medium	25.75	Light green	Much	Aus.	31	Medium	29.25	Mid Green	Much	Average
11	Medium	26.22	Mid Green	Much	Aus.	32	Large	26.50	Light green	Much	Average
12	Medium	29.50	Mid Green	Much	Average	33	Large	29.50	Mid Green	Much	Weak
13	Medium	38.22	Mid Green	Much	Weak	34	Medium	26.50	Mid Green	Much	Aus.
14	Large	27.52	Mid Green	Average	Weak	35	Medium	28.00	Mid Green	Few	Weak
15	Medium	26.52	Mid Green	Much	Weak	36	Medium	27.08	Dark Green	Much	Weak
17	Medium	26.72	Mid Green	Much	Weak	37	Medium	23.78	Mid Green	Much	Weak
18	Medium	26.72	Mid Green	Much	Weak	38	Medium	28.25	Mid Green	Much	Aus.
20	Medium	26.00	Mid Green	Much	Weak	39	Large	28.50	Dark Green	Much	Weak
21	Medium	27.25	Mid Green	Much	Aus.	40	Medium	26.08	Mid Green	Much	Weak
22	Medium	28.99	Mid Green	Much	Weak	41	Large	35.16	Dark Green	Much	Weak

[1]ALP = Plant Height; DPC = Diameter of the pseudo stem; CPC = Color of pseudo stem; CER = Waxiness of the pseudo stem; IAN = Intensity of anthocyanin.

The presence of large amount of waxiness was constant in most of the evaluated genotypes. One noted exception was genotype 35, which featured waxiness in the younger pseudo stems and, as they developed, lost this characteristic. Another attractive feature of genotype 35 was the change in the leaf limbus (Table 3).

Table 3. Characterization of *H. chartacea* genotypes for leaf morphological descriptors.

Gen.	CFO	LFO	LIM	CFI	CFS	TCN	CMP	CLD	CLV	FBL	CBL	CIB
1	70.87	Median	Torn	Dark	Dark	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
2	79.50	Median	Torn	Dark	Dark	Greenish-yellow	Green	Aus.	Aus.	Rounded	Uneven	Greenish-yellow
3	83.75	Median	Torn	Medium	Medium	Greenish-yellow	Green	Aus.	Aus.	Rounded	Uneven	Greenish-yellow
4	80.50	Median	Torn	Medium	Dark	Greenish-yellow	Green	Aus.	Aus.	Rounded	Uneven	Greenish-yellow
5	76.10	Median	Torn	Medium	Dark	Greenish-yellow	Green	Aus.	Aus.	Rounded	Variable	Light green
6	73.10	Wide	Torn	Dark	Dark	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green
7	79.75	Median	Torn	Dark	Dark	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green
9	67.77	Median	Torn	Medium	Medium	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green
10	85.75	Median	Torn	Medium	Medium	Greenish-yellow	Green	Aus.	Aus.	Rounded	Uneven	Greenish-yellow
11	82.69	Median	Torn	Medium	Medium	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green
12	85.00	Median	Torn	Dark	Dark	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green
13	65.74	Median	Torn	Medium	Medium	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green
14	67.82	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Variable	Greenish-yellow
15	85.82	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
17	87.24	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
18	82.24	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
20	71.00	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
21	80.50	Median	Torn	Dark	Dark	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green
22	80.43	Median	Torn	Medium	Dark	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green

23	79.25	Median	Torn	Dark	Light	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Uneven	Light green
24	81.00	Median	Torn	Medium	Medium	Light green	Green	Aus.	Aus.	Rounded	Uneven	Greenish-yellow
25	76.56	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
26	73.36	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
27	84.50	Median	Torn	Dark	Dark	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Uneven	Light green
28	87.75	Median	Torn	Medium	Medium	Greenish-yellow	Green	Aus.	Aus.	Rounded	Uneven	Light green
29	85.25	Median	Torn	Dark	Dark	Greenish-yellow	Green	Aus.	Aus.	Rounded	Uneven	Light green
30	83.50	Median	Torn	Dark	Dark	Greenish-yellow	Green	Aus.	Aus.	Rounded	Uneven	Light green
31	93.00	Median	Torn	Dark	Dark	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
32	87.00	Median	Torn	Dark	Dark	Light green	Purple	Aus.	Aus.	Rounded	Uneven	Greenish-yellow
33	91.00	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Uneven	Light green
34	94.50	Median	Torn	Medium	Medium	Greenish-yellow	Green	Aus.	Aus.	Rounded	Uneven	Light green
35	83.00	Median	Non-torn	Medium	Dark	Light green	Purple	Average	Average	Pointy	Variable	Mid Green
36	88.56	Median	Torn	Medium	Medium	Light green	Purple	Aus	Aus	Rounded	Variable	Light green
37	61.37	Median	Torn	Medium	Medium	Light green	Purple	Aus.	Aus.	Rounded	Variable	Light green
38	80.25	Median	Torn	Dark	Dark	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Mid Green
39	78.25	Median	Torn	Medium	Medium	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Uneven	Light green
40	57.56	Median	Torn	Medium	Medium	Greenish-yellow	Purple	Aus.	Aus.	Rounded	Variable	Light green
41	83.73	Median	Torn	Dark	Dark	Mid Green	Pink	Aus.	Aus.	Rounded	Uneven	Light green

CFO = Length of third leaf; LFO = Width of third leaf; LIM = Limbus; CFI = Green color of the underside of the leaf; CFS = Green color of the upper face off the leaf; TCN = Color shade of the underside of the main vein; CMP = Color of the edge of the petiole; CLD = Waxiness of the limbus on the dorsal surface of the leaf; CLV = Waxiness of the limbus on the ventral surface of the leaf; FBL = Shape of the limbus base; CBL = Comparison of the sizes of the limbus base; CIB = Inner color of the sheaths.

It was the only one that exhibited the edges of the entire limbus and had more vivid coloring, characteristics that increase the possibilities of using this genotype in landscaping, as it becomes something that is more visually attractive (Figure 3). The presence of wax on the upper and lower surfaces of the leaf blade was also observed exclusively in the individuals of this genotype, which gives them extra protection against water loss from transpiration.

Figure 3. Changes in leaf limb of *H. chartacea* var. Sexy Pink resulting from an in vitro polyploidy induction assay by somatic doubling using colchicine. (A) Genotype 12, (B) Genotype 35.

Among the descriptors corresponding to the leaf area (Table 3), there was no variation for the leaf position, presence of spots on the limbus of the sapling, shape of the petiole base, edge of the base and the shape of the petiole edge. In this case, all genotypes exhibited arched, non-spotted leaves with a narrow petiole with upright and closed edges and lacking scariosum base. Assessments of the length of the third leaf (CFO) ranged from 57.56 (genotype 40) to 94.50cm (genotype 34) and only genotype 6 displayed leaves with a width greater than 30cm. By and large, the leaf descriptors presented great variability, however, a series of characterizations are needed to standardize these for the producer and, as a result, the end consumer because parameters such as beauty, exoticism and ideal coloring are subjective.

Data shown in Table 4 again shows that the genotype 35 was detached from the others in relation to the position of the inflorescence. *H. chartacea* var. Sexy Pink normally exhibits drooping inflorescences and the genotype 35 produced erect and more compact inflorescences that are less than 40 cm in length which, according

to Albuquerque et. al. (2010) would classify this genotype as "suitable" for commercialization as a cut flower. The other genotypes would require individual packaging according to their size, which would require careful handling to avoid the rupturing the axis where the bracts are inserted. These genotypes also presented twisting of the spine, which makes the post-harvest preparation process excessively slow, including the use of protection for the bracts. All these resources include an additional component to the sale price.

Table 4. Characterization of *H. chartacea* genotypes for morphological descriptors of inflorescence.

Gen.	PIH	CI	TR	LI	FE	CEB	CRA	CBE	CBI	CSE	CPE	OCI	PF
1	Drooping	Large	Pres.	Wide	Variable	Much	Light pink	Dark Pink	Baby pink	Light green	Light green	Cream	Long
2	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
3	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
4	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
5	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
6	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Dark Green	Dark Green	Cream	Long
7	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Orange pink	Mid Green	Mid Green	Cream	Long
9	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
10	Drooping	Large	Pres.	Wide	Variable	Much	Light pink	Dark Pink	Baby pink	Light green	Light green	Cream	Long
11	Drooping	Large	Pres.	Wide	Variable	Much	Light pink	Dark Pink	Baby pink	Light green	Light green	Cream	Long
12	Drooping	Large	Pres.	Wide	Variable	Much	Light pink	Dark Pink	Baby pink	Light green	Light green	Cream	Medium
13	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID
14	Drooping	Large	Pres.	Wide	Variable	Few	Orange pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
15	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
17	Drooping	Large	Pres.	Wide	Variable	Much	Light pink	Dark Pink	Baby pink	Light green	Light green	Cream	Long
18	Drooping	Average	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
20	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID
21	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Orange pink	Baby pink	Mid Green	Mid Green	Cream	Long
22	Drooping	Large	Pres.	Wide	Variable	Much	Light pink	Baby pink	Baby pink	Light green	Light green	Cream	Long
23	Drooping	Large	Pres.	Wide	Variable	Few	Dark Pink	Light pink	Light pink	Light green	Light green	Cream	Long
24	Drooping	Large	Pres.	Wide	Variable	Much	Orange pink	Orange pink	Light pink	Mid Green	Mid Green	Cream	Long
25	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
26	Drooping	Large	Pres.	Average	Variable	Much	Dark Pink	Light pink	Light pink	Light green	Light green	Cream	Long
27	Drooping	Large	Pres.	Wide	Variable	Much	Light pink	Light pink	Light pink	Mid Green	Mid Green	Cream	Long
28	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Baby pink	Baby pink	Mid Green	Mid Green	Cream	Long
29	Drooping	Large	Pres.	Wide	Variable	Average	Dark Pink	Light pink	Baby pink	Mid Green	Mid Green	Cream	Long
30	Drooping	Large	Pres.	Wide	Variable	Few	Dark Pink	Orange pink	Baby pink	Mid Green	Mid Green	Cream	Long
31	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Orange pink	Baby pink	Mid Green	Mid Green	Cream	Long
32	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Orange pink	Baby pink	Greenish-yellow	Greenish-yellow	Cream	Long
33	Drooping	Large	Pres.	Wide	Variable	Few	Dark Pink	Light pink	Baby pink	Light green	Mid Green	Am.	Long
34	Drooping	Large	Pres.	Wide	Variable	Few	Dark Pink	Light pink	Baby pink	Dark Green	Dark Green	Cream	Long
35	Upright.	Average	Aus.	Average	Pres.	Few	Red	Red	Red	Greenish-yellow	Greenish-yellow	Cream	Long

36	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID
37	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID
38	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Orange pink	Baby pink	Light green	Light green	Cream	Long
39	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Light pink	Orange pink	Light green	Light green	Cream	Long
40	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID	NID
41	Drooping	Large	Pres.	Wide	Variable	Much	Dark Pink	Dark Pink	Baby pink	Dark Green	Dark Green	Cream	Medium

PIH = Position of inflorescence in relation to stem; CI = Length of the inflorescence ; TR = Twisting of the spine; LI = Width of the inflorescence; FE = Leaf at the tip (1st bract); CEB = Waxiness of the bracts; CRA = Color of the spine; CBE = External bract color; CBI = Color of the internal bract; CSE = Sepal Color; CPE = Petal Color; OCI = Ovary : Color of unripe fruit; PF = Flowering period. *NID = Unclassified – The genotypes did not output any flowering by the end of the evaluation.

For the inflorescences descriptors, there was no polymorphism for the length of the 2nd bract (average), width of the second bract (narrow), angle of the 1st bract relative to the peduncle (>90°), visualization of the soine (visible), apex shape (asymmetric), number of bracts in ripe inflorescence (average), perigone base color (cream), perigone lobes color (orange-yellow), presence of anthocyanin in perigonium (absent), presence of pollen (absent), ripe fruit color (purplish blue).

The length of the bract classified as medium (11 to 40 cm) is significant because it usually represents the most prominence in flower arrangements and, therefore, in customer appreciation. Because there were no changes in this characteristic, we can assert that there was no loss of one of the main commercial features in this species.

The absence of hairiness in the bracts is a positive characteristic because the presence of hair interferes greatly with the handling, preparation and transport. The damage to the handling is due to the fact that it occurs to the formation of a protective layer in the bracts, which can minimize the benefits of the conditioning treatment done for remove the heat from the field and restore the turgescence of harvested flowers. The hair layer can also host small insects, insecticide residues and dust particles, leading to intense handling and preparation and resulting in increasing production costs. Another negative aspect of the presence of hairiness with regards to transport is that the friction of the inflorescences causes hair to fall out in some bracts, damaging the appearance of the inflorescence and compromising its quality. Additionally, Broschat and Donselman (1983) warn of the necessity of packages that remain dry for suitable transport, and the hariness allows the bracts to be stored in excessive humidity, and can be transitioned to the packaging.

The period for outputting the first inflorescence varied greatly between the genotypes. Genotypes 12 and 41 exhibited inflorescence up to eight months after planting (seven and eight months, respectively), while the other genotypes showed a longer flowering period, flowering only after eight months of planting. Genotypes 13, 20, 36, 37 and 40 did not output inflorescences at the end of the two years of the evaluation period. Heliconias are cut tropical flowers and the floral stem is the end-product of commercial significance that is used in arranging and preparing bouquets. Hence, the largest number of flower stem represents a lower cost of production for the producer, along with higher competitiveness and greater profitability. According to Broschat and Donselman (1983), the neotropical species of heliconia produce terminal inflorescences after outputting 4 to 5 leaves, which was not noted in these genotypes. One use for plants that did not output inflorescences would be in constructing landscapes as foliage, since its exoticity enables this purpose.

Stomata of the genotypes offered tetrapeptic morphology and guard cells without significant changes. However, several genotypes displayed greater equatorial diameter and stomatal density compared to the mother-plant (Figure 4 and Table 5). These differences in the size and number of stomata per area have been common characteristics featured on several plant materials submitted to polyploidization, and are used as basic characteristics in indentifying polyploid individuals in various studies (CAVALCANTE FILHO, 2011; GŁOWACKA et al., 2010; WANG; LEI, 2012).

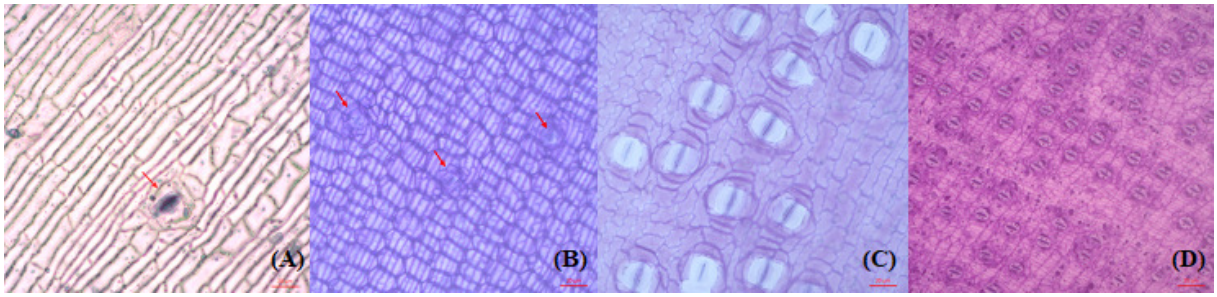

Figure 4. Format of stomata and guard cells in genotypes of *H. chartacea* var. Sexy Pink, induced polyploidy, in the Abaxial (A and B) and Adaxial (C and D) part of the leaves, in the genotypes 14 and mother-plant respectively. Bar = 20 µm.

On average, the stomatal density ("SD") was 94.73% higher in the abaxial part compared to the adaxial part (Table 5). The adaxial epidermis' SD may be less responsive to environmental variations due to radiation being directly incidental upon it (CASTRO et al., 2009) and transpiration occurs mainly through the epidermis of the abaxial face (STRECK, 2003).

Table 5. Average estimates of stomatal density (DE), polar diameter of stomatal (DP), equatorial diameter of the stomatal (DQ) and stomatal functionality (FUN), evaluated in 38 *H. chartacea* var. Sexy Pink genotypes in different regions of the leaf.

Genotypes	Leaf regions							
	Adaxial				Abaxial			
	DE	DP	DQ	FUN	DE	DP	DQ	FUN
1	28.57 a[1]	21.78 a	10.24 b	2.13 a	249.52 d	17.20 b	9.35 b	1.95 a
2	11.27 d	20.84 a	11.34 a	1.85 b	260.79 c	19.73 a	10.54 a	1.88 a
3	18.73 b	20.30 b	11.93 a	1.72 b	264.13 c	17.45 b	9.19 b	1.90 a
4	10.00 d	18.66 b	10.31 b	1.83 b	268.41 c	16.84 b	8.91 b	1.89 a
5	9.43 e	21.59 a	12.21 a	1.77 b	287.30 b	20.09 a	11.43 a	1.76 b
6	10.95 d	19.09 b	10.73 b	1.78 b	239.37 d	17.22 b	8.86 b	1.96 a
7	1.43 f	22.73 a	11.38 a	2.00 a	150.79 g	19.35 a	10.21 a	1.90 a
9	16.67 c	19.56 b	10.66 b	1.84 b	299.05 b	16.23 b	7.93 b	2.06 a
10	20.16 b	20.50 a	10.23 b	2.01 a	385.87 a	18.78 a	10.89 a	1.73 b
11	15.56 c	19.94 b	11.29 a	1.77 b	289.37 b	19.08 a	10.75 a	1.78 b
12	16.03 c	19.47 b	10.01 b	1.95 a	365.56 a	17.13 b	8.77 b	1.95 a
13	11.11 d	20.56 a	10.72 b	1.94 a	159.05 g	19.18 a	9.34 b	2.07 a
14	31.90 a	20.54 a	10.40 b	1.98 a	358.57 a	18.73 a	9.91 b	1.89 a
15	6.67 e	19.09 b	8.90 b	2.19 a	207.46 e	17.85 b	8.87 b	2.01 a
17	14.13 d	19.69 b	11.52 a	1.71 b	276.03 c	16.56 b	8.92 b	1.86 a
18	7.14 e	20.09 b	10.62 b	1.90 a	258.10 c	18.26 b	9.73 b	1.89 a
20	17.94 b	20.78 a	12.63 a	1.65 b	268.57 c	19.90 a	11.54 a	1.72 b
21	11.75 d	19.79 b	9.82 b	2.02 a	287.46 b	15.37 b	7.32 b	2.11 a
22	7.62 e	22.18 a	12.38 a	1.80 b	260.63 c	17.57 b	10.98 a	1.62 b
23	11.59 d	21.20 a	12.45 a	1.70 b	218.57 e	19.85 a	11.98 a	1.66 b
24	18.73 b	20.98 a	10.88 b	1.93 a	309.68 b	19.08 a	10.85 a	1.76 b
25	7.94 e	21.30 a	11.28 a	1.89 a	237.78 d	18.03 b	10.66 a	1.69 b
26	18.89 b	21.09 a	11.00 b	1.92 a	242.38 d	16.03 b	8.27 b	1.94 a
27	14.76 c	18.19 b	10.43 b	1.76 b	224.29 e	17.82 b	9.08 b	1.98 a
28	17.94 b	21.17 a	11.88 a	1.80 b	275.24 c	19.62 a	11.07 a	1.77 b
29	11.07 d	20.77 a	11.30 a	1.86 b	244.13 d	18.82 a	9.57 b	1.97 a
30	16.35 c	21.53 a	11.85 a	1.82 b	269.05 c	18.84 a	10.97 a	1.72 b
31	19.37 b	21.79 a	11.83 a	1.84 b	264.60 c	19.80 a	11.19 a	1.79 b
32	18.57 b	21.29 a	11.30 a	1.88 a	251.43 d	18.59 a	10.33 a	1.82 b
33	15.87 c	21.78 a	10.62 b	2.06 a	273.33 c	18.89 a	8.99 b	2.10 a
34	8.25 e	21.10 a	11.73 a	1.80 b	307.46 b	19.87 a	12.20 a	1.63 b

35	0.79 f	20.75 a	12.74 a	1.64 b	183.97 f	20.97 a	12.48 a	1.68 b
36	11.59 d	21.28 a	12.59 a	1.70 b	196.35 f	21.24 a	12.31 a	1.73 b
38	13.65 d	22.17 a	11.75 a	1.89 a	258.89 c	20.24 a	11.09 a	1.83 b
39	9.84 d	23.62 a	11.72 a	2.02 a	270.95 c	17.80 b	8.37 b	2.13 a
40	19.84 b	20.23 b	11.40 a	1.78 b	246.35 d	20.16 a	11.65 a	1.73 b
41	11.59 d	18.85 b	10.25 b	1.88 a	209.05 e	17.70 b	8.46 b	2.09 a
Mother-plant (control)	10.81 d	20.48 a	10.88 b	1.90 a	260.10 c	18.46 a	9.88 b	1.89 a
Overall mean	13.88	20.71	11.20	1.86	259.99	18.54	10.08	1.86
CVe (%)[2]	14.87	6.34	9.23	8.5	6.56	6.33	9.89	8.4

[1]Averages followed by the same letter in each column pertain to the same group according to the Scott and Knott grouping criterion at 5% probability; [2]Coefficient of experimental variation.

The highest SD were observed in genotypes 1 and 14 for the adaxial part of the leaves, whereas genotypes 10, 12 and 14 were the ones with the highest values for the abaxial part. The lowest values were found in genotypes 7 and 35 for the adaxial part and in genotypes 7 and 13 for the abaxial part of the leaves, differences caused by the induced mutation. Castro et al. (2009) relate that a higher number of stomata per unit of leaf area may confer a high adaptability to environments that are dry or have a lack of available water. Therefore, a higher SD can enable a stomatal opening in a shorter period of time, permitting an adequate capture of CO_2 and reducing the time in which these stomata become open. This reduces transpiration and allows for the plants to better adapt to conditions in which water is scarce (MIGLIORANZA; OLIVEIRA, 2013).

A higher stomatal functionality (FUN) may be associated with reduced transpiration because the stomata becomes more elliptical (CASTRO et al., 2009; BATISTA et al., 2010), the reduced transpiration may also be associated with a greater SD, which is frequently observed under conditions with a greater amount of radiation and less available water (SOUZA et al., 2007; CASTRO et al., 2009). Therefore, among the varieties with the highest FUN and SD values, genotypes 1, 12 and 14 stand out, and the best averages were obtained by genotype 14, both for the adaxial as well as the abaxial part, showing that this genotype presents the most functional stomata.

CONCLUSIONS

The induction of polyploidy in *H. Chartacea* var. Sexy Pink allowed for clones to be obtained with a high variability for leaf characteristics, pseudostem and inflorescence, with various attributes that confer a more efficient postharvest management to some genotypes, in addition to favorable aspects for commercialized purposes as a cut flower.

The presence of genotypes with changes in the position, coloring and size of the inflorescence, in addition to modifications in the leaf limb that left them with whole edges, showed promise for future projects geared towards the production of cut flowers and landscaping in *H. chartacea* var. Sexy Pink.

The evaluated genotypes present stomata with tetracytic morphology and guard cells that do not have significant changes. However, some genotypes show a larger equatorial diameter and stomatal density in relation to the mother-plant.

The next steps of this research are the analysis of the DNA content by flow cytometry for polyploidy identification of the genotypes.

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RESUMO: A expansão do mercado de flores tropicais tem demandado uma constante procura por novas variedades, principalmente aquelas dotadas de perfil exótico, mas ainda apresentando beleza e durabilidade. Dentre as flores tropicais de maior destaque, se encontram as do gênero *Helicônia*, sendo estas naturalmente encontradas na região Amazônica. Visando aumentar a variabilidade genética disponível em *Heliconia chartacea* var. Sexy Pink, pesquisas biotecnológicas foram realizadas com a aplicação de colchicina para indução a poliploidia em plantas da espécie. Deste modo, o objetivo do presente trabalho foi avaliar as plantas estabelecidas em campo, provenientes dos ensaios de indução à poliploidia in vitro para determinar as

características morfológicas e fisiológicas de 38 clones de *H. chartacea* var. Sexy Pink. As análises de caracterização foram realizadas por meio de 49 descritores morfológicos e avaliação da densidade estomática por microscopia. O genótipo 35 foi o que apresentou as maiores variações morfológicas, com alterações na posição e coloração da inflorescência, além de possuir as bordas do limbo foliar inteiras. O genótipo 18 apresentou os menores valores para altura da planta e tamanho das inflorescências, mostrando-se promissor para pesquisas voltadas ao uso em ambientes reduzidos. Alguns genótipos não tiveram floração, sendo recomendada a sua utilização exclusivamente para composição paisagística como folhagens, já que sua exoticidade permite esta finalidade. Os genótipos avaliados apresentaram estômatos com morfologia tetracítica e células-guarda sem alterações significativas, porém, foram obtidos genótipos com maior diâmetro equatorial e densidade estomática em relação a planta matriz. De modo geral, a indução a poliploidia permitiu a obtenção de clones com alta variabilidade para características da folha, pseudocaule e inflorescência, sendo vários os atributos que conferiram a alguns genótipos um manejo pós-colheita mais eficiente, além de aspectos favoráveis para comercialização como flor de corte.

PALAVRAS-CHAVE: Floricultura. Heliconiaceae. Colchicina. Descritores morfológicos. Densidade estomática.

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