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## Physiological development of zygotic embryos of heliconias propagated *in vitro* and conventionally

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### ABSTRACT

The aim of this study is to compare physiological development of *Heliconia bihai* cv. Lobster Claw Two plants derived from *in vitro* culture of zygotic embryos and conventional propagation. Heliconias obtained from rhizomes and from *in vitro* multiplication were evaluated every 30 days during ten months under greenhouse conditions. The experimental design was completely randomized, with ten repetitions, and the 2x10 factorial arrangement consisted of two plant multiplication methods and ten evaluations performed at different times. The analyzed biometric parameters were plant height, number of leaves, number of tillers, leaf area, and color intensity in the bracts. Plants derived from *in vitro* culture showed significant differences in the development of the evaluated physiological parameters in comparison to plants derived from rhizomes, and they also showed early flowering. Although the *in vitro* cultured plants were derived from zygotic embryos, no morphological changes were found in the vegetative and reproductive parts (inflorescence) of the plants or in the colorimetry. It shows that the *in vitro* cultures of zygotic embryos may be used as a technique to produce seedlings on a large-scale, thus allowing the floriculture sector to grow in the region and all over the country.

**Keywords:** *Heliconia bihai*, micropropagation, rhizome, vegetative propagation.

### RESUMO

#### Desenvolvimento fisiológico de embriões zigóticos de heliconias propagados por cultura *in vitro* e convencionalmente

O objetivo deste trabalho foi comparar o desenvolvimento fisiológico em casa de vegetação de plantas de *Heliconia bihai* cv. Lobster Claw Two provenientes do cultivo *in vitro* de embriões zigóticos e de propagação convencional. Para tanto, helicônias obtidas a partir de rizomas e de multiplicação *in vitro* foram avaliadas a cada 30 dias durante dez meses. O delineamento experimental utilizado foi inteiramente casualizado, com dez repetições, sendo o arranjo fatorial 2x10, composto por 2 condições de cultivo e dez avaliações em tempos distintos. Os parâmetros biométricos analisados foram: altura da planta, número de folhas, de perfilhos, área foliar e posteriormente intensidade da cor das brácteas. As plantas obtidas do cultivo *in vitro* apresentaram diferença significativa no desenvolvimento dos parâmetros fisiológicos avaliados em comparação com as plantas provenientes de rizoma, como também, apresentaram precocidade na floração. Apesar das plantas provenientes do cultivo *in vitro* procederem de embrião zigótico, não foram observadas alterações morfológicas na parte vegetativa e reprodutiva (inflorescências) das plantas e nem na colorimetria, evidenciando que a técnica de cultivo *in vitro* de embriões zigóticos, pode ser utilizada para produção de mudas em larga escala, possibilitando incremento no setor da floricultura na região e no país.

**Palavras-chave:** *Heliconia bihai*, micropropagação, rizoma, propagação vegetativa.

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The cultivation of ornamental plants has been constantly expanding and standing out in the national agribusiness, especially in the states of the Brazilian Northeastern and Northern regions (Farias *et al.*, 2013). Besides aggregating high potential for future expansion, it also represents one of the main activities generating employment and income for micro and small producers across the country (Junqueira & Peetz, 2014).

In order to reduce the negative

impacts of the economic crisis on the international markets since 2008, the Brazilian floriculture sector is mainly focused on the domestic consumption to which it directs 96.5% of its production. Thus, the flower production chain moved R\$ 5.22 billion in 2013, and it grew over 38% in comparison to 2012. This result is considered to be positive against the economic growth prone to stagnation (Junqueira & Peetz, 2014).

The country has many native

or introduced species that fit the benchmarks and, in this scenario, heliconias stand out mainly for their beauty and rusticity (Albuquerque *et al.*, 2014).

The genus *Heliconia* encompasses a wide diversity of ornamental plant species, varieties, hybrids and cultivars. The number of existing species is uncertain, but it comprises between 150 and 250 species. Approximately 40 species occur naturally in Brazil

being known by several common names (Castro *et al.*, 2007; Nascimento *et al.*, 2014).

Heliconias may be conventionally propagated from rhizomes. However, this vegetative propagation system presents serious risks of spreading pests and diseases that may compromise the production and, consequently, marketing of the final product (Santos *et al.*, 2006). Thus, the absence of phytosanitary-quality seedlings may compromise the production of heliconia seedlings by conventional methods.

Thus, the *in vitro* propagation is an alternative to produce heliconia seedlings with high physiological quality standard and free from diseases, fact that satisfactorily enables the economic development of such cultivation. The *in vitro* culture allows producing plants throughout the year regardless of weather conditions. In addition, it allows early and uniform flowering, thus enabling reducing the harvest period of higher yields (Álvares & Caldas, 2002). Although there are no published scientific reports on the physiological and productive development of heliconias derived from *in vitro* culture, micropropagated seedlings may usually be more productive than conventional ones (Drew & Smith, 1990).

Therefore, the aim of the current study is to compare the physiological development of *H. bihai* cv. Lobster Claw Two plants derived from *in vitro* culture of zygotic embryos and from conventional propagation (rhizome) in greenhouse.

## MATERIAL AND METHODS

### Plant material

*H. bihai* cv. Lobster Claw Two plants derived from *in vitro* culture of zygotic embryos measuring approximately 5 cm were acclimatized in 250 mL pots containing commercial substrate (Golden Mix). These plants were kept in greenhouse during 60 days at Rural Federal University of Pernambuco (UFRPE), under controlled temperature and light conditions. Plants were daily irrigated and received nutrient solution containing 742.86 mg L<sup>-1</sup> soluble fertilizer every fifteen days (Kristalon,

3% N; 11% P<sub>2</sub>O<sub>5</sub>; 38% K<sub>2</sub>O; 4% MgO; 11% S; 0.025% B; 0.004% Mo; 0.01% Cu-EDTA; 0.025% Zn-EDTA; 0.07% Fe-EDTA and 0.04% Mn-EDTA) and 840 mg L<sup>-1</sup> calcium nitrate (BarcoViking, 15.5% N and 19% Ca).

After acclimatization period (60 days), plants were transferred to the greenhouse (8°1'5''S, 34°56'42''W, 6.4 m altitude). Plants were planted in substrate composed by washed sand, coconut powder and sugarcane filter cake (sugar-ethanol industry residue derived from juice extracted from the mills in the rotary filter) as substrate, at the ratio of 2x1x1. The same procedure was performed in a 15-cm long conventional propagation unit (rhizome and pseudostem) derived from a commercial plantation in Paulista (07°56'27''S, 34°52'23''W, 13 m altitude), Pernambuco State, Brazil. Irrigation was performed on alternate days, and each plant received 200 mL of nutrient solution every 15 days.

### Evaluation of biometric parameters of the plants

During the experimental period (10 months), the following biometric parameters of plants were evaluated every 30 days: plant height (from the neck to the curvature of the fully expanded youngest leaf), number of leaves, number of tillers, and leaf area (all the leaves of the evaluated plants were measured). Leaf area (LA) was calculated according to methodology of Moreira (1987) based on the following formula:  $LA = LCW \times LML \times 0.8$ , wherein LCW = leaf central width and LML = leaf midrib length.

### Statistical analysis of biometric parameters

The experiment was distributed in a completely randomized design with 10 repetitions. Treatments were distributed in a 2x10 factorial arrangement consisting of two plant multiplication methods, characterized by plants derived from *in vitro* culture of zygotic embryos and from conventional vegetative cultivation (VC) and ten evaluations performed at different times (30, 60, 90, 120, 150, 180, 210, 240, 270

and 300 days). Data were analyzed using statistical software (SISVAR, v. 4.0) and subjected to analysis of variance. The treatment means were compared using the Student's t-test (P<0.05) for the methods of plant multiplication factor. The variables evaluated according to the cultivation time were analyzed by regression, and the adjustment degree of the models was evaluated by coefficient of determination and by the significance of regression coefficients using t-test (P<0.05). Pearson's correlations were performed in pairs at 5% significance level in order to analyze relationship between the variables. The number of leaves, tillers and leaf area variables were normalized by transformation, before the analysis of variance. Flower stems were harvested to determine bracts color. They were cut 20 cm above ground and placed in 10-liter capacity containers containing 2.5 liters of distilled water where they were kept during 15 days.

### Analysis of color intensity of the bracts

Color intensity was measured every three days using four replications. Bracts color was determined using a colorimeter (Konica Minolta Sensing, Inc.) operating in the CIE Lab system. Color measurement was performed with the sensor on the last inflorescence bract base surface (closed bract), based on the methodology proposed by McGuire (1992) and He *et al.* (2011). The following data were collected: L\*, lightness, representing interval between black and white; a\*, interval between green (-) and red (+); and b\*, interval between blue (-) and yellow (+). Data were subjected to analysis of variance (ANOVA) and means were compared by Student's t-test (P<0.05), using the statistical software (SISVAR, v. 4.0).

## RESULTS AND DISCUSSION

Throughout the evaluation period (10 months) we observed that plants derived from *in vitro* culture showed better vegetative growth than those grown from rhizomes (Figure 1A, 1B). Micropropagated plants may

have advantages in comparison to the conventional propagation units, namely: faster establishment, early shoot emission, higher production and vigor during growth (Drew & Smith, 1990; Teixeira & Bettiol Neto, 2011). In addition, they may produce approximately 30% more in the first cycles and allow synchronized harvests due to seedling homogeneity (Sanada, 1993).

Plant height increased up to the 180<sup>th</sup> day in both treatments and stopped at

approximately 210 days, when plants were 1 meter tall. Plant height remained until the end of the experiment (300 days) (Figure 2A), which may be related to the space limitation in the pots. These plants usually reach 2 to 3 meters tall, but plants grown in containers frequently have limited space to grow their roots (Kämpf, 2000) and this root restriction can reduce plant height and leaf area (Leles *et al.*, 1998). In addition, the container shape and size influence the water movement in the soil volume

(Kämpf, 2000; Vallone *et al.*, 2010).

The positive correlation between number of tillers and plant height (0.95\*\*) in plants derived from *in vitro* culture (Figure 2B) indicates that, in general, the plants producing many tillers show faster clump expansion. These results are consistent with those obtained by Rodrigues *et al.* (2006) who studied the same culture and observed the largest number of tillers as well as more flower stems in the treatment with micropropagated plants, which presented higher productivity in comparison to the conventional method. The number of tillers may actually help increasing the number of leaves and, therefore, lead to greater solar energy uptake, organic matter production and, consequently, to increased production of inflorescences (Assis *et al.*, 2009).

There was significant difference among the treatments regarding number of leaves and leaf area. The difference in number of leaves started decreasing from the 150<sup>th</sup> day on and that in leaf area started decreasing in the 180<sup>th</sup> day (Figure 2C, 2D). Although the number of leaves is not always an appropriate criterion to estimate plant growth among heliconias, this variable has direct importance in the clump development and in flower stem quality, since the leaves are responsible for producing energy in plant due to their high photosynthetic efficiency (Albuquerque *et al.*, 2010; Farias *et al.*, 2013).

At approximately 250 days, the presence of a reduced-limbo leaf (flag leaf) was observed, preceding the inflorescence. According to Criley & Broschat (1992), heliconia usually starts flowering after the plant reaches a considerable energy supply in order to start the reproduction process. The reproductive apex giving rise to the heliconia inflorescence is formed by the transformation of the terminal vegetative apex. This transition happens without external evidence until inflorescence becomes apparent.

Twenty percent (20%) of plants derived from *in vitro* culture issued the inflorescence tip at the 266<sup>th</sup> day (8 months and 13 days into the experiment). At the 275<sup>th</sup> day, the first bract was already open (Figure 1E). The harvest



**Figure 1.** Development of *H. bihai* cv. Lobster Claw Two plants in the greenhouse: rhizome-derived plants at 240 days in the experiment (A) and plant derived from the *in vitro* cultivation of zygotic embryos (B); Pseudostem of rhizome-derived plant (C) and of plant derived from the *in vitro* cultivation of zygotic embryos at 240 days into the experiment (D); Inflorescence obtained in the plant derived from the *in vitro* cultivation of zygotic embryos at 279 days into the experiment (E); Inflorescence at harvest point at 291 days into the experiment (F). (Photos by Ulysses, C.). Recife, UFRPE, 2014



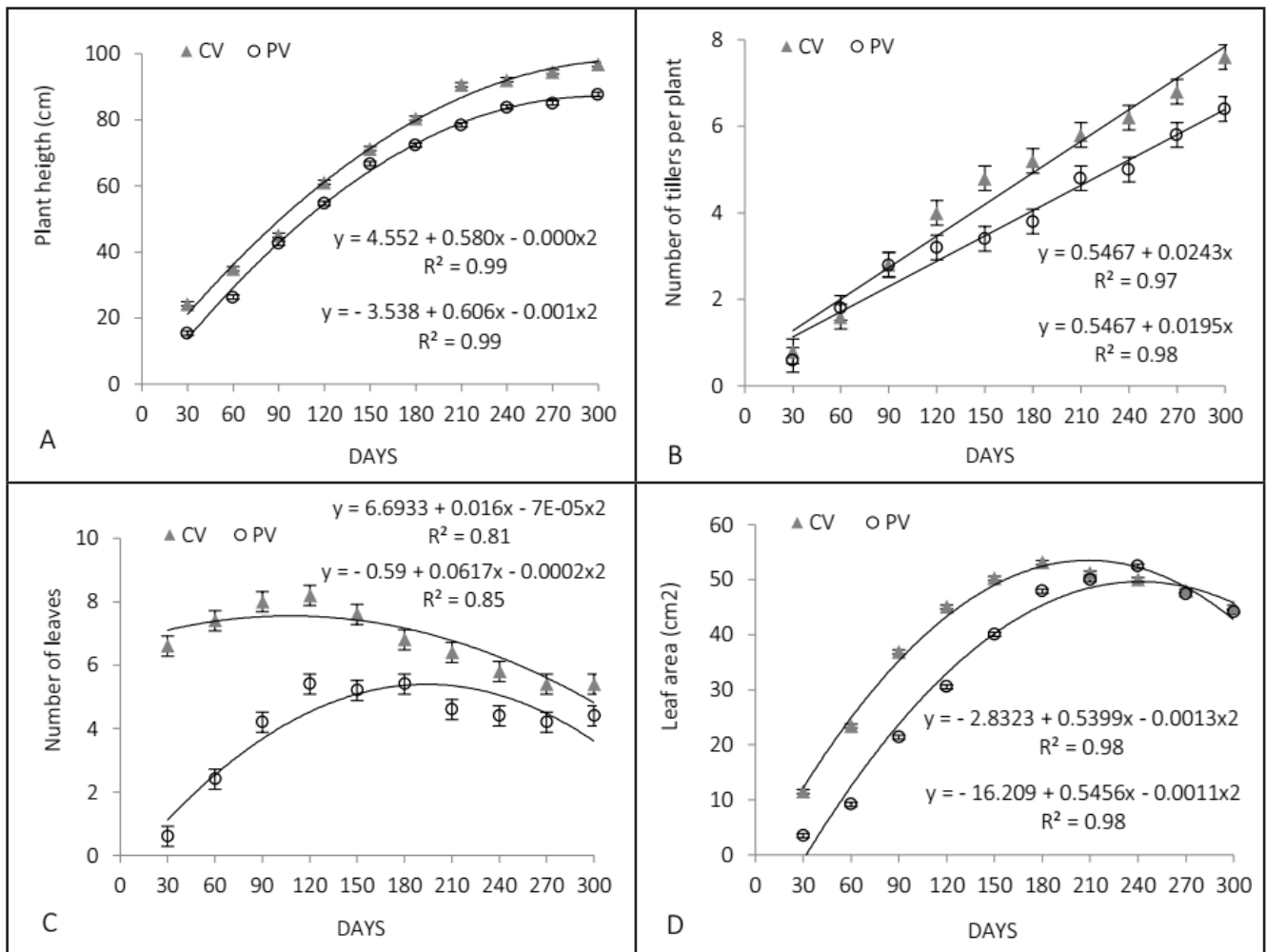
**Table 1.** Colorimetry for deployment of the post-harvest time variable in each multiplication method of *H. bihai* cv. Lobster Claw Two. (Summary ANOVA). Recife, UFRPE, 2014.

Variation sources	GL	Fc value	Prob.>Fc
<b>*L value</b>			
Post-harvest time	5	1.232	0.3145 ns
Multiplication methods	1	324.531	0.0000 **
Post-harvest time x Cultivation types	5	1.187	0.3347 ns
<b>*a value</b>			
Post-harvest time	5	0.850	0.5236 ns
Multiplication methods	1	338.901	0.0000 **
Post-harvest time x Cultivation types	5	0.809	0.5512 ns
<b>*b value</b>			
Post-harvest time	5	0.551	0.7365 ns
Cultivation types	1	94.000	0.0000 **
Post-harvest time x Cultivation types	5	0.411	0.8381 ns

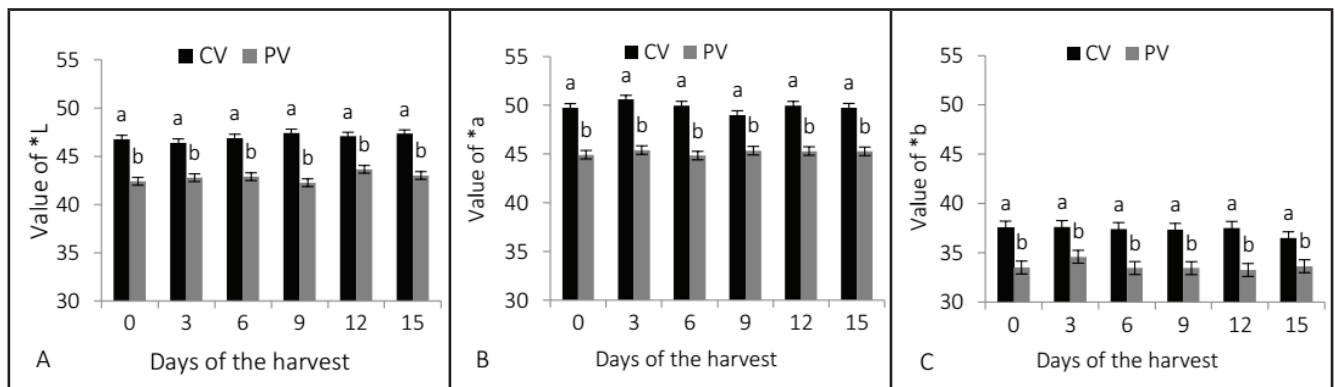
\*\*Significant at 1% probability; ns= non-significant.

point for the marketing (3 open bracts) of these inflorescences was achieved 25 days after inflorescence tip emergence. The obtained inflorescences showed mean size of 22.5 cm and the flower stems (inflorescence + stem) showed mean size of 55 cm (Figure 1F). Plants derived from rhizome propagation started issuing the inflorescence tip at the 310<sup>th</sup> cultivation day. Inflorescences showed mean size of 24.7 cm and flower stem showed mean size of 58 cm. Some authors (Drew & Smith, 1990; Álvares & Caldas, 2002) also observed that micropropagated banana plants flowered earlier than plants propagated by the conventional method.

Quality standard of flower stems



**Figure 2.** Biometric evaluation of *Heliconia bihai* cv. Lobster Claw Two plants according to cultivation time: VC= *in vitro* culture; VP= conventional vegetative propagation. Plant height (A), number of tillers (B), number of leaves (C), and leaf area (D). Standard error: A= 0.63563; B= 0.28460; C= 0.31780; D= 0.36804. Recife, UFRPE, 2014.



**Figure 3.** Colorimetry of *Heliconia bihai* cv. Lobster Claw Two plant preserved in distilled water 15 days after the harvest. L\* (A), a\* (B)\* and b\* (C) values. Equal letters do not statistically differ according to Student's t-test  $P < 0.01$ . OM - VC (L\*= 46.9; \*a= 49.8; \*b= 37.3), VP (\*L= 42.8; \*a= 45.2; \*b= 33.7). Standard error: \*L= 0.39921; \*a= 0.43813; \*b= 0.65560. Recife, UFRPE, 2014.

is very appreciated by producers, since the stem is the final product. Thus, the greatest vigor observed in heliconias derived from *in vitro* culture may be linked to phytosanitary status of seedlings, which reflects the quality of these plants and may justify productivity gains (Álvares & Caldas, 2002). The highest number of inflorescences and early flowerings represent lower production costs, greater competitiveness, more marketable products and, hence, higher profitability (Albuquerque *et al.*, 2010; Paulino, 2013). In addition, regarding the production process, precocity means opportunity for the producer to put the product on the market earlier, to anticipate the return of investment as well as to increase the flower productivity per area and per production period (Farias *et al.*, 2013).

About color intensity in the bracts, we observed that L\*, a\* and b\* values were not affected over time during the 15 post-harvest days, since no significant difference was found in the post-harvest time factor according to the F test, as observed in the analysis of variance (Table 1). In addition, no significant difference was found in the interaction between factors. Similar results were obtained by Costa *et al.* (2011) evaluating storage conditions of *Heliconia bihai* cv. Lobster Claw Two. The authors observed that color of bracts was not affected by storage time. However, it did not occur with the multiplication methods, which showed highly significant difference in

the same test.

The overall means (OM) observed for L\* were 42.8 (VP) and 46.9 (VC), showing intermediate shade between light and dark. Regarding a\*, the observed values were 49.9 (VP) and 45.19 (VC), thus indicating high red color intensity. For b\*, means were 33.6 (VP) and 37.2 (VC), showing slight tendency to yellow shade. Higher L\*, \*a and \*b values were observed in the colorimetry of plants derived from *in vitro* culture in comparison to those derived from vegetative propagation. It indicated that plants derived from *in vitro* culture showed more intense color (Figure 3). Although there was bract senescence, occurring in the form of dry tips, rather than in the form of color change. It was considered to be positive because tissue discoloration is undesirable, since color is one of the most used parameters after harvest and it is also the most attractive quality attribute to consumers (Kays, 1991).

These results allow inferring about the good growth and development of plants derived from *in vitro* culture, which showed higher development in all evaluated parameters when they were compared to plants derived from rhizome propagation. It showed that the *in vitro* method induced higher vegetative and reproductive growth. Similar results regarding plant vigor, derived from *in vitro* culture, were observed in other studies (Teixeira & Bettiol Neto, 2011; Rodrigues *et al.*, 2014). Seedlings derived from micropropagation showed active root

system and physiologically efficient leaf area from the time they were planted, fact that may justify the best initial plant performance (Drew & Smith, 1990).

Although plants derived from *in vitro* culture came from zygotic embryos, no changes were observed in the external morphology of the vegetative parts (pseudostem and leaves) or in the obtained inflorescences. Therefore, zygotic embryos could be used as an explant source to enable *in vitro* multiplication of this heliconia species.

In view of this, it is concluded that *H. bihai* cv. Lobster Claw Two plants derived from *in vitro* culture of zygotic embryos showed higher vegetative growth in all evaluated parameters, in comparison to the rhizome-derived plants. In addition, they did not show morphological changes in the vegetative and reproductive structures. Color intensity in the bracts was not affected during the 15-day-post-harvest period in the evaluated propagation methods and showed early flowering, and the first flowering showed small reduction in the reproductive structures size in comparison to that of rhizome-derived plants.

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