Ornamental Horticulture
ISSN 2447-536X | HTTPS://ORNAMENTALHORTICULTURE.EMNUVENS.COM.BR/RBHO
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

Scientific Article

Morphological and molecular characterization of native *Heliconia* sp. accessions of the Amazon region

Sarah Krause[1], Willian Krause[1], Eileen Azevedo Santos[1*], Ana Aparecida Rossi[2], Maria Helena Menezes Cordeiro[1], Celice Alexandre Silva[1]

[1] Universidade do Estado de Mato Grosso, Departamento Agronomia, Tangará da Serra-MT, Brasil.
[2] Universidade do Estado de Mato Grosso, Departamento Agronomia, Alta Floresta-MT, Brasil.

Abstract

Heliconias are tropical plants with ornamental potential. These plants are particularly used in the floriculture industry because of their exotic colors and shapes. Species characterization is important for the selection of genotypes for the ornamental plant market and subsequent application in studies of genetic improvement. The aim of this study was to estimate the genetic divergence of *Heliconia densiflora* and *Heliconia psittacorum* accessions based on quantitative morphological and molecular markers. The morphological and molecular descriptors revealed genetic variability among the accessions evaluated. The greatest genetic variability was observed among *H. psittacorum* accessions, whose sample number was also larger compared to *H. densiflora*. Morphological characterization was efficient in differentiating the two *Heliconia* species, especially to characteristics such as bract and inflorescence length, postharvest durability, and flower stem diameter, which contributed most to the divergence in this study. On the other hand, molecular characterization identified one *H. densiflora* individual that was grouped with the *H. psittacorum* genotypes. The results showed that ISSR markers can differentiate closely related *H. densiflora* and *H. psittacorum* individuals. The materials evaluated can contribute to the maintenance of local genetic diversity through the germplasm bank of the local breeding program of ornamental tropical plants.

Keywords: *Heliconia densiflora*, *Heliconia psittacorum*, genetic markers, molecular markers, tropical crops.

Resumo

Caracterização morfológica e molecular de acessos de *Heliconia* sp. nativas da região Amazônica

As Helicônias são plantas tropicais com potencial ornamental. Estas plantas são particularmente utilizadas na indústria da floricultura devido às suas cores e formas exóticas. A caracterização das espécies é importante para a seleção de genótipos para o mercado de plantas ornamentais e posterior aplicação em estudos de melhoramento genético. O objetivo deste estudo foi estimar a divergência genética de acessos de *Heliconia densiflora* e *Heliconia psittacorum* com base em marcadores morfológicos quantitativos e moleculares. Os descritores morfológicos e moleculares revelaram variabilidade genética entre os acessos avaliados. A maior variabilidade genética foi observada entre os acessos de *H. psittacorum*, cujo número amostral também foi maior em relação a *H. densiflora*. A caracterização morfológica foi eficiente na diferenciação das duas espécies de Heliconia, principalmente para as características comprimento das brácteas e inflorescências, durabilidade pós-colheita e diâmetro do caule da flor, que mais contribuíram para a divergência genética neste estudo. Por outro lado, a caracterização molecular identificou um indivíduo de *H. densiflora* que foi agrupado com os genótipos de *H. psittacorum*. Os resultados mostraram que os marcadores ISSR podem diferenciar indivíduos *H. densiflora* e *H. psittacorum* intimamente relacionados. Os materiais avaliados podem contribuir para a manutenção da diversidade genética local por meio do banco de germoplasma do programa de melhoramento local de plantas ornamentais tropicais.

Palavras-chave: culturas tropicais, *Heliconia densiflora*, *Heliconia psittacorum*, marcadores genéticos, marcadores moleculares.

*Corresponding author: eileen.azevedo@unemat.br

https://doi.org/10.1590/2447-536X.v29i2.2578
Received Dec 09, 2022 | Accepted June 14, 2023 | Available online June 27, 2023

Editor: Bruno Trevenzoli Favero

Introduction

Heliconias (Heliconiaceae) comprise approximately 450 species and approximately 200 hybrids. Of these, 25 species naturally occur in the Brazilian Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pantanal biomes (Flora do Brasil, 2022 under construction). *Heliconia densiflora* Verl. and *H. psittacorum* L.f. are adapted to the edaphoclimatic conditions found in the mid-west of Brazil and are widely distributed throughout the national territory. The characteristics of these species are considered adequate to serve the ornamental flower market: high productivity, color variety, and light stems (Gomes et al., 2016; Silva et al., 2017). The ornamental importance of heliconias is mainly related to their exotic colors and shapes, postharvest stem durability, and resistance to transport.

The possibility of evaluating the performance of genotypes in early stages allows breeders to concentrate efforts and resources on the combinations with the greatest breeding potential. This strategy is of great value for breeding programs. High experimental efficiency and appropriate selection methods allow the selection of superior individuals in early generations. The genetic variability in a population can be quantified by diversity analysis. The aim of such studies is to help identify genotypes that are genetically superior and distant from each other and that will be part of the next generation. Genetic distance can be assessed based on morpho-agronomic, morphological and molecular characteristics using different multivariate methods (Constantino et al., 2020).

Grouping individuals requires a measure of dissimilarity between the evaluated individuals. The standardized average Euclidean distance is the measure most widely used for the characterization of germplasms of perennial plants (Cruz et al., 2011; Pereira et al., 2016). Methods for morphological characterization estimate the genetic variability among genotypes present in germplasm banks using qualitative and/or quantitative descriptors of interest (Oliveira et al., 2022). However, these markers are modified by the environment, reducing the selection efficiency. With the advancement of molecular biology techniques, selection has become more accurate, especially by the use of molecular markers that allow the detection of polymorphisms at the DNA level (Marulanda et al., 2018).

Molecular markers, such as ISSRs have been used frequently in studies of genetic diversity in ornamental plants (Pereira et al., 2016; Villanueva-Viramontes at al., 2017). Inter-simple sequence repeats (ISSRs) are the molecular markers most widely used in studies of genetic diversity. The amplification of ISSRs does not depend on genomic knowledge of the evaluated species; it is also an informative technique that has higher temperature specificity and provides results with high reproducibility and polymorphism indexes.

The aim of this study was to estimate the genetic divergence of *Heliconia* sp. accessions conserved in the UNEMAT germplasm bank, based on morphological and molecular ISSR markers in order to enable the future use of the species in breeding programs.

Materials and Methods

Study location and population

Fourteen accessions of the Heliconiaceae family (Table 1) were evaluated: three *H. densiflora* accessions and eleven *H. psittacorum* accessions. These accessions were collected in 12 municipalities in the state of Mato Grosso. Seven of these municipalities are located in the northern region of the state (Alta Floresta, Carlinda, Colíder, Guarantã Norte, Matupá, Peixoto Azevedo, and Terra Nova do Norte), where vegetation of the Amazon biome predominates. The other five municipalities are located in the southwestern region of the state (Barra do Bugres, Nova Marilândia, Porto Estrela, Santo Afonso, and Tangará da Serra). Their vegetation corresponds to the transition between the Cerrado and Amazon Forest biomes and is also influenced by the Pantanal biome.

Table 1. Collection locations of *Heliconia densiflora* and *H. psittacorum* in the state of Mato Grosso, deposited in the Active Germplasm Bank of Ornamental Tropical Flowers at the State University of Mato Grosso, Tangará da Serra Campus/MT, 2020.

Species	Accession (Nº.)	Municipality	Latitude	Longitude	Altitude (m)
Heliconia densiflora	1	Alta Floresta	9° 51' 05"	56° 12' 31"	281
	2	Alta Floresta	9° 51' 47"	56° 12' 04"	271
	3	Carlinda	10° 10' 58"	55° 48' 53"	299
Heliconia psittacorum	4	Colíder	10° 46' 55"	55° 27' 00"	310
	5	Matupá	10° 12' 26"	54° 57' 39"	260
	6	Guarantã do Norte	9° 46' 02"	54° 53' 55"	348
	7	Peixoto Azevedo	10° 16' 59"	55° 01' 15"	324
	8	Terra Nova do Norte	10° 44' 45"	55° 08' 43"	295
	9	Santo Afonso	14° 35' 59"	57° 10' 56"	494
	10	Nova Marilândia	14° 21' 05"	57° 02' 01"	255
	11	Tangará da Serra	14° 42' 02"	57° 47' 31"	204
	12	Barra do Bugres	15° 07' 46"	57° 04' 34"	156
	13	Porto Estrela	15° 18' 51"	57° 10' 11"	168
	14	Porto Estrela	15° 24' 02"	57° 11' 51"	148

The collected accessions were deposited in the Active Germplasm Bank (BAG) of Ornamental Tropical Flowers (implemented in March 2014) in the experimental field of the State University of Mato Grosso, located in the municipality of Tangará da Serra, Mato Grosso state (14º39' S and 57º25' W, altitude of 321 m). The region's climate is classified as tropical, with an average annual rainfall of 1,800 mm. There are two well-defined climate seasons, a dry season from May to September and a rainy season from October to April (Martins et al., 2010).

Rhizomes were planted using a plant spacing of 3.0 m between rows and 1.5 m between plants in full sunlight. In fertilization of planting, we applied 50 g of monoammonium phosphate (MP) per pit. MP, urea and potassium chloride were applied monthly as fertilizers. A micro-sprinkler system, with one microjet per pit, was used for crop irrigation, which was performed three times per week. Pruning, insecticide and fungicide applications, and other crop treatments were performed according to recommendations for the cultivation of tropical flowers (Silva et al., 2017).

Morphological characterization

The morphological characteristics were evaluated in the first and second year after planting. Five flower stems per clump (plot) and eight clumps per accession were evaluated in March and April 2016. We followed the protocol of Silva et al. (2017) for morphological characterization; flower stems that had two or three open bracts were harvested 20 cm from the soil surface, twice a week between 7:00 and 8:00 am, and stored in containers with water for transportation to the postharvest laboratory.

For the evaluation of morphological characteristics, a tape measure was used to determine floral stem length (FSL), inflorescence width (IW), inflorescence length (IL), and bract length (BL). The floral stem diameter (FSD) was measured with a digital caliper. The fresh mass of the leafless floral stem (MLS) was measured using a digital scale. The number of inflorescence bracts (NIB) was recorded manually, counting each bract as corresponding to one unit. The postharvest durability of flower stems (PHD) was evaluated for 21 days as described by Silva et al. (2017). According to these authors, a temperature of 19 °C and relative humidity of 80% in a cold room are the best conditions for storing *H. densiflora* and *H. psittacorum* flower stems. The criterion adopted for the evaluation of floral longevity followed the four-point scale proposed by Costa et al. (2011) and adapted by Silva et al. (2017).

Molecular characterization

Samples of young leaves from the 14 *Heliconia* accessions were collected, wrapped in aluminum foil, identified, and dipped into dry ice to avoid DNA degradation. This material was stored in an ultra-freezer at a temperature of -86 °C in the Molecular Biology laboratory.

DNA was extracted according to the CTAB protocol (cationic hexadecyltrimethylammonium bromide) described by Doyle and Doyle (1987), with some modifications, including the addition of 2% polyvinylpyrrolidone and an increase from 2 to 5% in CTAB concentration and from 0.2% to 2% in β-mercaptoethanol concentration in the extraction buffer.

The following nine ISSR primers, developed by the University of British Columbia (UBC), Vancouver, Canada (Table 2), were used for DNA amplification at an annealing temperature of 52 °C: UBC 807 - Di (AG) 8 3'T; UBC 808 - Di (AG) 8 3'C; UBC 809 - Di (AG) 8 3'G; UBC 810 - Di (GA) 8 3'T; UBC 812 - Di (GA) 8 3'A; UBC 815 - Di (CT) 8 3'G; UBC 840 - Di (GA) 8 3'YT; UBC 841 - Di (GA) 8 3'YC, and UBC 842 - Di (GA) 8 3'YG.

Amplification by the polymerase chain reaction (PCR) was performed in a final volume of 13.5 µL containing 1.5 µL of 10x buffer (1 M KCl, 1 M Tris, pH 8.3, 10% Tween 20), 1.5 µL $MgCl_2$ (25 mM), 1.5 µL of each primer (0.2 mM), 1.5 µL of dNTP (1 mM of each dNTP), 0.5 µL DNA at a concentration of 5 ng, 0.12 µL Taq polymerase (5 U μL^{-1}), and ultrapure water.

Table 2. ISSR primers used for the molecular characterization of *Heliconia densiflora* and *H. psittacorum* deposited in the Active Germplasm Bank of Ornamental Tropical Flowers at the State University of Mato Grosso, Tangará da Serra Campus/MT, 2020.

Primers	*Heliconia densiflora*			*Heliconia psittacorum*		
	NL	%P	PIC (%)	NL	%P	PIC (%)
UBC 807	12	58.33	0.20	20	55.00	0.26
UBC 808	8	87.50	0.30	6	100.00	0.28
UBC 809	6	66.66	0.23	8	87.50	0.28
UBC 810	5	60.00	0.21	7	85.71	0.22
UBC 812	6	50.00	0.17	14	100.00	0.28
UBC 815	9	100.00	0.35	10	90.00	0.24
UBC 840	4	75.00	0.26	6	83.33	0.24
UBC 841	14	71.43	0.25	19	94.74	0.21
UBC 842	18	94.44	0.33	21	90.48	0.23
Mean	9.10	71.25	0.26	12.33	86.50	0.25
I	0.297				0.198	
H	0.198				0.262	

NL: number of loci; % P: percentage of polymorphism; PIC: polymorphic information content. I: Shannon-Weaver diversity index; H: genetic diversity of Nei index for *Heliconia densiflora* (N= 3) and *Heliconia psittacorum* (N=11) populations.

The reactions were carried out in an Eppendorf thermal cycler using the program proposed by Rocha et al. (2017). The amplification products were separated by electrophoresis on 1.5% agarose gel with 1X TBE buffer in an LCH 20x25 horizontal electrophoresis system (Loccus Biotecnologia®) at constant voltage of 80 V. The amplified fragments (bands) were analyzed using the 100-bp DNA Ladder as marker.

The gels were stained with ethidium bromide (0.6 µg mL^{-1}) for 20 minutes. The gels were then visualized, photographed, and edited using an LTB-20x20 STi UVB light transilluminator, a photo-documenter, and the L-Pix STi software (Loccus Biotecnologia®).

Statistical analysis

We used descriptive statistics (minimum, maximum, mean, standard deviation, and coefficient of variation) to assess the eight morphological characteristics with the Genes software (Cruz, 2013).

The most consistent and most evident bands were assessed visually for molecular characterization of the 14 accessions studied. A binary matrix was constructed for analysis of the molecular data, with 1 indicating the presence of a band and 0 indicating the absence of bands.

The Shannon-Weaver diversity index (I) (Shannon and Weaver, 1949), Polymorphic information content (PIC) and Nei genetic diversity (H) (Nei, 1978) were determined based on the information provided by the polymorphic primers. The analyses were performed using the Genalex 6.5 program (Peakall and Smouse, 2012).

Principal component analysis was performed using the PAST 3.12 software (Hammer et al., 2001). Data were divided into three groups: molecular (Jaccard index), morphological (standardized Euclidean distance), and simultaneous morphological and molecular data (variance-covariance matrix). The purpose of principal component analysis was to represent the distribution of species of the genus *Heliconia* in a two-dimensional Cartesian plane.

Results

Morphological characterization

The floral stems of *H. densiflora* were shorter and thinner and their inflorescences contained a smaller number of bracts when compared to the stems and inflorescences of *H. psittacorum*. The inflorescences of *H. densiflora* were arranged in aggregates, with an inflorescence width (IW) that was 46.77% smaller than that of *H. psittacorum* (Table 3). The lengths of *H. densiflora* inflorescences (IL) and bracts (BL) were approximately 69% and 75% greater than those of *H. psittacorum* (Table 3), respectively. The weight and postharvest durability (PHD) of *H. densiflora* stems were approximately 61% and 63% lower than those of *H. psittacorum* stems (Table 3).

Table 3. Mean values of floral morphological characteristics in the first and second year after planting populations of *Heliconia densiflora* and *H. psittacorum* obtained from the Active Germplasm Bank of Ornamental Tropical Flowers at the State University of Mato Grosso, Tangará da Serra Campus/MT, 2020

Characteristic	*Heliconia densiflora*			*Heliconia psittacorum*		
	Minimum-Maximum	Mean (SD)	CV (%)	Minimum-Maximum	Mean (SD)	CV (%)
FSL (m)	0.77 - 1.46	1.11 (0.04)	0.71	0.64 - 2.27	1.51 (0.04)	11.82
FSD (mm)	3.72 - 6.14	4.70 (0.16)	8.63	2.92 - 5.60	4.14 (0.07)	10.23
NBI (u)	2.00 - 5.20	3.23 (0.22)	14.55	2.00 - 8.00	4.45 (0.17)	16.36
IW (cm)	2.28 - 8.56	4.20 (0.32)	29.99	4.00 - 18.50	8.98 (0.31)	19.16
IL (cm)	13.80 - 26.20	17.80 (0.66)	9.20	8.70 - 20.50	12.28 (0.23)	9.45
BL (cm)	12.00 - 23.40	15.52 (0.63)	10.92	8.02 - 17.55	10.74 (0.18)	8.85
MLS (g)	36.40 - 142.20	63.48 (5.61)	27.54	24.00 - 188.60	104.33 (4.72)	20.07
PHD (days)	6.00 - 7.00	6.55 (0.06)	3.79	7.00 – 19.00	10.35 (0.31)	23.03

SD: standard deviation; CV: coefficient of variation; FSL: floral stem length; FSD: flower stem diameter; NBI: number of bracts in the inflorescence; IW: inflorescence width; IL: inflorescence length; BL: bract length; MLS: mass of leafless floral stem; PHD: postharvest durability of the stem.

The *H. densiflora* accessions showed coefficients of variation ranging from 0.71 to 29.99%; the greatest variations were observed for floral stem mass without leaves (MLS) and inflorescence width (IW). Regarding *H. psittacorum* accessions, the coefficients of variation for the same characteristics ranged from 8.85 to 23.03% (Table 2).

The coefficients of variation for floral stem mass without leaves (MLS) were high in both species (Table 3). The highest within-species coefficients of variation were found for inflorescence width (IW) of *H. densiflora* accessions and for postharvest durability (PHD) of *H. psittacorum* accessions (Table 3). The lowest within-species coefficients of variation were found for floral stem length (FSL) and postharvest durability (PHD) of *H. densiflora* accessions and for inflorescence length (IL) and bract length (BL) of *H. psittacorum* accessions (Table 3). Coefficients of variation between 20 and 30% were defined as high and indicated the degree of heterogeneity between the genotypes evaluated (Table 3).

Principal component analysis of the morphological characteristics showed the formation of four main groups and explained 83% of the total variation (Figure 1). The component 1 explained most of the variation (53.93%) and the variables IL (-0.42) and BL (-0.41) were the ones that most contributed to this variation, whose vector intensity increases in the direction of the *H. densiflora* genotypes, which stood out due to their larger size of bracts and inflorescence. In turn, the PHD (-0.58) and FSD (0.57) traits contributed most to explain the variation in the second component (29.08%). Note that the vectors for these traits, IW, MLS and NBI increase towards the species *H. psittacorum*, which share the highest mean values for these traits.

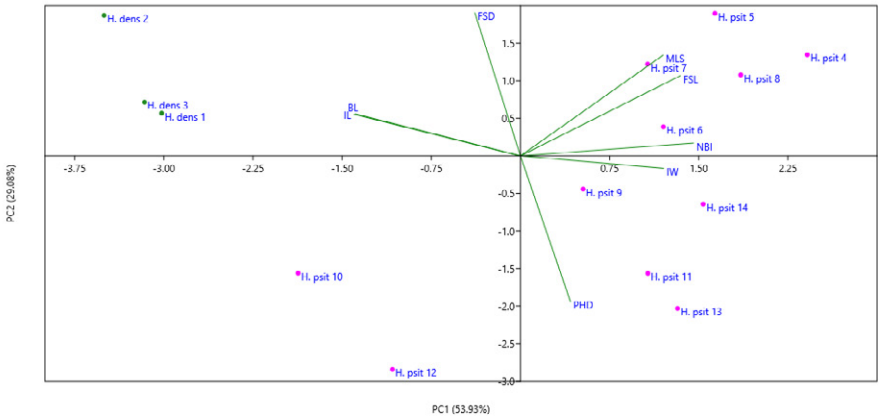

Figure 1. Principal components analysis among 14 Heliconia genotypes considering the morphological variables based on the standardized Euclidean distance, obtained by PAST software, version 3.12 (State University of Mato Grosso, Tangará da Serra Campus/MT, 2020).

All *H. densiflora* accessions (1, 2, and 3) were found in the first quadrant (Figure 1). The accessions were collected in the northern region of the state of Mato Grosso, in the municipalities of Alta Floresta and Carlinda. These municipalities are located 36 km apart inside the Amazon biome in an open rainforest environment.

The second quadrant was formed by five genotypes of *H. psittacorum* (4, 5, 6, 7, and 8). In this group, 55% of the accessions were from the northern region of the state of Mato Grosso, which is influenced by the Amazon biome (Figure 1). The third quadrant contained 45% of accessions from the southwestern region (9, 11, 13, and 14), which is characterized by transition forest between Cerrado and Amazon Forest. These two regions are located approximately 800 km apart.

Finally, accessions 10 and 12 of *H. psittacorum* were found in the fourth quadrant. These accessions were collected in the southwestern region of Mato Grosso, in the municipalities of Nova Marilândia and Barra do Bugres, respectively (Figure 1). The municipalities are located 102 km apart in regions with a seasonal semideciduous forest type vegetation. This structure was due to a shorter floral stem length (FSL), a smaller number of inflorescence bracts (NBI), lower fresh mass of the floral stem (MLS), and higher inflorescence length (IL), bract length (BL) and postharvest durability (PHD).

Molecular characterization

In *H. densiflora*, the nine ISSRs used as primers amplified 82 fragments, with a mean number of 9.10 loci per primer; of these, 76.83% were polymorphic. The number of amplified bands (number of loci) ranged from 4 to 18. The polymorphic information content ranged from 0.17 to 0.33%, with a mean of 0.26% (Table 2).

In *H. psittacorum*, the ISSR primers amplified 111 fragments, with a mean number of 12.3 loci per primer; of these, 87.39% were polymorphic. There were 26.13% more fragments than in the *H. densiflora* accessions. The number of loci ranged from 6 to 21. The polymorphic information content ranged from 0.21 to 0.283%, with a mean of 0.25% (Table 2).

The Shannon-Weaver (I) and Nei (H) indices indicated genetic differences in *H. densiflora* (I=0.297 and H=0.198) and *H. psittacorum* (I=0.401 and H=0.262) at the molecular level (Table 2).

Principal component analysis of the molecular data was efficient in discriminating *Heliconia* accessions in a two-dimensional plane. The first two components together explained 41.91% of the total variance, which was spread evenly among components (Figure 2). Accessions 1 and 2 of *H. densiflora* were close to *H. psittacorum* individuals 10 and 12, occupying the first and third quadrants. On the other hand, *H. densiflora* 3 was assigned to the fourth quadrant, closer to the other *H. psittacorum* accessions that occupied the second and fourth quadrants (Figure 2). There was greater dispersion of *H. psittacorum* accessions, which were distributed across all quadrants of the plane, indicating greater variability.

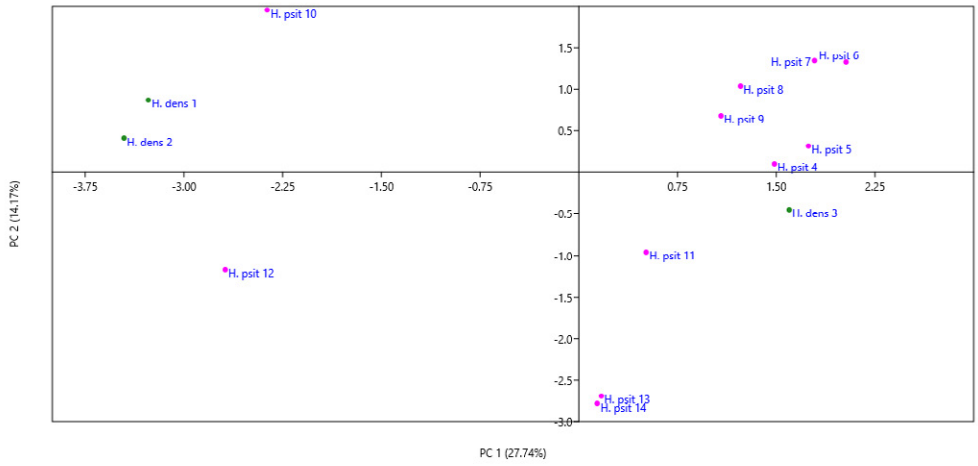

Figure 2. Principal components analysis among 14 Heliconia genotypes considering the ISSR molecular markers based on the Jaccard distance, obtained by PAST software, version 3.12

Simultaneous analysis of the molecular and morphological variables showed dispersion similar to the phenotypic traits. The first component explained 97.77% of the total variance, while component 2 only explained 1.15% (Figure 3). The *H. densiflora* accessions occupied the first quadrant and were close to individuals 10 and 12 of *H. psittacorum*, while the other *H. psittacorum* accessions were distributed in all quadrants (Figure 3). The MLS variable contributed 99.73% of the total variation in the first component and IL with 61.52% in the second. When analyzed together, the molecular variables were less important for explaining the total variation considering the whole data set.

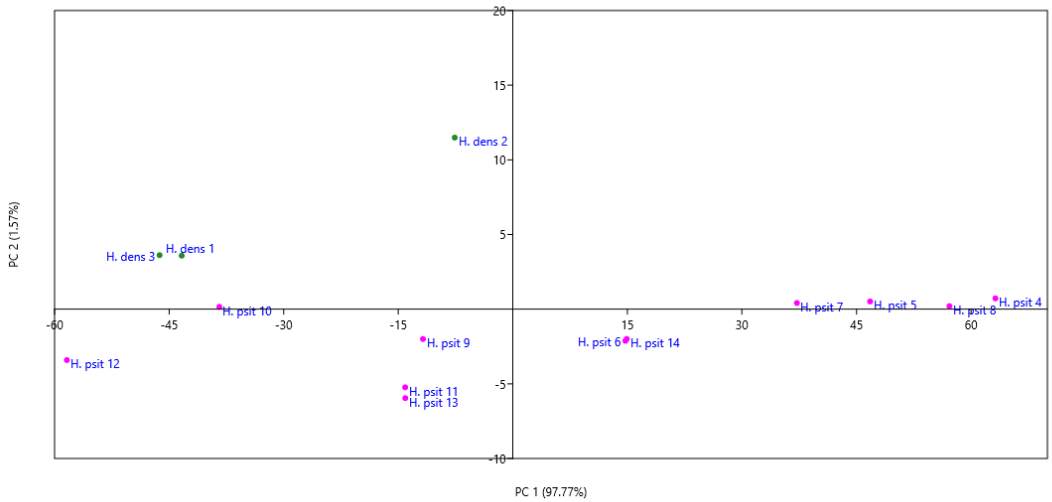

Figure 3. Principal components analysis among 14 Heliconia genotypes considering the morphological and molecular variables based on the variance-covariance matrix, obtained by PAST software, version 3.12 (State University of Mato Grosso, Campus of Tangará da Serra / MT, 2020).

Discussion

Morphological characterization

Morphological variability was found in all *Heliconia densiflora* and *H. psittacorum* accessions. The greatest morphological variability was observed among *H. psittacorum* accessions, whose sample number was also larger compared to *H. densiflora*. This fact may have influenced the biometric data since there were only three *H. densiflora* individuals.

The morphological variability among the *H. densiflora* and *H. psittacorum* accessions allows selection of genotypes with desirable characteristics for the ornamental flower market and for breeding programs of tropical

flowers (Silva et al., 2017). These characteristics include length and diameter of the floral stem, inflorescence length, fresh mass, and postharvest durability (Silva et al., 2019).

Stem resistance to lodging is related to the length and diameter of the floral stem. This desirable resistance condition also influences transport, treatment, selection, packaging, and postharvest durability. The latter is influenced by the carbon reserve of flower stems, which contributes to extend postharvest durability (Raizer et al., 2019). Floral stem length was the characteristic that showed the greatest variation among the accessions studied. Pocomucha and Rios (2017) reported a similar result in a study of 22 Heliconiaceae species, including *H. densiflora* and *H. psittacorum*. The selection of accessions with stems larger than 80 cm is desirable for the tropical flower market since this stem length facilitates the production of floral arrangements (Silva et al., 2019).

Inflorescence length is related to the use of species in floral arrangements and decorations and to appreciation by consumers (Avendaño-Arrazate et al. 2017). The length of inflorescences of the *H. densiflora* and *H. psittacorum* accessions was similar to that reported by Silva et al. (2017) and Raizer et al. (2019). The fresh mass of the floral stem found in the present study in the *H. densiflora* and *H. psittacorum* accessions also met the required commercial standards. The longevity of inflorescences is directly related to stem mass; flower stems with a greater mass contain a higher amount of carbohydrates and consequently exhibit longer postharvest durability (Silva et al., 2019).

The diversity of bract colors is another morphological characteristic that highlights *H. densiflora* and *H. psittacorum*. These morphological characteristics are important for the decoration and landscaping sectors. The bracts of *H. densiflora* have a red to orange color with orange flowers (Silva et al., 2017). The bracts of *H. psittacorum* are red, yellow, pink, and orange (Nascimento et al., 2018).

Molecular characterization

Dominant markers have been frequently used in the study of plant diversity. One of the most commonly employed methods to estimate within-population diversity is Nei's genetic diversity. The genetic variability among *H. densiflora* (0.198) and *H. psittacorum* (0.262) revealed by the molecular markers was higher than that reported for *Heliconia bihai*, *H. chartacea* and *H. wagneriana* in Pernambuco, Brazil, whose mean Nei index was 0.103 (Pereira et al., 2016).

The results showed that ISSR markers can differentiate individuals of closely related *H. densiflora* and *H. psittacorum*. The genetic similarities between the species studied may be related to their phylogenetic relationships of kinship (Iles et al. 2017). This may be due to the sharing of the same genomic regions accessed between species belonging to the same genus or family. Similar to the results of the present study, Marouelli et al. (2010) and Iles et al. (2017) observed that *H. densiflora* and *H. psittacorum* belonging to the same subgenus (*Stenochlamys*) were found in the same group. The present results also agree with Ángel et al. (2017) who evaluated *H. bihai*, *H. caribaea*, *H. orthotricha* and *H. stricta* hybrids belonging to the subgenus *Heliconia*. These hybrids were also found in the same group.

Comparison of the results of phenotypic, molecular and combined evaluation showed that morphological characterization separately grouped the two *Heliconia* species, with bract and inflorescence length, postharvest durability and flower stem diameter contributing most to the divergence in this study. On the other hand, molecular characterization grouped one *H. densiflora* accession with the *H. psittacorum* genotypes. Despite morphological differences, these individuals may have high genetic similarity since they share the same genomic regions analyzed in this study. Another factor that must be considered is the occurrence of natural interspecific hybridization since they are cross-pollinating and genetically compatible species (Marouelli et al., 2010). In this case, *H. densiflora* 3 would be a possible hybrid between *H. densiflora* x *H. psittacorum* as well *H. psittacorum* 10 and 12 which were closer to accessions 1 and 2 of *H. densiflora*. However, this possibility needs to be investigated.

Malakar et al. (2022) assert that there are no sufficient scientific reports on successful Heliconia hybrids production nor much genomic and cytogenetic characterization of them are available. Although there are no studies in the literature on natural hybrids of *H. psittacorum* x *H. densiflora*, natural hybrids of *H. bihai* x *H. caribaea* ('Bubble Gum' and 'Prince of Darkness') have been recorded in Puerto Rico (Heliconia International Society [HIS], 2015).

Different molecular cytogenetic techniques like in situ hybridization (Fluorescence and Genomic) can assist in chromosomal evaluation, cytogenetical classification, understanding in genomic constitution, polyploidy confirmation, gene introgression, and hybrid paternity confirmation (Malakar and Biswas, 2022). Costa et al. (2016) used FISH method using 45S and 5S rDNA probes to clarify the origin of different Heliconia hybrids (triploids and diploids) such as 'Sassy' (3n) (parent- diploid genotypes of *H. psittacorum*), 'Suriname Sassy' (3n) (parent- diploid genotypes of *H. psittacorum*), 'Golden Torch' (2n) (*H. psittacorum* x *H. spathocircinata*), 'Golden Torch Adrian' (2n) (*H. psittacorum* x *H. spathocircinata*) and 'Jacquinii' (2n) (*H. caribaea* x *H. bihai*). Therefore, different molecular cytogenetic techniques like in situ hybridization (Fluorescence and Genomic) could effectively assist in hybrid paternity confirmation of accessions 10 and 12 of *H. psittacorum* and *H. densiflora* 3.

Conclusions

The morphological and molecular descriptors are efficient in detecting genetic divergence between *H. densiflora* and *H. psittacorum* accessions available in the UNEMAT germplasm bank, which can provide valuable resources for breeding programs. The ISSR markers were able to differentiate closely related *H. densiflora* and *H. psittacorum* accessions. The hybrid paternity confirmation of accessions 10 and 12 of *H. psittacorum* and *H. densiflora*

3 needs to be investigated. The *H. densiflora* and *H. psittacorum* accessions can be used to maintain diversity in germplasm banks, as well as in genetic improvement programs of ornamental tropical plants.

Author Contribution

SK: Experimental execution, evaluation, literature review and writing. **WK**: Planning, experimental, field work literature review. **EAS**: Data analysis, literature review and writing. **AAR**: Data analysis, literature review. **MHM**: Field work and data capture, literature review. **CAS**: Research project coordinator, processing and data analysis, literature review and writing.

Acknowledgments

The authors would like to thank FAPEMAT for the scholarship of the first author, and the students of the botanical laboratory of the Center for the Study of Agricultural Research and Development (CPEDA) for helping in the field, and the Genetics and Plant Breeding Program (PGMP) of Mato Grosso State University.

References

AVENDAÑO-ARRAZATE, C.H.; ARRAZATE-ARGUETA, J.A.; ORTÍZ-CURIEL, S.; MORENO-PÉREZ, E.; IRACHETA-DONJUAN, L.; REYES-LÓPEZ, D.; CORTÉS-CRUZ, M. Morphological characterization in wild species of Heliconias (*Heliconia* spp) in Mexico. **American Journal of Plant Sciences**, v.8, n.6, p.1210, 2017.

ÁNGEL, M.L.M.; ISAZA, L.; LÓPEZ, P.A. Caracterización de la diversidad genética de cultivares comerciales de heliconias en el centro occidente de Colombia. **Revista de Ciências Agrícolas**, v.42, n.1, p.7-20, 2018.

COSTA, A.S.; NOGUEIRA, L.C.; SANTOS, V.F.; CAMARA, T.R.; LOGES, V.; WILLADINO, L. Storage of cut *Heliconia bihai* (L.) cv. Lobster Claw flowers at low temperatures. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.15, n.9, p.966-972, 2011.

COSTA, M.G.D.S.; LEITE, B.S.F.; LOGES, V.; SILVA, E.B.C.; COSTA, A.S.; GUIMARÃES, W.N.R.; BRASILEIRO-VIDAL, A.C. Chromosome markers confirm origin of *Heliconia* hybrids and triploids. **Euphytica**, v.212, p.501-514, 2016.

CRUZ, C.D.; FERREIRA, F.M.; PESSONI, L.A. **Biometria aplicada ao estudo da diversidade genética**. Viçosa: Produção Independente, 2011. 620p.

CRUZ, C.D. GENES - a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum**. v.35, n.3, p.271-276, 2013. http://doi.org/10.4025/actasciagron.v35i3.21251

DOYLE, J.J.; DOYLE, J.L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. **Phytochemical Bulletin** v.19, p.11-15, 1987.

FLORA E FUNGA DO BRASIL. Jardim Botânico do Rio de Janeiro. Available at: < http://floradobrasil.jbrj.gov.br/ >. Accessed on: December 06, 2022

GOMES, R.J., GUISELINI, C.; SIQUEIRA, G.M.; ALBUQUERQUE FILHO, J.C.C.; LOGES, V.; PANDORFI, H. Temporal stability of *Heliconia spp*. flower stem production. **Ornamental Horticulture**, v.22, n.3, p.318-325, 2016. https://doi.org/10.14295/oh.v22i3.943

HAMMER, Ø.; HARPER, D.A.T.; P.D. RYAN. PAST: Paleontological statistics software package for education and data analysis. **Paleontologia Electronica**, v.4, n.1, p.9, 2001.

HSI (Heliconia Society International) (2015) 12(1): 1–22. Available at: <http://www.cedaf.org.do/eventos/cfcs_2019/presentaciones/orales/RN_01.pdf>. Accessed on: Jun 12, 2023.

ILES, W.J.; SASS, C.; LAGOMARSINO, L.; BENSON-MARTIN, G.; DRISCOLL, H.; SPECHT, C.D. The phylogeny of *Heliconia* (Heliconiaceae) and the evolution of floral presentation. **Molecular Phylogenetics and Evolution**, v.117, p.150-167, 2017. https://doi.org/10.1016/j.ympev.2016.12.001

CONSTANTINO, L.V.; FUKUJI, A.Y.S.; ZEFFA, D.M.; BABA, V.Y.; CORTE, E.D.; GIACOMIN, R.M.; GONÇALVES, L.S.A. Genetic variability in peppers accessions based on morphological, biochemical and molecular traits. **Bragantia,** v.79, p.558-571, 2020.

MALAKAR, M.; BISWAS, S. Heliconias: dramatic flowers of the tropics and subtropics. In: DATTA, S.K.; GUPTA, Y.C. (eds). **Floriculture and Ornamental Plants. Handbooks of Crop Diversity: Conservation and Use of Plant Genetic Resources**. Singapore: Springer, 2022. 270p.

MALAKAR, M.; BERUTO, M.; BARBA-GONZALEZ, R. Biotechnological approaches to overcome hybridization barriers and use of micropropagation tool for further improvement in Heliconia: a review. **Plant Cell, Tissue and Organ Culture**, v.149, p.503-522, 2022. https://doi.org/10.1007/s11240-022-02300-w

MAROUELLI, L.P.; INGLIS, P.W.; FERREIRA, M.A.; BUSO, G.S. Genetic relationships among *Heliconia* (Heliconiaceae) species based on RAPD markers. **Genetics and Molecular Research**, v.9, n, 3, p.1377-1387, 2010. https://doi.org/10.4238/vol9-3gmr847

MARTINS, J.Á.; DALLACORT, R.; INOUE, M.H.; SANTI, A.; KOLLING, E.M.; COLETTI, AJ. Probabilidade de precipitação para a microrregião de Tangará da Serra, Estado do Mato Grosso. **Pesquisa Agropecuária Tropical**, v.40, p.291-296, 2010.

MARULANDA, M.L.; ISAZA, L.; LÓPEZ, P.A. Caracterización de la diversidad genética de cultivares comerciales de heliconias en el centro occidente de Colombia. **Agronomía Costarricense,** v.42, n.1, p.7-20, 2018.

NASCIMENTO, T.O.; SILVA, P.C.; LOGES, V.; MARIOTTO, S.; KRAUSE, W.; SILVA, C.A. Secondary pollen presentation and floral traits of *Heliconia psittacorum*. **Ornamental Horticulture**, v.24, n.4, p.451-458, 2018. http://dx.doi.org/10.14295/oh.v24i4.1227

NEI, M. Estimation of average heterozygozity and genetic distance from small number of individuals. **Genetics**, v.89, p.583-590, 1978.

OLIVEIRA, J.A.V.S.; SANTOS, E.A.; VIANA, A.P.; WALTER, F.H.B.; RIBEIRO, R.M. Genetic-molecular characterization in guava full-sib progeny. **Bragantia**, v.81, e3322, 2022. https://doi.org/10.1590/1678-4499.20210267

PEAKALL, R.; SMOUSE, P.E. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. **Bioinformatics**, v.28, n.19, p.2537-2539, 2012. https://doi.org/10.1111/j.1471-8286.2005.01155.x

PEREIRA, F.R.A.; MORAES FILHO, R.M.; MARTINS, L.S.S.; MONTARROYOS, A.V.V.; LOGES, V. Genetic diversity and morphological characterization of half-sib families of *Heliconia bihai* L., *H. chartacea* Lane ex Barreiros, and *H. wagneriana* Peterson. Genetics and **Molecular Research**, v.15, n.2, p.1-9, 2016. http://doi.org/10.4238/gmr.15028003

POCOMUCHA, V.S.; RÍOS, W. Caracterización morfológica de colecciones de heliconias en el centro de Investigación y Producción Tulumayo Anexo la Divisoria (CIPTALD)–unas. **Investigación y Amazonía**, v.6, p. 25-31, 2017.

RAIZER, M.D.M.; QUISEN, R.C.; VALENTE, M.S.F.; LOPES, R.; LOPES, M.T.G. Morphological and stomatal characterization of *Heliconia chartacea* var. Sexy Pink induced polyploidy. **Bioscience Journal**, v.35, n.1, p.222-235, 2019.

ROCHA, V.D.; TIAGO, P.V.; TIAGO, A.V.; PEDRI, E.C.M.; CARDOSO, E.S.; ROSSI, A.A.B. Genetic diversity among *Hymenaea courbaril* L. genotypes naturally occurring in the north of Mato Grosso State, Brazil. **Genetics and Molecular Research**, v.16, n.13, p.1-9, 2017. https://doi.org/10.4238/gmr16039706

SHANNON, C.E.; WEAVER, W. **The mathematical theory of communication**. Urbana: University of Illinois Press, 1949.

SILVA, C.G.; ZULLIAN, E.D.; LUZ, P.B.; KRAUSE, W.; LOGES, V.; SILVA. C.A. Genetic divergence of Heliconiaceae species in the Central West Brazil region. **Agronomía Colombiana**, v.35, n.3, p.285, 2017. https://doi.org/10.15446/agron.colomb.v35n3.67661

SILVA, C.G.D.; KRAUSE, S.; BOTINI, A.F.; FRANÇA, R.P.A.D.; SILVA, C.A. Postharvest durability of Heliconiaceae evaluated in a controlled environment in Mato Grosso state, Brazil. **Ornamental Horticulture**, v.25, p.80-86, 2019.

VILLANUEVA-VIRAMONTES, S.; HERNÁNDEZ-APOLINAR, M.; CARNEVALI FERNÁNDEZ-CONCHA, G.; DORANTES-EUÁN, A.; DZIB, G. R.; MARTÍNEZ-CASTILLO, J. Wild *Vanilla planifolia* and its relatives in the Mexican Yucatan Peninsula: Systematic analyses with ISSR and ITS. **Botanical Sciences**, v.95, n.2, p.169-187, 2017.