Genetics and Molecular Research

Genetic diversity and morphological characterization of half-sib families of *Heliconia bihai* L., *H. chartacea* Lane ex Barreiros, and *H. wagneriana* Peterson

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Genet. Mol. Res. 15 (2): gmr.15028003
Received August 8, 2015
Accepted November 26, 2015
Published June 21, 2016
DOI http://dx.doi.org/10.4238/gmr.15028003

ABSTRACT. The Neotropical *Heliconia* genus contains highly diversified plants and up to 220 species have been reported from the north of Mexico to the South of Brazil. *Heliconia* are cultivated as ornamental garden plants and as cut flowers. All species can be propagated by seeds or vegetatively, through rhizomes. Depending on the species, an individual plant can spread and form large clonal populations. *H. bihai* L., *H. chartacea* Lane ex Barreiros, and *H. wagneriana* Petersen are among the most cultivated *Heliconia* species. However, they still have undesirable characteristics that could be improved for the international market. This study aimed to characterize 15 half-sib families originating from commercial cultivations, by morphological and molecular

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markers. The genetic diversity ($\hat{H}_E$), considering all individuals of the three species was 0.103. For *H. bihai* half-sib families, the value of $\hat{H}_E$ was 0.242, showing high genetic diversity. The $\hat{H}_E$ value for *H. chartacea* was 0.068, indicating low genetic diversity. All individuals of *H. wagneriana* showed the same band patterns, suggesting that the two parental plants were propagated vegetatively from the same plant and may have undergone some endogamic crossings. These results showed that molecular characterization can differentiate individuals closely related as half-siblings for *H. bihai* and *H. chartacea*, despite the low variation observed with morphological descriptors. The high genetic diversity observed in *H. bihai* half-sibling genotypes can provide valuable resources for breeding programs.

Key words: ISSR markers; Morphological descriptors; Tropical flowers

INTRODUCTION

Heliconias are cultivated as ornamental garden plants. Their cut flowers are of great importance and are appreciated in the international market owing to their exotic nature and post-harvest durability. Under the rainforest conditions of northeast Brazil, most genotypes bloom throughout the year (Castro et al., 2007; Costa et al., 2009). *Heliconia* is the only genus in the Heliconiaceae plant family. However, up to 220 species have been reported from the north of Mexico to the South of Brazil (Kress, 1990; Betancur and Kress, 2007). The earliest species found in Brazil, *Heliconia bihai*, was described in 1771 by Linnaeus, and presently, 37 species have been described, which are distributed in two main areas, the Amazonic Region and the Atlantic Forest, and are the primary areas in which this genus is distributed in Brazil (Kress, 1990; Castro et al., 2007; Marouelli et al., 2010).

Heliconia species are propagated both by seeds and vegetatively, through rhizomes. Due to their vigorous vegetative growth, many monoclonal populations are observed. Depending on the species, an individual plant can spread and form large clonal clumps (Castro et al., 2011). *H. bihai* L., *H. chartacea* Lane ex Barreiros, and *H. wagneriana* Petersen are among the most cultivated *Heliconia* species. However, they still have undesirable characteristics that could be improved for the international market (Castro et al., 2007).

The great diversity of species, cultivars, and hybrids of this genus has caused uncertainty and confusion regarding species determination and the adequate use of synonyms. The inappropriate use of nomenclature, frequently due to incorrect identification, can lead to problems at both commercial and technical/scientific levels (Castro et al., 2007).

Morphologic descriptors have been frequently employed for the determination of phenotypic diversity and to differentiate some *Heliconia* cultivars and interspecific hybrids (Berry and Kress, 1991; Loges et al., 2007; Costa et al., 2009; Guimarães et al., 2014). These descriptors are usually multi-categorical and qualitative, related to plant structural features and morphology, or even binary when referring to the absence or presence of a certain characteristic (Guimarães et al., 2014). In this regard, morphological descriptors related to pseudostems and leaves are useful for preliminary genotype differentiation and selection for breeding purposes.

Molecular markers, such as inter-simple sequence repeats (ISSRs) (Zietkiewicz et

al., 1994), have been used frequently in studies of genetic diversity in plants (Kumar, 1999; Nybom, 2004; Wünsch and Hormaza, 2007; Isaza et al., 2012). DNA markers based on PCR using nonspecific primers have become extremely popular, because they require no sequence information on the species studied. Therefore, they are useful in studies involving genetically unknown species, and vast numbers of potential markers are made available with these methods (Nybom, 2004).

In this context, the present study aimed to characterize the genetic diversity of half-sib families of *H. bihai*, *H. chartacea*, and *H. wagneriana* from commercial cultivations in Pernambuco State, Brazil, using molecular and morphologic descriptors related to the pseudostem and leaves.

MATERIAL AND METHODS

Seeds and leaf samples from eight *H. bihai*, five *H. chartacea*, and two *H. wagneriana* (Figure 1) plants were collected from a commercial production area in the Paulista municipality in Pernambuco State, Brazil, between September 2009 and March 2010. Half-sib families containing 8-10 genotypes each were generated from the parental plants by the culture of zygotic embryos (Table 1).

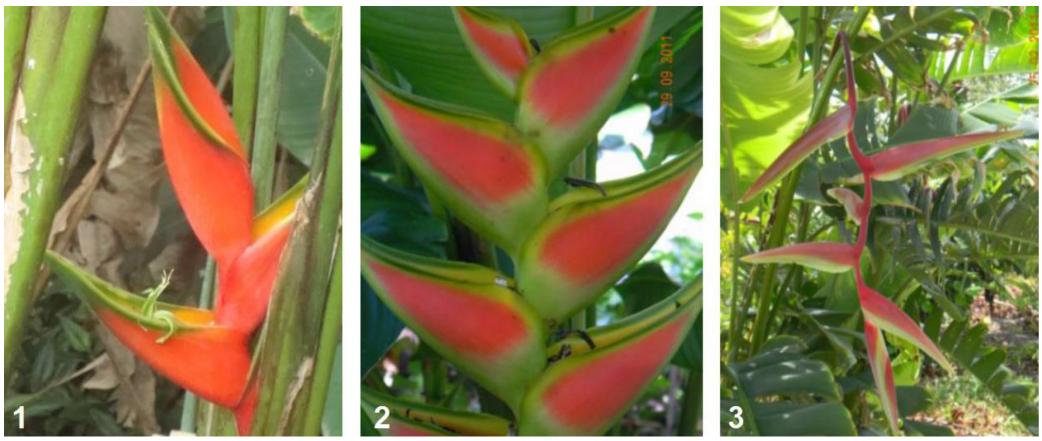

Figure 1. Evaluated *Heliconia* species. **1.** *H. bihai*; **2.** *H. wagneriana*; **3.** *H. chartacea*.

Table 1. Half-sib genotypes of *Heliconia* analyzed by morphological and molecular markers.

Species	Number of genotypes	Family (half-sibs)
H. bihai	65 half-sibs	HB9, HB11, HB13, HB14, HB15, HB16, HB17, HB18
H. chartacea	40 half-sibs	HC3, HC4, HC5, HC7, HC8
H. wagneriana	15 half-sibs	HW10, HW20

After 90-day acclimatization in a greenhouse, the genotypes were transplanted into 8-L pots. Following 120-day cultivation, the plants were transferred to an experimental field under full daylight conditions in a 0.3-ha experimental field in the Camaragibe municipality

(8°1'19" S, 34°59'33" W). The average annual temperature of the region is 25.1°C, and the average monthly precipitation is 176 mm, with a maximum 377 mm and a minimum 37 mm (ITEP, 2008).

Genotypes of each half-sib family were cultivated with 0.2-m spacing between plants in each row and 4.0 m between rows in three experimental areas, one for each species. Irrigation was applied as needed by a sprinkling irrigation system using 2.5-m high gun sprinklers.

Molecular characterization

DNA was extracted from the leaves of 15 parental plants and their respective half-sib families according to the methods described by Doyle and Doyle (1990).

DNA quantification was performed by visual comparison on a 0.8% agarose gel.

A set of nine primers (Table 2) was used and the amplification reactions were performed in a 25-μL final volume, containing 10 mM Tris-HCl at pH 8.0, 50 mM KCl, 2 mM $MgCl_2$, 100 mM dNTPs, 1 U Taq DNA polymerase, and 25 ng DNA primer at 0.4 mM. Amplifications carried out in an Eppendorf Mastercycler thermocycler were performed as follows: initial denaturation at 95°C for 15 min, followed by 30-35 cycles of denaturation at 94°C for 30 s, annealing temperature specific for each primer for 45 s, extension at 72°C for 2 min, and a final extension at 72°C for 7 min.

Table 2. Primers, sequences, number of loci, and polymorphic loci.

Primer	Sequence	Ta (°C)	No. of cycles
UBC#1	ACACACACACACACACT	50	35
UBC#2	GAGAGAGAGAGAGAGAT	50	35
UBC#812	GAG AGA GAG AGA GAG AA	50	35
UBC#813	CTC TCT CTC TCT CTC TT	50	35
UBC#817	CAC ACA CAC ACA CAC AA	50	35
UBC#820	GTG TGT GTG TGT GTG TC	50	35
UBC#827	ACA CAC ACA CAC ACA CG	50	35
UBC#862	AGC AGC AGC AGC AGC AGC	50	35
UBC#864	ATG ATG ATG ATG ATG ATG	50	35

The amplified products were separated by horizontal electrophoresis run at 100 V for 1.5 h, on 1.2% agarose gels containing SYBR gold and immersed in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0). The amplified fragments were visualized in a UV transilluminator and allele size was determined by using a 50-bp DNA ladder (Invitrogen™).

Polymorphisms identified by the ISSR technique were tabulated according to the presence (1) or absence (0) of bands. Each ISSR band was considered a single and bi-allelic locus, with an amplifiable allele and one null allele. The GenAlex Software 6.5 (Peakall and Smouse, 2012) was used to generate the genetic distance matrix according to the methods described by Nei (1972) and to calculate Nei's Genetic Diversity ($\hat{H}_E$) and percentage of polymorphic loci.

Morphological characterization

Sixteen morphological descriptors (Table 3) were analyzed fortnightly for 6 months. These descriptors were elaborated according to previous studies in the *Heliconia* genus (Loges

et al., 2007; Costa et al., 2009; Guimarães et al., 2014), and were also based on the catalog of *Musa* spp descriptors (Silva et al., 1999), with modifications to accommodate the typical *Heliconia* morphological descriptors. The 15 parental individuals were not included in this analysis.

Table 3. Evaluated qualitative descriptors related to the pseudostem and leaf.

Descriptors	Code	Categories	
		(0)	(1)
Pseudostem			
Pseudostem of dark green color**	PDG	Absence	Presence
Wax*	PW	Absence	Presence
Hair*	PH	Absence	Presence
Leaf		Absence	Presence
Wax in the petiole**	WP	Absence	Presence
Hair on petiole**	HP	Absence	Presence
Midrib underside shade of green**	MUG	Absence	Presence
Midrib upper shade of green **	MUSG	Absence	Presence
Wax midrib underside	WMU	Absence	Presence
Midrib underside hair	MUH	Absence	Presence
Leaf hair*	LH	Absence	Presence
Wax leaves*	WH	Absence	Presence
Leaf blade dark**	LD	Absence	Presence
Margin of leaves shade of purple**	MLP	Absence	Presence
Cut leaf blade**	CLB	Absence	Presence
Leaf blade stain	LS	Absence	Presence
Leaf blade basis	LB	Absence	Presence

*Description proposed by Loges et al. (2007) and Costa et al. (2009). **Description based on the catalog of *Musa* spp descriptors, with modifications.

Genetic distance matrices were generated in the GenAlex 6.5 software (Peakall and Smouse, 2012) and MEGA5 (Tamura et al., 2011) using both molecular and morphological data and were used to generate dendrograms of genetic similarity on the basis of the unweighted pair group method with arithmetic average (UPGMA) algorithm.

RESULTS AND DISCUSSION

Molecular characterization

The set of nine ISSR primers used in this study generated 44 loci, with a mean number of 4.9 loci per primer, ranging from 3 (UBC#820; UBC#864) to 7 (UBC#2) (Table 4). Considering all the species, 41 of the 44 observed loci were polymorphic (93.18%) and 3 were monomorphic. For *H. bihai*, 28 loci (63.64%) were polymorphic, and for *H. chartacea* only 13 (29.55%) were polymorphic. The 17 *H. wagneriana* individuals exhibited the same genotype, with 100% monomorphism.

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 ($\hat{H}_E$). Estimates derived using dominant inherited markers are very similar and may be directly comparable (Nybom, 2004). The genetic diversity was 0.03 when considering all individuals of the three species. For half-sib families of *H. bihai*, $\hat{H}_E$ was 0.242, showing high genetic diversity. The $\hat{H}_E$ value for *H. chartacea* was 0.068, indicating low genetic diversity. All *H. wagneriana* individuals generated the same band patterns, suggesting that the two

Table 4. Diversity estimates obtained from the individual primers and three evaluated species.

	LN	PL	ML	$\hat{H}_E$
UBC#1	4	4	-	-
UBC#2	7	7	-	-
UBC#812	6	6	-	-
UBC#813	6	6	-	-
UBC#817	5	3	2	-
UBC#820	3	2	1	
UBC#827	4	4	-	-
UBC#862	6	6	-	
UBC#864	3	3	-	
H. bihai	44	28 (63.64%)	16	0.242
H. chartacea	44	13 (29.55%)	21	0.068
H. wagneriana	44	-	44	0
Total	44	41 (93.18%)	3	0.103

LN = locus number; PL = polymorphic loci; ML = monomorphic loci; $\hat{H}_E$ = Nei's genetic diversity.

parental plants were propagated vegetatively from the same plant and may had undergone some endogamic crossings.

$\hat{H}_E$ values of *H. bihai* were lower than those observed in natural populations of *Musa acuminate* with ISSR markers (0.332-0.4; Padmesh et al., 2012), but consistent with the observations made by Meléndez-Ackerman et al. (2005) for *H. bihai* (0.13-0.30) using amplified fragment length polymorphism markers.

The results showed that ISSR markers can differentiate individuals of closely related *H. bihai* and *H. chartacea* as half-siblings (Figure 2). All parental individuals of the three species showed 100% genetic similarity, which can be explained by the vegetative propagation of genotypes by commercial producers. *H. bihai* half-sib genotypes showed high genetic diversity, which can provide valuable resources for breeding programs.

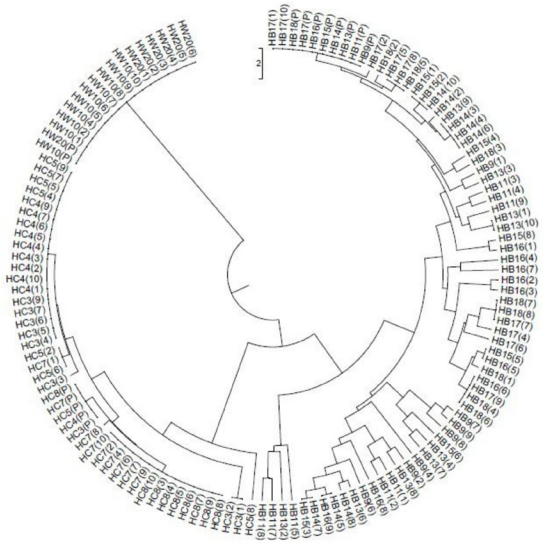

Figure 2. UPGMA dendrogram for the three species generated by ISSR markers. *Heliconia bihai* (HB); *H. chartacea* (HC); *H. wagneriana* (HW).

Morphologic characterization

With respect to the morphologic descriptors, none of the genotypes exhibited hairs on the pseudostem and leaves. The absence of hairs is a desirable characteristic for the cut flower market, but is not useful for differentiating the *Heliconia* genotypes evaluated, although these descriptors do occur in other species of the genus (Loges et al., 2007). All the other descriptors showed variation between the evaluated species, but were not suitable for early genotype differentiation.

Wax was observed in the pseudostem of the three species, but was absent in the petiole of leaves of *H. bihai* genotypes. The upper face of the leaves midrib showed a green coloration in *H. bihai* and *H. chartacea*, and purple coloration in *H. wagneriana.* The underside face of the leaves midrib was predominantly green in all three species, but some *H. bihai* genotypes exhibited the purple variation, which was also observed by Guimarães et al. (2014).

Despite the results obtained through the molecular characterization, one *H. wagneriana* genotype showed some phenotypic variation (Figure 3), indicating that these may not be clones, rather highly genetically similar individuals.

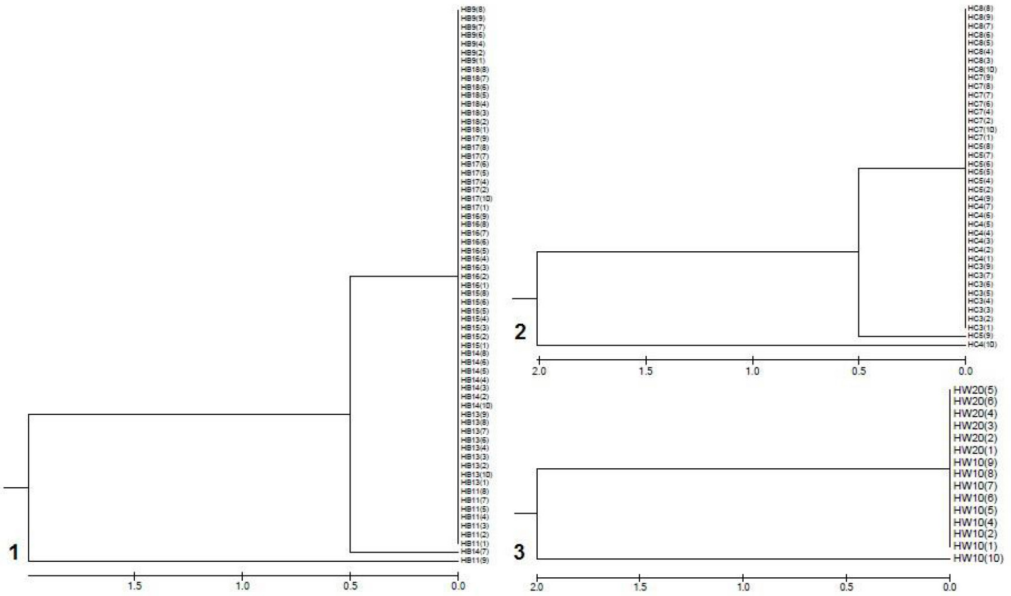

Figure 3. UPGMA dendrograms generated by 16 morphologic markers. **1.** *Heliconia bihai* (HB); **2.** *H. chartacea* (HC); **3.** *H. wagneriana* (HW).

Despite the genetic diversity of *H. bihai* and *H. chartacea* shown by ISSR markers, the evaluated genotypes exhibited little variation in the 16 morphologic markers used, which were not suitable for distinguishing genotypes within half-sib families. The results of the molecular characterization suggest that the evaluated *H. chartacea* genotypes may have undergone some genetic selection or crosses between genetically related individuals. The fact that all *H.*

wagneriana individuals generated the same pattern of bands indicates that the two parents were highly homozygous and the observed phenotypic variation may be of an environmental origin. To obtain more accurate results, studies involving more individuals and markers are needed.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by CNPq, CAPES and UFRPE resources. F.R.A. Pereira was supported by a Master Fellowship from CNPq and R.M. Moraes Filho was supported by a Post-Doctoral Fellowship PNPD-CAPES.

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