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Genetic diversity and molecular characterization of several *Heliconia* species in Colombia

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ABSTRACT. Researchers have classified the *Heliconia* genus as a group of highly variable and diverse plants. Species and cultivars are visually differentiated primarily on the basis of the color and size of inflorescence bracts. At taxonomic level, flower type (parabolic, sigmoid, or erect) and size are taken into account. The vast morphological diversity of heliconias at intra-specific, intra-population, and varietal levels in central-west Colombia prompted the present study. We characterized the genetic variability of 67 genotypes of cultivated heliconias belonging to *Heliconia caribaea* Lamarck, *H. bihai* (L.) L., *H. orthotricha* L. Andersson, *H. stricta* Huber, *H. wagneriana* Petersen, and *H. psittacorum* L. f., as well as that of several interspecific hybrids such as *H. psittacorum* L. f. x *H. spathocircinata* Aristeguieta and *H. caribaea* Lamarck x *H. bihai* (L.) L. We also created an approximation to their phylogenetic analysis. Molecular analysis using amplified fragment length polymorphism (AFLP) markers revealed a total of 170 bands. Two large, well-defined groups resulted: the first grouped cultivars of the very closely related *H. caribaea* and *H. bihai* species with those of *H. orthotricha* and *H. psittacorum*, and the second grouped *H. stricta* and *H. wagneriana* cultivars. The lowest percentage of polymorphism was found in *H. psittacorum* (17.65%) and the highest was in *H. stricta* (55.88%). Using AFLP, phylogenetic analysis of the

species studied revealed the monophyletic origin of the Heliconiaceae family, and identified the *Heliconia* subgenus as monophyletic while providing evidence of the polyphyletic origin of several representatives of the *Stenochlamys* subgenus.

Key words: *Heliconia*; Genetic diversity; Phylogenetic relationships; AFLP; Colombia

INTRODUCTION

In Colombia, the cultivation of native flowers such as heliconias for export undeniably constitutes a profitable and environmentally friendly enterprise, provided it is carried out in such a way that genetic diversity is respected and overexploitation of natural populations is avoided. However, not only is an understanding of the agronomic aspects and genetic base of the crop required, good agricultural practices are needed as well (Maza, 2004).

The Heliconiaceae family contains a single genus, *Heliconia* L., but nearly 220 species of heliconias have been reported, with the largest number of species (104) found in Colombia (Betancur and Kress, 2007), where many still grow in the wild and therefore offer great potential to diversify the international market. Although there is important market demand for this flower in different parts of the world, its potential is barely recognized in Colombia. Large-scale cultivation of *Heliconia* species, traditionally propagated through rhizomes and seeds, has been limited because of their very slow growth (Atehortúa and Valencia, 2002), which would render it difficult to satisfactorily meet international demand for this exotic flower. Cultivation of this tropical flower was recently proposed as a promising alternative that offers significant economic advantages for regions located in central-west Colombia. However, the few *Heliconia* species that are currently being cultivated have not undergone genetic selection, and as a result, their quality does not comply with international market standards.

The broad diversity of species and artificial groups of the *Heliconia* genus, such as the varieties, hybrids, and cultivars used for ornamental and commercial purposes, has caused confusion and uncertainty regarding the correct denomination of the species and the adequate use of synonyms, triggering problems at both commercial and technical/scientific levels. The inappropriate use of nomenclature helps disseminate incorrect identifications, thus perpetuating errors (Castro et al., 2007). Taxonomic traits not considered for diagnosis are staminodes, bract angle, indumentum, number of colonies, and auto-ecology. Genetic variability, genotypic plasticity, and level of natural hybridization are not taken into account either.

There is a clear need to study the existing germplasm of wild and cultivated *Heliconia* species in Colombia to better contribute to their conservation while advancing selection and multiplication programs to improve the genetic quality of planted materials. Amplified fragment length polymorphism (AFLP) markers have been widely used in genetic variation studies and are becoming increasingly popular in low-level systematization (Bussell et al., 2005). This study therefore aimed to analyze the genetic variability of several *Heliconia* species and cultivars using AFLP molecular markers (Vos et al., 1995) in an attempt to better understand and conserve the existing diversity of these genetic resources in Colombia. The results of the present study will serve as input for future breeding programs and compose an approximation to the analysis of the phylogenetic relationships between *Heliconia* species, hybrids, and cultivars.

MATERIAL AND METHODS

Sixty-nine genotypes consisting of 67 *Heliconia* species, cultivars, and hybrids, as well as two accessions of other families of the order Zingiberales (Musaceae and Strelitziaceae) used as contributors of diversity, *Musa coccinea* (MC32) and *Strelitzia reginae* (Sn95), were analyzed (Table 1). DNA was extracted from young leaves using a QIAGEN DNeasy Plant Mini Kit (QIAGEN, Germany). DNA was quantified by electrophoresis on 0.8% agarose gel.

The AFLPs were developed using the Invitrogen AFLP® Analysis System I kit. Two combinations of primers developed for Musaceae (Ude et al., 2002) were used for this study: *Eco*RI + ACC/*Mse*I + CAG, and *Eco*RI + AGC/*Mse*I + CAG. The DNA fragments were run on 6% polyacrylamide gel dyed with silver nitrate ($AgNO_3$) (Bassam et al., 1991). A binary matrix of presence/absence of bands was built based on the resulting data, recording band presence (1) and absence (0) for each of the 69 individuals studied.

The GenAIEx Version 6.1 package (Peakall and Smouse, 2006) was used to determine the average number of loci, the number of alleles per locus, the effective number of alleles (N_E), the percentage of polymorphic loci per individual and per population, the percentage of total polymorphism, and the expected heterozygosity (H_E) (Excoffier et al., 1992; Peakall et al., 1995; Peakall and Smouse, 2006).

Molecular variance between populations (species and cultivars) and individuals (Excoffier et al., 1992; Schneider et al., 1997) was calculated based on the genetic distance matrix (Nei, 1978). The Dice similarity coefficient (Dice, 1945) was calculated using the NTSYSpc 2.02 statistical package (Rohlf, 1997), and a dendrogram was constructed using UPGMA (unweighted pair group method with arithmetic mean) (Sneath and Sokal, 1973). Cladistic analysis was based on the criterion of maximum parsimony (MP) using the Palaeontological Statistics (PAST) 1.75 program (Hammer et al., 2001), which assigns the same weight to each trait. A heuristic search helped construct the most parsimonious tree. Consistency and retention indices (Kluge and Farris, 1969; Farris, 1989) were also determined.

RESULTS AND DISCUSSION

AFLP analysis yielded 170 bands with an overall polymorphism of 34.34%. Table 2 indicates the polymorphism for each *Heliconia* species and/or hybrid. The lowest percentage of polymorphism occurred in *H. psittacorum* (17.65%) and the highest was in *H. stricta* (55.88%). The high degree of polymorphism can be related to the diversity of the species and cultivars analyzed.

The use of AFLP markers enabled measurement of the genetic variation in the eight *Heliconia* populations (species, cultivars, and hybrids) as well as the intra-population variation in all individuals studied. The number of alleles per locus ranged from 3 to 12, with an overall average of 8.289; the number of different alleles per locus ranged from 0.488 to 1.124, with an overall average of 0.765, and the number of effective or polymorphic alleles per locus ranged from 1.091 in *H. caribaea* to 1.247 in *H. stricta*, with a total average of effective alleles of 1.154 (Brown and Weir, 1983) (Figure 1).

Table 1. *Heliconia* species and cultivars studied and their collection sites in central-west Colombia.

Sample	Specie and cultivar	Code of sample in herbarium
1H.b9	*H. bihai* (L.) L. cv. Arawak	
H.b75	*H. bihai* (L.) L. cv. Arawak	
H.b2	*H. bihai* (L.) L. cv. Aurea	Londoño, L. 033808 HUQ
H.b62	*H. bihai* (L.) L. cv. Lobster Claw 1 (black spot)	
H.b7	*H. bihai* (L.) L. cv. Lobster Claw 1 (black spot)	
H.b63	*H. bihai* (L.) L. cv. Lobster Claw 2 (green sport)	
H.b20	*H. bihai* (L.) L. cv. Lobster Claw 2 (green spot)	
H.b46	*H. bihai* (L.) L. cv. Lobster Roja	
H.b6	*H. bihai* (L.) L. cv. Lobster Salmón	Isaza, L. 033732 HUQ
H.b60	*H. bihai* (L.) L. cv. Lobster Salmón	
H.b30	*H. bihai* (L.) L. cv. Naranja	
H.b38	*H. bihai* (L.) L. cv. Yellow Dancer	
H.c25	*H. caribaea* Lamarck cv. Barbados	
H.c77	*H. caribaea* Lamarck cv. Barbados	
H.c15	*H. caribaea* Lamarck cv. Brazilian Bomber	
H.c40	*H. caribaea* Lamarck cv. Brazilian Bomber	
H.c56	*H. caribaea* Lamarck cv. Chartreuse	Londoño, L. 033818 HUQ
H.c26	*H. caribaea* Lamarck cv. Gold	Londoño, L. 033807 HUQ
H.c55	*H. caribaea* Lamarck cv. Gold	
H.c48	*H. caribaea* Lamarck cv. Purpurea	Isaza, L. 033730 HUQ
H.c69	*H. caribaea* Lamarck cv. Salmón	Londoño, L. 033825 HUQ
H.c16	*H. caribaea* Lamarck cv. Vulcano	Londoño, L. 033809 HUQ
H.c61	*H. caribaea* Lamarck cv. Vulcano	
H.cxb37	*H. caribaea* Lamarck x *H. bihai* (L.) L. cv. Kawauchi	
H.cxb14	*H. caribaea* Lamarck x *H. bihai* (L.) L. cv. Criswick	
H.cxb 59	*H. caribaea* Lamarck x *H. bihai* (L.) L. cv. Criswick	
H.cxb74	*H. caribaea* Lamarck x *H. bihai* (L.) L. cv. Gold	
H.cxb13	*H. caribaea* Lamarck x *H. bihai* (L.) L. cv. Jacquinii	Londoño, L. 033812 HUQ
H.cxb44	*H. caribaea* Lamarck x *H. bihai* (L.) L. cv. Jacquinii	
H.cxb42	*H. caribaea* Lamarck x *H. bihai* (L.) L. cv. Piton Point	
H.o11	*H. orthotricha* L. Andersson cv. Arcoiris	
H. 81	*H. orthotricha* L. Andersson cv. Arcoiris	
H.o1	*H. orthotricha* L. Andersson cv. Bicolor	
H.o27	*H. orthotricha* L. Andersson cv. Edge of Nite	Isaza, L. 033733 HUQ
H.o71	*H. orthotricha* L. Andersson cv. Edge of Nite	Londoño, L. 033929 HUQ
H.o80	*H. orthotricha* L. Andersson cv. Pintoresca	
H.o12	*H. orthotricha* L. Andersson cv. Pintoresca	
H.o 28	*H. orthotricha* L. Andersson cv. Roja	
H.o98	*H. orthotricha* L. Andersson cv. Roja	
H.o34	*H. orthotricha* L. Andersson cv. She	
H.o100	*H. orthotricha* L. Andersson cv. She	
H.o10	*H. orthotricha* L. Andersson cv. Tricolor	
H.p79	*H. psittacorum* L. f. cv. Choconiana	
H.p8	*H. psittacorum* L. f. cv. Opal Crema	
H.p49	*H. psittacorum* L. f. cv. Opal Crema	
H.pxs76	*H. psittacorum* L. f. x *H. spathocircinata* Aristeguieta cv. Opal Fire	
H.pxs 83	*H. psittacorum* L. f. x *H. spathocircinata* Aristeguieta cv. Golden Torch	
H.pxs84	*H. psittacorum* L. f. x *H. spathocircinata* Aristeguieta cv. Golden Torch Adrian	
H.s19	*H. stricta* Huber cv. Bucky	Isaza, L. 033731 HUQ
H.s47	*H. stricta* Huber cv. Bucky	
H.s23	*H. stricta* Huber cv. Giant Jamaican	
H.s97	*H. stricta* Huber cv. Giant Jamaican	
H.s50	*H. stricta* Huber cv. Las Cruces	Londoño, L. 033806 HUQ
H.s31	*H. stricta* Huber cv. Lone Lover	
H.s5	*H. stricta* Huber cv. Fire Bird	Londoño, L. 033827 HUQ

Continued on next page

Table 1. Continued.

Sample	Specie and cultivar	Code of sample in herbarium
H.s53	*H. stricta* Huber cv. Fire Bird	
H.s22	*H. stricta* Huber cv. Quito Gold Amarilla	
H.s21	*H. stricta* Huber cv. Quito Gold Naranja	
H.s17	*H. stricta* Huber cv. Tagami	
H.s85	*H. stricta* Huber cv. Tagami	
H.w86	*H. wagneriana* Petersen cv. Gorda	
H.w 58	*H. wagneriana* Petersen cv. Amarilla	
H.w65	*H. wagneriana* Petersen cv. Crema	Londoño, L. 033814 HUQ
H.w41	*H. wagneriana* Petersen cv. Verde	Isaza, L. 033734 HUQ
H.w57	*H. wagneriana* Petersen cv. Roja	
H.w24	*H. wagneriana* Petersen cv. Sharoni	
H.w29	*H. wagneriana* Petersen cv. Splendid	
Mc32	*Musa coccinea*	Londoño, L. 033826 HUQ
Sn95	*Strelitzia reginae*	

Table 2. Percentage of polymorphism of different *Heliconia* species.

Population/specie	Polymorphism (%)
H. bihai	28.24%
H. caribaea	32.35%
H. caribaea x *H. bihai*	37.06%
H. orthotricha	43.53%
H. psittacorum	17.65%
H. psittacorum x *H. spathatocircinata*	27.65%
H. stricta	55.88%
H. wagneriana	32.35%
Overall average	34.34%

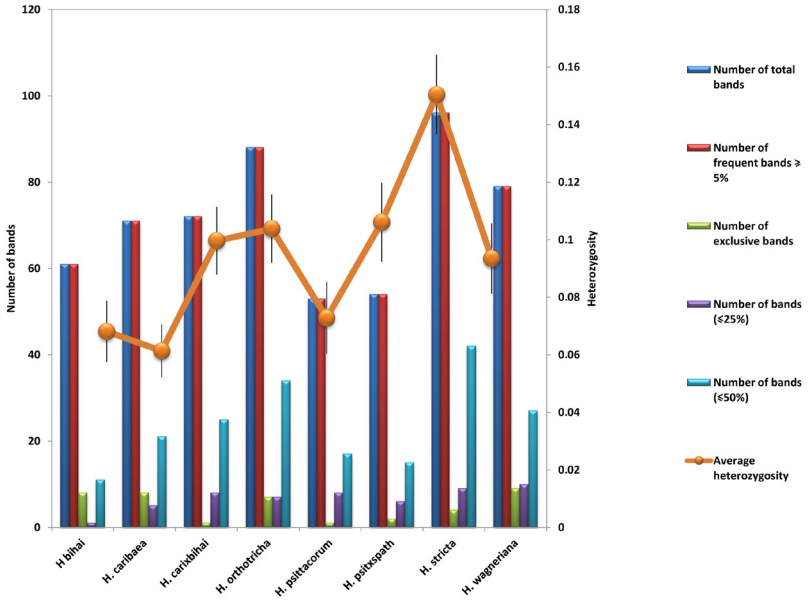

Figure 1. Distribution and frequency of total bands, common bands, rare bands, and exclusive bands per species based on average expected heterozygosity.

The N_E (1.247) and H_E (0.150) of *H. stricta* agree with the percentage of polymorphism (55.88%), which was the highest compared with all other species and cultivars. Therefore, it can be inferred that *H. stricta* presents the greatest intra-specific variability, followed by *H. orthotricha* with a polymorphism of 43.53%, N_E of 1.163, and H_E of 0.104. Corresponding polymorphism and relatively low H_E values were 32.35% and 0.061 for *H. caribaea*, 28.24% and 0.068 for *H. bihai*, and 37.06% and 0.1 for the *H. caribaea* x *H. bihai* hybrid, respectively, evidence of the smaller intra-specific genetic variability in these species as compared with *H. stricta* and *H. orthotricha* (Brown and Weir, 1983; Smouse and Peakall, 1999).

Based on Nei's genetic distance (Nei, 1978) between populations (species and cultivars), the smallest genetic distances occurred between *H. caribaea* x *H. bihai* and *H. caribaea* (0.031) and between *H. bihai* and *H. caribaea* (0.032). The largest distances occurred between *H. caribaea* x *H. bihai* and *H. wagneriana* (0.119) and between *H. caribaea* and *H. wagneriana* (0.114) (Figure 2). Similarly, the highest values of Nei's genetic identity (Nei, 1978) occurred between *H. caribaea* x *H. bihai* and *H. caribaea* (0.970) and between *H. caribaea* and *H. bihai* (0.968), while the lowest values occurred between *H. caribaea* x *H. bihai* and *H. wagneriana* (0.887) and between *H. caribaea* and *H. wagneriana* (0.89).

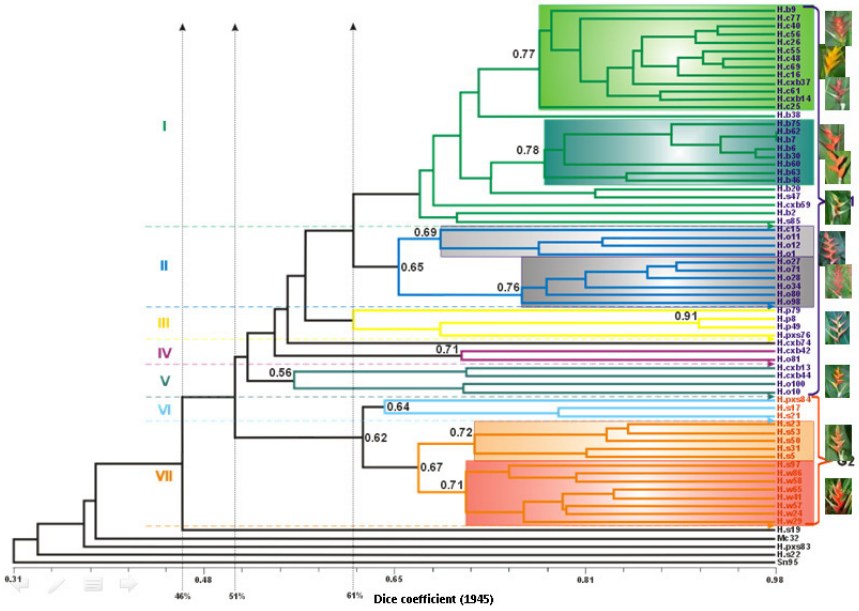

Figure 2. Dendrogram of 69 genotypes of the genus *Heliconia* developed using the Dice similarity index (Dice, 1945).

The above data reveal a close genetic relationship between *H. caribaea* and *H. bihai*, and between both these species and the interspecific hybrid *H. caribaea* x *H. bihai*. This genetic proximity was also demonstrated by the Dice similarity index (Dice, 1945) (Figure 1).

The dendrogram in Figure 2 was built based on the results of the present study. Two very well-defined groups could be distinguished, allowing the degree of genetic similarity between the species studied to be established. The *H. caribaea* and *H. bihai* species were most

closely related, with a genetic similarity of 0.75. Completely separated from the above were *H. stricta* and *H. wagneriana*, which were more closely related to one another, presenting a genetic similarity of 0.67.

As shown in the dendrogram (Figure 2), almost all of the plants studied were grouped at 46% similarity. Two large groups were formed, presenting 51% similarity, and each group was in turn divided into the following subgroups:

Group 1, which presented 52% similarity, was formed by the following five subgroups:

- Subgroup I: with 67% similarity, contained all cultivars belonging to the *H. bihai* species and most of the cultivars of the *H. caribaea* species. Also included three *H. caribaea* x *H. bihai* hybrids and two genotypes belonging to the *H. stricta* species. This subgroup comprised 27 genotypes.
- Subgroup II: with 65% similarity, contained most of the *H. orthotricha* cultivars studied and a single *H. caribaea* genotype.
- Subgroup III: with 61% similarity, contained most of the *H. psittacorum* plants studied, including genotype H.p79 (*H. psittacorum* cv. Choconiana) and H.pxs76 (*H. psittacorum* x *H. spathocircinata* cv. Opal Fire). With 91% similarity, this subgroup contained another subgroup comprising two *H. psittacorum* cv. Opal Crema individuals. Genotype H.cxb74 (*H. caribaea* x *H. bihai* cv. Gold), collected in Manizales, was alone and not related to the other study individuals.
- Subgroup IV: with 71% similarity, comprised only two individuals, *H. caribaea* x *H. bihai* cv. Piton Point and *H. orthotricha* cv. Arcoiris, collected in Manizales.
- Subgroup V: with 71% similarity, divided into two more subgroups: one formed by two *H. caribaea* x *H. bihai* cv. Jacquinii hybrids collected at two localities (Manizales and Salónica), and the other by genotypes H.o10 (*H. orthotricha* cv. Tricolor), collected in Salónica, and H.o100 (*H. orthotricha* cv. She), collected in Manizales.

Group 2, which presented 62% similarity, was formed by two subgroups:

- Subgroup VI: with 64% similarity, contained one hybrid, *H. psittacorum* x *H. spathocircinata* cv. Golden Torch, and two *H. stricta* individuals, cv. Quito Gold Amarilla and cv. Tagami, both collected in Salónica.
- Subgroup VII: with 67% similarity, contained two subgroups: one formed exclusively by *H. stricta* genotypes (H.s23, H.s53, H.s50, H.s31, and H.s5), the other with a single *H. stricta* genotype (H.s97) together with all *H. wagneriana* genotypes included in this study.

Several genotypes were outliers and were not included in any of the groups. As expected, these were *M. coccinea* and *S. reginae* species, but also included *H. stricta* cv. Bucky and cv. Quito Gold Amarilla, both collected in Salónica, and the H.pxs83 sample (*H. psittacorum* x *H. spathocircinata* cv. Golden Torch) collected in Manizales.

Based on the use of AFLP molecular markers and genomic DNA, the close relationship between *H. bihai* and *H. caribaea* and the greater similarity between *H. stricta* and *H. wagneriana* observed in this study agree with the phylogenetic relationships discovered by Lagomarsino and Kress (2007) that were based on chloroplast sequences and detailed characterization of floral morphology. This means that species in the same clade share a group of monophyletic DNA sequences and therefore have descended from a common ancestor.

The genetic proximity between *H. caribaea* and *H. bihai* found in this study, and demonstrated by Lagomarsino and Kress (2007), agrees with the existence of numerous natural

hybrids, although the morphology of their bracts apparently differs. Both species are native to the Lesser Antilles and have a common pollinator - the hummingbird (*Eulampis jugularis*). This flower-pollinator association, also called co-evolution, has been determinant in the evolution of the floral morphology of these two species (Temeles et al., 2000; Temeles and Kress, 2003; Yoshioka, 2003; Meléndez-Ackerman et al., 2005; Lagomarsino and Kress, 2007).

The existence of interspecific hybrids, such as *H. caribaea* x *H. bihai*, can be determined by the fact that hummingbirds are the sole pollinators of heliconias in the Americas, influencing the hybridization phenomenon between heliconias. The bills of hummingbird species represent sexual dimorphism: females have long, curved bills while males have short, straight bills (Temeles et al., 2000). An example of this is the report of the female purple-throated hummingbird of the Caribbean island of Saint Lucia possessing long, curved bills, while males have straight and short bills. In most Caribbean islands, *H. bihai* is pollinated by females of this hummingbird species, whereas *H. caribaea* is pollinated by males. Lagomarsino and Kress (2007) demonstrated that the flower morphology of each of these species has evolved to adapt to the size and shape of the bills of females and/or males of this hummingbird species. These studies explain the close relationship found between *H. caribaea* and *H. bihai*.

The number of exclusive bands per species was also determined, as were the bands most common to all genotypes, and species exhibiting rare and exclusive bands were identified (Figure 2). The study identified exclusive bands for *Heliconia* genotypes and species as well as the bands found in *H. wagneriana*, *H. orthotricha*, *H. bihai*, *H. caribaea*, hybrid *H. caribaea* x *H. bihai* cv. Jacquinii, and hybrid *H. psittacorum* x *H. spathocircinata* cv. Golden Torch.

The highest number of bands was found in *H. stricta* (96), followed by *H. orthotricha* (88) and *H. wagneriana* (79). The highest number of exclusive bands per species occurred in *H. wagneriana* (9), followed by *H. bihai* and *H. caribaea* (both 8), *H. orthotricha* (7), and *H. stricta* (4) (Figure 2).

Similarly, the identification of an important number of bands that differentiate outlying groups such *S. reginae* and *M. coccinea*, which presented very low similarity indexes (0.30-0.39), as well as the grouping of all heliconias into a larger group, clearly reveal the taxonomic and phylogenetic separation of heliconias regarding the other two botanical families included in the analysis: Musaceae and Strelitziaceae. These results corroborate the findings of Andersson (1992), Kress (1994), Kress et al. (2001, 2002), and Lagomarsino and Kress (2007) regarding the existence of sufficient characteristics and phylogenetic and taxonomic criteria to designate heliconias a separate botanical family.

Although the study population consisted of replicates of each genotype, albeit collected in different localities, the results obtained with the Dice similarity index (Dice, 1945) and Nei's genetic distance (Nei, 1978), illustrated in Figure 2, show that the individuals tagged as being the same were not replicates or clones of the same genotype, but different individuals and genotypes. Although most were located in the same subgroup, a few individuals were separated into different subgroups. Nonetheless, two genotypes of 69 presented a similarity index of 0.97: Hb62 and Hb7.

Another aspect that should be highlighted is the presence of *Heliconia aurea* Rodriguez, which was initially identified and labeled as "Hb2" (*H. bihai* cv. Aurea) because this is how it is known to farmers, and was recorded as such by Berry and Kress (1991). More recent reviews (Kress and Betancur, 1999; Castro et al., 2007; Lagomarsino and Kress, 2007) have determined that it is a different species, not a cultivar of *H. bihai.* Consequently, the discrimi-

nating power of the molecular marker used (AFLP) revealed that this genotype is in subgroup I of the dendrogram produced, but separated in a small cluster with a genetic similarity index of 0.7, while most of the *H. bihai* individuals studied are grouped together.

A consensus tree with 774 steps was selected based on MP analysis. The analysis also revealed a low consistency index (0.22) and retention index (0.51) (Figure 3).

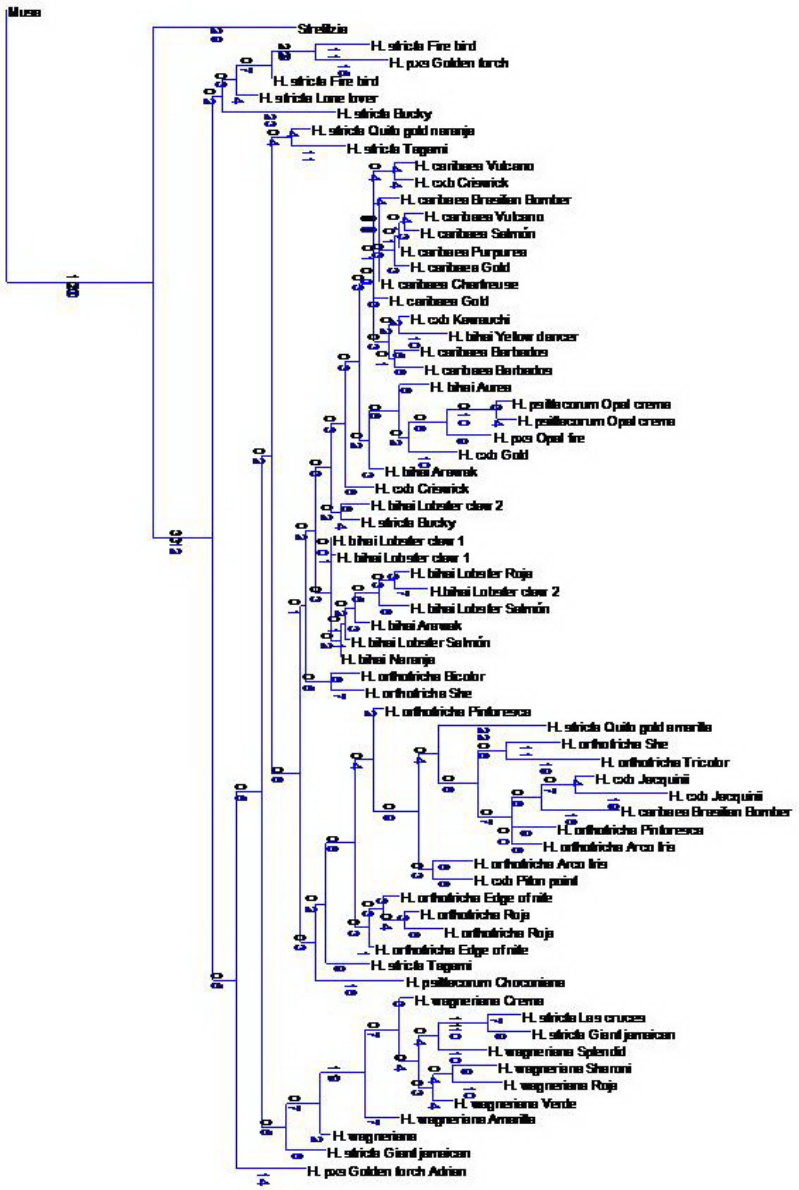

Figure 3. Cladogram based on the phylogenetic analysis of 67 *Heliconia* accessions, calculated with 170 bands yielded by AFLP markers using the PAST method (consistency index = 0.220; retention index = 0.510).

Although the use of AFLP markers to solve phylogenetic relationships is controversial (Després et al., 2003), the results of this study reflect the current classification of the order Zingiberales, taking into account that a representative of the Musaceae family and another of the Strelitziaceae family were used as an external group. Musaceae was presented as the basal family and Strelitziaceae as the family most closely related phylogenetically to the Heliconiaceae family; these results are similar to those found by Marouelli et al. (2010). The AFLP markers made it possible to identify the Heliconiaceae family as a monophyletic group, agreeing with the proposal by Kress et al. (2001), Angiosperm Phylogeny Group (2003), Kress and Specht (2006), and Marouelli et al. (2010). The *Heliconia* subgenus, to which most of the species included in this study belong, also proved to be broadly monophyletic.

Based on the results, the high interspecific, intra-specific, and intra-population variability present in *Heliconia* was corroborated, as evidenced in the analysis of molecular variance (AMOVA), which revealed 70% intra-population variation. Regarding intra- and interspecific variability, despite the large diversity of types found in the *Heliconia* species studied, cultivars exhibited high genetic similarity (Figures 1 and 3). High phenotypic and molecular variability has been found in other studies conducted on heliconias by Berry and Kress (1991), Kumar et al. (1998), Marques et al. (2004), Meléndez-Ackerman et al. (2005), and Marouelli et al. (2010).

This study also indicated that *H. psittacorum* and the hybrid *H. psittacorum* x *H. spathocircinata* do not belong to the *Heliconia* subgenus, although most of the species analyzed in this study (*H. bihai*, *H. caribaea*, *H. orthotricha*, *H. stricta*, *H. wagneriana*, and *H. aurea*) did; rather, they belonged to the *Stenochlamys* Baker subgenus, *Stenochlamys* (Baker) Schum section (Kress et al., 1999), and were revealed to be grouped with the *Heliconia* subgenus (Figure 2). This means that although these species belong to different subgenera, they possess several DNA bands in common, as identified by the AFLP markers. The MP analysis (Figure 3) also revealed that individuals of the *Stenochlamys* subgenus appear to be grouped into clades, separated as brother groups of species such as *H. stricta*, *H. orthotricha*, and *H. aurea*, or ungrouped, such as *H. psittacorum* x *H. spathocircinata* cv. Golden Torch Adrian, evidence that the *Stenochlamys* subgenus is clearly polyphyletic. Marouelli et al. (2010) reported the polyphyletic origin of the same subgenus in an analysis conducted with random amplified polymorphic DNA markers, which indicated that it was closely related to the *Griggsia* subgenus to which all species with pendular flowers belong. Andersson (1985, 1992), Kress (1994), and Castro et al. (2007) have suggested that the *Griggsia* subgenus is not monophyletic.

The AFLP markers proved to be a tool useful not only to measure and characterize genetic variability, but also to support and strengthen classical taxonomic classification because of their potential relationship with morphological characters, thus contributing to the understanding of inter-specific and phylogenetic relationships of the Heliconiaceae family.

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