Genetic Analyses of *Heliconia* Species and Cultivars with Randomly Amplified Polymorphic DNA (RAPD) Markers

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ABSTRACT. Many *Heliconia* species are polymorphic with a large number of cultivars. Cultivar identification has been primarily based on morphological differences of the flowers and inflorescences. A protocol was developed to extract DNA from *Heliconia* leaves and to analyze genetic variation using RAPD. The percentage genetic similarities among *Heliconia* species, cultivars, and hybrids were determined. Data from 11 primers indicated that the RAPD technique is useful in distinguishing species and cultivars of *Heliconia*. Using a single 10-mer primer (OPA18), we were able to generate distinct RAPD profiles for 16 cultivars of *H. psittacorum* L.f. grown at the Jurong BirdPark, Singapore, a *Heliconia* Society International depository. Hence, the characteristic profiles generated by RAPD may be used as additional DNA markers for classifying different species and cultivars of *Heliconia*. The phylogenetic tree derived from the RAPD data showed that all the 16 cultivars examined are closely related to each other, thus providing the first genetic evidence that this large group of cultivars has a common genetic background. Moreover, two triploid cultivars of *H. psittacorum*—‘Iris’ and ‘Petra’—showed identical RAPD profiles with 10 different primers in agarose and polyacrylamide gels, suggesting that they are of the same genotype.

Heliconias, which are native to South and Central America, are popular as ornamental plants and cut flowers because of their brilliant colors and exotic appearance. Many *Heliconia* species are polymorphic. It is estimated that the genus *Heliconia* consists of ≈200 to 250 species and at least that many cultivars (Berry and Kress, 1991). *Heliconia psittacorum* comprises ≈25 cultivars (Berry and Kress, 1991). Species and cultivar identification is primarily based on morphological differences and coloration of the flowers and inflorescences. Significant variations exist within and between species of *Heliconia*. However, natural variation among individuals and populations of heliconias has caused confusion among hobbyists and commercial growers (Berry and Kress, 1991). Variation may be due to geographical isolation, environmental influence (e.g., light), and nutrients. Clones of *H. psittacorum* growing close together tend to vary in vegetative and floral characteristics, e.g., size and color of bracts. In addition, postharvest characteristics can vary from clone to clone (Donselman and Broschat, 1986).

As *Heliconia* is a relatively new interest for taxonomists and horticulturists (Criley and Broschat, 1992), correct identification of the various species and cultivars is important. Although a few heliconias can be identified by their foliage features, an inflorescence is required to identify most accurately. For the different varieties of *H. psittacorum*, the subtle variations can be rather difficult to describe and follow. The easiest practical method of identification so far has been by comparing color photographs of the inflorescences (Berry and Kress, 1991). It is therefore desirable to have some independent, objective methods to identify species and hybrids accurately. This is also important for breeding programs.

RAPD markers have been found to be useful in characterizing and studying the diversity of various crop plants (Kaemmer et al., 1992; Koller et al., 1993). We have developed a protocol for RAPD analysis of *Heliconia* as part of our effort to study and develop the plants as an ornamental and cut-flower crop. We demonstrate the usefulness of such markers for identifying >16 cultivars of *H. psittacorum* available in Singapore. The RAPD profiles were used to investigate the relationships between them as well as among various *Heliconia* species.

Materials and Methods

PLANT MATERIALS. Leaf samples from a newly expanded flag leaf of *Heliconia* were collected in triplicate from the Jurong BirdPark, Singapore, a depository of the *Heliconia* Society International. The leaves were surface sterilized by continuously shaking in 15% Clorox with two to three drops of Tween-20 for 15 min. Subsequently, the leaves were rinsed with sterilized water and samples of 0.5 to 1.0 g fresh mass were wrapped in aluminum foil, frozen in liquid nitrogen, and stored at –80 °C. Three replicate samples from independent populations of each cultivar or species were analyzed.

The study included 16 diploid cultivars of *Heliconia psittacorum* and three triploid cultivars—‘Iris’, ‘Petra’, and ‘Sassy’. Other species of *Heliconia* used included *H. rostrata* Ruiz and Pavon, *H. rostrata* ‘Ten Day’, *H. chartacea* Lane ex Barreiros ‘Sexy Pink’, and *H. bihai* L. ‘Claw II’. The following hybrid species were also analyzed: *H. psittacorum* × *H. spathocircinata* Aristeguieta ‘Golden Torch’ (two forms: red and yellow), *H. psittacorum* × *H. spathocircinata* ‘Red Torch’ (also known as Golden Torch Adrian), *H. psittacorum* × *H. spathocircinata* ‘Alan Carle’, and *Heliconia* × *nickeriensis* Maas & de Rooij (*H. psittacorum* × *H. marginata*; two forms: red and yellow). Five taxa from the order Zingiberales, to which *Heliconia* belongs—*Alpinia purpurata* (Vieill.) Schum. (red ginger), *Ravenala madagascariensis* Sonn. (traveller’s palm), *Musa* sp. (cultivated banana), and two cultivars of *Canna indica* L.

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(red and yellow flowered)—were used as outgroup plants in the analysis.

DNA EXTRACTION. DNA extraction protocol was modified from that of Honda and Hirai (1990) with the additional steps of RNase treatment and phenol-chloroform extraction. Briefly, 0.5 g leaf material was pulverized in liquid nitrogen before the addition of 2.5 mL of extraction buffer and incubating at 65 °C for 15 min. The extraction buffer contained 100 mM Tris-HCl (pH 8), 50 mM EDTA (pH 8), 500 mM NaCl, and 10 mM β -mercaptoethanol. The homogenate was transferred to ice for 15 min and centrifuged at 4 °C and 13,000 rpm for 20 min. The supernatant was collected and DNA was precipitated at -20 °C with 0.7 volume isopropanol. After $\approx$ 1 h, the mixture was centrifuged as before to collect DNA pellet. The pellet was dried in the speed vac for 5 min, resuspended in 100 mL of Tris-EDTA (pH 8), treated with RNase A (1 mL of 10 mg·mL⁻¹), and incubated at 37 °C for 30 min. The mixture was extracted with phenol-chloroform and subjected to ethanol precipitation. The resulting DNA pellet was resuspended in 100 mL Tris-EDTA and the concentration of DNA was determined by spectrophotometry. The DNA solution was stored at -20 °C until used for RAPD analysis.

RAPD ANALYSIS. The RAPD reaction mixture consisted of the following ingredients in a final volume of 50 μ L. Autoclaved water, 10 $\times$ PCR buffer (Promega), 0.4 mM of each dNTP (NEBiolabs), 1 mM of primer, 5 units of *Taq* DNA polymerase (Promega), 3 mM MgCl₂, and 100 ng template DNA. The reaction mixture was overlaid with 40 μ L of mineral oil before DNA amplification.

All the primers used, except primer NUSZG3, were from Operon Technologies (Alameda, Calif.), and each of them generated not less than six scorable bands per species. They were 10-

mers of the following sequences (5' to 3' direction):

(OPA1) CAGGCCCTTC, (OPA4) AATCGGGCTG, (OPA7) GAAACGGGTG, (OPA8) GTGACGTAGG, (OPA10) GTGATCGCAG, (OPA12) TCGGCGATAG, (OPA13) CAGCACCCAC, (OPA15) TTCCGAACCC, (OPA18) AGGTGACCGT, and (OPA20) GTTGCGATCC. The 9-mer primer, NUSZG3 (GGTAACGCC) was synthesized based on Dinesh et al. (1993). These primers were selected after a preliminary screening of >20 random primers.

RAPD analysis was carried out using a PTC-100 programmable thermal cycler (MJ Research). The amplification conditions were as follows: 2 min predenaturing at 95 °C followed by 30 cycles of 1 min denaturation at 95 °C, 3 min primer annealing at 37 °C, and 2 min extension at 72 °C with a final extension of 10 min. The RAPD products were separated by agarose (1.5% w/v) gel electrophoresis at 85 to 90 v for $\approx$ 3 h. The gels were viewed and photographed under UV light after staining with ethidium bromide.

The RAPD products of two triploid cultivars of *H. psittacorum*—'Iris' and 'Petra'—were also separated by denaturing polyacrylamide gel (4% stacking and 8% separating) electrophoresis (PAGE) and visualized by silver staining (Dinesh et al., 1993). Electrophoresis in the 1-mm-thick gels was carried out at 100 v constant voltage for 3.75 h.

DATA ANALYSIS. The bands were visually scored as present (1) or absent (0) and the percent genetic similarities between samples were derived from the similarity index of Nei and Li (1979). Only reproducible bands in multiple, independent runs were used for analysis. The phylogenetic tree was generated using the pairwise association coefficient and unweighted pair-group method using arithmetic average (UPGMA) cluster analysis.

Table 1. The percentage genetic similarity (%GS) for the various *Heliconia* species, cultivars and outgroup plants. The %GS values shown in the table were calculated by pairwise comparisons between samples according to Nei and Li (1979) and converting the factors to percentage values. The data are based on RAPD bands from all the random primers tested. The first 19 are cultivars of *H. psittacorum*.

Cultivar or species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1 Andromeda	100.0																																	
2 Tay	85.7	100.0																																
3 Suriname Sassy	73.9	80.0	100.0																															
4 Lady Di	82.8	83.3	77.3	100.0																														
5 Sheila	81.4	75.2	74.1	86.0	100.0																													
6 Susi	83.3	78.6	73.9	82.8	83.2	100.0																												
7 Emerald	73.7	71.2	73.5	78.7	84.0	79.0	100.0																											
8 Lilian	75.7	76.5	73.7	80.7	75.9	72.1	71.8	100.0																										
9 Yuki	75.6	78.1	80.3	80.3	79.0	74.0	78.4	83.6	100.0																									
10 St Vincent Red	81.1	78.2	78.9	79.0	75.7	73.0	73.2	77.1	78.6	100.0																								
11 Coverden Red	86.3	81.1	80.0	81.8	82.2	80.4	77.8	78.1	81.4	82.0	100.0																							
12 Fuchsia	79.3	73.3	75.6	80.7	79.3	74.1	73.8	80.7	83.5	80.7	81.8	100.0																						
13 Black Cherry	77.6	76.7	77.3	79.0	72.7	74.1	72.1	82.4	83.5	77.2	81.8	88.7	100.0																					
14 Claire	86.3	79.3	74.3	78.2	78.5	84.3	74.1	72.4	76.1	84.0	85.4	74.6	74.6	100.0																				
15 Barbara	79.6	71.4	64.8	72.4	74.3	77.8	71.9	72.1	75.6	81.1	78.4	79.3	75.9	84.3	100.0																			
16 Sybille	78.3	82.4	78.0	81.3	78.3	76.5	72.7	83.1	84.1	77.9	82.6	86.2	86.2	75.2	76.5	100.0																		
17 Iris	87.4	86.2	80.3	85.0	82.3	80.7	73.6	77.1	80.0	76.9	83.2	80.3	78.7	77.9	74.0	84.1	100.0																	
18 Petra	87.4	86.2	80.3	85.0	82.3	80.7	73.6	77.1	80.0	76.9	83.2	80.3	78.7	77.9	74.0	84.1	100.0	100.0																
19 Sassy	81.4	78.6	75.9	82.6	81.4	81.4	74.0	74.1	74.2	77.5	80.4	79.3	74.4	76.6	76.1	78.3	87.1	87.1	100.0															
20 H. xRed Torch	80.7	80.7	76.3	84.6	81.7	80.0	79.3	76.3	74.6	76.1	78.9	71.5	74.8	75.2	69.6	75.4	81.0	81.0	80.0	100.0														
21 H. xAlan Carle	79.0	76.4	77.1	81.9	77.4	77.3	76.8	77.1	76.9	75.2	79.7	74.0	77.2	77.9	72.3	74.6	78.5	78.5	77.4	79.4	100.0													
22 H. xGolden Torch (red)	78.4	80.6	75.0	81.2	75.4	76.8	71.8	79.7	79.4	78.1	74.0	75.2	75.2	72.3	72.0	77.3	83.8	83.8	78.5	87.9	80.9	100.0												
23 H. xGolden Torch (yellow)	78.5	80.6	75.0	81.2	75.4	76.8	71.8	79.7	79.4	78.1	74.0	75.2	75.2	72.3	72.0	77.3	83.8	83.8	78.5	87.9	80.9	100.0	100.0											
24 H. xnickeriensis (red)	72.6	73.4	69.3	75.8	69.8	69.4	69.2	75.6	78.5	67.2	72.9	77.3	70.3	67.8	72.6	76.3	74.1	74.1	66.7	68.7	72.6	73.8	73.8	100.0										
25 H. xnickeriensis (yellow)	72.6	73.4	69.3	75.8	69.8	69.4	69.2	75.6	78.5	67.2	72.9	77.3	70.3	67.8	72.6	76.3	74.1	74.1	66.7	68.7	72.6	73.8	73.8	100.0	100.0									
26 H. rostrata	53.0	47.9	48.3	46.4	45.9	51.3	48.8	48.3	48.4	53.9	50.5	48.0	48.0	52.3	49.6	45.2	50.0	50.0	47.5	50.0	51.6	49.3	46.3	49.6	49.6	100.0								
27 H. rostrata Ten Day	60.6	54.9	51.8	54.7	54.4	56.9	52.2	53.6	55.0	59.8	54.4	51.3	49.6	56.3	55.1	51.7	58.3	58.3	57.9	55.2	51.7	55.6	55.6	54.4	54.4	72.9	100.0							
28 H. chartacea Sexy Pink	46.0	42.7	41.4	46.3	49.2	46.0	45.4	51.7	45.2	45.1	43.0	47.9	44.6	43.0	40.7	43.3	43.6	43.6	44.1	50.0	51.6	53.9	53.9	46.5	46.5	47.5	50.9	100.0						
29 H. bhari Claw II	47.0	45.4	47.5	52.0	43.3	45.2	46.3	44.1	47.6	47.8	47.7	47.2	48.8	42.2	43.5	44.3	47.6	47.6	48.3	44.3	46.2	48.5	48.5	42.8	42.8	48.4	48.3	45.0	100.0					
30 Alpina	2.6	2.5	2.5	2.4	2.4	2.6	2.4	2.5	4.6	2.7	2.8	2.4	2.4	2.8	2.6	2.4	2.3	2.3	2.4	0	2.3	2.1	2.1	6.5	6.5	11.6	10.3	4.9	2.4	100.0				
31 Musa	12.7	12.3	12.4	17.0	13.9	12.7	13.8	15.9	16.5	12.7	11.5	13.6	15.3	15.4	9.1	12.0	11.6	11.6	12.2	15.4	18.2	15.8	15.8	11.1	11.1	11.8	7.2	12.2	12.0	10.1	100.0			
32 Ravenala	10.5	8.1	8.2	11.7	10.0	4.2	11.9	12.2	11.3	17.2	11.2	13.6	11.7	11.2	14.7	7.8	9.4	9.4	10.0	9.8	15.1	14.3	14.3	14.4	14.4	5.8	6.3	4.0	7.8	6.3	10.3	100.0		
33 Canna (red)	4.7	4.4	6.7	8.5	8.7	4.7	8.7	11.2	10.3	4.8	5.0	8.5	10.6	2.5	4.7	8.6	6.2	6.2	4.4	8.6	10.3	11.7	11.7	7.8	7.8	4.2	2.3	13.2	6.5	0	13.6	13.7	100.0	
34 Canna (yellow)	4.7	4.4	6.7	8.5	8.7	4.7	8.7	11.2	10.3	4.8	5.0	8.5	10.6	2.5	4.7	8.6	6.2	6.2	4.4	8.6	10.3	11.7	11.7	7.8	7.8	4.2	2.3	13.2	6.5	0	13.6	13.7	100.0	100.0

Fig. 1. RAPD products (three independent sets of amplification) generated with the primer OPA8 and separated on 1.5% agarose gels. Lanes M, 1-kb DNA ladder (Gibco BRL); lanes 1–3, *Heliconia* 'Golden Torch' (red); lanes 4–6, *Heliconia* 'Golden Torch' (yellow); lanes 7–9, *H. xnickeriensis* (red); lanes 10–12, *H. xnickeriensis* (yellow); lanes 13–15, negative control (without DNA templates).

Results and Discussion

In the preliminary experiments, DNA was extracted according to Honda and Hirai (1990) without RNase treatment. The amplified DNA bands were either faint or not reproducible when such a template was used. After RNase treatment and phenol–chloroform extraction, the DNA was cleaner and there was no visible background smear in such extracts subsequent to agarose gel electrophoresis compared to the DNA without RNase treatment. Also, satisfactory amplification and reproducible DNA band profiles were obtained with RNase-treated DNA templates. It is possible that the contaminating RNA in the extracts might have interfered with DNA amplification (Ellsworth et al., 1993).

The optimum amount of DNA template for *Heliconia* was 100 ng of total DNA. No DNA bands were amplified in the absence of $MgCl_2$. The relative intensities of the amplified DNA fragments increased as $MgCl_2$ concentration increased from 1 mM to an optimum of 3 mM. Beyond 3 mM, the intensities of the bands decreased, and the bands at 8 mM $MgCl_2$ were the faintest (data not shown). The Mg^{2+} ion is known to affect the primer–template interactions (Welsh and McClelland, 1990), polymerase activity, and the melting temperature of double-stranded DNA (Rolfes et al., 1992).

Table 2. Using DNA markers to identify hybrids with *Heliconia psittacorum* as a common parent. The primer used for amplification was OPA18. Markers were scored as present (1) or absent (0).

Marker (bp)	Hybrid cultivar ^a			
	HGT	HRT	HAC	HN
850	0	0	0	1
820	1	1	1	0
800	1	0	1	1
760	0	0	1	0
730	1	0	0	0

^aHGT = *Heliconia* 'Golden Torch', HRT = *Heliconia* 'Red Torch', HAC = *Heliconia* 'Alan Carle', HN = *H. xnickeriensis*.

The optimal primer annealing temperature used was 37 °C. At this temperature, the number of bands varied from 4 to 20 with different templates and primers. The size of amplified DNA fragments ranged from ≈300 to >3800 bp.

The genetic similarities (GSs) between replicates of a particular sample ranged from 93.3% to 100%. Table 1 shows the percentage GS between pairwise comparisons of the samples calculated using Nei and Li's coefficient (Nei and Li, 1979). The highest GS values were between *H. psittacorum* cultivars (71.2% to 87.9%). GS was lowest between *Heliconia* spp. and the control outgroup plants

Fig. 2. (a) RAPD profiles generated using the primer OPA8. Lanes 1 and 20, molecular weight markers (1-kb ladder); lane 2, *Heliconia rostrata* 'Ten Day'; lane 3, *H. rostrata*; lane 4, *H. chartacea* 'Sexy Pink'; lane 5, *H. bihai* 'Claw II'; lane 6, *H. psittacorum* 'Andromeda'; lane 7, *H. psittacorum* 'Tay'; lane 8, *H. psittacorum* 'Iris'; lane 9, *H. psittacorum* 'Petra'; lane 10, *H. psittacorum* 'Sassy'; lane 11, *H. psittacorum* 'Suriname Sassy'; lane 12, *Heliconia* 'Golden Torch' (red); lane 13, *Heliconia* 'Red Torch'; lane 14, *Heliconia* 'Alan Carle'; lane 15, *H. xnickeriensis* (red); lane 16, *H. psittacorum* 'Lady Di'; lane 17, *H. psittacorum* 'Sheila'; lane 18, *H. psittacorum* 'Susi'; lane 19, *H. psittacorum* 'Emerald'. (b) RAPD profiles generated using the primer OPA8. Lanes 1 and 19, molecular weight markers (1-kb ladder); lane 2, *Heliconia* 'Golden Torch' (yellow); lane 3, *H. xnickeriensis* (yellow); lane 4, *H. psittacorum* 'Lilian'; lane 5, *H. psittacorum* 'Yuki'; lane 6, *H. psittacorum* 'St. Vincent Red'; lane 7, *H. psittacorum* 'Coverden Red'; lane 8, *H. psittacorum* 'Fuchsia'; lane 9, *H. psittacorum* 'Black Cherry'; lane 10, *H. psittacorum* 'Claire'; lane 11, *H. psittacorum* 'Barbara'; lane 12, *H. psittacorum* 'Sybille'; lane 13, *Alpinia purpurata* (red ginger); lane 14, *Musa* spp.; lane 15, *Ravenala madagascariensis* (Traveller's Palm); lane 16, *Canna indica* (yellow); lane 17, *Canna indica* (red); lane 18, negative control (without DNA template).

Fig. 3. RAPD products generated with the primer OPA18 and separated on 1.5% agarose gels. Lane markings are as in Fig. 2a.

(0% to 18.2%), followed by that between different *Heliconia* species (40.7% to 60.6%) (Table 1).

COMPARISON BETWEEN *Heliconia* AND ITS DISTANT RELATIVES. *Heliconia* species exhibited relatively low GS to the outgroup plants. The average GS value obtained was 12.3% with *Musa* sp., followed by *Ravenala madagascariensis* at 10.7%, the two *Canna indica* cultivars at 7.4%, and finally *Alpinia purpurata* at 3.4% (Table 1). Although no genetic polymorphisms were observed between the two cultivars of *Canna* in this study, they served as adequate controls.

COMPARISON AMONG HYBRIDS WITH *H. psittacorum* AS A COMMON PARENT. Three cultivars of the hybrid *H. psittacorum* × *H. spathocircinata*, namely, 'Golden Torch', 'Red Torch' and 'Alan Carle', showed only slight differences in RAPD profiles which were distinct from that of *H. xnickeriensis* (*H. psittacorum* × *H. marginata*) (Fig. 1).

Taxa of the same parentage showed higher GS values compared

to plants of different parentage. For example, between 'Golden Torch', 'Red Torch', and 'Alan Carle', GS varied from 79.4 to 87.9 (Table 1). The pairwise comparison between 'Golden Torch' and 'Red Torch' gave a particularly high GS value of 87.9%. This agrees with the fact that the morphologies of the inflorescences of these two plants were very similar. The phenotypic differences between 'Golden Torch' and 'Red Torch' were only in the colors of the bracts, florets, and ovaries. When the above mentioned three hybrids were compared with *H. xnickeriensis*, GS was reduced to between 68.7 to 74.3%. Also, the GS for these hybrids was <50% with other species of *Heliconia* (Table 1). Such observations confirm the use of RAPD markers to identify the parentage of plants. Table 2 gives the DNA markers that may be used to identify and distinguish these different hybrids.

Two types of 'Golden Torch' are known. One is uniformly golden-yellow, while the other is golden-yellow with a small patch of red at the base of each of the bracts. Also, there are two forms of *H. xnickeriensis*, namely one with more red on the bracts and rachis and another has more yellow in place of the red. The RAPD analysis of both forms of these two pairs of cultivars showed identical profiles in band numbers, fragment size, and band intensities (Fig. 1). Furthermore, the GS values obtained for the two 'Golden Torch' cultivars (pairwise comparison with the other cultivars tested) were identical to each other, as were the GS values for the two *H. xnickeriensis* cultivars (Table 1). Therefore, both the forms of these two pairs of cultivars belong to the same genotype. It is possible that such morphological differences might be solely due to environmental influences on the plants. Environmental factors such as the amount of irradiance (e.g., comparing plants grown in the shade to plants grown in full sunlight) and mineral nutrition (e.g., plants fed with adequate levels of fertilizers compared to plants given insufficient nutrients) affected the physiological processes of *Plantago* species (Kuiper and Bos, 1992). It is likely that such environmental influence might have affected the red pigmentation on *Heliconia* inflorescences, resulting in the variations of inflorescence colors without any genetic changes.

Alternatively, the differences may be due to the parentage of the plants. Until now, the true parents of these hybrids have not been

Table 3. The use of multiple DNA markers to identify *H. psittacorum* cultivars using primer OPA18. Bands were scored according to their presence (1) or absence (0).

Marker (bp)	<i>Heliconia psittacorum</i> cultivar ²															
	An	Ta	SS	LD	Sh	Su	Em	Li	Yu	SV	CR	Fu	BC	Cl	Ba	Sy
2150	1	1	1	1	0	1	0	1	0	1	1	1	1	0	0	1
2000	0	1	1	0	0	0	0	1	1	1	0	0	0	0	0	1
1850	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1480	0	0	1	0	0	1	1	0	1	0	0	0	0	1	0	1
1450	0	0	0	1	0	0	1	1	1	1	0	1	1	0	1	0
1400	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1370	0	0	1	0	0	1	1	0	1	1	0	0	1	0	0	0
1200	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1060	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0
1050	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1
900	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1
880	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
850	0	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1
820	1	1	0	1	1	1	0	0	0	1	0	0	0	1	1	0
800	1	0	0	1	1	1	1	0	1	1	1	1	0	1	1	0
760	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

²An = 'Andromeda', Yu = 'Yuki', Ta = 'Tay', SV = 'St. Vincent Red', SS = 'Suriname Sassy', CR = 'Coverden Red', LD = 'Lady Di', Fu = 'Fuchsia', Sh = 'Sheila', BC = 'Black Cherry', Su = 'Susi', Cl = 'Claire', Em = 'Emerald', Ba = 'Barbara', Li = 'Lilian', Sy = 'Sybille'.

Fig. 4. RAPD profiles of the 16 diploid cultivars of *H. psittacorum* generated with the primer OPA18 and separated on 1.5% agarose gels. The samples were run in the same sequence (from left to right) as the data presented in Table 3.

confirmed. Some workers reported that *H. psittacorum* is the female parent (Berry and Kress, 1991), while others suggest that *H. psittacorum* is the male parent (Criley and Broschat, 1992). Confirming the female parent of the plants is important because the mother plant may have contributed more to the genetic makeup of the offspring due to cytoplasmic inheritance (Gardner et al., 1991).

COMPARISON AMONG *Heliconia* SPECIES. Significant differences in RAPD profiles were observed among different species of *Heliconia* and some distantly related plants. Single, unique DNA markers

could be used to identify different species of *Heliconia* studied. For example, using primer OPA8, *H. chartacea* 'Sexy Pink' could be identified by the absence of a band at 2700 bp, which was present in all other *Heliconia* species tested (Fig. 2 a and b). The band at 1130 bp was found in all *H. psittacorum* cultivars except 'Sassy'. For *H. bihai* 'Claw II', the absence of band at 1050 bp can be used as a marker. Finally, the band at 625 bp was present only in *H. rostrata* cultivars.

- a) ***Heliconia rostrata*.** When comparing *H. rostrata* with *H. rostrata* 'Ten Day', polymorphism in banding patterns was observed and the GS was only 72.9. It was possible to distinguish *H. rostrata* 'Ten Day' using primer OPA18 with a band at 2200 bp, which was absent in *H. rostrata*. Similarly, a band at 1630 bp was present in *H. rostrata* and not in 'Ten Day' (Fig. 3).
- b) ***Heliconia psittacorum*.** Polymorphisms were also observed in RAPD profiles in *H. psittacorum* cultivars. With these variations in RAPD profiles, it is evident that RAPD markers can be used to distinguish and identify cultivars within a particular species. It may also be possible to confirm the relationship between cultivars with more extensive studies. Also, the homology of shared bands between different species of *Heliconia* can be confirmed with further analyses as described earlier.

COMPARISON BETWEEN DIPLOID AND TRIPLOID CULTIVARS. Between the diploid and triploid cultivars of *H. psittacorum* there were no noticeable differences in fragment intensities (e.g., Fig. 2 a and b). Perhaps because the template concentration was the same for all the samples studied, the resulting amplifications all had uniform intensities. However, Wu and Lin (1994) were able to distinguish between diploid and hexaploid lines of buffalograss

Fig. 5. Phylogenetic tree based on data from the RAPD profiles generated with the primer OPA18.

Fig. 6. RAPD products generated with the primers OPA8, OPA15, OPA18, NUSZG3—separated on 1.5% agarose gels (a–d), and with the primers OPA1, OPA10, OPA18—separated by SDS-PAGE (e–g). Three independent sets of amplification were carried out from different templates for each cultivar. Lane 1, molecular weight marker (1-kb DNA ladder); lanes 2–4, *Heliconia psittacorum* 'Iris'; lanes 5–7, *H. psittacorum* 'Petra'; lane 8, negative control (without DNA template).

[*Buchloe dactyloides* (Nutt.) Engelm.] using RAPD analysis. This suggests that, by further analysis using different primers or by densitometric estimation of the intensities of the various bands, it may be possible to distinguish between the triploid and diploid cultivars of *H. psittacorum*.

RAPD polymorphisms can provide DNA markers in genomic regions that are not accessible to RFLP analysis due to the presence of repetitive DNA sequences (Williams et al., 1990). Also, because both techniques visualize different regions of the genome, they are more likely to complement each other (Kaemmer et al., 1992).

COMPARISON AMONG DIPLOID CULTIVARS OF *H. psittacorum*. In diploid cultivars of *H. psittacorum*, polymorphism of bands was extensive, and these polymorphisms in the RAPD profiles enabled the identification of all 16 diploid cultivars by using multiple markers (Table 3). Hu and Quiros (1991) found that two and three primers were sufficient to distinguish each of the broccoli (*Brassica oleracea* L. var. botrytis) and cauliflower (*Brassica oleracea* L. var. Cauliflora) cultivars, respectively. Similarly, using two separate 10-mer primers, Mulcahy et al. (1993) obtained distinctive fingerprints for each apple (*Malus domestica* Borkh.) cultivar studied. Also, four primers were enough to produce RAPD profiles that differed for all three races of *Bipolaris maydis* (Nisikado & Miyake) Shoemaker (Nicholson et al., 1993). However, with the amplification conditions used, we were able to distinguish and identify all 16 diploid cultivars of *H. psittacorum* using only one primer, namely OPA18 (Table 3, Fig. 4). This indicates that RAPD markers provide a quick and reliable alternative to morphological markers for identifying cultivars of *H. psittacorum*.

The phylogenetic tree derived from data presented in Table 3

and that of the outgroup plants shows that the various cultivars tested are relatively closely related to each other (Fig. 5). Compared with these, the other species of *Heliconia* and the outgroup plants clearly form a distinct cluster. Data from the other primers tested also followed the same trend (not shown).

COMPARISON AMONG TRIPLOID CULTIVARS OF *H. psittacorum*. 'Iris', 'Petra', and 'Sassy' are triploid cultivars belonging to *H. psittacorum* (Lee et al., 1994). The triploid plants are more robust, with larger leaves and longer fleshier florets than those of the diploids. Morphologically, 'Iris' and 'Petra' plants are similar to each other. In both the cultivars, plants are ≈ 1.5 m tall, with a distichous leaf arrangement. Their inflorescences have four to six bracts, with the proximal parts shaded orange and the distal portions pinkish. The rachis is orange proximally and pinkish distally. The sepals (perianth) are orange with a dark greenish band below the tip. The ovary is yellowish but deepened to orange in the distal half. The pedicels are orange.

Although 'Petra' was reported to produce more inflorescences and slightly fewer florets per inflorescence than 'Iris', sporad formation after meiotic division in the microsporocytes in both the cultivars is identical and both are sterile (Lee et al., 1994). Since the number of inflorescence and florets can vary depending on the environment (Criley, 1986), we further examined their similarities or differences at the DNA level using RAPD analysis.

From an analysis of the RAPD amplification products with 10 random primers in agarose and polyacrylamide gels, our results show that the genetic fingerprints of 'Iris' and 'Petra' are identical (Fig. 6), but distinct from that of 'Sassy' (Fig. 2a, lanes 8–10).

Hence, 'Iris' and 'Petra' can be considered as the same genotype.

To date, there has been no concerted effort on mounting a breeding program for *Heliconia*, probably due to the relatively low efficiency of fruit set (<5%) among popular cultivars (Lee et al., 1994). Cytological observations indicated that many of the *H. psittacorum* cultivars, except triploids, are fertile and produce normal viable pollen tetrads (Lee et al., 1994). However, fruit set was limited by the pollen germination rates on the stigma. The genetic similarity indices obtained from RAPD analyses such as that reported here may aid future breeding schemes for *Heliconia*. Once valuable hybrids are generated, they can be rapidly propagated by tissue culture. Efficient methods suitable for large-scale shoot multiplication by bud culture (Goh et al., 1995, Nathan et al., 1992) and via callus intermediate (Nathan et al., 1993) have been established for *Heliconia*.

Our results indicate that the RAPD technique is useful in distinguishing species and cultivars of *Heliconia*. Therefore, the characteristic profiles generated by RAPD may be used as additional DNA markers for classifying the different species and cultivars of *Heliconia*. In view of the observed identical RAPD profiles and similarities in cytology and morphology, we recommend that the cultivar names 'Iris' and 'Petra' be treated as synonyms.

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