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Molecular characterization of *Heliconia* by RAPD assay

V.L. Sheela*, P.R. Geetha Lekshmi, C.S. Jayachandran Nair, and K. Rajmohan

Department of Pomology and Floriculture, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram 695 522, Kerala.

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Abstract

Seventeen *Heliconia* species and varieties were analyzed using RAPD markers. Eight primers, which produced the highest number of bands, were used for DNA amplification. The genetic similarity matrix constructed with Jaccard's coefficient using RAPD marker scores showed that the highest value was between Petra Orange and Parakeet, while the lowest was between Golden Torch and *H. humilis*. The 17 species and varieties of *Heliconia* formed nine distinct clusters at similarity coefficient value of 0.42, implying a strong parallelism between genetic and morphologic/taxonomic variability of *Heliconia* genotypes. Petra Orange, Deep Orange, Parakeet, Pascal, and Alan Carle formed a big cluster within which Petra Orange and Parakeet formed a more cohesive entity.

Keywords: Cut flower trade, DNA marker, Genetic similarity.

Introduction

Heliconias (F. Heliconiaceae) are excellent cut flowers in the international and national markets due to their striking shape, attractive colour, long vase life, and prolific flower producing capacity. Importance of *Heliconia* as a newly introduced cut flower is also increasing. However, variations abound in the morphology of flowers and inflorescences of this crop, which hitherto formed the basis for cultivar identification. Recent developments in molecular biology have made DNA fingerprinting of plants feasible and an array of techniques are available today to characterize DNA sequence/polymorphism. Several of these have been used in phylogenic studies, genetic diversity analysis, forensic science, paternity determination, plant varietal characterization, and the like (Williams et al., 2004). In *Heliconia* too, a protocol to extract DNA from leaves and analyze the genetic variations using RAPD has been developed (Kumar et al., 1998). And it is thus possible to group species and varieties using such tools to facilitate further improvement. The objective of the present study was to classify selected locally important species and varieties of *Heliconia* based on their molecular characteristics.

Materials and Methods

The experiment was conducted during 2003–06 in which four species and 13 varieties of *Heliconia* (*H. psittacorum* × *H. spathocircinata* cv. Golden Torch, *H. psittacorum* cv. Lady Di, *H. latispatha*, *H. psittacorum* × *H. marginata* cv. De Rooij, *H. psittacorum* cv. Petra Orange, *H. psittacorum* cv. Deep Orange, *H. rostrata*, *H. collinsiana* × *H. bourgena* cv. Pedro Ortiz, *H. Humilis*, *H. psittacorum* × *H. spathocircinata* cv. Guyana, *H. wagneriana* cv. Wagneriana Red, *H. wagneriana* cv. Wagneriana Yellow, *H. psittacorum* cv. Parakeet, *H. psittacorum* cv. Pascal, *H. collinsiana*, *H. psittacorum* cv. St.Vincent Red, and *H. aristegueta* cv. Alan Carle) were characterized.

For isolation of the genomic DNA, tissues from emerging leaves before they were fully unfurled were

*Author for correspondence: Phone 0471-2381002 (extn. 222); Fax 0471-2382239; E-mail <sheela_jayagopan@yahoo.com>.

used. The leaf sample was placed in a pre-chilled mortar and pestle, to which 2.5 ml of 2 % CTAB and 300 µl of 1% poly vinyl pyrolidone (PVP) were added and the leaf tissues macerated. The contents were transferred to 15 ml polypropylene centrifuge tube containing 5 ml of pre-warmed CTAB extraction buffer [2% (w/v) CTAB (hexa decyl trimethyl ammonium bromide), 100 mM Tris HCL (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 0.2% mercaptoethanol] using a sterile spatula. To this, 1 ml of PVP solution (1%) was added and incubated at 60°C for 30 min. in a water bath with occasional mixing by gentle swirling. After 30 min., the samples were taken out and kept at room temperature for 10 min. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and the contents mixed by inversion, and centrifuged at 15,000 rpm for 10 min. at 5°C. The aqueous phase was separated with a wide-bore pipette and transferred to a clean tube, to which equal volume of phenol chloroform isoamyl alcohol was added and centrifuged at 15,000 rpm for 10 min. (5°C). The upper phase was collected and extracted with chloroform: isoamyl alcohol (24:1), and 0.1 volume of 3 M sodium acetate (pH 4.8) and 2/3 volume of isopropanol were added to the aqueous phase. It was mixed by quick gentle inversion to precipitate the DNA, which was pelleted by centrifugation at 10,000 rpm for 5 min. at 5°C. The supernatant was decanted carefully and the DNA pellet washed with 70% cold ethanol and air-dried. The DNA pellet was dissolved in 100 µl TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and stored at 4°C.

For purification, equal volumes of DNA and phenol: chloroform isoamyl alcohol (25: 24: 1) were mixed properly and centrifuged at 10,000 rpm for 10 min. at room temperature. The aqueous phase was removed and transferred to a fresh microfuge tube and extracted twice with equal volume of chloroform and isoamyl alcohol (24: 1). After centrifugation, the aqueous phase was transferred to a fresh microfuge tube with 0.1 volume of 3 M sodium acetate (pH 5.2). Absolute alcohol (2.5 volume) was added and mixed by quick gentle inversion to precipitate the DNA which was kept in a freezer for 1 h, and centrifuged at 10,000 rpm for 10 min. to pellet the DNA.

For DNA quantification, the absorbance of DNA dissolved in TE buffer was measured at 260 nm and 280 nm in a UV-vis spectrophotometer (Genesys 5, Spectronic Instruments, USA). The quantity of DNA in the sample was estimated as A260 x 50 x dilution factor, where, A260 = absorbance at 260 nm.

DNA amplification was done using 40 arbitrarily designed decamer primers (Operon Inc, CA, USA.) adopting the procedure of Lim et al. (1999). Polymerase chain reaction (PCR) was carried out in a volume of 25 µl containing 2.5 µl 10 x buffer [10 mM Tris HCI (pH 9.0), 1.50 mM $MgCl_2$, 50 mM KCl, and 0.01% gelatin], 10 pM primer, 250 µM each of deoxynucleotides (dNTPs), 0.2 units of Taq DNA polymerase, and 20 ng of genomic DNA. Amplification was performed in a programmable Thermal Controller (MJ Research Inc., USA) for an initial denaturation of 1 min. at 95°C, followed by 35 cycles of denaturation at 95°C for 1 min., annealing at 35°C for 2 min., and extension for 2 min. at 72°C. A final extension for 10 min. at 72°C was included after the last cycle. The products of amplification were cooled at 4°C. A negative control containing sterile water was included in each reaction set instead of templates.

Amplified products along with DNA molecular weight markers (US Biochemicals) were separated in a horizontal gel electrophoresis unit using 1.4 % agarose gel, stained with ethidium bromide and visualized on a UV transilluminator. The number of monomorphic bands, number of polymorphic bands, and intensity of bands were recorded. Primers, which on amplification produced the maximum number of bands, were used to amplify the DNA and the amplification profile using eight primers were taken with the help of gel documentation system. The RAPD bands were represented as '+' (present) and '–' (absent). The PCR was repeated at least twice in order to check reproducibility. The amplification profiles of those primers, which produced amplification for most of the species and varieties, only were used in the final analysis.

A genetic similarity matrix was constructed with Jaccard's coefficient (Jaccard, 1908) using the score of

RAPD marker bands. Jaccard's coefficient, Sj = a/(a+b+c), where, a = number of bands present in both the species and varieties in a pair, b = number of bands present in the first variety but not in the second one, and c = number of bands present in the second but not in the first. Based on the similarity coefficient, the association between the species and varieties was computed using the software NTSYS (version 2.02) and a dendrogram was constructed following the Unweighted Pair Group Method with Arithmetic Average (UPGMA) method. The data obtained from eight primers (OPA 01, OPA 14, OPA 17, OPA 18, OPB 07, OPB 12, OPB 18, and OPB 20) that gave reproducible bands were used for statistical analysis.

Results and Discussion

Purity and yield of DNA from the selected 17 *Heliconia* species and varieties are presented in Table 1. Genomic DNA yield ranged from 360 to 1170 µg ml^{-1} with the highest for Deep Orange and lowest for Wagneriana Yellow. Purity values (A_{260}/A_{280}) were between 1.5 and 2.0 implying good quality of the DNA extracted. Electrophoretic assay revealed the integrity of DNA samples with no smearing. As evident from the banding pattern of primers in PCR reactions, the number of bands resolved per amplification was primer-dependent. The primer, OPA 01, yielded four bands (Fig. 1) and OPA 14 gave six bands (Fig. 2). OPA 14 was also monomorphic for all species and varieties except Pedro Ortiz. Six bands were obtained on amplification with the primer OPA 17, while OPA 18 gave seven bands, the highest (Fig. 3) with a monomorphic band for all the species and varieties except *H. humilis*. Five bands were obtained for OPB 07 and four bands with OPB 12. The primers OPB 18 and 20 yielded the highest of four bands.

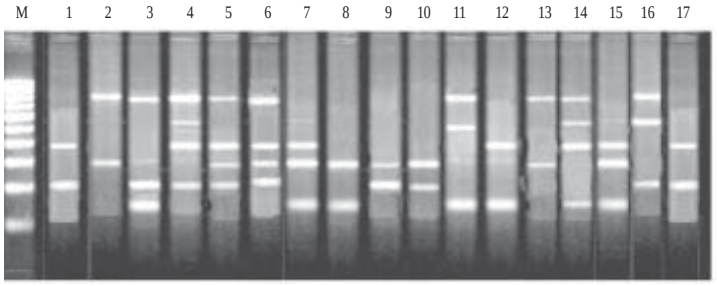

Figure 1. Amplification profile of the DNA of 17 *Heliconia* species and varieties with the primer OPA-01 (M-Molecular marker; see Table 1 for the varietal names of items 1 to 17 listed in that order)

Table 1. Quantity and yield of DNA from 17 *Heliconia* species and varieties.

Treatments	Purity[1] (A_{260}/A_{280})	Yield of DNA (µg ml^{-1})
Golden Torch	1.94	1110
Lady Di	1.88	900
H. latispatha	1.90	630
De Rooij	1.80	540
Petra Orange	1.88	510
Deep Orange	1.95	1170
H. rostrata	1.80	540
Pedro Ortiz	1.72	570
H. humilis	1.60	840
Guyana	1.93	930
Wagneriana Red	1.85	390
Wagneriana Yellow	1.50	360
Parakeet	1.70	510
Pascal	1.88	450
H. collinsiana	1.90	570
St. Vincent Red	1.82	870
Alan Carle	1.94	930

[1]Absorbance at 260 and 280 nm.

The similarity coefficient was least (0.08) for Golden Torch and *H. humilis*, followed by Pedro Ortiz and Alan Carle (Table 2). The highest value for similarity index (0.60) was for Petra Orange and Parakeet followed by Petra Orange and Deep Orange. The 17 species and varieties of *Heliconia* formed nine homogenous clusters (similarity coefficient value of 0.42; Fig.4). The biggest cluster was produced by Petra Orange, Deep Orange, Parakeet, Pascal, and Alan Carle. Within this cluster, Petra Orange and Parakeet formed a sub-cluster. Varieties Lady Di, Pedro Ortiz, and *H. humilis* formed separate clusters, while Wagneriana Red and Wagneriana Yellow formed one cluster at a similarity index of 0.49. *H. rostrata* and *H. collinsiana* were similar at a similarity coefficient of 0.57. This is consistent with the fact that both are producing hanging type inflorescence.

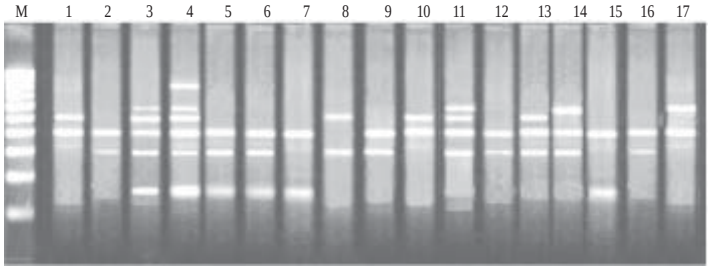

Figure 2. Amplification profile of the DNA of 17 *Heliconia* species and varieties with the primer OPA-14 (M-Molecular marker; see Table 1 for the varietal names of items 1 to 17 listed in that order).

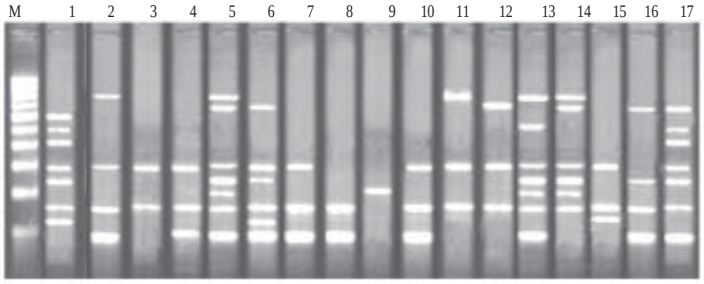

Figure 3. Amplification profile of the DNA of 17 *Heliconia* species and varieties with the primer OPA-18 (M-Molecular marker; see Table 1 for the varietal names of items 1 to 17 listed in that order).

Likewise, the varieties Golden Torch and Guyana formed another distinctive cluster and both these are derived from crosses between *H.psittacorum* and *H. spatho-circinata*.

As expected, taxonomically related entries were clustered together and the distant ones segregated (Fig 4). For example, the varieties Wagneriana Red and Wagneriana Yellow belonging to *H. wagneriana* species, which is similar to *H. humilis* (Timothy, 1996) assembled together. Results of the present investigation thus re-confirm that taxonomic and genetic similarities coincide with one another. The present RAPD analysis is also useful in detecting genetic variations and similarities among the *Heliconia* genotypes. Similar work was reported earlier by Goh et al. (1995) in *Heliconia*, Lim et al. (1999) in Vanda, and Jau-Yueh et al. (1999) in anthurium.

Table 2. Matrix of similarity coefficients for 17 *Heliconia* species and varieties obtained by random amplified polymorphic DNA analysis.

	G. Torch	Lady Di	*H. latis patha*	*De* Rooij	Petra Orange	Deep Orange	*H. rostrata*	Pedro Ortiz	*H. humilis*	Guy ana	Wag. Red	Wag. Yellow	Para keet	Pas cal	*H. collin siana*	St. Vin Red	Alan Carle
G. Torch	1.00																
Lady Di	0.27	1.00															
H. latispatha	0.17	0.19	1.00														
De Rooij	0.27	0.33	0.45	1.00													
Petra Orange	0.33	0.51	0.31	0.39	1.00												
Deep Orange	0.45	0.41	0.23	0.40	0.57	1.00											
H. rostrata	0.26	0.24	0.30	0.32	0.32	0.35	1.00										
Pedro Ortiz	0.15	0.21	0.27	0.22	0.26	0.18	0.20	1.00									
H. humilis	0.08	0.31	0.29	0.24	0.34	0.19	0.22	0.27	1.00								
Guyana	0.45	0.24	0.38	0.31	0.45	0.38	0.34	0.37	0.21	1.00							
Wag. Red	0.17	0.32	0.43	0.36	0.38	0.22	0.31	0.29	0.30	0.23	1.00						
Wag. Yellow	0.26	0.32	0.23	0.23	0.45	0.32	0.36	0.29	0.34	0.27	0.47	1.00					
Parakeet	0.28	0.37	0.35	0.33	0.60	0.39	0.25	0.34	0.31	0.39	0.39	0.33	1.00				
Pascal	0.21	0.27	0.41	0.34	0.47	0.37	0.16	0.23	0.24	0.22	0.45	0.33	0.54	1.00			
H. collinsiana	0.26	0.37	0.30	0.29	0.32	0.35	0.56	0.20	0.30	0.26	0.35	0.31	0.22	0.19	1.00		
St. Vin. Red	0.36	0.45	0.25	0.48	0.44	0.52	0.23	0.23	0.21	0.29	0.24	0.21	0.43	0.36	0.23	1.00	
Alan Carle	0.43	0.33	0.19	0.38	0.43	0.41	0.24	0.13	0.19	0.27	0.25	0.29	0.26	0.30	0.21	0.31	1.00

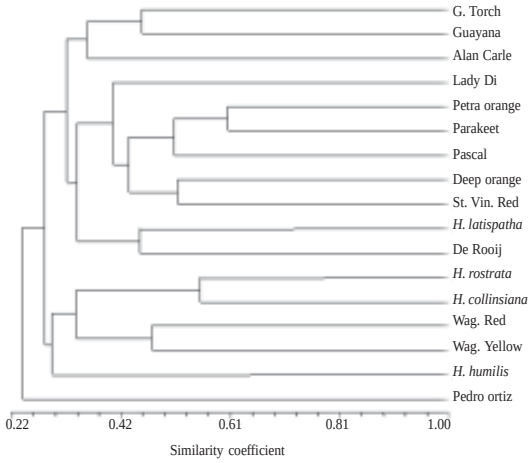

Figure 4. Dendrogam of 17 Heliconia species and varieties.

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