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A study on phylogenetic relationship among *Heliconia* and related genera

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ABSTRACT

Heliconia varieties/types along with related genera were subjected to RAPD analysis for studying the genetic diversity and to construct phylogenetic tree based on banding pattern. Twenty primers were selected from 120 RAPD primers screened initially. The selected primers produced a high degree of polymorphism. A total of 156 amplified bands were produced, in which, 154 were polymorphic, with an average of 7.7 polymorphic bands per primer and accounting for 99.05% polymorphism. Number of bands per primer varied from 2 (OPN-17) to 13 (OPB-17). Polymorphic Information Content ranged from 0.06 in OPN-17 to 0.50 in OPY-19 with an average of 0.31. The highest resolving power of 9.58 was calculated for OPQ-03, which was closely followed by 9.52 for OPY-19. The lowest resolving power (*Rp*) of 0.12 was calculated for OPN-17. Average *Rp* for 20 primers was found to be 4.29. In the dendrogram, the genotypes which belong to related genera species, *viz.*, *Canna indica* (Red), *Canna indica* (Orange), *Musa* spp. (Grand Naine), *Maranta arundinacea*, *Alpinia purpurata*, *Zingiber officinale* and *Curcuma longa* are all out groups, while all the 26 *Heliconia* genotypes grouped in clusters E and F at coefficient value of 0.19. Thus, it proved that *Heliconia* forms a distinct genus in the botanical order Zingiberales.

Key words: *Heliconia*, genetic diversity, DNA markers, phylogenetic relationship.

INTRODUCTION

The Heliconiaceae family contains a single genus, *Heliconia* L., with approximately 200 to 250 species of Neotropical origin, ranging from north Mexico to the south of Brazil. Only a small paleotropical group, with approximately six species, is endemic to the Pacific Islands (Andersson, 1; Berry and Kress, 2). In Brazil, there are about 40 species distributed in two main areas, the Amazon Basin and the Atlantic Forest, which correspond to the primary areas of distribution of the genus in the country (Kress, 7). Originally, *Heliconias* were included in the family Musaceae, but the genus was always considered to be homogeneous and with its own characteristics, such as inverted flowers, the presence of a single staminode and drupe-type fruits. Then currently *Heliconia* is raised to the family level (Heliconiaceae) (Nakai, 11), this family has only one genus (*Heliconia*), belonging to the order Zingiberales, which comprises eight families: Musaceae (bananas), Strelitziaceae (bird of paradise), Lowiaceae, Heliconiaceae (heliconias), Zingiberaceae, Costaceae (costos), Cannaceae, and Marantaceae.

The genus *Heliconia* L. contains a great diversity of species, varieties, hybrids, and cultivars of ornamental and commercial interest. However, there is confusion and uncertainty about the number of species and the relationships among them. Therefore, molecular studies may help to increase our understanding of the genetic variability in the genus and its speciation process (Marouelli *et al.*, 10). *Heliconia psittacorum* one of the major species comprises about 25 cultivars. Species and cultivar identification is primarily based on morphological differences and colouration 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, 2). Variations 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 colour of bracts. In addition, postharvest characteristics can vary from clone to clone (Donselman and Broschat, 5).

As *Heliconia* is a relatively new interest for taxonomists and horticulturists (Criley and Broschat, 4), correct identification of the various species and cultivars is important. The easiest practical method of identification so far has been by comparing colour photographs of the inflorescences (Berry and Kress, 2). In the present study, we present the results of genetic diversity among the different types/ varieties of *Heliconia* and related genera and phylogenetic relationship among the types.

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MATERIALS AND METHODS

The present experiment was carried out during 2011-2012 at the Plant Molecular Biology lab (Horticulture) of ICAR Research Complex for Goa. A total of 45 heliconias and 15 types of related genera are maintained, of which 26 heliconia and 12 related genera were included in the genetic diversity study (Table 1). Tissues from emerging leaves before they were fully unfurled were used. The leaf sample

Table 1. Details of *Heliconia* types/ varieties and other genera used in the study.

Code	Botanical name	Variety / Type
H-1	*Heliconia bihai*	Lobster Claw One
H-2	*Heliconia psittacorum*	Golden Torch
H-3	*Heliconia psittacorum*	Lady Di
H-4	*Heliconia rostrata*	Pendent type
H-5	*Heliconia psittacorum*	Choconiana
H-6	*Heliconia psittacorum* × *spathocircinata*	Guyana
H-7	*Heliconia bihai*	Lobster Claw Two
H-8	*Heliconia chartacea*	Sexy Pink
H-9	*Heliconia wagneriana*	Erect type
H-10	*Heliconia orthotricha*	Macas Pink
H-11	*Heliconia psittacorum*	Petra Orange
H-12	*Heliconia psittacorum*	Guyana
H-13	*Etlingera elatior**	Red Torch
H-14	*Etlingera elatior**	Pink Torch
H-15	*Tapienochilos ananassae**	Indonesian Was Ginger
H-16	*Heliconia psittacorum* × *spathocircinata*	Lobster Claw Three
H-17	*Heliconia psittacorum*	Alan Carle
H-18	*Heliconia psittacorum*	Sassy
H-19	*Heliconia psittacorum* × *spathocircinata*	Tropics
H-20	*Heliconia psittacorum*	Adrian
H-21	*Heliconia psittacorum*	St. Vincent Red
H-22	*Heliconia psittacorum*	Kenya Red
H-23	*Heliconia stricta*	Tagami
H-24	*Heliconia collisiana* × *bourgeana*	Pedro ortiz
H-25	*Heliconia latispatha*	Latispatha
H-26	*Heliconia hirsuta*	Erect type
H-27	*Heliconia chartacea*	Sexy Scarlet
H-28	*Musa laterita**	Ornamental Banana
H-29	*Heliconia bihai*	Erect type
H-30	*Heliconia chartacea* × *platystachys*	Temptress
H-31	*Canna indica**	Red
H-32	*Canna indica**	Orange
H-33	*Musa* spp*	Grand Naine
H-34	*Maranta arundinacea**	Arrowroot
H-35	*Strelitzia reginae**	Bird of Paradise
H-36	*Alpinia purpurata**	Red Ginger
H-37	*Zingiber officinale**	Ginger
H-38	*Curcuma longa**	Turmeric

*Related genera of *Heliconia*

was placed in a pre-chilled mortar and pestle and macerated and quality DNA was prepared. The purification procedure was repeated twice to get high quality DNA. Initially 120 RAPD primers were screened against the genomic DNA of two heliconia cultivars for their ability to amplify DNA fragments. Three replicate DNA extractions (pool of five plants) from leaves of two different heliconia types, *viz.*, Golden Torch and Lobster Claw One were used to assess the consistency of the band profiles. Of all the primers, 20 primers produced robust amplification patterns and no band was detected in any negative control. The reproducibility of the RAPD amplifications were assessed using selected primers (OPR-15, OPK-04 and OPZ-16) with different DNA samples isolated independently from same cultivars (Golden Torch and Lobster Claw One) and amplified at different times.

PCR was carried out using 3 µl of template DNA, 2 µl of dNTP mix, 2 µl of 0.05 µM primer, 2 µl of 10X assay buffer, 0.30 µl of 3U *Taq* DNA polymerase and sterile water (10.7 µl) was used as reaction mixture. Amplification was performed on a programmable thermal controller (MJ Research Inc., USA) for an initial denaturation of 1 min. at 95°C, followed by 36 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. Amplified products along with DNA molecular weight markers were separated in a horizontal gel electrophoresis unit using 1.5% agarose gel, stained with ethidium bromide and visualized on a UV transilluminator and documented using GBOX-F^3 (Syngene) (GeneSys Software, v). 120 random decamer primers were screened and the primers that produced amplification were used for all the accessions. Sizes of the identified bands were determined by referring to the super mix DNA ladders (Chromous Biotech) used. By Neighbour Joining method, a dendrogram was constructed using NTSYS version 2.02 (Rohlf, 15) with the developed tree matrix based on the distance scores as input. Estimates of similarity were based on Jaccard's similarity coefficient. Cluster analysis was based on the unweighted paired-group method using arithmetic means (UPGMA).

RESULTS AND DISCUSSION

Twenty primers were selected (OPB-17, OPH-08, OPI-03, OPI-07, OPI-12, OPK-04, OPM-06, OPM-09, OPN-03, OPN-06, OPN-17, OPO-14, OPP-12, OPQ-03, OPQ-15, OPR-15, OPW-20, OPY-19, OPZ-16, and OPBF-12) from the screening of 120 primers. The selected primers produced a high degree of polymorphism, where of a total of 156 amplified bands, 154 were polymorphic (Table 2), with each primer giving an average of 7.8 polymorphic bands and accounting for 99.05% polymorphism. Number of bands per primer varied from 2 (OPN-17) to 13 (OPB-17). This high polymorphism may be related to the diversity of species and genera included in this study.

Table 2. Polymorphism details of RAPD markers in heliconia.

Parameter	RAPD
No. of bands obtained	156
No. of polymorphic bands	154
Polymorphic bands (%)	99.05
Av. No. of bands per primer	7.8

Total No. of bands (TNB) recorded for all the 20 RAPD primers was 156. The highest No. of bands (NPB) was 13 recorded in OPB-17 and the lowest No. was 2 recorded in OPN-17. On an average 7.8 bands were produced per primer. Total No. of polymorphic bands for 20 primers was 154 with an average of 7.7 bands. Percentage of polymorphic bands was calculated from the values of TNB and NPB. The lowest value obtained was 90.91% in OPR-15, followed by 90% in OPY-19 (Table 3). All the others, *i.e.* 18 primers showed 100% polymorphism. The highest polymorphism may be due to diversity in the materials used which comprised heliconia and related genera also. The same has been reported earlier on heliconia using RAPD markers (Kumar *et al.*, 8; Sheela *et al.*, 16; Loges *et al.*, 9).

The Marker Index values ranged from 2 for OPN-17 to 12.50 for OPB-17. Average for 20 primers was found to be 6.76. The Mean Marker Index values were highest for OPN-17 and OPP-12, which was 1.00 and lowest were for OPQ-03 (0.60). Average for 20 primers was found to be 0.88. Polymorphic Information content ranged from 0.06 in OPN-17 to 0.50 in OPY-19 with an average of 0.31. The highest resolving power of 9.58 was calculated for OPQ-03, which was closely followed by 9.52 for OPY-19. The lowest resolving power of 0.12 was calculated for OPN-17. Average for 20 primers was found to be 4.29. The Rp values were high compared to earlier published works, but it was pointed out that Rp values could also vary between taxa for a selected set of primers (Prevost and Wilkinson, 14). The dendrogram depicting the phylogenetic relations among the 23 genotypes of *Heliconia* out of 26 and 10 out of 12 related genera genotypes was constructed using the TREE and GRAPH programs of NTYSYS 2.2, utilizing the genetic coefficient values.

The genotypes *Tapienochilos ananassae* - Indonesian Was Ginger (H-15), *Heliconia psittacorum*

Table 3. List of RAPD primers selected for study with their efficiency.

Primer	Primer sequence (5'–3')	Total No. of bands	No. of polymorphic bands	Percentage of polymorphic bands	Marker Index (MI)	Mean Marker Index (MMI)	Polymorphic Information Content (PIC)	Resolving power (Rp)
OPB-17	AGGGAACGAG	13	13	100	12.50	0.96	0.25	3.88
OPH-08	GAAACACCCC	6	6	100	5.89	0.98	0.21	1.39
OPI-03	CAGAAGCCCA	10	10	100	9.41	0.94	0.31	3.76
OPI-07	CAGCGAGAAG	10	10	100	8.74	0.87	0.42	6.12
OPI-12	AGAGGGCACA	8	8	100	7.39	0.92	0.36	3.82
OPK-04	CCGCCCAAAC	9	9	100	6.56	0.73	0.49	7.94
OPM-06	CTGGGCAACT	9	9	100	8.09	0.90	0.35	4.12
OPM-09	GTCTTGCGGA	10	10	100	8.62	0.86	0.39	5.21
OPN-03	GGTACTCCCC	3	3	100	2.98	0.99	0.15	0.48
OPN-06	GAGACGCACA	9	9	100	8.73	0.97	0.22	2.30
OPN-17	CATTGGGGAG	2	2	100	2.00	1.00	0.06	0.12
OPO-14	AGCATGGCTC	5	5	100	4.65	0.93	0.27	1.58
OPP-12	AAGGGCGAGT	5	5	100	4.98	1.00	0.10	0.55
OPQ-03	GGTCACCTCA	8	8	100	4.77	0.60	0.06	9.58
OPQ-15	GGGTAACGTG	6	6	100	5.36	0.89	0.42	3.58
OPR-15	GGACAAGGAG	11	10	90.91	8.60	0.78	0.47	8.42
OPW-20	TGTGGCAGCA	8	8	100	7.78	0.97	0.22	2.06
OPY-19	TGAGGGTCCC	10	9	90	7.09	0.71	0.50	9.52
OPZ-16	TCCCCATCAC	9	9	100	7.16	0.80	0.48	7.27
OPBF-12	CTTCGCTGTC	5	5	100	3.88	0.78	0.48	4.06

x *H. spathocircinata*- Tropics (H-19), *H. psittacorum* - Adrian (H-20), *H. psittacorum* - St. Vincent Red (H-21) and *Strelitzia reginae* - Bird of paradise (H-35) showed no bands during PCR based RAPD.

The coefficient was plotted in the range of 0.00 to 0.78. Cluster A was formed at 0.00 and further branched at 0.12 into two sub-clusters. Sub-cluster A1 branched at 0.568 into H-31 (*Canna indica* - Red) and H-32 (*C. indica* - Orange), while sub-cluster A2 at 0.12 comprised of H-33 (*Musa* spp.). Cluster B separated from Cluster A at 0.027 and comprised of only one genotype, *i.e.* H-36 (*Alpinia*). Cluster C separated from Cluster B at 0.086 and branched at 0.104 to give H-37 (*Zingiber officinale*) and H-34 (*Maranta arundinacea*). Cluster D separated from Cluster C at 0.123 and comprised of only one genotype, *i.e.* H-38 (*Curcuma longa*). Cluster E and Cluster F were separated from Cluster D at 0.19. Cluster E formed two branches, *i.e.* sub-cluster E1 and E2 at 0.257 (Fig. 1).

Sub-cluster E1 branched into two groups say 1 and 2 at 0.35. Group 1 branched at 0.485 to give H-22 (*H. psittacorum* - Kenya Red) and H-23 (*H. stricta* - Tagami), while Group 2 branched at 0.368 to give H-26 (*H. psittacorum* - Kawauchi) and at 0.439 to H-24 (*H. psittacorum* x *H. spathocircinata*- H-01) and H-25 (*H. latispatha* -H-05). Sub-cluster E2 branched into two groups say 1 and 2 at 0.285. Group 1 branched into 1a and 1b at 0.35. 1a at 0.408 divided into H-27 (*Heliconia* spp. - Cut leaves) and H-30 (*H. chartacea* - Temptress) and 1b at 0.35 comprised of H-28 (*Musa laterita* - Ornamental banana).

Cluster F formed two sub-clusters say F1 and F2 at 0.276. Sub-cluster F1 further divided at 0.294 and formed 5 groups say 1, 2, 3, 4 and 5. Group 1 branched at 0.325, which comprised of H-1 (*Heliconia bihai* - Lobster Claw One) and H-4 (*H. rostrata*). Group 2 branched into 2 groups say 2a and 2b at 0.325. 2a branched out at 0.359 to H-5 and at 0.436 to H-2 (*H. psittacorum* - Golden Torch) and H-3 (*H. psittacorum* - Lady Di). 2b comprised of H-6 (*H. psittacorum* x *H. spathocircinata*) only. Group 3 at 0.325 comprised of H-11 (*H. psittacorum* - Petra Orange). Group 4 branched at 0.568 into H-13 (*Etlingera elatior* - Red Torch) and H-14 (*Etlingera elatior* - Pink Torch). Group 5 branched at 0.35 into 5a and 5b. 5a comprised of H-7 (*Heliconia bihai* - Lobster Claw Two) and H-8

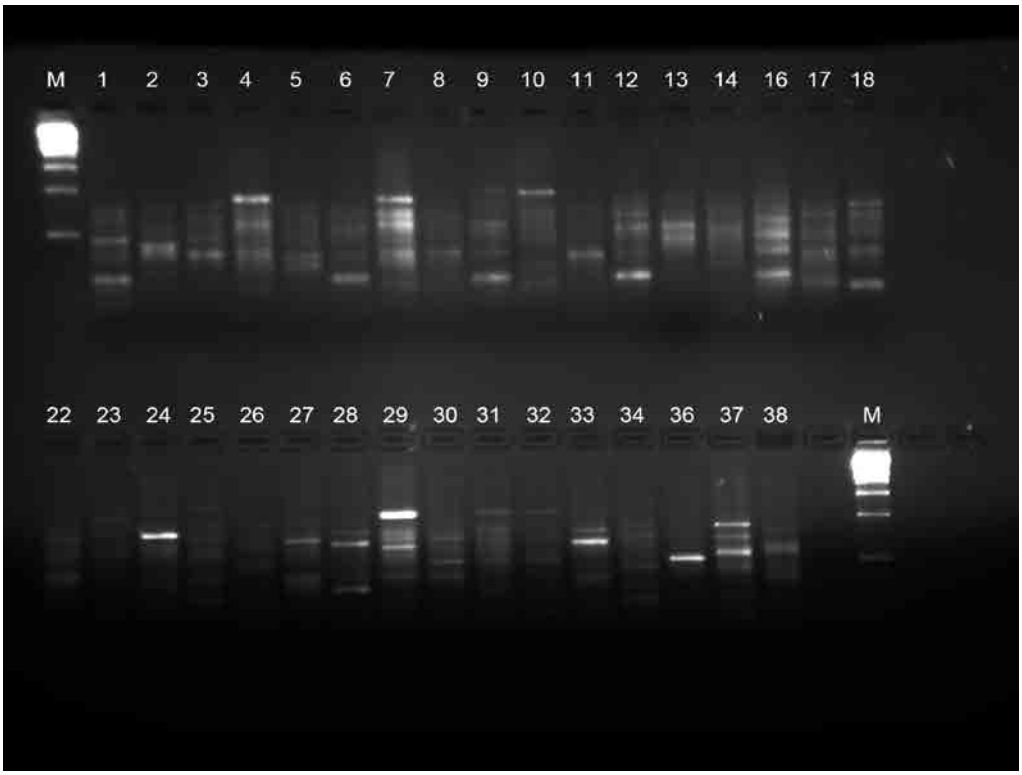

Fig. 1. RAPD profile of *Heliconia* varieties/ types and related genera for RAPD primer OPR-15 (Lane1 = marker, lane 2 = 38 *Heliconia* samples, lane 39 = negative control and lane 40 = marker).

(*Heliconia chartacea* - Sexy Pink) at 0.368, while 5b comprised of H-9 (*H. wagneriana*) and H-10 (*H. orthotricha* - Pink) at 0.417. Sub-cluster F2 branched at 0.313 in two groups say 1 and 2. Group 1 comprised of H-12 (*H. psittacorum* - Guyana) at 0.313. Group 2 formed two branches at 0.362 to give H-16 (*H. psittacorum* - H-02) and at 0.417 to give H-17 (*H. psittacorum* - Alan Carle) and H-18 (*H. psittacorum* - Sassy).

The dendrogram generated for the RAPD indicated distinct types in separate cluster like cultivated banana and Canna (Red and orange type) grouped in one major cluster. Hence, it is inferred that this group forms the out group with heliconia types and varieties used in this study and it got separated from other heliconia types (Kumar *et al.*, 8). The genotypes, which belong to related genera, *viz. Canna indica* - Red (H-31), *Canna indica* - Orange (H-32), *Musa* spp. - Grand Naine (H-33), *Maranta arundinacea* - Arrowroot (H-34), *Alpinia* (H-36), *Zingiber officinale* - ginger (H-37), and *Curcuma longa* - turmeric (H-38) are all out-groups, while all the 26 genotypes of *Heliconia* were present in the Clusters E and F at coefficient value of 0.19. Thus, it proves that *Heliconia* forms a distinct genus in order Zingiberales (Fig. 2).

It was also interesting to note that majority of the heliconia types derived from the species psittacorum grouped together irrespective of morphological features like plant height, spike length and bract colour *etc.* Since it is already reported that number of inflorescence and florets can vary depending on the environment, the RAPD analysis in this study also corroborated the earlier findings on morphological diversity in heliconia (Criley, 3).

The results observed with the RAPD markers are considered consistent, without any environmental interference, which is common in phenotypic data in addition to provide broad genome coverage (Williams *et al.*, 18). Such information are important on the selection of genotypes in order to support uniform and also stable production and serves as guidelines for studies on heliconia cut flower breeding programme (Loges *et al.*, 9). The genetic similarity and dissimilarity indices derived from RAPD studies may aid future breeding schemes on *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*., 6; Nathan *et al.*, 12) and *via* callus intermediate (Nathan *et al.*, 13) have been established for *Heliconia*. Although the present study

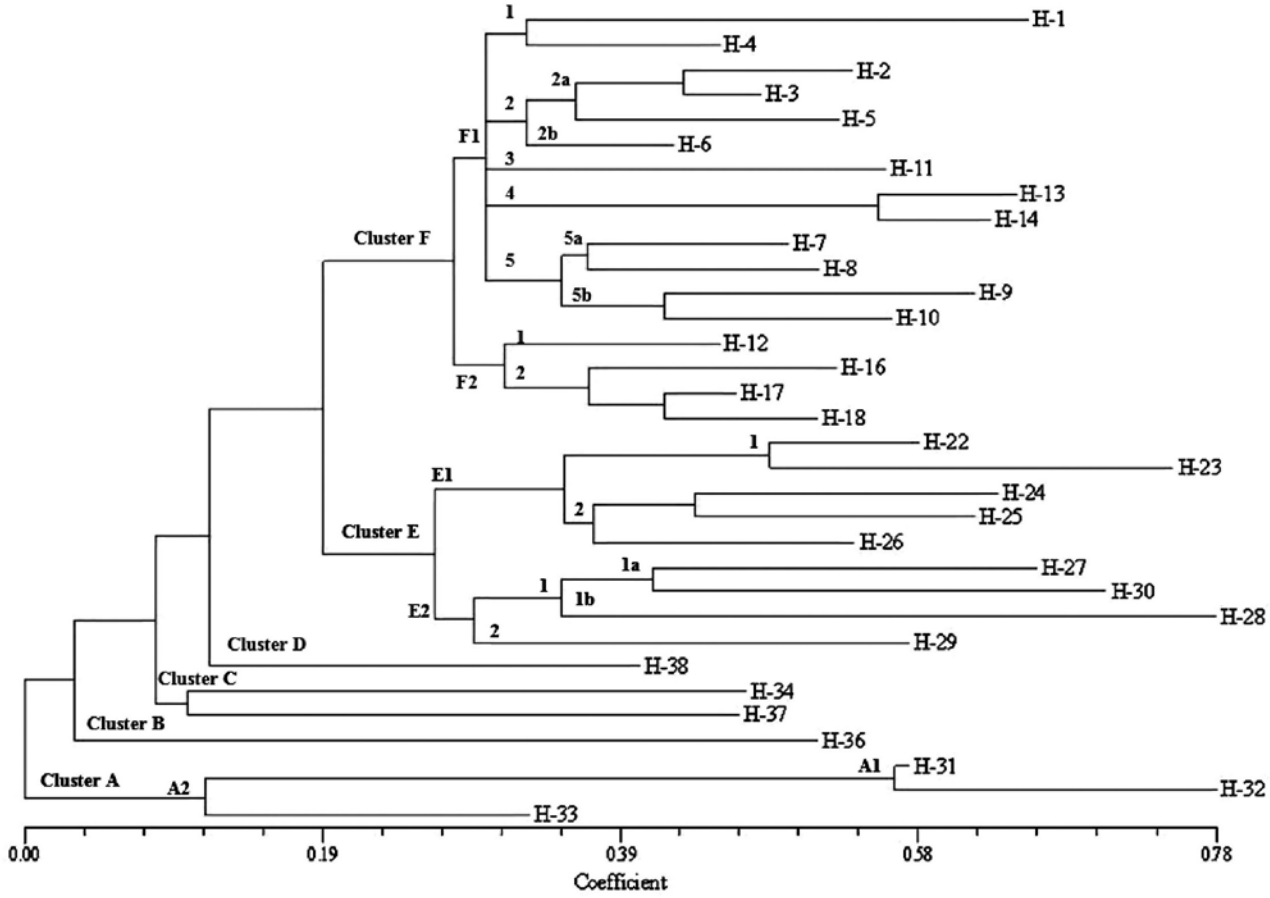

Fig. 2. UPGMA dendrogram based on Nei's genetic distance for *Heliconia* and related genera.

with RAPD primers able to distinguish heliconia and related genera clearly, show there are clusters with out-groups like torch lilly in the heliconia cluster. Hence, clear clustering and identification of variety/ type specific markers can be achieved by employing other types of DNA markers like ISSR and/ or microsatellite markers. It is also supported by others that it is essential to use two different markers for establishing the genetic similarity and distance rather than drawing conclusion with one set of markers (Vengatachalam *et al.*, 17).

Nevertheless, it is the first study on higher number of *heliconia* types and also related genera to find out the genetic diversity and phylogenetic relationships among the different in varieties and genera from India. In summary, the RAPD is having high resolution power and appear to offer many advantages in establishing genetic distances. It is effective and promising marker for assessing the genetic diversity in *Heliconia* types and the derived information will be useful in correct identification of varieties/ types apart from its uses in the genome conservation in *Heliconia*.

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