Genetic diversity of the ornamental plant *Anthurium andraeanum* in Vietnam

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Abstract
Anthurium sp. is an imported ornamental plant with high economic value in Vietnam. Consumer preferences and demand depend greatly on the colour of the spathes, so understanding the genetic diversity of varieties is necessary to create novel flowers. In this work, we aimed to characterise genetic diversity among Anthurium accessions collected from four provinces in Vietnam (Ha Noi, Thua Thien Hue, Lam Dong and Can Tho). DNA of eighteen Anthurium accessions was extracted using the cetyl trimethyl ammonium bromide (CTAB) method. Then, genomic DNA was amplified with matK and rbcL primers in PCR and sequencing to determine sequence parameters using DNAsp v6 and MEGA11 software. Three phylogenetic trees including the Neighbor-joining (NJ) tree, Maximum likelihood (ML) tree and Bayesian inference (BI) tree were created to separate Anthurium accessions. In addition, ten morphological traits were also described and were cluster-analysed (UPGMA) using MVSP v3.22 software.

The matK yielded the highest diversity information with 54 variable (polymorphic) sites, 52 parsimony informative sites, 6 Insertion-Deletion (InDel) sites and nucleotide diversity at 0.01195. Besides, intraspecific distances ranged from 0.0000 to 0.0058 and interspecific genetic distances ranged from 0.0000 to 0.0645. Furthermore, phylogenetic tree analysis using DNA barcoding results and morphological description data classified accessions into two groups corresponding to the Anthurium and Caladium species. In particular, HUIB_AA06 was distinct from the remaining accessions.

Keywords: *Anthurium andraeanum*, DNA barcode, Genetic diversity, *mat*K, *rbc*L.

Introduction

Anthurium is a perennial herb genus in the family *Araceae*. In this genus, *Anthurium andraeanum* is a common cut flower and an indoor ornamental plant that brings important commercial value. Its flower is prized for the bright, heart-shaped spathes[3,14,20]. Plant breeders created flowers with novel spathe colours and shapes through interspecific hybridization[14]. Knowledge of genetic diversity between flower varieties is useful to support breeding efforts[18]. Meanwhile, current studies on *Anthurium* mainly focus on propagation, disease control and stress response[25].

DNA barcoding is a common molecular technique that helps to detect, identify and evaluate genetic diversity of plant germplasms based on standardised DNA markers[8,15]. The genetic region known as the DNA barcode includes a small portion of the nuclear genome (e.g. nuclear internal transcribed spacer, ITS) or the chloroplast genome (e.g. *rbc*L, *mat*K, *trn*H-*psb*A, *rpo*B, *rpo*C1, *ycf*1,…)[6]. Plant chloroplast genomes are more commonly used since they contain highly conserved sequences, while the ITS region is highly conserved at the species level[2,8,12].

For *Anthurium*, the *rpo*B, *rpo*C1, *mat*K and *rbc*L genomic regions and the *trn*H-*psb*A, *atp*F-*atp*H and *psb*K-*psb*I intergenic regions have been used to determine species, origin, interrelationships, their evolution and diversification[26]. Among them, *rbc*L and *mat*K (chloroplast coding regions) were useful to construct phylogenetic relationships between angiosperms[8,10,21]. The combination of *rbc*L and *mat*K was recommended as the standard plant barcode by the Consortium for the Barcode of Life[4]. Based on *rbc*L sequence data, the average genetic distances between 10 *A. andreanum* cultivars collected in Thailand were calculated[5]. *A. acaule* was identified by Elansary et al[10] based on *mat*K and *rbc*L, with GenBank accession numbers KX783630 and KX783822 respectively[10].

On the other hand, the *mat*K sequence was recommended for species identification in the genus *Anthurium*, as it shows genetic differences between *A. plowmani* and *A. ravenii*[17]. In Vietnam, planting *Anthurium* is gaining more and more popularity. There are various varieties of *Anthurium* imported into Vietnam with diverse flower forms and colours. In this study, two barcoding loci including *mat*K and *rbc*L were used to identify *Anthurium* species and to study their genetic diversity.

Material and Methods

Plant materials: Eighteen varieties of *Anthurium* and two varieties of *Caladium* were collected from gardens and plant shops in 4 different provinces of Vietnam (Ha Noi, Thua Thien Hue, Lam Dong and Can Tho) (Table 1). Among them, two varieties of *Caladium* (HUIB_CB01 and HUIB_CH05) belonging to the same *Araceae* family with *Anthurium* were used as the controls for the analysis.

DNA extraction and PCR: Genomic DNA was extracted from young leaves, using the modified cetyl trimethyl

ammonium bromide (CTAB) method[19]. The quality of genomic DNA was examined using gel electrophoresis (1% agarose), visualised with a gel documentation system (Vilber, France). Plant DNA were amplified with *matK* and *rbc*L primers in PCR (Applied Biosystems, USA). Each 15 µL PCR mixture contained 20 ng of genomic DNA, 7.5 µL of 2x MyFi Mix (Meridian Bioscience, US) and 10 pmol primers. The thermocycling program for each barcode primer was listed in table 2. DNA products were sequenced using the Sanger sequencing method and analysed with BLAST.

Sequence data and phylogenetic analysis: Parameters of sequence including the number of polymorphic sites, the number of parsimony informative sites, the total number of Insertion-Deletion (InDel) sites, sequence conservation, nucleotide diversity, number of Haplotypes and Haplotype diversity, were evaluated by DNAsp v6[24]. Intraspecific and interspecific genetic distances were evaluated using a pairwise distance matrix (Kimura-2-parameter (K2-P) model) in MEGA11 software[27]. For calculating small distances, K2-P was considered the most favourable model[13].

The species discriminatory power of each single and multi-locus barcode was assessed by three methods including the Neighbour-joining (NJ) tree, Maximum likelihood (ML) tree and Bayesian inference (BI) tree. The analysis of NJ trees was conducted using MEGA11 with 1000 bootstraps[27]. The ML trees were constructed in raxmlGUI v2.0.10, following the study of Gogoi et al[13]. For BI analysis, the phylogenetic trees were conducted in MrBayes v3.2.7[23] and were visualised in FigTree v1.4.4[22]. The species resolution rates were calculated from reconstructed trees to express genetic distances among varieties.

Morphological characteristic analysis: Ten qualitative morphological traits were assessed including spathe colour, spathe shape, spadix colour, spadix tip colour, spadix orientation, peduncle colour, petiole colour, leaf colour, leaf shape and scale leaf. The colours of the spathe, spadix, leaf, peduncle and petiole were recorded using a horticultural colour chart (Wilson Color Ltd., UK)[11]. For each accession, the traits were standardised. Then, UPGMA cluster analysis based on the Gower general similarity coefficient was performed in MVSP v3.22[30].

Results and Discussion

Sequence analysis: Polymorphisms in the chloroplast genome are useful data for distinguishing commercial cultivars and identifying genetically close species for breeding[1,7]. In our study, two chloroplast genome regions (*mat*K, *rbc*L) and their combination were used as plant DNA barcoding for species identification purposes. A total of 36 new sequences from *Anthurium andraeanum* and 4 new sequences from *Caladium* sp. were submitted to GenBank (Table 1). The sequence length of *mat*K (836-879 bp) was higher than *rbcL* (558-562 bp). However, the GC content of *mat*K ranged from 31.98 to 32.52%, which was lower than *rbc*L (41.68 - 41.86% GC). A combination of *mat*K and *rbc*L provided maximum variability (69 sites) and parsimony informative characters (66 sites), followed by *mat*K. Both *mat*K and the combination contained 6 InDels (1-3 bp), whereas *rbc*L did not. All barcodes had 3 haplotypes (*h*) and 0.279 haplotype diversities (*Hd*).

In addition, nucleotide diversity (*Pi*) of *mat*K at 0.01195 was the most prominent. Intraspecific distances of two barcodes and their combination ranged from 0.0000 to 0.0057, while interspecific distances ranged from 0.0000 to 0.0645. *mat*K reported the highest mean intraspecific and interspecific distances (Table 3). Consequently, *mat*K and the combination of *mat*K and *rbc*L produced greater diversity information (number of variable sites, parsimony informative sites, InDel sites and nucleotide diversity) than *rbc*L. Besides, the highest values of intraspecific and interspecific distances indicated the species identification advantage of *mat*K (Table 3). This was similar to previous reports by Elansary et al[10] and Kolondam et al[17].

Phylogenetic analysis: Three tree-based methods (NJ, ML and BI) were used to determine genetic distance and evaluate species discrimination efficiency[13,29]. Sequences of the *mat*K region and the combination of *mat*K and *rbc*L were selected to build phylogenetic trees including BI, ML and NJ. In general, the phylogenetic tree of matK showed the highest genetic distance (Fig. 1). As expected, *Anthurium* and *Caladium* sp. were well separated and considered to be monophyletic. In the matK-based phylogenetic tree, *C. bicolor* and *C. humboldtii* were in clade 1 with BI, ML and NJ distance values of 0.0219, 0.0412 and 0.0323 respectively. Clade 2 consisted of 18 accessions of *A. andraeanum* (BI - 0.0188, ML - 0.0376, NJ - 0.0287). In this clade, HUIB_AA06 was singled out, whereas the remaining *Anthurium* accessions belonged to another group with BI/ML/NJ distance values close to 0.

Remarkably, the genetic difference indices between accessions remained low (< 0.05). Although the genetic distance value in the ML method was the largest, the difference between ML, NJ and BI was not significant[13,28]. This was because the collected samples belonged to only one species of *A. andraeanum*. In summary, *mat*K and the combination of *mat*K and *rbc*L were useful primer pairs to evaluate genetic diversity among *Anthurium* accessions.

Analyses of morphological characteristics: Scale, spadix and spathe were absent in *Caladium* sp. while these parts in *Anthurium andraeanum* were diverse in this collection (Table 4). In terms of morphology, the colours of spathes and spadices were highly correlated. Spathe and spadix colour varied from red (3 spathe colour-based accessions, 1 spadix colour-based accession), dark red (2 spathe colour-based accessions), yellow (4 spadix colour-based accessions), pink (2 accessions), light green (1 accession), cream (1 accession), white (3 accessions), lavender (1 accession),

light lavender (1 accession) to multi-colour (4 accessions). Among qualitative traits, spathe shape (heart) and scale leaf colour (brown) were shared by all *Anthurium* accessions.

Most of the colours of the spadix tip were yellow or dark yellow (10 accessions). Some accessions had red (HUIB_AA06), light green (HUIB_AA10), cream (HUIB_AA12), white (HUIB_AA07, HUIB_AA08 and HUIB_AA09), lavender (HUIB_AA04) or dark lavender (HUIB_AA02) spadix tips. Furthermore, spadix orientation of 17 *A. andraeanum* accessions was erect and only HUIB_AA06 demonstrated arched growth. Peduncle colour of the rest was red (HUIB_AA06), dark green (HUIB_AA15), greenish-violet (HUIB_AA02 and HUIB_AA04) and green. The wide colour range of spathe and spadix of accessions may reflect their complex origin through interspecific hybridisation[11,16].

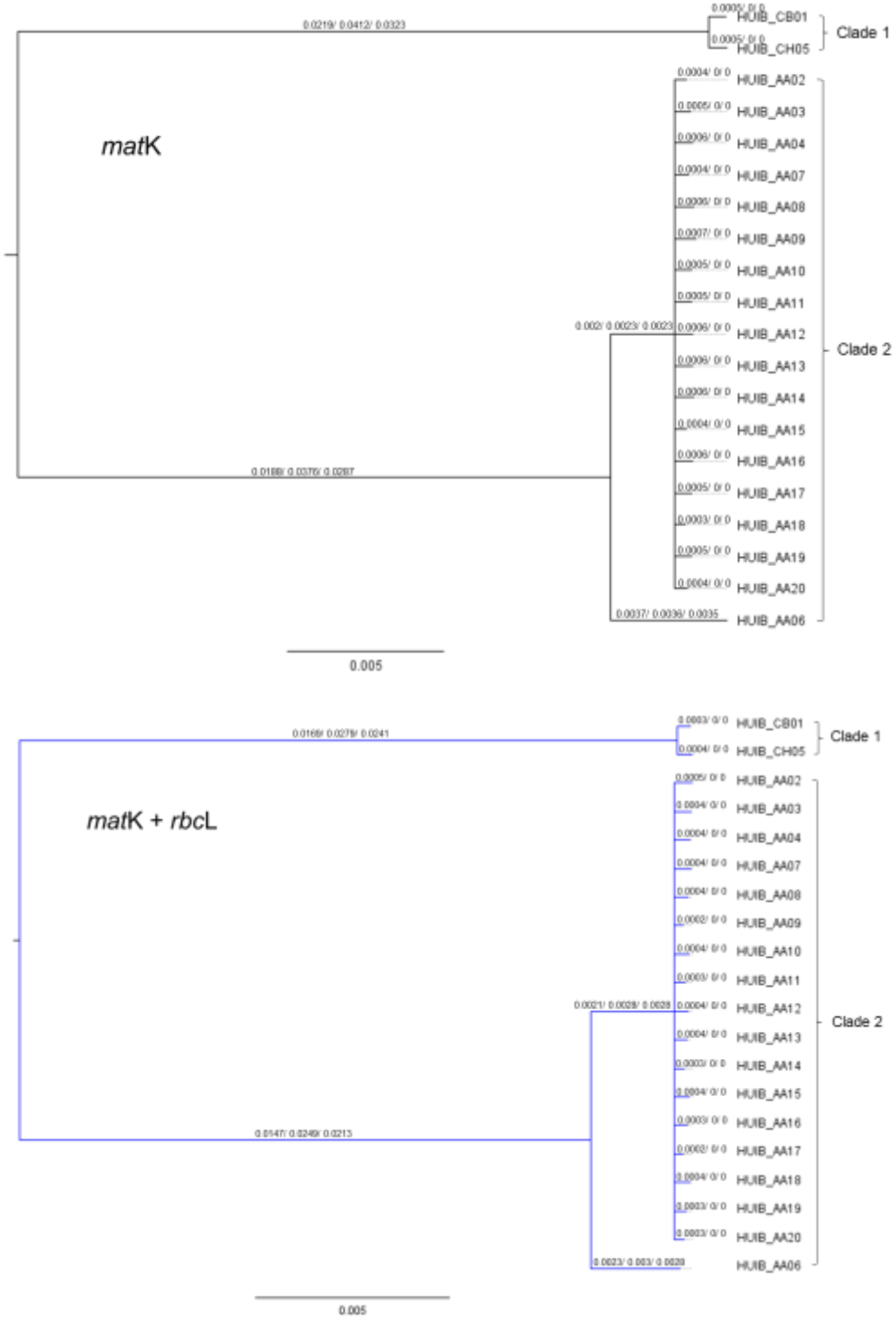

Fig. 1: Phylogenetic tree of *mat*K (upper panel) and the combined *mat*K and *rbc*L barcodes (lower panel) using BI methods. Results for ML and NJ analysis were mapped onto BI tree. The numbers on each branch represented BI/ML/NJ genetic distance values

Table 1
***Anthunium* and *Caladium* accessions collected across Vietnam**

S.N.	Accession code	Species name	GenBank accession (*mat*K/*rbc*L)	Place of collection
1	HUIB_CB01	*Caladium bicolor*	PP265947/ PP265967	Sieu Thi Cay Canh Shop, Bao Loc, Lam Dong
2	HUIB_AA02	*Anthurium andraeanum*	PP265948/ PP265968	Sieu Thi Cay Canh Shop, Bao Loc, Lam Dong
3	HUIB_AA03	*Anthurium andraeanum*	PP265949/ PP265969	Sieu Thi Cay Canh Shop, Bao Loc, Lam Dong
4	HUIB_AA04	*Anthurium andraeanum*	PP265950/ PP265970	Sieu Thi Cay Canh Shop, Bao Loc, Lam Dong
5	HUIB_CH05	*Caladium humboldtii*	PP265951/ PP265971	Havaca Shop, Can Tho
6	HUIB_AA06	*Anthurium andraeanum*	PP265952/ PP265972	Sieu Thi Cay Canh Shop, Bao Loc, Lam Dong
7	HUIB_AA07	*Anthurium andraeanum*	PP265953/ PP265973	Havaca Shop, Can Tho
8	HUIB_AA08	*Anthurium andraeanum*	PP265954/ PP265974	Go Supermarket, Hue City, Thua Thien Hue
9	HUIB_AA09	*Anthurium andraeanum*	PP265955/ PP265975	Huong Loc Garden Conpany limited, Hue City, Thua Thien Hue
10	HUIB_AA10	*Anthurium andraeanum*	PP265956/ PP265976	Huong Loc Garden Conpany limited, Hue City, Thua Thien Hue
11	HUIB_AA11	*Anthurium andraeanum*	PP265957/ PP265977	Havaca Shop, Can Tho
12	HUIB_AA12	*Anthurium andraeanum*	PP265958/ PP265978	Havaca Shop, Can Tho
13	HUIB_AA13	*Anthurium andraeanum*	PP265959/ PP265979	Havaca Shop, Can Tho
14	HUIB_AA14	*Anthurium andraeanum*	PP265960/ PP265980	Havaca Shop, Can Tho
15	HUIB_AA15	*Anthurium andraeanum*	PP265961/ PP265981	Caycanh789 Shop, Bat Trang, Gia Lam, Ha Noi
16	HUIB_AA16	*Anthurium andraeanum*	PP265962/ PP265982	Huong Loc Garden Conpany limited, Hue City, Thua Thien Hue
17	HUIB_AA17	*Anthurium andraeanum*	PP265963/ PP265983	Huong Loc Garden Conpany limited, Hue City, Thua Thien Hue
18	HUIB_AA18	*Anthurium andraeanum*	PP265964/ PP265984	Huong Loc Garden Conpany limited, Hue City, Thua Thien Hue
19	HUIB_AA19	*Anthurium andraeanum*	PP265965/ PP265985	Caycanh789 Shop, Bat Trang, Gia Lam, Ha Noi
20	HUIB_AA20	*Anthurium andraeanum*	PP265966/ PP265986	Caycanh789 Shop, Bat Trang, Gia Lam, Ha Noi

Table 2
Primer pairs used in this study

Regions	Primer	Sequence (5'-3')	PCR program
*rbc*L[26]	Aa.*rbc*L-F	GTAAAATCAAGTCCACCGCG	94 °C - 3 min; 35 cycles (94 °C - 30 s, 50 °C - 30 s, 72 °C - 40 s); 72 °C - 5 min
	Aa.*rbc*L-R	ATGTCACCACAAACAGAAACTAAAGC	
*mat*K[17]	*mat*K-3F-KIM	CGTACAGTACTTTTGTGTTTACGAG	95 °C - 3 min; 30 cycles (95 °C - 30 s, 58 °C - 30 s, 72 °C - 45 s); 72 °C - 5 min
	*mat*K-1R-KIM	ACCCAGTCCATCTGGAAATCTTGGTTC	

Petiole's colour ranged from red (HUIB_CB01), light green (HUIB_CH05), dark green (HUIB_AA15) to green (the remaining 17 accessions). In terms of leaf colour, green (11 accessions), dark green (4 accessions), light green (2 accessions) and purplish green (HUIB_AA15) were observed. On the other hand, red leaves and leaves with green-white spots were shown in *C. bicolor* and *C. humboldtii* respectively. Leaf shape also varied from cordate (14 accessions), heart (2 accessions), lanceolate (1 accession) to deltoid (3 accessions) (Table 4).

The morphological traits were used to classify accessions in a UPGMA cluster analysis with Gower general similarity coefficient (Fig. 2). Here, clade 1 consisted of 18 *Anthurium* accessions and HUIB_AA15 and HUIB_AA06 showed the highest morphological differences. In clade 2, HUIB_CB01 and HUIB_CH05 displayed a similarity coefficient of over 85%. The two clades were separated on the phylogenetic tree, showing clear morphological differences between *Anthurium* and *Caladium* sp.

Conclusion

This study successfully identified *A. andraeanum* species in Vietnam based on *mat*K and *rbc*L regions. Overall, *mat*K and the combination of *mat*K and *rbc*L were useful to evaluate the genetic differences of *Anthurium* species. Genetic polymorphism among *Anthurium* accessions remained relatively low. Besides, HUIB_AA06 accesstion had the most different morphology and genetics when compared to the rest.

Table 3
Assessment of two barcodes (*mat*K, *rbc*L) and combination

	matK	***rbc*L**	***mat*K + *rbc*L**
Sequence length (bp)	863-879	558-562	-
GC content (%)	31.98-32.54	41.68-41.86	-
No. of variable (polymorphic) sites	54	15	69
No. of parsimony informative sites	52	14	66
Total number of InDel sites (length)	6 (1-3 bp)	0	6 (1-3 bp)
Nucleotide diversity, *Pi*	0.01195	0.00522	0.00930
Number of haplotypes, *h*	3	3	3
Haplotype diversity, *Hd*	0.279	0.279	0.279
Intraspecific distances (mean±SE)	0.0000-0.0058 (0.0006±0.0003)	0.0000-0.0054 (0.0006±0.0003)	0.0000-0.0057 (0.0006±0.0002)
Interspecific distances (mean±SE)	0.0000-0.0645 (0.0633±0.0090)	0.0000-0.0257 (0.0256±0.0068)	0.0000-0.0483 (0.0482±0.0058)

In which, Insertion - Deletion: InDels; dashes indicate not data

Table 4
Qualitative morphological traits observed in 20 studied accessions

S.N.	Accessions	Spathe colour	Spathe shape	Spadix colour	Spadix tip colour	Spadix orientation	Peduncle colour	Petiole colour	Leaf colour	Leaf shape	Scale leaf colour
1	HUIB_CB01	-	-	-	-	-	-	1	1	2	-
2	HUIB_AA02	8	1	8	8	1	4	2	2	4	1
3	HUIB_AA03	9	1	9	2	1	2	2	2	4	1
4	HUIB_AA04	7	1	7	7	1	4	2	2	4	1
5	HUIB_CH05	-	-	-	-	-	-	3	5	2	-
6	HUIB_AA06	1	1	1	1	2	1	2	3	3	1
7	HUIB_AA07	6	1	6	6	1	2	2	3	1	1
8	HUIB_AA08	6	1	6	6	1	2	2	3	1	1
9	HUIB_AA09	6	1	6	6	1	2	2	3	1	1
10	HUIB_AA10	4	1	4	4	1	2	2	2	1	1
11	HUIB_AA11	3	1	3	2	1	2	2	2	1	1
12	HUIB_AA12	5	1	5	5	1	2	2	4	1	1
13	HUIB_AA13	1	1	2	3	1	2	2	4	1	1
14	HUIB_AA14	9	1	9	2	1	2	2	2	1	1
15	HUIB_AA15	2	1	2	2	1	3	4	6	1	1
16	HUIB_AA16	2	1	2	2	1	2	2	2	1	1
17	HUIB_AA17	1	1	2	3	1	2	2	2	1	1
18	HUIB_AA18	3	1	3	2	1	2	2	2	1	1
19	HUIB_AA19	9	1	9	2	1	2	2	2	1	1
20	HUIB_AA20	9	1	9	2	1	2	2	2	1	1

Spathe colour: red (1), dark red (2), pink (3), light green (4), cream (5), white (6), lavender (7), light lavender (8), multi-coloured (9), not applicable (-).
Spathe shape: heart (1), not applicable (-).
Spadix colour: red (1), yellow (2), pink (3), light green (4), cream (5), white (6), lavender (7), dark lavender (8), multi-coloured (9), not applicable (-).
Spadix tip colour: red (1), yellow (2), dark yellow (3), light green (4), cream (5), white (6), lavender (7), dark lavender (8), not applicable (-).
Spadix orientation: erect (1), arched (2), not applicable (-).
Peduncle colour: red (1), green (2), dark green (3), greenish-violet (4), not applicable (-).
Petiole colour: red (1), green (2), light green (3), dark green (4).
Leaf colour: red (1), green (2), dark green (3), light green (4), green-white spots (5), purplish green (6).
Leaf shape: cordate (1), heart (2), lanceolate (3), deltoid (4).
Scale leaf colour: brown (1), not applicable (-).

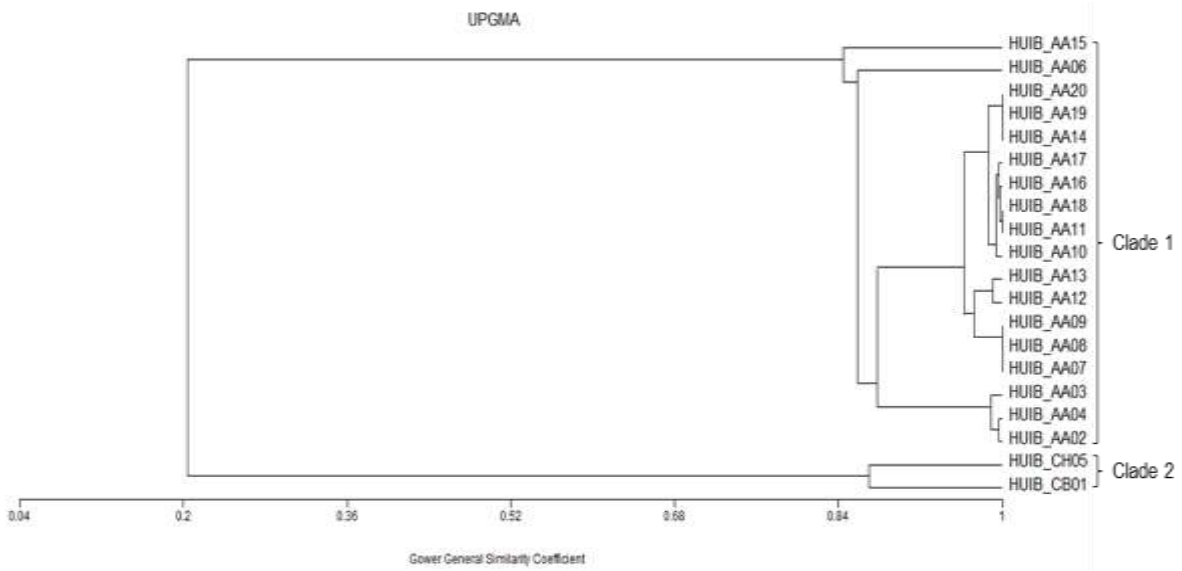

Fig. 2: Cluster analysis showed the relationship among the studied accessions based on morphological traits.

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