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Genetic Variation of *Hippeastrum* Accessions in Vietnam

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The present study was conducted to clarify the distribution and genetic diversity of *Hippeastrum* accessions cultivated in Vietnam. Twenty–five *Hippeastrum* accessions collected from 17 provinces in the northern, central, and southern regions of Vietnam were included. Results of floral observation and RAPD analysis indicate that there are four distinct groups of *Hippeastrum* being grown in Vietnam. These include *cam dại* (*H. puniceum* (Lam.) Kuntze syn. *H. equestre* Herbert), *đỏ dại* (*H.* x 'Johnsonii'), *đỏ cam dại* (*H. striatum* Lamarck), and the hybrid cultivars. In the RAPD analysis result, 25 primers produced a total of 230 distinct bands, of which 167 were polymorphic (about 72.6%). The number of polymorphisms per primer ranged from 1 to 13, and one primer did not reveal any polymorphisms. Our phylogeny indicated four separate groups, with dissimilarity values ranging from 0.2 to 0.3 according to the phylogeny based on RAPD results. Some variation was observed between northern, central, and southern *H. puniceum* populations. Extensive variation in flower color, shape, and size was detected among accessions of the hybrid group. While *H. puniceum* is the most widely distributed species from the northern to the southern regions, *H.* x 'Johnsonii' has been observed only in the northern region, but not in the central or southern regions, so the distribution of *Hippeastrum* within Vietnam might depend on adaptation to environmental conditions. Our results have distinguished the *đỏ dại* and the *cam dại* groups, although a single scientific name has been applied to both extant groups in Vietnam. The present study is the first report on the presence and distribution of, and genetic variation among *Hippeastrum* accessions grown in Vietnam, which will be valuable information for *Hippeastrum* breeding programs.

Key words: genetic variation, *Hippeastrum*, RAPD analysis, Vietnam

INTRODUCTION

Hippeastrum is an ornamental flowering bulb that belongs to a genus *Hippeastrum* which comprised of more than 60 species (Dole and Wilkins, 2004; Banerji *et al.*, 2011) in the Amaryllidaceae family (Traub, 1949; Rees, 1992, Meerow, 1988; Banerji *et al.*, 2011). Because they originated in the subtropical Americas, from eastern Brazil to the southern central Andes of Peru, Argentina, and Bolivia (Traub, 1949; Meerow, 1988; Okubo, 1993), members of this genus are adapted to growth in tropical and subtropical regions. Under natural conditions, *Hippeastrum* grows in the summer, goes dormant in the winter, and bears beautiful flowers from the end of spring to summer. *Hippeastrum* is not only well adapted to growth in pot culture, but also for use as a cut flower due to the longevity of its blooms (about 14 d at 20–22˚C), which enables them to compete commercially with other popular cut flowers (Read, 2004). Many species in this genus and their hybrids have large, colorful flowers that are prized as Christmas and New Year ornamentals (Silberbush *et al.*, 2003).

In Vietnam, *Hippeastrum* (commonly referred to as hippeastrum or amaryllis in English, or as *loa kèn đỏ*, *mạc chu lan*, *tứ diện*, or *lan huệ* in local languages) has been introduced and grown as an ornamental plant in home gardens, as a landscape plant, or as a potted plant in many regions. With advancements in the diversity of many characters such as flower color, longevity, and ease of planting and care, *Hippeastrum* is becoming one of the most valuable commercial flowers. There is a huge diversity of different hybrids of *Hippeastrum* plants on the market today (Robert *et al.*, 2006); however, there is little documentation of *Hippeastrum* growing in Vietnam. Recently, there have been many attempts to breed new *Hippeastrum* varieties, but a lack of important information regarding the distribution of and genetic variation within the genus can increase the time and costs of the breeding process.

To date, several molecular techniques have been developed to complement traditional methods for evaluating the diversity of plant populations. RAPD is such a technique that has been widely applied for genetic analysis of many genera of flowering bulb. Lee *et al.* (1996) successfully applied RAPD markers to establish a classification system for lilies. In another study, Hamada and Hagimori (1996) used 60 RAPD primers to identify variation among 12 lily varieties while Chakrabarty *et al.* (2007) traced the relatedness among *Hippeastrum* varieties in the germplasm collection at the National Botanical Research Institute in Lucknow, India using RAPD analysis. RAPD has also been used for cultivar identification (fingerprinting) (Swoboda and Bhalla, 1997). The efficiency RAPD as a technique for identifying polymorphisms tends to increase with increasing primer length (Yamagishi *et al.*, 2002). Recently, many authors reported that the combination of RAPD analysis and morphological observations can be very effective for studying plant genetic variation (Geraldine *et al.*, 1995; Helena *et al.*,

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1998; Perleb *et al.*, 2000; Pham *et al.*, 2006; Chakrabarty *et al.*, 2007). Thus, in the present study, the RAPD method was used in combination with the observation of floral characteristics to evaluate genetic variation among and between *Hippeastrum* accessions cultivated in Vietnam.

MATERIALS AND METHODS

Plant materials

Bulbs of 25 *Hippeastrum* accessions were collected from 17 provinces in Vietnam including Caobang, Haiduong, Hagiang, Langson, Sonla, Laichau, Hungyen, Ninhbinh, Hanam, Hanoi, Hatinh, Thuathienhue, Daklak, Hochiminh, Bentre, Soctrang and Lamdong. The sites from which the bulbs were collected are shown in Fig. 1. At the collection sites, the plants were not identified by a species or varietal name. The growers had classified them only according to major flower characteristics such as color red (*đỏ*), red–orange (*cam đỏ*), orange (*cam*), and whether the specimen was a hybrid. The collected bulbs were maintained in the germplasm collection at the Department of Horticulture, Faculty of Agronomy, Hanoi University of Agriculture (HUA), Hanoi, Vietnam. Floral characteristics such as flower shape, size, color, and flower throat color were recorded at each collection site. Subsequent DNA analysis was carried out in the laboratory at the Applied Biological Sciences, Faculty of Agriculture, Saga University, Japan.

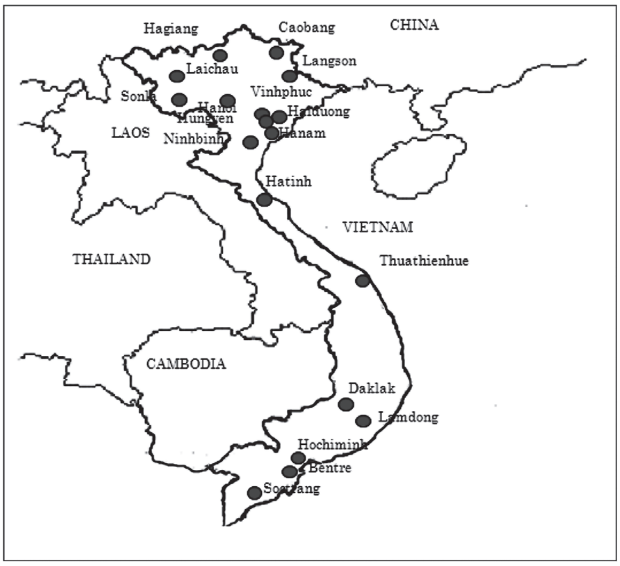

Fig. 1. Collection sites of of *Hippeastrum* accessions in Vietnam.

RAPD analysis

Total DNA was extracted from 1–2 g of fresh young leaves of each *Hippeastrum* accession using the CTAB method, as described by Murray and Thompson (1980), with minor modifications, as described by Yamashita *et al.* (2000). The PCR was programmed as: initial denaturation for 30 s at 94°C, followed by 45 cycles of 30 s at 94°C, 2 min at 40°C, and 3 min at 72°C; the final extension for 7 min at 72°C. The final reaction mixture was cooled down to 4°C.

One hundred RAPD primers were tested for the amplification of genomic sequences; 60 random arbitrary 10–bp primers were obtained from Operon Technologies, Inc., (Alameda, California) (designated as primers OPA through OPF), and 40 arbitrary 12–bp primers were included in the A primer set from BEX (Tokyo, Japan) (designated as primers A00 through A40). Amplification of RAPD markers from DNA samples from each of six accessions was carried out in triplicate for each PCR run. Only primers that consistently produced clear bands were selected for further experiments.

DNA amplification products were separated by agarose gel electrophoresis. Agarose (1% w/v) gel with 1X TAE (40 mM Tris–acetate, 1 mM EDTA (pH 8.5) buffer, and electrophoresis was conducted at 70 V/cm for 1.30 h. Gels were stained with ethidium bromide (EtBr), visualized under UV light, and images were captured using a digital camera FAS–Ⅲ (Toyobo Co. Ltd., Osaka, Japan). All reactions were repeated at least three times and only those bands that were reproducible on all runs were used for further analysis. Molecular Weight Marker Ⅱ (*Hind*Ⅲ/*Eco*RI digested Lambda DNA) was used as a reference to estimate the size of the DNA fragments.

RAPD bands on gels were scored as present (+) or absent (–) for each accession studied. To estimate the genetic distance among the strains, the genetic similarity measure (F) for each of the RAPD fragments was calculated using the formula of Nei and Li (1979), $F=2\,Mxy/(Mx+My)$, where Mxy is the number of shared fragments between the strains 'x' and 'y'; Mx is the number of fragments scored as strain 'x'; and My is the number of fragments scored as strain 'y'. Distance values were calculated as $1 - F$. The UPGMA clustering method in MEGA version 5 (Tamura *et al.*, 2011) was used to generate a phylogeny.

RESULTS

Floral observation

Considerable variation in the floral characters was detected among the accessions we examined (Table 1, Fig. 2). Based on floral characteristics, four different groups of *Hippeastrum* were distinguished, and were designated *cam dại* (orange), *đỏ dại* (scarlet red), *đỏ cam dại* (red orange), and hybrids, respectively.

Seven *cam dại* accessions (H37, H53–2, H59, H96, H142, H147, and H56) had small orange trumpet–shaped flowers with a yellowish green throat and a large white star at the base. No variation in the floral characters was observed among *cam dại* accessions collected from different geographical locations. Some of the floral characteristics of the *đỏ cam dại* accessions (H6 and H72) were similar to those of the cam *dại* accessions, except for the flower and throat color. The flowers of these accessions were red–orange with a yellowish green throat, and a large white star and a red ring at the base. The *đỏ dại* accessions (H4, H22, H53–1, H5, and H79) had trumpet–shaped, scarlet red flowers with a yellow–

Table 1. Collection sites and floral characteristics of the *Hippeastrum* accessions in Vietnam

Accession	Vietnamese name	Collection site (Town, Province)	Flower shape	Flower size	Petal color	Throat color
H6	*Cam đỏ*	Thuanchau, Sonla	Trumpet	Small	Red orange	Yellowish green, large white star, red ring at the base
H72	*Cam đỏ*	Mochau, Sonla	Trumpet	Small	Red orange	Yellowish green, large white star, red ring at the base
H4	*Đỏ dại*	Thuanchau, Sonla	Trumpet	Medium	Scarlet red, short white stripe	Yellowish green
H22	*Đỏ dại*	Duytien, Hanam	Trumpet	Medium	Scarlet red, short white stripe	Yellowish green
H53–1	*Đỏ dại*	Gialoc, Haiduong	Trumpet	Medium	Scarlet red, short white stripe	Yellowish green
H54	*Đỏ dại*	Caophong, Langson	Trumpet	Medium	Scarlet red, short white stripe	Yellowish green
H79	*Đỏ dại*	Honglinh, Hatinh	Trumpet	Medium	Scarlet red, short white stripe	Yellowish green
H37	*Cam đỏ*	Baolac, Caobang	Trumpet	Small	Orange	Yellowish green, large white star
H53–2	*Cam đỏ*	Gialoc, Haiduong	Trumpet	Small	Orange	Yellowish green, large white star
H59	*Cam đỏ*	Bacquang, Hagiang	Trumpet	Small	Orange	Yellowish green, large white star
H96	*Cam đỏ*	Cuchi, Hochiminh	Trumpet	Small	Orange	Yellowish green, large white star
H142	*Cam đỏ*	Trande, Soctrang	Trumpet	Small	Orange	Yellowish green, large white star
H147	*Cam đỏ*	Chauthanh, Bentre	Trumpet	Small	Orange	Yellowish green, large white star
H56	*Cam đỏ*	Hue, Thuathienhue	Trumpet	Small	Orange	Yellowish green, large white star
H104–1	*Cam sọc*	Dalat, Lamdong	Triangular	Medium	Orange, small white vein	Yellowish green, red ring at the base
H31–2	*Cam sọc*	Tamduong, VinhPhuc	Triangular	Medium	Orange, small white vein	Yellowish green, red ring at the base
H52	*Đỏ sọc trắng*	Bacson, Langson	Star	Large	Red, large white stripe	Yellowish green, red ring at the base
H84	*Đỏ sọc trắng*	Phongtho, Laichau	Star	Large	Red, large white stripe	Yellowish green, red ring at the base
H27	*Trắng*	Vangiang, Hungyen	Star	Large	White	Yellowish green
H77	*Trắng sọc tím*	Nhoquan, Ninhbinh	Star	Large	White, dark red vein	Yellowish green, red ring at the base
H85	*Trắng sọc tím*	Mochau, Sonla	Star	Large	White, dark red vein	Yellowish green, red ring at the base
H101	*Hồng đào*	Dalat, Lamdong	Star	Large	Pale pink and white	Yellowish green, red ring at the base
H67	*Đỏ gáo*	Krongbong, Daklak	Star	Medium	Red	Dark red star
H28	*Hồng sọc*	Dongla, Hanoi	Trumpet	Medium	Pale pink netted vein	Yellowish green
H88	*Hồng sọc*	Cholach, Bentre	Trumpet	Medium	Pale pink netted vein	Yellowish green

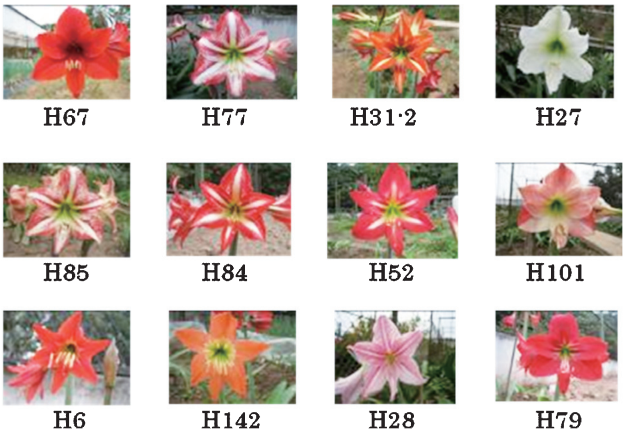

Fig. 2. Flowers of the *Hippeastrum* accessions collected in Vietnam.

green throat.

Extensive variation was observed in all the floral characters of the hybrid group. Hybrid flower size was either medium or large. Among hybrid accessions, two (H31–2 and H104) had triangular–shaped flowers, whereas the others had flowers that were star–shaped. Hybrid flower colors varied from dark red (accession H67), to pure white (accession H27), or bi–color (accessions H101, H84, H104, H31–2, H77, and H85). Differences were also observed in the coloration of the flower throats of hybrid accessions. Six of nine accessions had a red ring at the base of the flower throat while accession H67 had a large dark red star throat. Accessions H28 and H88 were easy to distinguish from other accessions due to their pale pink flowers with netted venation.

RAPD analysis

From the 100 RAPD primers tested, 25 that consistently produced clear bands were chosen for genotyping the *Hippeastrum* accessions. RAPD analysis revealed a high degree of genetic diversity among these accessions. The number of bands generated by each primer varied from 3 (for primers A06 and A21) to 14 (for primers OPB–05 and A15), with an average of 9.2 bands per primer. In this study, 24 out of 25 primers produced polymorphic amplification products. A total of 230 distinct bands were produced, of which 167 were polymorphic (about 72.6%). The number of polymorphisms

Table 2. Total band number and number of polymorphic bands detected in RAPD analysis of the *Hippeastrum* accessions in Vietnam

Order	Primer	Sequence	Company	Total no. of bands	No. of poly– morphic bands
1	OPA–01	5'–CAGGCCCTTC–3'	Operon	10	9
2	OPA–04	AATCGGGCTG	Operon	10	7
3	OPA–17	GACCGCTTGT	Operon	12	10
4	OPA–12	TCGGCCATAG	Operon	9	6
5	OPB–01	GTTTCGCTCC	Operon	13	11
6	OPB–05	TGCGCCCTTC	Operon	14	11
7	OPB–12	CCTTGACGCA	Operon	9	8
8	OPE–01	CCCAAGGTCC	Operon	12	11
9	OPF–03	ACGGATCCTG	Operon	9	6
10	OPF–12	ACGGTACCAG	Operon	13	9
11	OPF–13	GGCTGCAGAA	Operon	5	3
12	OPF–16	GGAGTACTGG	Operon	9	4
13	OPF–18	TTCCCGGGTT	Operon	9	7
14	OPF–19	CCTCTAGACC	Operon	9	7
15	A00	ATCAGCGCACCA	BEX	4	2
16	A03	TGCCTCGCACCA	BEX	5	2
17	A06	ACTGGCCGAGGG	BEX	3	1
18	A12	CTCCTGCTGTTG	BEX	7	4
19	A13	CTCAGCGATACG	BEX	7	5
20	A15	ATCGCGGAATAT	BEX	14	13
21	A17	GGTTCGGGAATG	BEX	7	5
22	A19	AGGCGCGAACG	BEX	7	6
23	A21	GTGACCGATCCA	BEX	3	2
24	A22	TTCAAGCTACCA	BEX	8	8
25	A26	GGTGAGGATTCA	BEX	11	10
	Total			230	167

detected per primer ranged from 1 (primer A06) to 13 (primer A15), while primer A22 revealed no polymorphism (Table 2). Some primers produced identical RAPD banding patterns for the *Hippeastrum* accessions with similar physical floral characters, especially flower color. One RAPD marker (about 2000 bp in size) was detected in all the *đỏ cam dại* and *đỏ dại* accessions using primer OPF–03. The primer OPA–04 yielded a RAPD marker (about 830 bp in size) only in the *đỏ cam dại* accessions (Fig. 3). The RAPD marker detected using primer OPF–13 (about 400 bp in size) could distinguish the *đỏ dại* accessions from the other accessions. Moreover, one RAPD marker (about 1375 bp in size) was revealed only in the *cam dại* accessions collected from the southern and central regions, but was not detected in accessions collected from the northern region by using primer OPF–16.

The results from RAPD analysis were used to calculate genetic dissimilarity coefficients in the pair–wise comparisons among the accessions. Genetic distances ranged from a minimum value of 0.008 between accession H142 and accession H147, to a maximum value of 0.357 between accessions H53 and H5 (Fig. 4). The dendrogram developed from the genetic dissimilarity values revealed by RAPD analysis is shown in Fig. 5. At genetic dissimilarity values greater than 0.3, two distinct clusters of accessions were observed. The first cluster included 16 *Hippeastrum* accessions of the *đỏ dại* and the hybrid groups, while the second cluster included nine accessions from the *cam dại* and the *đỏ cam dại* groups. Within the first cluster, two subgroups were clearly identified with a dissimilarity value greater than 0.2. Accessions with similar flower color, shape, and size were always in

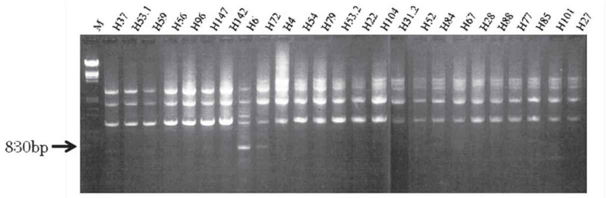

Fig. 3. RAPD profiles of the *Hippeastrum* accessions in Vietnam generated by primer OPA–04.
(M: DNA Size Standard: λ/*Hind*III–*Eco*RI)

[	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]
[1]																									
[2]	0.024																								
[3]	0.032	0.024																							
[4]	0.031	0.056	0.031																						
[5]	0.028	0.044	0.028	0.019																					
[6]	0.043	0.052	0.028	0.019	0.024																				
[7]	0.035	0.052	0.028	0.012	0.023	0.008																			
[8]	0.189	0.192	0.190	0.186	0.192	0.184	0.191																		
[9]	0.180	0.167	0.173	0.185	0.183	0.183	0.181	0.031																	
[10]	0.344	0.357	0.346	0.338	0.339	0.339	0.336	0.357	0.346																
[11]	0.317	0.331	0.311	0.296	0.304	0.304	0.301	0.331	0.312	0.128															
[12]	0.323	0.336	0.325	0.317	0.317	0.317	0.315	0.336	0.325	0.119	0.033														
[13]	0.312	0.325	0.306	0.299	0.306	0.306	0.304	0.333	0.315	0.139	0.021	0.021													
[14]	0.320	0.333	0.314	0.306	0.314	0.306	0.303	0.342	0.331	0.135	0.030	0.038	0.034												
[15]	0.315	0.320	0.325	0.333	0.325	0.325	0.331	0.288	0.278	0.262	0.248	0.238	0.243	0.249											
[16]	0.288	0.293	0.275	0.277	0.284	0.284	0.281	0.279	0.255	0.232	0.241	0.255	0.252	0.258	0.209										
[17]	0.290	0.311	0.300	0.301	0.309	0.301	0.298	0.255	0.253	0.261	0.247	0.261	0.258	0.265	0.170	0.169									
[18]	0.278	0.290	0.280	0.289	0.296	0.296	0.286	0.268	0.258	0.273	0.236	0.250	0.247	0.253	0.177	0.161	0.069								
[19]	0.303	0.308	0.305	0.314	0.322	0.322	0.320	0.283	0.273	0.273	0.233	0.256	0.236	0.242	0.205	0.202	0.126	0.160							
[20]	0.336	0.342	0.339	0.347	0.355	0.355	0.344	0.317	0.298	0.266	0.235	0.242	0.230	0.227	0.225	0.251	0.173	0.189	0.165						
[21]	0.333	0.339	0.336	0.336	0.344	0.352	0.341	0.322	0.296	0.279	0.232	0.247	0.227	0.233	0.238	0.256	0.169	0.185	0.179	0.021					
[22]	0.320	0.325	0.322	0.322	0.331	0.331	0.320	0.284	0.265	0.265	0.243	0.257	0.254	0.252	0.215	0.195	0.103	0.104	0.145	0.159	0.155				
[23]	0.325	0.339	0.336	0.328	0.336	0.328	0.325	0.290	0.279	0.255	0.241	0.238	0.244	0.241	0.197	0.194	0.102	0.111	0.144	0.183	0.179	0.026			
[24]	0.314	0.319	0.316	0.300	0.308	0.308	0.305	0.303	0.283	0.242	0.209	0.233	0.221	0.218	0.207	0.219	0.162	0.195	0.162	0.219	0.198	0.156	0.137		
[25]	0.320	0.325	0.322	0.331	0.339	0.347	0.336	0.301	0.275	0.251	0.237	0.235	0.231	0.246	0.185	0.191	0.175	0.174	0.150	0.188	0.202	0.169	0.160	0.169	

Fig. 4. Dissimilarity values based on RAPD analysis among the *Hippeastrum* accessions in Vietnam.

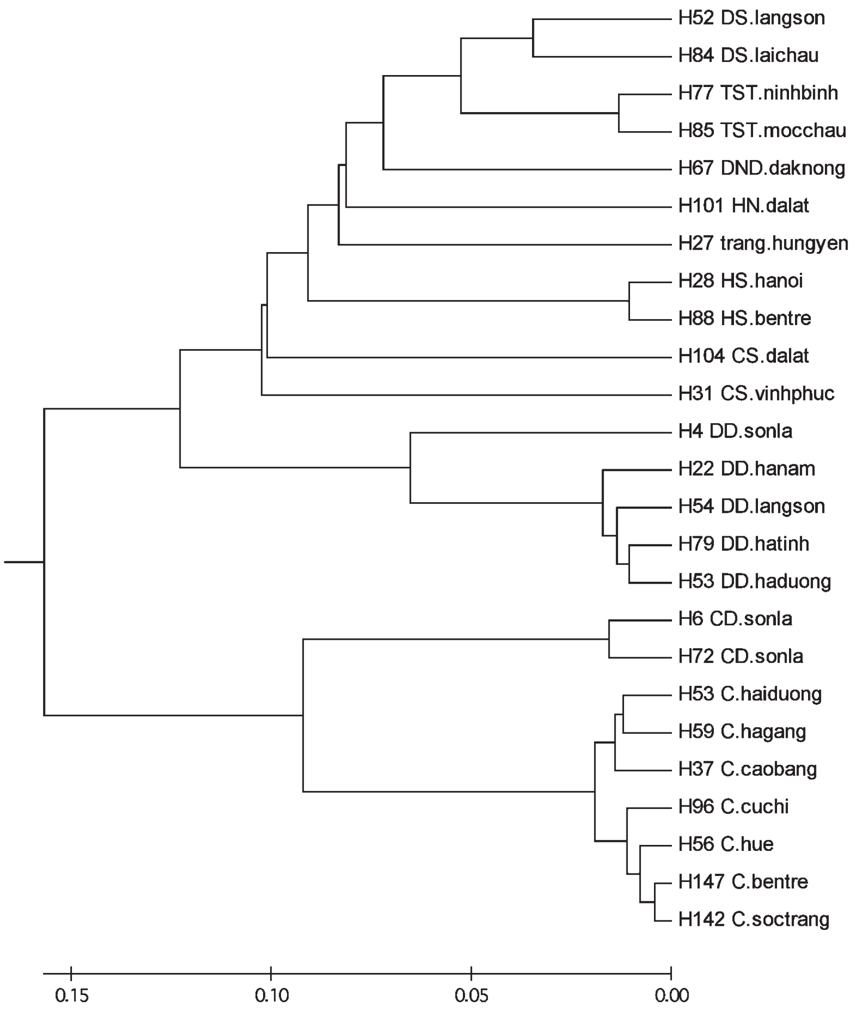

Fig. 5. Dendrogram of the *Hippeastrum* accessions in Vietnam developed from RAPD data.

close proximity in the dendrogram (e.g., accessions H52 and H84, or H28 and H88, or H77 and H85). These accessions clustered into three small branches of the dendrogram, which indicates their relatively close relationship. In a second cluster, two different subgroups could be distinguished. One included the *cam dại* accessions, and the other included two ***đỏ cam dại*** accessions. Although all of the *cam dại* accessions were relatively close, they were separated into two small branches on the dendrogram. The accessions H53, H59, and H37 collected from the northern region (Haiduong, Hagiang, and Caobang provinces, respectively) were grouped into one branch. However, the accessions H96, H56, H147, and H142 collected from the central and southern regions (Thuathienhue, Hochiminh, Bentre, and Soctrang provinces, respectively) were clustered on another short branch, indicating their close relationship.

DISCUSSION

Based on floral observation and results of the RAPD analysis in the present study, four different groups of *Hippeastrum* cultivated in Vietnam were revealed. Those groups could identified as *cam dại*, or *Hippeastrum puniceum* (Lamk.) Kuntze (syn. *Hippeastrum equestre* Herb.); ***đỏ dại***, or *Hippeastrum* x 'Johnsonii'; ***đỏ cam dại***, or *Hippeastrum striatum* Lamarck ; and the *lai* or hybrid group. These findings are not in agreement with those of other authors as to the number of extant *Hippeastrum* species and cultivars currently in Vietnam. Previous reports had indicated only two *Hippeastrum* species cultivated in Vietnam as *H. reticulatum* Herb. (a subspecies *H. reticulatum* Herb. var. striatifolia Herb.), and *H. puniceum* (Lamk.) Kuntze (syn. *H. equestre* Herb.) (Ho, 1999; Do *et al.*, 2007).

There is also an apparent contradiction in classification based on the flower color of *H. puniceum* (syn. *H. equestre* Herb.) in Vietnam. According to Do *et al.* (2007), the flowers of *H. puniceum* are red or orange; however, Ho (1999) reported flowers of this species to be red. In our study, accessions that were identified as *H. puniceum* had orange flowers with yellowish green throats and a large white star at the base, the same as described for *H. puniceum* by Traub (1949), Okubo (1993), and Read (2004).

Among these *Hippeastrum* groups, *H. puniceum* is the most widely distributed in Vietnam. It is found in home gardens from the northern region to the southern region. It is also important to note that Vietnam has two distinct climate regions: a northern region that is characterized by four distinct seasons including a cold winter, and the southern and central regions that are characterized by two distinct seasons, hot and rainy or hot and dry. *H. puniceum* flowers at the beginning of the rainy season in the southern and central regions, but flowers at end of spring in the northern region, when the weather is still cold and dry. It seems that flower initiation in *H. puniceum* in Vietnam does not appear to depend on vernalization, but on exposure to dry conditions. Therefore, the study on the flower initiation of *H. puniceum* is essential. Moreover, RAPD analysis identified some genetic variation between the *H. puniceum* populations from the northern region compared to those from the southern and central regions. All of the accessions collected from the southern and central regions (H96, H56, H147, and H142) could be phylogenetically distinguished from those collected in the northern region (H53, H59, and H37) with this RAPD data. Thus, the variation observed between *H. puniceum* populations of the northern region and those of the southern and central regions could have resulted from the adaptation of each population to these different climactic conditions.

To date, the name *H. equestre* (syn. *H. puniceum*) has been applied to accessions belonging to both the ***đỏ dại*** and the *cam dại* groups in Vietnam. However, our study indicates that accessions of the ***đỏ dại*** and *cam dại* groups are likely separate, as depicted in the dendrogram developed from analysis of this RAPD data. There is significant genetic variation (genetic distance value >0.3) between these two groups. Also, based on observation of the floral characteristics of each accession, the ***đỏ dại*** accessions could be redesignated as *H.* x 'Johnsonii', which is known to be a hybrid of *H. reginae* x *H. vittatum* (Traub, 1949; Meerow *et al.*, 1990, and Read, 2004). *H.* x 'Johnsonii' is cultivated in the northern region for its scarlet red flowers, but is not grown in the southern or central regions. Its flowering season is at the end of spring, although it does flower rarely in summer or winter.

Plant classifications based on morphological characteristics can sometimes present problems for taxonomists, but RAPD analyses can help to resolve such ambiguities (Swoboda and Bhalla, 1997). It is not uncommon for incorrect scientific names to persist locally, as in the case of the classification of *Allium* species occurring in Vietnam. Pham *et al.* (2006) had previously distinguished the wakegi onion (*Allium wakegi* Araki) from the shallot (*Allium cepa*, Agregatum group) using cytological, morphological, and RAPD analysis, although both had previously been classified as part of the *A. cepa* Agregatum group. Similarly, the present study is the first report distinguishing *H. puniceum* and *H.* x 'Johnsonii' in Vietnam.

H. striatum has not been officially reported to occur in Vietnam, but has only been informally observed in some northern provinces in Vietnam, including Hanoi, Thanhhoa, and Sonla provinces (personal observation). Although the flower size and shape of *H. striatum* are similar to those of *H. puniceum*, the genetic diversity between these two species is considerable (approximately 0.2). Due to small sample numbers, genetic variation among the *H. striatum* accessions has not yet been determined. Further studies of the morphological, cytological, and physiological characteristics of *H. striatum* are necessary to confirm whether those accessions are distinct at the species or lower level.

The demand for the new *Hippeastrum* cultivars is increasing in Vietnam and worldwide. Consequently, a number of new cultivars have been imported into the country for cultivation. The present study has revealed significant genetic variation among *Hippeastrum* accessions of the hybrid group. The available diversity of flower color, shape, and size of these new cultivars will be useful for *Hippeastrum* breeding in Vietnam. Among the hybrid accessions, *hồng sọc*, or *H. reticulatum* Herb. var. striatifolia Herb., is particularly easy to identify due to its pale pink flowers with netted venation and a white stripe on the leaves (visual observation). This accession blooms in September, while others flower from March to May. According to Meerow (1988) the flower emergence of these groups may be affected by a photoperiodic response more than by low temperatures. In particular, the variety *hồng sọc* could become an important genetic resource for breeding late–blooming *Hippeastrum* varieties.

In addition to providing valuable information on the relationships among and distribution of *Hippeastrum* species and varieties in Vietnam, our report also agrees with that of Chakrabarty *et al*. (2007) in that the RAPD method is useful for identifying new and existing hybrids, and for determining the relatedness of *Hippeastrum* accessions. The useful RAPD primers and the RAPD markers developed in the present study could be used in further research to analyse the relationships between *Hippeastrum* species, hybrids, and varieties not only in Vietnam, but also in other countries.

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