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Preliminary chromosome studies on *Hippeastrum* species from Vietnam

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Hippeastrum plants collected from different provinces of Vietnam and grown in the greenhouse at the Vietnam National University of Agriculture exhibit diversity in several morphological characteristics, especially in flower size and color. In order to understand the karyotypes and the phylogenesis of *Hippeastrum* species in Vietnam, somatic chromosome counts and karyotyping were performed for 97 *Hippeastrum* accessions. Chromosome counts showed that most (62 of 97 accessions, or 63.9%) *Hippeastrum* accessions in this collection are diploid with somatic chromosome number $2n$ = 2x = 22. Three accessions, or 3.1%, are triploid ($2n$ = 3x = 33). This collection includes 32 tetraploids, or 33%, that have chromosome number $2n$ = 4x = 44. The karyotypes of three *Hippeastrum* species in Vietnam, including *H.* x 'Johnsonii', *H. puniceum*, and *H. reticulatum* var. *striatifolia* Herb. were analyzed. Karyotype variation among these three species included differences in chromosome lengths, long arm to short arm ratios, centromere positions, and chromosomes types. These species carry one to three unequal pair(s) of chromosomes in their chromosome complements. The results of this research will be useful for identification and classification of *Hippeastrum* species and subspecies.

Key words: chromosome, germplasm, *Hippeastrum*, karyotype analysis

INTRODUCTION

Hippeastrum species are ornamental bulb–bearing flowering plants that belonging to the genus *Hippeastrum* in the Hippeastreae tribe of the Amaryllidaceae family. According to Okubo (1993), they are native to Central and South America, and therefore adapt easily to tropical and subtropical regions. More than 70 *Hippeastrum* species in tropical and subtropical regions of South America have been reported (Poggio *et al.*, 2007).

In Vietnam, *Hippeastrum* plants are widely grown in many home gardens. They were imported to Vietnam long ago and have become distributed throughout the country. Due to their easy cultivation and vegetative propagation, many *Hippeastrum* plants currently growing in Vietnam are generally local cultivars that have been propagated mainly by home or commercial growers for many years. Due to increasing demand for *Hippeastrum* in Vietnam, a number of new *Hippeastrum* varieties have been imported and introduced for production. However, the main limitations of using imported seeds are their limited supply and expense. Therefore, is has become necessary to perform *Hippeastrum* breeding in Vietnam in order to satisfy the demand for new cultivars for domestic production. Researchers at the Vietnam National University of Agriculture have collected 97 Vietnamese *Hippeastrum* accessions from all over the country to a develop germplasm collection to use for selecting and breeding improved varieties. To effectively exploit this germplasm collection, it will be necessary to evaluate the genetic diversity of its accessions, including by karyotype analysis.

The genus *Hippeastrum* contains taxa with a variety of chromosome numbers and ploidy levels that affect the utility of each taxon for breeding purposes (Williams and Dudley, 1984). Therefore, many chromosome studies have been carried out previously on *Hippeastrum* species (Baldwin and Speese, 1947; Guha, 1979; Arroyo, 1982; Naranjo and Poggio, 1988; Shafiq and Vahidy, 1998). These researchers have reported the basic chromosome number of *Hippeastrum* species as x = 11, with ploidy levels ranging from diploid to hexaploid. Interestingly, Arroyo (1982) discovered aneuploid *Hippeastrum* species. Although *Hippeastrum* cultivation has long been popular in Vietnam, as yet there have been no studies on the regional genetic diversity and or karyotypes of the genus. This study aims to discover the karyotype diversity of *Hippeastrum* species in Vietnam with an initial focus on diploid species.

MATERIALS AND METHODS

Plant materials

A total of 97 *Hippeastrum* accessions have been collected from various provinces in Vietnam since 2010 and grown in the greenhouse of Agronomy Faculty at the Vietnam National University of Agriculture. Chromosome numbers were evaluated in all of these accessions. Chromosome measurement and karyotyping were carried out on individual plants from three accessions (H141, H142, and H128) previously identified as diploid accessions that belong to the species *H.* x 'Johnsonii', *H. puniceum*, and *H. reticulatum* var. *striatifolia* Herb (Phuong *et al.*, 2014).

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Somatic chromosome preparation

Five young root tips of accessions H141, H142, and H128 were collected and pretreated with 0.05% colchicine at 20°C for 3 h. Root tips were then fixed in the Carnoy's solution (1 part acetic acid, 3 parts ethanol, v/v) for 24 h. Fixed root tips were then hydrolyzed in 1N HCl at 60°C for 6 min and stained with leucobasic fuchsine at 10°C for 6 h.

Chromosome observation

Treated root tips were squashed in 45% acetic acid and observed under the microscope. Somatic chromosomes were counted and lengths of the long and short arms of each chromosome were measured.

Karyotyping

Karyotypes were constructed from a photograph of the best chromosome spread for each accession. Individual chromosomes were excised from the photograph, then arranged and adhered onto a sheet of paper after classifying each chromosome according to its length and centromere position. The chromosome pairs of each of these three diploid accessions (H141, H142, and H128) were numbered from I to XI based on the lengths of each chromosome from longest to shortest. Chromosome pairs arranged in the karyoidiograms were divided into three groups following Baldwin and Speese (1947): group A (including chromosomes with the shortest short arm), group B (including chromosomes with a longer short arm), and group C (including chromosomes that are approximately isobrachial). Chromosome arm ratios were calculated as the long arm length divided by the short arm length. The centromeric index (CI) for each chromosome was calculated by dividing the long arm length by the total chromosome length. Chromosome types were identified according to Chaikla *et al*. (2011) as metacentric (m, CI = 0.500–0.599), submetacentric (sm, CI = 0.600–0.699), subacrocentric (sa, CI = 0.700–0.799), acrocentric (a, CI = 0.800–0.899), or telocentric (t, CI = 0.900–1.000).

RESULTS

Chromosome number

The results of chromosome counts are shown in Table 1. All 97 of the *Hippeastrum* accessions in this collection have a basic chromosome number x = 11. All are euploid with ploidy levels varying from diploid to tetraploid. Most *Hippeastrum* accessions in this collection were found to be diploid with a somatic chromosome number $2n$ = 2x = 22 (62 accessions, or 63.9% of total studied accessions). Three of the 97 (3.1%) accessions collected are triploids ($2n$ = 3x = 33). There are 32 (33%) tetraploid accessions with a chromosome number $2n$ = 4x = 44.

This collection shows diversity in several morphological characteristics, particularly in flower size and color. Based on their morphological characteristics, these three *Hippeastrum* species from Vietnam have previously been classified in *Hippeastrum* collections as *H*. x 'Johnsonii', *H. puniceum*, and *H. reticulatum* var. *striatifolia* Herb. (Table 2). In order to examine the karyotypes and phylogenesis of these three species, karyotyping was performed using three individual accessions (H141, H142, H128, further defined below) to represent these three species.

Flower characteristics of *H*. x 'Johnsonii', *H. puniceum*, and *H. reticulatum* var. *striatifolia* Herb.

In Vietnam, local *Hippeastrum* cultivars have usually been traditionally named base on their flower color. Accessions that belong to the species *H*. x 'Johnsonii' and *H. puniceum* that have become naturalized throughout Vietnam are named "wild red" (Do dai in Vietnamese) and "wild orange" (*Cam dai* in Vietnamese), respectively. The *H*. x 'Johnsonii' accession H141 was collected from Hanoi. *H*. x 'Johnsonii' is known by its scarlet red flower with short white stripes. This accession has flowers 10.8 cm in diameter, which are smaller than those of the other two species in the present study. The *H. puniceum* accession H142 was collected from Soctrang (Southern Vietnam) and has orange flowers 12.3 cm in diameter, which are larger than those of the other two species in the present study. The *H. reticulatum* var.

Table 1. Chromosome number of *Hippeastrum* accessions in Vietnam

Chromosome number	Number of accessions	Percentage of total accessions (%)
$2n$ = 2x = 22	62	63.9
$2n$ = 3x = 33	3	3.1
$2n$ = 4x = 44	32	33.0
Total	97	100.0

Table 2. Location of collection and flower characteristics of three typical diploid *Hippeastrum* species

Species	Accession	Vietnamese name	Locality (District, Province)	Flower diameter (cm)*	Flower color
H. x 'Johnsonii'	H141	Đỏ dại	Gialam, Hanoi	10.8 ± 0.2	Scarlet red with short white stripes
H. puniceum	H142	Cam dại	Trande, Soctrang	12.3 ± 0.7	Orange
H. reticulatum var. *striatifolia* Herb.	H128	Hồng sọc	Dongla, Hanoi	11.5 ± 1.0	Pale pink with netted veins

* The number of flowers measured: n = 10.

striatifolia Herb. accession H128 (from Northern Vietnam) has pale pink, 11.5 cm flowers with netted veins. This variety is named *Hong soc* in Vietnamese because of its pink flower color.

Karyotypes of *H.* x 'Johnsonii', *H. puniceum*, and *H. reticulatum* var. *striatifolia* Herb

The results presented in Table 3 and Fig. 1 show the variation in chromosome size and chromosome arm ratio between individual plants of these three species. Among these three species, the chromosomes of *H.* x 'Johnsonii' (accession H141) were 126.6 μm in total length, while those of *H. reticulatum* var. *striatifolia* Herb. (accession H128) were 63.1 μm in total length. The chromosomes of pair I of *H.* x 'Johnsonii' were 8.1 μm in length, while those of *H. puniceum* were 6.9 μm, and those of *H. reticulatum* var. *striatifolia* Herb. were only 4.2 μm in length. The differences in the lengths of the 10 remaining chromosome pairs of these three species were proportionally similar. The lengths of all 11 of the chromosome pairs of *H. reticulatum* var. *striatifolia* Herb. were approximately half those of their corresponding chromosome pairs in *H.* x 'Johnsonii'. Except for pairs V and VI, nine chromosome pairs of *H. puniceum* are of average length in comparison with the others. The chromosome arm ratios also differ among these three species. The chromosome arm ratios of chromosome pairs I, III, and X in these three species are nearly the same. The chromosome arm ratios of the eight remaining chromosome pairs in these three species also differ. All of the three species in the present study have unequal chromosome pairs. However, the number and order of unequal pairs differ between the three species. Chromosome pair II exhibits unequal chromosome pair length in *H.* x 'Johnsonii'. The two chromosomes of this pair clearly differ in length (8.1 μm and 6.9 μm), but do not differ in chromosome arm ratio (2.3 and 2.4). There are two unequal chromosome pairs, II and VIII, in *H. puniceum*. The two chromosomes of these two pairs differ in both total length and chromosome arm ratio. Three unequal chromosome pairs, IV, VIII, and XI, were observed in *H. reticulatum* var. *striatifolia* Herb. The two chromosomes in pair IV differ in length, but are similar in chromosome arm ratio. The two chromosomes of pair VIII differ in both length and chromosome arm ratio. Meanwhile, two chromosomes of pair XI are similar in length but different in chromosome arm ratio.

Karyotype formulas of *H.* x 'Johnsonii', *H. puniceum*, and *H. reticulatum* var. *striatifolia* Herb.

As shown in the Fig. 1, three different chromosome types corresponding to groups A (chromosomes with the shortest short arm), B (chromosomes with a longer short arm), or C (chromosomes that are approximately isobrachial) can be observed in the chromosome complements of *H.* x 'Johnsonii', *H. puniceum* and *H. reticulatum* var. *striatifolia* Herb. To distinguish the karyotypes of these species, chromosome lengths and centromeric indices were used to define the karyotype formula of each species (Table 4). Accession H141 (*H.* x 'Johnsonii' species) bears four pairs of subacrocentric chromosomes

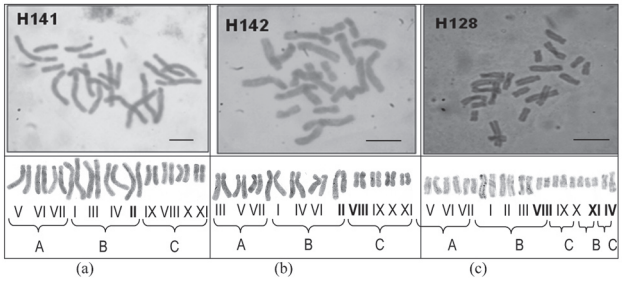

Fig. 1. Mitotic metaphase chromosomes and karyotype of *H.* x 'Johnsonii' (a), *H. puniceum* (b), and *H. reticulatum* (c). Roman numerals (I – XI): chromosome pair number. Bold Roman numeral: unequal pair of chromosomes. A: group of chromosomes with the shortest short arm; B: group of chromosomes with a longer short arm; C: group of approximately isobrachial chromosomes. Scale bar: 5 μm.

Table 3. Chromosome size of three typical diploid *Hippeastrum* species

Accession - Species	Chromosome size	Chromosome pair number											Total chromosome length (μm)
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
H141 *H.* x 'Johnsonii'	Chromosome length (μm)	8.1	8.1 (6.9)*	7.5	7.2	5.6	5.6	5.3	4.4	4.2	4.1	3.8	126.6
	Chromosome arm ratio	2.4	2.3 (2.4)	2.5	2.3	5.5	4.7	6.0	1.1	1.8	1.0	1.0	
H142 *H. puniceum*	Chromosome length (μm)	6.9	6.6 (4.9)*	6.3	6.1	6.1	5.6	4.7	3.7 (3.4)*	3.4	3.0	2.4	107.9
	Chromosome arm ratio	2.4	2.5 (1.7)	2.5	2.7	3.3	4.2	3.0	1.4 (1.0)*	1.0	1.0	1.0	
H128 *H. reticulatum* var. *striatifolia* Herb.	Chromosome length (μm)	4.2	3.8	3.4	3.4 (2.6)*	3.3	3.0	2.7	2.4 (1.9)*	2.3	2.0	1.7 (1.7)*	63.1
	Chromosome arm ratio	2.3	2.2	2.4	1.0 (1.0)	3.8	3.6	5.7	2.3 (1.8)*	1.0	1.0	1.5 (1.0)*	

* The values given in parentheses are of the smaller chromosomes in unequal pairs of chromosomes.

Table 4. Centromeric index of three typical diploid *Hippeastrum* species

Accession - Species	Chromosome pair number										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
H141 *H.* x 'Johnsonii'	0.706	0.700 (0.708)*	0.714	0.701	0.846	0.825	0.857	0.516	0.636	0.500	0.500
Chromosome type	sa	sa	sa	sa	a	a	a	m	sm	m	m
H142 *H. puniceum*	0.706	0.714 (0.630)*	0.714	0.730	0.767	0.808	0.750	0.583 (0.500)*	0.500	0.500	0.500
Chromosome type	sa	sa sm	sa	sa	sa	a	sa	m	m	m	m
H128 *H. reticulatum* var. striatifolia Herb.	0.697	0.688	0.706	0.500 (0.500)*	0.792	0.783	0.851	0.697 (0.643)*	0.500	0.500	0.600 (0.500)*
Chromosome type	sm	sm	sa	m	sa	sa	a	sm	m	m	sm m

* The values given in parentheses are the CI for the smaller chromosomes in unequal pairs of chromosomes.

Table 5. Karyotype formulas of three typical diploid *Hippeastrum* species

Species	Accession	Number of chromosomes							
		Long chromosome (L)				Short chromosome (S)			
		a	sa	sm	m	a	sa	sm	m
H. x 'Johnsonii'	H141	8	6	0	0	0	0	2	6
H. puniceum	H142	2	11	1	0	0	0	0	8
H. reticulatum var. *striatifolia* Herb.	H128	2	6	4	2	0	0	3	5

(pairs I, II, III, and IV), three pairs of acrocentric chromosomes (pairs V, VI, and VII), one pair of submetacentric chromosomes (pair IX), and three pairs of metacentric chromosomes (pairs VIII, X, and XI). Five pairs of subacrocentric chromosomes (pairs I, III, IV, V, and VII), four pairs of metacentric chromosomes (pairs VIII, IX, X, and XI), and one pair of acrocentric chromosomes (pair VI) were found in the chromosome complement of accession H142 (*H. puniceum*). The longer of the unequal chromosomes in chromosome pair II in accession H142 is subacrocentric, while the shorter chromosome is submetacentric. Three pairs of subacrocentric chromosomes (pairs III, V, and VI), three pairs of metacentric chromosomes (pairs IV, IX, and X), three pairs of submetacentric chromosomes (pairs I, II, and VIII), and one pair of acrocentric chromosomes (pair VII) were observed in the chromosome complement of accession H128 (*H. reticulatum* var. *striatifolia* Herb.). The smallest unequal pair of chromosomes (pair XI) of this accession is comprised of one submetacentric and one metacentric chromosome.

The results shown in Tables 4 and 5 indicate that karyotype formulas of these three accessions from different species are differ from each other. The karyotype formulas of accessions H141, H142 and H128 are $8L_a + 6L_{sa} + 2S_{sm} + 6S_m$, $2L_a + 11Ls_a + 1L_{sm} + 8S_m$, and $2L_a + 6L_{sa} + 4L_{sm} + 2L_m + 3S_{sm} + 5S_m$, respectively.

DISCUSSION

Many studies have confirmed that most *Hippeastrum* species have a basic chromosome number of x = 11. The results of the present study agree with this finding. However, some species with basic chromosome numbers of x = 10 for *H. blumenavia* (Arroyo, 1982), or x = 12 for *H. iguazuanum* (Williams and Dudley, 1984) have been discovered. However, in our collection no accession had a basic chromosome number different from x = 11. Aneuploidy has also previously been discovered in some *Hippeastrum* species (Mookerjea, 1955; Arroyo, 1982), but has not been observed in the present collection. Several authors have also previously reported variation in ploidy levels among *Hippeastrum* species. For example, diploids ($2n$ = 2x = 22), triploids (3x = 33), tetraploids (4x = 44), pentaploids (5x = 55), and hexaploids (6x = 66) have been identified previously and occur in most of the *Hippeastrum* species studied (Arroyo, 1982; Naranjo and Poggio, 1988; Brandham and Bhandol, 1997; Poggio *et al.*, 2014). We have identified only diploids, triploids, and tetraploids in our *Hippeastrum* collection in Vietnam. Similarly, most other *Hippeastrum* accessions from Vietnam are diploid or tetraploid, and there are very few triploid accessions. The *H. puniceum* plant in our collection was found to be diploid, although other examples of this species with different ploidy levels have been identified (Poggio *et al.*, 2014).

The chromosome lengths of two diploid *Hippeastrum* species and one cultivar described in the present paper differ from each other as well as from those of other *Hippeastrum* species studied previously. Brandham and

Bhandol (1997) reported that the total chromosome length of diploid genomes varied from 126.1 μm to 200.4 µm among six different *Hippeastrum* species (*H. aulicum*, *H. cybister, H. forgetii* Worsley, *H. pardinum*, *H. puniceum*, and *H. reginae*). The total chromosome length of *H. parodii* was measured as 158.5 μm (Naranjo and Poggio, 1988). The three diploid accessions in the present study have shorter chromosomes relative to those other species. In particular, accession H128 (*H. reticulatum* var. *striatifolia* Herb.) has very short chromosomes that are 63.1 μm in total chromosome length. Brandham and Bhandol (1997) hypothesized that, just as for *Aloe* species studied by Brandham (1983), the more primitive *Hippeastrum* species might have smaller genomes. By similar reasoning, we speculate that *H. reticulatum* could be the most primitive species among the species we have examined.

Karyotypic differences between the three diploid *Hippeastrum* species in this study were clearly observed. As for other *Hippeastrum* species, diploid genomes of these species are also comprised of 14 long chromosomes and eight short chromosomes; however, the karyotype formulas of these species differ from each other as well as from those of other species published previously. Shafiq and Vahidy (1998) observed a basic karyotype of two metacentric, two submetacentric, four subacrocentric, and three acrocentric chromosomes in their analysis of *Hippeastrum vittatum* plants. Five of six *Hippeastrum* species studied by Brandham and Bhandol (1997) had 14 long acrocentric and eight short metacentric chromosomes in their diploid karyotypes, while other species (*H. reginae*) had 12 long acrocentric, one long metacentric, and eight short metacentric chromosomes. These authors did not define subacrocentric and submetacentric chromosomes in their analyses. *Hippeastrum* parodii was found to have basic karyotype of three subtelocentric (or subacrocentric), four submetacentric, and four metacentric chromosomes (Naranjo and Poggio, 1988). The karyotypic dissimilarities observed have previously been attributed to possible amphiplasty, pericentric inversions, or unequal reciprocal translocations (Shafiq and Vahidy, 1998). Thus, karyotype formulas are quite specific to different plants and species. The karyotype formula of diploid *H. puniceum* in our study differed clearly from those of triploid and hexaploid *H. puniceum* studied by Poggio *et al*. (2014). We found unequal or mismatched chromosome pairs in our study in numbers varying from one in *H*. x 'Johnsonii' to three in *H. reticulatum* Herb. var. *striatifolia* Herb. Mismatches were observed in *H. reticulatum* var. *striatifolia* Herb. chromosome pairs Ⅱ, Ⅳ, Ⅷ, and Ⅺ. Shafiq and Vahidy (1998) found mismatches for two chromosome pairs (Ⅶ and Ⅺ) in diploid *H. vittatum* and in two chromosome pairs (Ⅳ and Ⅺ) in tetraploid *H. vittatum*. The mismatched chromosomes in their study were assumed to provide evidence of extensive hybridization during the evolution of the species. By similar reasoning, we assume that the *Hippeastrum* accessions in the present study are probably natural hybrids.

CONCLUSION

The *Hippeastrum* germplasm collection in Vietnam includes diploid, triploid, and tetraploid accessions. A large proportion of the entire collection consists of diploids. The differences in chromosome lengths, long arm to short arm ratios, centromere positions, and chromosome types among the three plants observed from *H*. x 'Johnsonii', *H. puniceum*, and *H. reticulatum* var. *striatifolia* Herb. revealed the variation in their karyotypes. These species carry from one to three unequal pair(s) of chromosomes in their chromosome complements. The results of this study will be useful for the identification and classification the *Hippeastrum* species for breeding purposes.

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