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Chromosomal Heteromorphy in the Karyotypes of Three Local Cultivars of *Hippeastrum vittatum* (Amaryllidaceae)

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Abstract
Cytological studies of three cultivars viz., 'Apple Blossom', Cardinal and `Minerva' of *H. vittatum* were undertaken for the evaluation of Karyomorphological variations. On the basis of length, the chromosomes were classified in to three groups viz., large, medium and small. Within each group, chromosomes were arranged in the ascending order of heterobrachy. Basic Karyotype observed was composed of 2 metacentric, 2 submetacentric, 4 subacrocentric 3 acrocentric chromosomes. Chromosomes exhibited heteromorphism for size due to loss of parts of long or short arms in one of the members in varying degrees. Pronounced difference in length was regarded as mismatching and lesser degree of variation between counterpart members as heteromorphy. In diploid plants, chromosomes VII and XI exhibited variation, which was regarded as mismatching. Three cultivars could not be differentiated on the basis of chromosomal morphology. In tetraploid plants chromosomes were arranged in to eleven linkage group of four members each. In general, the morphology of chromosomes was similar to that of diploid cultivars. Among the Karyotypes of tetraploid eva. Cardinal and 'Minerva' there was no set with mismatched chromosomes, however, in cv. 'Apple Blossom' mismatched chromosomes were observed in linkage group IV and XI. Heteromorphy of varying degree was found in several chromosomes of diploid and tetraploid plants.

Key words: Amaryllis, karyotyping, chromosomal changes, hybridization, anenploidy

Introduction

Generally genus *Hippeastrum* is considered synonym to *Amaryllis*, however, according to Moore (1963) the former is applied on South American and later to South African species. Goldblatt (1984) os of the opinion that the name *Hippeastrum* is applied to those which belong to those which belong to the Caribbean species cited by the Linneaus, while *Amaryllis* to the South African plants in almost all major herbaria of the world. The first hybrid of *Amaryllis* was produced between *A. reginae* X *A. vittata* in 1799 followed by extensive hybridization for improvement of various morphological characters of plant and flower (Narain and Khoshoo, 1977). The process of cross breeding for improvement of required characters, continued for almost two centuries. Thus the resent day cultivars are the result of indiscriminate hybridization and selection followed by multiplication by vegetative means, though genotypically these usually carry reproductive sterility and high degree of heterozygosity.

The chromosome count of *Hipeastrum* is based on X = 11, with the most common cytotype being diploid 2n = 22; though different ploidy levels ranging from triploidy to heptaploidy have reported (Guha, 1979; Arroyo, 1982). According to Brandham (1986) the relative frequency of the various ploidy levels has become established as a result of artificial selection that has occurred largely in absence of deliberate chromosomes manipulation in *Narcissus*, which is a related taxa of *Hippeastrum*. In Pakistan the distribution of one species of the genus i.e., *Amaryllis* Vittata with two ploidy levels, 2n = 22 & 44 has been reported to occur in nature (Stewart, 1972), however, many cultivated forms are available in local nurseries and home gardens. Several basic karyotypes of various species and cultivars have been proposed by different workers, many showing heteromorphism of different degree in one more homologous chromosomes. Present investigation deals with the evaluation of heteromorphism in locally grown cultivars. The chromosomes were identified on the basis of length, morphology and heterobrachy, such that the heteromorphy of chromosomes be identified.

Materials and Methods

The material used comprised of three cultivars of *H. vittatum* the VIZ. 'Apple Blossom', 'Cardinal' and 'Muberva'. A total number of one hundred seventy five bulbs obtained from local nurseries and home gardens were multiplied at the Department of Genetics, University of Karachi for this study. Each bulb was individually planted in a mixture of sand and clay in earthen flower pot. Active growing roots tips were collected and subjected to pretreatment mixture, comprised of 0.01 g colchicine, 0.005 g 8-hydroxyquinoline and 5 drops of DMSO in 20 ml of distilled water. Fixation in 45% acetic acid was followed by staining in 20% aceto orcien. Slides were prepared by squash technique. The coverslips were removed by treating in liquid nitrogen, followed by dehydration in absolute alcohol for 1 hour, before mounting in Canada balsam. The preparations were scanned and at least five cells per bulb were selected for photomicrography and karyotyping. Photomicrographs were enlarged to 10.5 X 12.0 inches prints to form karygrams.

Individual chromosome were cut and pasted on a sheet of paper after classifying on the basis of length and heterobrechy of chromosomes. Chromosomal arm ratios were calculated. For the nomenclature of chromosomes terms used by Sybenga (1992) were followed.

Results

Distribution of ploidy levels in three cultivars is shown in Table 1. On the basis of length, the chromosomes were divided into three groups VIZ., large, medium and small. Within each group, chromosomes were arranged in ascending order of heterobrachy. This, in general coincided with the decreasing total length of the chromosomes within the group. Group large consisted of three, numbered I-III pairs, while medium and small groups comprised of four pairs of chromosomes each numbered IV-VII and VIII-XI respectively. Chromosomes VII and IX were matecentric, X and XI submetacentric, I and II subacrocentric, III subacrocentric to acrocentric and IV-VII acrocentric in nature. Structural variation within pairs, of two degrees was, observed one was considered as heteromorphy' and other as 'mismatching' between the homomorphic chromosomes. Mismatching was observed in VII and XI chromosomes of diploid plants. Besides these pairs, some homomorphic chromosomes showed small degree of heteromorphism. The mismatched and the heteromorphic chromosomes were larger in size than the presumed actual members.

Diploids

Within group large, pair I was least heterobrachial. Short arm of pair II was always smaller than pair 1. Pair III was subacrocentric in cv. `Apple Blossom` (Fig. 1) and acrocentric in cvs. Cardinal' (Fig. 2) and Minerva' (Fig. 3). Pair IV was classified in medium group. In cv. `Cardinal` (Fig. 2) short arm of pair V was equal to that of pair IV. Pair VII contained mismatched chromosomes, which was larger in length than presumed actual member, chromosome VII was smaller than the presumed actual member of pair VII. Pair XI also contained mismatched chromosome similar to pair VII. Its morphology did not match to any of the chromosome of the complement. In some of the plants, only short arm of this chromosome was similar to that of chromosome XI (Fig. 2). The heteromorphic member of the pair XI was large subacrocentric to acrocentric in nature. Its long arm was at least double in length than the presumed actual member of pair XI. In cvs. `Apple Blossom` (Fig. 1) and Minerva (Fig. 3) length of short arm of heteromorphic chromosome of VII and XI pairs were larger than those arms of their respective members. In these cultivars, the heteromorphic chromosome of XI pair was subacrocentric in nature. While in cv. Cardinal` (Fig.2) length of short arm of heteromorphic chromosome of pair XI was equal to its counterpart member and acrocentric in nature.

Tetraploids

For the karyotype of the tetraploids, the chromosomes were classified into eleven linkage groups of four chromosomes each. Unlike diploids, there was no set with distinct heteromorphism in karyorypes of cvs. Cardinal` (Fig. 4) and Minerva` (Fig. 5). However, minor variation in chromosomal length or their arm was observed among some of the members within sets. Pronounced heteromorphy was found in karyotype of cv. Apple blossom` (Fig. 6), in sets IV and XI. In general, the morphology of the chromosomes was similar to that of the diploids. The size of chromosomes in three groups viz., large, medium and small ranged from 12.0-10.0, 9.5-7.0 and 6.0-3.0 microns (Table 2) respectively.

Discussion

The chromosome counts of many cultivars of *Hippeastrum* are based on x = 11. The present findings were in agreement to those of (Mookerjea, 1955; Narain and Khoshoo, 1968; Guha, 1979; Naranjo and Poggio, 1988; Khaleel *et al*., 1991). However, certain deviation from this number are also on record. For example, the chromosomes of some of the species are based on n= 8, Fernandez, 1970), x = 10 (Arroyo, 1982) and x = 12, Williams and Dudley, 1984). According to Rees and Jones, 1977) any change in basic number results into alterations in number of linkage group that in turn changes the amount of recombination through independent segregation which ultimately causes genetic variability in a group 0.

In general, the plants studied had the basic Karyotype of 2 metacentric, 2 submetacentric, 4 subacrocentric and 3 acrocentric chromosomes. Different basic Karyotypes have reported by various workers for the species and cultivars with x = 11. Narain and Khoshoo (1968) proposed a basic Karyotype of 2 metacentric, 5 submetacentric and 4 acrocentric chromosomes in diploid and tetraploid cultivars. Naranjo and Andrada (1975) found 4 metacentric, 4 submetacentric and 3 acrocentric chromosomes in different diploid species. Khaleel *et al*. (1991) suggested that the most frequent basic karyotype comprised of 4 metacentric, 5 submetacentric and 2 acrocentric chromosomes in majority of tetraploid cultivars studied by them. Such variations among the Karyotypes of allied species indicates prominent role of chromosomal structural changes operating in the evolution. Amphiplasty, pericentric inversions and unequal reciprocal translocations have been suggested as the principle causes for such Karyotypic dissimilarities.

No gross morphological differences except certain minor variations in the chromosomes among the complements of three cultivars `Apple Blossom`, Cardinal and `Minerva` were found. Structural variation of two degrees viz., heteromorphy and mismatching was observed. In all diploid plants mismatching in pairs VII and XI was observed. Short arms of mismatched chromosomes VII and XI were either

Table 1: Distribution of ploidy levels in three cultivars of *Hippeastrum* studied

Cultivar	Diploid	Tetraploide	Total
Apple Blossom	35 (70)	15 (30)	50 (100)
Cardinal	39 (60)	26 (40)	65 (100)
Minerva	43 (72)	17 (28)	60 (100)

Figures in parenthesis are percentages within the cultivars

Table 2: Total length (L+S) and Long/Short arm ratios (L/S) in microns of somatic chromosomes in three cultivars of *Hippeastrum*. The values are means of 200 and 150 cells from diploid and tetraploid plants respectively

Chromosome Number	2X		4X	
	L+S	L/S	L+S	L/S
I	12.5	1.5	12.0	1.9
II	12.0	2.4	11.5	2.1
III	10.5	2.9	10.0	2.9
IV	10.0	3.0	9.5	2.3
	(5.0)*	(1.5)*		
V	9.5	3.5	8.5	3.6
VI	9.0	4.8	8.0	4.0
VII	7.5	4.1	7.0	5.2
	(7.8)*	(4.7)*		
VIII	7.0	1.0	6.0	1.1
IX	6.0	1.2	6.0	0.9
X	4.0	1.2	5.0	1.1
XI	3.0	2.2	3.0	1.5
	(8.5)*	(3.7)*	(2.0)*	(1.0)*

*Values given in parentheses are of mismatched chromosomes

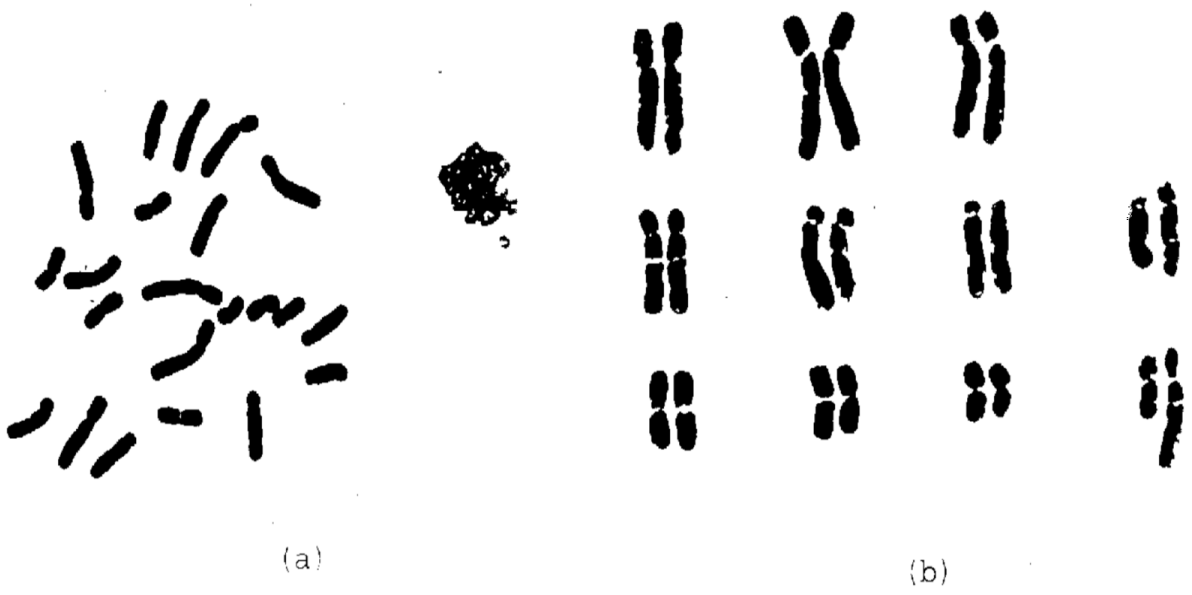

Fig. 1: (a) Representative cell of diploid cv. Apple Blossom. (b) Karyogram of same cell

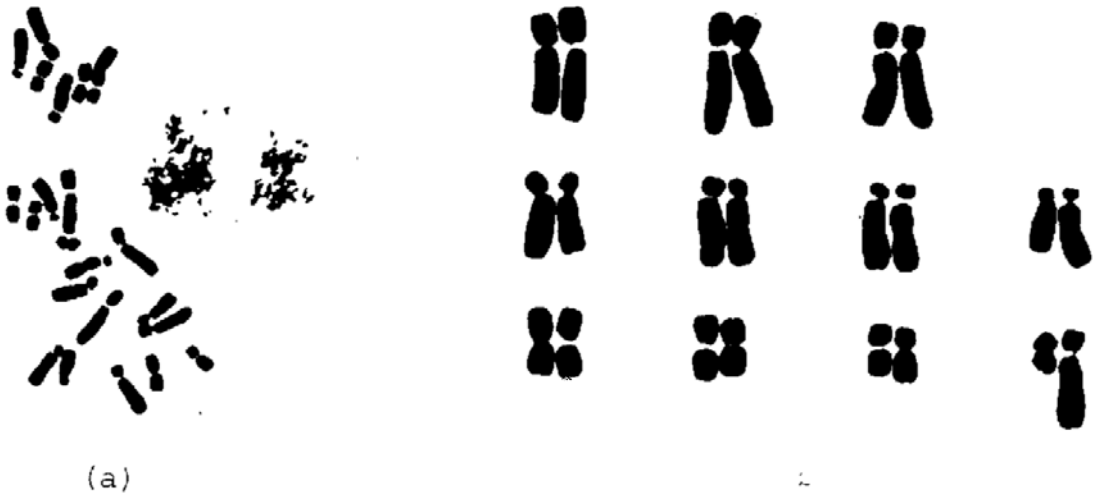

Fig. 2: (a) Representative cell of diploid cv. `Cardinal (b) Karyogram of same cell

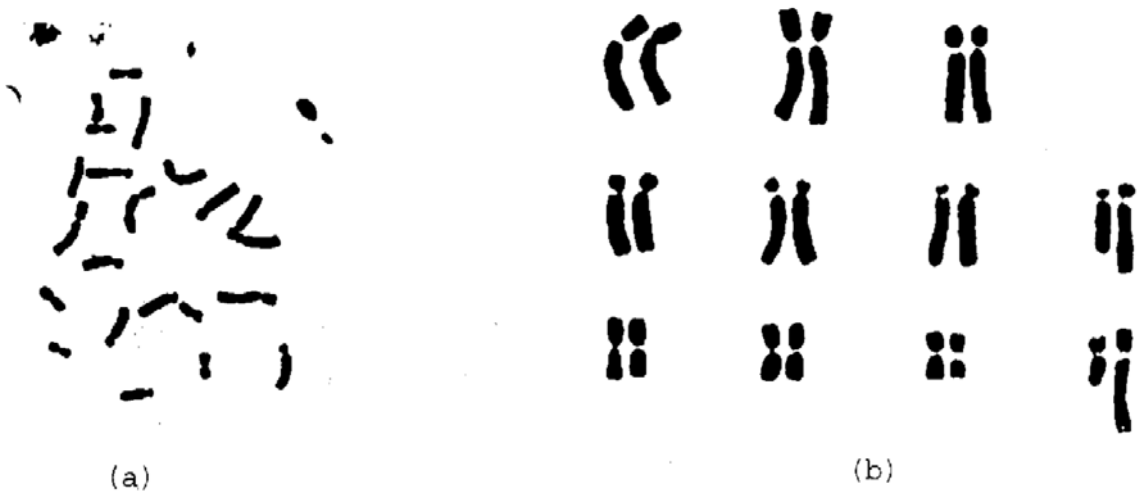

Fig. 3: (a) Representative cell of diploid cv. `Minerva` (b) Karyogram of same cell

larger or equal ton their counterpart members. Long arm of mismatched chromosome VII was substantially larger than that of its counterpart. Long arm of mismatched chromosome XI being about 2.5 times larger than that of its counterpart revealed that either addition or translocation took place, which became persistent due to vegetative mode of propagation of plants. Presence of mismatched and heteromorphic chromosomes provides evidence of extensive hydridization in past and hybrid nature of genus. Diploid cv. `Adulles` of *A. belladonna* contained four median, ten submedian and eight subterminal chromosomes. Narain and Khoshoo (1968) arranged them in six pairs while rest of the ten chromosomes were not classified in to five homomorphic pairs due to mismatching. Presence of heteromorphic chromosomes is considered as the clue to the structural hybridity (Sharma and Bal, 1956). Naranjo and AAandrada (1975) reported chromosomal complement in four species viz *H. argentinum*, *H. rutilum*, *H. aglaiar* and *H. vitamin* more or less similar to those found in three cultivars investigated in present study. In *H. argentinum*, a small submetacentric pair contained mismatched chromosome, that was formed due to large subacrocentric chromosome. Two diploid species. A belladonna and *H. parodii* studied by Naranjo and Poggio (1988), also contained three large, four medium and small

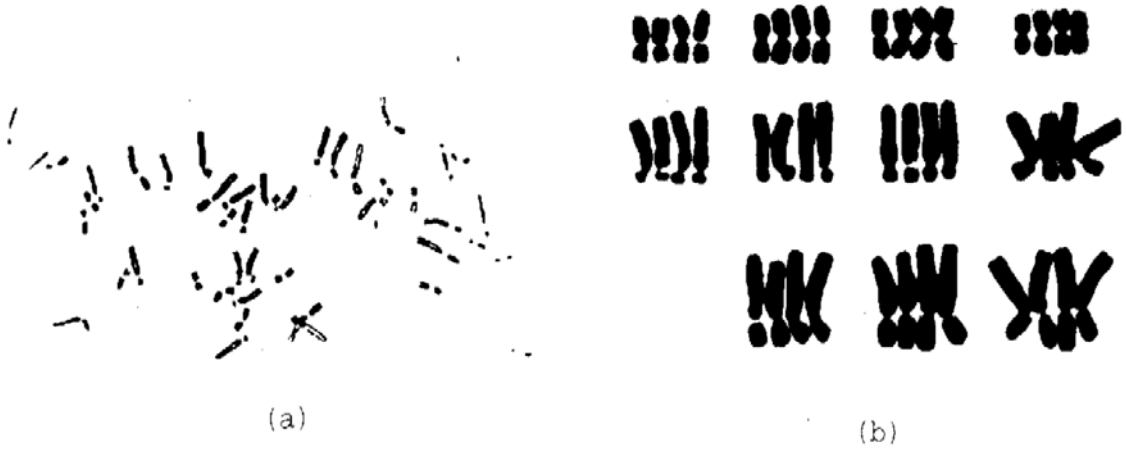

Fig. 4: (a) Representative cell of tetraploid cv. `Cardinal` (b) Karyogram of same cell

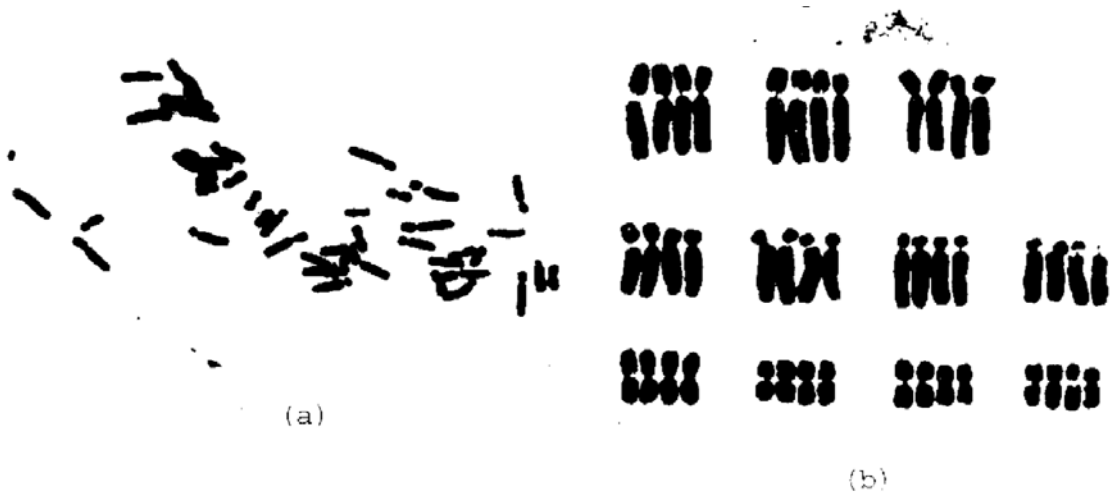

Fig. 5: (a) Representative cell of tetraploid cv. `Minerva` (b) Karyogram of same cell

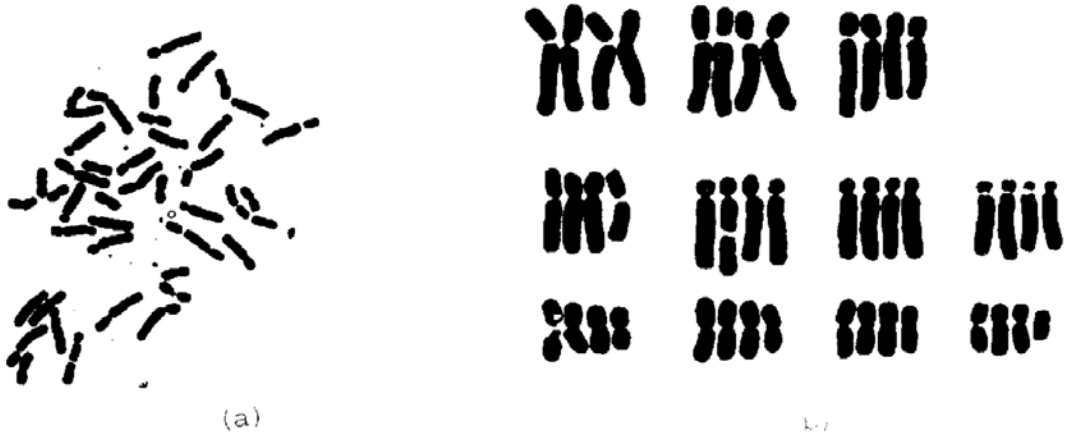

Fig. 6: (a) Representative cell of tetraploid cv. `Apple Blossom` (b) Karyogram of same cell

Chromosomes each, as reported in the present findings. Narain (1977) reported two mismatched chromosomes along with nine homomorphic pairs in *A. stylosa*, that contained a pair of satellited chromosomes.

Tetraploidy may be the result of hybridization between two species and then doubling of the diplid genome or it may be doubling of diploid genome directly. There is no confirmation that tetraploid plants have chromosomes of autotetraploid or allotetraploid origin. Among tetraploid plants only cv. `Apple Blossom` has shown pronounced heteromorphy of chromosomes similar to diploid plants. The heteromorphic chromosomes were observed in two sets, viz., IV and XI. In earlier reports too, heteromorphic chromosomes were found in tetraploid species and cultivars. High degree of heteromorphy or mis matching have been reported by Narain and Khoshoo (1968) in cv. `Andromeda` contained seven sets with four chromosomes each, two sets with six chromosomes each and two pairs with chromosomes each. Vij *et al*. (1978) arranged in to six sets with four chromosomes each, three sets with three chromosomes each, two pairs and four single chromosomes in a cultivar of A. belladonna; thus showing very high degree of mismatching. Khaleel and Siemsin (1989) and Khaleel *et al*. (1991) classified chromosomes into twenty-two pairs and reported some mismatched pairs in tetraploid cultivars of *Amaryllis* hybrids. No mismatched chromosomes was oserved in tetraploid species, H. petiolatum by Naranjo and Andrada (1975). However, various degree of heteromophy was reported by them in most of chromosomes similar to our Karyotypes of cvs. `Cardinal` and `Minerva,` H. petiolatum investigated by them, revealed the general morphology of chromosomes that was similar to the three cultivars reported here. Generally less heteromorphy was reported in small metacentric chromosomes in various species and cultivars of *Hippeastrum* including the cultivars studied in present investigation.

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