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Research article

Karyomorphological studies on seven variants of *Clitoria terantea* L. (Fabaceae)

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Abstract: Mitosis of seven variants of *Clitoria ternatea* was carried out and the number of chromosomes in all the variants were 16. Karyotype indicated that all the variants consist of nearly median and sub-median chromosomes. Total and absolute chromosome length were higher in double pink and lower in light blue. The analysis of total form percentage showed that 'double pink' possess lowest value and represented as the most advanced type whereas 'single blue' possess highest value and represented as the most primitive karyotype. The variation in chromosomal characters coincides with the morphological variability within the species.
Keywords: Karyotype - Butterfly pea - Asymmetrical - Idiogram.

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INTRODUCTION

Clitoria ternatea L. commonly known as 'Butterfly Pea' (Shankupushpam) belongs to the tribe Phaseolae of the family Fabaceae. It is probably a native to South America (Upadhyaya & Pachauri 1983, Parrotta 2001, Harinarayanan 2005) and widely distributed in tropical and sub-tropical countries. The plant is a perennial, tall and branched with white to blue/pinkish flowers. The butter-fly pea is a deep-rooted, slender climbing legume. The 'butterfly pea' is reported to have a wide range of medicinal properties. Besides being an ornamental, it is also cultivated for medicinal properties (Srivastav & Raina 1982, Mukherjee *et al.* 2008). Its roots are aperient, laxative, diuretic and demulcent and are given in fever, croup, chronic bronchitis, ascites, dropsy and enlargement of the abdominal viscera (Khory & Katrak 1984).

A perusal of available literature reveals that the cytological reports of this species are rather meagre and are mostly chromosome number reports (Fedorov 1974, Sanjappa & Dasgupta 1977, Bir & Kumari 1978, George & George 1978, Lackey 1980, Srivastav & Raina 1980, 1982). Detailed studies on the karyomorphology of different variants of *Clitoria ternatea* are lacking. The present communication concerns karyomorphology of seven variants.

MATERIALS AND METHODS

Sample preparation

Root tips of germinated seeds of seven variants of *Clitoria ternatea* were collected in morning hours between 10.00 to 11.00 am and fixed in 3:1 Carnoy's fluid. The root tip materials were pre-treated in 0.002 M solution of 8-hydroxyquinoline, and kept at 4ºC for 3 hours before fixation. The preparations were stained using 2% acetocarmine, and well spread chromosome were photomicrographed using Leica DM 450 digital camera under Leica DM 2500 microscope.

Statistical analysis

The following parameters were estimated in each metaphase plate to characterize the karyotype numerically. Long arm (LA), short arm (SA), total length (TL) [LA+SA], arm ratio (AR) [LA/SA] and karyotypic formula. Karyotype asymmetry was estimated using the total form percentage (TF %) [(ΣSA/ΣTL) ×100]. Karyotypic characters have been determined using the symmetry classes of Stebbins (1971). Karyotype formula was determined from chromosome morphology based on centromere position in accordance with the classification of

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Levan *et al.* (1964) and Abraham & Prasad (1983). Karyograms were drawn based on the length of chromosome size.

RESULTS

Mitotic observations using root tip cells of the seven variants of *Clitoria ternatea* were documented. The detailed features in each variant were given below.

Single blue: There were 16 chromosomes (Fig. 1A) ranged in length from 1.24 to 4.42 µm (Fig. 2A). Total chromosome length was 48.31 µm. Absolute chromosome length and TF% were 3.02 and 38.13 µm respectively. The karyotype consisted of 5 pairs of nearly median chromosomes and 3 pairs of nearly sub median chromosomes (Table 1). The karyotype belonged to 3B category.

Single light blue: There were 16 chromosomes (Fig.1B) ranged in length from 1.49 to 4.63 µm (Fig. 2B). Total chromosome length was 46.47 µm. Absolute chromosome length and TF% were 2.90 and 34.76 µm respectively. The karyotype consisted of 4 pairs of nearly median chromosomes and 4 pairs of nearly sub median chromosomes (Table 1). The karyotype belonged to 3B category.

Single white: There were 16 chromosomes (Fig. 1C) ranged in length from 2.17 to 4.98 µm (Fig. 2C). Total chromosome length was 54.75 µm. Absolute chromosome length and TF% were 3.42 and 35.85 µm respectively. The karyotype consisted of 5 pairs of nearly median chromosomes and 3 pairs of nearly sub median chromosomes (Table 1). The karyotype belonged to 2B category.

Single violet: There were 16 chromosomes (Fig. 1D) ranged in length from 2.34 to 5.19 µm (Fig. 2D). Total chromosome length was 57.95 µm. Absolute chromosome length and TF% were 3.62 and 34.16 µm respectively. The karyotype consisted of 2 pairs of nearly median chromosomes and 6 pairs of nearly sub median chromosomes (Table 1). The karyotype belonged to 2B category.

Double blue: There were 16 chromosomes (Fig. 1E) ranged in length from 1.61 to 4.54 µm (Fig. 2E). Total chromosome length was 46.85 µm. Absolute chromosome length and TF% were 2.93 and 34.84 µm respectively. The karyotype consisted of 4 pairs of nearly median chromosomes and 4 pairs of nearly sub median chromosomes (Table 1). The karyotype belonged to 2B category.

Double pink: There were 16 chromosomes (Fig.1F) ranged in length from 2.20 to 5.58 µm (Fig. 2F). Total chromosome length was 59.05 µm. Absolute chromosome length and TF% were 3.69 and 32.26 µm respectively. The karyotype consisted of 7 pairs of nearly median chromosomes and 1 pair of nearly sub median chromosomes (Table 1). The karyotype belonged to 2B category.

Double white: There were 16 chromosomes (Fig. 1G) ranged in length from 2.19 to 4.46 µm (Fig. 2G). Total chromosome length was 55.02 µm. Absolute chromosome length and TF% were 3.44 and 36.72 µm respectively. The karyotype consisted of 5 pairs of nearly median chromosomes and 3 pairs of nearly sub median chromosomes (Table 1). The karyotype belonged to 2B category.

Table 1. Comparison of the chromosomes of seven variants of *Clitoria ternatea* L.

Variants	Symmetry class	Karyotype formula	Chromosome length range (µm)	Total chromosome length (µm)	Absolute chromosome length (µm)	TF%
Single blue	3B	5nm+3nsm(-)	1.24–4.42	48.31	3.02	38.13
Single light blue	3B	4nm+4nsm(-)	1.49–4.63	46.47	2.90	34.76
Single white	2B	4nm+3nsm(-)	2.17–4.98	54.75	3.42	35.85
Single violet	2B	2nm+6nsm(-)	2.34–5.19	57.95	3.62	34.16
Double blue	2B	4nm+4nsm(-)	1.61–4.54	46.85	3.02	34.84
Double pink	2B	7nm+1nsm(-)	2.20–5.58	59.05	3.69	32.26
Double white	2B	5nm+3nsm(-)	2.19–4.66	55.02	3.44	36.72

Note: nm- Nealy median; nsm- Nearly sub median; µm- Micro meter; TF- Total form percentage.

Comparison of karyomorphology

Though the diploid somatic chromosome number was 16 (2n=16) in all the 7 variants of *Clitoria ternatea*, they showed in karyotype morphology. Details of karyotype analysis of the metaphase stage were summarized in table 1 and the idiograms in figure 2. Karyotype analysis indicated that chromosome length ranged from 1.24 to 5.58 µm and the 7 variants consist of both the nearly median (nm) and nearly sub median (nsm) type chromosomes in varying number. All the variants exhibited variation with respect to total chromosome length,

absolute chromosome length and TF% (Table 1). Total chromosome length and absolute chromosome length were higher in 'double pink' (59.05 µm & 3.69 µm respectively) and lower in 'single light blue' (46.47 µm & 2.90 µm respectively). In general, karyotypes of all the variants fell in the 2B category except that in 'single light blue' and 'single blue' which come under the 3B category (Table 1). The analysis of TF% showed that 'double pink' possess lowest value and represented as the most asymmetrical karyotype whereas 'single blue' possessed highest value and represented as the most symmetrical karyotype (Table 1).

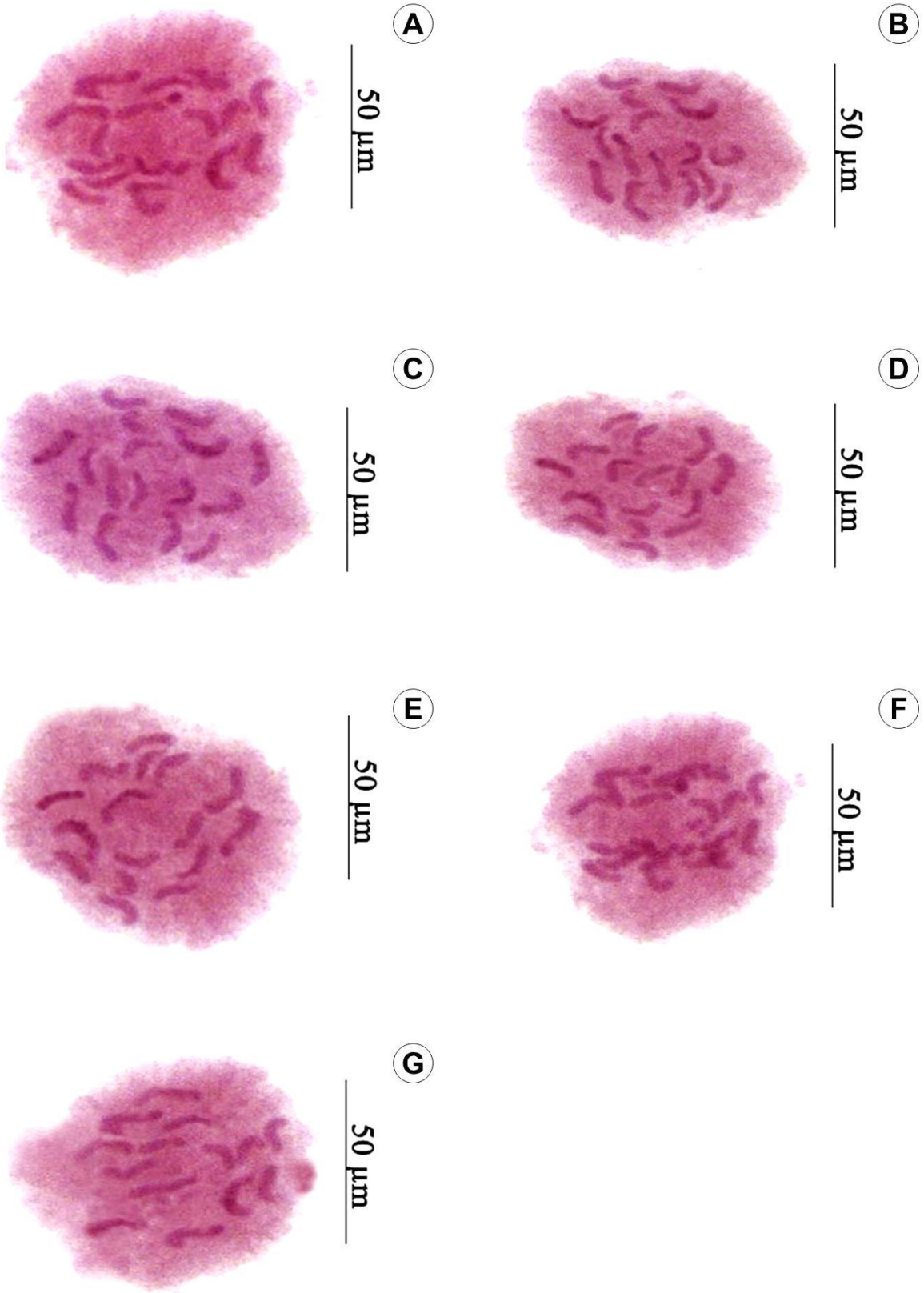

Figure 1. Metaphase stage of root tip cells of *Clitoria ternatea* L.: **A**, Single blue; **B**, Single light blue; **C**, Single white; **D**, Single violet; **E**, Double blue; **F**, Double pink; **G**, Double white.

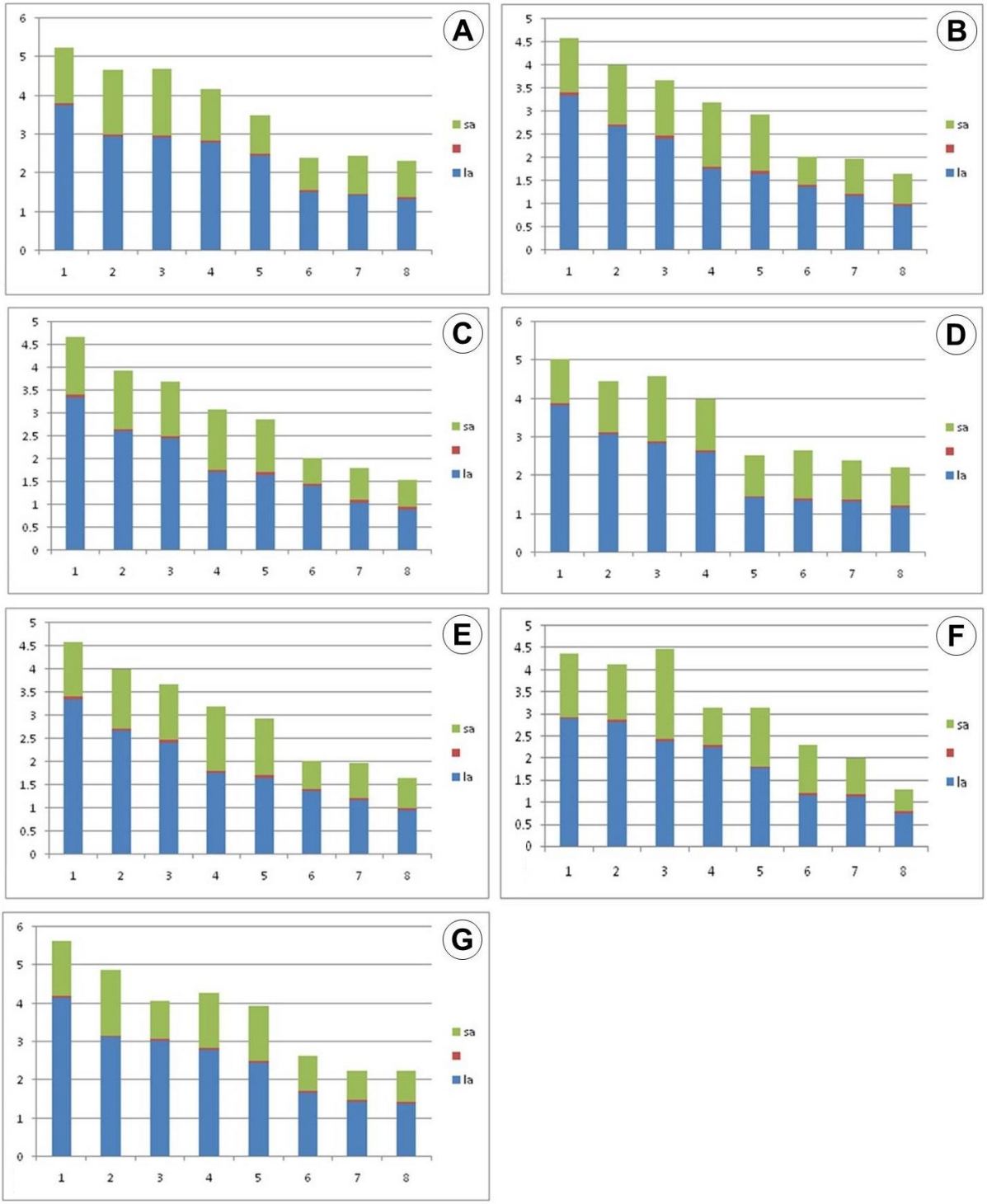

Figure 2. Idiograms: **A**, Single blue; **B**, Single light blue; **C**, Single white; **D**, Single violet; **E**, Double blue; **F**, Double pink; **G**, Double white.

DISCUSSION

In the present investigation, no alteration in the chromosome number has been found and mitotic metaphase revealed that the haploid and diploid chromosome number were 8 and 16 respectively in all the 7 variants of *Clitoria ternatea*. It perfectly matches by the reports of earlier workers (Fedorov 1974, Sanjappa & Dasgupta 1977, George & George 1978, Lackey 1980, Srivastav & Raina 1980, Mathew & Ninan 1989). It appears therefore that the basic chromosome number of *Clitoria ternatea* is 8. However, Bir & Kumari (1979), reported that n=7 and 2n=14 in specimens of this species from Punjab, whereas Jacob (1940) recorded the basic chromosome number in this species as 4. This suggests the probable existence of other basic numbers in this species. Singh & Gupta (1978), Srivastav & Raina (1980) and Gandhi & Patil (1994) observed that the chromosome variation can occur in this species during different climatic conditions.

If the length between the smallest chromosomes to the largest chromosome ranges between 0.0–0.5 µm, that specimen belong to 2B category whereas if it is between 0.51–0.99 µm, it belong to 3B category (Levan *et al.* 1964). Karyomorphological studies of the 7 variants of *Clitoria ternatea* revealed that, most of them belong to 2B category except 'single blue and 'light blue' which come under 3B category. The finding is similar to the observations in four variants made by Stebbins (1971) and Mathew & Ninan (1989). Each variant however, has its own distinctiveness with respect to the finer details of the morphology of their chromosomes as shown in table 1 'Double white' and 'blue' consist of 5 nearly median and 3 nearly sub median chromosomes whereas 4 each of both the category were observed in 'single' blue and 'light blue' (Table 1). 'Double pink' possess 7 nearly median and 1 nearly sub median chromosomes which makes it quite distinct from others. Similarly, 2 nearly median and 6 nearly sub median chromosomes in 'single violet' was also a distinct condition which supplements to the uniqueness of the 'single violet' variant. Among the 7 variants studied, a sub-terminal pair of chromosomes was observed only in 'single white' whereas all the others were having median and sub-median chromosomes (Table 1). Previous report by Mathew & Ninan (1989) on four variants mentioned similar result which perfectly coincides the present study. Total chromosome length and absolute chromosome length were maximum in 'double pink' (59.05 & 3.69 µm respectively) followed by 'single violet' (57.95 & 3.62 µm respectively), 'double white' (55.02 & 3.44 µm respectively), 'single white' (54.75 & 3.42 µm respectively), 'double blue' (48.31 & 3.02 µm respectively), 'single blue' (46.85 & 2.93 µm respectively) and 'light blue' (46.47 & 2.90 µm respectively) (Table 1).

Karyotype asymmetry was estimated using the Total Form percentage (TF%), the value obtained by dividing total short arm length to the total chromosome length. Besides the differences mentioned above, slight variations were noticed in TF% among the 7 variants. 'Double pink' with maximum total chromosome length has minimum TF% value. Sharma *et al*. (1999) pointed out that, plant with maximum total chromosome length and minimum TF% can be considered as advanced type. This statement indicated that, the 'double' pink is more advanced than the other variants.

Thombre & Atale (1974) pointed that, 'single' variants are wild types and the 'doubles' are of mutant origin from the singles. George & George (1978) also agreed that alteration in floral morphology from 'single' to 'double' is due to spontaneous mutation. The present karyomorphological studies thus strongly support the mutant origin of 'double' variants from 'singles'.

CONCLUSION

Comparison of karyomorphological features of the seven variants revealed that the 'single forms' are cytologically allied to their respective 'double' forms. 'Single' and 'double white', 'single violet' and 'double pink' and 'single' and 'double blue' were resembling each other. The 'light blue' indicates its cytological affinity with the other two blue forms.

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