



CYTOTAXONOMY OF FOUR FLORAL VARIANTS OF *IMPATIENS BALSAMINA* L.

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ABSTRACT

Karyomorphological characters were investigated in four floral variants of *Impatiens balsamina* L. viz. white, pink, violet and red for proper characterization and inferring relationships among them. All four floral variants of *I. balsamina* were found to possess $2n = 14$ chromosomes, and 'Simple Chromocenter Type' of interphase nuclei was observed in all variants with many small heterochromatin blocks after orcein staining. 'Continuous Type' of prophase chromosomes was found in red variant, whereas both 'Continuous and Gradient Type' were found in other three variants. The red variant could easily be distinguished from the remaining three variants by total chromosome length, centromeric formula, average chromosome length, total form value, and karyotype asymmetry plus symmetry index. Based on karyomorphology, the white, pink and violet variants of *I. balsamina* were found very closely related and constituted with similar genome, whereas red variant was found to possess slightly modified genome and distinct from other three floral variants.

Key words: Cytotaxonomy, Karyomorphology, Asymmetric indices, *Impatiens balsamina* L.

INTRODUCTION

Impatiens balsamina L., commonly known as rose balsam or garden balsam, belonging to the family Balsaminaceae is distributed in North America, Africa, Asia and Europe (Willis 1973). This species is commonly used as ornamental plants due to possessing attractive flower colors such as white, pink, violet and red. Presence of three anthocyanidins viz. pelargonidin, peonidin and malvidin might be responsible for different pigmentations in this species. However, there is no available report on correlation between these pigmentations with genetic structure (Meenu *et al.* 2015). Traditionally, the whole plant is used in the treatment of thorn or glass-puncture wounds, abscesses, carbuncles, rheumatism, fractures, dysentery, superficial infections, tumor and puerperal pain (Kang and Moon 1992, Yang *et al.* 2001, Sakunphueak and Panichayupakaranant 2012). The dried plant is also used either in the form of tea (boiled with water) for treatment of systemic bacterial and fungal infections or used externally on the skin or nails in a plaster form to treat local infections (Lee and Lee 2010, Kim *et al.* 2011). *Impatiens* species are extremely variable and difficult to

identify and characterize morphologically due to similar characters. When morphological approaches fail to identify the species or varieties in that case some other approaches viz. cytological studies offer powerful tool to overcome this constraint and proper identity of taxa (Pico and Dematteis 2014, Kaur 2018). Since four floral variants are evident in *I. balsamina* and these variants cannot be distinguished easily without flower color it is necessary to investigate and identify these floral variants employing some other tools, such as cytology and cytogenetics, as chromosome number and additional karyotypic features provide more precise information about a species.

The importance of cytological studies is many-fold, and even simple chromosome counts quite often add grander value for recognizing the distinctness of a species (Love and Love 1966). Karyosystematics is well established as one of the most important parameter in determination of the genetic relationship and degree of divergence among species or populations (Guerra 2008). The nature of interphase nuclei,

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prophase and metaphase chromosomes, such as different stages of mitotic cell division also share useful information about the genome of that species. The karyomorphological analysis enables to characterize even different forms and varieties of a species (Dash *et al.* 2017).

Previous studies revealed that *Impatiens balsamina* possess different chromosome numbers (Sharma 1970, Govindarajan and Subramanian 1986, Huang *et al.* 1989). Various reports regarding somatic chromosome number indicated the presence of different cytotypes of this species which might be originated through different types of chromosome aberrations, thus this species gains interest for cytogenetical study. Though several authors paid attention in counting the chromosome number for *Impatiens balsamina* (Sharma 1970, Chatterjee and Sharma 1970, Raghuvanshi and Mahajan 1982, Iwatsubo and Naruhashi 1999, Momtaz *et al.* 2007), and a cytotaxonomic study on five species of *Impatiens* [e.g. *I. javensis* Steud., *I. platyptala* Lindl., *I. radicans* Zoll., *I. kinabaluensis* S. Akiyama & H. Ohba and *I. choneceras* Hassk. (wrongly spelled as 'chonoceras')] was carried out from Java and Borneo (Ikeda *et al.* 2005), no such study was made on *Impatiens balsamina* so far. Since four floral variants of *Impatiens balsamina*, viz. white, pink, violet and red are found in nature and there is complexity among these variants, it is important to examine these floral variants using cytological investigation. Therefore, the present study aimed at investigating different cytological characters for proper identification and characterization of the floral variants of *Impatiens balsamina* available in Bangladesh, and to evaluate these characters as taxonomic tool for inferring relationships among these variants.

MATERIALS AND METHODS

Four floral variants of *Impatiens balsamina* L., viz. white, pink, violet and red variants were collected and maintained in the Botanic garden, Department of Botany, University of Dhaka,

Bangladesh. The plant specimens were critically studied morphologically and identified with special reference to taxonomic values (Ahmed *et al.* 2008).

For cytological study, healthy roots were collected and pre-treated with 8-hydroxyquinoline (0.002%) for 1 h at 18 °C followed by 15 min fixation in 45% acetic acid at 4 °C and preserved in 70% alcohol for further use. They were then hydrolyzed in a mixture of 1N HCl and 45% acetic acid (2:1) at 65 °C for 2 min. The root tips were stained and squashed in 1% aceto-orcein for 2 h (Sultana *et al.* 2011). Afterwards, these slides were observed under a compound microscope (Nikon eclipse 100). The well spread and clear mitotic metaphase chromosomes were taken to analyze different karyotypic parameters. Centromeric formula was determined based on the position of centromere and the number of chromosomes (Levan *et al.* 1964). Different karyo-morphological parameters, asymmetry and symmetry indexes were calculated, such as range of chromosomal length, total chromosome length, average chromosome length, total form (TF value) percent (Huziwara 1962), mean centromeric asymmetry (Peruzzi and Eroglu 2013), karyotype asymmetry index (Arano 1963), karyotype symmetry index (Greilhuber and Speta 1976), intrachromosomal and interchromosomal asymmetry index (Zarco 1986), degree of asymmetry of karyotypes (Watanabe *et al.* 1999) and karyotypes category (Stebbins 1971).

RESULTS

TAXONOMIC EVALUATION

Impatiens balsamina L., Sp., Pl. : 938 (1753).
Synonym: *Impatiens cornuta* L. (1753) (Fig. 1).

An erect, annual, succulent herb, pubescent or glabrate, attaining up to 100 cm tall. Leaves are usually simple, alternate, sometimes lowest ones opposite, up to 13 cm long; leaf blade lanceolate or elliptic, both surfaces glabrous or sparsely pubescent, lateral veins 4-6 pairs, margin serrate.

Inflorescences 1-3 flowered. Flowers are single cup-shaped or double camellia-like shaped, white, pink, violet and red in color, axillary, pubescent; pedicels up to 2 cm long, densely pubescent. Stamens 5, with linear filaments and ovoid anthers. Ovary fusiform, densely pubescent. Fruits are capsules, ellipsoid and narrowed at both ends, tomentose, with rigid yellow bristles, 1-2 cm long. Seeds are globose, tubercled and 0.4-0.6 cm in diameter. *Flowering and fruiting period*: March to October.

A comparative morphological account of taxonomic importance in four floral variants of *Impatiens balsamina* is summarized in Table 1.

CYTOLOGICAL INVESTIGATION

Somatic chromosome number and karyomorphology

In the present study, four floral variants, *i.e.*

white, pink, violet and red variants of *Impatiens balsamina* were found to possess $2n=14$ chromosomes (Fig. 2). The centromeric formulae of white, pink and violet variants were $10m+4sm$, whereas $8m+6sm$ was observed in the red variant (Fig. 3). The individual chromosomal length ranged from 3.10-4.62 μm in white variant, 2.73-5.17 μm in pink, 2.87-5.28 μm in violet, and 3.49-5.65 μm in red variant. The total chromosome length was found nearly similar in white (54.25 ± 2.09 μm), pink (54.82 ± 3.14 μm) and violet variants (56.03 ± 3.32 μm), while larger in red variant (64.25 ± 1.56 μm).

The average chromosomal length was found to be bigger in red variant than those of white, pink and violet variants. Karyotype asymmetry was higher in red variant (59.42%) and relatively lower in white (56.72%), pink (55.62%) and violet variants (55.82%). In contrast, karyotype

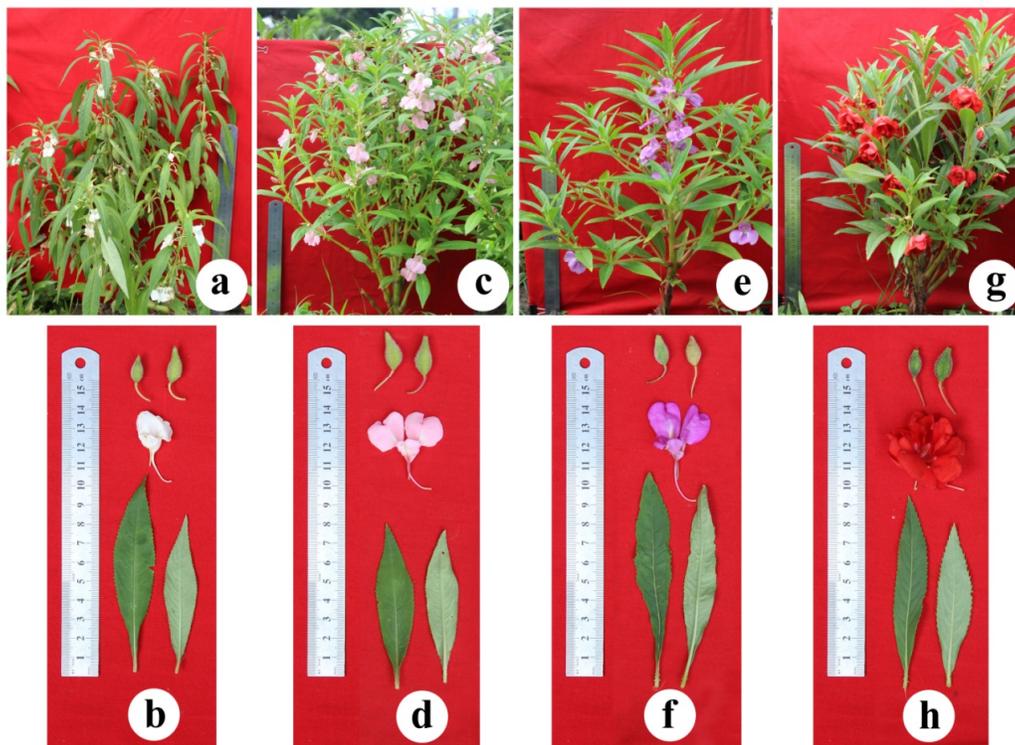


Fig. 1. Plant morphology of four floral variants of *Impatiens balsamina* L. (a) Habit of white variant; (b) Leaves, flower and pods of white variant; (c) Habit of pink variant; (d) Leaves, flower and pods of pink variant; (e) Habit of violet variant; (f) Leaves, flower and pods of violet variant; (g) Habit of red variant; (h) Leaves, flower and pods of red variant.

symmetry was found lower in red variant (68.28%) and relatively higher in white (76.31%), pink (79.80%) and violet variants (79.15%). The mean centromeric asymmetry in white, pink, violet and red variants was observed as 12.36, 11.15, 11.43 and 18.06, respectively. Total form (TF) value among these types ranged from 40.58% to 44.38%. The intrachromosomal asymmetry index and interchromosomal asymmetry

index were found same in pink variant, however, the former was higher than the latter in white and red variants, but lower in violet variant. In the red floral variant of *I. balsamina*, the degree of asymmetry of karyotype was higher than the remaining three variants (Table 2). No satellite was observed in these four floral variants. Karyotypes of all these four variants could be categorized as '1A'.

Table 1. Comparative morphological account of taxonomic importance among four floral variants of *Impatiens balsamina* L.

Characters	White	Pink	Violet	Red
Plant height (cm)	50-75	60-80	60-85	70-90
Leaf size (cm)	6-12×1.5-2.5	6-11.5×1.5-2	7-15×1.5-2.5	9-16×2-3
Leaf shape*	Lanceolate	Lanceolate	Lanceolate	Lanceolate to elliptic
Stem color	Green	Green, light reddish stripe present at the base	Green, reddish stripe present at the base	Green, reddish stripe present at the base
Stem nature	Erect, sparsely branched	Erect, sparsely branched	Erect, sparsely branched	Erect, densely branched
Flower shape*	Single cup-shaped	Single cup-shaped	Single cup-shaped	Double camellia-like shaped
Number of petals*	5	5	5	10
Petal size (mm)	5-18×10-25	5-20×9-26	5-20×10-25	20-22×20-25
Inflorescence*	Solitary	Solitary	Solitary	Fascicles in 1-3 flowers
Fruit color*	Light green	Light green	Light green	Deep green in young stage, light green at maturity
Seed size (mm)	2-3×1.5-2	2-3×1.5-2	2-3×1.5-2	3-4.5×1.5-2.5

* Taxonomic importance

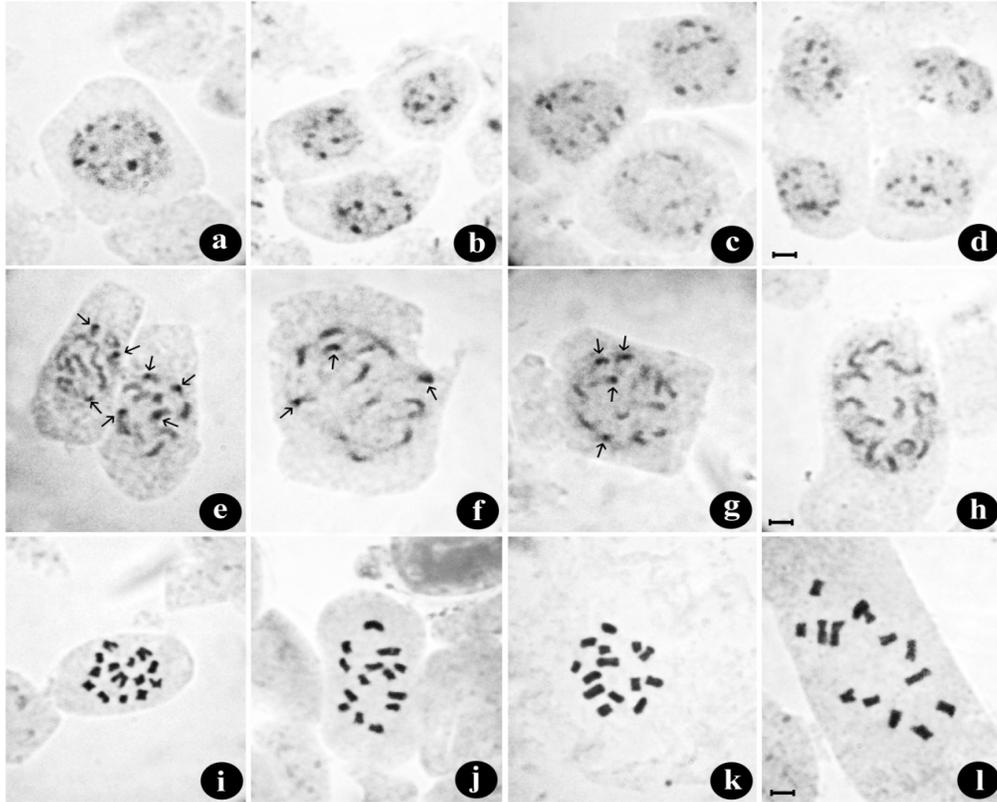


Fig. 2. Orcein-stained interphase, prophase and metaphase stages of mitotic cell division of four floral variants of *Impatiens balsamina* L. (a) Interphase nuclei of white variant; (b) Interphase nuclei of pink variant; (c) Interphase nuclei of violet variant; (d) Interphase nuclei of red variant; (e) Prophase chromosomes of white variant; (f) Prophase chromosomes of pink variant; (g) Prophase chromosomes of violet variant; (h) Prophase chromosomes of red variant; (i) Metaphase chromosomes of white variant; (j) Metaphase chromosomes of pink variant; (k) Metaphase chromosomes of violet variant; (l) Metaphase chromosomes of red variant (Arrow indicates gradient type). Bar = 5 μ m.

Nature of heterochromatin distribution

The present study revealed the presence of seven to fourteen prominent darkly stained heterochromatic blocks in the interphase nuclei of four variants of *I. balsamina* and they were scatteredly distributed (Fig. 2a-d). The prophase chromosomes of red variant were stained homogeneously along the entire length of chromosomes, and were considered as 'Continuous Type' (Fig. 2h).

On the other hand, co-existence of continuous and gradient (darker in one end and gradually faint to the other end) types of prophase chromosomes were observed in white, pink and violet variants (Fig. 2e-g). The findings indicated that the facultative heterochromatin were aggregated in the interphase nuclei and then somehow were homogeneously or gradually distributed in the prophase chromosomes.

Table 2. Comparison among four floral variants of *Impatiens balsamina* L. based on karyomorphological analyses

Karyotype parameters	White	Pink	Violet	Red
Chromosome number	2n=14	2n=14	2n=14	2n=14
Centromeric formula	10m+4sm	10m+4sm	10m+4sm	8m+6sm
Mean centromeric asymmetry	12.36	11.15	11.43	18.06
Range of chromosomal length (μm)	3.10–4.62	2.73–5.17	2.87–5.28	3.49–5.65
Total chromosome length (μm)	54.25 \pm 2.09	54.82 \pm 3.14	56.03 \pm 3.32	64.25 \pm 1.56
Average chromosome length (μm)	3.87	3.92	4.00	4.59
Total Form (TF) value (%)	43.28	44.38	44.18	40.58
Karyotype asymmetry index (%)	56.72	55.62	55.82	59.42
Karyotype symmetry index (%)	76.31	79.80	79.15	68.28
Intrachromosomal asymmetry index	0.21	0.19	0.19	0.30
Interchromosomal asymmetry index	0.13	0.19	0.22	0.17
Degree of asymmetry of karyotypes	0.12	0.11	0.11	0.18
Karyotype category	1A	1A	1A	1A

m = metacentric chromosome, sm = sub-metacentric chromosome

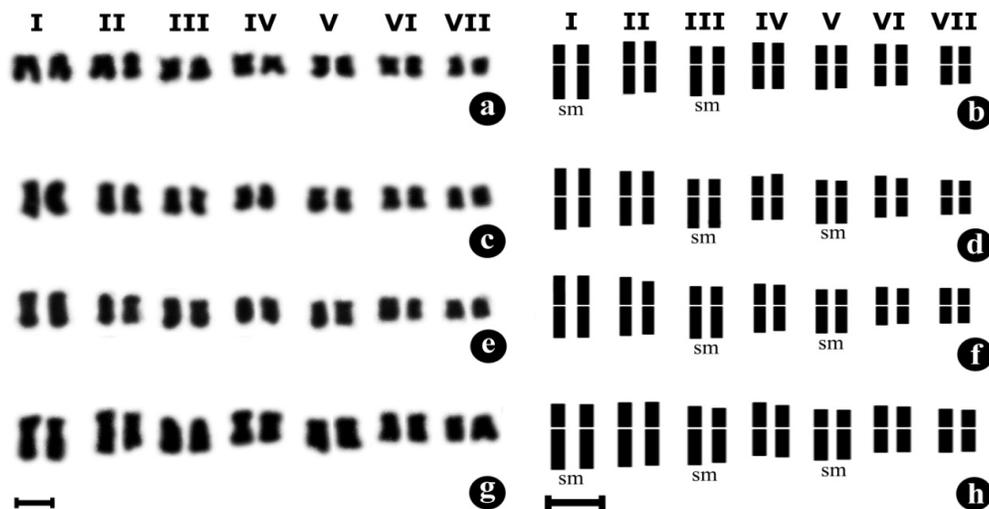


Fig. 3. Karyotype and ideogram made from metaphase chromosomes of four floral variants of *Impatiens balsamina* L. (a) Karyotype of white variant; (b) Idiogram of white variant; (c) Karyotype of pink variant; (d) Idiogram of pink variant; (e) Karyotype of violet variant; (f) Idiogram of violet variant; (g) Karyotype of red variant; (h) Idiogram of red variant. sm = sub-metacentric chromosome. Bar = 5 μm .

DISCUSSION

In the present study, we investigated karyomorphological features among four floral variants of *I. balsamina* and all the variants were found to possess $2n = 14$ chromosomes with basic chromosome number $x = 7$ (Fig. 2i-l, Table 2). The results were found to be consistent with earlier studies, where $2n = 14$ chromosomes were reported (Sharma 1970, Chatterjee and Sharma 1970, Raghuvanshi and Mahajan 1982, Govindarajan and Subramanian 1986, Huang *et al.* 1989, Iwatsubo and Naruhashi 1999, Momtaz *et al.* 2007). However, some other studies showed different somatic chromosome number of $2n = 12, 18, 20$ and 24 in *I. balsamina* (Sharma 1970, Chatterjee and Sharma 1970). If the basic chromosome number of *I. balsamina* is $x = 6$, then $2n = 2x = 12$, $2n = 3x = 18$, $2n = 4x = 24$, which might be regarded as diploid, triploid and tetraploid, respectively. On the other hand, $2n = 20$ could be originated through aneuploidy.

The staining properties of interphase nuclei and prophase chromosomes sometimes provide additional karyomorphological features that help to characterize different specimens (Tanaka 1971). Darkly stained heterochromatic blocks were observed in the interphase nuclei of all floral variants of *I. balsamina* (Fig. 2a-d), and this type of staining of interphase nuclei could be regarded as 'Simple Chromocenter Type' (Tanaka 1971). Generally, the localized heterochromatins, as observed in the interphase nuclei are not homogeneously distributed in the prophase chromosomes rather occupy different locations. The present findings did not support the usual regulation regarding distribution of heterochromatin in prophase chromosomes. Presence of facultative heterochromatin might be one of the reasons for this disagreement. Constitutive heterochromatin block along with facultative heterochromatin were found in white, pink and violet variants, whereas most of the heterochromatin were facultative in the red variant. Therefore, four floral variants of

Impatiens balsamina could be categorized on the basis of these characters.

Although all floral variants of *I. balsamina* were found to possess $2n = 14$ chromosomes, they differed in many other karyomorphological parameters (Table 2). The centromeric formulae of white, pink and violet variants were $10m+4sm$, whereas it was found to be $8m+6sm$ in the red variant. Variation in chromosomal length in these four variants displayed that the largest chromosome was almost double than that of the smallest chromosomes. Therefore, a gradual decrease of chromosomal length was observed in these four *Impatiens balsamina* variants (Fig. 3). The total chromosome length was nearly similar in white, pink and violet variants, while larger in the red variant. The average chromosomal length and karyotype asymmetry was found bigger in red variant than those of white, pink and violet variants. In contrast, karyotype symmetry was lower in the red variant and relatively higher in white, pink and violet variants (Table 2). The red variant also showed significant higher values in intrachromosomal asymmetry index and degree of asymmetry of karyotypes than the remaining three variants. Results obtained from the present investigation revealed that the white, pink and violet variants shared similar genome indicating a very close relationship cytotaxonomically among them, whereas the red floral variant is distantly related from other variants with cytogenetical dissimilarity.

Though floral variants of *I. balsamina* employed in this study showed similar morphological characters, but they differed from one another in color and shape of the flowers, inflorescence and pod color characters. Among the studied materials, double camellia-like shaped flower was found in red variant, whereas single cup-shaped flowers were evident in rest three variants. In addition, the pod color was darker in red variant than in other three variants (Fig. 1, Table 1).

According to karyotype analyses, red floral variant showed different heterochromatic condensation nature, distinct centromeric formulae, higher total chromosomal length, higher asymmetry index and lower symmetry index than other three variants (Figs. 2-3, Table 2). Karyotype analyses revealed that the white, pink and violet variants of *I. balsamina* are closely allied and constituted with similar genome. In contrast, the red variant was found to possess slightly modified genome and distinct from the remaining three variants. Differences in various cytological parameters in red floral variant might be correlated with the formation of distinct flower shape (double camellia-like shape) from other floral variants (single cup shape) of *Impatiens balsamina*. On the basis of morphological characters of taxonomic importance and cytogenetical variation all the four *I. balsamina* variants could easily be classified into two groups, one with single cup shaped flower and other with double camellia-like shaped flower indicating the significance of cytotaxonomical studies of *I. balsamina* L.

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