

Karyomorphological Analysis of Different Varieties of *Tabernaemontana coronaria*

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Summary *Tabernaemontana coronaria* of Apocynaceae is an economically important medicinal plant, distributed throughout the tropics of the world. The different alkaloids produced by this species are effective against various disorders like abdominal tumors, asthma, diarrhoea, epilepsy, eye infections and fever. Morphological characters including internode length, petiole length, leaf area and leaf index varied between these varieties. Cytological analysis revealed three diploid varieties with $2n=22$ chromosomes (Wild type *T. coronaria*, *T. coronaria* var. Variegata, *T. coronaria* var. Dwarf) and a triploid variety with $3n=33$ chromosomes (*T. coronaria* var. Flore-pleno). A variation was recorded in total chromosome length and volume among the diploid varieties of *T. coronaria*. Most of the centromeric chromosomes were either median or median region. The present study indicated that cryptic structural changes of chromosomes might be responsible in the evolution of different varieties of *T. coronaria* except the Flore-pleno variety. Moreover, differential condensation of chromosomes attributing to variable chromosome length and volume has been suggested.

Key words Apocynaceae, Chromosome, Karyotype, Morphology, *Tabernaemontana*.

Tabernaemontana coronaria (syn. *T. divaricata*, *Ervatamia coronaria*), commonly known as Tagor, belongs to the family Apocynaceae, Plumeroidae subfamily and Tabernmontanae tribe. Approximately 100 species of *Tabernaemontana* are widely distributed in tropical countries of the world including Brazil, Egypt, India, Sri Lanka, Vietnam, Malaysia and Thailand. In India, it occurs in the upper Gangetic plain of West Bengal, Khasi Hills, Assam and Hills of Vishakapatnam. A number of varieties/cultivars of *Tabernaemontana coronaria* are available. It is cultivated as an ornamental plant and grows wild in hedges and shady forests.

Wild type *Tabernaemontana coronaria* is evergreen, large, shrub, often attaining height of a small tree to 8-m high and is dichotomously branched with grey bark having lenticels. The leaves are elliptic or elliptic oblong, acuminate, and narrowed into petioles which are shining green in colour. The flowers are white and arranged in cyme inflorescence with flowers having single whorl of fan-shaped petals. *T. coronaria* var. Variegata is a medium sized plant and leaves are variegated with single flowers. *T. coronaria* var. Dwarf is a dwarf and compact plant with single flowers having flat and round petals. *T. coronaria* var. Flore-pleno is a comparatively large plant with flowers having multiple whorls of thick petals (Bose *et al.* 2011).

It is an important medicinal plant with anti-ulcer, anti-bacterial, anti-inflammatory properties

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and is also used as an anti-helminthic, anti-hypertensive, diuretic, hair growth promoter, purgative, (Suffredini *et al.* 2002, Neimkhum *et al.* 2010, Ashikur *et al.* 2011, Khan 2011), aphrodisiac, remedy against poisons and tonic for brain, liver and spleen (Nadkarni and Nadkarni 1996). This plant is important as a natural synthesizer of different alkaloids including indole alkaloids. Phytochemical studies on various parts of this plant reveal that it contains at least 66 indole alkaloids, as well as non-alkaloid constituents like enzymes, flavonoids, hydrocarbons, phenolic acids, phenyl propanoids, steroids and terpenoids (Pratchayasakul *et al.* 2008). The extracts of the root, leaves, stems and even flowers of this plant show anti-tumor activity (Khan 2011). Growing evidences suggest that this plant has medicinal benefits and its extract could possibly be used as pharmacological intervention in various diseases. *Tabernaemontana* is one of the genus that is used in Ayurveda, Chinese and Thai traditional medicine for the treatment of fever, pain and dysentery (Van Beek *et al.* 1984, Boonyaratanakornkit and Supawita 2005).

The chromosome complement of this species has been found to be homogeneous with $2n=22$ and $n=11$ (Roy Tapadar and Sen 1960, Roy Tapadar 1964, Dutta and Maity 1972) except one report by Van der Laan and Arends (1985), where they found $2n=33$ chromosomes. The different species of this genus contain $2n=22$ chromosomes revealing basic number to be $x=11$ (Van der Laan and Arends 1985). Chromosome analysis of *T. coronaria* was carried out earlier which involved meiotic behavior in pollen mother cells (Raghuvanshi and Chauhan 1969, 1970, Chauhan and Raghuvanshi 1976).

However, no reports are so far available on detailed analysis on morphological characters and karyotypes in varieties of *T. coronaria*. In view of scanty records, the present investigation was undertaken to study the genomic diversity of different varieties of *T. coronaria*.

Materials and methods

Materials

In the present study, four varieties of *Tabernaemontana coronaria* of Apocynaceae were selected for morphological and cytological investigations. These are:

- i) *Tabernaemontana coronaria* (Jacq.) Willd (Wild type)
- ii) *Tabernaemontana coronaria* var. *Variegata*
- iii) *Tabernaemontana coronaria* var. *Dwarf*
- iv) *Tabernaemontana coronaria* var. *Flore-pleno*

Plants were collected from the local nursery of Howrah district of West Bengal and grown in the experimental garden of the Department of Botany, University of Calcutta.

Methods

Morphological study

For a detailed morphological study on *Tabernaemontana*, the different morphological characters were considered including inter-node length, petiole length, leaf area and leaf index. For each plant, 20 readings were taken at random, and mean values as well as standard errors were calculated. Leaf area was calculated using graph paper. Leaf index was obtained by dividing the length of the leaf by its breadth (Mukhopadhyay and Sharma 1987).

Cytological study

Somatic chromosomes were studied from root tip cells. The time for maximum meristematic activity was found to be between 10:40 a.m. to 12:30 p.m. Fresh healthy root tips were collected and washed thoroughly in running tap water. Roots were pretreated in a mixture of saturated aqueous solutions of *p*-dichlorobenzene (PDB) and 2.0mM 8-hydroxyquinoline (1:1). Pretreatment was carried out at 12°C for 5h, after an initial shock treatment at 0°C for 5min

(Mukhopadhyay and Banerjee 1989). The root tips were then fixed in chilled Carnoy's fixative (glacial acetic acid:chloroform: absolute ethanol, 1:3:6) at 12°C for 24h, followed by overnight fixation in a mixture of propionic acid: absolute ethanol (1:3). To remove dense cytoplasmic content, a treatment with either only 1N HCl or 5N HCl, or 1N HCl followed by NaCl treatment was tried and the best result was obtained when the root tips were hydrolyzed in 1N HCl for 12 min at 60°C. After thorough washing in distilled water to ensure complete removal of traces of acid, root tips were stained in 2% propionic-orcein at room temperature for 3h before squashing in 45% propionic acid.

Karyotype analysis was carried out by critically examining about 20 clear and well-scattered metaphase plates from different root tips of each plant. The chromosomes were drawn with the help of a drawing prism and were classified into different types based on their '*i*'-values (Levan *et al.* 1964). The total chromosome length was calculated by adding the whole lengths of all the chromosomes present in a complement. For determination of chromosome volume, the breadth of individual chromosome was measured from the drawn metaphase plates. Assuming the chromosome a cylinder, the volume was calculated from the formula: chromosome volume (v)= $\pi r^2 h$, where r =radius of the chromosome=breadth/2 and h =whole length of the chromosome (Mukhopadhyay and Sharma 1987). Total chromosome volume was calculated by summing up the volumes of all the chromosomes of a complement.

Results and discussion

Morphological data

Out of the morphological characters internode length, petiole length, leaf area and leaf index were taken into account for genome analysis. The petiole length ranged from 1.5 mm in the variety Dwarf to 7.3 mm in Variegata variety of this species. The internode length, on the other hand, did not vary to a large extent except in Dwarf variety (Table 1). Leaf area ranged from 0.62 to 9.03 cm² in case of small leaves, from 0.83 to 16.37 cm² in case of medium leaves, and from 1.02 to 30.50 cm² in case of large leaves. The leaf indices values did not differ remarkably between these varieties except the variety Flore-pleno which showed lower value (Table 1). Such differences in morphological characters might be under genetic control and are probably related to the environmental conditions where these plants grow for their adaptation.

Cytological data

The somatic chromosome number was found to be $2n=22$ in three varieties of *T. coronaria*, except *T. coronaria* var. Flore-pleno where it was $3n=33$ (Table 3; Fig. 1). The previous reports revealed 22 somatic chromosomes in wild type *T. coronaria* (Roy Tapadar and Sen 1960, Roy Tapadar 1964, Dutta and Maity 1972). The chromosomes are mostly medium in size in three varieties ranging between 1.45 to 4.04 μm in length. However, chromosomes of the Dwarf variety are extremely small where it ranges from 1.45 μm to only 2.12 μm (Table 3). The longest chromosome was observed in the wild type. The chromosome complements revealed a gross morphological similarity and types were described on the basis of such similarity. The different chromosome types were noted to be common between these species and classified on the basis of number and position of the constrictions (Table 2; Fig. 2). Three different types of centromeric chromosomes were observed and these were median, sub-median and median region types. On the other hand, four types of nucleolar chromosomes were present in these varieties of *T. coronaria* (Table 2; Fig. 2). The number and types of these different types of chromosomes varied between these varieties (Table 3). Karyotype formula was expressed in numerical form on the basis of type of chromosome and number of chromosomes in each type, present in the chromosome complement of a particular variety (Table 3, Fig. 1a-l). Among the diploids, the highest total chromosome

length was obtained in the wild type and the lowest was found in the variety Dwarf. The total chromosome volume was least in Dwarf variety and highest in wild type *T. coronaria* (Table 3). Among the diploids, the chromosomes were comparatively larger. The differences in total chromosome length and total chromosome volume might be attributed to differential chromosome condensation as well as association of variable levels of histone and non-histone proteins entering into chromosome composition, respectively, which are under genetic control (Mukhopadhyay and Sharma 1987).

Chromosome characteristics have shown a distinct variation between the number and types of different chromosomes among these species. The different species have graded karyotypes ranging from medium to short in size. The number of chromosomes with secondary constrictions varied

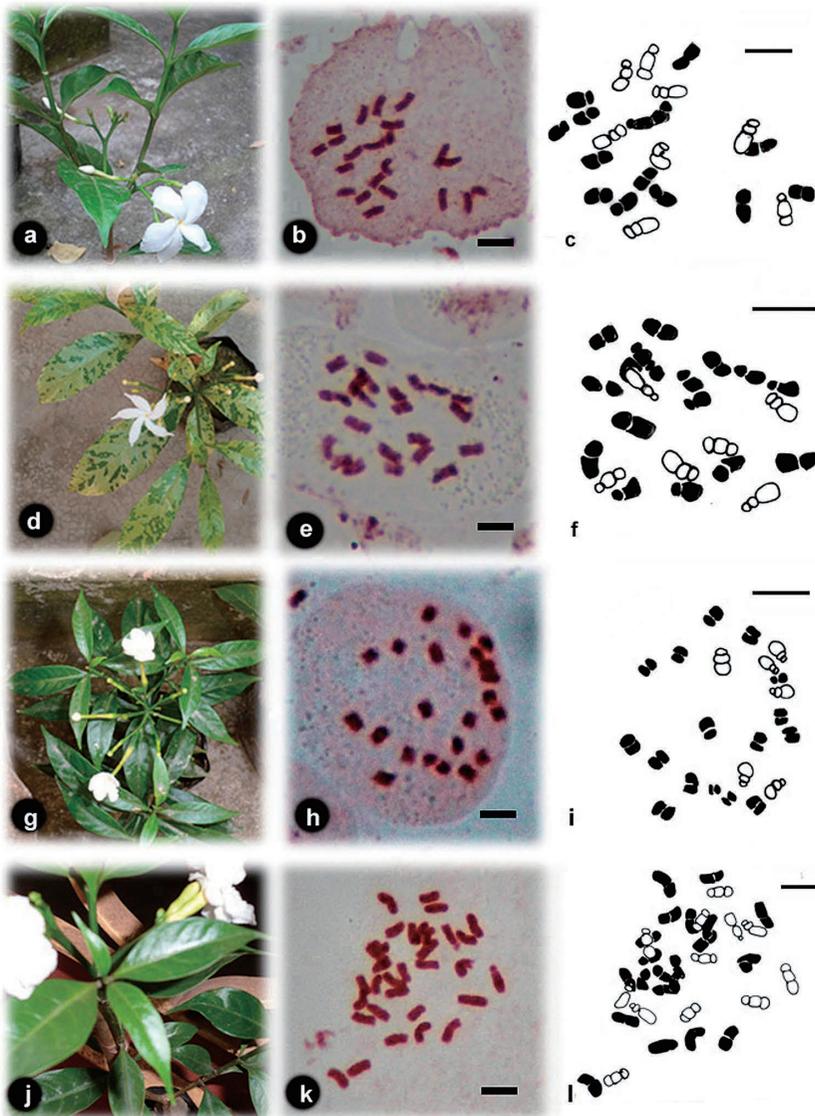


Fig. 1. Photograph of the plants, somatic metaphase plates and drawing of the same plates. (bar=5 μ m). a-c: *T. coronaria*. d-f: *T. coronaria* var. Variegata. g-i: *T. coronaria* var. Dwarf. j-l: *T. coronaria* var. Flore-pleno.

Table 1. A comparative representation of morphological features in three varieties of *Tabernaemontana coronaria*.

Species/Varieties	Internode length (cm)	Petiole length (mm)	Leaf area (cm ²)			Leaf index*		
			Small leaf	Medium leaf	Large leaf	Small leaf	Medium leaf	Large leaf
<i>T. coronaria</i> Wild type	2.7±0.8	7.0±2.0	6.33±1.70	12.67±1.10	15.60±2.60	2.80±0.71	3.08±0.31	3.43±0.10
<i>T. coronaria</i> var. Variegata	2.3±0.7	7.3±1.7	3.48±0.90	6.94±1.20	8.04±1.96	2.76±0.06	3.21±0.11	3.41±0.71
<i>T. coronaria</i> var. Dwarf	0.5±0.2	1.5±0.5	0.62±0.04	0.83±0.05	1.02±0.05	2.80±0.17	3.00±0.18	3.40±0.16
<i>T. coronaria</i> var. Flore-pleno	2.5±0.2	6.0±1.0	9.03±0.51	16.37±2.12	30.50±1.75	2.03±0.12	2.31±0.20	2.66±0.09

*Data represent $\bar{x} \pm SE$ from 20 replicates.

Table 2. Different types of chromosome present in different varieties of *Tabernaemontana coronaria*.

Chromosome type	Size (µm)	Constriction type
Type A	1.81 to 4.04	One constriction sub terminal and the other median region.
Type B	3.78	One constriction sub terminal and the other sub median.
Type C	2.39 to 3.46	Both constrictions sub median.
Type D	2.18 to 3.23	One constriction sub median and the other median.
Type E	1.81 to 3.23	Median
Type F	1.81 to 3.23	Median region
Type G	1.45 to 2.52	Sub median

Table 3. A comparative representation of different chromosomal parameters in different varieties of *Tabernaemontana coronaria*.

Species	SCN	RCL (µm)	TCL (µm)	RCV (µm ³)	TCV (µm ³)	Range of <i>i</i> value	No. of centromeric chromosome	No. of nucleolar chromosome	Karyotype formula
<i>T. coronaria</i> Wild type	22	2.42–4.04	68.58	2.73–4.56	77.48	20.00–50.00	14	8	A ₄ C ₂ D ₂ E ₂ F ₁₀ G ₂
<i>T. coronaria</i> var. Variegata	22	1.70–2.73	50.46	1.37–2.21	40.84	18.75–50.00	16	6	A ₄ C ₂ E ₄ F ₆ G ₆
<i>T. coronaria</i> var. Dwarf	22	1.45–2.18	36.98	1.32–1.98	33.64	16.66–50.00	16	6	A ₄ D ₂ E ₈ F ₄ G ₄
<i>T. coronaria</i> var. Flore-pleno	33	2.52–3.78	102.93	1.74–4.61	109.67	16.66–50.00	21	12	A ₃ B ₃ C ₆ E ₃ F ₁₅ G ₃

SCN, Somatic chromosome number; RCL, Range of chromosome length; TCL, Total chromosome length; RCV, Range of chromosome volume; TCV, Total chromosome volume.

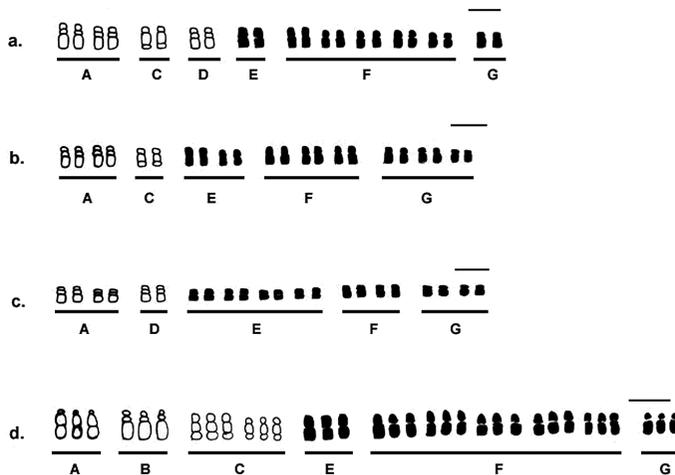


Fig. 2. a–d Karyogram of *T. coronaria*, *T. coronaria* var. Variegata, *T. coronaria* var. Dwarf, and *T. coronaria* var. Flore-pleno. (bar=5µm).

from four to six in these varieties. The increase or decrease in number of secondary chromosomes might be responsible for duplication of such chromosomes or translocation between the chromosomes with or without secondary constrictions at a very early stage of evolution (Mukhopadhyay and Sharma 1987, Mukhopadhyay and Ray 2013). Notwithstanding gross similarities, the karyotype formula is distinct for every species as they differ in minute details. Thus the importance of cryptic structural alterations of chromosomes in the evolution of different varieties of *Tabernaemontana* is indicated (Mukhopadhyay and Sharma 1987, Lahiri *et al.* 2010, Mukhopadhyay and Ray 2013). Each species shows a distinct karyotype, total chromosome length, and total chromosome volume with different chromosome number. Therefore, these characters which are under genetic control may be utilized as suitable parameters for classification and identification (Mukhopadhyay and Sharma 1987).

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