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KARYOMORPHOLOGICAL STUDY OF *ARISTOLOCHIATAGALA*CHAM.-A RARE MEDICINAL PLANT FROM ASSAM, INDIA

Bhaskar Sarma[1]* and Nilakshi Kalita[2]

[1&2]Cytogenetics and Plant Breeding Laboratory, Department of Botany, Gauhati University, Guwahati-781014, Assam, India

ABSTRACT: A karyomorphological analysis of *Aristolochiatagala* Cham. from Assam, India was conducted. The study included determination of somatic chromosome number, total chromosome length, volume, arm ratio and centromeric position. Chromosome classification was done on the basis of the position of the centromere. *Aristolochiatagala* Cham. possesses 2n=22 chromosomes of which 8 median chromosomes, 10 metacentric chromosomes and 4submetacentric chromosomes. The chromosome length varied from 1.36 µm to 2.21 µm while their volumes ranged from 0.001 μm^3 to 0.018 μm^3. The relative length of the chromosomes varied from 2.97 µm to 4.83 µm.
Key Words: *Aristolochiatagala* Cham**.** Karyomorphology, Chromosome classification, Rare medicinal plant

*Corresponding author: Bhaskar Sarma, [2]Cytogenetics and Plant Breeding Laboratory, Department of Botany, Gauhati University, Guwahati- 781014, Assam, India E-mail: bhaskarsarma252@gmail.com
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INTRODUCTION

*Aristolochia (*Aristolochiaceae) is an important genus widely used in traditional medicine [1]. During the past two decades, this genus has attracted much interest and has been the subject of numerous chemical and pharmacological studies. It is a rich source of aristolochic acids, which are unique to this genus, and of terpenoids [2].

The family Aristolochiaceae comprises of 8 genera and 450-600 species distributed in tropical and subtropical regions. The members of the genus *Aristolochia* are mostly distributed in tropical, subtropical, and mediterranean regions of the world [3; 4,5, 6]. In India, eight species of *Aristolochia* has so far been reported and five species of *Aristolochiaviz.*,*A. tagala* Chamisso, *A. cathcartii* Hooker.F, *A. indica*Linn.,*A. saccata* Wall. and*A. Platanifolia* Duchart Vern. are available in Assam [7; 8]. The species of *Aristolochia* are shrubby or perennial herbs, usually climbing.

Aristolochiatagala Cham.is quite glabrous, shrubby, twining, leaves large cordate upper often narrow subsagittatelylanceolate lower or all ovate or broadly ovate-oblong pedately 5-7 nerved, upper with the 2 principal nerves produced far beyond the middle, lower with all the nerves spreading, flowers in racemosepuberulous cymes, lip of parianth villous.

The plant is used as medicines. The Malays pound the leaves apply it to the head to treat fever. In Indonesia, poultice is made with leaves of the species; it is applied to the swollen abdomen or limbs. In the Philippines, snake bites and malaria is treated with the plant. In India, the roots are considered a tonic, carminative and emmenagogue [7].

Karyotype analysis can be used for many purposes, such as to study chromosomal aberrations, cellular functions, taxonomic relationships, and to gather information about past evolutionary events. An examination of karyotypic differences is often of great value in understanding the nature of plant variations, particularly from the level of population to genus. Karyotypes also show differences in absolute chromosome size, indicating changes in nuclear DNA in evolution [9]. Karyotype is a morphological aspect of the chromosome complement of an organism or species as seen at mitotic metaphase. The number, size and shape of chromosomes constitute the karyotype.

The karyotype is an important biological attribute which provides individuality and a cyto-taxonomic status of species. Karyotypes are useful as they permit rapid recognition of any aberrations either in chromosome number or morphology or both. Karyotype can also help in establishing evolutionary relationship between different species [10].
Here, karyomorphological analysis has been conducted for *Aristolochiatagala* available in Assam as adequate karyomorphological information is not available for this species.

MATERIALS AND METHODS

Collection of plants

A. tagala Cham.was collected from Karbi Anlong district (25°54′20.22″ N, 93°39′41.16″ E) of Assam, India. The collected experimental plants were grown and maintained in the experimental garden of Botany Department, Gauhati University.

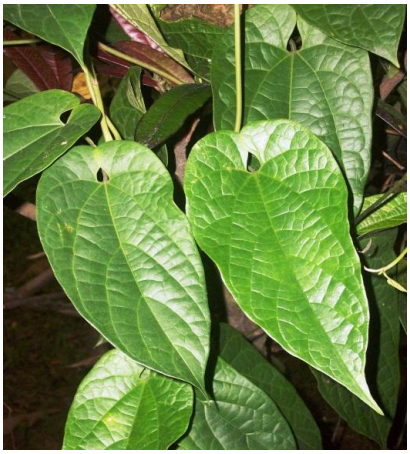

Fig. 1. *A.tagala*

Karyomorphological analysis

All cytological investigations pertaining to chromosome characterization were carried out in the meristematic cells of the newly emerged root tips.

Collection of root tips

For karyomorphological analysis, healthy root tips with active cell divisions were collected from the plants between 7.00 - 7: 30 a.m. The collected fresh root tips were thoroughly washed with distilled water and about 1 cm long were cut and pretreated in saturated solution of Para-dichloro Benzene (PDB) at 4°C for 3 hours, then fixed in Carnoy's fluid (Ethyl alcohol-Glacial acetic acid 3: 1) at 4°C for 6 hours. After fixation, root tips were thoroughly washed and preserved in 70% ethyl alcohol [11]. The pretreatment and fixing periods as well as the treatment temperature were standardized previously through trial and error method.

Preparation of slides

During the preparation of slides the following procedures were followed.

Hydrolysis and staining of chromosomes

For preparation of slides the root tips were first hydrolyzed in 1N HCl and 45% acetic acid (v/v) at 60°C for 2-3 min. after hydrolysis, root tips were stained with 1.5% aceto-orcein solution for half an hour with gentle warming.

Squash preparation

Now, from the root tips, the meristematic parts were cut out and transferred to a small drop of 45% glacial acetic acid on a grease free clean slide, then covered with a cover slip and squashed for microscopic observation. The temporary slides thus prepared were observed under compound microscope at a magnification of 1600x using oil immersion (16x × 100x, oil immersion). Well scattered metaphase plates were selected for karyomorphological analysis of the chromosomes and perfectly stained chromosomes were photographed. The drawings of the chromosomes were made with the help of a prism type camera lucida apparatus.
Total chromosome length was measured by adding the length of all the chromosomes in the karyotype. Volume of the chromosome was measured by using the formula $\pi r^2 h$, where 'r' is the radius of the chromosome and 'h' is the length of the chromosomes. Arm ratio 'R' of each chromosome was calculated as:

$$R = \frac{\text{Length of the long arm (L)}}{\text{Length of the short arm (S)}}$$

The relative length of the chromosome represents the ratio in percentage of the length of the individual chromosome to the total chromatin length of the diploid set [12]; thus

$$\textit{Relative chromosome length} = \frac{\text{Length of the individual chromosome x 100}}{\text{Total chromatin length of the diploid set}}$$

Depending upon the total length, the chromosomes were grouped into different categories. These were Type A= 3.51µm and above, Type B = 3.01µm – 3.50µm, Type C = 2.51µm – 3.00µm, Type D = 2.01µm – 2.50µm, Type E = 1.51µm – 2.00µm, Type F = 1.50µm and below.

Location of the centromere on the chromosome was expressed as a percentage of ratio between the arms and was calculated as a centromeric index or F% [13].

$$\text{F \%} = \frac{\text{Length of the short arm x 100}}{\text{Total length of the chromosome}}$$

The total form percent of TF% was calculated by using the formula given by Huziwara [14]. Thus,

$$\text{TF \%} = \frac{\text{Total sum of the short arm length x 100}}{\text{Total sum of the chromosome length}}$$

On the basis of the centromeric position, the chromosomes were classified into metacentric, sub - metacentric, sub - telocentric, and telocentric [13]. Then the karyotypes were prepared by arranging the chromosomes in descending order of size keeping their centromeres in a straight line. Thus the longest chromosome is placed on the extreme left and the smallest one on the extreme right. The karyotype of a species may be represented diagrammatically (in contrast to the actual photographs of the chromosomes in karyotype) showing all the morphological features of the chromosomes, such a diagram is known as idiogram. The idiogram was prepared by arranging the chromosomes in such a way that the largest chromosome is placed on extreme left at number 1 position and the smallest one is placed on the extreme right position [15].

RESULTS

The chromosome no. of *Aristolochiatagala* was found to be 2n = 22 in the somatic cells. The chromosome length varied from 1.36 µm to 2.21 µm while their volumes ranged from 0.001 μm^3 to 0.018 μm^3. The relative length of the chromosomes varied from 2.97 µm to 4.83 µm. On the basis of the length, the chromosomes were classified into Type A, Type B, Type C, Type D, Type E, and Type F [Table 1; Fig. 2-3]. The total genomic chromosome length was found to be 40.46 µm. The different types of chromosomes categorized on the basis of the length are represented as: $D_8 + E_{10} + F_4 = 2n = 22$.

These 22 somatic chromosomes comprised of 8 median chromosomes, 10 metacentric chromosomes and 4 sub-metacentric chromosomes. The karyotypic formula for *Aristolochiatagala* is represented as: $M_8 + m_{10} + sm_4 = 2n = 22$.

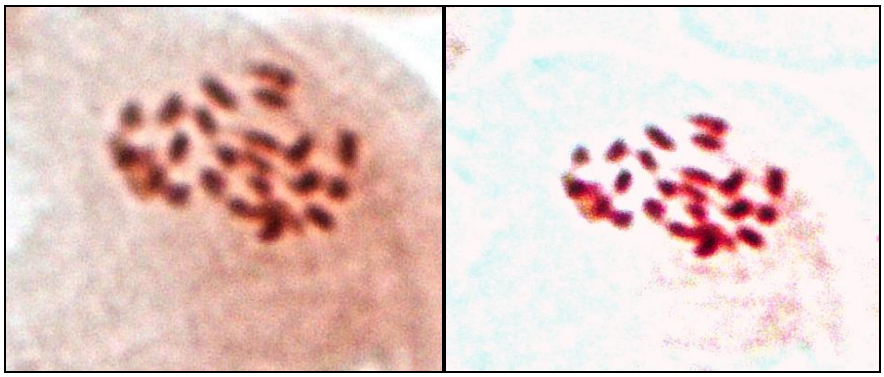

Fig-2. Microphotograph of the chromosomes of *A.tagala*(1600× magnification)

Fig.3. Karyotype of *A. tagala*

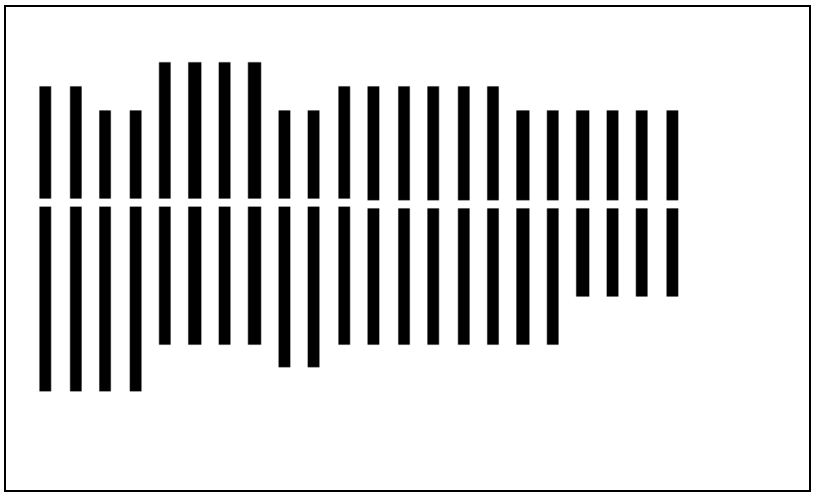

Fig.4. Idiogram of the chromosomes of *A. tagala*

Table 1. Karyomorphological characteristics of *A. tagala*

Chr. Type	Chr. No.	Chromosome Length		Total Length (l+s) µm	Relative Chr. Length (µm)	Arm Ratio (l/s)	Chromosome		Centro-meric Index (F%)	Position of Centro-mere	Nomenclature of chromosome
		Long Arm (l) µm	Short Arm (s) µm				Radius (r) µm	Vol. ($\pi r^2 h$) μm^3			
E	1	1.02	0.68	1.70	3.71	1.5	0.017	0.0015	40	m	Metacentric
E	2	1.02	0.68	1.70	3.71	1.5	0.017	0.0015	40	m	Metacentric
E	3	1.19	0.68	1.87	4.08	1.75	0.051	0.0152	36.36	sm	Submetacentric
E	4	1.19	0.68	1.87	4.08	1.75	0.051	0.0152	36.36	sm	Submetacentric
E	6	1.02	0.85	1.87	4.08	1.2	0.051	0.0152	45.45	m	Metacentric
E	6	1.02	0.85	1.87	4.08	1.2	0.051	0.0152	45.45	m	Metacentric
E	7	1.02	0.85	1.87	4.08	1.2	0.051	0.0152	45.45	m	Metacentric
E	8	1.02	0.85	1.87	4.08	1.2	0.051	0.0152	45.45	m	Metacentric
E	9	1.02	0.85	1.87	4.08	1.2	0.051	0.0152	45.45	m	Metacentric
E	10	1.02	0.85	1.87	4.08	1.2	0.051	0.0152	45.45	m	Metacentric
F	11	0.68	0.68	1.36	2.97	1	0.017	0.0012	50	M	Median
F	12	0.68	0.68	1.36	2.97	1	0.017	0.0012	50	M	Median
F	13	0.68	0.68	1.36	2.97	1	0.017	0.0012	50	M	Median
F	14	0.68	0.68	1.36	2.97	1	0.017	0.0012	50	M	Median
D	15	1.36	0.85	2.21	4.83	1.6	0.051	0.0180	38.46	m	Metacentric
D	16	1.36	0.68	2.04	4.46	2	0.051	0.0166	33.33	sm	Submetacentric
D	17	1.36	0.68	2.04	4.46	2	0.051	0.0166	33.33	sm	Submetacentric
D	18	1.36	0.85	2.21	4.83	1.6	0.051	0.0180	38.46	m	Metacentric
D	19	1.02	1.02	2.04	4.46	1	0.034	0.0074	50	M	Median
D	20	1.02	1.02	2.04	4.46	1	0.034	0.0074	50	M	Median
D	21	1.02	1.02	2.04	4.46	1	0.034	0.0074	50	M	Median
D	22	1.02	1.02	2.04	4.46	1	0.034	0.0074	50	M	Median

DISCUSSION

In the present work, karyotypic analyses of *Aristolochiatagala* available in Assam has been carried out, aiming to understand the chromosome evolution and the taxonomic relationships among the other species of Aristolochia. The importance of karyotype analysis in distinguishing plants species is well known. The role of alteration of chromosome morphology in speciation and in determining inter-relationships between species, varieties and even strains has been reviewed earlier by Sharma and Varma, [16]. It has been reported that species often show similarity in gross chromosome morphology but they differ from each other in small details in chromosome morphology like the centromere, secondary constriction, number and size of satellites and variation in total chromatin length [17, 18,19; 20; 21; 22]. Therefore, in order to understand the taxonomy and evolution among very closely related species, karyomorphological study is still considered asan essential parameter.

Aristolochiatagala Cham. revealed the chromosome number 2n = 22 in the somatic cells. These 22 somatic chromosomes comprised of 8 median chromosomes, 10 metacentric chromosomes and 4 sub-metacentric chromosomes. The chromosomes were more or less homomorphic in length, showing no gradual decrease in chromosome length. The proportions of metacentric and median chromosomes were high in the karyotypes of this species. Hence, the nature of chromosomes occurred here is an indication of a primitive character.

CONFLICT OF INTEREST: None

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