Annals of Plant Sciences
ISSN: 2287-688X
OPEN ACCESS
www.annalsofplantsciences.com

Original Research Article

Karyomorphological Study of *Stephania hernandifolia* - A Rare Medicinal Plant from Assam, India

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Received for publication: August 21, 2014; **Accepted:** September 17, 2014.

Abstract: A karyomorphological analysis of *Stephania hernandifolia* Walp 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. *Stephania hernandifolia* possesses 2n=26 chromosomes of which12 median chromosomes, 8 metacentric chromosomes and 4 submetacentric chromosomes. One pair of metacentric chromosome possesses secondary constriction at sub-terminal region. The chromosome length varied from 1.36 µm to 3.74 µm while their volumes ranged from 0.004 μm^3 to 0.294 μm^3. The relative length of the chromosomes varied from 2.17 µm to 5.97 µm.

Key Words: *Stephania hernandifoli* Walp., Karyomorphology, Chromosome classification, Medicinal plant

Introduction

The genus *Stephania* belongs to the family Menispermaceae, comprises about 350 species; distributed native to eastern and southern Asia and Australia. Among them *Stephania hernandifolia* is a climbing, leaves ovate or sub-deltoid acute obtuse or acuminate, umbels capitate. This plant has lots of medicinal value. It is used to treat laryngitis, arthritis, rheumatism and toothache, abdominal problems, diabetes, cholera, dysentery, antimycobacterial, anti-inflammatory, induces apoptosis in human T-cell lines, lung carcinoma and hepatoblastoma cells, antiproliferative and apoptosis-inducing activity (1-4).

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 (5). This paper deals with karyomorphological aspects of *Stephania hernandifolia* from Assam, India, because adequate karyomorphological data is not available for this species.

Material and Methods

The plant was collected from Kamrup district of Assam, India and was grown in the experimental garden of Botany Department, Gauhati University, Assam.

All cytological observations were made from root tips. Fresh root tips about 1 cm long were cut and pretreated in saturated solution of Para-dichloro Benzene (PDB) at 4°C for 3 hrs, then fixed in Carnoy's fluid (Ethyl alcohol-Glacial acetic acid 3:1) at 4°C for 6 hrs. After fixation root tips were thoroughly washed and preserved in 70% ethyl alcohol. After maceration in 1N HCl and 45% acetic acid (v/v) at 60°C for 2-3 min, materials were stained with 1.5% aceto-orcein and squashed for microscopic observation (6-9). Karyomorphological studies were made on chromosomes at metaphase stage. The following parameters were considered for the karyomorphological analysis:

(i) Total chromosome length (ii) Arm ratio = Length of long arm/Length of short arm (iii) Volume of the chromosome ($\pi r^2 h$) (iv) Relative length of the chromosome = (Length of the individual chromosome/Total chromatin length of the diploid set) X 100. (v) On the basis of length, chromosomes will be categorized as: Type A, Type B, Type C, Type D, Type E and Type F (vi) F% = (Length of the short arm / Total length of individual

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chromosome) X 100. (vii) Total form percent or TF % = (Total sum of short arm length / Total sum of chromosome length) X 100. (viii) On the basis of the centromeric position, the chromosomes may be classified into metacentric, sub-metacentric, sub- telocentric and telocentric (10).

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

Results

The detail karyomorphological analysis of *Stephania hernandifolia,* collected from Kamrup district of Assam, India was carried out. The chromosome number was found to be 2n=26 in the somatic cells. The chromosome length varied from 1.36 µm to 3.74 µm while their volumes ranged from 0.004 μm^3 to 0.294 μm^3. The relative length of the chromosomes varied from 2.17 µm to 5.97µm. 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). The total genomic chromosome length was found to be 62.56µm. The different types of chromosomes categorized on the basis of the length are represented as: $A_2 + B_6 + C_4 + D_6 + E_2 + F_6 = 2n = 26$

These 26 somatic chromosomes comprised of 12 median chromosomes, 8 metacentric chromosomes and 4 submetacentric chromosomes (Fig.1a and 1b). One pair of chromosome with two constrictions, primary and secondary was observed of which one constriction was found at metacentric region and other at subterminal region. The karyotypic formula for *Stephania hernandifolia* is represented as: $m.st_2 + M_{12} + m_8 + sm_4 = 2n = 26$

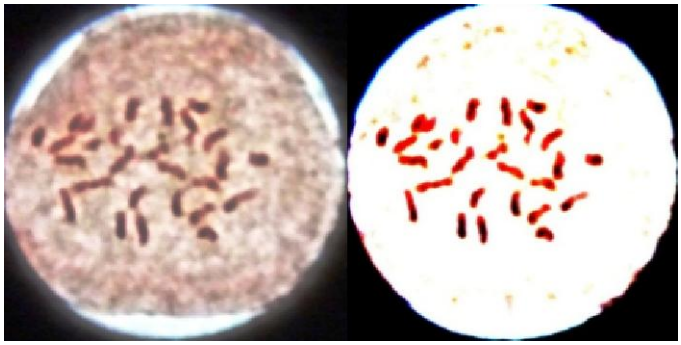

Figure 1(a): Microphotograph of the chromosomes *Stephania hernandifolia* Walp

Figure 1(b): Karyotype of *Stephania hernandifolia* Walp.

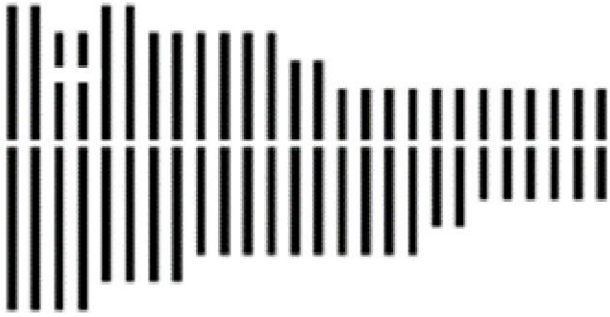

Figure 1(c): Idiogram of *Stephania hernandifolia* Walp.

Discussion

Chromosome size and morphology may help indicate evolutionary relationship among plant species (11). *Stephania hernandifolia* contain diploid chromosome number. The chromosomes were small in length. The chromosome complements only consisting of median, metacentric and submetacentric chromosomes. The chromosomes were more or less homomorphic in length, showing no gradual decrease in chromosome length.

Table 1: Karyomorphological features of *Stephania hernandifolia* Walp

Chr. Type	Chr. No.	Chromosome Length		Total Length (l+s) µm	Relative Chr. Length (µm)	Arm Ratio (l/s)	Chromosome		Centromeric Index (F%)	Position of Centromere	Nomenclature Of chromosome
		Long Arm (l) µm	Short Arm (s) µm				Radius (r) µm	Vol. ($\pi r^2 h$) μm^3			
D	1	1.36	0.68	2.04	3.26	2	0.068	0.294	33.33	sm	Submetacentric
D	2	1.36	0.68	2.04	3.26	2	0.068	0.294	33.33	sm	Submetacentric
D	3	1.36	0.68	2.04	3.26	2	0.068	0.294	33.33	sm	Submetacentric
D	4	1.36	0.68	2.04	3.26	2	0.068	0.294	33.33	sm	Submetacentric
F	5	0.68	0.68	1.36	2.17	1	0.034	0.004	50	M	Median
F	6	0.68	0.68	1.36	2.17	1	0.034	0.004	50	M	Median
F	7	0.68	0.68	1.36	2.17	1	0.034	0.004	50	M	Median
F	8	0.68	0.68	1.36	2.17	1	0.034	0.004	50	M	Median
F	9	0.68	0.68	1.36	2.17	1	0.034	0.004	50	M	Median
F	10	0.68	0.68	1.36	2.17	1	0.034	0.004	50	M	Median
E	11	1.02	0.68	1.70	2.71	1.5	0.068	0.024	40	m	Metacentric
E	12	1.02	0.68	1.70	2.71	1.5	0.068	0.024	40	m	Metacentric
C	13	1.36	1.36	2.72	4.34	1	0.102	0.088	50	M	Median
C	14	1.36	1.36	2.72	4.34	1	0.102	0.088	50	M	Median
C	15	1.36	1.36	2.72	4.34	1	0.102	0.088	50	M	Median
C	16	1.36	1.36	2.72	4.34	1	0.102	0.088	50	M	Median
D	17	1.36	1.02	2.38	3.80	1.33	0.068	0.034	42.85	m	Metacentric
D	18	1.36	1.02	2.38	3.80	1.33	0.068	0.034	42.85	m	Metacentric
B	19	1.70	1.70	3.40	5.43	1	0.136	0.196	50	M	Median
B	20	1.70	1.70	3.40	5.43	1	0.136	0.196	50	M	Median
B	21	1.70	1.36	3.06	4.89	1.25	0.136	0.176	44.44	m	Metacentric
B	22	1.70	1.36	3.06	4.89	1.25	0.136	0.176	44.44	m	Metacentric
B	23(st)	2.04	1.36	3.40	5.43	1.5	0.136	0.196	40	m	Metacentric
B	24(st)	2.04	1.36	3.40	5.43	1.5	0.102	0.111	40	m	Metacentric
A	25	2.04	1.70	3.74	5.97	1.2	0.136	0.216	45.45	m	Metacentric
A	26	2.04	1.70	3.74	5.97	1.2	0.136	0.216	45.45	m	Metacentric

Stephania hernandifolia Walp possesses 2n=26 chromosomes. Among them 12 median, 8 metacentric, 4 submetacentric and 2 metacentric chromosomes having secondary constrictions at subterminal regions. Chromosomes showing the abundance of median chromosomes in its karyotype.

Previous cytological work reported 2n=22 chromosomes and no secondary constrictions in any of the chromosomes in *Stephania hernandifolia*, but in the present investigation we found 2n=26 chromosomes, having a pair of metacentric chromosomes with secondary constrictions in sub terminal regions (12).

The proportion of metacentric and median chromosomes was high in the karyotype of this species. Therefore, a tendency of symmetric karyotypes exists in this species, indicating a primitive character.

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Source of support: Nil
Conflict of interest: None Declared