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SHORT RESEARCH ARTICLE

Karyomorphological studies in three species of *Smilax* L. found in Assam

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Abstract

The present cytological investigation was carried out on the karyomorphology of three species, *Smilax zeylanica* L., *S. ovalifolia* Roxb. ex D. Don and *S. arisanensis* Hayata. The results showed that the somatic number of *S. zeylanica* is 2n = 30 and the somatic number of other two species, *S. ovalifolia* were 2n = 32 and *S. arisanensis* 2n = 36, respectively. It is reported for the first time. The information on karyophology may be very useful for the genetic improvement of *Smilax*.

Keywords: Karyomorphology, *Smilax* spp., somatic number, chromosome

The genus *Smilax* L. belongs to the Smilacaceae family with 200 to 350 species found in tropical and subtropical regions (Takhtajan 1997). Out of 33 species (Hooker 1886), 17 are from Northeast India with the majority from the eastern Himalayan region (Baruah et al. 2018). *Smilax zeylanica* L. is a large climbing shrub with smooth stems, listed as vulnerable in the IUCN Red Data Book (Balaguru et al. 2006; Baruah et al. 2014). The stems of *S. zeylanica* are used for urinary complaints and dysentery, while its roots are used as a tonic (Das et al. 2012). *S. ovalifolia* Roxb. ex D. Don. is a coarse vine found in tropical India, used for venereal disease treatment (Anonymous 2010), and *Smilax arisaensis* Hayata is a short-spined climber. Although it is a well established fact that chromosomal morphology in higher plants is of considerable evolutionary and taxonomic significance (Stebbins1958; Love and Love 1975), the karyomorphology of the genus *Smilax* L. is obscure (Vijayavalli et al. 1989). Therefore, the present study was undertaken.

Smilax saplings were collected from Numligarh and Khetri, Assam for karyomorphological studies. The plant species was authenticated through herbaria at Gauhati University. The chromosomes were characterized using karyotypic studies and root tips squash technique (Sharma and Sharma 1980; Boro and Das 2020). The karyotype study involved collecting root tips between 7:45 and 8 am and pre-treating them with para-dichlorobenzene for 4 hours at 4° ± 2°C. Young tips were fixed in Carnoy's fuild for 24 to 28 hours, washed with 70% ethanol, and stored for cytological work after fixation. The karyomorphological analysis involved selecting metaphase plates, viewing them under oil immersion, photographing them, and measuring them using Scope Image software. The chromosomes were drawn using the Camera Lucida apparatus, and ideograms were constructed. Parameters such as length of a long arm, short arm, total length, arm ratio, relative length (Khosla and Sobti 1985), the volume of the chromosome ($\pi r^2 h$), total form percent (TF%) (Huziwara 1962), centromeric position (Levan et al. 1964), coefficient gradient (CG), coefficient of variation for chromosome length (CV), dispersion index (DI) (Lavania and Srivastava 1992), and Asymmetry Index (AI) (Paszko et al. 2006) were calculated for precision and sensitivity for accessing asymmetries in the karyotype (Boro and Das 2019, 2020; Ravindran and Das 2022).

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$$\text{Centromeric gradient (CG)} = \frac{\text{Length of median short arm}}{\text{Length of median chromosome}} \times 100$$

$$\text{Coefficient of variation for chromosome length (CV)} = \frac{S_{CL}}{X_{CL}}$$

where S_{CL} = Standard deviation of chromosome length and X_{CL} = Mean of chromosome length

$$DI = \frac{CG \times CV}{100}$$

$$CV_{CL} = \frac{S_{CL}}{X_{CL}} \times 100$$

$$CV_{CI} = \frac{S_{CI}}{X_{CI}} \times 100$$

$$Asymmetry\ index\ (AI) = \frac{CV_{CL} \times CV_{CI}}{100} \times 100$$

where, CV_{CL}- a relative variation in chromosome length, CV_{CI}- a relative variation in centromeric index (Ravindran and Das 2022).

Karyotyping

Based on length, chromosomes have been classified into the following types: Type A - chromosome with a length of 6.1 µm and more, Type B -chromosome with a length of 4.1 to 6.0 µm, Type C - chromosome with a length of 2.1 to 4.0 µm, Type D - chromosome with a length of 0 to 2.0 µm. The total volume of the chromosomes was determined by adding the volume of all chromosomes. Based on centromere position, chromosomes were divided into median metacentric (M), metacentric (m), sub-metacentric (Sm), sub-telocentric (St) and telocentric (t) according to the nomenclature system of Levan et al. 1964.

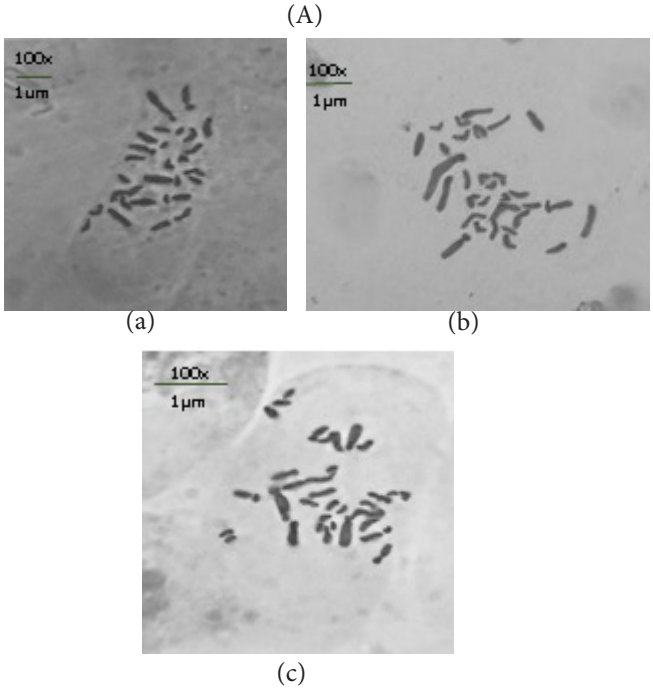

Fig. 1. (A) Microphotograph of somatic chromosomes at metaphase stage (10x100x oil immersion); (B) Cameralucida diagram (10x100x magnification (C) Karyotype and (D) Ideogram; (a) *S. zeylanica* L. (b) *S. ovalifolia* Roxb. ex D. Don (c) *S. arisanensis* Hayata

Tables 1 and 2 provide a summary of the karyotypic data, and Figure 1 shows the morphology of the karyotype in the form of a microphotograph, camera lucida diagram, constructed ideogram, and chromosome karyogram. The study focuses on three *Smilax* species in Northeast India, *S. zeylanica*, *S. ovalifolia* and *S. arisanensis*. The karyomorphological data reveal that *S. zeylanica* has 2n = 30, *S. ovalifolia* has 2n = 32, and *S. arisanensis* has 2n = 36 chromosomes. The somatic number of *S. zeylanica* is 2n = 30, earlier reports reveal 2n = 32 (Vijayavalli et al. 1989). *S. zeylanica* has two chromosomes under type A with two secondary constrictions, two chromosomes under type B with one secondary constriction, 20 chromosomes under type C and six chromosomes under type D.*S. ovalifolia* has two chromosomes under type A with two secondary constrictions, six chromosomes under type B,12 chromosomes under type C and 12 chromosomes undertype D. *S. arisanensis* has 4 chromosomes under type B with three secondary constrictions, 20 chromosomes under type C and 12 chromosomes under type D.

S. arisanensis has more chromosomes than *S. ovalifolia* and *S. zeylanica*. The difference in karyotype in different species may have occurred due to minor changes of chromosomal repatterning or changes in genes or gene complexes, which are responsible for genetic variability (Boro and Das, 2019).The DI value in *S. ovalifolia* is higher than in *S. zeylanica* and *S. arisanensis*, indicating a more specialized karyotype (Lavania and Srivastava 1992). The asymmetric index (AI) indicates higher heterogeneity in *S. arisanensis*, allowing for a more accurate depiction of the organism's karyotype nature. Table 1 shows a high proportion of median and metacentric chromosomes in the karyotype, indicating a primitive character due to a tendency towards symmetric karyotype (Stebbins 1971). Even their morphological characters, which are more or less generalized, prove their primitiveness (Ravindran and Das 2022).

The identity of the different species of *Smilax* is often difficult due to its close morphological similarity, making karyotype analysis crucial for identifying species related to morphological variants adapted to different eco-climate regions (Das et al. 1998). Therefore, a detailed cytological study of the three species of *Smilax* has been conducted, which will be crucial for future phylogenetic and taxonomic research as well as bioprospecting, as *Smilax* is a medicinal plant with immunomodulatory, antioxidant, antibacterial, antifungal, and diuretic properties.

Table 1: Details of numerical karyotypic parameters of three species of *Smilax* L

Taxa	Chromosome no. (2n)	Range of chromosome					Total genomic chromosome length (± SE) (µm)	Total genomic chromosome volume (± SE) (µm)	TF%	Centromeric index mean (F%) (±SE)	Dispersion index (DI)	Type of chromosome	Karryotype formula
		Length (µm)	Relative length (µm)	Radius (µm)	Volume (πr²h)	Arm ratio							
S. zeylanica L.	30	6.54 - 0.84	7.66- 0.98	0.50- 0.38	5.13 -0.38	1.6 - 6.25	85.361 ± 0.23	61.28 ± 0.19	42.15	42.43 ± 1.9	19.51	$A_2+B_2+C_{20}+D_6$	$M_9+m_{16}+Sm_2+St_3$
S.ovalifolia Roxb. ex D. Don	32	7.352 - 0.246	7.78- 0.26	0.50- 0.38	5.77 -0.2	1.0005 - 5	94.445 ± 0.3	71.55 ± 0.25	46	45.7 ± 1.46	29.06	$A_2+B_6+C_{12}+D_{12}$	$M_{16}+m_{12}+Sm_3+St_1$
S. arisanensis Hayata	36	5.944 - 0.84	6.17- 0.87	0.50- 0.38	4.66 -0.4	1 - 8.3	96.223 ± 0.21	70.37 ± 0.17	41.24	41.8 ± 2.14	18.48	$A_0+B_4+C_{20}+D_{12}$	$M_{18}+m_8+Sm_3+St_6+t_1$

Table 2. Chromosome statistics for three species of *Smilax* L.

Name of the species	Coefficient of variation for chromosome length (CV_{CL})	Coefficient of variation for the centromeric index (CV_{CI})	Karyotype asymmetry (AI)
S. zeylanica L.	39	25.57	9.97
S. ovalifolia Roxb. ex D. Don	58.4	18.08	10.55
S. arisanensis Hayata	45.56	30.81	14.04

Authors' contribution

Conceptualization of research (BND); Designing of the experiments (FB); Contribution of experimental materials (FB); Execution of field/ lab experiments and data collection (FB); Analysis of data and interpretation (FB, BND); Preparation of the manuscript (FB, BND).

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