



SHORT RESEARCH ARTICLE

Karyomorphological studies in two species of genus *Strychnos* L. significant in ethno-pharmacological aspects

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Abstract

In the present investigation, *Strychnos nux vomica* L. and *Strychnos wallichiana* Steud. ex A. DC. have been selected for karyotypic investigation to strengthen genetic relationships. Chromosome number recorded in *S. nux vomica* is $2n=32$ while in *S. wallichiana* is $2n=40$. In both species A-type, B-type and C-type chromosomes were observed with 28 metacentric chromosomes and 4 sub-metacentric in *S. nux vomica* and 38 metacentric and 2 sub-metacentric chromosomes in *S. wallichiana*. The total form per cent (T.F %), Dispersion Index (DI), Asymmetry index (AI) depict the symmetric karyotype of chromosomes which represents their primitive level in the family.

Keywords: Ethno-botanical importance, karyomorphology, strychnine, *Strychnos* spp., vulnerable

The genus *Strychnos* L. is an ethno-pharmacologically known member of the family Loganiaceae, a well-established group of 13 genera under Gentianales (Backlund et al. 2000). Globally, 200 species of this genus are distributed through the warm regions of Asia, America, and Africa (Chuang 2009; Adebowale et al. 2016). In India, they are found throughout tropical regions, deciduous forests upto an altitude of 1200 m (Chowdhury 2010). These species are found in the wild and domesticated near sacred places as a part of Hindu traditions. In North East India, specifically in Assam, species like *Strychnos nux vomica* L., *S. potatorum*, *S. wallichiana* and *S. laurina* are some of the species rarely spotted in Silghat- Nowgong, Naga Hills, Khasi Hills, Lakhimpur, Garo Hills (Kanjilal et al. 1939) and Kaziranga. Various authors have documented bioprospecting in Indian *Strychnos*.

Strychnos nux vomica L. commonly known as Snake wood tree, Strychnine tree (English), Kuchla (Hindi) and *Strychnos wallichiana* Steud. ex A. DC. often known as Snakewood (English), Nagamusti (Kannada), are medically very important species. Karyotyping is categorized under classical genetics, where the theory of inheritance can be related to evolutionary genetics and the findings can play a role to firm genetic evidence. (Tamarin 2002). In several instances, studies of karyotype morphology have led the way to a new and fuller understanding of the systematic relationships within a major group of plants and to a complete reorganization of the taxonomic system of the group (Stebbins 1971; Nabis Das 2007; Boro and Nabis Das 2020). In the present study, *Strychnos nux vomica* and *S.*

wallichiana were subjected for karyotypic investigation.

Experimental plant saplings of *Strychnos nux vomica* L. were collected from Recreation Park, Diphu, Assam and Pathanamthitta district in Kerala, whereas *S. wallichiana* were collected from the wild of Vallicode, Pathanamthitta district, Kerala in the month of January 2020. The collected plants were properly identified by KFRI Thrissur, Kerala and maintained in earthen pots to facilitate harvesting of new young leaves for the experiment in the garden of Handique Girls' College, Guwahati. For chromosome characterization, leaf tip squash technique was followed (Sharma and Sharma 1980). Well scattered metaphase plates were selected after observing under oil immersion (10 x 100x). Photos were

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taken by N-400M Trinocular microscope by Lawrence & Mayo with Lynx 9 MP CMOS camera with image analyzing system. Measurements were done by using version 9.0 Scope Image software. The chromosome drawings were prepared using prism type camera lucida apparatus. For constructing karyotypes, the chromosomes were arranged in order of decreasing size and increasing asymmetry (Figs. 1 and 2).

The following parameters have been taken into consideration to draw conclusion over the karyotypic characters: (a) Total chromosome length, (b) Volume of the chromosome ($\pi r^2 h$), (c) Arm ratio of each chromosome, (d) The relative length of the chromosome (Khosla and Sobti 1985), (e) Centromeric Index or F% locates the centromere on the chromosome, (f) The total form per cent (TF %) (Huziwara 1962) to describe the karyotypic relationships, (g) A dispersion index parameter to compare phylogenetic differentiation and origin in the genus *Papaver* (Lavania and Srivastava 1992) as given below:

$$CG = \frac{\text{Median length of short arm}}{\text{Median length of the chromosomes}} \times 100$$

$$CV = \frac{S_{CL}}{x_{CL}} \times 100,$$

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

Where S_{CL} is the standard deviation of the chromosome length, x_{CL} is the mean of chromosome length, and (h) Asymmetry index (AI) (Paszko et al. 2006,) which have the advantage of allowing a high degree of precision and sensitivity to access karyotype asymmetry, i.e., higher the

value of AI, higher the levels of karyotypic heterogeneity; as the index gets lower, it indicates greater karyotype symmetry as per standard procedure given below:

$$CV_{CL} = \frac{S_{CL}}{x_{CL}} \times 100,$$

$$CV_{CI} = \frac{S_{CI}}{x_{CI}} \times 100,$$

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

Where, CV_{CL} - relative variation in chromosome length, CV_{CI} - relative variation in Centromeric index

(i) The types of chromosomes were determined on the basis of the length (Nabis Das 2007; Boro and Nabis Das 2020): Type A- chromosomes having length 0.8 μm to 1 μm ; Type B- chromosomes having length 0.6 μm to 0.799 μm ; Type C- chromosomes having length 0.4 μm to 0.599 μm and Type D- chromosomes having length 0.2 μm to 0.399 μm and (j) based on centromere position, the chromosomes were classified into metacentric, sub metacentric, sub-telocentric and telocentric following the nomenclature system (Levan et al.1964).

Details of numerical karyotypic parameters

The karyotypic details are summarized in Table 1 and Table 2 and the morphology of the karyotype have been depicted in the form of microphotograph; camera lucida diagram; the idiogram constructed; karyogram of the chromosome in Fig. 1 and Fig. 2 for *S. nux vomica* L. and *S. wallichiana*, respectively. Leaf tip cells of *S. nux vomica* L. L. showed chromosomes no. $2n=32$, which ranged from 0.885 μm -

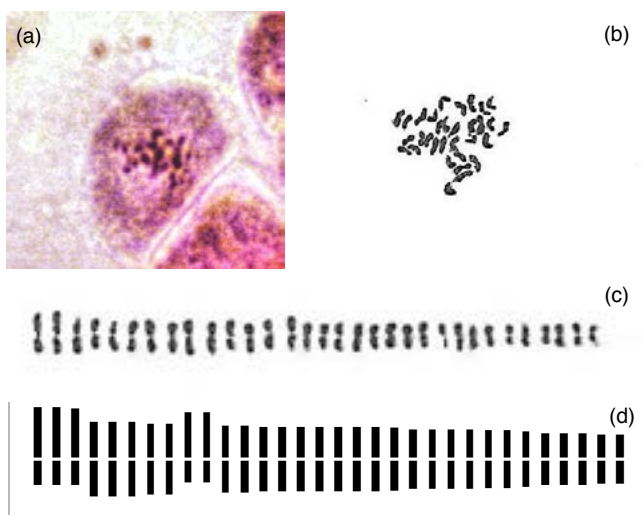


Fig. 1. Illustrations for karyological work in *Strychnos nux vomica* L.
(a) Microphotograph of somatic chromosomes ($2n=32$) at metaphase (10x100x, oil immersion).
(b) Camera lucida diagram of metaphase chromosome (10x100x, oil immersion). (c) Karyotype and (d) Idiogram

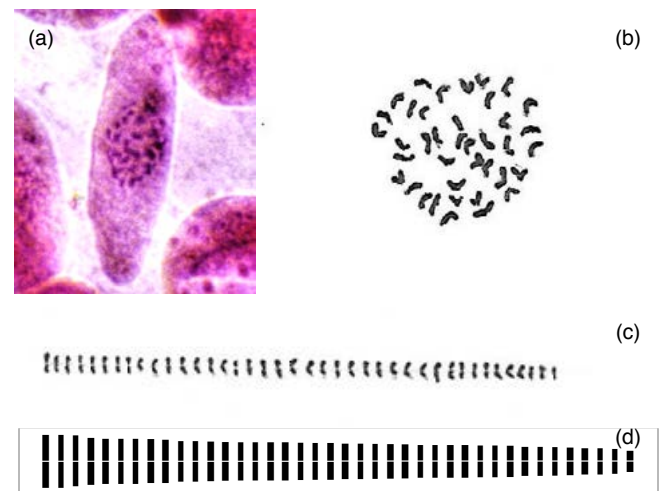


Fig. 2. Illustrations for karyological work in *Strychnos wallichiana* Steud. ex A. DC.,
(a) Microphotograph of somatic chromosomes ($2n=40$) at metaphase (10x100x, oil immersion),
(b) Camera lucida diagram of metaphase chromosome (10x100x, oil immersion),
(c) Karyotype and (d) Idiogram

Table 1. Details of numerical karyotypic parameters of *Strychnos nux vomica* L. and *Strychnos wallichiana* Steud. ex A. DC.

Taxa	Chromosome no. (2n)	Range of chromosome				Arm ratio	Total Genomic chromosome length (± SE) (µm)	Total Genomic chromosome Volume (± SE) (µm)	TF%	Centromeric index mean (F %) (± SE)	Dispersion Index (DI)	Type of chromosome	Karyotype formula
		Length (µm)	Relative length (µm)	Radius (µm)	Volume (µm ³)								
<i>Strychnos nux vomica</i> L.	32	0.885-0.56	3.79-2.4	0.25-0.23	0.173-0.085	2.03-1	23.32 ± 0.093	3.647 ± 0.022	46.82	47.38 (±6.17)	6.375	A ₈ +B ₁₈ +C ₆ =2n=32	m ₂₈ +sm ₄ =2n=32
		1.43-0.56	3.63-1.44	0.25-0.22	0.28-0.18	1.04-1	39.39 ± 0.202	7.528 ± 0.04	49.89	49.94(±0.225)	10.2	A ₂₆ +B ₁₂ +C ₂ =2n=40	m ₃₈ +sm ₂ =2n=40

Table 2. Chromosome statistics for *Strychnos nux vomica* L. and *Strychnos wallichiana* Steud. ex A. DC.

Name of the species	Coefficient of variation for Chromosome length (CVCL)	Coefficient of variation for the Centromeric index (CVCI)	Karyotype asymmetry (AI)
<i>Strychnos nux vomica</i> L.	12.75	13.02	1.66
<i>Strychnos wallichiana</i> Steud. ex A. DC.	20.4	0.45	0.0918

0.56µm in length. 8 A- type chromosomes, 18 B- type chromosomes and 6 C- type chromosomes were recorded under this species with 28 metacentric chromosomes and 4 sub-metacentric chromosomes. In somatic cells of *S. wallichiana* chromosomes no. 2n=40 were recorded, which ranged from 1.43 µm – 0.568 µm. Twenty-six chromosomes were recorded under A- type, 12 chromosomes came under B- type and 2 chromosomes under C- type with nomenclature of 38 metacentric chromosomes and 2 sub-metacentric chromosomes.

TF% value ranged between 46.82 in *S. nux vomica* L. and 49.89 in *S. wallichiana*, which depicts the indices of symmetry. Dispersion indexes (DI) were recorded as 6.375 and 10.2 for *S. nux vomica* L. and *S. wallichiana* followed by Asymmetric index (AI), which proved to be the most practical and allowed a high degree in depicting the karyotypic nature of the organism was recorded as 1.66 in *S. nux vomica* L. and 0.0918 in *S. wallichiana*.

Very little variations were noted between the chromosomes regarding size and volume in each species. In terms of no. of chromosomes, more of them were spotted in *S. wallichiana* than in *S. nux vomica*. In between the species, the chromosomes of *S. wallichiana* were longer and prominent than that in the *S. nux vomica*. T.F %, DI values also describes the advancement of species *S. wallichiana* than in comparisons of *S. nux vomica* L. (Lavania and Srivastava 1992; Boro and Nabis Das 2020) in contrast, only asymmetric index (AI) showed higher value in *S. nux vomica* than in *S. wallichiana* which indicates higher level of heterogeneity in the former one. Therefore, values of the above parameters describe the symmetrical nature of the karyotypes among the two species which represents a very primitive level in the family (Stebbins 1971). Among the two species *S. nux vomica* appears to be more advanced than *S. wallichiana*. Such expressions depict the adaptive value correlated with the physiological characteristics of the individuals (Nabis Das 2007). Even after such long domestication and cultivation, the primitiveness in them is a point to highlight. Even their morphological characters, which are more or less generalized, prove their primitiveness. On the other hand, the different adaptability among the family in these

members stands against this statement, as in the same genus we can find them as big deciduous trees, medium-sized tree to long woody and shrubby climbers.

They are unspecialized progenitors of the family and are morphologically more or less generalized. Therefore, such members of the family have conserved genome and have close genetic relations with each other (Stebbins 1971). Over exploitation of seeds, woods, deforestation, and high commercial demand are some challenges faced by these species at present. Preserving such commercially and traditionally well-known species will lead to ex-situ conservation and germplasm regeneration.

Authors' contribution

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

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