PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.3407

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Unveiling the chromosomal architecture of *Stemona tuberosa* Lour.: first report of its karyotype and intra-generic investigation

Supriya Adhikari[1], Ashok Kumar Sutradhar[1], Shibananda Sharma[1], Kripamoy Chakraborty[2]* & Rabindra Kumar Sinha[1]

[1]Cytogenetics and Plant Biotechnology Laboratory, Department of Botany, Tripura University (A Central University), Suryamaninagar 799 022, Tripura, India

[2]Department of Botany, Tripura University (A Central University), Suryamaninagar 799 022, Tripura, India

*Email: kripachakraborty@gmail.com

OPEN ACCESS

ARTICLE HISTORY

Received: 19 February 2024
Accepted: 07 October 2024

Available online
Version 1.0 : 21 January 2025

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Adhikari S, Sutradhar A K, Sharma S, Chakraborty K, Sinha R K. Unveiling the chromosomal architecture of *Stemona tuberosa* Lour.: first report of its karyotype and intra-generic investigation . Plant Science Today (Early Access). https://doi.org/10.14719/pst.3407

Abstract

The present investigation aims to provide a comprehensive understanding of the chromosomal architecture of *Stemona tuberosa* Lour, a plant species of high ethnobotanical significance belonging to the family Stemonceae. The study entailed a thorough examination of the chromosome number, which was determined to be 2n=14, with a basic number of X=7. Based on Stebbins's categorization, the karyotype of *S. tuberosa* falls under 1B category, which indicates a relatively lower degree of asymmetry in chromosome complements. Moreover, the study also comprises various other karyomorphological indices, which may act as a significant key for intra-generic investigation of other species in the future. These findings offer new insights into the chromosomal makeup of *S. tuberosa* and could have potential implications for the conservation and utilization of this plant species.

Keywords

chromosomal architecture; chromosomal asymmetry; karyotype; Stemonaceae; *Stemona tuberosa*

Introduction

Stemonaceae is a plant family with significant ethnomedicinal importance, having a group of monocotyledonous plants having four genera and over 30 species primarily found in Southeast Asia, tropical Australia and the United States (1, 2). The genus *Pentastemona* has later drawn out of the Stemonaceae and assigned to a monotypic family, i.e., the PentaStemonaceae (3). For decades in South Asian countries, *Stemona* sp. were popularly used as a traditional medicine in the treatment of cough and various respiratory disorders, like pulmonary tuberculosis and bronchitis (4-6).

Stemona tuberosa Lour. found in Tripura, North East India, has high ethno-pharmaceutical significance due to its antiseptic, anti-inflammatory, anti-cancerous, antitussive and insecticidal activity (7-11). Tripura extends from 22°56'-24°34' N and 91°10'-92°20' E and lies within the Indo-Burma biodiversity hotspot region of the world (12, 13). Many plant species found in this region are either endemic to the state or to the north-eastern part of India (14). The antecedent pharmacological investigations have revealed a group of chemical compounds that represent the main bioactive constituents of these plants, i.e., alkaloids named *Stemona* alkaloids (15-18). *Stemona* sp. are very popular among researchers for their bioactive components,

like *Stemona* alkaloids (19). Unlike the phytochemical data, cytological information about this small monocotyledonous family, Stemonaceae is very scarce to date.

Only a few members in the family Stemonaceae have been characterized using somatic chromosome numbers so far, but no such karyological information has yet been documented. The somatic chromosome number of some *Stemona* sp. (i.e., *Stemona colinsae*, *S. japonica, S. kerrii, S. sessilifolia, S. tuberosa, S. aphylla, S. curtisii, S. involunta, S. mairei* and *S. phyllantha*) was reported as 2n = 14 with basic chromosome number x=7 (20-22). For some other members of Stemonaceae, such as in the case of *Croomia* and *Stichoneuron*, the chromosome number has also been reported. Many researchers found the mitotic chromosome number of *Stichoneuron caudatum* and *S. membranaceum* to be 2n=18, with the basic chromosome number x = 9 (20, 24). Whereas all three *Croomia* species have chromosome numbers 2n = 24 with basic chromosome number x = 12 (20), except for *C. japonica* with a somatic chromosome number 2n = 26 (23).

In this present study, a detailed karyomorphological investigation has been done to decipher the intricate chromosomal characteristics of this species, which may add more valuable information to the previous data and of course, play a significant role in better understanding of this species and other members of this family both at the sub-cellular and genetic level as well.

Materials and Methods

Collection of plant materials

Plants (Fig. 1a) were collected from Ishan chandranagar (23°45'35"N 91°15'26"E) and are grown in the Botany Experimental Garden at Tripura University for future experimentation.

Somatic chromosome study

The somatic chromosomal study was conducted utilizing a modified aceto-orcein staining technique as described by Sharma and Sharma (25). Since it was quite a difficult task to avail root tips from this particular plant, young and healthy shoot tips of 2 mm size were carefully selected and pre-treated respectively with a solution containing 0.002M of 8-hydroxyquinoline and Para dichloro benzene (PDB) in a ratio of 1:1 for 6-8 h at 12-15 °C. Subsequently, the pre-treated shoot tips were thoroughly rinsed with distilled water (DH_2O) and immersed in a mixture of acidulated alcohol (1:1 ratio of 1N HCL: alcohol) for 1 h. After swift rinsing of those shoot tips with distilled water, they were then allowed to soak overnight in Carnoy's solution (ethanol: acetic acid; 3:1, v:v). Then, these shoot tips were exposed to 1 N HCL for 15 min at room temperature and were rinsed thoroughly 2-3 times with distilled water repetitively, then treated with 45% acetic acid for 10 min. Finally, treated shoot tips were stained with aceto-orcein (2% w/v):1 N HCl in a ratio of 9:1 for 2h, following which they were

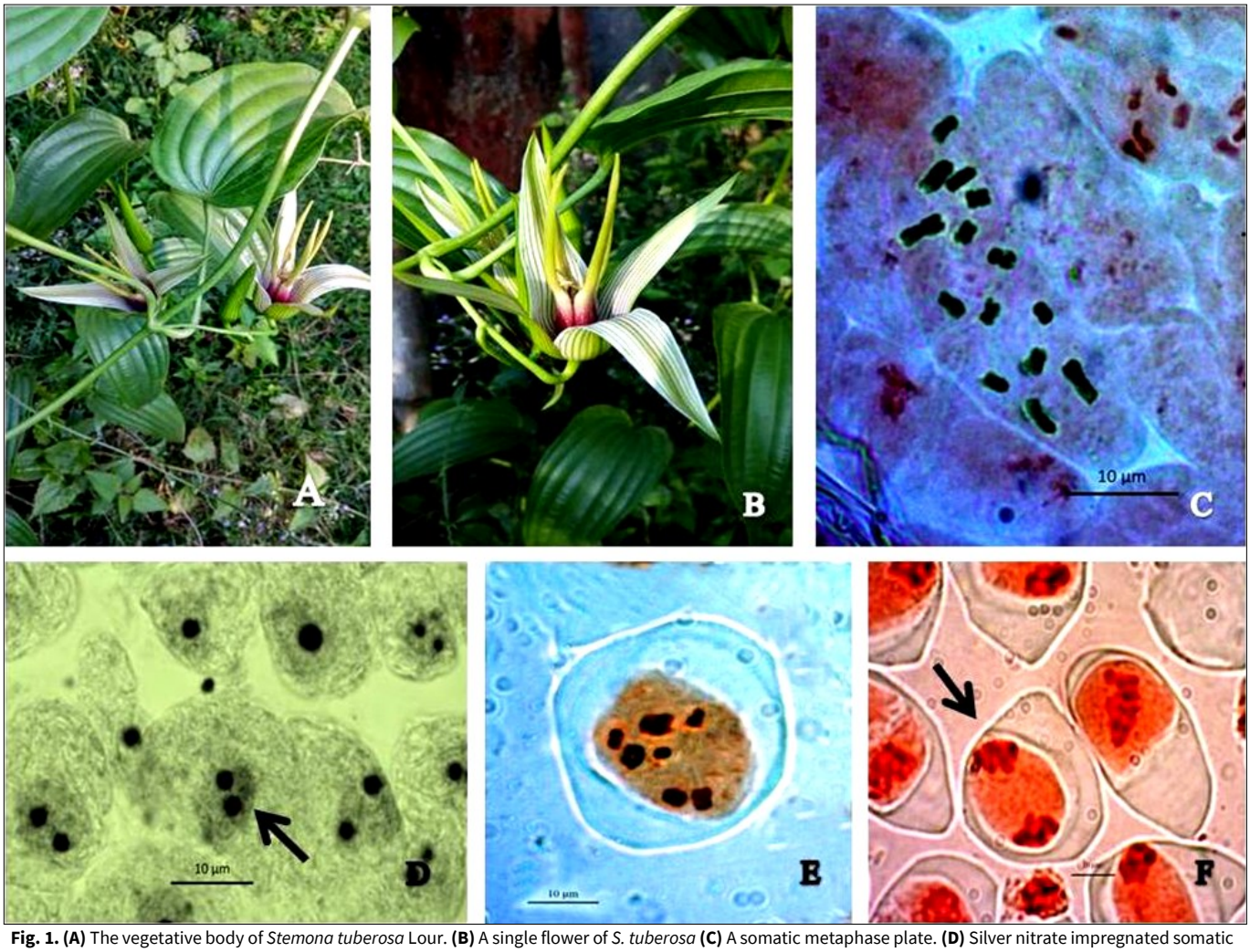

Fig. 1. (A) The vegetative body of *Stemona tuberosa* Lour. **(B)** A single flower of *S. tuberosa* **(C)** A somatic metaphase plate. **(D)** Silver nitrate impregnated somatic cells showing two nucleoli (**E)** Meiotic metaphase plate of *Stemona tuberosa* **(F)** Anaphase plate showing normal meiotic behaviour.

squashed and examined at 100 X oil immersion objective, of Axio Carl Zeiss Lab A.1 microscope for finding few well spread metaphase plates for our analysis.

Meiotic Chromosome Study

To investigate the meiotic chromosomes, flower buds that were pre-fixed in Carnoy's solution (ethanol: acetic acid; 3:1, v:v) overnight, following which the anthers collected from the flower were stained with 2% aceto-carmine.

Nucleoli study of the somatic cells

The nucleolar staining procedure followed the method (26). Initially, shoot-tips were placed in a fixative solution consisting of a 1:1 mixture of 10% Formol and 1% Hydroquinone for 2h. Subsequently, they were thoroughly rinsed in distilled water and then submerged in a 2% solution of $AgNO_3$ (Silver Nitrate) at 60°C in darkness overnight. The shoot-tips treated with $AgNO_3$ were then subjected once again to the Formol-Hydroquinone (1:1) solution for 1h before being ultimately flattened in 45 % acetic acid.

Data analysis

Five adequately dispersed chromosomal metaphase plates were studied in detail to analyse the mitotic and meiotic chromosomes. Karyo-morphological analysis was carried out on the mitotic chromosome, where the numerical data of the karyotype were obtained by comparing 5 well-spread mitotic metaphase plates. In the case of the length and p/q arm ratio exhibiting variability, the mean value was computed to find the Centromeric Index (F%). Karyological parameters used in this analysis were calculated with the help of the following formulae (Table 1).

Results

Table 1. Different karyological parameters used to analyze the chromosomal characteristics

Karyological parameter	Formula	Reference
Covariance of Centromeric Index (CV_{CI})	$\frac{S\ CI}{X\ F\%}$	(25)
Total Form factor (TF %)	$\left(\frac{\sum S}{\sum CL}\right) x\ 100$	(26)
Inter-chromosomal asymmetry index (A_2)	$S_{CL}/\ \underline{X}_{CL}$	(27)
Intra-chromosomal asymmetry index (A_1)	$1-\frac{\sum_{i=1}^{n}\ \frac{bi}{Bi}}{n}$	(27)
Degree of karyotype asymmetry (A)	$\frac{\left\lfloor\frac{\sum L-S}{L+S}\right\rfloor}{n}$	(28)
Coefficient of variation of chromosome length (CV_{CL})	A_2 x 100	(25)
Mean Centromeric Asymmetry (M_{CA})	A x 100	(29)
Karyotype asymmetry index (Ask %)	$\frac{(N1-N2)}{(N1+N2)}$ x 100	(30)
The index of karyotype symmetry (Syi %)	[(2n - D) / (2n)] x 100	(31)
The index of chromosomal size resemblance (Rec)	1 - Σ(|ai - bi|) / (2n)	(31)
The dispersion index (DI)	Σ(d2i) / [(n-1) x Σ(Li)2]	(32)

In the present investigation, the somatic chromosome number of *Stemona tuberosa* Lour. was recorded as 2n = 14 (Fig. 1a) having 2 chromosomes bearing secondary constriction. The Ag-NOR study shows a maximum of 2 nucleoli present in somatic cells (Fig. 1d).

Furthermore, a meiotic chromosome study also reveals seven bivalents (Fig. 1e) in pollen mother cells.

Based on the size and centromeric position, chromosomes are classified into 2 distinct morphological types (33) (Fig. 2a and 2b).

Type A: Chromosomes are medium in size, ranging from (4.96 µm to 3.52 µm), bearing 2 constrictions, the primary, which is median in position (m) and the secondary, which is sub-terminal (st) in position.

Type B: Medium to small-sized chromosomes range from (3 µm to 2.08 µm) with nearly median centromeric (m) position.

The numerical data of the karyotype analysis (Table 2)

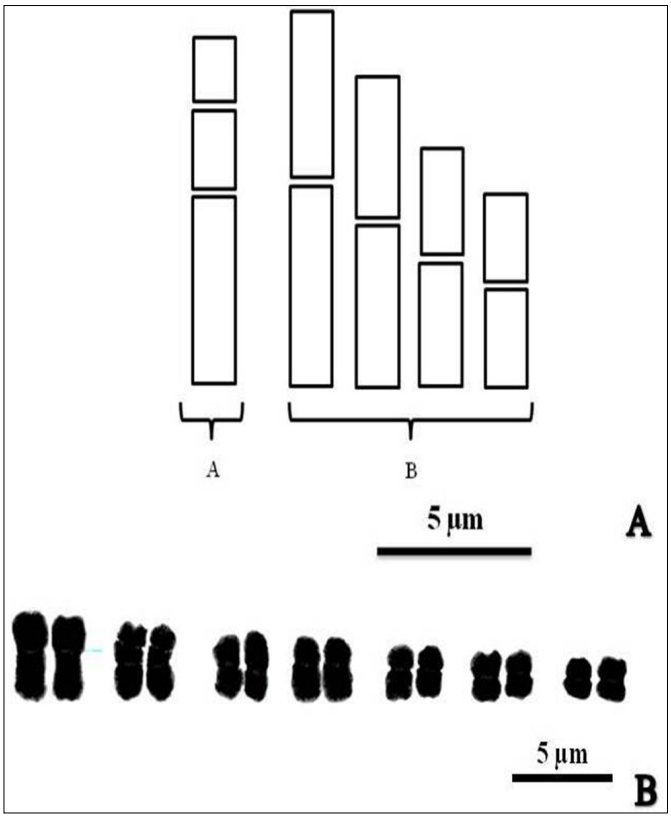

Fig. 2. (A) Ideogram of somatic metaphase chromosomes showing chromosome types of *S. tuberosa*. **(B)** Karyogram of the corresponding mitotic metaphase plate of *Stemona tuberosa* Lour. Karyotype formula : $\mathbf{A_2}$(2m) $\mathbf{B_{12}}$(12m).

are summarized below to understand the chromosomal characteristics of this species.

Somatic chromosome number 2n=14, Number of chromosomes having secondary constriction -2; Ranges of chromosome length - (2.08 µm to 4.26 µm); Total chromosome length- 38.84 µm; Ratio of largest and smallest chromosome- 2.05; Mean arm ratio (L/S) -; Karyotype formula $\mathbf{A_2}$ (2m) $\mathbf{B_{12}}$ (12m); Stebbins categorisation-1B; TF% -; CV_{CL} – 26.61; M_{CA} - 11.52.

Table 2. Numerical data of the karyotype of *Stemona tuberosa* Lour

Chromosome pair	Long arm length (µm)	Short arm length (µm)	Total Length (µm)	F %	Centromeric position	Arm ratio (L/S)	Relative length (%)	Types of chromosome
1	2.4 ± 0.40	1.86 ± 0.29	4.26 ± 0.59	43.66	m	1.29	21.97	A
2	1.57 ± 0.25	1.43 ± 0.17	3.00 ± 0.28	47.67	m	1.10	15.47	B
3	1.59 ± 0.14	1.26 ± 0.10	2.85 ± 0.09	44.21	m	1.26	14.70	B
4	1.54 ± 0.15	1.06 ± 0.09	2.60 ± 0.07	40.77	m	1.45	13.41	B
5	1.25 ± 0.09	1.09 ± 0.11	2.34 ± 0.07	46.58	m	1.15	12.07	B
6	1.19 ± 0.13	1.07 ± 0.05	2.26 ± 0.13	47.35	m	1.11	11.66	B
7	1.24 ± 0.10	0.84 ± 0.03	2.08 ± 0.09	40.38	m	1.48	10.73	B

Table 3. Detail karyotype parameters of *S. tuberosa* Lour

Karyological Indices	CV_{CI}	CV_{CL}	M_{CA}	Ask%	TF%	Syi%	Rec	A_1	A_2	A	DI	AI
S. tuberosa	9.46 ± 1.21	26.61 ± 6.62	11.52 ± 0.67	55.77 ± 0.39	44.23 ± 0.39	79.32 ± 1.27	64.77 ± 6.22	0.20 ± 0.01	0.27 ± 0.07	0.11 ± 0.01	11.64 ± 3.32	2.49 ± 0.54

Discussion

The observed somatic chromosome counts for *Stemona tuberosa* Lour. is 2n = 14, with a basic chromosomal number of X = 7, aligning with findings from other *Stemona* species and corroborating previous research (20, 22, 34, 35). In contrast, *S. curtisii*, another species within the genus, exhibits a variable somatic chromosome count of 2n = 13-16 (21).

In *Stemona tuberosa*, all chromosomes were characterized as nearly metacentric (m) with an arm ratio (L/S) of less than 2:1. The ratio of the largest to the smallest chromosome was determined to be 2.05, exceeding the 2:1 threshold, thus categorizing the karyotype within Stebbins' 1B category. The inter- and intra-chromosomal asymmetry, evaluated using Stebbins' quali-quantitative method (36, 37), along with the absence of acrocentric or telocentric chromosomes, indicates a high degree of chromosomal homology. This homology contributes to the symmetrical nature of the karyotype.

Despite limited reports on the somatic chromosome count of this species and other members of the Stemonaceae family, detailed karyo-morphological data on chromosomal architecture remains sparse. In our present study, most chromosomes were found to be short in size, with a single pair of medium-sized chromosomes exhibiting secondary constriction (Fig. 2a). Additionally, Ag-NOR studies revealed the presence of 2 nucleoli in silver-impregnated somatic cells, providing concrete evidence of one pair of chromosomes containing satellite DNA.

Meiotic chromosome studies conducted on pollen mother cells revealed the presence of seven bivalents, as depicted in Fig. 1e, indicating a haploid chromosome number, n = 7. This finding suggests that *Stemona tuberosa* Lour. exhibits regular chromosome pairing and segregation during meiosis. The presence of 7 bivalents and their orderly segregation during anaphase demonstrates normal meiotic behavior in this species.

The association of meiotic chromosomes provides insights into chromosomal homology. Synchronous disjunction during meiosis is crucial for genome stability (38, 39). Several researchers have reported that improper alignment and abnormal segregation of chromosomes during gamete formation can lead to sterility or polyploidy (40, 41). Additionally, meiotic abnormalities can induce morphological and genetic variations, influencing the evolution and intraspecific reproductive barriers (38, 42).

Similar to *Stichoneuron membranaceum* (24), the karyotype of *Stemona tuberosa* Lour. is nearly identical, with a karyotype formula of A2 (2 m) B12 (12 m), indicating its symmetrical nature. This symmetry suggests stable karyomorphological characteristics without structural alterations in the genome. Beyond the Stebbins asymmetry index, additional intra-chromosomal and inter-chromosomal asymmetry indices have been presented to provide a more comprehensive characterization of the chromosome makeup of this species (Table 3). Karyological parameters such as CVCL, CVCI, MCA, A1, TF%, ASK% and Syi% are essential for elucidating the evolutionary position of a species (43) and provide a foundation for comparative studies within the genus.

From the detailed karyotype data, CVCI, MCA and A1 were found to be 9.46 ± 1.21, 11.52 ± 0.67 and 0.20 ± 0.0 respectively, indicating a low degree of variation at the intra-chromosomal level. These findings are supported by other asymmetry indices like TF%, ASK % and Syi%, which showed higher values of 44.23 ± 0.39, 55.77 ± 0.39 and 79.32 ± 1.27 respectively. However, some degree of inter-chromosomal asymmetry was observed with elevated values of CVCL and Rec, at 26.61 ± 6.62 and 64.77 ± 6.22 respectively. This study represents the first comprehensive illustration of the karyomorphology of *Stemona tuberosa*, employing various symmetry and asymmetry parameters, which may serve as a baseline for understanding the chromosomal architecture and karyotype formulas of related genera.

Conclusion

The genus, *Stemona* has very limited karyological information. Given its threatened status, a comprehensive and

well-formulated set of data is of significant value. The current study determines to provide an accurate and precise delineation of the karyomorphological data of this species. In this present study, *Stemona tuberosa* Lour. has been found to have somatic chromosome number 2n=14, having the karyological formula A_2B_{12}. This is the first report on the detailed karyomorphological characteristics of *Stemona tuberosa* Lour. Although it is not a conclusive explanation, this observation could suggest that the plant has not undergone significant evolutionary changes and may be at risk of extinction. These findings contribute to our understanding of the genetic makeup and cytological features of this species, which can have implications for its taxonomy, evolutionary relationships and breeding programs, among other areas of research.

Acknowledgements

The authors of the paper extend profound gratitude to the UGC-Non-NET fellowship for the funding of the work. They also would like to acknowledge gratefulness to the head of the department, department of Botany, Tripura University, for providing laboratory support.

Authors' contributions

SA and RKS have designed the experiment. SA, AKS and SS have carried out the karyotype experiments. AKS and SA did the imaging and measurement work. SA, AKS and KC worked on the data analysis. All the authors were involved in the writing of the article.

Compliance with ethical standards

Conflict of interest: Authors declare there is no conflict of interest.

Ethical issues: None

References

1. Duyfjes B. Stemonaceae. Flora Malesiana-Series 1, Spermatophyta. 1993 Jan 1;11(2):399-409.
2. Kubitzki K. Stemonaceae. In: Flowering Plants· Monocotyledons: Lilianae (except Orchidaceae). Berlin, Heidelberg: Springer Berlin Heidelberg; 1998.pp. 422-25. https://doi.org/10.1007/978-3-662-03533-7_53
3. Duyfjes B. Stemonaceae and PentaStemonaceae; with miscellaneous notes on members of both families. Blumea: Biodiversity, Evolution and Biogeography of Plants. 1991 Jan 1;36(1):239-52.
4. Greger H. Structural relationships, distribution and biological activities of *Stemona* alkaloids. Planta Medica. 2003;72:99-113. https://doi.org/10.1055/s-2005-916258
5. Kaltenegger E, Brem B, Mereiter K, Kalchhauser H, Kählig H, Hofer O, et al. Insecticidal pyrido [1, 2-a] azepine alkaloids and related derivatives from *Stemona* species. Phytochemistry. 2003 Aug 1;63(7):803-16. https://doi.org/10.1016/S0031-9422(03)00332-7
6. Pilli RA, Rosso GB, de Oliveira MD. The chemistry of *Stemona* alkaloids: An update. Natural Product Reports. 2010;27(12):1908-37. https://doi.org/10.1039/c005018k
7. Singh B, Kumar Borthakur S, Phukan SJ, Kumar Sinha B. Assessing ethnobotanical values and threat status of wild asparagus (*Stemona tuberosa* Lour.): A case study in Eastern Himalaya, India. International Journal of Conservation Science. 2012 Oct 1;3(4). https://doi.org/10.11609/JoTT.o2751.2277-94
8. Song Y, Wu Y, Li X, Shen Y, Ding Y, Zhu H, et al. Protostemonine attenuates alternatively activated macrophage and DRA-induced asthmatic inflammation. Biochemical Pharmacology. 2018 Sep 1;155:198-206. https://doi.org/10.1016/j.bcp.2018.07.003
9. Ramli RA. Phytochemical and biological studies on selected *Stemona* and *Stichoneuron* species (Stemonaceae). Doctor of Philosophy Thesis, School of Chemistry, University of Wollongong, 2015;2015. https://ro.uow.edu.au/theses/4441
10. Chung HS, Hon PM, Lin G, But PP, Dong H. Antitussive activity of *Stemona* alkaloids from *Stemona tuberosa*. Planta Medica. 2003 Oct;69(10):914-20. https://doi.org/10.1055/s-2003-45100
11. Fang L, Song XQ, He TT, Zhu KK, Yu JH, Song JT, et al. Two new polyketides from the roots of *Stemona tuberosa*. Fitoterapia. 2018 Sep 1;129:150-53. https://doi.org/10.1016/j.fitote.2018.06.025
12. Mao AA, Hynniewta TM, Sanjappa M. Plant wealth of Northeast India with reference to ethno botany. 2009;8(01):96-103.
13. Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. Biodiversity hotspots for conservation priorities. Nature. 2000 Feb 24;403(6772):853-58. https://doi.org/10.1038/35002501
14. Deb DB. The Flora of Tripura state: Vegetation and Ophioglossaceae-Staphyleaceae. Today and Tomorrow's Printers and Publishers; 1981.
15. Sakata K, Aoki K, Chang CF, Sakurai A, Tamura S, Murakoshi S. Stemospironine, a new insecticidal alkaloid of *Stemona japonica* Miq. Isolation, structural determination and activity. Agricultural and Biological Chemistry. 1978 Feb 1;42(2):457-63. https://doi.org/10.1080/00021369.1978.10862996
16. Xu YT, Hon PM, Jiang RW, Cheng L, Li SH, Chan YP, et al. Antitussive effects of *Stemona tuberosa* with different chemical profiles. Journal of Ethnopharmacology. 2006 Nov 3;108(1):46-53. https://doi.org/10.1016/j.jep.2006.04.022
17. Yang YE, Guo-Wei Q, Ren-Sheng X. Alkaloids from *Stemona tuberosa*. Phytochemistry. 1994 Nov 7;37(4):1201-03. https://doi.org/10.1016/S0031-9422(00)89558-8
18. Schinnerl J, Brem B, But PP, Vajrodaya S, Hofer O, Greger H. Pyrrolo-and pyridoazepine alkaloids as chemical markers in *Stemona* species. Phytochemistry. 2007 May 1;68(10):1417-27. https://doi.org/10.1016/j.phytochem.2007.03.002
19. Xu YT, Shaw PC, Jiang RW, Hon PM, Chan YM, But PP. Antitussive and central respiratory depressant effects of *Stemona tuberosa*. Journal of Ethnopharmacology. 2010 Apr 21;128(3):679-84. https://doi.org/10.1016/j.jep.2010.02.018
20. Oginuma K, Horiuchi K, Fukuhara T. Karyomorphology of two genera in Stemonaceae. Acta Phytotaxonomica et Geobotanica. 2001 Jul 30;52(1):57-63. https://doi.org/10.18942/apg.KJ00003256634
21. Li LC. Chromosome observations of some plants of China. Guihaia. 1986;6:99-105.
22. Adhikari S, Sinha S, Sinha RK. Chromosomal characteristics in two different sexual phenotypes of *Stichoneuron membranaceum*. Vegetos. 2020 Mar;33:26-30. https://doi.org/10.1007/s42535-019-00077-6
23. Sharma AK, Sharma A. Chromosome technique theory and practical. Third Edition. Butter works Ltd. London. 1980. https://doi.org/10.1016/B978-0-408-70942-2.50003-1
24. Morenodiazdelaespina S, Fernandezgomez M, Risueno M. Occurrence of nucleolar material in the cytoplasm of plant cells.

Cell Biology International Reports. 1979;3(3):215-25. https://doi.org/10.1016/0309-1651(79)90034-1

25. Paszko B. A critical review and a new proposal of karyotype asymmetry indices. Plant Systematics and Evolution. 2006 Apr;258:39-48. https://doi.org/10.1007/s00606-005-0389-2
26. Huziwara Y. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster. American Journal of Botany. 1962 Feb;49(2):116-19. https://doi.org/10.1002/j.1537-2197.1962.tb14916.x
27. Zarco CR. A new method for estimating karyotype asymmetry. Taxon. 1986 Aug;35(3):526-30. https://doi.org/10.2307/1221906
28. Watanabe K, Yahara T, Denda T, Kosuge K. Chromosomal evolution in the genus *Brachyscome* (Asteraceae, Astereae): statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information. Journal of Plant Research. 1999 Jun; 112:145-61. https://doi.org/10.1007/PL00013869
29. Peruzzi L, Eroğlu HE. Karyotype asymmetry: again, how to measure and what to measure? Comparative Cytogenetics. 2013;7(1):1. https://doi.org/10.3897/compcytogen.v7i1.4431
30. Arano H. Cytological studies in subfamily Carduoideae (Compositae) of Japan. IX. The karyotype analysis and phylogenetic considerations on *Pertya* and *Ainsliaea*. Botanical Magazine (Tokyo). 1963;76:32-39. https://doi.org/10.15281/jplantres1887.76.32
31. Greilhuber J, Speta F. C-banded karyotypes in the *Scilla hohenackeri* group, *S. persica* and *Puschkinia* (Liliaceae). Plant Systematics and Evolution. 1976 Jun;126:149-88. https://doi.org/10.1007/BF00981669
32. Lavania UC, Srivastava S. A simple parameter of dispersion index that serves as an adjunct to karyotype asymmetry. Journal of Biosciences. 1992 Jun;17:179-82. https://doi.org/10.1007/BF02703503
33. Levan A, Fredga K, Sandberg AA. Nomenclature for centromeric position on chromosomes. Hereditas. 1964 Dec; 52(2):201-20. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x
34. Hartl M, Kiehn M. Chromosome numbers and other karyological data of four *Stemona* species (Stemonaceae) from Thailand. Blumea-Biodiversity, Evolution and Biogeography of Plants. 2004.Dec.10;49(2-3):457-60. https://doi.org/10.3767/000651904X484405
35. Kiehn M, Temsch EM, Pernausl LA, Hofbauer M, Chen G, Vajrodaya S, Schinnerl J. New chromosome counts and other karyological data for members of the Stemonaceae. Blumea-Biodiversity, Evolution and Biogeography of Plants. 2021 Jul 31;66(1):53-56. https://doi.org/10.3767/blumea.2021.66.01.02
36. Stebbins GL. Chromosomal evolution in higher plants. Chromosomal Evolution in Higher Plants. 1971 December 1;216. https://doi/full/10.5555/19711606614
37. Phukan HD, Saha K. A study on genetic relationship between *Allium sativum* L. and *Scadoxus multiflorus* (Martyn) Raf. of Amaryllidaceae. Plant Science Today. 2019 Oct 6;6(4):491-94. https://doi.org/10.14719/pst.2019.6.4.605
38. Kaur D, Singhal VK. Meiotic abnormalities affect genetic constitution and pollen viability in dicots from Indian cold deserts. BMC Plant Biology. 2019 January 7;19(10): https://doi.org/10.1186/s12870-018-1596-7
39. Sybenga J. Meiotic configurations. Berlin: Springer-Verlag. 1975;1:220-29. https://doi.org/10.1007/978-3-642-80960-6_4
40. Sharma SK, Bisht MS, Pandit MK. Synaptic mutation-driven male sterility in *Panax sikkimensis* Ban. (Araliaceae) from Eastern Himalaya, India. Plant Syst Evol. 2010 April 9;287:29-36. https://doi.org/10.1007/s00606-010-0286-1
41. Bernardo Filho RA, Santos ACC, Souza FHD, Valls JFM, Pagliarini MS. Complete asynapsis resulting in 2n pollen formation in *Paspalum jesuiticum* Parody (Poaceae). Genet Mol Res. 2014 January 17;13:255-61. https://doi.org/10.4238/2014.January.17.9
42. Wani AA, Bhat TA. Asynaptic and desynaptic in plants, Chapter in book, Chromosome Structure and Aberrations; 2017. 127-40. https://doi.org/10.1007/978-81-322-3673-3_6
43. Basu S. Elucidating karyotype structure and affinity through application of karyomorphological parameters and multivariate analysis, as discerned from the study of four important legumes. The Nucleus. 2023 Apr; 66(1):39-46. https://doi.org/10.1007/s13237-023-00416-8 .