



ISSN: 2348-1900

Plant Science Today

<http://www.plantsciencetoday.online>



Research Communication

Karyotype analysis of *Solanum torvum* Sw. - an ethnobotanical Solanaceous species of Tripura, North East India

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Article history

Received: 06 September 2018
Accepted: 14 October 2018
Published: 12 November 2018

Editor

Dr Nishikant Wase, University of Nebraska Lincoln, USA

Publisher

Horizon e-Publishing Group

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Abstract

Solanum torvum Sw. is a wild Solanaceous plant species, commonly used by the indigenous people of Tripura. Cytological study of the species was carried out to determine the somatic chromosome number and to construct the karyotype formula. The detailed karyomorphological analysis revealed $2n=24$ somatic chromosomes having haploid number $n=12$. The size of chromosomal complement was found to range from 2.14 ± 0.21 to 4.02 ± 0.26 μm with a pair of chromosomes bearing secondary constrictions. Strictly median primary constriction was recorded in two pairs of chromosomes. In general, karyotype formula was found to be $A_2B_4C_{18}$. The detailed karyotype analysis revealed that chromosomes are generally small in size and fall under the Stebbins category of "2A" indicating symmetrical nature of the karyotype. The present study could be utilised in understanding the cytogenetic nature of the species and for future crop improvement programme.

Keywords

Solanum torvum; ethnobotany; chromosome number; karyomorphology

Citation

Singha H R, Chowdhury B D, Sinha S, Sinha R K. Karyotype analysis of *Solanum torvum* Sw. - an ethnobotanical Solanaceous species of Tripura, North East India. Plant Science Today 2018;5(4):191-195. <https://dx.doi.org/10.14719/pst.2018.5.4.421>

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Indexing: Plant Science Today is covered by Scopus, CAS, AGRIS, CABI, Google Scholar, etc. Full list at <http://www.plantsciencetoday.online>

Introduction

Solanum torvum Sw. belonging to the family Solanaceae, is one of the important ethnobotanical wild species of Tripura, North East India. The *S. torvum* is mostly distributed in road sides and waste places. Fruits of the species are used as vegetables by the local people and infrequently available in some local markets of Tripura (1, 2, 3, 4) since there is as such no organised cultivation.

Fruits are also used in traditional medicine to cure cough, bronchial asthma, liver and spleen enlargement (5, 6, 7, 8, 9). The species also has pharmaceutical importance due to the presence of a good source of secondary metabolites such as alkaloids, flavonoids, saponins, tannins and glycosides in their wild fruits (10, 11).

Karyotype studies have been extensively carried out in plant phylogenetics and diversity

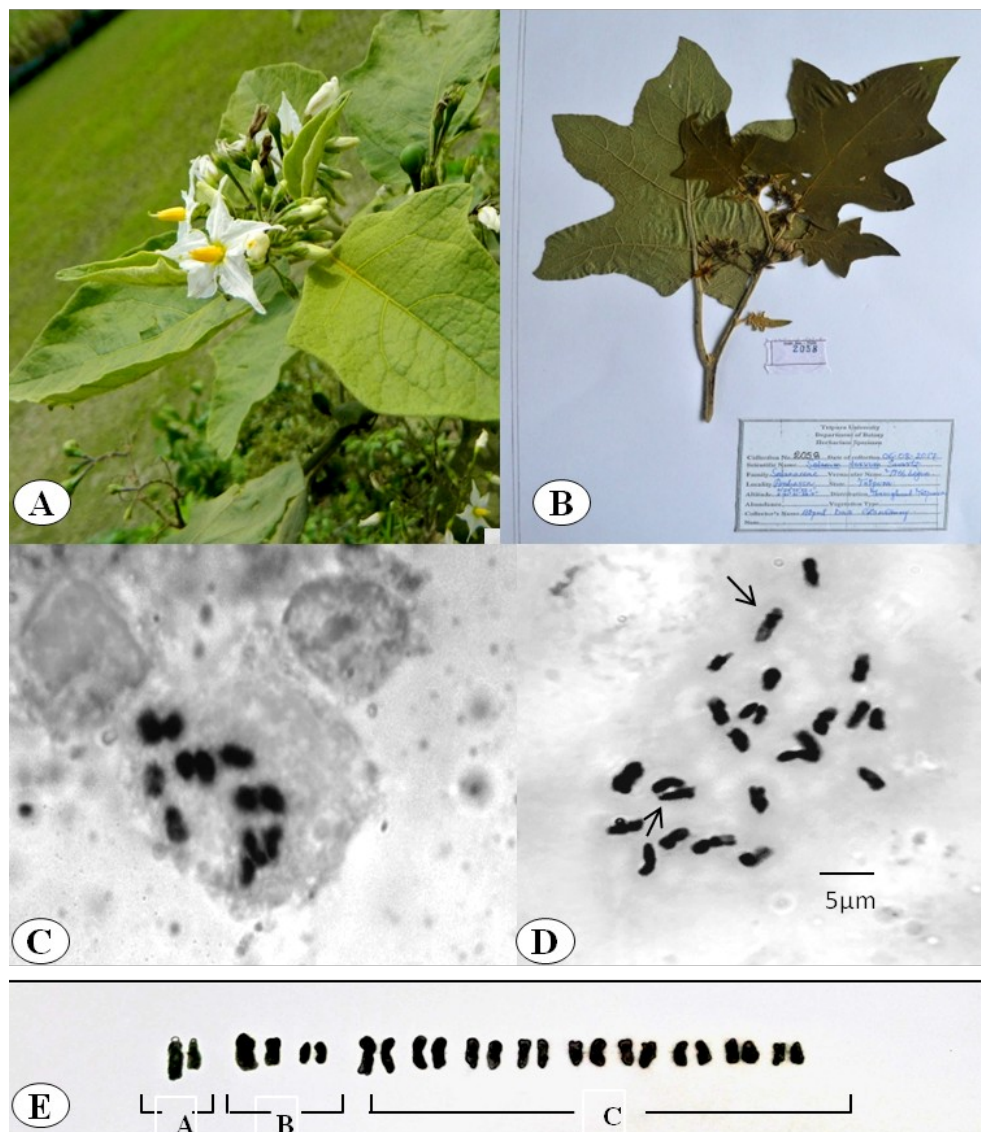


Fig. 1: A. Flowering twig of the *Soanum torvum* Sw.; B. Herbarium of the same; C. Meiotic metaphase plate showing $n=12$ bivalents; D. Mitotic metaphase plate showing $2n=24$ chromosomes; E. Karyogram showing different chromosome types of *S. torvum* (arrow indicating secondary constriction bearing chromosomes)

research. In this era of modern molecular techniques, cytology is still a valuable method for taxonomy and diversity studies (12). The information on the karyotypic features of the species *i.e.* the chromosome number, size, morphology and behaviour of the chromosomes during meiosis and mitosis are important for comparative assessment of the genomic compatibility between species/ varieties for future crop improvement programmes (13). Karyotype analyses have been regarded as an important method for exploring the genomic information of organisms, endowed with the length of chromosome, arm ratio, relative length of chromosome and position of centromeres (14). Chromosomal study and karyotype analysis have also been regarded as an important step for genetic improvement programmes and conservation of wild gene pool (15).

In spite of some ethnobotanical (16), reproductive biology (17) and comparative cytological analyses (18) there is as such no

detailed record on karyomorphological characteristics of the *S. torvum* growing wild in this region. Therefore the present study has been carried out to explore the karyomorphological details of *S. torvum* – a useful Solanaceous wild species of Tripura, North East India.

Materials and Methods

Plant material was collected from the wild population of Ambasa, Dhalai Tripura (Fig. 1A) with a geographical coordinate latitude N $23^{\circ}55'52.1''$ and longitude E $091^{\circ}51'38.9''$ having 91 m elevation. After the collection, the species was identified by detailed morphometric workout and finally compared with the species characters of the sample through consultation with “Flora of Tripura” (19) and herbarium was prepared (Fig. 1B) along with definite collection number TUH-2058 and deposited at the herbarium centre of Department of Botany, Tripura University.

Table 1: Karyomorphological characteristics of *Solanum torvum* Sw.

No of chromosome complements in pairs	*Average total length of chromosomes (μm)	*Average length of short arm (μm)	RLC	Average F%	Nature of primary constriction	Chromosome type
1	4.02 \pm 0.26	0.61 \pm 0.13 1.10 \pm 0.09	10.98	15.20 \pm 3.28 28.06 \pm 1.40	st sm	A
2	3.14 \pm 0.08	1.57 \pm 0.04	8.58	50.00 \pm 0.00	M	B
3	2.14 \pm 0.21	1.07 \pm 0.10	5.84	50.00 \pm 0.00	M	B
4	3.90 \pm 0.40	1.55 \pm 0.03	10.65	40.18 \pm 4.32	m	C
5	3.41 \pm 0.21	1.38 \pm 0.21	9.31	40.48 \pm 5.34	m	C
6	3.20 \pm 0.27	1.45 \pm 0.13	8.74	45.28 \pm 1.35	m	C
7	3.08 \pm 0.00	1.47 \pm 0.14	8.41	47.99 \pm 4.49	m	C
8	2.95 \pm 0.12	1.32 \pm 0.21	8.06	47.72 \pm 5.46	m	C
9	2.95 \pm 0.33	1.21 \pm 0.19	8.06	40.96 \pm 3.14	m	C
10	2.69 \pm 0.23	1.05 \pm 0.05	7.35	39.49 \pm 4.06	m	C
11	2.58 \pm 0.21	1.00 \pm 0.21	7.05	39.13 \pm 5.30	m	C
12	2.55 \pm 0.22	1.01 \pm 0.20	6.96	39.37 \pm 5.94	m	C

Note: *Average of five metaphase plates. RLC - Relative length of chromosome. F% - Forma percentage.

Table 2: Summarized karyomorphological details and karyotype category of *S. torvum* Sw.

Ratio of Longest and shortest chromosome length (μm)	Number of chromosomes with arm ratio <2:1	TCL in haploid set (μm)	TF%	Stebbins karyotype category
1.88	22	36.61	41.46	2A

Note: TCL -Total chromosome length. TF% - Total forma percentage.

Meiotic chromosome study of *S. torvum* was carried out by 2% aceto-carmine staining technique using prefixed (overnight fixed) flower buds (0.5 mm in size) between 9 a.m. to 10 a.m. in glacial acetic acid and alcohol mixture in 1:3 ratio. For mitotic chromosome study, root tip meristem was generated by keeping the seedling in normal tap water using conical flask and grown in normal room temperature. After 5-10 days, fresh root tips were emerges out from the seedling and root tips measuring 1-1.5 cm were collected between 10 a.m. to 11 a.m. and pretreated with saturated para-Dichlorobenzene (PDB) and 8-Hydroxyquinoline in 1:1 ratio for 4.30 h. Then the pretreated root tips were fixed in 1:3 acetic acid and ethyl alcohol mixture for overnight. Next day, root tips were treated with 45% acetic acid for 30 min and subsequently root tips were hydrolysed in 1N HCl for 20 min. After proper softening, staining of the root tip cells was done with 2% aceto-orcein (20). After 2 h of staining, root tips were squashed in 45% acetic acid and observed under compound microscope. At least 50 metaphase plates were observed and five well spread metaphase plates were drawn using camera lucida under oil immersion objective and photographs were taken using Sony Cyber shot DSC-W320 camera. Measurements of different chromosomal complements like size of short arm, long arm, forma percentage, relative length of chromosomes (RLC), arm ratio, total chromosome length in

haploid set (TCL), etc were done from camera lucida drawn pictures and presented in a tabular form.

Results and Discussion

Cytological analysis of *S. torvum* clearly revealed $2n=24$ chromosomes (Fig. 1D). Moreover, the presence of 12 regular bivalents during meiosis indicates that its haploid chromosome number is $n=12$ (Fig. 1C). This observation is in accordance with the previous reports (21). The different karyotypic parameters such as total length of chromosomes, length of short arms, total haploid chromosome length, total forma percentage (TF%) and position of centromere were determined and summarized in tabular form (Table 1 and 2). The average length of the largest and smallest chromosomes were found to be $4.02 \pm 0.26 \mu\text{m}$ and $2.14 \pm 0.21 \mu\text{m}$ respectively with a total forma percentage of 41.46%. The range of relative length of chromosome (RLC) varied from $6.96 \mu\text{m}$ to $10.98 \mu\text{m}$ and the total length of the haploid chromosomes was found to be $36.61 \mu\text{m}$. Although there is no difference in somatic chromosome number with the earlier report but the critical examination revealed a characteristic difference in different karyotypic features like size of the chromosome, which ranges from $0.60 \mu\text{m}$ to $3.10 \mu\text{m}$ with a 45.9 total forma percentage (TF%). The relative length of chromosome varies from 1.37

μm to $7.08 \mu\text{m}$ (22). Whereas, according to Oyelana (23), the size of the chromosome of *S. torvum* ranges from $0.94 \mu\text{m}$ to $1.29 \mu\text{m}$. Such kind of variation in different karyomorphological accounts of the species at different locations was also reported by Krishnappa and Chennaveeraiah (24). The species *S. torvum* was characterized with one pair of secondary constriction in the largest chromosome pair and the secondary constriction is associated with the short arm of the chromosome. On the basis of the size of chromosomes and nature of constrictions the chromosomes are classified into following categories (25).

Type A: Chromosome bearing a secondary constriction with an average size $4.02 \pm 0.26 \mu\text{m}$ having one sub-telomeric constriction and another sub-metacentric constriction.

Type B: Chromosomes with an average size range $2.14 \pm 0.21 \mu\text{m}$ to $3.14 \pm 0.08 \mu\text{m}$ having typical metacentric (M) constrictions.

Type C: Chromosomes with an average size range $2.55 \pm 0.21 \mu\text{m}$ to $3.90 \pm 0.40 \mu\text{m}$ having nearly metacentric (m) primary constrictions.

The present karyotype study of the species reveals two pair of strictly median chromosomes (M), 9 pair of nearly median chromosomes (m) and one pair of sub-median chromosome (sm) having sub-telomeric (st) secondary constriction. Thus the karyotype formula of the *S. torvum* is $A_2B_4C_{18}$ (Fig. 1E). In the present study, out of 24 somatic chromosomes, 22 are found to have arm ratio (L/S) less than 2:1 (<2:1). On the other hand the ratio of longest to shortest chromosome was $1.88 \mu\text{m}$, i.e. less than 2:1. Thus according to the Stebbins (26) degree of symmetry karyotype of the *S. torvum* belongs to the 2A category suggesting its symmetrical nature.

Acknowledgement

Authors are highly thankful to the University Grants Commission (UGC), New Delhi, for providing BSR fellowship (No. F. TU/FIN(233)/(P)/12) to carry out the present research work.

Conflict of interest

The authors have declared that there is no conflict of interest.

Authors' contribution

SHR carried out the whole experimental work, data analysis and manuscript preparation, CBD collected the plant material and helped in identification of species, SS and SRK supervised and structured the manuscript for final submission.

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