



RESEARCH COMMUNICATION

Karyomorphological study of *Houttuynia cordata* Thunb.: an ethnobotanical species of Manipuri community of Tripura, India

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ABSTRACT

Houttuynia cordata Thunb. (Saururaceae), has the chromosome number of $2n = 112$ with karyotype formula $A_2+B_{98}+C_{12}$. The size of the chromosomal complement was found to range from 1.52 μm to 3.00 μm with one pair of chromosomes bearing secondary constrictions. The detailed karyotype analysis revealed that chromosomes fall under the Stebbins category of 1A, which indicating slightly asymmetric nature of chromosome. The chromosome tally and conformity of the karyotype in the present study corroborated as a new cytotype being adapted in this area, the north-eastern region of India.

Introduction

Houttuynia cordata Thunb. is the only species in the genus *Houttuynia* and a perennial, aromatic herb belonging to the family Saururaceae. The herb is restricted to moist habitats and propagated by underground stems and through parthenogenesis, sexual reproduction has not been reported (1, 2). The plant is mainly distributed in the Sino-Japanese regions of eastern Asia, ranging from Japan to the Himalayas through the Ryukyu Islands, Taiwan and China to Southeast Asia. It shows morphological variations and is commonly used for both vegetable and medicinal purposes (3). Its extract contain active compounds such as houttuynin and flavonoids (4). *H. cordata* is a common ethnobotanical species to Manipuri community and called as 'Toningkhokin', cosmopolitan in the north-eastern part of India.

Previous cytological work on *H. cordata* by different workers recorded variable somatic chromosome numbers $2n = 24$ (5), 84 (6), 96 (2, 7), 100–104 (8), 36–126 (9), 24–128 (3) having variable basic chromosome number $x = 8, 9, 12$ (3, 10) mostly from China and India. These observations led to the idea for us on the existence of new ecotypes in the taxon. In view of the ethnomedicinal (11) importance as well as an indication of the existence of new cytotypes, a precise cytological study was carried out to explore

the karyomorphological analysis of *H. cordata*, a useful ethnobotanical species of Tripura.

Materials and Methods

Wild germplasm of *Houttuynia cordata* (Fig. 1) in the form of stoloniferous rootstocks was collected from five different parts of the rural and Manipuri community-based areas of Bishalgarh, Sepahijala, Tripura with a geographical coordinate latitude $N23^{\circ}6'39.54''$ and longitude $E91^{\circ}8'16.81''$ having 21.87 m elevation. After a detailed morphological study, the plant species was identified following "Flora of Tripura" (12). Voucher specimens were prepared (RTM/BOT-1106, 1109, 1142, 1217 and 1288) and deposited in the Herbarium Centre (RTMHC) Department of Botany, Rabindranath Thakur Mahavidyalaya, Bishalgarh.

For mitotic chromosome study, shoot tip meristem from five different populations of Bishalgarh was pre-treated with saturated pDB (para-dichlorobenzene) for 5.30 hrs. At 12 °C temp. Then the treated shoot tips were fixed in 1:3 acetic acid and ethyl alcohol mixture at room temperature for overnight. Next day, the shoot tips were kept in 45% acetic acid for 15 mins and followed by hydrolysis in 1N HCl at cold for 15 mins, finally, stained with 2%



Fig. 1. *Houttuynia cordata* Thunb. flowering twig.

aceto-orcein (13). After overnight staining, the shoot tips were squashed in 45% acetic acid and observed under a compound microscope. At least ten metaphase plates were drawn using camera lucida under oil immersion objective (x1460) for each population. Measurements of different chromosomal complements like the size of the short and long arm, relative length of chromosomes (RCL), arm ratio, total chromosome length in the haploid set, TF% (14) etc. were done from camera lucida drawing (Table 1). Meiotic chromosome study of *H. cordata* was carried out by 2% aceto-carmin staining technique using prefixed flower buds. Study of nucleoli in somatic

studied was found to be two (Fig. 2c), which corresponds with the number of somatic chromosomes bearing secondary constriction found in the present investigation. The different karyotypic parameters such as length of the short arm, length of the whole chromosome, total forma percent (TF %) and position of the centromere were determined and summarized in Table 1. The average length of the largest and smallest chromosomes was found to be $3.00 \pm 0.12 \mu\text{m}$ and $1.52 \pm 0.27 \mu\text{m}$ with a total forma percent as 42.21. The total length of the haploid chromosomes was found to be 136.30 ± 2.33 (Table 2).

Although, an earlier report (9) suggested that the *Houttuynia* populations from the Sichuan Province in southwest China present $2n = 36-126$ (36, 54, 72, 80, 81, 82, 83, 84, 86, 88, 90, 126) with $2n = 81$ having the highest rate and $x = 9$ the basic chromosome number. In the populations from Japan, Nepal, Taiwan, Thailand and unknown locations (3), various chromosome numbers have been identified ($2n = 24-128$) (3) and $x = 12$ is first proposed and $x = 8$ has recently been suggested basic chromosome number (3). The present observation on somatic chromosome number in *H. cordata*, $2n = 112$, did not corroborate with the previous findings (3, 9). This may be due to the phenomenon of cytotoxicity observed in the populations of *H. cordata* (4).

Moreover, *H. cordata* populations from different regions showed wide variations of chromosome numbers ($2n = 36-126$ or $24-128$) and then intraspecific polyploidy, but its basic chromosome number is controversial, with $x = 8, 9, 12$ proposed for the samples studied (3). These cytotypes possibly originated from the cytotoxicity reported for the species (4, 9) because it has been shown to be a potential means to conserve the genetic

Table 1. Karyomorphological characteristics of *Houttuynia cordata* Thunb.

Type of Chromosome	No. of Chromosome	*Length of whole arm ($\mu\text{m} \pm \text{SD}$)	*Length of short arm ($\mu\text{m} \pm \text{SD}$)	F%	Nature of primary constriction	Stebbins karyotype category
A	2	2.82 ± 0.31	0.42 ± 0.21 0.63 ± 0.00	14.89 ± 0.37 22.34 ± 0.37	st sm	1A
B	32	3.00 ± 0.12	1.25 ± 0.39	41.66 ± 0.32	nm	
	36	2.52 ± 0.18	1.20 ± 0.52	47.61 ± 0.35	nm	
	30	2.00 ± 0.24	0.80 ± 0.42	40.00 ± 0.37	nm	
C	10	1.72 ± 0.34	0.62 ± 0.42	41.05 ± 0.57	nm	
	2	1.52 ± 0.27	0.62 ± 0.41	40.75 ± 0.63	nm	

Note: *Average of twenty-five metaphase plates (including all the five populations), F%-Forma percentage

cells is confirmed by following slightly modified method of Fernandez-Gomez technique to remove chlorophyll from shoot tips were initially treated with glacial acetic-ethanol mixture (1:3) for 2 hrs (15), to determine the nuclear organizer region of chromosome complement of this taxon.

Results and Discussion

The somatic chromosome complement of *H. cordata* was found to be constant with $2n = 112$ (Fig. 2a, 3) in all the populations studied. Moreover, a meiotic study from flower buds showed 56 pairs of distinct bivalents (Fig. 2b). The maximum number of nucleoli

heterozygosity of gametes (16), additional means for phylogenetic evolution of karyotypes by reducing or increasing the basic series (17, 18), creation of aneuploids and polyploids (19, 20).

In the present investigation, *H. cordata* has the chromosome number of $2n = 112$, characterized by one pair of secondary constriction in one pair of chromosome having the length of $2.82 \pm 0.31 \mu\text{m}$ and secondary constriction is associated with the short arm of the chromosome. Based on the size of the chromosomes and nature of constrictions, the chromosomes are classified into three distinct categories (Table 1). The present study of the species reveals 55 pairs of nearly median chromosomes (nm)

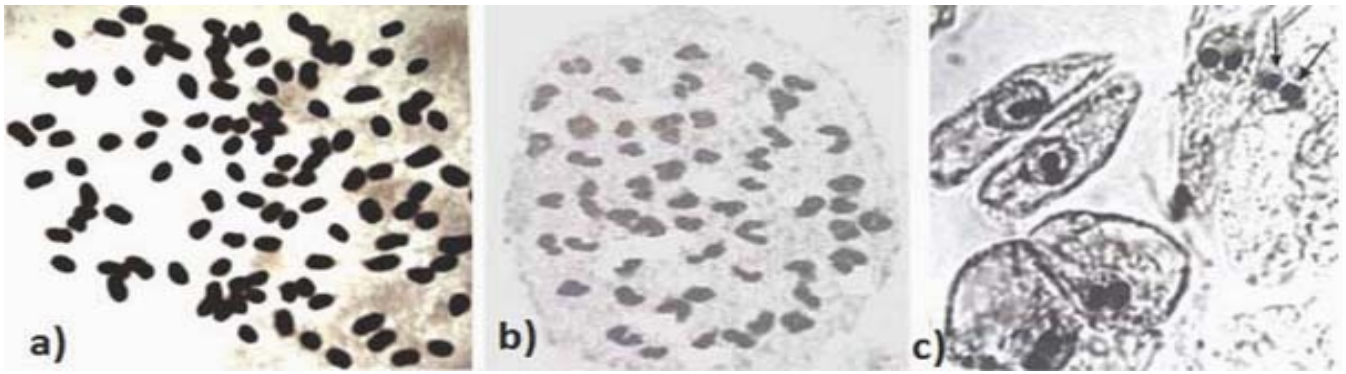


Fig. 2. a) Somatic chromosome $2n=112$ of *Houttuynia cordata* Thunb., b) Meiotic metaphase-I showing 56 distinct bivalents, c) $AgNO_3$ -NOR staining (arrow indicates two distinct nucleoli) .

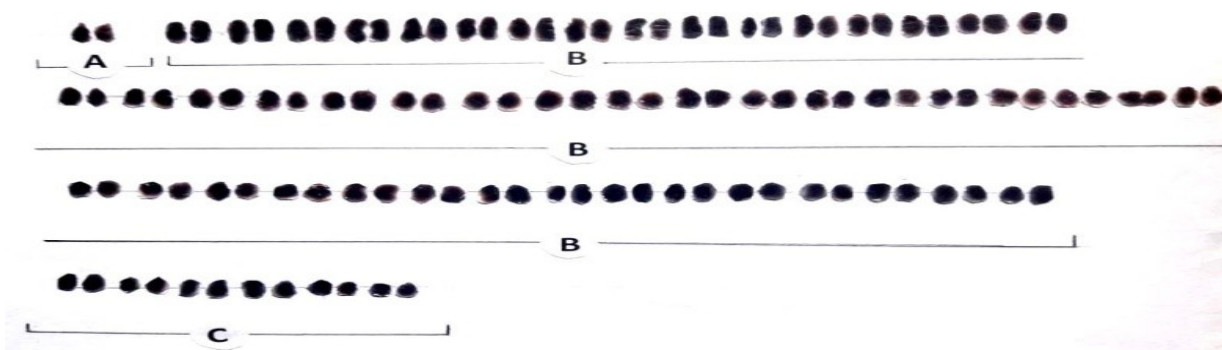


Fig. 3. Karyogram of somatic chromosomes.

Table 2. Summarized karyomorphological details of *Houttuynia cordata* Thunb.

Long / short arm ratio of chromosome (μm)	Chromosome number with arm ratio < 2:1	Total chromosome length in haploid set (μm)	Total forma percentage (TF%)
1.37	110	136.30 ± 2.33	42.21

and one pair with the sub-median chromosome (sm) having sub-telomeric (st) secondary constriction. Therefore, the present cytological investigation on *H. cordata* clearly reveals the somatic chromosome number $2n = 112$ with its characteristic karyotype formula $A_2+B_{98}+C_{12}$. Further, the ratio of long/short arm of the chromosome complements was 1.37 as well as the ratio of longest to shortest chromosome was 1.97 which corresponds the Stebbins karyotype category in *H. cordata* belongs to 1A and to be considered as slightly asymmetric (21). Thus, the present investigation suggests the existence of new cytotype of *H. cordata* growing in Indian sub-continent, particularly in this north-eastern region of India.

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Authors' contributions

MRS and PB carried out the whole experimental work and data preparation. AR supervised the

experimental work and AG supervised overall work and structured the manuscript for final submission.

Conflict of interests

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

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