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CYTOGENETICS OF *HERITIERA FOMES* BANKS: THE DOMINANT PLANT SPECIES OF THE SUNDARBANS MANGROVE FOREST, BANGLADESH

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Heritiera fomes Banks is a dominant mangrove species of the Sundarbans and plays a crucial ecological and economic role. However, its chromosomal characteristics remain poorly understood. In this study, cytogenetic analysis was performed on seedlings collected from different regions of the Sundarbans, Bangladesh. Mitotic chromosome analysis revealed a stable somatic chromosome number of 2n = 38, supporting earlier findings and suggesting a basic chromosome number of x = 19. Karyotype analysis showed a predominantly symmetric complement with a karyotype formula of 2n = 34m + 4sm and total chromosome length of 110.16 ± 2.54 µm. Fluorochrome banding using CMA and DAPI revealed 10 CMA-positive and 12 DAPI-positive bands, mainly distributed in centromeric and terminal regions at the short arms. Heteromorphicity observed in several chromosome pairs indicates minor structural variations such as deletions or duplications. These findings provide the first detailed karyotypic profile of *H. fomes* in Bangladesh and offer valuable cytogenetic markers for future phylogenetic, evolutionary, and conservation studies.

Keywords: Tropical moist forest, chromosomes, karyotypes, CMA, DAPI

INTRODUCTION

Mangroves are salt-tolerant phanerogams with different plant types ranging from shrubs to trees that form a highly specialised forest ecosystem in the world's tropical and subtropical climates abutting protected sea beaches and estuaries. The Sundarbans are the world's biggest mangrove forests, located between India and Bangladesh. These are located on the northern border of the Bay of Bengal, and the Bangladesh portion of the Sundarbans is located between 89°00' –89°55'E and 21°30'–22°30'N (Islam & Bhuiyan 2018). The Sundarbans' flora is diverse, with 66 plant species from 37 families recorded (Chaffey & Sandom 1985). There are four dominant plant species among the flora of the Sundarbans, namely, *Heritiera fomes*, *Excoecaria agallocha* L., *Ceriops decandra* (Griff.) W.Theob. and *Sonneratia apetala* Banks (Anjum et al. 2025). Additionally, some other frequent species are *Nypa fruticans* Wurmb, *Bruguiera* sp. and *Xylocarpus moluccensis* (Lam.) M.Roem. (synonym of *Xylocarpus mekongensis* Pierre) (Anjum et al. 2025). *Heritiera fomes* is the main dominant species, which alone constitutes 64% of the forest area (Chaffey et al. 1985, Rahman 1995). Notably, *H. fomes* has been categorised as "Near Threatened" according to the latest Plant Red List of Bangladesh, highlighting the conservation importance of this keystone mangrove species within the Sundarbans ecosystem (Sultana 2024).

The chromosomal study of mangrove species is challenging due to the difficult soil conditions in which they grow. These conditions make it hard to collect root tips from seedlings, which may explain the limited cytological data available in the literature. Previous studies have reported varying somatic chromosome numbers for different *Heritiera* species. For instance, *H. fomes* and *H. littoralis* were found to have 2n = 28 and 38 chromosomes, suggesting two distinct basic chromosome numbers, x = 14 and x = 19 (Pal 1973, Das et al. 1995, Das et al. 2001). However, Poty and Hamel (1968) reported a different count of 2n = 20 chromosomes for *H. littoralis.* Meanwhile, *H. macrophylla* has consistently shown a stable somatic chromosome number of 2n = 38 in earlier studies (Pal 1973, Das et al. 1995, Das et al. 2001).

Advance research is essential on these ecological and economically important mangrove species for a clearer understanding of their genetic makeup. Earlier cytological studies on the genus *Heritiera* were limited to basic chromosome counts and did not incorporate fluorescence banding techniques. Given the ecological significance of *Heritiera* species in Bangladesh's mangrove forests, it is crucial to investigate them using a comprehensive cyto-molecular approach. Cytological analysis plays a vital role in resolving taxonomic ambiguities and elucidating the interrelationships, affinities, and evolutionary history of plant taxa (Salwana et al. 2025). In such studies, detailed chromosomal morphology is as important as chromosome numbers. Since morphological structures can vary between species, combining conventional cytogenetic methods with fluorescence chromosome banding offers a powerful tool for assessing phylogenetic relationships. Banding techniques using Chromomycin A_3 (CMA) and 4',6-diamidino-2-phenylindole (DAPI) can reveal distinct genomic characteristics and highlight genomic diversity (Schweizer 1976, Alam & Kondo 1995). These banding patterns provide valuable information for species characterisation. However, no comprehensive cytological study has yet been conducted on *H. fomes* in Bangladesh. Therefore, a cytogenetic characterisation of this species using both conventional staining and DNA base-specific banding techniques represents a pioneering effort that will support future conservation and management strategies.

MATERIALS AND METHODS

Ten young seedlings of *H. fomes* were collected from various locations (Hiron Point, Katka, Karamjal) within the Sundarbans mangrove forest, Bangladesh. The seedlings were cultivated in experimental plots at the Botanical Garden of the Department of Botany, University of Dhaka. Each of the ten individual plants was maintained separately and used as experimental material. Healthy root tips were collected and subjected to a sequence of cytological treatments. Initially, the roots were pretreated with 2 mM 8-hydroxyquinoline for 2 hours at 20–25 °C, followed by fixation in 45% acetic acid for 15 minutes at 4 °C. The fixed roots were then hydrolysed for 1 minute at 65°C in a 2:1 mixture of 1 M HCl and 45% acetic acid. One drop of 1% aceto-orcein was applied to the root tips, and the slides were incubated in an acetic acid chamber overnight. Chromosome spreads were prepared using the squash technique with 1% aceto-orcein and examined under a Nikon Eclipse 50i microscope.

As for the CMA and DAPI banding, the procedure from Alam and Kondo (1995) was used with minor modifications on treatment durations. After hydrolysis, root tips were squashed in 45% acetic acid. Cover slips were removed using the dry ice method, and the slides were left to air dry for at least 24 hours. The air-dried slides were pre-incubated in McIlvaine buffer (pH 7.0) for 30 minutes, and gently rinsed with the same buffer supplemented with 5 mM $MgSO_4$ for 15 minutes. For CMA staining, 0.1 mg mL^{-1} CMA was applied for 3 hours in a humid chamber, followed by a 10-minute rinse in the buffer with $MgSO_4$. The slides were then mounted in 50% glycerol and stored at 4 °C overnight before observation using a Nikon Eclipse 50i fluorescence microscope equipped with a blue-violet filter. For DAPI staining, slides were pre-incubated in buffer for 30 minutes after 24 hours of air drying, then immersed in 0.01 mg mL^{-1} DAPI solution for 3 hours. After staining, the slides were rinsed in buffer for 10 minutes, mounted in 50% glycerol, and examined under a fluorescence microscope using an ultraviolet filter cassette.

Centromere positions were determined following the method of Levan et al. (1964). Karyomorphological parameters, including symmetry and asymmetry indices, were calculated using KaryoType software (Altinordu et al. 2016) based on the methodologies of Huziwara (1962), Arano (1963), Greilhuber and Speta (1976), Zarco (1986), Lavania and Srivastava (1992), Watanabe et al. (1999), Paszko (2006) and Peruzzi and Eroğlu (2013).

RESULTS AND DISCUSSION

This study presents a detailed cytogenetic and karyomorphological characterisation of *H. fomes*, a major mangrove tree species native to the Sundarbans of Bangladesh. Utilising both conventional orcein staining and advanced fluorochrome banding (CMA and DAPI), this investigation offers novel insights into the chromosomal organisation and genome structure of this ecologically and economically significant species.

Mitotic analysis confirmed the somatic chromosome number of *H. fomes* as 2n = 38 (Figure 1), which is consistent with earlier reports (Pal 1973, Das et al. 1995, Das et al. 2001). However, Pal (1973) reported 2n = 28 chromosomes, which was different from the present observation. This stability is notable when compared to *H. littoralis*, a closely related species that exhibits more variation in somatic chromosome numbers, ranging from 2n = 20 to 38 (Poty & Hamel 1968, Pal 1973, Das et al. 1995, Das et al. 2001). In contrast, *H. macrophylla* has consistently shown a somatic chromosome number of 2n = 38 across multiple studies (Das et al. 1995, Das et al. 2001), further supporting the trend of karyotypic stability within certain *Heritiera* species. The findings of the present study provide strong evidence that the basic chromosome number for *H. fomes* is x = 19, consistent with its stable diploid count.

The karyotypic analysis revealed a total chromosome length (TCL) of 110.16 ± 2.54 µm, with individual chromosomes ranging in size from 4.95 µm (pair 1) to 1.31 µm (pair 19), and an average chromosome length (ACL) of 2.90 µm (Table 1 & 2). Das et al. (1995, 2001) reported the total chromosome length 72.24 ± 0.15 µm with an average chromosomal length of 1.95 ± 0.06 µm. They also reported the karyotype of *H. fomes* as 2A + 2B + 26C + 8D based on the chromosome size, position of centromere and presence of secondary constriction. In this research, based on centromeric positions, the chromosomes were classified into 34 metacentric (m) and 4 submetacentric (sm) types, resulting in a karyotype formula of 2n = 34m + 4sm (Table 1). Chromosome pairs I and II were classified as submetacentric, with arm ratios exceeding 1.5 and centromeric indices of ~35–37%. All remaining chromosomes were metacentric with centromeric indices near 50% (Table 1). These findings point to a predominantly symmetric karyotype with limited structural differentiation, suggesting that *H. fomes* retains a relatively primitive karyotype organisation. Such symmetry is generally indicative of evolutionary stability and a lack of major chromosomal rearrangements (Stebbins 1971).

The karyotype symmetry was quantitatively assessed using several indices. The intra-chromosomal asymmetry index (A_1) and inter-chromosomal asymmetry index (A_2) were calculated to be 0.08 and 0.26, respectively, suggesting a relatively low level of chromosomal

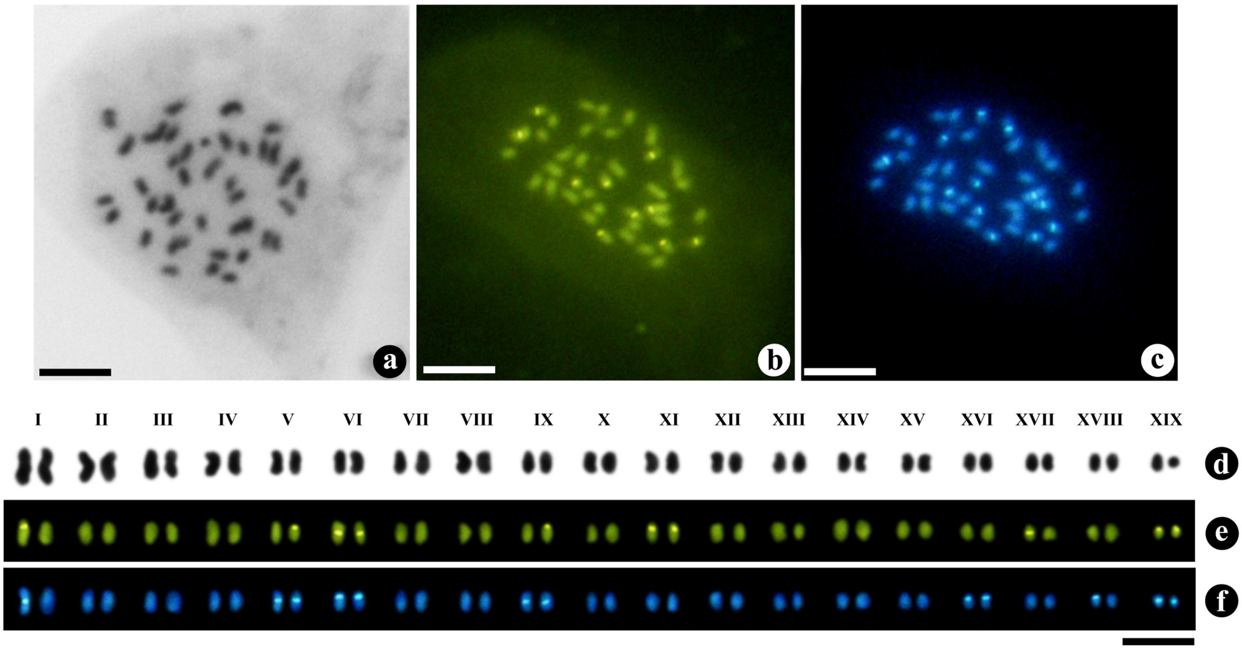


Figure 1 Orcein-, CMA- and DAPI- stained mitotic metaphase chromosomes and karyotypes of *Heritiera fomes,* (a) orcein, (b) CMA, and (c) DAPI-stained mitotic metaphase chromosomes; (d) orcein, (e) CMA, and (f) DAPI-stained karyotype, Bar = 10 µm

Table 1 Length, arm ratio, centromeric index, relative length, centromeric index and centromeric type of mitotic metaphase chromosomes of *Heritiera fomes*

Chromosome pair	Long arm (l) µm	Short arm (s) µm	Total length (T) µm	Arm ratio l/s	Relative length (RL)%	Centromeric index (CI)	Centromeric type (CT)
I	3.09	1.86	4.95 ± 0.22	1.66	4.50	37.57	sm
	3.09	1.86	4.95 ± 0.12	1.66	4.50	37.57	sm
II	2.67	1.43	4.10 ± 0.13	1.87	3.72	34.82	sm
	2.57	1.36	3.93 ± 0.19	1.88	3.57	34.67	sm
III	2.16	1.60	3.76 ± 0.18	1.35	3.41	42.51	m
	2.07	1.55	3.62 ± 0.11	1.34	3.28	42.75	m
IV	1.83	1.57	3.41 ± 0.10	1.17	3.09	46.15	m
	1.76	1.65	3.41 ± 0.14	1.06	3.09	48.46	m
V	1.66	1.60	3.26 ± 0.15	1.04	2.96	49.00	m
	1.66	1.60	3.26 ± 0.17	1.04	2.96	49.00	m
VI	1.56	1.56	3.12 ± 0.15	1.00	2.83	50.00	m
	1.56	1.56	3.12 ± 0.15	1.00	2.83	50.00	m
VII	1.56	1.56	3.12 ± 0.12	1.00	2.83	50.00	m
	1.56	1.55	3.10 ± 0.11	1.01	2.82	49.79	m
VIII	1.47	1.47	2.93 ± 0.09	1.00	2.66	50.00	m
	1.45	1.45	2.91 ± 0.11	1.00	2.64	50.00	m
IX	1.43	1.43	2.86 ± 0.10	1.00	2.59	50.00	m
	1.43	1.43	2.86 ± 0.06	1.00	2.59	50.00	m
X	1.43	1.43	2.86 ± 0.08	1.00	2.59	50.00	m
	1.36	1.34	2.70 ± 0.11	1.02	2.45	49.51	m
XI	1.36	1.34	2.70 ± 0.10	1.02	2.45	49.51	m
	1.36	1.34	2.70 ± 0.16	1.02	2.45	49.51	m
XII	1.36	1.34	2.70 ± 0.11	1.02	2.45	49.51	m
	1.35	1.32	2.67 ± 0.17	1.02	2.43	49.51	m
XIII	1.36	1.27	2.63 ± 0.10	1.07	2.39	48.26	m
	1.36	1.27	2.63 ± 0.14	1.07	2.39	48.26	m
XIV	1.22	1.22	2.44 ± 0.15	1.00	2.21	50.00	m
	1.22	1.22	2.44 ± 0.09	1.00	2.21	50.00	m
XV	1.18	1.05	2.23 ± 0.08	1.13	2.02	47.06	m
	1.18	1.05	2.23 ± 0.05	1.13	2.02	47.06	m
XVI	1.11	1.07	2.19 ± 0.06	1.04	1.99	49.10	m
	1.11	1.07	2.19 ± 0.08	1.04	1.99	49.10	m
XVII	1.11	1.07	2.19 ± 0.05	1.04	1.99	49.10	m
	1.11	1.07	2.19 ± 0.08	1.04	1.99	49.10	m
XVIII	1.11	1.07	2.19 ± 0.04	1.04	1.99	49.10	m
	1.11	1.07	2.19 ± 0.05	1.04	1.99	49.10	m
XIX	1.07	1.07	2.15 ± 0.05	1.00	1.95	50.00	m
	0.66	0.66	1.31 ± 0.12	1.00	1.19	50.00	m
		Total length =	110.16 ± 2.54				

m = metacentric chromosome, sm = submetacentric chromosome

Table 2 Karyological features of *Heritiera fomes*

Karyological features	Unit
Somatic chromosome number (2n)	38
Karyotype formula (KF)	34m + 4sm
Total chromosome length (TCL), µm	110.16 ± 2.54
Average chromosome length (ACL), µm	2.90
Range of chromosomal length (RCL), µm	4.95-1.31
Coefficient of variation of centromeric index (CV_{CI})	9.23
Coefficient of variation of chromosome length (CV_{CL})	26.11
Mean centromeric asymmetry (M_{CA})	4.96
Karyotype asymmetry index (AsK), %	53.30
Total form value (TF), %	46.70
Karyotype symmetry index (Syi), %	87.60
The index of chromosomal size resemblance (Rec), %	58.55
Intra-chromosomal asymmetry index (A_1)	0.08
Inter-chromosomal asymmetry index (A_2)	0.26
Degree of asymmetry of karyotypes (A)	0.05
The asymmetry index (AI)	2.41
The dispersion index (DI)	13.01
Karyotype category	1B
No. of CMA- positive bands	10
Total length of CMA- positive banded region (µm)	4.70 ± 0.19
% of CMA- positive banded region	4.27
CMA- banded karyotypic formulae	$3\alpha+7\varphi+28\delta$
No. of DAPI- positive bands	12
Total length of DAPI- positive banded region (µm)	5.14 ± 0.22
% of DAPI- positive banded region	4.66
DAPI-banded karyotype formulae	$7\alpha+5\varphi+26d$

m = metacentric chromosome, sm = sub-metacentric chromosome, α = Band in centromeric region, φ = Band in short arm, δ = No band

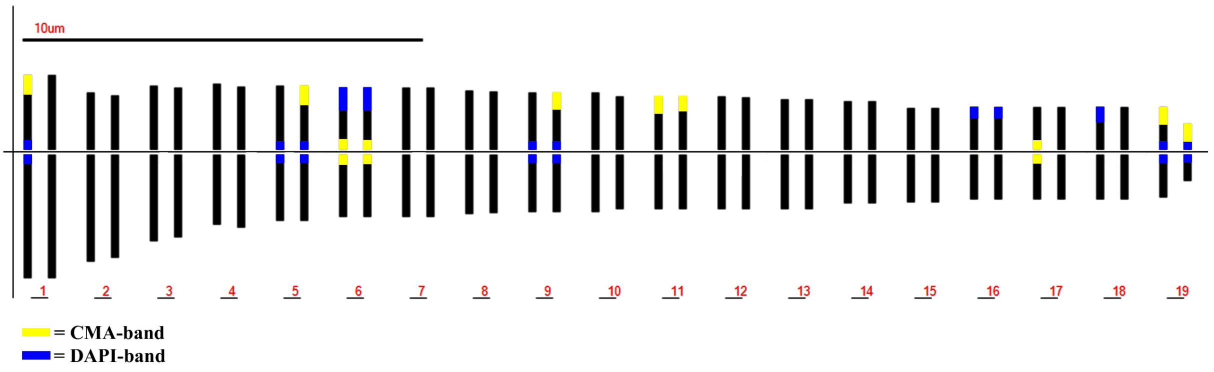
asymmetry (Table 2). The mean centromeric asymmetry (M_{CA}) was 4.96, and the asymmetry index (AI) was only 2.41, which further supports the conclusion of a moderately symmetric karyotype. According to Stebbins' (1971) classification, the karyotype falls under category 1B, reflecting a high level of symmetry with minor asymmetry introduced by a few submetacentric chromosomes. Additionally, the karyotype symmetry index (Syi) was calculated at 87.60%, and the total form value (TF) was 46.70%, both of which emphasise a conserved chromosomal architecture. The coefficient of variation of chromosome length (CV_{CL}) and centromeric index (CV_{CI}) were recorded at 26.11% and 9.23%, respectively, supporting the morphological consistency among most chromosomes (Table 2). The low degree of asymmetry, coupled with the uniformity in centromere positions and chromosome lengths, indicates that *H. fomes* may represent a more ancestral or less derived lineage within the genus.

Fluorochrome banding provided insight into the distribution of GC-rich and AT-rich regions across the chromosomes. The CMA-banding revealed 10 CMA-positive bands (Figure 1), collectively measuring 4.70 ± 0.19 µm, which accounted for 4.27% of the total chromatin length. These bands were primarily located in the centromeric (3α) and short arm (7φ) regions. The CMA-banded karyotypic formula was determined as $3\alpha + 7\varphi + 28\delta$, where δ denotes chromosomes without any detectable bands (Table 2). The DAPI staining, which targets

AT-rich regions, exhibited 12 DAPI-positive bands with a total length of 5.14 ± 0.22 µm, comprising 4.66% of the total chromosome length. The DAPI-banded karyotypic formula was 7α + 5φ + 26δ, indicating that the AT-rich heterochromatin is also prominently distributed in the centromeric and short arm regions of the chromosomes (Figure 1, Table 2). These results suggest a modest but discernible degree of heterochromatin compartmentalisation in the genome of *H. fomes*.

Both CMA and DAPI staining patterns revealed heteromorphicity, suggesting the presence of structural chromosomal rearrangements such as deletions or tandem duplications. These structural variations are typically indicated by asymmetric or unmatched banding patterns between homologous chromosome pairs. The idiogram generated from orcein-, CMA-, and DAPI-stained chromosomes (Figure 2) effectively illustrates the banding profiles and morphometric features, facilitating the accurate identification of homologous pairs. CMA-banding revealed heteromorphicity in chromosome pairs 1, 5, 9, and 17, where one homologue displayed a terminal CMA-positive band, while the other lacked it. A similar pattern of heteromorphicity was observed in the DAPI-banding, particularly at the terminal region of the short arms in chromosome pairs 1 and 18. These differences in banding patterns between homologues likely result from deletion or tandem duplication of heterochromatic sequences, indicating minor structural alterations within the genome of *H. fomes*. The distinct fluorescence banding patterns provide cytological landmarks that may be used in future genomic or phylogenetic studies, especially for distinguishing *H. fomes* from closely related taxa or for studying intraspecific diversity.

The results of this study provide a foundational cytogenetic framework for *Heritiera fomes*. The combination of classical staining and fluorescence banding techniques provides comprehensive information about chromosome morphology and genome organisation. These findings are especially valuable given the scarcity of cytological data for this species in Bangladesh. This work lays the groundwork for further studies into genome evolution, taxonomic relationships, and conservation genetics of mangrove species. Moreover, the clear identification of GC- and AT-rich chromosomal regions can assist in identifying genome-specific markers that may be used in breeding, molecular mapping, and evolutionary studies. The present analysis also contributes to the chromosomal database of mangrove species and reinforces the importance of cytogenetic tools in understanding plant biodiversity and systematics.



Bar = 10 µm

Figure 2 Idiogram of *Heritiera fomes* representing the differential banding patterns in terms of orcein, CMA, and DAPI staining

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REFERENCES

Alam SS & Kondo K. 1995. Differential staining with orcein, Giemsa, CMA and DAPI for comparative chromosome study of 12 species of Australian *Drosera* (Droseraceae). *American Journal of Botany* 82: 1278–1286. https://doi.org/10.2307/2446251.

Altinordu F, Peruzzi L, Yan Y & HE X. 2016. A tool for the analysis of chromosomes: KaryoType. *Taxon* 65: 586–592. http://dx.doi.org/10.12705/653.9.

Anjum S, Hossen N, Islam T & Kamruzzaman M. 2025. Stand structure, species composition and carbon accumulation in the polyhaline zone of the Sundarbans mangrove forest, Bangladesh. *Journal of Tropical Forest Science* 37: 118–129. https://doi.org/10.26525/jtfs2025.37.1.118.

Arano H. 1963. Cytological studies in subfamily Carduoideae (Compositae) of Japan. IX. The karyotype analysis and phylogenetic considerations on *Pertya* and *Ainsliaea*. *Botanical Magazine* 76: 32–39. https://doi.org/10.15281/jplantres1887.76.32.

Chaffey DR & Sandom JH. 1985. *Sundarbans forest inventory project, Bangladesh. A glossary of vernacular plant names and a field key to the trees.* Main report. Overseas Development Administration, Land Resources Development Centre, England.

Chaffey DR, Miller FR & Sandom JH. 1985. *A forest inventory of the Sundarbans, Bangladesh.* Main report. Overseas Development Administration, Land Resources Development Centre, England.

Das AB, Basak UC & Das P. 1995. Karyotype diversity and genomic variability in some Indian tree mangroves. *Caryologia* 48: 319–328. https://doi.org/10.1080/00087114.1995.10797341

Das AB, Mukherjee AK & Das P. 2001. Molecular phylogeny of *Heritiera* Aiton (Sterculiaceae), a tree mangrove: variations in RAPD markers and nuclear DNA content. *Botanical Journal of the Linnean Society* 136: 221–229. https://doi.org/10.1006/bojl.2000.0440

Greilhuber J & Speta F. 1976. C-banded karyotypes in the *Scilla hohenackeri* group, *S. persica* and *Puschkinia* (Liliaceae). *Plant Systematics and Evolution* 126: 149–188. https://doi.org/10.1007/BF00981669.

Huziwara Y. 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of *Aster*. *American Journal of Botany* 49: 116–119. https://doi.org/10.1002/j.1537-2197.1962.tb14916.x.

Islam Smdu & Bhuiyan Mah. 2018. Sundarbans mangrove forest of Bangladesh: causes of degradation and sustainable management options. *Environmental Sustainability* 1: 113–131. https://doi.org/10.1007/s42398-018-0018-y

Lavania UC & Srivastava S. 1992. A simple parameter of dispersion index that serves as an adjunct to karyotype asymmetry. *Journal of Biosciences* 17: 179–182.

Levan A, Fredga K & Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x.

Pal Rk. 1973. Cytotaxonomy of the family Sterculiaceae. *Journal of Cytology and Genetics* 7–8: 98–105.

Paszko B. 2006. A critical review and a new proposal of karyotype asymmetry indices. *Plant Systematics and Evolution* 258: 39–48. https://doi.org/10.1007/s00606-005-0389-2

Peruzzi L & Eroğlu HE. 2013. Karyotype asymmetry: again, how to measure and what to measure. *Comparative Cytogenetics* 7: 1–9. https://doi.org/10.3897/CompCytogen.v7i1.4431.

Poty J & Hamel JL. 1968. Contribution à l'étude caryotaxonomique des Sterculiacées. *Mémoires du Musée d'Histoire Naturelle, Série B* 18: 3–36.

Rahman MA. 1995. *Mangrove plant pathology of the Sundarbans Reserved Forest in Bangladesh.* Field Document No. 3. FAO/UNDP Project BGD/84/056 – Integrated Resource Development of the Sundarbans Reserved Forest, Khulna, Bangladesh.

Salwana HS, Norwati M, Rasip AGA, Mahani MC & Emyza SS. 2025. Morphological and cytological differentiation of *Endospermum diadenum* and *Endospermum quadriloculare*: implications for forest plantation selection. *Journal of Tropical Forest Science* 37: 373–388. https://doi.org/10.26525/jtfs2025.37.4.373.

Schweizer D. 1976. Reverse fluorescent chromosome banding with Chromomycin and DAPI. *Chromosoma* 58: 307–324. https://doi.org/10.1007/BF00292840.

Stebbins GL. 1971. *Chromosomal evolution in higher plants.* Addison-Wesley Publishing Company, California, USA.

Sultana M. 2024. *Heritiera fomes.* Pp 262 in Rahman MO (ed) *IUCN Bangladesh. Plant Red List of Bangladesh.* Volume 2. Bangladesh National Herbarium, Forest Department, Ministry of Environment, Forest and Climate Change and IUCN, International Union for Conservation of Nature and Natural Resources.

Watanabe K, Yahara T, Denda T & Kosuge K. 1999. 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* 112: 145–161. https://doi.org/10.1007/PL00013869.

Zarco RC. 1986. A new method for estimating karyotype asymmetry. *Taxon* 35: 526–530. https://doi.org/10.2307/1221906.