

KARYOTYPE PROFILE OF *HOLARRHENA ANTIDYSENTERICA* (ROXB. EX FLEMING) WALL**CHANDAN KUMAR DASH, NOWSHIN ANJUM, PRASENJIT ROY AND SYEDA SHARMEEN SULTANA****Department of Botany, University of Dhaka, Dhaka 1000, Bangladesh**Keyword: Chromosomes, Karyotypes, Idiograms, CMA, DAPI, Medicinal plants***Abstract**

The karyomorphological features of *Holarrhena antidysenterica* (Roxb. ex Fleming) Wall. through the application of differential staining techniques (using orcein, CMA, and DAPI) were studied. After applying orcein staining, the interphase nuclei of this species exhibited a 'simple chromocenter type' with numerous small heterochromatin blocks and the prophase chromosomes were 'interstitial type'. The karyotype analysis revealed that this species possesses diploid chromosome number of $2n = 22$, with all metacentric chromosomes. The total length of the chromosome complement in the diploid set was $29.91 \mu\text{m}$. A total of four CMA-positive bands were observed in this species whereas seven bands were found after DAPI-staining. Heteromorphicity was observed in the DAPI stained metaphase stage indicating the probable occurrences of chromosomal aberrations such as deletion and inversion. The karyotype profile of *H. antidysenterica* obtained from orcein and fluorescent banding was determined for the first time in Bangladesh.

The useful medicinal plant *Holarrhena antidysenterica* (Roxb. ex Fleming) Wall. belonging to Apocynaceae. This plant has been used for addressing ailments including bronchitis, epilepsy, asthma, piles, leprosy, eczema, diarrhea, fevers, jaundice, scabies (Prajapati *et al.* 2004) and also have antibacterial and antidiarrheal attributes (Bhattacharjee 2000, Ganapathy *et al.* 2008). However, classical cytogenetic analysis focused on characterizing *H. antidysenterica* species was limited to somatic chromosome count and attempted by only a small number of earlier researchers. According to earlier chromosome records, the gametophytic chromosome number is $n = 11$ and somatic chromosome number is $2n = 22$ for this species (Sharma and De 1976, Chauhan and Raghuvanshi 1977, De 1978, Sumita and Roy 2003). Most of these reports were primarily focused on chromosome counts and did not include comprehensive karyotype analysis. Due to its significance and popularity as a medicinal plant, this species proves to be noteworthy for cytogenetic analysis. In this context, understanding the genetic makeup is important for creating effective plans to manage and multiply the species. Despite the medicinal importance of *H. antidysenterica*, no cytogenetic research has been conducted in Bangladesh to characterize this species. Thus, constructing a cytogenetic characterization of this plant is a pioneer attempt that will assist in future conservation and improvement initiatives.

Seedlings of *H. antidysenterica* were collected from Dhaka University campus and maintained in the Botanical Garden, Department of Botany, University of Dhaka. Healthy root tips were collected and treated with 8-hydroxyquinoline (0.002 M) for 4 hrs and then fixed with aceto-alcohol (1:3) solution at 4°C . Slides were prepared by orcein squashed method and examined under Euromax Axion microscope. Alam and Kondo's (1995) method was used with slight modification for CMA- and DAPI banding. Slides were observed under Nikon (Eclipse 50i)

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fluorescent microscope with a blue-violet (BV) filter cassette for CMA-staining and an ultraviolet (UV) filter cassette for DAPI-banding. Idiogram was prepared from combined orcein, CMA and DAPI-staining patterns.

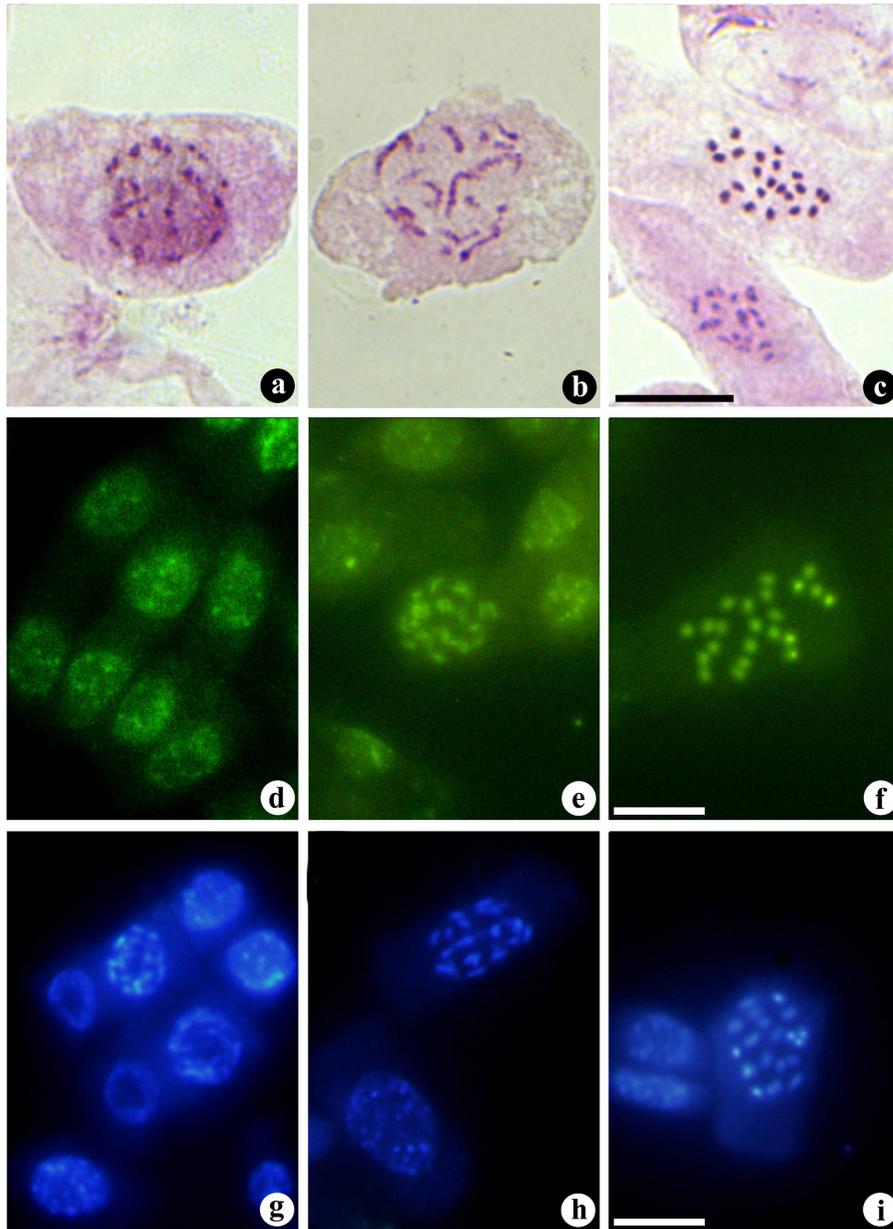


Fig. 1. Orcein-, CMA- and DAPI- stained mitotic interphase nuclei, prophase chromosomes and metaphase chromosomes of *Holarrhena antidysenterica*. Orcein-stained mitotic a. interphase nuclei, b. prophase chromosomes, c. metaphase chromosomes. CMA-stained mitotic d. interphase nuclei, e. prophase chromosomes, f. metaphase chromosomes. DAPI-stained mitotic g. interphase nuclei, h. prometaphase chromosomes, i. metaphase chromosomes. Bar = 10 μ m.

In *H. antidysenterica*, 20-22 small heterochromatic regions were scatteredly distributed around the nucleus (Fig. 1a). A prominent nucleolus occupying about 1/3 portion of nucleus was found in the interphase nuclei of this species (Fig. 1a). The prophase chromosomes were stained darkly at interstitial and terminal regions of the chromosomes with orcein-staining. In the interphase nuclei of *H. antidysenterica*, a range of 11 to 14 CMA-stained blocks were observed (Fig. 1d). Additionally, during the prophase stage, 5 to 8 small, intensely fluorescent CMA bands were seen within the chromosomes of this plant (Fig. 1e). Brightly fluoresced areas (14-18) were observed in the nucleus of the DAPI-stained interphase stage (Fig. 1g). The prophase chromosomes of *H. antidysenterica* were found to possess 3-4 DAPI bands at the interstitial regions of chromosomes (Fig. 1h). There was no visible nucleolus at this stage (Fig. 1b). According to Tanaka's classification (1971), the observed types of interphase nuclei and prophase chromosomes were labeled as 'simple chromocenter' and 'interstitial' type, respectively. This pattern indicated that there was a tendency for the heterochromatin to aggregate in the interstitial regions of the prophase chromosomes. In this investigation, *H. antidysenterica* was found to possess $2n = 22$ chromosomes. This finding supported previous report on $2n$ chromosome count reported by different scientists (Mehra and Bawa 1969, Datta and Maiti 1972, Sarkar 1975, Mehra 1976, Sharma and De 1976, Chauhan and Raghuvanshi 1977, De 1978, Sumita and Roy 2003). Therefore, this species has strict $2n$ chromosome number with basic number $x = 11$. The combined length of the diploid chromosome set in this species measured $29.91 \mu\text{m}$. The relative length of chromosomes varied between 3.70 and 5.69%. Individual chromosome lengths ranged from 1.11 to $1.70 \mu\text{m}$. Chauhan and Raghuvanshi (1977) reported the length of the chromosomes which was found to range from 1.18 to $1.79 \mu\text{m}$. This observation is more or less similar to the results of the present study. The centromeric formula for this species was identified as $22m$. No satellite was observed in this species (Fig. 1c). Considering these parameter *H. antidysenterica* could be suggested as primitive in nature.

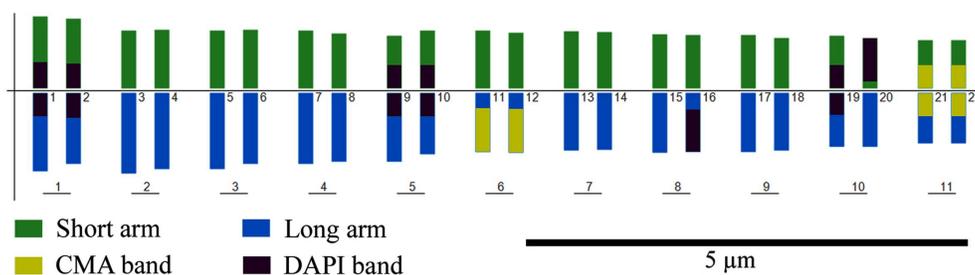


Fig. 2. Idiogram of *Holarrhena antidysenterica* representing the differential karyotype in terms of orcein, CMA and DAPI-banding patterns. CMA and DAPI karyotypic formula were $2\beta + 2\phi + 18\delta$ and $1\alpha + 1\beta + 5\phi + 15\delta$, respectively. α = Band in short arm, β = Band in long arm, ϕ = Band in centromere, δ = No band. Bar = $5 \mu\text{m}$.

Table 1. Karyomorphology of *Holarrhena antidysenterica* L.

2n	Total length of the metaphase chromosomes (μm)	Range of individual chromosomal length (μm)	Range of relative length (%)	Centromeric formula	No. of CMA bands	% of GC-rich repeats	No. of DAPI bands	% of AT-rich repeats
22	29.91	1.11–1.70	3.70–5.69	22m	4	7.09	7	13.17

m = Metacentric chromosome.

A total of four CMA-positive bands were found in four different chromosomes of *H. antidysenterica* (Fig. 1f). These bands indicate the GC-rich regions of the respective chromosomes. The total length of CMA-banded region was 2.12 μm which occupied about 7.09% of the total chromatin length (Fig. 2, Table 1). The CMA banded karyotypic formula for this plant was $2\beta + 2\phi + 18\delta$ as two of the CMA bands were situated in long arms of the chromosomes, other two were observed in the centromeric regions and the rest of the chromosomes did not have any CMA bands (Fig. 2). No heteromorphism was seen regarding CMA banding patterns. In this species, seven DAPI positive bands were found. The DAPI-banded region had a total length of 3.94 μm , which accounted for approximately 13.17% of the entire chromatin length. (Fig. 1i, Table 1). Among the 22 chromosomes, one showed DAPI band in the short arm, one in the long arm, five in centromeric regions and 15 chromosomes had no bands at all. So the DAPI banded karyotypic formula for this plant was $1\alpha + 1\beta + 5\phi + 15\delta$. DAPI-banded chromosomes indicate heteromorphism due to having seven DAPI bands (Fig. 2). Heteromorphism was seen in the 8th and 10th pairs of chromosomes. In chromosome pair 8, presence of one DAPI band in one chromosome and absent in other homologue might be due to deletion of AT rich region from the respective chromosome. On the other hand, the 10th pair of this plant displayed DAPI bands in two different positions, indicating a probable inversion (Fig. 2). All the chromosomes showed either intercalary or terminal bands in both the cases with CMA and DAPI. The present study contributes to enhancing the chromosomal database by providing detailed karyomorphological information specific to this plant species in Bangladesh. The findings of this research can be used as a valuable resource for future research and understanding of the species' chromosomal characteristics in the region.

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