

PATTERNS OF HETEROCHROMATIN DISTRIBUTION IN SIX SPECIES OF ACANTHACEAE

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Abstract

Six medicinally and economically important species of Acanthaceae namely, *Asystasia gangetica* (L.) T. Anderson, *Barleria cristata* L., *B. prionitis* L., *Ruellia simplex* C. Wright, *Thunbergia erecta* (Benth.) T. Anderson and *Thunbergia mysorensis* (Wright) T. Anderson were cytogenetically investigated for authentic characterization based on heterochromatin condensation and chromosomal behavior. *Asystasia gangetica* and *R. simplex* exhibited 'Diffuse type' of interphase nuclei, characterized by evenly distributed heterochromatin, whereas *B. cristata* and *B. prionitis* showed 'Complex chromocenter type' with large, distinct heterochromatic blocks; in contrast, *T. erecta* and *T. mysorensis* displayed 'Simple chromocenter type' with numerous small heterochromatic spots. The prophase chromosomes of *A. gangetica* and *R. simplex* showed 'Continuous type', whereas the remaining species exhibited 'Interstitial type', reflecting the typical trend of heterochromatin condensation. The species belonging to the same genus showed similar heterochromatic condensation nature with orcein, CMA and DAPI-staining. The CMA and DAPI staining of metaphase chromosomes further revealed distinct species-specific banding patterns. The findings suggest close cytogenetic relationships within the genera based on chromosomal behavior.

The Acanthaceae family is a large pantropical family of herbs and shrubs belonging to the order Lamiales (Scotland *et al.* 1995). This family consists of approximately 240 genera and 3250 species throughout the world (Wasshausen and Wood 2004). So far 39 genera and 107 species under the Acanthaceae have been reported from Bangladesh (Ahmed *et al.* 2008). Among these, *Asystasia gangetica* (L.) T. Anderson, *Barleria cristata* L., *B. prionitis* L., *Ruellia simplex* C. Wright, *Thunbergia erecta* (Benth.) T. Anderson and *Thunbergia mysorensis* (Wright) T. Anderson are most widely distributed in Bangladesh with promising medicinal and ecological values (Ahmed *et al.* 2008). A recent study by Anjum *et al.* (2024) reported the somatic chromosome numbers and karyotype formulae of six Acanthaceae species: *Asystasia gangetica* ($2n = 2x = 24; 22m + 2sm$), *Barleria cristata* ($2n = 4x = 40; 38m + 2sm$), *B. prionitis* ($2n = 4x = 40; 36m + 4sm$), *Ruellia simplex* ($2n = 2x = 36; 34m + 2sm$), *Thunbergia erecta* ($2n = 4x + 6 = 62; 56m + 6sm$), and *T. mysorensis* ($2n = 2x = 34; 34m$). However, distinguishing individual chromosomes using conventional methods remains challenging. Staining features observed in prophase chromosomes and interphase nuclei along with fluorescence banding with Chromomycin A₃ (CMA) and 4',6-diamidino-2-phenylindole (DAPI) fluorochromes have become essential for comparative cytogenetic studies (Tanaka 1971, Schweizer 1976, Sultana and Alam 2016, Dash *et al.* 2018). This study is the first to assess staining properties, heterochromatin condensation, and metaphase banding patterns using orcein, CMA, and DAPI, demonstrating that these techniques can effectively distinguish six studied Acanthaceae species based on cytogenetic features.

This study involved the collection of *Asystasia gangetica* (L.) T. Anderson, *Barleria cristata* L., *B. prionitis* L., *Ruellia simplex* C. Wright, *Thunbergia erecta* (Benth.) T. Anderson and *Thunbergia mysorensis* (Wright) T. Anderson from nurseries in Dhaka City and the Dhaka

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University campus. Obtained root tips were pretreated with 0.002 M 8-hydroxyquinoline for 2 hr at 28–30°C, and fixed in Carnoy's solution (3 : 1 absolute alcohol : glacial acetic acid) at 4°C. Root tips were hydrolyzed in 1N HCl : 45% acetic acid (2 : 1) at 65°C for 8 min, stained with 1% aceto-orcein for 2.5 h, and squashed in order to perform orcein-based cytological analysis. CMA and DAPI staining were performed following the protocol described by Alam and Kondo (1995). CMA- and DAPI-treated slides were observed under a Nikon Eclipse 50i fluorescent microscope with a blue-violet and ultra-violet filter, respectively.

The interphase nuclei and prophase chromosomes of six Acanthaceae species were analyzed using differential staining, examining at least 200 cells per species. In this study, *A. gangetica* and *R. simplex* exhibited 'Diffuse type' of interphase nuclei according to Tanaka (1971), where heterochromatin is homogeneously distributed throughout the nucleus (Figs 1A, D). In contrast, the interphase nuclei of *B. cristata* and *B. prionitis* showed a few large, darkly stained heterochromatic blocks and can be classified as 'Complex chromocenter type' (Figs 1C, D). Moreover, numerous small, dot-like heterochromatic blocks were scattered throughout the nuclei of *T. erecta* and *T. mysorensis*, indicating 'Simple chromocenter type' of interphase nuclei (Figs 1E, F). A clearly visible nucleolus was observed in the nuclei of *A. gangetica*, *B. cristata*, *B. prionitis*, and *R. simplex* following orcein staining (Figs 1A–D). In *A. gangetica*, *T. erecta*, and *T. mysorensis*, several small, brightly fluorescing CMA bands were observed, irregularly distributed throughout the nucleus (Figs 1G, K, L). In *B. cristata*, only a few large regions of the interphase nucleus exhibited strong CMA fluorescence (Fig. 1H). In contrast, *R. simplex* showed only a limited number of faint CMA bands (Fig. 1J), while no distinct CMA bands were detected in *B. prionitis* (Fig. 1I). With DAPI staining, numerous small bright bands were observed throughout the nuclei of *A. gangetica* and *R. simplex* (Figs 1M, P). However, *B. cristata* and *B. prionitis* displayed only a few larger DAPI-positive regions (Figs 1N, O). No prominent DAPI bands were detected in the interphase nuclei of *T. erecta* and *T. mysorensis* (Figs 1Q, R). Overall, the CMA- and DAPI-banding patterns are consistent with the observations obtained from orcein staining.

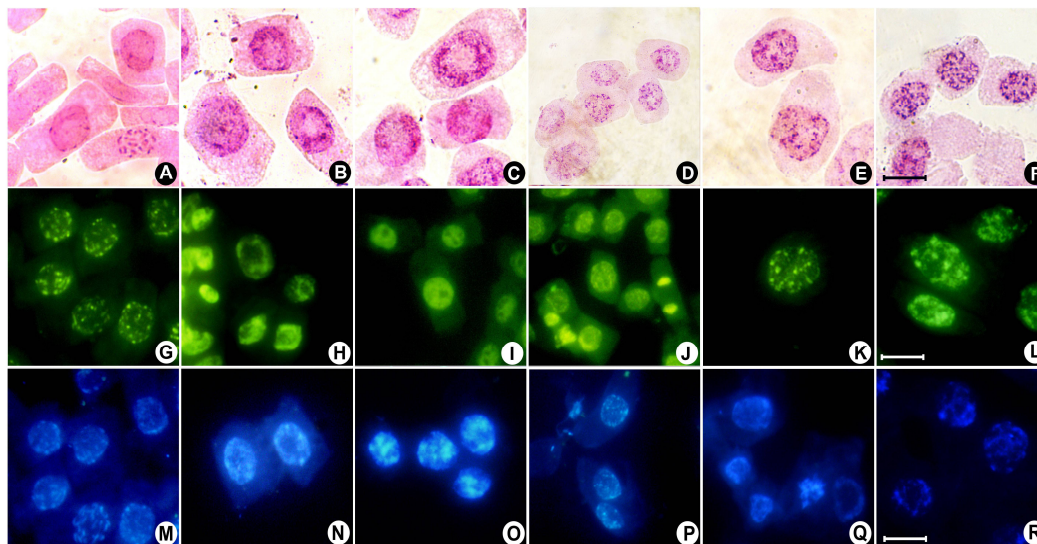


Fig. 1. Orcein-, CMA- and DAPI- stained mitotic interphase nuclei of six species of Acanthaceae; A, G, M: *Asystasia gangetica*, B, H, N: *Barleria cristata*, C, I, O: *Braleria prionitis*, D, J, P: *Ruellia simplex*, E, K, Q: *Thunbergia erecta*, and F, L, R: *Thunbergia mysorensis*. Bar = 10 μ m.

Regarding prophase chromosomes, orcein staining showed that in *A. gangetica* and *R. simplex*, heterochromatin was uniformly distributed along the entire chromosome length (Figs 2A, D). In contrast, the interstitial regions of prophase chromosomes were darkly stained in *B. cristata*, *B. prionitis*, *T. erecta*, and *T. mysorensis* (Figs 2B, C, E, F). According to Tanaka (1971), the prophase chromosomes of *A. gangetica* and *R. simplex* can be classified as the 'Continuous type,' whereas those of *B. cristata*, *B. prionitis*, *T. erecta* and *T. mysorensis* are considered the 'Interstitial type' (Figs 2A-R, Table 1). Bright, dot-like CMA bands were sporadically distributed along the prophase chromosomes of *A. gangetica*, *R. simplex*, *T. erecta* and *T. mysorensis* (Figs 2G, J, K, L).

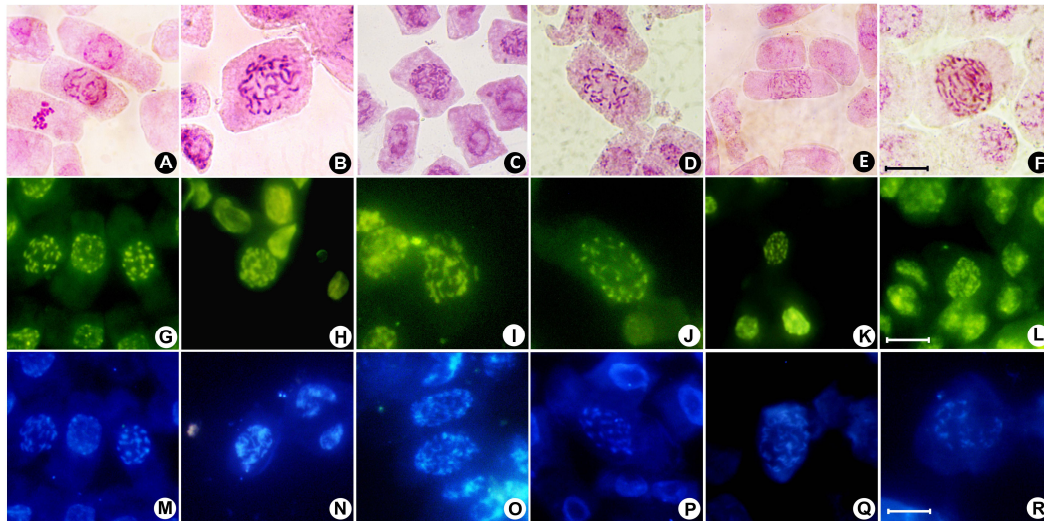


Fig. 2. Orcein-, CMA- and DAPI- stained mitotic prophase chromosomes of six species of aAcanthaceae; A, G, M: *Asystasia gangetica*, B, H, N: *Barleria cristata*, C, I, O: *Braleria prionitis*, D, J, P: *Ruellia simplex*, E, K, Q: *Thunbergia erecta*, and F, L, R: *Thunbergia mysorensis*. Bar = 10 μ m.

However, such bands were absent in *B. cristata* and *B. prionitis* (Figs 2H, I). DAPI staining revealed several small, bright bands in the prophase chromosomes of *A. gangetica*, *B. prionitis*, *R. simplex* and *T. erecta* (Figs 2M, O, P, Q). In *B. cristata*, comparatively larger DAPI bands were observed (Fig. 2N), whereas no prominent DAPI bands were detected in *T. mysorensis* (Fig. 2R).

Table 1. Types of interphase nuclei, prophase chromosomes and fluorescence banding patterns of six species of aAcanthaceae.

Species	Somatic chromosome number (2n)	Types of interphase nuclei	Types of prophase chromosomes	Number of CMA band	Number of DAPI band
<i>Asystasia gangetica</i>	24	Diffuse	Continuous	4	4
<i>Barleria cristata</i>	40	Complex chromocenter	Interstitial	2	5
<i>B. prionitis</i>	40	Complex chromocenter	Interstitial	5	2
<i>Ruellia simplex</i>	36	Diffuse	Continuous	6	3
<i>Thunbergia erecta</i>	62	Simple chromocenter	Interstitial	5	-
<i>T. mysorensis</i>	34	Simple chromocenter	Interstitial	7	5

Usually, species with 'Diffuse type' of interphase nuclei retain 'Continuous type' of prophase chromosomes with homogenous distribution of heterochromatins. In this study, *A. gangetica* and *R. simplex* followed the general rule of heterochromatin distribution. On the other hand, species with 'Simple chromocenter type' of interphase nuclei usually show 'Gradient' or 'Interstitial type' of prophase chromosome. Therefore, *T. erecta* and *T. mysorensis* followed the general rule of heterochromatin distribution. However, heterochromatins are firmly aggregated at large portions of interphase nuclei of *B. cristata* and *B. prionitis* and somehow distributed in several small interstitial regions along the prophase chromosomes which indicated the presence of facultative heterochromatin (Figs 1A-F). Two species of the genus *Barleria* possessed similar nature, while *T. erecta* and *T. mysorensis* were found to have similar tendencies regarding the heterochromatic condensation pattern (Figs 1, 2). The aforementioned patterns represent the closeness of the species of the respective genus. The CMA and DAPI banding further highlighted GC- and AT-rich regions, respectively. Small, strongly fluorescing CMA bands in the nuclei of *A. gangetica*, *T. erecta*, and *T. mysorensis* aggregated into dot-like patterns that were present in their prophase chromosomes (Figs 2G, K, L). In contrast, *R. simplex* exhibited only 4-6 weak CMA bands (Fig. 2J), whereas no CMA bands were detected in *B. prionitis* (Fig. 2I). Small DAPI bands were prominent in the interphase nuclei and prophase chromosomes of *A. gangetica*, *B. prionitis*, and *R. simplex* (Figs 1, 2), while larger DAPI-fluorescing areas characterized *B. cristata* (Figs 1N, 2N). No significant DAPI bands were detected in *T. erecta* and *T. mysorensis* (Figs 1Q, 1R, 2Q, 2R).

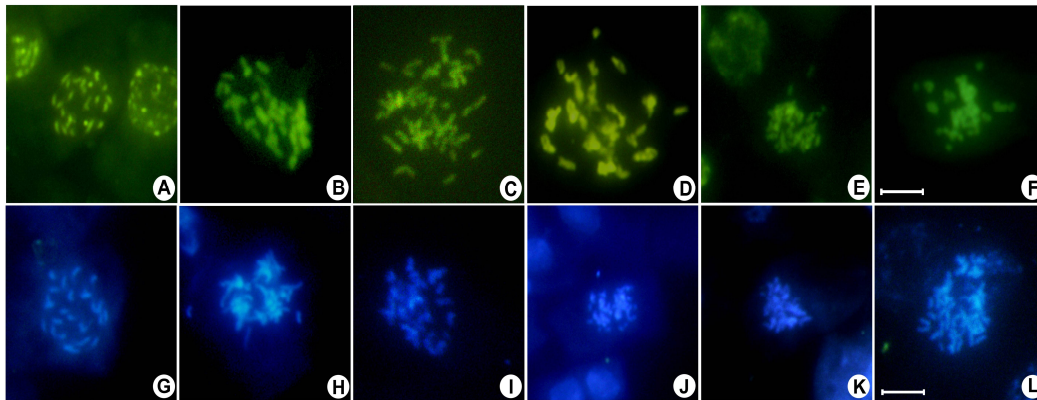


Fig. 3. CMA- and DAPI- stained mitotic metaphase chromosomes of six species of aAcanthaceae family; A, G: *Asystasia gangetica*, B, H: *Barleria cristata*, C, I: *Barleria prionitis*, D, J: *Ruellia simplex*, E, K: *Thunbergia erecta*, and F, L: *Thunbergia mysorensis*. Bar = 10 μ m.

In this investigation, two base-specific fluorochromes (CMA and DAPI) were used, which preferentially bind to GC- and AT-rich regions, respectively (Schweizer, 1976). Four CMA-banded chromosomes were observed in *A. gangetica*, whereas *B. cristata* and *B. prionitis* exhibited four and ten CMA-positive chromosomes, respectively (Figs 3A-C). In *R. simplex*, six CMA bands were detected on four chromosomes, while *T. erecta* showed three distinct CMA-banded chromosomes. No prominent CMA bands were observed in *T. mysorensis* (Figs 3D-F). Four DAPI-positive chromosomes were observed in *A. gangetica*, whereas two bright bands were detected in *B. prionitis* (Figs 3A, 3C). The metaphase chromosomes of the other species were not sufficiently well-resolved to allow accurate band identification (Figs 3A, 3C-F). The variation in CMA and DAPI banding patterns among the studied species reflects differences in the distribution

and proportion of GC- and AT-rich repetitive DNA segments. Such heterogeneity in fluorochrome binding patterns may indicate species-specific chromosomal organization and provide useful cytological markers for comparative genomic and taxonomic studies. These findings contribute valuable insights into the chromosomal architecture and genetic affinities of Acanthaceae members. Future studies integrating molecular cytogenetics and genome sequencing are recommended to further elucidate the evolutionary relationships and functional significance of heterochromatin organization in this family.

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