Bangladesh J. Bot. **55**(2): 427-431, 2026 (June) **- Short communication**
DOI: https://doi.org/10.3329/bjb.v55i2.91206

COMPARATIVE ANALYSIS OF HETEROCHROMATIN DISTRIBUTION IN FOUR *OXALIS* L. SPECIES

ISHRAT JAHAN BONNA AND SYEDA SHARMEEN SULTANA*

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Keywords: Interphase nuclei, Prophase chromosomes, CMA, DAPI, *Oxalis*

Abstract

Cytogenetic analysis was carried out in *Oxalis corymbosa*, *O. corniculata*, *O. latifolia* and *O. triangularis* for precise characterization. Several small orcein-stained heterochromatic regions were found in the interphase nuclei of *O. corymbosa* and *O. triangularis* while large heteropycnotic blocks were observed in *O. corniculata* and *O. latifolia*. Darkly stained heterochromatic regions were scattered in the prophase chromosomes of *O. corymbosa*. In contrast, *O. corniculata* and *O. triangularis* possessed prophase chromosomes with homogenously staining along the entire length while *O. latifolia* stained gradually from one end to another. In four species, the nature of staining indicates the probable presence of facultative heterochromatin. The four *Oxalis* species showed discrete and species-specific CMA- and DAPI banding patterns based on the distribution of GC- and AT-repetitive sequences.

Modern cytogenetic techniques with base-specific fluorochromes have greatly helped in understanding heterochromatin and euchromatin distribution patterns within genome (Alam and Kondo 1995, Merita *et al*. 2015, Lamo *et al.* 2016).

The family Oxalidaceae comprises of about five genera and nearly 570 species which are mostly distributed in the tropical, subtropical and temperate regions of the world (Xu and Deng 2017) but propagated vegetatively by bulbs and have no natural viable seeds. As a consequence, the genetic variability is limited. Among these species, *Oxalis corniculata* is a perennial invasive plant, found in tropical and temperate regions. The leaf extracts of *O. corniculata* are reported to exhibit antifungal, antimicrobial, antioxidant, antidiabetic, anticancer, anthelmintic, hepatoprotective, astringent and cardioprotective effects (Lubna *et al.* 2020, Sharma *et al.* 2024). *O. corymbosa*, *O. latifolia* and *O. triangularis* are also well known for their beautiful pink, white, and purple flowers and foliage (Ahmed *et al.* 2009). The extensive medicinal uses make this genus vulnerable and threatened. For proper conservation, knowledge of genomic information is necessary. In this context, analysis of heterochromatin composition and type provides valuable insights into genome organization, including the distribution of repetitive sequence families across plant species. With crucial cellular function and rapid evolutionary changes, heterochromatin contributes to chromosomal arrangement, segregation and species diversification (Lamo *et al.* 2016, Jahan *et al.* 2025). Work on detailed chromosome number was carried out in these four species previously but heterochromatin distribution was not reported earlier (Bonna *et al.* 2017). Patterns of heterochromatin condensation in interphase nuclei and prophase chromosomes provide additional genomic information for authentic characterization of species (Merita *et al*. 2015).

This research aimed to compare the degree of heterochromatic condensation of the interphase nuclei and prophase chromosomes in four *Oxalis* species *viz. O. corymbosa, O. corniculata, O. latifolia* and *O. triangularis* after staining with orcein, CMA and DAPI for proper characterization.

Oxalis triangularis was collected from a nursery in Agargaon and *O. corymbosa, O. corniculata* and *O. latifolia* were taken from the Botanical Garden, Department of Botany, University of Dhaka (Fig. 1). The identification of studied plants was done by a taxonomist.

*Author for correspondence: <syedasharmeensultana@du.ac.bd>.

These were planted in the Botanical Garden of Botany Department. For chromosome preparation, healthy roots were cut and treated with 8-hydroxyquinoline (0.002 M) at 18°C for 1 hr and fixed at 4°C in acetic acid (45%) for 30 min. Root hydrolysis was carried out with 1 N HCl: 45% acetic acid (2:1) at 60°C for 20 sec. The root tips were squashed in 1% aceto-orcein. For fluorescent staining procedure, Alam and Kondo's (1995) method was undertaken with trivial modifications. Images of mitotic interphase and prophase chromosomes were examined using the microscope equipped with blue-violet and ultraviolet fluorescence filter sets sequentially. At least 50 clear interphase and prophase cells were observed in all the species to determine banding pattern.

Fig. 1. Morphology of four *Oxalis* species. Leaf (A), flower (B) and propagative organ (C) of *O. corymbosa*; Leaf (D), flower (E) and propagative organ (F) of *O. corniculata*; Leaf (G), flower (H) and propagative organ (I) of *O. latifolia* and Leaf (J), flower (K) and propagative organ (L) of *O. triangularis.*

In *O. corymbosa* and *O. triangularis*, several small darkly stained regions were fused in interphase nuclei, forming small heterochromatic bodies (Figs 2A and D). These were scattered around the nucleus. Tanaka (1971) termed this as 'Simple chromocenter type' interphase nuclei. On the other hand, *O. corniculata* and *O. latifolia* were found to possess some darkly stained large heterochromatic regions mostly at the periphery of nucleus. This is considered as 'Complex chromocenter type' (Tanaka 1971) (Figs 2B, C and Table 1).

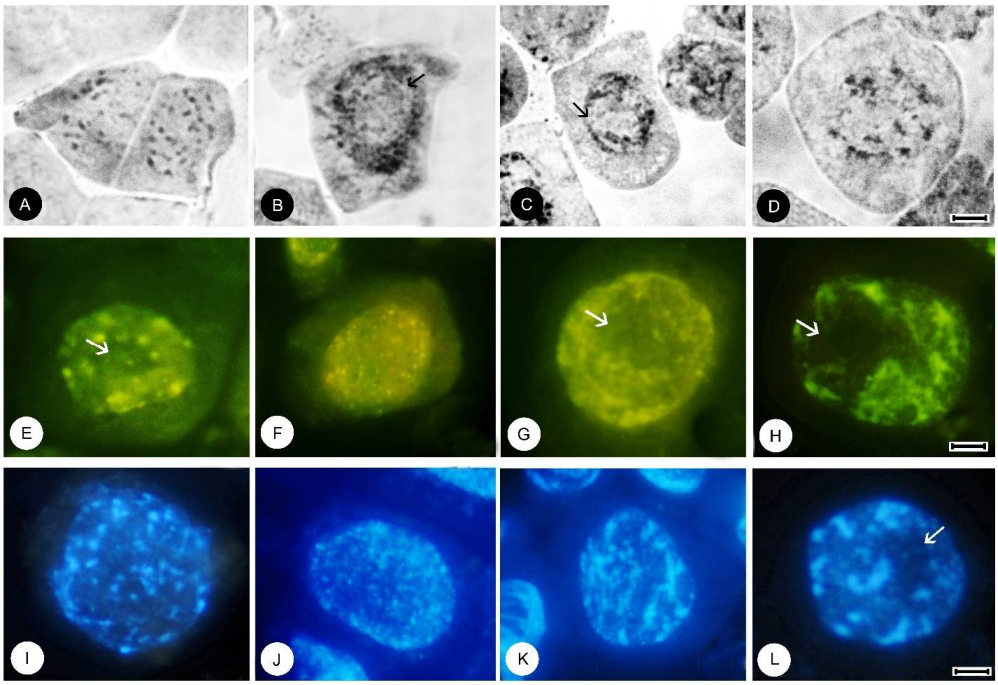

Fig. 2. Orcein, CMA and DAPI-stained mitotic interphase nuclei of four *Oxalis* species. Orcein-stained *O. corymbosa* (A), *O. corniculata* (B), *O. latifolia* (C) and *O. triangularis* (D); CMA-stained *O. corymbosa* (E), *O. corniculata* (F), *O. latifolia* (G) and *O. triangularis* (H); DAPI-stained *O. corymbosa* (I), *O. corniculata* (J), *O. latifolia* (K) and *O. triangularis* (L). Arrows indicate nucleolus. Bar= 10 µm.

Table 1. Type of orcein stained interphase nuclei and prophase chromosomes in *Oxalis* L.

Oxalis species	Interphase nuclei type	Prophase chromosomes type
O. corymbosa	Simple chromocenter	Interstitial
O. triangularis	Simple chromocenter	Continuous
O. corniculata	Complex chromocenter	Continuous
O. latifolia	Complex chromocenter	Gradient

The prophase chromosomes were homogeneously stained along the entire length in *O. corniculata* and *O. triangularis* and these are termed as 'Continuous type' prophase chromosome according to Tanaka (1971) (Figs 3B, D and Table 1). In *O. corymbosa*, prophase chromosomes were stained at the interstitial regions of heterochromatin and known as 'Interstitial type' prophase chromosomes (Fig. 3A). The prophase chromosomes of *O. latifolia* were stained darkly at one end but gradually faded on the other end and known as 'Gradient type' according to Tanaka (1971) (Fig. 3C and Table 1).

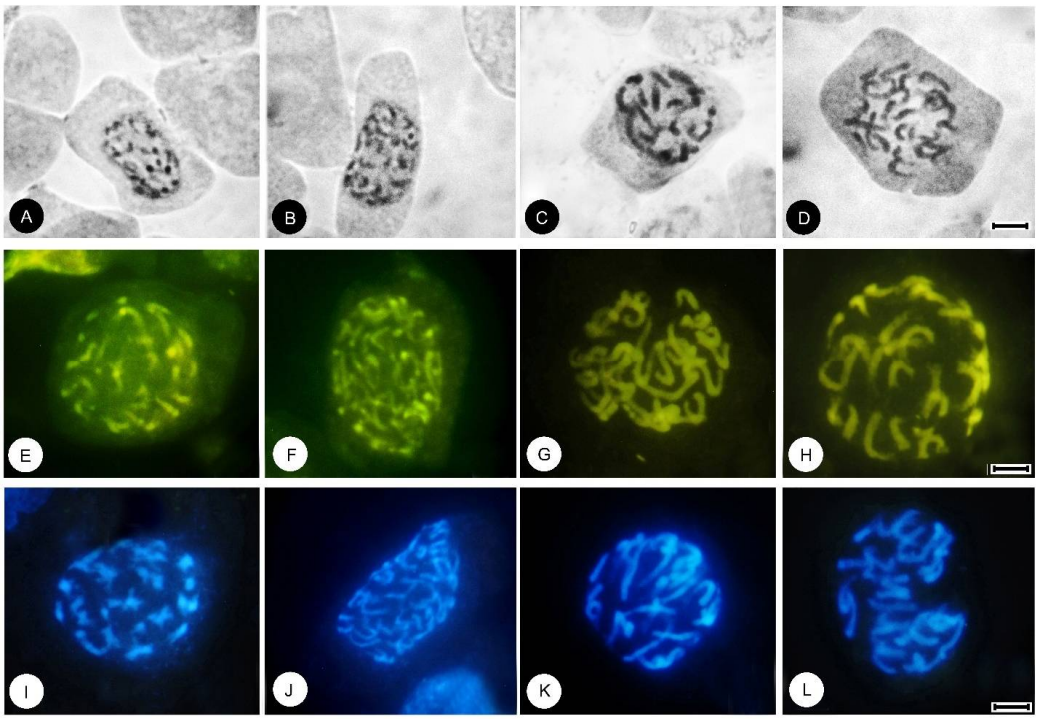

Fig. 3. Orcein, CMA and DAPI-stained mitotic prophase chromosomes of four *Oxalis* species. Orcein-stained *O. corymbosa* (A), *O. corniculata* (B), *O. latifolia* (C) and *O. triangularis* (D); CMA-stained *O. corymbosa* (E), *O. corniculata* (F), *O. latifolia* (G) and *O. triangularis* (H); DAPI-stained *O. corymbosa* (I), *O. corniculata* (J), *O. latifolia* (K) and *O. triangularis* (L). Bar= 10 µm.

Oxalis corymbosa and *O. latifolia* followed the usual distribution pattern of heterochromatin but *O. corniculata* and *O. triangularis* did not show the regular arrangement due to the presence of facultative heterochromatin which was decisively indicated in the interphase nuclei and later homogeneously disseminated in the prophase chromosomes. Previously, *O. corniculata*, showed complex chromocenter heterochromatin distribution but *O. triangularis* represented simple chromocenter type interphase nuclei (Warasy 2024) which differs from the current study. However, both *O. corniculata* and *O. triangularis* showed continuous type of prophase chromosomes which is in line with the current research (Warasy 2024).

A number of CMA-stained interphase nuclei bodies were found in the four species (Fig. 2). In *O. corymbosa*, 10-15 CMA fluoresced bands were detected in interphase nuclei (Fig. 2E). In contrast, numerous dots like CMA bands were observed in the interphase nuclei of *O. corniculata* (Fig. 2F). CMA bands were less fluoresced in *O. latifolia* (Fig. 2G). On the other hand, few bigger CMA-fluoresced area were observed in *O. triangularis* (Fig. 2H). A non-staining region was observed in the nucleus of *O. corymbosa*, *O. latifolia* and *O. triangularis* (Figs 2E, G, H). In *O. corymbosa*, the prophase chromosomes had 8-10 CMA fluoresced bands (Fig. 3E). In contrast, numerous dots like CMA-bands were found in *O. corniculata* (Fig. 3F). CMA bands were less fluoresced in *O. latifolia* (Fig. 3G). In contrast, a few bigger CMA-fluoresced areas were seen in the prophase chromosomes of *O. triangularis* (Fig. 3H).

After DAPI-staining, the interphase nuclei of *O. corymbosa* and *O. corniculata* was found to have a huge number of small brightly stained bodies (Figs 2I and J). In *O. latifolia*, relatively big

DAPI-stained bodies were found around the nucleus (Fig. 2K). In contrast, the DAPI-banded regions were aggregated forming blocks in the nuclei of *O. triangularis*. Prominent non-staining regions were observed in the nuclei of *O. triangularis* (Fig. 2L). About 8-10 DAPI bands were seen at the interstitial areas of prophase chromosomes of *O. corymbosa* (Fig. 3I). Some dots like DAPI-bands were found in the prophase chromosomes of *O. corniculata* (Fig. 3J). DAPI bands were less fluoresced in *O. latifolia* and *O. triangularis* (Figs 3K and L).

The mitotic interphase nuclei and prophase chromosomes features of *Oxalis* species after orcein, CMA and DAPI staining have not been documented earlier in the literature or online sources and thus could be considered as a novel attempt for characterization. The comparative account of patterns of heterochromatin condensation in interphase nuclei and prophase chromosomes in these *Oxalis* species will be helpful to provide additional genomic information for authentic characterization.

References

Ahmed ZU, Hassan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA and Haque EU 2009. Encyclopedia of flora and fauna of Bangladesh. Angiosperms: Dicotyledons (Magnoliaceae - Punicaceae). Asiat. Soc. Bangladesh **9**: 354-356.

Alam SS and Kondo K 1995. Differential staining with orcein, Giemsa, CMA and DAPI for comparative chromosome study of 12 species of Australian *Drosera* (Droseraceae). Am. J. Bot. **82**(10): 1278-1286.

Bonna IJ, Afroz M, Sultana SS and Alam SS 2017. Comparative karyotype and RAPD analysis of four *Oxalis* L. species. Cytologia **82**: 527-533.

Jahan N, Bonna IJ and Sultana SS 2025. Heterochromatin distribution pattern in five varieties of *Trichosanthes anguina* L. from Bangladesh. Bangladesh J. Bot. **54**(1): 49-52.

Lamo JM, Devi SP, Devi KM, Anju S and Rao SR 2016. Heterochromatin distribution pattern in aid of understanding plant species inter-relationships. J. Biol. **12**: 246-247.

Lubna AS, Jan R, Khan AL and Lee IJ 2020. Complete chloroplast genome characterization of *Oxalis corniculata* and its comparison with related species from family Oxalidaceae. Plants **9**(8): 928.

Merita K, Kattukunnel JJ, Yadav SR, Bhat KV and Rao SR 2015. Comparative analysis of heterochromatin distribution in wild and cultivated *Abelmoschus* species based on fluorescent staining methods. Protoplasma **252**: 657-664.

Sharma S, Bhuyan N and Mohanty J 2024. A pharmacognostic, phytochemical and antioxidant potential of *Oxalis triangularis*. J. Appl. Pharm. Res. **12**: 133-145.

Tanaka R 1971. Type of resting nuclei in Orchidaceae. Bot. Mag. Tokyo **84**: 118-122.

Warasy AA 2024. Chromosomal characterization of two medicinal plants from Bangladesh. J. Bangladesh Acad. Sci. **48**(1): 135-140.

Xu Z and Deng M 2017. Oxalidaceae. *In*: Identification and Control of Common Weeds, Springer, Netherlands. **2**: 617-628.

(Manuscript received on 25 September, 2025; revised on 29 November 2025)