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Research Article

Chromosomal characterization of two medicinal plants from Bangladesh

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ABSTRACT

Two medicinal plants, *O. corniculata* and *O. triangularis*, were investigated cytogenetically for proper characterization. Both plants had Complex Chromocenter interphase nuclei and Continuous prophase chromosomes. *O. corniculata* was discovered to have 2n=48 chromosomes and 2n=30 in *O. triangularis*. The chromosomal length range was 0.82±0.02-1.61±0.02 in *O. corniculata* and 1.02±0.02-2.52±0.03 in *O. triangularis.* The total length of the 2n chromosome complement was 53.52±1.19 and 46.76±0.41 for *O. corniculat*e and *O. triangular,* respectively. The two species differed in centromeric formulas, such as 48m for *O. corniculata* and 28m+2sm for *O. triangularis*. In their karyotype, no steady decrease in chromosomal length was seen. These characteristics showed that *O. corniculata* and *O. triangularis* could be considered primitive species. Therefore, compiling the cytogenetic features will be helpful to ensure the genuine identification and characterization of the two medicinally important *Oxalis* species.

Introduction

Oxalis is an extensive flowering plant genus that belongs to the Oxalidaceae family, consisting of eight genera with herbaceous plants, shrubs, small trees, and about 570 species (Christenhusz and Byng, 2016). Some species are yellow or pink sorrels, while others are called fake shamrocks or sourgrasses. They are characterized by their divided, palmate leaves that resemble clover leaves-, and funnel- or bowl-shaped flowers that often close at night or in dull weather. Several species, cultivars, and hybrids are popular in rock gardens, raised beds, an alpine house, or as houseplants. Some are considered very invasive weeds. Most of the members of this family are distributed across the world's warmer regions (Mathew, 1958). *Oxalis corniculata* L. is one of these species and possesses well-known traditional medicinal uses like- diarrhea, dysentery, stomachache, Datura poisoning, scorpion sting, giddiness, curing fever, coughs, cold, mouth ulcers, eczema, headache, expulsion of gastrointestinal worms, jaundice, and hepatitis (Muhammad and Mir, 2000). *O. triangularis* are largely used as medicinal plants. Other *Oxalis* spp. are grown as food (tuberous roots of *O. tuberosa* are used as food, leaves of *O. acetosella* are used as salads, and stems of *O. pescaprae* are used as a vegetable), and because of their magnificent pink, purple, and white blooms and lovely leaves, they have potential as an ornamental herb (Sharma and Chatterji, 1960). Different members of this genus contain oxalic acid, giving the leaves and blossoms an acidic flavor that can be refreshing to chew (Ahmed et al., 2009). So, the members of this genus are under threat because of their extreme medicinal uses. The consequences would be worse if they were not managed or conserved at this stage.

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In such a situation, genetic diversity analysis is essential in order to achieve appropriate conservation, multiplication and management options for the *Oxalis* spp. that exist in Bangladesh. Authentic characterization of *Oxalis* spp. is required for this purpose. Karyotype analysis is a must for authentic characterization. Other karyotypical criteria, such as heterochromatin distribution, should also be studied to obtain further information about different germplasm. Tanaka (1971) used orcein staining to classify distinct types of heterochromatin distribution. Later, other researchers attempted to describe heterochromatin distribution using different cytogenetical methods (Shahla and Alam, 2011). These investigations found that staining features in interphase nuclei and prophase chromosomes may differentiate distinct taxa, including variations of many germplasm karyotypes, a consistent and dependable characteristic unique to each specimen. Several cytological studies have been done on this genus abroad, but locally, it is very rare. The cytological work in the genus *Oxalis* has revealed several exciting features. Firstly, an aneuploid series of chromosome numbers have been found in this genus, such as x=5, 6, 7, 9, and 11 (Bonna et al., 2017). In addition to direct aneuploid sets, chromosome numbers other than the diploid sets have been recorded, such as 2n=24 chromosomes in *O. corniculata* (Rutland, 1941) and *O. stricta* (Bonna et al., 2017). High polyploidy sets such as 2n=66 and other higher numbers of chromosomes in *O. tuberosa* are also on record. Further, even in the same species, diploid and polyploid individuals are recorded in *O. brasillienses* (2n=14, 28).

The members of this genus are interesting for chromosome study because of their variability in chromosome number. The study of karyotypes is critical in cases where the basic chromosomal number is variable. Such research helps to describe each species and sheds light on how one form evolved from another.

Therefore, a conventional cytogenetic method through karyotype analysis was used to compare the degree of heterochromatic condensation, determine the chromosomal number, create a karyotype, and characterize two *Oxalis* species, *O. corniculata* and *O. triangularis*. This characterization is constructive for improving and conserving these germplasm.

Materials and Methods

Materials

This study investigated the following two *Oxalis* species: *O. corniculata* and *O. triangularis.* The plant materials were obtained from the conservatory and old Arts building of Jahangirnagar University, Savar, Dhaka, Bangladesh.

Methods

Healthy roots were collected. Only cold water at room temperature was used to pre-treat the collected root, followed by a 15-minute fixation in 45% acetic acid at 4^{o}C. The roots were hydrolyzed for 8-9 seconds. These roots were placed on a slide, and the meristematic area was cut. Then, 1% aceto-orcein was added to it. A clean cover glass was applied to the substance and then lightly tapped and squished. Finally, the slides were observed under a microscope and photographed using a digital camera.

Results and Discussion

Orcein-stained interphase nuclei and prophase chromosomes.

Diverse karyomorphological traits were observed in heterochromatin distribution, which aids in characterizing diverse germplasm. In the current investigation, some big heterochromatic areas were found in the interphase nuclei of both cultivars. These were scattered around the nucleus. Few heterochromatic areas grouped together in these two species, generating larger heterozygotic patches in the interphase nucleus. A prominent nucleolus occupying more than $1/3^{rd}$ of the nucleus was found in both cases (Figs. 1, 2). Both cultivar's prophase chromosomes were stained uniformly along their full length (Figs. 3, 4).

Tanaka (1971) was the first to provide karyomorphological criteria for interphase nuclei and prophase chromosomes. He divided them into five separate types based on each case's staining property. Later, several workers used these criteria to characterize various plant materials. (Sultana and Alam, 2016; Saha and Begum, 2020).

The present study discovered "Complex Chromocenter Type" interphase nuclei and "Continuous Type" prophase chromosomes in both *Oxalis corniculata* and *O. triangularis* (Figs. 1, 2, 3, 4; Table 1). The current findings do not support the common feature of heterochromatin distribution in prophase chromosomes. The observations above suggested the presence of facultative heterochromatin. These features have been limitedly considered as cytological parameters in *Oxalis* spp. Therefore, this is a critical strategy for characterizing the two *Oxalis* species.

Table 1. Types of heterochromatin distribution.

***Oxalis* species**	**Interphase nuclei**	**Prophase chromosomes**
O. corniculata	Complex chromocenter	Continuous
O. triangularis	Complex chromocenter	Continuous

2n chromosomes number

Two *Oxalis* species had distinct 2n chromosome numbers in this investigation. In the current study, *Oxalis corniculata* was discovered to have 2n=48 chromosomes (Fig. 5, 7; Table 2). Another scientist previously reported the same chromosomal number (Mathew, 1958). Furthermore, different chromosomal numbers were reported, including 2n=24 (Rutland, 1941) and 2n=44 (Chatterjee and Sharma, 1970). Several scientists believed that the primary chromosomal number for this species was x=11. Hence, specimens with 2n=44 may be called tetraploid. If x=7 is also included, then 2n=28 and 2n=42 could be tetraploid and hexaploid, respectively. However, the observed 2n=48 in this investigation did not correspond to the basic numbers of x=11 and x=7. In this scenario, aneuploid aberration may have evolved the 2n=48 from a higher ploidy level. In the case of *O. triangularis,* 2n=30 diploid chromosome number was determined in the present investigation (Fig. 6, 8; Table 2). Bonna et al. (2017) reported the same chromosome number for this species. Thus, the present report of 2n=30 chromosomes supports that of Bonna et al. (2017). However, the 2n=30 chromosome number confused the basic chromosome numbers x=11 and x=7. In this case, the 2n=30 might have evolved from a nearer ploidy level by aneuploid aberration. 2n=48 for *Oxalis corniculata* and 2n=30 for *O. triangularis* indicated that the species has octaploid or pentaploid nature if x=6, respectively. 2n=42 for *O. corymbosa*, x=6 is most likely the primary chromosome number for these species.

The cytological work carried out locally (only Bonna et al. (2017) and abroad on the genus *Oxalis* has revealed several interesting features. Several aneuploid series of chromosome numbers have been reported, such as x=5, 6, 7, 9, and 11 (Bonna et al., 2017). In addition, chromosome numbers other than the diploid sets have been recorded, such as 2n=24 chromosomes in *O. corniculata* (Rutland, 1941). High polyploidy sets like 2n=66 and other high diploid chromosome numbers in *O. tuberosa* are also recorded (Bonna et al., 2017). Moreover, Diploid and polyploid individuals in the same species (2n=14, 28 in *O. brasillienses*) were also reported by different scientists (Bonna et al., 2017).

Chromatin length

The chromosomal length range was 0.82±0.02-1.61±0.02 µm for *O. corniculata* and 1.02±0.02-2.52±0.03 for *O. triangularis*, where there was no discernible steady decrease in chromosomal length in either case (Figs. 7, 8, 9, 10; Table 2). The average chromosomal length for *O. corniculata* was 1.12 µm and 1.56 µm for *O. triangularis.* The overall length of the 2n chromosomal complement was determined to be 53.52±1.19 µm for *O. corniculata* and 46.76±0.41 µm for *O. triangularis*. The total length

of *O. corniculata* was almost nearer than that of *O. triangularis*. Bonna et al. (2017) did not find this type of observation regarding total chromatin length among the two species. As a result, the current findings clearly demonstrated the variety of chromatin length across the specimens tested in this study.

Centromeric feature

Two *Oxalis* species were discovered to have more or less comparable centromeric index ranges of 45.90-50.00 for *O. corniculata* and 45.63-49.02 μm for *O. triangularis.* In the instance of the centromeric formula, all metacentric chromosomes in *O. corniculata* were shown to be strictly symmetric. Bonna et al. (2017) also found all metacentric chromosomes in this species. As a result, the current findings demonstrate that this species has a strictly symmetric karyotype. On the other hand, *O. triangularis* possessed 28 metacentric and 2 sub-metacentric chromosomes, representing an almost symmetric karyotype. Bonna et al. (2017) observed 26 metacentric and 4 submetacentric chromosomes in this species. There is quite a difference between Bonna et al. (2017) and the present investigation. The reason for this differential observation may be some chromosomal abnormalities, such as terminal deletion, pericentric inversion, unequal translocation, could have resulted in the formation of sub-metacentric chromosomes from metacentric chromosomes.

According to Stebbins (1971), two species of *Oxalis* are primitive plants, while *O. triangularis* is relatively advanced.

Karyotype symmetry and asymmetry index

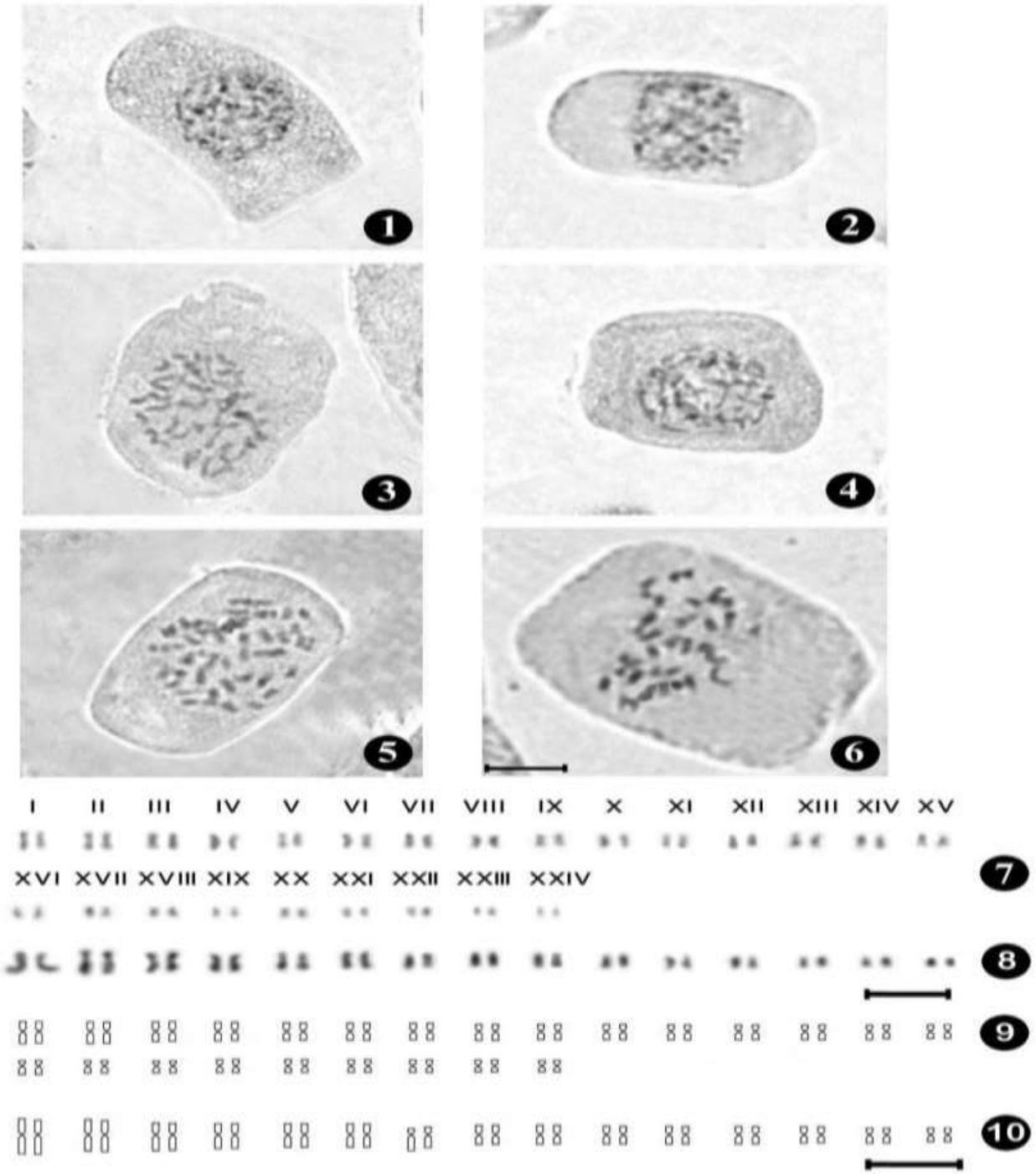
The Karyotype asymmetry index (AsK %) was 52.90% for *O. corniculata* and 54.60% for *O. triangularis*, as observed in the present study (Table 2). On the other hand, 89.83% karyotype symmetry index (Syi %) was found in case of *O. corniculata* and 83.53 % was found in *O. triangularis* (Table 2). Karyotype symmetry index values decreased with increasing asymmetry, indicating both species' symmetric karyotype nature. No reports about these karyotypic parameters of *Oxalis* were available in earlier studies. So these features are essential for characterization of selected *Oxalis* species.

Therefore, the two medicinal plants, *O. corniculate* and *O. triangular* were karyomorphologically investigated in this study. They showed the above characteristic features after staining with orcein, which is very helpful for improving and conserving this germplasm.

Table 2. Comparative karyotype analysis.

Cytogenetical Parameters	***O. corniculata***	***O. triangularis***
2n	48	30
Total length of chrom. complement (μm)	53.52±1.19	46.76±0.41
Range of individual chromosome length (μm)	0.82±0.02-1.61±0.02	1.02±0.02-2.52±0.03
Range of CI	45.90-50.00	45.63-49.02
Centromeric formula	48m	28m+2sm
Karyotype symmetry index (Syi %)	89.83	83.53
Karyotype Asymmetry index (AsK %)	52.90	54.60

m = metacentric, sm = sub-metacentric chromosome, CI = Centromeric index



Figs. 1-10. Orcein-stained cytogenetical analysis of two species of *Oxalis* L. 1. Interphase nuclei of *O. corniculata*. 2. Interphase nuclei of *O. triangularis*. 3. Prophase chromosome of *O. corniculata,* 4. Prophase chromosome of *O. triangularis*. 5. Mitotic metaphase of *O. corniculata.* 6. Mitotic metaphase of *O. triangularis*. 7. Karyotype of *O. corniculata*. 8. Karyotype of *O. triangularis*. 9. Idiogram of *O. corniculata*. 10. Idiogram of *O. triangularis*. Bar=5µ.

References

Ahmed ZU, Hassan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA and Haque EU. In: Encyclopedia of Flora and Fauna of Bangladesh. Angiosperms: Dicotyledons (Fabaceae-Lythraceae). *Asiat. Soc. Bangladesh.* 2009; pp. 305-309.

Bonna IJ, Afroz M, Sultana SS and Alam SS. Comparative karyotype and RAPD analysis of four oxalis L. species. *Cytologia*. 2017; 82(5): 527-533.

Chatterjee A and Sharma AK. Chromosome study in Gera- niales. *Nucleus*. 1970; 13: 179-200.

Christenhusz MJM and Byng JW. The number of known plants species in the world and its annual increase. *Phytotaxa.* 2016; 261(3): 201-217.

Mathew PM. Cytology of Oxalidaceae. *Cytologia*. 1958; 23: 200-210.

Muhammad IS and Mir AK. Folk use of medicinal herbs of Margalla Hills National Park, Islamabad. *J. Ethnopharmacol*. 2000; 69: 45-56.

Rutland JP. The merton catalogue. *New Phytol.* 1941; 40: 210-214.

Saha S and Begum KN. A comparative analysis on mitotic interphase and prophase among twelve varieties of *Brassica* L. from Bangladesh: Brassicaceae. *International J. Bios*. 2020; 17(4):73-82.

Shahla S and Alam SS. Comparative fluorescent banding in two forms of *Leonurus sibiricus* L. *Cytologia*. 2011; 76(3): 361-366.

Sharma AK and Chatterji T. Cytological studies on three species of Oxalis. *Caryologia.* 1960; 13: 755-765.

Stebbins GL. Chromosomal evolution in higher plants. Edward Arnold, London. 1971; p. 216.

Sultana SS and Alam SS. Differential fluorescent banding in 11 varieties of *Gossypium hirsutum* L. from Bangladesh. *Cytologia.* 2016; 81(1): 111-117.

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