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- Short communication

Karyomorphology of *Centella asiatica* (L.) Urban

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Centella asiatica (L.) Urban, commonly known as Thankuni is a small, herbaceous, annual plant of Apiaceae. This plant is indigenous to Bangladesh, India, Pakistan, China, Japan, America and the pacific (Koh *et al*., 2009) and commonly seen in moist, sandy or clay soils and waste places (Jamil *et al*., 2007). It has a long history in ancient Ayurvedic remedy, used in wound healing cleansing for skin problem and digestive disorders (Chopra *et al*., 1956, Chevallier 2001). Moreover, it is used in effective treatment of stomach ulcers, mental fatigue, diarrhea, epilepsy, hepatitis, urethritis, allergy, leucorrhea , toxic fever, syphilis and asthma (Kan 1986, Hong *et al*., 2005, Shetty *et al*., 2006, Goldstein & Goldstein, 2012). Due to its medicinal importance the species has crossed over the boundary limit of this sub-continent and now extensively used in the West (Chevallier 2001, Meulenbeld & Wujastyk, 2001).

Since this species is available in different countries, it is necessary to characterize the germplasm found in Bangladesh. Genomic characterization is more dependable than morphology because due to phenotypic plasticity, same species may show different morphology. Karyomorphology is a reliable method that gives a preliminary idea about the genome of a specimen. This method includes the properties of interphase nuclei and prophase chromosomes including the karyotype. In this investigation an attempt was undertaken to characterize *Centella asiatica* by orcein staining karyomorphological study.

C*entella asiatica* was used as experimental material in this study. The plant materials were collected from plant conservatory of Botany Department, Jahangirnagar University.

Young healthy roots were cut ca. 0.5 cm away from the tip by a sharp blade. The optimum time of collection was 10.15 am during summer. The collected root tips were soaked on a filter paper to remove surface water and pre-treated with 8-Hydroxyquinoline (0.002M) for 2 hour at room temperature (28 - 30°C). Root tips were fixed in 45% acetic acid for 15 minutes at 4°C. The pretreated root tips were hydrolyzed in a mixture of 1N Hydrochloric Acid and 45% Acetic acid (2:1) at 60°C for 10 seconds.Then the hydrolyzed roots were soaked on a filter paper and taken on a clean slide. The meristematic region was cut with a fine blade under a dissecting microscope. A drop of 1% aceto orcein was added to the material. A clean cover glass was placed on the material. At first the materials were tapped gently by a tooth pick and then squashed by

placing thumbs. During tapping and squashing care was taken so that the cover glass did not move because minute displacement of it could damage the entire preparation. The slides were observed under advanced research microscope (Olympus DP72) with a digital camera.

Orcein stained interphase nuclei and prophase chromosomes: A big and prominent nucleolus was observed in each interphase nucleus indicating the active transcription of rDNA. Several large heterochropycnotic blocks were found in the nucleus (Fig. 1). These are due to aggregation of heterochromatic bodies. According to Tanaka (1971) this type of interphase nucleus may be regarded as Complex Chromocentric Type.

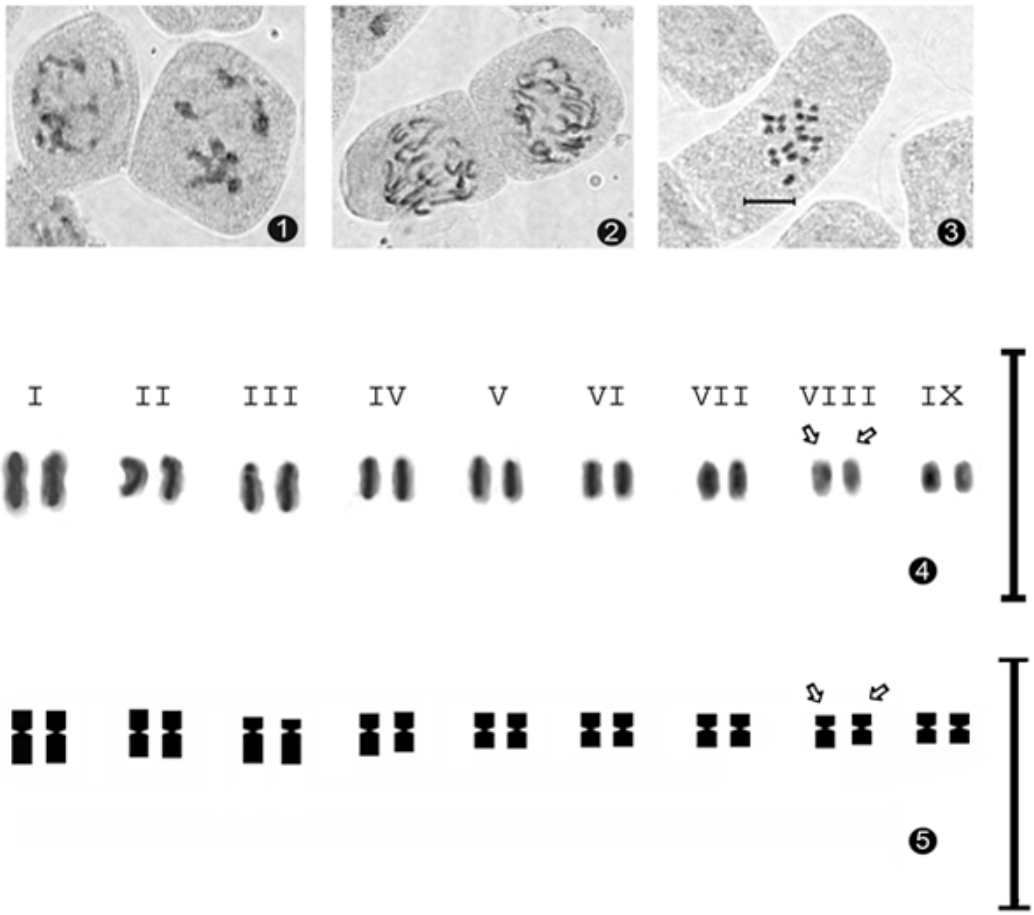
The prophase chromosomes stained along the length revealing the uniform distribution of heterochromatins (Fig. 2). The nature of staining properties of prophase chromosomes may be considered as Continuous Type (Tanaka, 1971). In general, the specimen shows Complex Chromocenter Type of interphase nucleus possess Interstitial type of prophase chromosomes- i,e. the heterochromatin are present in certain parts of prophase chromosomes. However, in this study the prophase chromosomes stained uniformly, therefore did not follow the general rule. The probable reason was that this species might have facultative heterochromatin, which aggregation in interphase while uncoiled and uniformily distributed in prophase chromosomes.

Karyotype analysis: This was found to possess 2n=18 chromosomes (Figs. 3, 4). Similar chromosome count was reported earlier (Bell *et al.*, 1960, Liu *et al.*, 1961). Therefore, the present findings support the earlier report on 2n chromosome number.

The total length of 2n chromosome complement was 14.28 µm. Individual chromosome length ranged from 0.63 – 1.11 µm. No satellite or secondary constriction was found. The relative length and centromeric index of each chromosome was ranging from 0.04 - 0.08 and 20.0-50.0 µm, respectively (Table 1). The centromeric formula of this species was 15m+1sm+2ac (Table 1). This species possessed metacentric, sub metacentric and acrocentric chromosomes representing a heterogeneous karyotypes. According to Stebbins (1971) the heterogeneous karyotype was an advanced character. *Centella asiatica* is therefore, relatively advanced.

This species showed heteromorphicity in pair VIII. One member of this pair was sub-metacentric and other metacentric chromosome (Figs. 4, 5, Table 1). The probable reason for this heteromorphicity was deletion of chromosomal part from the long arm of sub-metacentric chromosome resulting the formation of metacentric chromosome.

Although the 2n chromosome number of this speceis was reported earlier, no information about other karyotype parameter are available. In this study, a more detail karyotype features together with staining property of interphase nuclei and prophase chromosomes were described. These karyomorphological information would be useful for characterization of this species.



Figs. 1-5. Karyomorphology of *Centella asiatica*. 1. Interphase nuclei. 2. Prophase chromosomes. 3. Metaphase chromosomes. Bar = 5 µm. 4. Karyptype. Bar = 5 µm. 5. Idiogram. Bar = 5µm.
→= Heteromorphicity in respect of centromeric position

Table 1. Karyotype analysis of C*entella asiatica*

2n Chromosome number	Range of chromosomal length (µm)	Total length of 2n chromosome complements (µm)	Range of relative length (RL)	Range of Centromeric Index (CI)	Centromeric formula
18	0.63 – 1.11	14.28	0.04-0.08	20.00-50.00	15m+1sm+2ac

m = metacentric chromosome, sm = sub-metacentric chromosome, ac = acrocentric chromosome.

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