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KARYOTYPE ANALYSIS WITH ORCEIN AND CMA FROM LEAF BASE CELLS OF *RAUVOLFIA SERPENTINA* BENTH. *ET* KURZ

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Abstract

Many prominent darkly stained heterochromatic blocks were found in the interphase nuclei of *Rauvolfia serpentina* following orcein staining. The prophase chromosomes of this species became stained homogeneously throughout the entire length. This species was found to possess 2n = 20 metacentric chromosomes revealing symmetric karyotype but if the chromosome length (6.67 - 3.17 µm) is considered, it indicates asymmetric karyotype. Total GC-rich region was 15.62 % of the total chromatin length. Eight CMA-positive bands on different locus revealed the accumulation of GC-rich repeats. The two entirely CMA-banded chromosomes were so unique that could be used as marker for this species.

Rauvolfia serpentina Benth. *et* Kurz (Apocynaceae) is available in Bangladesh and is being used as medicine (Ghani 1998). The authors felt it essential to have an idea about the genetic constituent of the organism. Karyotype analysis gives a preliminary idea of its genome. In addition, fluorescent banding with differential fluorochrome helps to identify even a single chromosome. Unfortunately, there is no such report for *R. serpentina* found in Bangladesh. Therefore, the aim of this investigation is to characterize the karyotype of *R. serpentina* using conventional and fluorescent banding technique.

Rauvolfia serpentina was collected from Dhaka district and maintained in the Botanical Garden, Department of Botany, University of Dhaka, Bangladesh.

Healthy and young upper leaves and shoot apices (3 - 5 mm) were collected and pretreated with 0.002 M 8-hydroxyquinoline for one hour at room temperature (28 -30º C) followed by 15 min fixation in 45% acetic acid at 4º C. These were then hydrolysed in a mixture of 1 N HCl and 45% acetic acid (2:1) at 60º C for 30 sec. The shoot apices were stained and squashed in 1% aceto orcein. For fluorescent banding method of Alam and Kondo's (1995) was followed with slight modification. After hydrolysing and dissecting, the materials were squashed with 45% acetic acid. The cover glasses were removed quickly on dry ice and allowed to dry in air for at least 48 h before study. The air-dried slides were first pre-incubated in McIlvaine's buffer (pH 7.0) for 30 min followed by distamycin A (0.1 mg/ml) treatment for 10 minutes. The slides were rinsed mildly in McIlvaine's buffer supplemented with $MgSO_4$ (5 mM) for 15 min. One drop of CMA (0.1 mg/ml) was added to the materials for 15 min and rinsed with McIlvaine's buffer with $MgSO_4$ for 10 minutes. Slides were mounted in 50% glycerol and kept at 4º C for overnight before observation. These were observed under Nikon (UFX-IIA) fluorescent microscope with Blue Violet (BV) filter cassette.

Orcein staining: Many prominent darkly stained heterochromatic blocks were observed in the interphase nuclei of *R. serpentina* (Fig. 1). The prophase chromosomes were found to be stained homogeneously throughout the entire length (Fig. 2). According to Tanaka (1971) this type of interphase nuclei and prophase chromosomes are called complex chromocenter type and continuous type, respectively. Generally the species has complex chromocenter type of interphase nuclei and possess interstitial type of prophase chromosomes (Tanaka 1971). In this study, the

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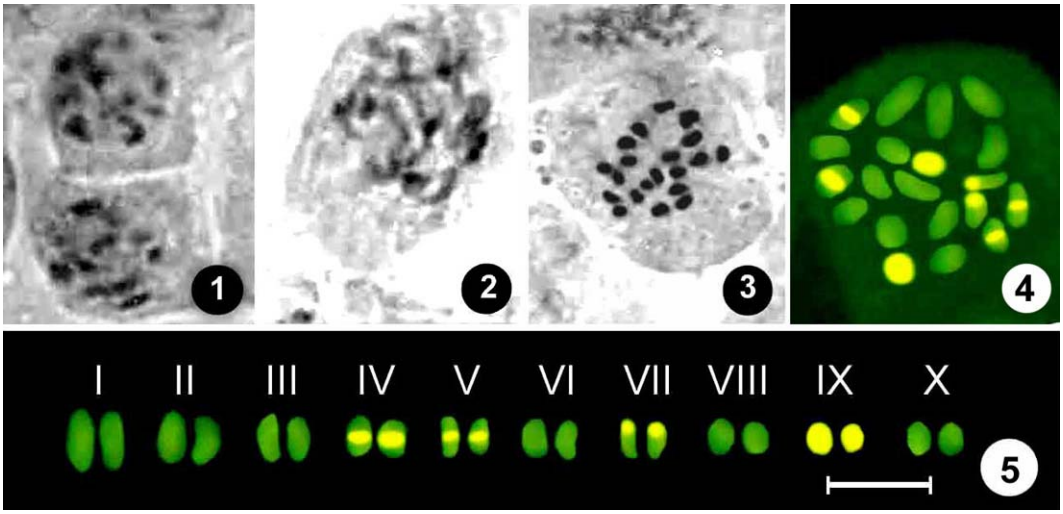
prophase chromosomes should show interstitial type of staining since the interphase nuclei were complex chromocenter type. The probable reasons for this disagreement are perhaps the presence of facultative heterochromatin which aggregated in the interphase nuclei and diffused in prophase chromosomes as the cell cycle proceed.



Figs. 1-5. Different stages of mitotic cell division in *Rauvolfia serpentina* stained with orcein and CMA. 1. Orcein stained interphase nuclei, 2. Orcein stained prophase chromosomes, 3. Orcein stained metaphase chromosomes, 4. CMA stained metaphase chromosomes and 5. CMA stained karyotype prepared from mitotic metaphase chromosomes. Bar = 10 µm.

This species was found to possess 2n = 20 metacentric chromosomes (Fig. 3). The individual chromosome length ranged from 3.17 to 6.67 µm and showed a gradual decrease in chromosomal length. This species possesses an interesting karyotype. The 20 metacentric chromosomes revealed symmetric karyotype but if the chromosome length is considered, it indicates asymmetric nature. Stebbins (1971) mentioned that the asymmetric karyotype points out advance character. Therefore, *Rauwolfia serpentina* might be considered as an advanced species in respect of chromosomal length and primitive on the basis of centromeric type. 2n = 20 for this species was reported earlier (Singh 1961, Raghavan 1957). The present findings thus confirm diploid chromosome number of *R. serpentina* found in Bangladesh. Moreover, different chromosome number such as 2n = 22 (Tapadar *et al.* 1960, Tapadar and Roy 1964 and Koul 1964), 2n = 24 (Chandra 1957) and 2n = 44 (Koul 1964) were also reported indicating the presence of different cytotypes and polyploidy of this species.

CMA banding: In *R. serpentina* the total GC-rich region was 39.62 µm, which is about 15.62 % of total chromatin length. Eight CMA-positive bands were observed in four different pairs of chromosomes (Fig. 4). Pair IX was entirely fluoresced. Centromeric bands were found in each member of the pairs IV and V. Chromosome pair VII contained two upper terminal CMA-positive bands (Fig. 5). It revealed the accumulation of GC-rich repeats at those portions of the respective chromosomes.

CMA-banding pattern of pair IX was unique since both the members were entirely fluoresced. This type of staining is rare. It might due to be the repetitive tandem duplication of GC-rich bases along the length of chromosome. These chromosomes were so distinct that these could be used as marker for this species.

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