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PACHYTENE CHROMOSOMES OF COLEUS FORSKOHLII

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COLEUS FORSKOHLII Briq. (family Lamiaceae) is an important source of Coleonol (Forskolin) which is being developed as a drug for glaucoma, congestive cardiomyopathy and asthma[1–4]. Its somatic chromosomes are relatively small and lack discernible morphological markers for identification. On the other hand at pachytene stage of meiotic prophase, where the chromosomes are paired in an extended condition, each pair is identifiable by distinct morphological features. Detailed pachytene analysis of the chromosome complement in this species is of considerable importance as it will help in understanding the evolutionary relationship and preparing the linkage groups for use in genetic and cytogenetic investigations. The present communication reports on the morphology of 15 chromosome pairs in *C. forskohlii*. This is, as far as we know, the first report presenting complete pachytene analysis in this species.

The young flower buds from a healthy plant of *C. forskohlii* were fixed in a freshly prepared acetic-alcohol mixture (1:3) for 24 h. The anthers were stained and squashed in 2% acetocarmine. For all measurements and the study of morphological details five intact cells at mid-pachytene stage in which all the 15 bivalents could be traced from one end to the other were selected from temporary slides. The chromosomes were numbered one to fifteen following the method adopted by McClintok[5]. For describing the position of centromeres on the chromosome, the method of Leven *et al*[6] was followed.

At pachytene, the chromosome complement resolved itself into a haploid set of 15 bivalents. All the chromosomes were differentiated into euchromatic and heterochromatic regions along their lengths. The centromeres were located within the heterochromatic regions and the chromosome ends were rarely terminated by a prominent telomere. The mean observations relating to the characteristic features of the chromosome identified as 1 to 15 are summarized in table 1. The photomicrographs of a single cell showing the entire pachytene chromosome complement and its interpretive drawing are shown in figures 1 and 2 respectively. The *C. forskohlii* karyotype with distinguishing features of each chromosome is shown in figure 3.

The length of the haploid complement was measured at 515.88 μ. The chromosome length varied between 50.45 μ and 15.00 μ and the arm ratio between 0.09 and 0.90. The chromosomes were comprised of largely euchromatic regions (EUL: HCL = 1:0.286). The variation in arm ratio demonstrates that the chromosomes of *C. forskohlii* are acrocentric to metacentric (table 1).

Chromosomes 9 and 13 are the nucleolus organizing chromosomes which could be readily identified by virtue of their constant association with the nucleolus and presence of nucleolus organizing region in their short arms which unlike rest of the chromosomes were totally heterochromatic. In addition to length, centromere position and extent of differentiation into euchromatic and heterochromatic regions, size, number and position of chromomeres formed the diagnostic criteria on the basis of which the 15 chromosomes of *C. forskohlii* could be identified at pachytene.

Table 1 *Morphological features of pachytene chromosomes of C. forskohlii*

Chromosome	Chromosome length (μ)				Arms ratio (S/L)	Centromere position	HCL/EUL
	Total	Relative	Short arm (S)	Long arm (L)			
1	50.45 ± 0.396	100.00	9.09 ± 0.128	41.36 ± 0.352	0.22 ± 0.089	st	0.169 ± 0.003
2	49.54 ± 1.276	98.19	21.36 ± 0.389	28.18 ± 0.439	0.76 ± 0.002	m	0.164 ± 0.003
3	45.91 ± 1.945	91.00	16.82 ± 0.664	29.09 ± 1.281	0.57 ± 0.007	sm	0.159 ± 0.059
4	38.64 ± 1.141	76.59	15.91 ± 0.454	22.73 ± 0.703	0.70 ± 0.007	m	0.382 ± 0.011
5	38.17 ± 0.556	75.66	10.45 ± 0.174	27.72 ± 0.382	0.38 ± 0.002	sm	0.265 ± 0.013
6	38.17 ± 0.713	75.66	10.90 ± 0.208	27.27 ± 0.505	0.40 ± 0.001	sm	0.323 ± 0.005
7	36.82 ± 1.091	72.98	13.18 ± 0.320	23.64 ± 0.787	0.56 ± 0.008	sm	0.308 ± 0.008
8	34.99 ± 0.886	69.35	14.09 ± 0.346	20.90 ± 0.540	0.67 ± 0.003	m	0.179 ± 0.006
9*	31.82 ± 1.058	63.07	2.73 ± 0.256	29.09 ± 0.919	0.09 ± 0.008	t	0.415 ± 0.015
10	31.81 ± 0.649	63.05	5.45 ± 0.098	26.36 ± 0.552	0.21 ± 0.002	st	0.355 ± 0.009
11	30.00 ± 1.039	59.46	4.09 ± 0.378	25.91 ± 0.694	0.16 ± 0.011	st	0.098 ± 0.007
12	26.82 ± 1.102	53.16	12.73 ± 0.281	14.09 ± 0.949	0.90 ± 0.055	m	0.695 ± 0.021
13*	25.46 ± 0.582	50.46	3.64 ± 0.076	21.82 ± 0.516	0.17 ± 0.003	st	0.578 ± 0.032
14	22.28 ± 1.060	44.16	4.55 ± 0.418	17.73 ± 0.699	0.26 ± 0.015	st	0.558 ± 0.033
15	15.00 ± 1.165	29.73	5.45 ± 0.459	9.55 ± 0.707	0.57 ± 0.007	sm	0.364 ± 0.008

*SAT chromosome; t–terminal; st–sub-terminal; sm–sub-median; m–median; HCL–heterochromatin length; EUL–euchromatin length; ±–standard deviation.

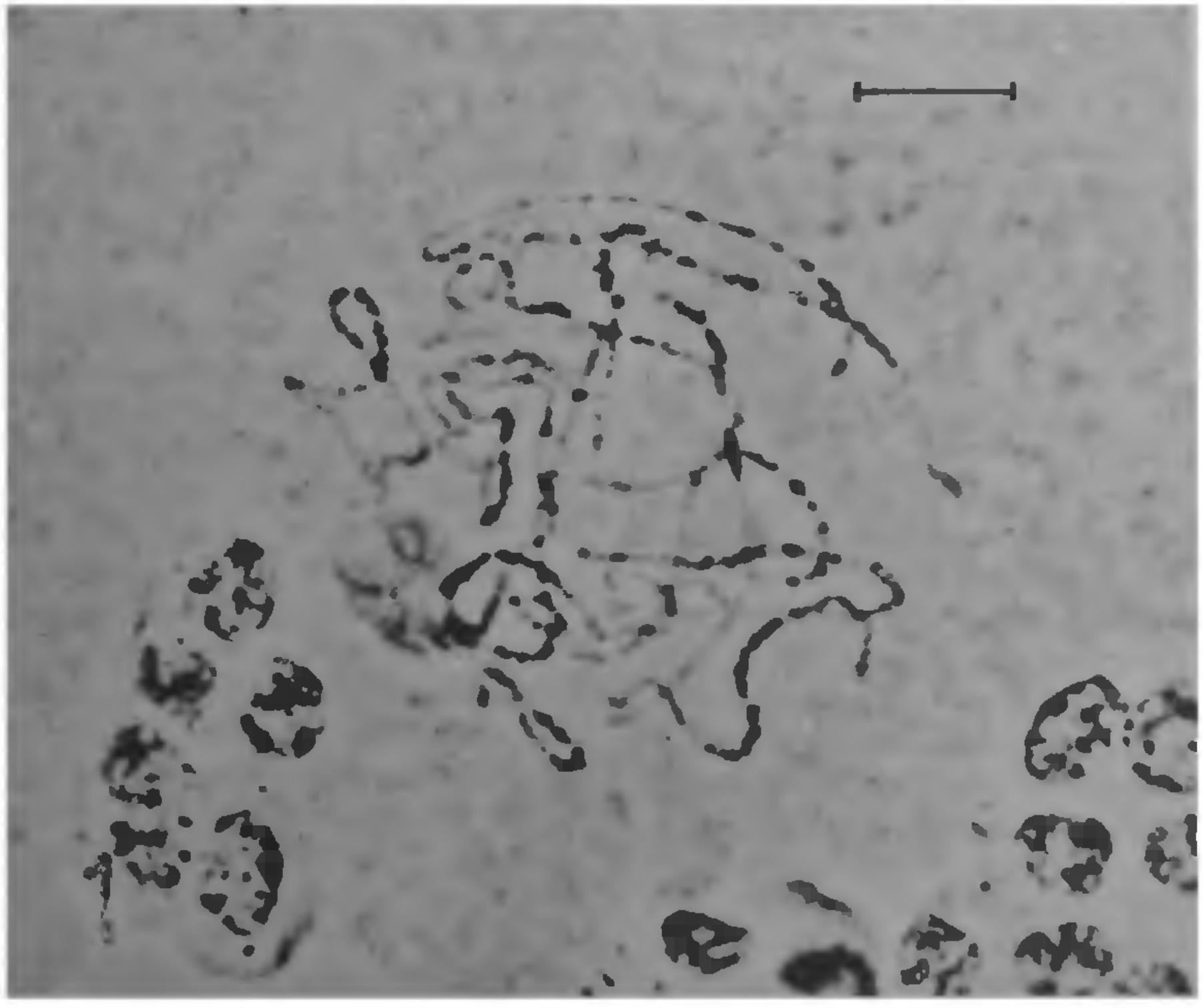
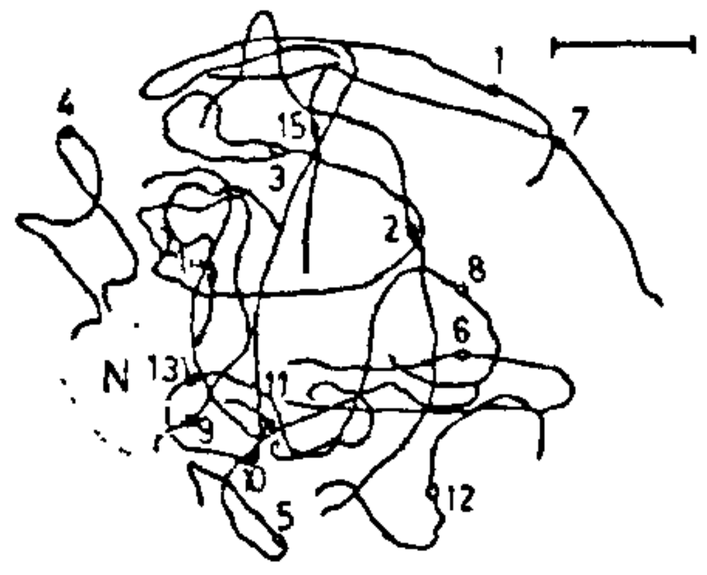
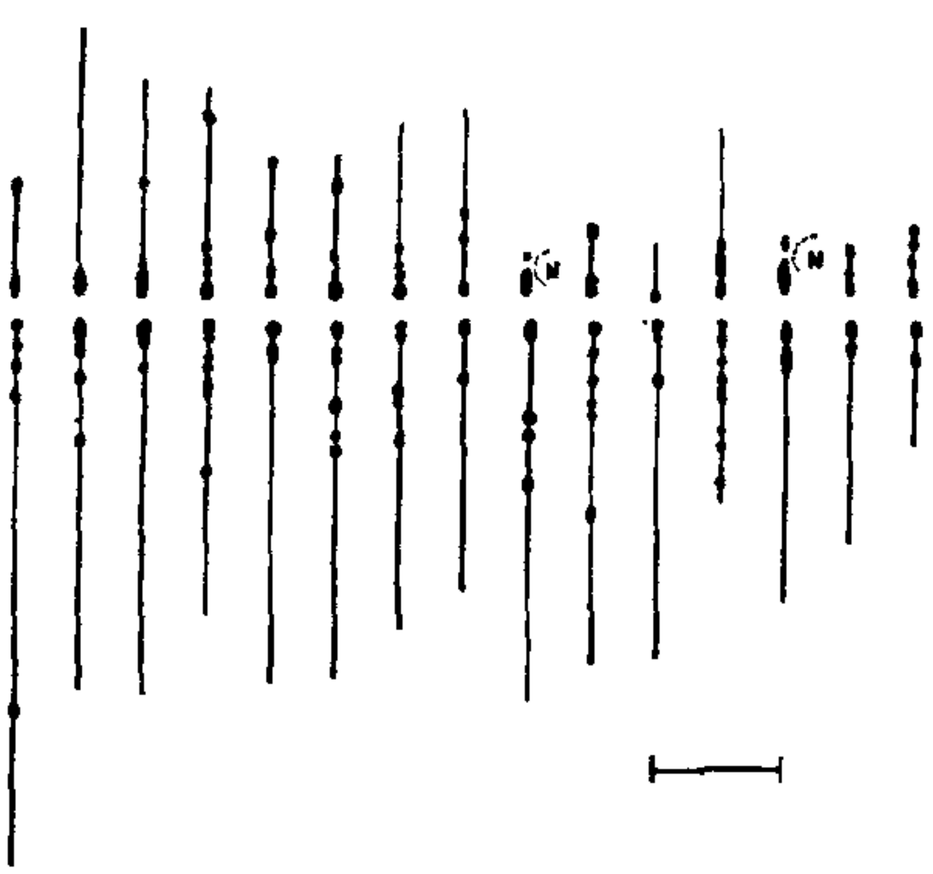


Figure 1. A photomicrograph of pachytene chromosome complement of *C. forskohlii*. Bar represents 10 μ.



2



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

3

Figures 2 and 3. 2. An interpretive drawing of the chromosome of figure 1. Centromere of each chromosome is marked by its number which corresponds to that of idiogram. Bar represents 10 μ; **3.** An idiogram of *C. forskohlii* pachytene chromosomes; the nucleolus (N) is not drawn to the scale. Bar represents 10 μ.

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TISSUE LEVELS OF GLUTATHIONE, CYSTEINE AND COENZYME A IN EXPERIMENTAL MERCURIALISM

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THE toxicity of mercurials has been known for centuries but the precise biochemical mechanism of their toxic action is not known with certainty. Since mercurials have an exceptionally high affinity for sulphydryl groups[1] and predominant low molecular weight intracellular sulphydryl compounds are cysteine, coenzyme A, glutathione, lipoate and thioglycolate[2], it is argued that if binding of mercury with these compounds is responsible for toxicity, it should be possible to demonstrate their depleted tissue levels on mercurial administration and their protective effect against mercurial toxicity. The protective effect of cystine[3] and lipoic acid amide[4] against mercury compounds has been reported. Recently, we had found significant protection of toxicities of mercuric chloride and methylmercuric chloride by cysteine and coenzyme A[5], but the tissue levels of these thiols could not be measured on account of small organ size of goldfish. The aim of the present study was to measure the tissue content of coenzyme A, glutathione and cysteine after administration of mercuric chloride in rats.

Twenty male albino rats (*Rattus norvegicus*) (weighing 200–250 g) were divided into two groups, equalising them for litter-mates and weight. The experimental group rats were intraperitoneally injected a dose (5 mg/kg) of mercuric chloride. The control rats received 0.2 ml of normal saline. As the peak values of mercury in blood were obtained at 3 h post-injection[6] and the renal distribution of mercury was also quite extensive at this time (unpublished observations) these rats were sacrificed under ether anesthesia after 3 h. The blood was collected directly from heart in heparinized vials. Liver and kidneys were then removed, washed in ice-cold normal saline, blotted dry, and a weighed piece was homogenized in ice-cold 5% perchloric acid in glass homogenizer. The homogenate was centrifuged at 3000 r.p.m. for 20 min at 5°C in a refrigerated centrifuge.

The homogenate was immediately assayed for its coenzyme A (CoASH), reduced glutathione (GSH) and cysteine content. CoASH was assayed by phosphotransacetylase method[7]. Reduced GSH was