binding protein and hence can be considered as an environmental indicator of cadmium pollution. Further work on purification and metal-binding properties of this protein is in progress.

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Note added in proof: Metal binding proteins in algae now reported to be poly(γ-glutamylcysteinyl) glycines as in higher plants (see Gekler *et al.*, *Arch. Microbiol.*, 1988, **150**, 197).

REGENERATION OF SHOOT BUDS FROM CALLUS CULTURES OF *PAPAVER SOMNIFERUM*

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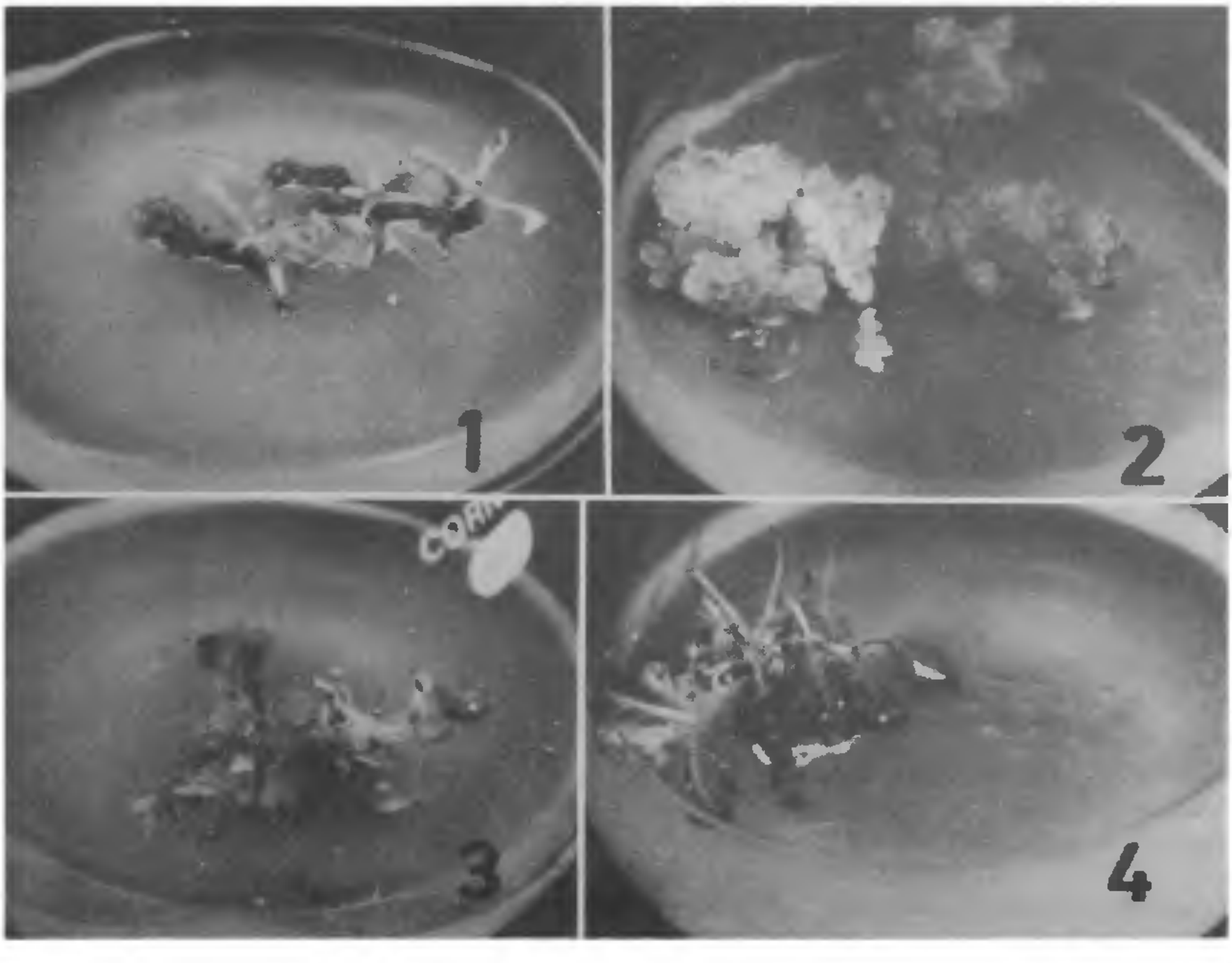
TISSUES from opium poppy have been grown in culture as a callus[1-3], but most of these studies were aimed at studying alkaloid metabolism. In the present investigation, efforts have been made to culture various explants from the opium poppy, *Papaver somniferum* L., and induce organogenesis *in vitro*.

Hypocotyl segments, cotyledons, seedling roots, stem segments and leaf discs of *P. somniferum* were inoculated onto Murashige and Skoog[4] (MS) basal medium supplemented with sucrose (3%) and various concentrations (0.05–5.0 mg/l) of kinetin or benzyladenine singly or in combination with indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D) or α-naphthaleneacetic acid. Callus initiation was observed only from seedling root explants on MS medium containing 0.5–1.0 mg/l of kinetin and 0.5–3.0 mg/l of IAA (figure 1). Other combinations of growth regulators failed to evoke any response in any of the other explants. The callus initiated on medium containing 0.5 mg/l of kinetin and 0.5 mg/l of IAA was isolated and maintained as stock callus on medium supplemented with 0.5 mg/l of kinetin and 3.0 mg/l of 2,4-D (figure 2). This callus was creamy-white, friable and actively growing, and was subcultured regularly every 30th day. The stock callus in its 5th passage of subculture was transferred onto medium containing kinetin or benzyladenine singly or in combination with auxins for differentiation. Shoot bud regeneration was observed only on medium containing kinetin (0.05–1.0 mg/l) alone or kinetin (0.05–3.0 mg/l in combination with 0.5 mg/l of IAA (table 1). Other growth regulators singly or in combinations did not evoke any response. Shoot buds (2–5 in number) were regenerated on MS medium containing 0.05–1.0 mg/l of kinetin. Further increase in kinetin concentration (3.0–5.0 mg/l) inhibited shoot bud regeneration completely. Addition of IAA (0.5 mg/l) to medium containing 0.05–3.0 mg/l of kinetin improved the shoot bud regeneration response of the callus. The maximum number of shoot buds (6–8) was obtained



Figures 1–4. Tissue culture of opium poppy. **1,** Callus initiation from seedling root on MS + kinetin (0.5 mg/l) + IAA (0.5 mg/l). **2,** Stock callus (30 days old) on MS + kinetin (0.5 mg/l) + 2,4-D (3.0 mg/l). **3,** Shoot buds regenerated on MS + kinetin (0.5 mg/l) + IAA (0.5 mg/l). **4,** Isolated shoot buds on MS + kinetin (0.5 mg/l) + IAA (0.5 mg/l).

Table 1 *Effect of various concentrations of kinetin singly or in combination with IAA on regeneration of shoot buds in callus cultures of P. somniferum*

Kinetin (mg/l)	Response: IAA in mg/l				
	0	0.5	1.0	3.0	5.0
0.05	S(2–3)	S(4–6)	NR	NR	NR
0.5	S(2–5)	S(6–8)	NR	NR	NR
1.0	S(2–3)	S(4–5)	NR	NR	NR
3.0	NR	S(2–5)	NR	NR	NR
5.0	NR	NR	NR	NR	NR

S, Shoot buds; NR, no response. Numbers in parentheses give numerical range of shoot buds per five flasks.

on medium containing 0.5 mg/l of kinetin and 0.5 mg/l of IAA (figure 3). This complete elimination/ replacement of 2,4-D with IAA favoured shoot bud regeneration. These results indicate the inhibitory effect of 2,4-D on shoot bud regeneration in *P. somniferum*, as observed in cereals[5].

Shoot buds differentiated on these media were 4–5 mm long. They were isolated and transferred into the same medium or other media containing various combinations of auxins, cytokinins and gibberellic acid for elongation and/or rooting. Shoot buds elongated up to 15 mm on media containing 0.5–1.0 mg/l of IAA and 0.05–3.0 mg/l of kinetin (figure 4). Gibberellic acid did not cause elongation of shoot buds. Under no circumstances could rooting be induced in isolated shoot buds. Further experiments are in progress to induce rooting and to obtain complete plantlets.

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KARYOTYPE ANALYSIS OF *PAPAVER DUBIUM* L.

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PAPAVER DUBIUM L. is an important medicinal plant of the family Papaveraceae. The latex from its immature capsules is known to possess two alkaloids, viz. aporein and aporeidin. Aporein has a physiological action resembling thebaine. The petals of *P. dubium* are sudorific and contain cyanidin-B and pelargonidin-C (ref. 1). The chromosome numbers reported[2] in this species are $2n=28$ and $2n=42$ (ref. 2). However, in a literature survey we could not find any report on chromosome morphology of *P. dubium*. The karyotype of this medicinally important plant species is described in this communication.

Actively growing roots (1–2 cm long) originating from germinated seeds were fixed in Carnoy's solution (6 parts absolute alcohol, 3 parts chloroform and 1 part glacial acetic acid) after pretreatment with saturated solution of *p*-dichlorobenzene at 15°C for 3 h, and transferred to 70% alcohol. After fixation, they were hydrolysed in 1 N HCl at 60°C for 5–10 min and then stained and squashed in 2% acetocarmine. Photographs of ten well-spread metaphase plates were made using phase contrast optics on an Olympus Vanox-S microscope from temporary slides and used for analysis. The chromosomes of the haploid complement were numbered 1 to 14 in decreasing order of length. Centromere positions were described following the nomenclature of Levan *et al.*[3] The centromeric index was calculated as per cent ratio of short arm length to long arm length.

The morphological characteristics of *P. dubium* chromosomes are presented in table 1. A single metaphase cell and its karyogram are shown in figure 1. The somatic chromosome number of *P. dubium* was found to be $2n=28$, which is in conformity with an earlier report[2]. The haploid complement was measured at 41.46 μm. An easily descernible secondary constriction was associated with the long arm of chromosome 2. The chromosome length varied from 2.45 to 3.52 μm. A narrow range of variation in arm ratio (0.26 to 0.47) clearly indicated that all the 14 chromosomes of the haploid complement are acrocentric with subterminally located centromeres. The centromeric index ranged between 19.05 and 31.40, suggesting that the karyotype is symmetric.

Table 1 *Details of chromosome morphology in Papaver dubium L.*

Chromosome number	Chromosome length (μm)				Arm ratio (s/l)	Centromere position	Centromeric index
	Total	Relative	Short arm	Long arm			
1	3.52 ± 0.03	100.00	1.03 ± 0.02	2.41 ± 0.01	0.43	st	29.26 ± 0.29
2*	3.38 ± 0.02	96.02	0.97 ± 0.01	2.36 ± 0.01	0.41	st	28.69 ± 0.18
3	3.28 ± 0.01	93.18	1.03 ± 0.01	2.17 ± 0.02	0.47	st	31.40 ± 0.20
4	3.21 ± 0.02	91.19	0.97 ± 0.01	2.17 ± 0.01	0.45	st	30.22 ± 0.22
5	3.14 ± 0.03	89.20	0.67 ± 0.02	2.38 ± 0.01	0.28	st	21.34 ± 0.32
6	3.07 ± 0.02	87.22	0.62 ± 0.01	2.41 ± 0.01	0.26	st	20.19 ± 0.20
7	2.97 ± 0.02	84.37	0.67 ± 0.01	2.24 ± 0.01	0.30	st	22.56 ± 0.15
8	2.89 ± 0.02	82.10	0.62 ± 0.01	2.24 ± 0.02	0.28	st	21.45 ± 0.22
9	2.86 ± 0.01	81.25	0.76 ± 0.02	2.07 ± 0.01	0.37	st	26.57 ± 0.24
10	2.79 ± 0.02	79.26	0.72 ± 0.01	2.00 ± 0.01	0.36	st	25.81 ± 0.21
11	2.72 ± 0.02	77.27	0.69 ± 0.02	2.00 ± 0.01	0.35	st	25.37 ± 0.38
12	2.66 ± 0.03	75.57	0.55 ± 0.01	1.96 ± 0.01	0.28	st	20.68 ± 0.54
13	2.52 ± 0.03	71.59	0.48 ± 0.02	1.72 ± 0.02	0.28	st	19.05 ± 0.39
14	2.45 ± 0.03	69.60	0.48 ± 0.01	1.55 ± 0.01	0.31	st	19.59 ± 0.26

st, Subterminal.
*Satellited chromosome.