has also affected sex expression in mulberry[1]. Similarly interspecific grafting has been employed to change the sex of dioecious plants. Often, to change the sex of a particular strain, it is grafted on a stock bearing the desired sex.

It is observed that when interspecific/intraspecific grafting is done with different scions and stocks, sometimes the scion gets some of the features of the stock. Change of sex in scion has been reported[2] in mulberry through grafting of strains of different sex.

This note reports occurrence of a variegated mutant in mulberry when axillary buds from exotic strains were grafted on to branches of a local strain. To establish a mulberry germplasm of temperate strains, which are difficult to root, bud grafting of exotic strains on the established local trees was undertaken. Nearly 60 varieties were grafted on local trees. Among them 18 buds of Kokuso-21, an improved Japanese mulberry variety of *M. multicaulis*, were grafted on a *M. indica* tree of 6–7 years (figure 1).

A week after grafting, the top portion above the grafted bud was pruned to facilitate quick sprouting of scion buds. When the buds sprouted, among 12 sprouts one was found producing variegated leaves. This branch was allowed to grow till December of the same year. It attained a growth of 127 cm with 55 leaves. All the leaves were variously variegated (figure 2). The variegation pattern was irregular (figure 3). The mutated scion bears features present in neither the stock nor the scion. The leaves are also thin and differ in size from those of both the parents. The length of the stock branch was 242 cm, with 22 leaves per metre and internodal distance of 4.5 cm; the girth of the branch was 1.5 cm; the area of the leaf was 638 cm^2. The length of nonmutated scion was 218 cm, with 30 leaves per metre and internodal distance of 3.3 cm; the girth of the branch was 2 cm; the area of the leaf was 231.25 cm^2. The length of the mutant branch was 127 cm, with 43 leaves per metre and internodal distance of 2.3 cm; the girth of the branch was 1 cm; the area of the leaf was 187 cm^2.

From the above, it is clear that the length of the mutant branch, internodal distance and leaf area are reduced when compared to those of the nonmutant scion and stock.

The mutant is being multiplied and observations being made to assess its value to sericulture. The variegated nature of the leaves can serve as a marker in genetic studies. In addition, the variegated leaves can be of horticultural importance.

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CADMIUM INDUCIBLE PROTEINS IN *SCENEDESMUS QUADRICAUDA*

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HEAVY metals like cadmium, lead, mercury, copper, etc. are increasing in the biosphere through pollution, agricultural runoff and mining activities. They have direct bearing on various physiological and biochemical functions of organisms. The pronounced effects of heavy metal toxicity are reduction in growth and photosynthesis, inhibition of enzyme activities, and degradation of chloroplasts and mitochondria. Heavy metal tolerance in plants has been explained by several views: exclusion from plant parts[1], accumulation of metals in vacuoles[2] and on cell walls[3], evolution of metal-tolerant enzymes[4], and induction of specific metal-binding proteins similar to metallothioneins[5]. Metals like cadmium, zinc, lead, silver, antimony, copper, mercury, gold, beryllium, tin and nickel induce these proteins[6], which are thought to be involved in metal ion homeostasis and many other functions, such as regulation of cellular metabolism, control of cellular growth, detoxification of free radicals and excess metal ions, and protection against ionizing radiation[7].

Algae and cyanobacteria were studied for metal toxicity[8]. The first report of cadmium-inducible binding proteins was in the freshwater blue-green alga *Anacystis nidulans*[9]. Metal-inducible proteins were also reported in some other algae, viz. *Chlorella ellipsoida*[10], *C. pyrinoidosa*[11], *Dunaliella bioculata*[12] and *Synechococcus* sp.[13]

The present report deals with cadmium-inducible proteins in the common freshwater alga *Scenedesmus quadricauda*. *S. quadricauda* cells were grown in modified Chu medium[14]. Cultures were maintained at 25±2°C in a 16 h light and 8 h dark cycle. Cultures were exposed to different concentrations of cadmium chloride (1, 10, 50, 100, 200 and 400 μM Cd) and growth was measured as increase in optical

density at 678 nm and chlorophyll *a*. Figure 1A shows growth curves of unexposed and cadmium-exposed cells. Growth (as chl *a*) was increased at low concentration of cadmium (1 μM), but inhibited about 18% in 10 μM, 63% in 50 μM, 68% in 100 μM, 90% in 200 μM and totally in 400 μM cadmium (figure 1B). Cadmium causes inhibition of chlorophyll synthesis, decreases the chl *a/b* ratio, and causes disorganization of grana[15]. We also found decreased synthesis of chl *a* and chl *b* in cadmium-exposed cells (table 1). At 100 μM cadmium chl *a* and chl *b* were decreased by about the same extent. However, at higher concentration (200 μM), chl *b* synthesis was inhibited to a greater extent.

Cadmium-exposed (200 μM) and unexposed cells were collected by centrifugation (6000 *g*, 10 min), subjected to sonication in 0.5 M Tris–HCl buffer (pH 8.6), and centrifuged at 4°C (12,000 *g*, 10 min). The supernatant was acetone-precipitated and electrophoresis was done on an 18% SDS-polyacrylamide gel[16]. Cadmium-exposed cells showed an additional band co-migrating with a molecular mass marker of 8 kDa (figure 2). This band was not observed in unexposed cells. This protein may be similar to metal-binding proteins reported from other algae. Cadmium-binding protein from *Synechococcus* sp. has a molecular mass of 8.1 kDa, whereas in *Dunaliella* it is 10 kDa. The molecular mass of purified metal-binding proteins from different algae ranges from 8 to 10 kDa.

The reported cadmium-inducible protein (8 kDa) in *S. quadricauda* is presumed to be a cadmium-

Table 1 *Effect of cadmium on chl a and chl b synthesis in S. quadricauda*

Cadmium (μM)	Chl *a*	Chl *b*
None (control)	4.54 ± 0.54	1.46 ± 0.23
100	1.52 ± 0.42	0.58 ± 0.17
200	1.00 ± 0.3	0.27 ± 0.12

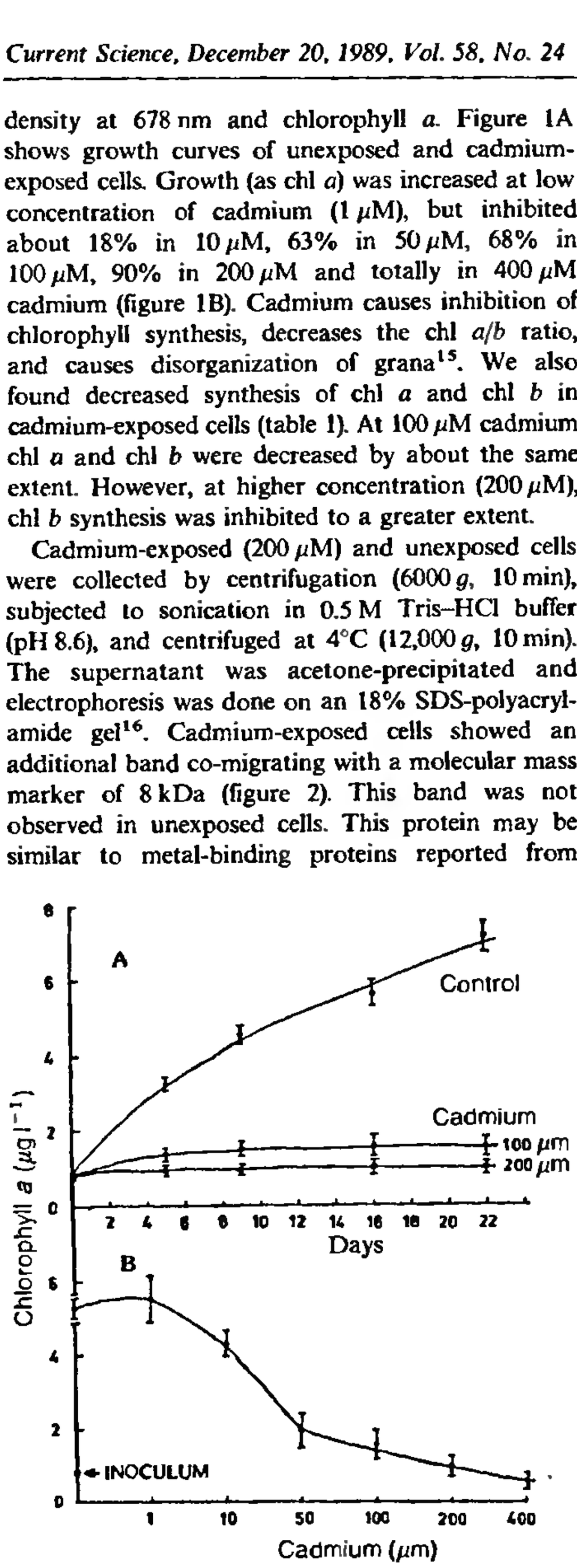

Figure 1. A, Growth curves (as chl *a*) of cadmium-exposed and unexposed cells of *Scenedesmus quadricauda*. B, Effect of different concentrations of cadmium on *S. quadricauda*.

Figure 2. Electrophoretic protein profile of cadmium exposed (left) and unexposed (right) *S. quadricauda*.

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