the target organs of toxicity are kidney, mucus membranes and central nervous system, and for lead, erythropoietic system and unstriated muscles[13].

Since it is the first time the presence of lead and cadmium in the groundwaters of Hubli city is reported an immediate quality monitoring of groundwater in this area is suggested to know the trend and degree of groundwater quality deterioration, which enables one to take precautionary measures to overcome future hazards.

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Regeneration and transformation of *Nasturtium indicum*: a wild crucifer

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***Nasturtium indicum* DC (*cruciferae*) is a common weed growing in India. It was successfully and reproducibly regenerated from leaf explants. When transferred to soil the regenerated plants looked morphologically normal and bore flowers and seeds. When leaf discs were cocultivated with an engineered *Agrobacterium* T$_i$ plasmid vector containing plant expressable kanamycin resistance gene as a selection marker and glucuronidase (GUS) gene as reporter, transformed plants were produced. This tropical crucifer weed can be used as a model plant in studies related to the improvement of oilseed mustard by modern biotechnology.**

RAPESEED mustard is grown as an important oil seed crop in the Indian subcontinent and some other countries of Asia, Europe and North America. Mustard seed is not only a source of oil but also a rich source of protein, and the seed meal after extraction of oil can provide nutrient proteins for food and feed purpose[1]. With a view to improving oil seed mustard using modern biotechnology, work has been initiated in this laboratory, on the isolation of genes expressed in seeds and the analysis of their regulatory sequences[2,3]. We use tobacco as a model plant for the analysis of function of mustard seed protein gene regulatory domains. However, tobacco being a plant that takes long duration from seed sowing to maturation, the experiments take more than a year from tissue culture transformation to maturing seed. The experimental favourite is *Arabidopsis thaliana* because of its short life cycle, small plant size, small genome size and much of its genetics being known[4]. However, being a temperate plant, it cannot be maintained in open environment in warm tropical climate. Moreover, in tissue culture or pot culture, *Arabidopsis* does not grow and set seeds properly above a temperature of 20°C, a condition which is expensive in tropical countries.

While searching for a suitable tropical alternative model of oil seed *Brassica*, we picked up *Nasturtium indicum* DC, commonly known as water cress (syn. *Rorippa montana* Small), which grows readily in Indian climatic condition almost throughout the year. Though the growth is more vigorous in moderate winter, the plant has been observed to grow well even in summer months in partially shaded areas when maximum temperature rises up to 38°C. Its seeds are tiny (1000 seeds, weight 59 mg) and a large number of seeds can be collected for analysis from individual plant. The seeds were analysed for total protein and oil content and were found to contain 21.4% protein and 42.2% oil on the basis of seed dry weight, values being close to mustard[1]. The plant is photoperiod insensitive so that it flowers throughout the year. The haploid DNA content (C value) as measured by cytophotometry after Feulgen staining[5] was found to be 1.4 pg, compared to 1.5 pg in tetraploid *Brassica juncea*. The growth duration from germination to mature seeds is 2–3 months depending on the season. Thus *Nasturtium* resembles cultivated *Brassica* in many ways and having some advantages as mentioned, it can be used as a model plant for expressing mustard genes.

Seeds were collected from the *Nasturtium* weeds

growing in the Institute garden, surface sterilized by 2% sodium hypochlorite (commercial bleach), washed with sterile water and germinated in half strength Murashige and Skoog[6] (MS) agar medium. Of the several media tested, Linsemeir and Skoog[7] (LS) medium supplemented by 3 mg l^{-1} 6-benzylaminopurine gave almost 100% regeneration of multiple shoots from cotyledon or leaf disc explants[8]. The regenerated plants were rooted in half strength MS without any growth regulators. The rooted plants when transferred to soil grew normally. The regenerated plants in tissue culture also flowered and set seeds in less than four months, when maintained at 25 ± 1°C and a light intensity of 2000 lux from fluorescent lamp (Figure 1) with 16 h light and 8 h dark cycles.

For transformation, the binary vector pGSGLUCl with the kanamycin resistance (*kan*r) gene driven by PTR1 promoter as selectable marker and glucuronidase (GUS) gene under PTR2 promoter as a reporter was used[9]. The vector is schematically shown in Figure 2. The engineered *Agrobacterium* pGV2260::pGSGlucl was cocultivated with leaf disc according to Horsch *et al.*[8] and transformed plants were selected on kanamycin plate.

Portions of leaves and roots from the control and transformed plant were stained for GUS activity by the *in situ* histochemical staining methods of Jefferson[10] using 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-gluc) as substrate. Blue violet staining of the root and leaves indicated transformation and expression of

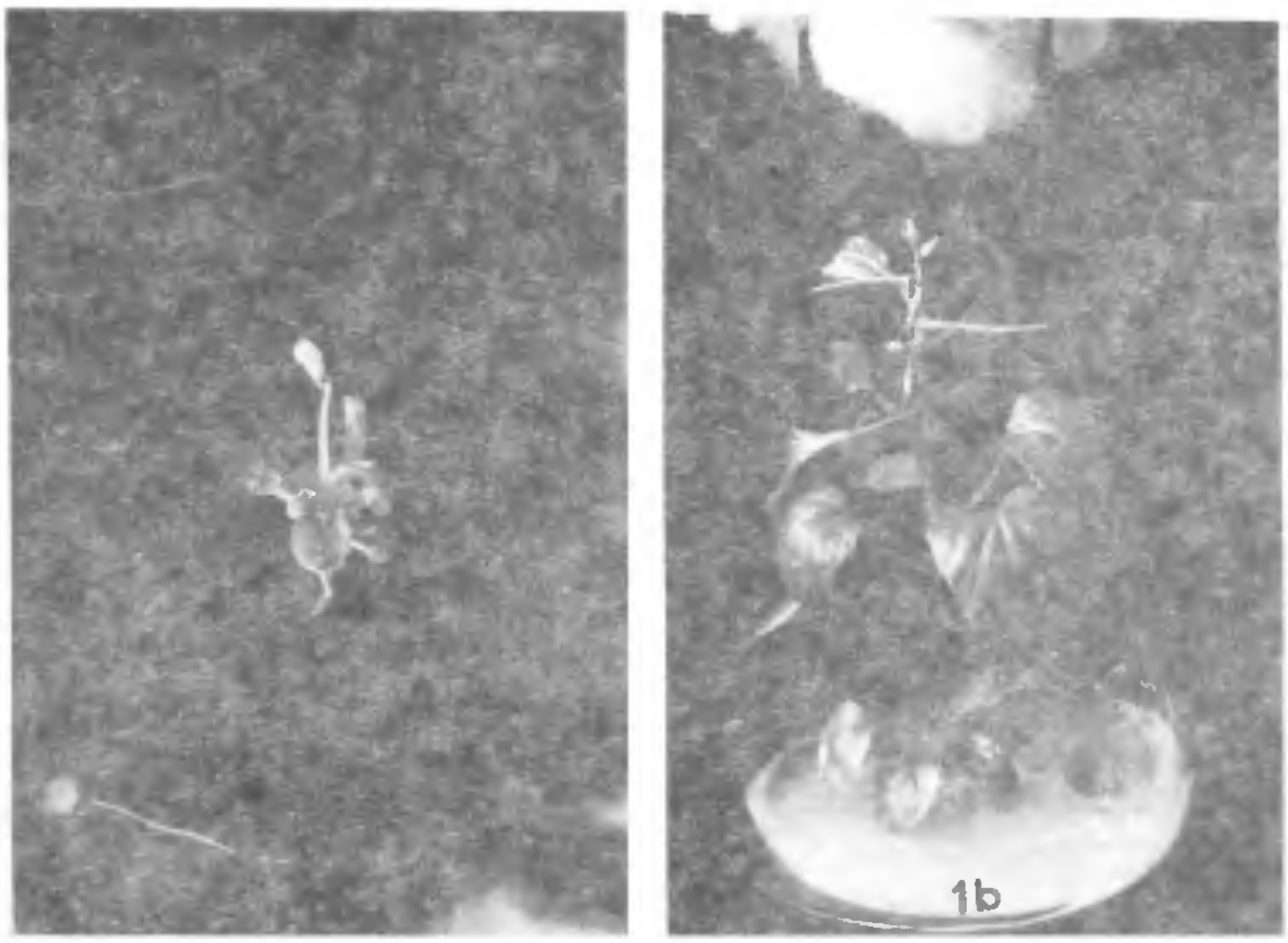

Figure 1. *a*, Multiple shoot regeneration from cotyledonary leaf explant. Note that shoots regenerate not only from the cut end of the petiole but also from other points of the cotyledon. *b*, Regenerated plant showing flowers and maturing siliqua in culture. *c*, Normal-looking regenerated plant growing in soil

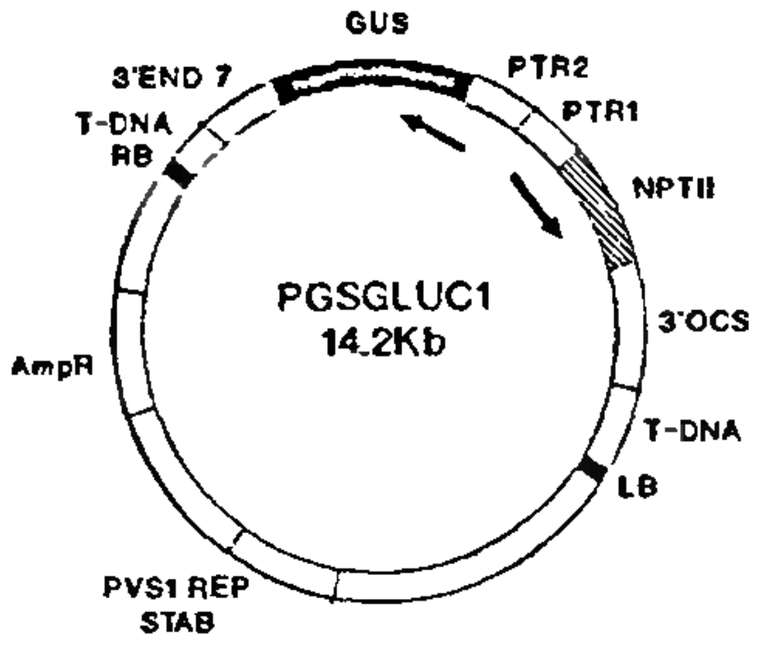

Figure 2. Schematic diagram of the vector pGSGlucl, showing the selectable marker *NPTII* and the reporter *GUS* gene. RB, right border; LB, left border; NPTII, neomycin phosphotransferase II; OCS, octopine synthase; GUS, β-D-glucuronidase; PTR1 and PTR2, promoters of TR1 and TR2 genes; PVS1Rep Stab, PVS1 replication stabilizer. Arrows indicate the direction of transcription. Drawn after Deblaere *et al.*[8].

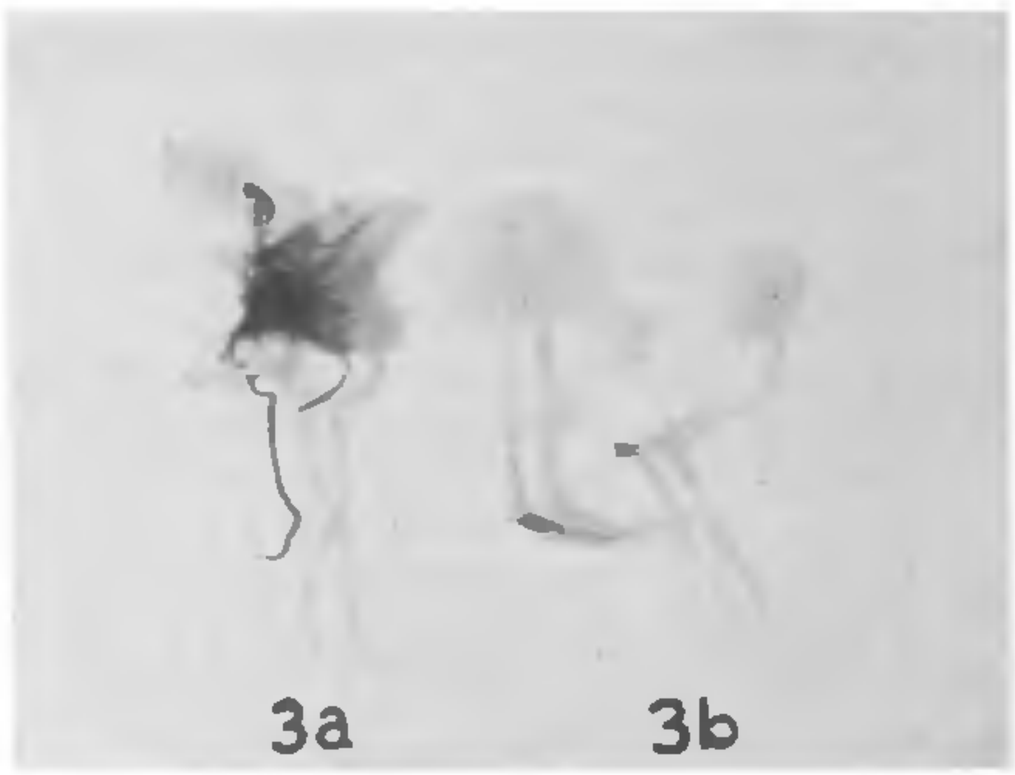

Figure 3. Histochemical staining for GUS activity of regenerated plants. *a*, Transformed plant showing GUS activity; *b*, control plant.

gus gene. As PTR1 promoter is constitutive the enzyme β-D-glucoronidase was expressed both in root and leaf (Figure 3*b*).

Thus *N. indicum* can be used as a convenient tropical plant model for expressing foreign genes/promoters used for oil seed improvement as also of other transgenes for basic plant molecular biology. The use of this plant will require less stringent condition in tissue culture and plant rooms.

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