Theor Appl Genet (1989) 78: 161–168

Heavy metal tolerant transgenic *Brassica napus* L. and *Nicotiana tabacum* L. plants

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Received October 26, 1988; Accepted March 29, 1989
Communicated by P. Maliga

Summary. A chimeric gene containing a cloned human metallothionein-II (MT-II) processed gene was introduced into *Brassica napus* and *Nicotiana tabacum* cells on a disarmed Ti-plasmid of *Agrobacterium tumefaciens*. Transformants expressed MT protein as a Mendelian trait and in a constitutive manner. Seeds from self-fertilized transgenic plants were germinated on media containing toxic levels of cadmium and scored for tolerance/susceptibility to this heavy metal. The growth of root and shoot of transformed seedlings was unaffected by up to 100 μM $CdCl_2$, whereas control seedlings showed severe inhibition of root and shoot growth and chlorosis of leaves. The results of these experiments indicate that agriculturally important plants such as *B. napus* can be genetically engineered for heavy metal tolerance/sequestration and eventually for partitioning of heavy metals in non-consumed plant tissues.

Key words: *Brassica napus* – Transgenic – Heavy metal tolerance – Human metallothionein gene – Ti-plasmid

Introduction

Over the past years, modern agricultural practices such as the excessive use of phosphatic fertilizers (Varma and Katz 1978; Friberg et al. 1974) and sewage sludge (Council for Agricultural Science and Technology 1980, Report No. 83) has resulted in contamination of agricultural soils with heavy metals. The passive uptake of metals such as cadmium (Cd) moves them into the food chain, and consumption of such contaminated food and tobacco result in chronic exposure, which poses a serious threat to human health (Sherlock 1984). In addition, industrial activities such as mining and smelting operations have produced large areas with copper (Cu) and Zinc (Zn) contaminated soils, where climatic factors are otherwise favourable for crop production (Petolino and Collins 1984). The increasing levels of toxic metals in the soils warrants the production and use of plant varieties capable of: (a) heavy metal tolerance, (b) sequestration of toxic metals in non-consumed plants parts.

A logical approach to this problem is through the expression of gene(s) coding heavy metal binding/sequestration proteins derived from vertebrates and fungi into transgenic plants. Heavy metals in vertebrates and fungi are detoxified by the metallothioneins (MTs), which are low-molecular-weight cysteine-rich, and heavy metal binding proteins (Kagi and Nordberg 1979). Their synthesis is regulated at the transcriptional level in response to stress. These proteins are, however, not found in plants. Instead, phytochelatins (small peptides, which are not gene products), have been shown to sequester heavy metals (Grill et al. 1985; Grill et al. 1987; Jackson et al. 1987). Despite the presence of these peptides, plants are generally susceptible to enhanced levels of Cd (Rauser 1986).

Agrobacterium tumefaciens, a soil bacterium has been used widely as a vehicle for stable integration and transfer of DNA into the genome of transgenic plants (Horsch et al. 1984; DeBlock et al. 1984; Rogers et al. 1986). Genes for several agriculturally relevant traits have been transformed and expressed in plants using Ti-plasmid vectors. The most promising examples involve genes protecting crop plants against non-selective herbicides (Shah et al. 1986; Fillati et al. 1987), insect control (Vaeck et al. 1987) and protection against viral disease (Powell et al. 1986; Harrison et al. 1987).

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In order to confer tolerance to toxic levels of heavy metals, we have introduced a chimeric human MT gene into *B. napus* and *N. tabacum* cells on a disarmed Ti-plasmid of *A. tumefaciens*. Our results show that growth of transgenic seedlings was unaffected by up to 0.1 m*M* $CdCl_2$ in the media, whereas control seedlings showed severe inhibition of root and shoot growth. Inheritance studies on transformed seeds show that the tolerance phenotype segregated in a manner consistent with Mendelian inheritance. This is the first report of stable integration and expression of a heavy metal resistance phenotype in two distinct genera, oilseed rape and tobacco.

Materials and methods

Construction of chimeric gene and introduction into Agrobacterium tumefaciens

An Rsa1-Rsa1 fragment (290 bp) was derived from a 4.8-kb EcoRI fragment of human genomic DNA that encoded a processed metallothionein-II gene (Varshney and Gedamu 1984; Fig. 1). The excised fragment which contained the translation initiation and termination sites (truncated gene) was cloned into the Sma1 site of PGEM-2 plasmid (Promega, Biotech). For cloning of the metallothionein gene into the intermediary transformation vector, a 320-bp fragment containing the entire MT coding sequence was excised from the pGEMhMT-IIpg plasmid by restriction digestion with EcoRI and Xba1. The fragment was made blunt-ended by T4 DNA polymerase and was then ligated into the expression cassette vector pMON316 (Sanders et al. 1987) at the BglII site, which was also made blunt-ended. The structure of the resultant plasmid with respect to orientation of the gene was confirmed by Southern blot analysis of rapid plasmid digests of pMONhMT-IIpg, using ^{32}P-labelled, nick-translated, BamH1-PvuII fragment and by DNA sequence analysis using the dideoxy method (Sanger et al. 1977). The resultant plasmid (pMONhMT-IIpg), carrying the chimeric MT-gene (CaMV35S:hMT-IIpg:NOS 3′), in addition to the chimeric neomycin phosphotransferase II (NOS:NPT:NOS) gene for selection of transformed tissue on kanamycin and an intact nopaline synthase gene as a reporter gene (Sanders et al. 1987), was introduced into *A. tumefaciens* strain GV3111 harboring the disarmed plasmid pTiB6S3-SE (Fraley et al. 1985). Recombinants between the Ti-plasmid and the intermediary vector were selected for resistance to spectinomycin and streptomycin, as described.

Transformation, selection and regeneration

Transformation and regeneration of tobacco (*Nicotiana tabacum* L. cv W38) was essentially as described by Horsch et al. (1985). For co-cultivation of *B. napus* L. cv Westar, stem epidermal explants were prepared according to conditions described by Klimaszewska and Keller (1985). For transformation, after 3 days of culture, explants were dipped for 5 min in an overnight bacterial culture. The explants were blotted on filter paper and then co-cultivated for 24–48 h on media containing Murashige and Skoog's basal (MS) salts (Murashige and Skoog 1962), B5 vitamins, 3% sucrose, 0.65% Phytagar (Gibco), pH 6.8, 10 mg/l BA and 0.5 mg/l NAA. After co-cultivation, the epidermal explants were transferred to fresh media to which 0.5 mg/l carbenicillin (Ayerst Labs, Montreal, Canada) was added to inhibit further growth of bacteria and 0.1 mg/ml kanamycin for selection. At 2–3 weeks, the explants were transferred to fresh plates containing the same medium. In order to induce roots, well-developed shoots were transferred to B5 media lacking growth regulators but containing 0.5 mg/ml carbenicillin and 0.1 mg/ml kanamycin. Culture of explants and rooting of regenerated shoots were in a growth room under cool white light, with a 14 h photoperiod and constant temperature of 24 °C. Plantlets were transferred to Jiffy-7 peat pellets, kept in Magenta jars for a gradual hardening process and later transplanted to 4″ pots and transferred to a growth chamber with a day/night temperature of 22°/18 °C and 18 h photoperiod.

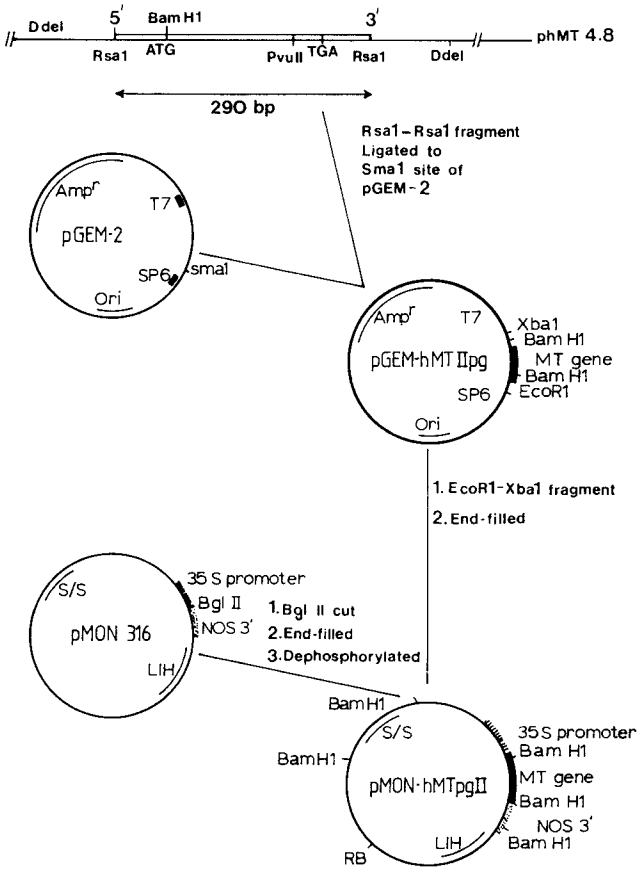

Fig. 1. Cloning strategy for the construction of the chimeric human-MT gene

Analysis of MT gene sequences in DNA of transformed tissue

Plant DNA was isolated from leaves of control and transformed plants according to the method described by Lichtenstein and Draper (1985). Restriction endonuclease-digested DNA was subjected to electrophoresis in an 0.8% agarose gel and transferred to nitrocellulose (Maniatis et al. 1982). Prehybridization and hybridization conditions were according to Varshney and Gedamu (1984). Briefly, prehybridization was done for 2–6 h at 60°–65 °C in buffer consisting of 3XSET, 10X Denhardt's, 0.1% SDS, 0.1% sodium pyrophosphate, 10 μg/ml Poly (A), 85 μg/ml yeast RNA, 0.1 mg/ml sheared and denaturated *E. coli* DNA. Filters were hybridized to ^{32}P-RNA complementary to hMT-IIpg mRNA, synthesized from EcoRI-linearized pGEMhMT-IIpg plasmid, using a Riboprobe kit according to the manufacturer's instructions (Promega, Biotech).

Gel electrophoresis and fluorography of metallothionein

In order to investigate the expression of the metallothionein gene under the CaMV 35S promoter in transformed plants, 20 leaf discs each from transformed and control plants were incubated with L-[^{35}S] cysteine (250 μCi/ml; specific activity 1200 Ci/mmole; Amersham) for 4 h. Tissue was homogenized in 20 m*M* ammonium acetate (pH 8.6) and centrifuged for 10 min in an Eppendorf centrifuge to collect the supernatant. Extracts containing equal amounts of acid-precipitable radioactivity were carboxymethylated according to Durnam et al. (1980) and precipitated by adding 2.5 volumes of 95% ethanol. The pellets were resuspended in 15 ml of sample buffer (62.5 m*M* TRIS pH 6.8, 10% glycerol, 5% β-MSH and 0.001% Bromophenol blue) and electrophoresed on a 20% polyacrylamide slab gel with 5% stacking gel, in the complete absence of SDS as described by Price-Haughey et al. (1987). The gels were fixed in acetic acid and methanol, treated with Amplify (Amersham) as described by the supplier and dried. Fluorography was carried out as described previously.

Seedling test

Seeds of *B. napus* were surface-sterilized with 0.2% w/v mercuric chloride for 20 min followed by 6% sodium hypochlorite (commercial Javex bleach) for 30 min. Seeds of tobacco were surface-sterilized with 6% sodium hypochloride for 10 min. After rinsing in distilled water, the seeds were aseptically germinated on one-tenth MS agar media with or without $CdCl_2$, ranging in concentration from 0.025 to 1.0 m*M*. Fresh growth and root length of seedlings were recorded after 3 weeks.

Results

Construction of chimeric gene encoding the MT protein

A metallothionein-processed gene (hMT-IIpg) was isolated from a human genomic library and its complete sequence was determined (Varshney and Gedamu 1984). The gene represents a full-length perfect copy of its corresponding mRNA. The strategy for constructing the chimeric gene (pMONhMT-IIpg) containing this human metallothionein gene is shown in Fig. 1. For insertion of MT gene into the intermediary transformation vector, a 320-bp fragment was excised from the pGEMhMT-IIpg plasmid (Fig. 1) by restriction digestion with EcoRI and XbaI. This fragment was cloned at the BglII site of the expression cassette vector pMON316 (Sanders et al. 1987), by blunt-end ligation. The insert and its proper orientation with respect to the CaMV 35S promoter was confirmed by Southern blot analysis of the BamH1 digests of pMONhMT-IIpg using ^{32}P-labelled nick-translated BamH1-PvuII fragment and by DNA sequence analysis (data not shown).

Transformation, selection and regeneration of B. napus and N. tabacum

Leaf discs of *Nicotiana tabacum* cv W38 and stem epidermal explants of *Brassica napus* cv Westar were inoculated with *Agrobacterium tumefaciens* cells containing the pMONhMT-IIpg:pTiB6S3-SE cointegrate plasmid. The intermediary vector pMON316 carried a chimeric neomycin phosphotransferase II (NOS:NPT:NOS) gene for selection of transformed tissue on kanamycin and an intact nopaline synthase gene as a screenable marker (Sanders et al. 1987). The transformed cells of *B. napus* and *N. tabacum* were selected and regenerated on media containing 0.1 mg/ml and 0.25 mg/ml of Kanamycin, respectively. Finally, regenerated shoots that rooted on B5 media containing 0.1 mg/ml kanamycin were screened for expression of nopaline synthase and neomycin phosphotransferase II activities. A total of 5 different transgenic plants of *B. napus* and 15 different transgenic plants of *N. tabacum* were regenerated. Finally only 3 plants of *N. tabacum* and 2 plants of *B. napus* that showed significantly higher levels of nopaline synthase and NPT-II enzyme activities were selected for further analysis (data not shown).

Integration and expression of the chimeric MT gene in transgenic plants

Protein analysis was conducted on putative transformants to detect the presence of the metallothionein protein and to provide a conservative estimate of the frequency at which the non-selectable MT gene was co-expressed with the selectable kan^R gene. Proteins were labelled with [^{35}S]cysteine, carboxymethylated and electrophoresed on a 20% PAGE as described in 'Materials and methods'. [^{35}S]labelled proteins from a $ZnCl_2$-induced rainbow trout hepatoma (RTH) cell line were used as a standard reference. In the complete absence of SDS, the low-molecular-weight carboxymethylated MTs migrate rapidly just behind the solvent front while other proteins are insoluble in the sample buffer (Fig. 2 A). Using this separation technique, we detected MT protein in all five of the kan^R resistant plants. In transformed *N. tabacum* (nos. 1010, 1045, 1015) and in *B. napus* (nos. 2001, 2002), a band was observed that co-migrated with MT band from $ZnCl_2$-induced RTH cell extracts. This band was absent in non-transformed cells of *N. tabacum* and *B. napus*.

To confirm chromosomal integration of chimeric MT gene, genomic DNA was isolated from control and transformed leaves. For Southern-blot analysis, BamH1 restriction enzyme-digested DNA was fractionated on a 0.8% agarose gel, transferred to nitrocellulose and hybridized to a ^{32}P-labelled cRNA probe for MT-IIpg gene. As expected, the probe hybridized to an internal BamH1 fragment of about 0.258 kb in the leaf DNA from *B. napus* (nos. 2001, 2002) and *N. tabacum* (nos. 1010, 1015, 1045) transformants (fig. 2 B). No hybridization was observed in control *N. tabacum* and *B. napus* leaf DNA. The NPT-II gene was also shown to be integrated in the DNA from the transformants (data not shown).

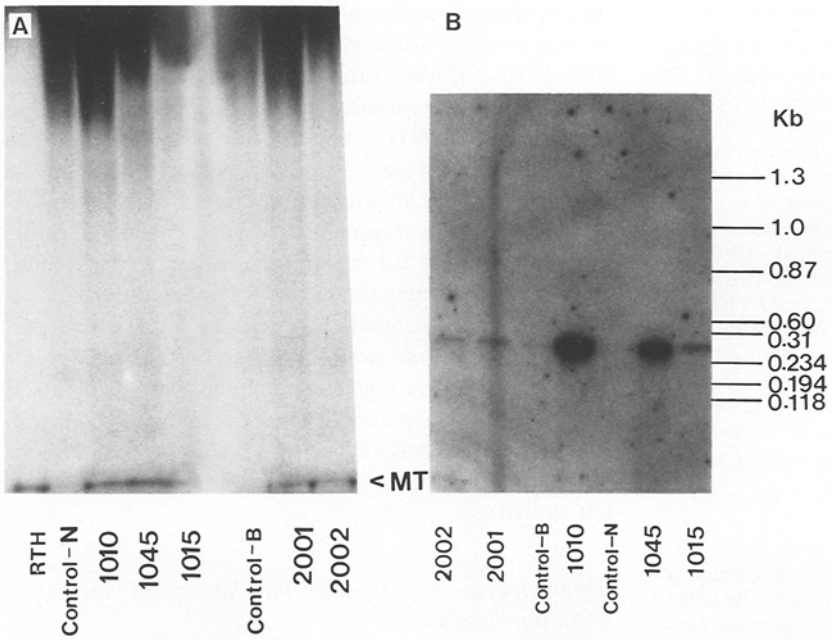

Fig. 2 A and B. Integration and expression of metallothionein gene. **A** Expression of metallothionein gene in primary *B. napus* and *N. tabacum* transformants. Comparison of proteins isolated from control and transformed leaf tissue. Metallothionein induced in response to $ZnCl_2$ treatment in rainbow trout hepatoma cells was used as standard reference (RTH). *Arrowhead* indicates position of metallothionein. Plant nos. 1010, 1045, 1015 are transgenic tobacco and nos. 2001, 2002 are transgenic *B. napus*. Controls are non-transformed *N. tabacum* (control-N) and *B. napus* (Control-B) plants. **B** Southern-blot analysis of primary *B. napus* and *N. tabacum* transformants. Leaf genomic DNA from control and transformed plants was digested with BamH1. The control plants are non-transformed *B. napus* (control-B) and *N. tabacum* (control-N) plants. Plant nos. 1010, 1015, 1045 are transgenic *N. tabacum* and nos. 2001, 2002 are transgenic *B. napus*, respectively. The blot was probed with a ^{32}P-labelled RNA complementary to hMT-IIpg mRNA. The sizes of the molecular weight markers are indicated

Heavy metal tolerance of transgenic plants

In the initial experiments, cuttings of control and transgenic plants were tested for their tolerance to cadmium. Stem cuttings with three top-expanding leaves attached were placed, for 10 h, in solutions which ranged in concentration from 0.1 m*M* to 10 m*M* $CdCl_2$. In solutions containing up to 0.1 m*M* $CdCl_2$, the leaves of control as well as transformed plants remained turgid for up to 7 days. In contrast, 5–10 m*M* $CdCl_2$ solutions proved to be lethal to control as well as transformed leaves. A 10-h exposure to 1 m*M* $CdCl_2$ resulted in wilting and browning of control leaves, whereas transformed leaves remained turgid and green for up to 7 days (data not shown).

Inheritance of the cadmium-tolerant phenotype

The need for a sufficient number of uniform plants in order to obtain statistically significant results places limitations on experiments with cuttings (Powell et al. 1986). Therefore, all other experiments on heavy metal tolerance were performed with seed progeny of self-fertilized transgenic plants.

A simple assay method was developed which was used to identify tolerant/susceptible phenotypes. The assay is based on the fact that exceedingly low concentrations of heavy metal ions in the order of a few parts per billion cause inhibition of root growth in solution cultures (Gemmell 1977). Similar root inhibitory effects are also observed in soil-grown plants. The root system of afflicted plants become coralloid and stumpy in appearance. Based on this information, root length of seedlings was employed as a criterion for heavy metal toxicity. Seed progeny from self-pollinated transformants (S_1 generation) and control (non-transformed) plants were germinated on MS media containing 0.025–1 m*M* $CdCl_2$. The seedlings were then scored for root length and general growth for 3–4 weeks after germination. On media containing 1 m*M* $CdCl_2$, growth of non-transformed *B. napus* and *N. tabacum* seedlings was completely inhibited. However, when control *N. tabacum* and *B. napus* seedlings were germinated on media containing up to 0.1 m*M* $CdCl_2$, sensitivity to Cd was clearly indicated by inhibiton of root growth. The addition of 0.1 m*M* $CdCl_2$ to the medium reduced the fresh weight and root length of the seedlings dramatically (Table 1). The fresh weight of *N. tabacum* and *B. napus* was reduced by 83% and

Table 1. Heavy metal tolerance of transgenic *B. napus* and *N. tabacum* S_1 progeny. Seeds from self-pollinated control and transformed plants were germinated on MS media containing 0.1 m*M* $CdCl_2$. Root length and fresh wt. of seedlings were recorded 3 weeks after germination of seed. Data presented are mean values of 100 seed samples of *N. tabacum* and 15 seeds of *B. napus* each. In case of the transformants, only Cd-resistant seedlings were taken into account

Seed sample	Media	Fresh wt. (mg)	Percent of control	Root length (cm)	Percent of control
N. tabacum					
Non-transformed	− Cd	28.6 ±5.6		5.5 ±0.02	
	+ Cd	5.0 ±0.15	17.5	0.91±0.18	16.3
Transformed 1010	− Cd	28.75±3.5		4.87±0.2	
	+ Cd	30.00±5.5	104.3	3.72±0.21	76.4
1045	− Cd	27.1 ±2.1		4.01±0.5	
	+ Cd	25.5 ±1.8	94.0	3.25±0.16	81.04
1015	− Cd	27.9 ±1.5		4.5 ±0.35	
	+ Cd	26.2 ±1.3	93.90	3.5 ±0.40	77.7
B. napus					
Non-transformed	− Cd	176 ±63		10 ±3.1	
	+ Cd	90 ± 2.4	51.1	1.7 ±0.03	17
Transformed 2001	− Cd	192 ± 2.3		8.0 ±1.3	
	+ Cd	170 ± 3.6	88.5	7.0 ±2.0	87.5
2002	− Cd	191 ± 1.5		9.0 ±0.5	
	+ Cd	170 ± 1.2	89.0	7.75±1.2	86.1

Table 2. Genetic inheritance of heavy metal tolerance in *N. tabacum* plants containing the metallothionein gene. The ratio of tolerant to susceptible plants was compared to the expected ratio using a Chi-square analysis. The genetic inheritance of the MT gene was determined by scoring the progeny of the transformed plants for tolerance to 0.1 m*M* $CdCl_2$ as was observed by the root growth. The ratio of tolerant to suceptible plants for the expected 3:1 ratio in the three cases tested indicate that MT gene is inherited as a single locus

Transformant	Observed ratio		Expected ratio		χ^2 (3:1)	Significance level
	T	S	T	S		
1010	52	15	51	17	0.078	0.7−0.8
1045	32	7	29	9	1.04	0.3−0.5
1015	36	6	31	10	2.5	0.1−0.2

χ^2 – Chi-square value for 3:1 ratio
T – Tolerant
S – Susceptible

49%, respectively. Similarly, in each case the root length showed 83%−84% reduction relative to the seedlings growing on control media. In contrast, the progeny of the selfed transformants segregated for root growth. Some of the seedlings grew normally on media containing 0.1 m*M* Cd (Fig. 3A and B). After 3 weeks on 0.1 m*M* Cd, the average root length of Cd-tolerant *N. tabacum* seedlings was 3.5±0.25 cm compared to 0.9±0.1 cm of control seedlings (Table 1). In *B. napus*, the average root length of Cd-tolerant seedlings was 7.0 cm compared to 1.7 cm of control seedlings. The fresh weight of Cd-tolerant seedlings of *N. tabacum* and *B. napus* also showed slight reduction, if any, whereas the control seedlings were all severely affected (Table 1).

The genetic inheritance pattern of the MT gene was determined by scoring the progeny of the transformed tobacco plants for tolerance to cadmium. As shown in Fig. 4A, the seed progeny segregated into two distinct populations. The smaller population of seedlings had small, stumpy roots with an average root length of 0.5±0.11 cm. In the larger population, the seedling growth appeared to be unaffected by cadmium up to a concentration of 0.1 m*M* $CdCl_2$. The average root length of this group of seedlings was 3.5±0.25 cm. A Chi-square analysis was conducted on data from the three *N. tabacum* transformants and it demonstrated that the ratio of tolerant to susceptible plants was 3:1. This ratio indicates that the MT gene was inherited as a single locus (Table 2). These seed populations also segregated in a 3:1 ratio on media containing kanamycin (data not shown).

The first leaf of Cd-resistant tobacco plants was also assayed for kanamycin resistance by its ability to callus on media containing 0.1 mg/ml kanamycin. The leaves from Cd-resistant plants were resistant to kanamycin as demonstrated by callusing on kan^R media (Fig. 4B). Results of such experiments showed that, at least in the three *N. tabacum* plants which have been tested to date, the MT gene segregated with the kanamycin resistance gene.

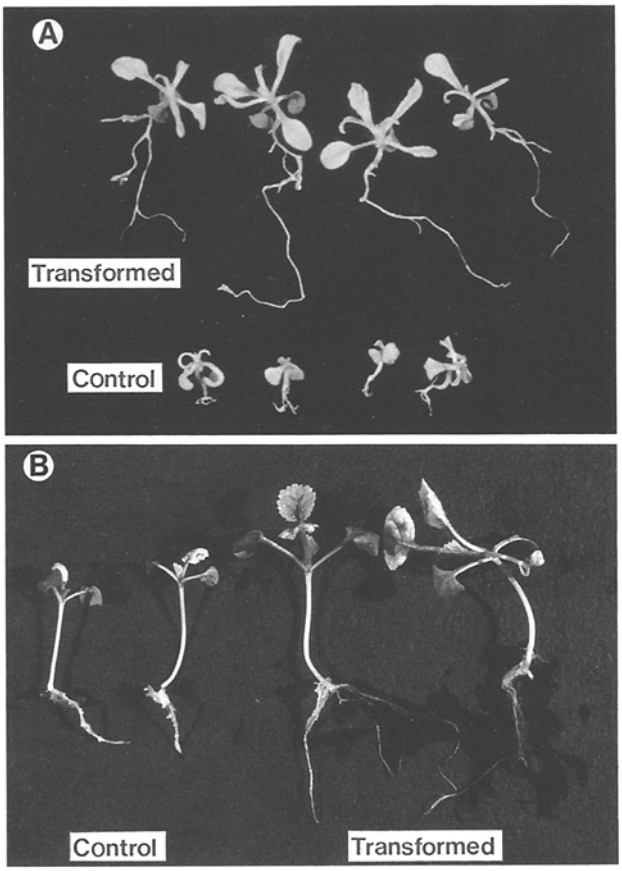

Fig. 3 A and B. Cadmium tolerance of S_1 transgenic seedlings. Surface-sterilized seeds from self-pollinated plants were germinated on MS media containing 0.1 m*M* $CdCl_2$. Germination and seedling growth were scored 3 weeks after germination of seeds. **A** Non-transformed and transformed *N. tabacum* (no. 1010) seedlings. **B** Non-transformed and transformed *B. napus* (no. 2001) seedlings. Differences in root length and seedling growth are apparent in each case

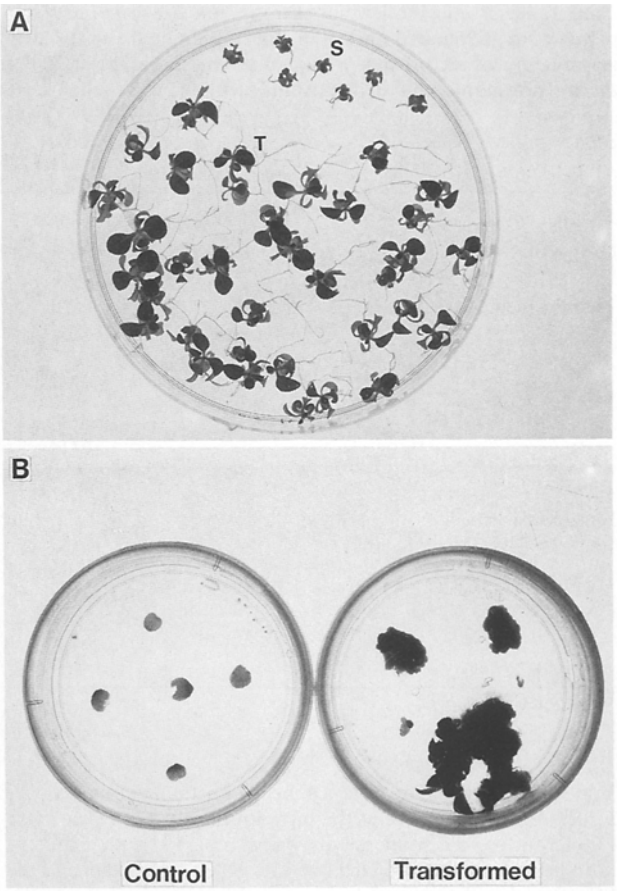

Fig. 4. **A** Segregation of Cd-tolerant and susceptible phenotype in a S_1 population of transgenic *N. tabacum* seed sample (no. 1010). Seeds were germinated on MS media with 0.1 m*M* $CdCl_2$ and scored for segregation 3 weeks after germination. T – Tolerant; S – susceptible. **B** Leaf disc test for kanamycin resistance of Cd-tolerant (transformed, right) and Cd-susceptible (left) *N. tabacum*. First cotyledonary leaf of transformed plants selected on 0.1 m*M* $CdCl_2$ was used in this test. Leaf discs were cultured on media containing 0.1 mg/ml kanamycin and scored for their ability to form callus on the selective medium after 2 weeks

Discussion

In this report we show, for the first time, that a human MT-II processed gene is stably integrated and expressed in *B. napus* and *N. tabacum* seedlings. These transgenic plants show tolerance to toxic levels of cadmium, suggesting that the MT protein synthesized in *B. napus* and *N. tabacum* may be involved in heavy metal detoxification/sequestration.

In addition, the heavy metal tolerance trait showed Mendelian inheritance and co-segregated with kanamycin resistance. To our knowledge, this is the first demonstration of inheritance and expression of a human MT gene in transgenic plants.

In the past, a cDNA clone of a Chinese hamster metallothionein-II gene was expressed as a recombinant cauliflower mosaic virus in systemically infected *B. campestris* tissue and was shown to protect the plant against cadmium (Lefebvre et al. 1987). Although the function of MT in this species is demonstrated, the approach has a limitation, since the gene is neither integrated nor stably inherited. Recently, a mouse MT cDNA has also been expressed in transformed tobacco tissue. However, the stable inheritance of this MT cDNA and heavy metal tolerance was not demonstrated (Maiti et al. 1988). Our approach of conferring heavy metal tolerance by a stable integration and expression of a single gene coding for a heavy metal binding and/or sequestering protein clearly demonstrated that plants can be genetically engineered for heavy metal tolerance. In this regard, genes of bacterial and viral origin resulting in resistance to, e.g., herbicides, insect and viral attacks have been

reported to function in transgenic plants. In each case the single gene trait is inherited in a Mendelian fashion.

Other approaches in producing metal tolerance plants using in vitro selection have limited value (Petolino and Collins 1984). Although cell lines with improved abilities to grow under high concentrations of metal such as aluminum, mercury, zinc and cadmium have been produced, in only a few cases has the acquired trait shown to be stably expressed in plants regenerated from such cells. Also, in nature, plants differ in their ability to grow in soils containing elevated levels of toxic metals. Natural selection on mine sites has resulted in ecotypes within species capable of growth in the presence of high concentrations of toxic ions (Gemmell 1977; Foy et al. 1978). Highly tolerant (Cu, Zn) populations of some grasses (*Agrostis, Festuca*) have evolved in response to metal contamination. However, such tolerant ecotypes have inherently slow growth rates. Also, seed multiplication of these ecotypes is difficult because of selection against tolerance on uncontaminated soils (Gemmell 1977).

The Ti-plasmid mediated genetic transformation of MT gene in plants provides a valuable method of generating metal tolerant varieties, which could be useful for reclamation of wastelands and mine spoils. Also, this approach has a potential of regulating MT synthesis in a tissue-specific manner, thereby partitioning toxic metals in non-consumed parts of the plant. Analysis of plants grown on agricultural soils contaminated with sewage sludge and phosphatic fertilizers, which may contain high levels of Cd and other heavy metals, has shown that the highest concentration of these metals accumulate in leaf tissue. It is not surprising, therefore, to find high levels of cadmium in leafy vegetables, such as lettuce, spinach and even tobacco leaves (Sherlock 1984; Van Bruwane et al. 1984). Expression of MT in root tissue specifically may overcome this problem to some extent. Efforts are now underway to express MT in roots of *B. napus* and *N. tabacum* and examine its effect in partitioning of Cd between various plant parts.

Acknowledgements. This research was supported in part by the Natural Sciences and Engineering Research Council of Canada. S. Misra was a recipient of NSERC Postdoctoral and NSERC Industrial Research Fellowships. We would like to thank R. Fraley and S. Rogers (Monsanto, St. Louis) for providing the transformation vectors. We also thank Dr. W. R. Scowcroft for his encouragement, helpful discussions and critical review of the manuscript.

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