# Cadmium induces DNA damage in tobacco roots, but no DNA damage, somatic mutations or homologous recombination in tobacco leaves

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## Abstract

The heavy metal cadmium ($Cd^{2+}$) applied on tobacco roots in the form of cadmium chloride, induced significantly higher levels of DNA damage as measured by the cellular Comet assay than did treatment of isolated root nuclei, analyzed by use of the acellular Comet assay. DNA damage induced by $Cd^{2+}$ in roots of a transgenic catalase-deficient tobacco line (CAT1AS) was higher than in wild-type tobacco (SR1) roots. In contrast to treatment with the positive control ethyl methanesulphonate, $Cd^{2+}$ induced no significant DNA damage in leaf nuclei, and neither somatic mutations, nor homologous recombination as measured by the GUS genereactivation assay, were observed in leaves. Analysis of the accumulation of cadmium by inductively coupled plasma optical emission spectrometry demonstrates that roots accumulate almost 50-fold more cadmium than above-ground parts of the tobacco seedlings. This may explain the absence of $Cd^{2+}$ genotoxicity in leaves.
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## 1. Introduction

Cadmium is a widespread heavy metal, released into the environment by power stations, heating systems, waste incinerators, metal working industries and from many other sources. Accumulation of cadmium in soils can become dangerous to all kinds of organ-isms, including plants. Even though the toxic effects of cadmium compounds in plants have been studied over many years, inconsistent results have been obtained with respect to their genotoxic properties [1–4]. The possible pathway(s) of cadmium-induced genotoxicity are still unknown, but may involve the interaction of this metal with DNA, either directly or indirectly [5].

In the work presented here we have treated tobacco seedlings or isolated tobacco nuclei with cadmium chloride ($Cd^{2+}$) and measured and compared: (1) the level of DNA damage induced by in vivo treatment of roots (cellular Comet assay) and isolated root nuclei (acellular Comet assay), (2) the DNA damage

in roots and leaves measured by the cellular Comet assay, (3) the somatic mutation frequency, and (4) the homologous recombination frequency as measured by the GUS gene-reactivation assay. Comparing the effects of $Cd^{2+}$ in the cellular and acellular Comet assay, and in tobacco leaves and roots, with different levels of reactive oxygen scavengers, may help to elucidate the mechanism of genotoxicity of $Cd^{2+}$. As a positive control we have applied the monofunctional alkylating agent ethyl methanesulphonate. In addition, studies were performed to analyze the accumulation of cadmium in tobacco roots and leaves.

## 2. Materials and methods

### 2.1. Chemicals and media

Cadmium chloride, hemi(pentahydrate), ($Cd^{2+}$, CAS No. 7790-78-5), ethyl methanesulphonate (EMS, CAS No. 62-50-0), 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide cyclohexylamine salt (CAS No. 114162-64-0), the plant growth medium (Phytagel, MS salts), reagents for electrophoresis, normal melting point (NMP) and low melting point (LMP) agarose, and general laboratory reagents were purchased from Sigma Chemical Co., St. Louis, MO.

### 2.2. Plant material

(1) Double heterozygous *Nicotiana tabacum* var. xanthi ($a_1^+/a_1$; $a_2^+/a_2$) plants [6] were used for the Comet assay and for the detection of somatic mutations.
(2) *N. tabacum* var. Petit Havana SR1-wild type and *N. tabacum* CAT1AS (a transgenic line that expresses only 10% of wild-type catalase activity in the leaves and only 40% in the roots due to the antisense expression of the *cat1* gene), was derived from *N. tabacum* Petit Havana SR1 [7,8]. The seeds of the transgenic tobacco line were kindly provided by Dr. F. Van Breusegem (Gent, Belgium).
(3) The tobacco line N9, derived from *N. tabacum* Petit Havana SR1 [9] containing overlapping sequences of the *uidA* gene in inverted orientation, separated by a hygromycin phosphotransferase

gene was used for the GUS gene-reactivation assay. The seeds of line N9 were kindly provided by Dr. I. Kovalchuk (University of Lethbridge, Canada)

### 2.3. Tobacco growth and $Cd^{2+}$ treatment conditions

Tobacco seeds were sterilized by immersion in 70% ethanol for 2 min followed by 20 min in a sterilizing solution (4.5 ml distilled water, 0.5 ml 5.25% sodium hypochlorite, 5 µl 10% Triton X-100). The sterilizing solution was aspirated and the seeds were washed five times in sterile distilled water. Each seed was placed in a vented container that contained 50 ml of sterile, solid growth medium and the plants were grown in a plant growth chamber at 26 °C with a 16 h per day photo-period to the four to five true-leaf stage. At this stage the roots of seedlings were carefully rinsed in water and immersed in glass vials containing 22 ml of a defined concentration of $Cd^{2+}$. The plants were treated in the dark at 26 °C for 24 or 72 h. A detailed description of plant growth conditions was published previously [10].

### 2.4. Cellular and acellular Comet assay

The alkaline version of the Comet assay (single-cell gel electrophoresis, SCGE) can quantitatively measure DNA damage, including single-strand breaks, double-strand breaks, alkali labile sites (primarily apurinic and apyrimidinic sites), incomplete excision repair sites and DNA cross-links (for review see [11]).

After seedling treatment, *N. tabacum* var. xanthi roots or leaves were placed in a 60 mm petri dish kept on ice and spread with 250 µl of cold 400 mM Tris buffer, pH 7.5. Using a fresh razor blade, roots or leaves were gently sliced. The plate was kept tilted in the ice so that the isolated nuclei would collect in the buffer. Regular microscope slides were dipped into a solution of 1% NMP agarose prepared with water at 50 °C, dried overnight at room temperature and kept dry in slide boxes until use. Onto each slide, nuclear suspension (50 µl) and 1% LMP agarose (50 µl) prepared with phosphate-buffered saline were added at 40 °C. The nuclei and the LMP agarose were gently mixed by repeated pipetting using a cut micro-pipet tip, and a coverslip was placed on the mixture. The

slide was cooled in a steel tray on ice for a minimum of 3 min, the coverslip was removed and a final layer of 0.5% LMP agarose (100 μl) was placed on the slide, which was covered again with a coverslip. The slides were placed in a horizontal gel-electrophoresis tank containing freshly prepared cold electrophoresis buffer (1 mM $Na_2EDTA$ and 300 mM NaOH, pH > 13). The nuclei were incubated for 15 min to allow the DNA to unwind prior to electrophoresis at $0.72\,V\,cm^{-1}$ (26 V; 300 mA) for 30 min at $4\,°C$. After electrophoresis, the slides were rinsed three times with 400 mM Tris buffer, pH 7.5, stained with 80 μl ethidium bromide ($20\,μg\,ml^{-1}$) for 5 min, dipped in ice-cold water to remove the excess stain, and covered with a coverslip. For each slide, 25 randomly chosen nuclei were analyzed using a fluorescence microscope with an excitation filter of BP 546/10 nm and a barrier filter of 590 nm.

A computerized image-analysis system (Komet version 3.1, Kinetic Imaging Ltd., Liverpool, UK) was employed. The tail moment (integrated value of tail DNA density multiplied by the migration distance) and in some cases the % of tail DNA were used as the primary measure of DNA damage. Twenty-five nuclei were scored per slide, three slides were evaluated per treatment and each treatment was repeated at least twice. From the repeated experiments, the averaged median tail moment value and averaged median % of tail DNA from each slide were calculated [12].

In the acellular Comet assay, SCGE slides with nuclei from untreated tobacco root cells were prepared as outlined above and immersed in solutions of 400 mM Tris buffer, pH 7.5, containing different concentrations of $Cd^{2+}$ for 2 h at $26\,°C$. After the treatment period, the slides were rinsed three times for 5 min by immersion in cold distilled water, electrophoresed and analyzed as described above.

Plants have a cell wall that cannot be removed by a lysing step as used in the Comet assay protocol for animal and human cells to remove the cell membrane and to denature proteins. The nuclei for the plant cellular and acellular Comet assay have to be isolated mechanically. Thus the plant DNA on the slide is not free of DNA-associated protein. However, analysis of the DNA damage induced by EMS without or with exposure for 1 or 24 h in a lysing solution demonstrated no difference in the tail-moment values [13].

### 2.5. Detection of somatic mutations

The *N. tabacum* var. xanthi seedlings were cultivated as described above. For each experiment, eight seedlings at the stage of four to five leaves were treated with $Cd^{2+}$ or EMS for 24 or 72 h in dark. Then the seedlings were rinsed, and cultivated in glass vials with a 50% Hoagland's solution in a growth chamber at $22–26\,°C$ with an 18 h per day photo-period for 2–3 weeks. The dark green, yellow and green/yellow twin sectors [6] were identified on the pale green leaves newly formed after the treatment (on the first newly formed leaves 14 days and on second leaves 21 days after the treatment). Each experiment was repeated twice.

### 2.6. GUS gene-reactivation assay

The GUS gene-reactivation assay utilizes N9 transgenic tobacco plants that carry in their genome two non-functional disrupted overlapping copies of the *uidA* (GUS) gene. This was shown to effectively monitor the frequency of homologous recombination events [9].

For each experiment, 14 tobacco N9 seedlings at the stage of four to five leaves were treated with $Cd^{2+}$ or EMS for 24 h. After the treatment, the roots of the seedlings were rinsed and cultivated in glass vials with a 50% Hoagland's solution in a growth chamber at $22–26\,°C$ with an 18 h per day photo-period. After 14 days the first and after 21 days the second newly formed leaves were histochemically stained [14–16]. The leaves were placed in a beaker with a staining buffer containing 100 mg 5-bromo-4-chloro-3-indolyl beta-D-glucuronide cyclohexylamine salt, 150 mg $NaN_3$, and 300 μl Triton X-100 in 300 ml phosphate buffer adjusted to pH 7.3. To support the penetration of the staining buffer, the leaves were vacuum infiltrated (twice 1 min with a 30 s interval at 100 mbar) and incubated while shaking at $37\,°C$ for 48 h. Then the leaves were incubated in ethanol for 5 h at $65\,°C$ to remove chlorophyll. After enzymatic hydrolysis, the water-soluble indoxyl intermediate undergoes an oxidative dimerization to produce a blue indigo precipitate. The recombination events were scored as blue sectors on the bleached leaves. The average numbers of recombination events on the first and second leaves formed newly after treatment were summed up.

### 2.7. Determination of total cadmium content in roots and leaves of tobacco

Aliquots (1 g) of the dried and powdered leaves or roots, prepared from control and $Cd^{2+}$-treated 8 weeks old seedlings, were decomposed in borosilicate glass test-tubes at $400\,^{\circ}C$ for 8 h in a mixture of oxidizing gases ($O_2 + O_3 + NO_x$) in a Dry Mode Mineralizer Apion (Tessek, Czech Republic) [17]. The ash was dissolved in 20 ml of 1.5% $HNO_3$ (electronic grade purity, Analytika Ltd., Czech Republic) and kept in glass tubes until measurement. Aliquots of the certified reference material V-10 Hay powder were mineralized under the same conditions for quality assurance of the analytical data. In this reference material, containing $0.02$–$0.05\,mg\,Cd\,kg^{-1}$, a total amount of $0.048\,mg\,Cd\,kg^{-1}$ was detected.

Total cadmium concentrations in the digests were determined by inductively coupled plasma optical emission spectrometry with axial plasma configuration (ICP-AES—Varian VistaPro, Australia, equipped with an autosampler SPS-5, at spectral line $\lambda = 226.5\,nm$).

### 2.8. Statistical analysis

Data were analyzed using the statistical and graphical functions of SigmaPlot 4.01 and SigmaStat 2.03 (SPSS Inc., Chicago, IL, USA). The median tail moment values were used in a one-way analysis of variance test. If a significant $F$-value of $P \leq 0.05$ was obtained, a Dunnett's multiple comparison versus the control group analysis was conducted. Differences between two groups were statistically evaluated by the Paired $t$-test. The values of S.D. for the mean total Cd-accumulation data in the roots and leaves represent % values. To compare the $Cd^{2+}$ dose–response data and DNA damage expressed by the tail moment and % of tail DNA, a Pearson correlation test was conducted, using also the SigmaStat 2.03 program.

### 3. Results

#### 3.1. Cellular and acellular Comet assays in tobacco roots

Fig. 1A illustrates the DNA-damaging effect of $Cd^{2+}$ applied for 2 h on roots of *N. tabacum* var. xan-thi seedlings. The data of the cellular Comet assay with increasing concentrations of $Cd^{2+}$ (0.2–1.6 mM) clearly demonstrate a significant increase in the averaged median tail moment (TM) values ($\pm$S.E.) from $6.1 \pm 0.5\,\mu m$ (negative control) to $42.0 \pm 2.2\,\mu m$ after a 1.6 mM $Cd^{2+}$ treatment ($F_{5,35} = 65$, $P < 0.001$).

By contrast, in the acellular Comet assay, the treatment of isolated root nuclei for 2 h with 0.2–1.6 mM $Cd^{2+}$ yielded much less DNA damage. The TM values ($\pm$S.E.) after the 1.6 mM $Cd^{2+}$ treatment of isolated root nuclei increased only to $12.1 \pm 1.2\,\mu m$ (Fig. 1A) compared to the similar treatment analyzed in the cellular assay, with TM $= 42.0 \pm 2.2\,\mu m$. The latter value was significantly higher ($t = 9.1$, $P < 0.001$).

Isolated nuclei embedded in agarose on a slide are more sensitive to stress than nuclei surrounded with cytosol and protected by the cell wall. Thus treatments longer than 2 h cannot be applied in the acellular Comet assay as even control nuclei are severely damaged, resulting in increased tail moment values.

After 2 h treatment of roots of the transgenic CAT1AS line and wild-type SR1 tobacco with 0.2–1.6 mM $Cd^{2+}$, nuclei were isolated and a concentration-response analysis was conducted with the cellular Comet assay (Fig. 1B). In CAT1AS tobacco, the average median TM values ($\pm$S.E.) increased significantly with increasing concentrations of $Cd^{2+}$, from $2.8 \pm 0.3\,\mu m$ (negative control) to $64.6 \pm 2.4\,\mu m$ after treatment with 1.6 mM $Cd^{2+}$ ($F_{5,71} = 107$, $P < 0.001$). Treatment of roots of the wild-type tobacco SR1 with concentrations of 0.2–1.6 mM $Cd^{2+}$ also resulted in a significant increase of the TM values compared with the negative control ($F_{5,50} = 59$, $P < 0.001$), however the DNA damage was less than that induced in the CAT1AS tobacco line. With 1.6 mM $Cd^{2+}$ the TM value in the CAT1AS line reached a value of $64.6 \pm 2.4$, whereas in SR1 the TM value was significantly lower, reaching only $42.0 \pm 3.1\,\mu m$ ($P = < 0.001$).

To determine the direct effect of $Cd^{2+}$ on DNA, the acellular Comet assay was applied, in which the DNA is no longer under the regulation of any metabolic pathway or protected by the cell-wall barrier (Fig. 1B). In contrast to the root treatments with $Cd^{2+}$, treatments of isolated CAT1AS and SR1 root nuclei resulted in a significant increase in the TM values only at the concentration of 1.6 mM $Cd^{2+}$ ($F_{5,53} = 16$, $P < 0.001$ for CAT1AS; $F_{5,50} = 59$, $P < 0.001$ for SR1).
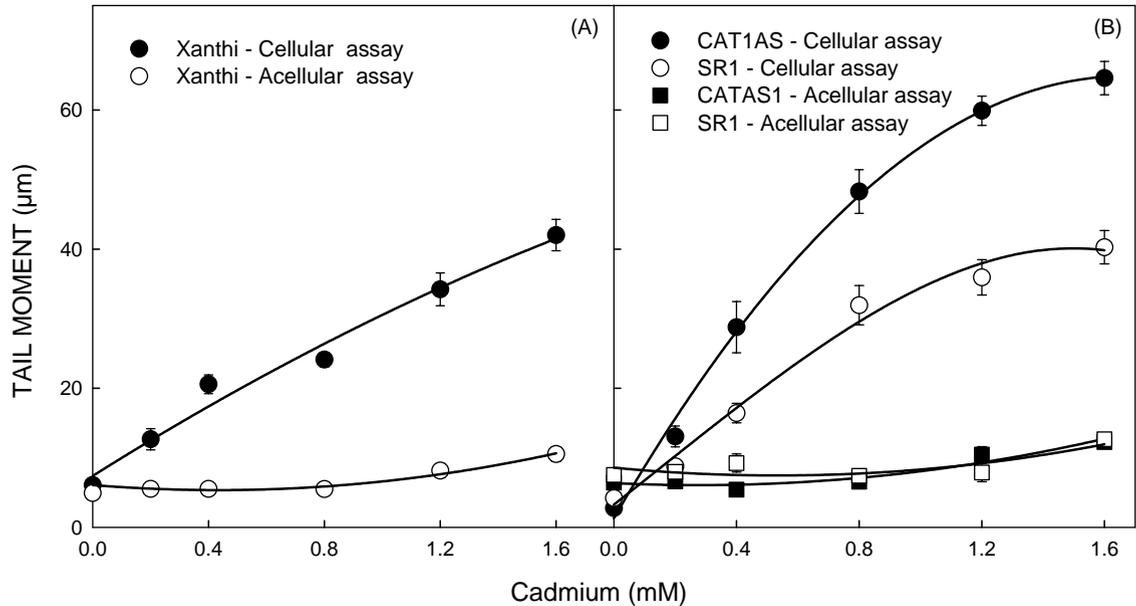
Fig. 1. Dose–response curves of the average median tail moment values as a function of cadmium ($Cd^{2+}$) treatment for 2 h at $26\,°C$ of *N. tabacum* var. xanthi [Xanthi] (A) or the catalase-deficient line (CAT1AS) and wild-type (SR1) tobacco (B). Cellular Comet assay: nuclei isolated from $Cd^{2+}$-treated roots. Acellular Comet assay: isolated root nuclei treated with $Cd^{2+}$. The error bars represent the standard error of the averaged medians.

### 3.2. Cellular Comet assay in roots and leaves

After a 24 h treatment period in the dark with $Cd^{2+}$, nuclei were isolated from roots of *N. tabacum* var. xanthi and a concentration–response analysis in the Comet assay was conducted (Fig. 2A). With increasing concentrations of $Cd^{2+}$ in the range of 0.02–0.1 mM, the average median tail moment values ($\pm$S.E.) increased significantly from $4.7 \pm 0.6\,\mu m$ (negative control) to $76.6 \pm 3.1\,\mu m$ after treatment with 0.08 mM $Cd^{2+}$ ($F_{5,33} = 149$, $P < 0.001$). Treatment with 0.1 mM $Cd^{2+}$ resulted in highly damaged nuclei that could not be evaluated.

By contrast, analysis of DNA damage in nuclei isolated from the leaves of tobacco seedlings treated with 0.02–0.1 mM $Cd^{2+}$ for a period of 24 h did not result in a significant increase in the TM values ($F_{5,33} = 0.6$, $P = 0.714$).

As there is no consensus among investigators as to the most appropriate manner in which to express the DNA damage in the Comet assay, the data on $Cd^{2+}$-induced DNA damage were expressed both as the TM value and as % of tail DNA (Fig. 2A). The results demonstrate that both values can be used to express the DNA damage as measured by the Comet assay. The control values of the DNA damage in the roots when expressed as % of tail DNA, were found to be higher ($7.9 \pm 0.7\%$) than those expressed as TM ($4.7 \pm 0.6\,\mu m$). The Pearson product moments values, expressing the correlation between the concentration of $Cd^{2+}$ and the DNA damage in the roots was about the same when the DNA migration was expressed as the TM ($r = 0.98$) or as the % of tail DNA ($r = 0.96$) values. Due to the lower control values, we have used in our experiments the TM value to express DNA damage measured by the Comet assay.

In nuclei isolated from the roots after a 72 h treatment of tobacco seedlings with $Cd^{2+}$ (0.004–0.02 mM) (Fig. 2B), the average median TM values ($\pm$S.E.) increased from $4.7 \pm 0.6\,\mu m$ (negative control) to $76.6 \pm 3.1\,\mu m$ ($F_{4,27} = 108$, $P < 0.001$). On the other hand, in nuclei isolated from the leaves of the tobacco seedlings subjected to the same treatment
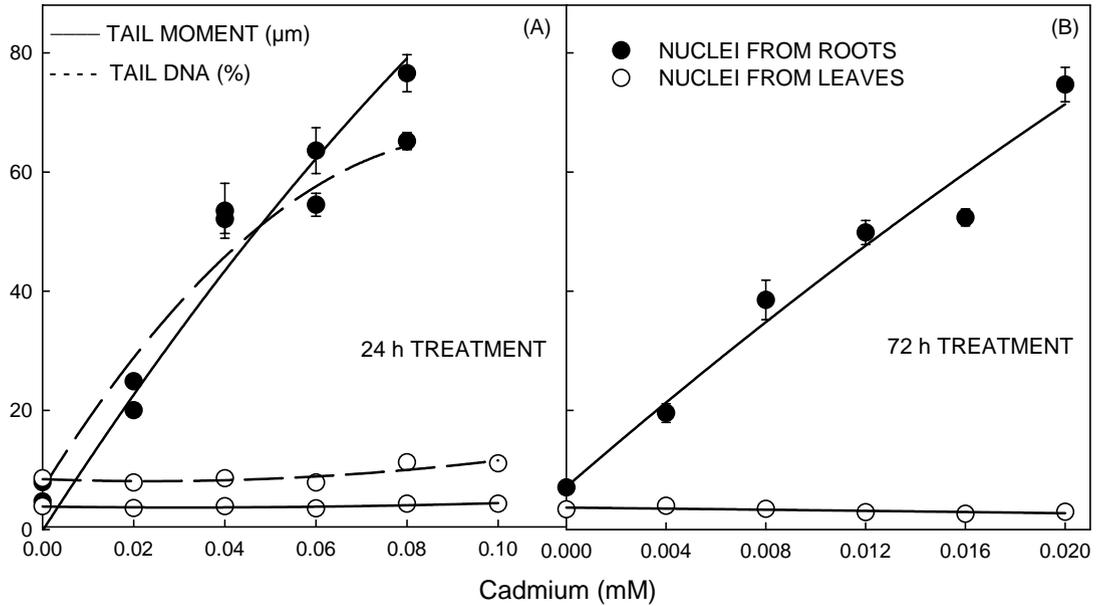
Fig. 2. Dose–response curves of the DNA damage in nuclei of *N. tabacum* var. xanthi roots and leaves as a function of cadmium ($Cd^{2+}$) treatment for 24 h (A) or 72 h (B) at 26 °C. DNA damage expressed by the tail moment (—) or by the % of tail DNA (— —). The error bars represent the standard error of the averaged medians.
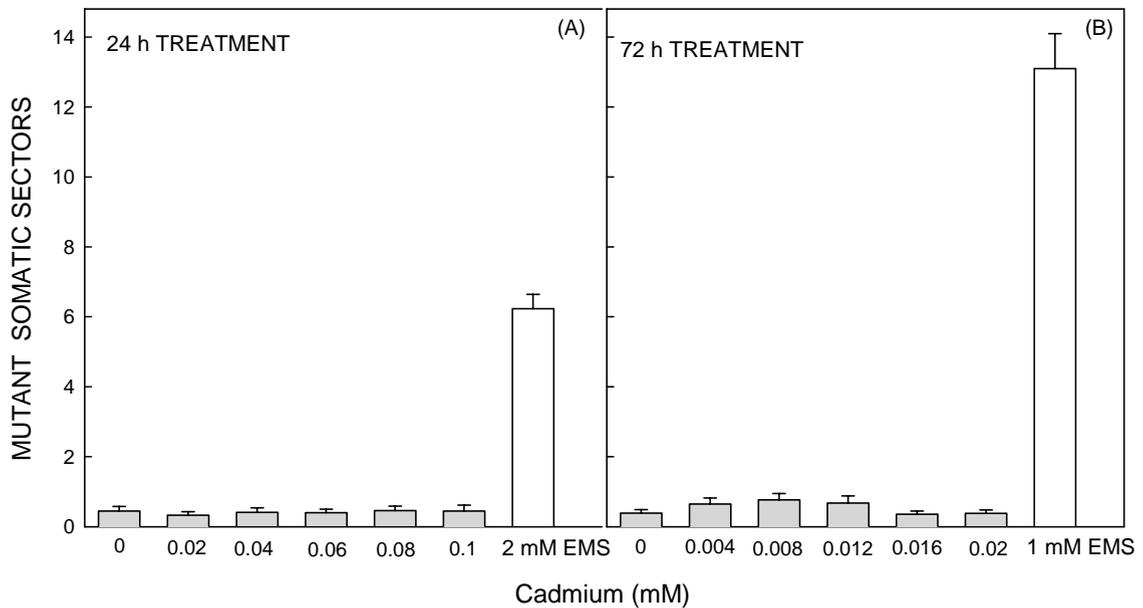


Fig. 3. The average frequency of somatic mutant sectors on the first and second newly formed leaves after treatment of *N. tabacum* var. xanthi seedlings with cadmium ($Cd^{2+}$) for 24 h (A) or 72 h (B) at 26 °C. Ethyl methanesulphonate (EMS) was used as a positive control. The error bars represent the standard error of the mean.

schedule, no significant increase in the TM values was demonstrated ($F_{5,35} = 0.6$, $P = 0.714$).

### 3.3. Yield of somatic mutations

The mutagenic activity of $Cd^{2+}$ in the chlorophyll-deficient tester strain *N. tabacum* var. xanthi was studied after 24 and 72 h treatments of seedlings. The mono-functional alkylating agent EMS was used as a positive control. Treatment of seedlings for 24 h in a concentration range from 0.02 to 0.1 mM $Cd^{2+}$ induced a mean number of mutant sectors per leaf ($\pm$S.E.) that ranged from $0.3 \pm 0.1$ to $0.5 \pm 0.1$. These values were not significantly different ($F_{5,142} = 0.14$, $P = 0.981$) from the negative control ($0.5 \pm 0.1$) (Fig. 3A). Higher concentrations could not be applied as they inhibited the growth of the apical meristem and no new leaves, where the mutant sectors could be scored, were formed. The positive control EMS (2 mM) induced $6.2 \pm 0.4$ mutant sectors per leaf.

Treatment of seedlings for 72 h with concentrations ranging from 0.004 to 0.02 mM $Cd^{2+}$ resulted in a frequency of somatic mutant sectors ranging from $0.4 \pm 0.1$ to $0.8 \pm 0.2$ per leaf. This was not significantly different ($F_{5,162} = 1.6$, $P = 0.164$) from the negative control. Treatment with 1 mM EMS (positive control) for 72 h induced $13.1 \pm 1.0$ mutant sectors per leaf (Fig. 3B).

### 3.4. Homologous recombination frequency

The frequency of blue spots per first and second leaves formed after a 24 h treatment of transgenic N9 tobacco seedlings with $Cd^{2+}$ was a measure of homologous recombination (Fig. 4). Concentrations of $Cd^{2+}$ in the range of 0.02–0.08 mM did not significantly increase ($F_{4,139} = 0.86$, $P = 0.491$) the average frequency of blue spots ($\pm$S.E.) compared to the negative control ($1.8 \pm 0.3$). The frequency of blue spots induced by 4 mM EMS was $8.5 \pm 0.6$ per leaf.

### 3.5. Accumulation of total cadmium in tobacco roots and leaves

Treatment of 8 weeks old tobacco seedlings with 0.1 mM cadmium chloride for 24 h resulted in about
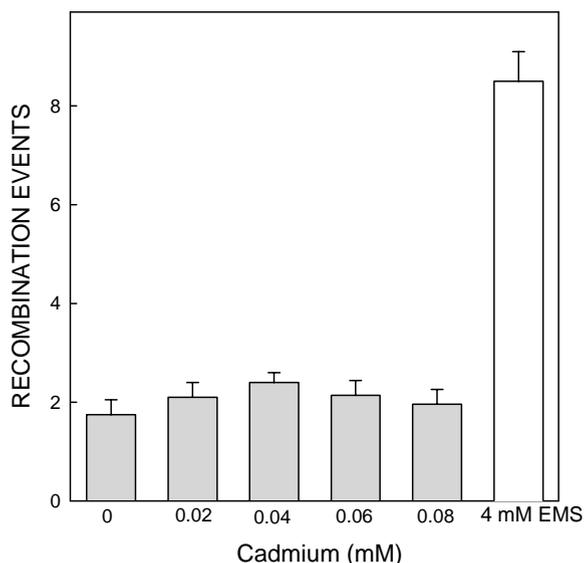


Fig. 4. The average frequency of homologous recombination events in the GUS recombination assay on the first and second newly formed leaves after treatment of the transgenic *N. tabacum* line N9 seedlings with cadmium ($Cd^{2+}$) for 24 h at 26 °C. Ethyl methanesulphonate was used as a positive control. The error bars represent the standard error of the mean.

a three-fold increase in the total Cd content of the leaves ($40.4 \pm 1.8$) compared to the negative control ($13.2 \pm 2.8$ mg kg$^{-1}$ dry mass) (Table 1). By contrast, after the same treatment the roots accumulated almost 50-fold more cadmium than leaves and showed nearly a 1000-fold increase in Cd content ($1963 \pm 0.6$) compared to the control ($2.4 \pm 1.3$ mg kg$^{-1}$ dry mass).

Table 1

Comparison of total cadmium accumulation in above-ground biomass and roots of tobacco (*N. tabacum* var. xanthi) after treatment of 8 weeks old seedlings with $Cd^{2+}$

| $Cd^{2+}$ (mM), 24 h treatment | Cd in above-ground biomass (mg kg$^{-1}$ dry mass) | Cd in roots (mg kg$^{-1}$ dry mass) |
|---|---|---|
| 0 | $13.2 \pm 2.8$ | $2.4 \pm 1.3$ |
| 0.02 | $27.1 \pm 1.5$ | $497 \pm 0.7$ |
| 0.04 | $26.3 \pm 1.2$ | $789 \pm 1.1$ |
| 0.06 | $34.8 \pm 1.4$ | $1635 \pm 0.3$ |
| 0.08 | $30.8 \pm 0.5$ | $1668 \pm 0.9$ |
| 0.1 | $40.4 \pm 1.8$ | $1963 \pm 0.6$ |

The results represent means of five measurements $\pm$ S.D.

## 4. Discussion

### 4.1. The possible mechanism of cadmium genotoxicity

The molecular mechanism responsible for the genotoxicity of cadmium remains largely unclear, but it has been suggested that it may involve either direct or indirect interaction of $Cd^{2+}$ with DNA [5]. The direct reaction may involve binding of Cd with DNA possibly at G, A and T bases [18]. However, the cellular toxicity and genotoxicity of Cd may be also mediated indirectly. This metal depletes the cell's major antioxidants, particularly thiol-containing antioxidants and enzymes and thus may cause an increase in production of reactive oxygen species (ROS) such as the hydroxyl radical, the superoxide radical or hydrogen peroxide. Enhanced generation of ROS can overwhelm the cell's intrinsic antioxidant defenses, and result in a condition known as "oxidative stress". Cells under oxidative stress display various dysfunctions due to lesions caused by ROS to lipids, proteins and DNA [19].

The low level of $Cd^{2+}$-induced DNA damage observed in the acellular Comet assay after treatment of isolated nuclei without cytosol (Fig. 1) supports the assumption that the DNA damage induced by $Cd^{2+}$ is preferentially mediated indirectly. Apparently, the metabolic products of $Cd^{2+}$-induced stress in the cytosol, i.e. ROS, mainly participate in the production of DNA damage, and the direct binding of $Cd^{2+}$ with nuclear DNA is of a less importance. The present experimental findings with transgenic catalase-deficient tobacco (Fig. 1B) lend support to this interpretation.

### 4.2. Why is $Cd^{2+}$-induced DNA damage detected in tobacco roots and no $Cd^{2+}$-induced genotoxicity in the leaves

Analytical studies by inductively coupled plasma optical emission spectrometry demonstrate (Table 1) that after treatment of tobacco seedlings with $Cd^{2+}$, the accumulation of the heavy metal is markedly higher in the roots than in the leaves. This lower accumulation may explain the absence of $Cd^{2+}$ genotoxicity in leaves. The role of thiol-rich phytochelatins in binding of cadmium and accumulating it within vacuolar compartments of root cells is well known

[3] and perhaps responsible for the high Cd-content in roots.

$Cd^{2+}$ generates various ROS in plant cells, including $H_2O_2$ [20,21]. The higher DNA-damaging effect of $Cd^{2+}$ in the CAT1AS line compared to that in the SR1 tobacco roots (Fig. 1B) may thus be associated with the lower catalase activity in the catalase-deficient tobacco line.

Compared to roots, leaf cells are better equipped with an antioxidant defense system that might protect the nuclear DNA in leaf cells from Cd-induced oxidative stress. In previous studies [8] it was demonstrated that the activity of catalases, the principal $H_2O_2$-scavenging enzymes, is about 30 times higher in tobacco leaves than in roots. Consequently, the high content of catalases and other enzymes inactivating ROS in leaves, prevents their reaction with DNA. For example, $H_2O_2$ was shown to induce DNA damage as measured by the Comet assay in tobacco roots, but did not cause DNA damage in leaves [8].

In conclusion, both the lower accumulation of Cd in the leaves and the fact that the leaf cells are better equipped with antioxidant defense system contribute to the protection of the nuclear DNA in leaf cells from Cd-induced oxidative stress.

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