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Induced DNA damage measured by the Comet assay in 10 weed species

T. GICHNER and Z. MÜHLFELDOVÁ

Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Karlovce 1a, CZ-16000 Praha 6, Czech Republic

Abstract

For most plant species growing in polluted areas no mutagenicity assays are available. We have studied the possibility of using the alkaline protocol of the Comet assay as a method for detecting induced DNA damage in wildly growing weeds. The monofuctional alkylating agent ethyl methanesulphonate (EMS) was applied on leaves of 10 weed species (ordered according to the diameter of the nuclei): *Arabidopsis thaliana, Convolvulus arvensis, Bellis perennis, Urtica dioica, Lamium album, Chenopodium rubrum, Plantago media, Poa annua, Taraxacum officinale,* and *Agropyron repens*. With increasing concentrations of EMS (2 to 10 mM) the DNA damage, expressed by the averaged median tail moment values, significantly increased in nuclei of all weeds studied. Using the Head Extent parameter of the Komet version 3.1, we have measured the diameter size of the nuclei of the 10 weed species either immediately after the isolation of the nuclei or after 20 or 45 min of treatment with alkaline buffer (pH > 13). According to the increase of the diameter of the nuclei (including the formed "halo") resulting from the to alkaline buffer treatment, electrophoretic conditions (unwinding and electrophoresis time) for the Comet assay can be selected for the individual weed species.

Additional key words: *Arabidopsis thaliana, Agropyron repens, Bellis perennis, Chenopodium rubrum, Convolvulus arvensis,* ethyl methanesulphonate, *Lamium album, Plantago media, Poa annua,* single cell gel electrophoresis, *Taraxacum officinale, Urtica dioica.*

Introduction

Plants are exposed to various types of environmental xenobiotics, either deliberately as in the case of agricultural pesticides and plant growth regulators, or accidentally as compounds present in polluted air, soil or water. There are three primary benefits of using plant systems in addition to microbial and animal biomarkers to monitor for environmental mutagens: *1*) Plants can store and metabolize non-mutagenic pollutants to mutagenic products that may then be introduced into the human food chain and the affected environment; *2*) environmental pollutants may evoke genotoxic responses in the phytosphere and damage global ecological systems; and *3*) plants can be employed as sensitive *in situ* biological indicators for environmental toxicology.

For most plant genetic bioassays only specific tester lines can be used and these testers are at present not available for most plant species. This limitation hampers or prevents the detection of the genotoxicity of environmental mutagens in plants growing, for example, on polluted soil. To overcome this limitation we used a plant-based molecular assay - the Single Cell Gel Electrophoresis (SCGE) assay, also called the Comet assay, to detect induced DNA damage in 10 weed species, growing wild in large areas of Europe. With the exception of *Arabidopsis thaliana*, no mutagenicity assays are available at present for these weed species (Gichner *et al.* 1994).

The Comet assay is a powerful genetic assay for the analysis of DNA damage in eukaryotic cells (Tice *et al.* 2000). The alkaline version of the Comet assay 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 crosslinks. Although this

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Abbreviations: EMS - ethyl methanesulphonate; TM - tail moment; SCGE - single cell gel electrophoresis.
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*Corresponding author; fax: (+420) 2 24310113, e-mail address: gichner@ueb.cas.cz

technique has been primarily applied to animal cells, the incorporation of the Comet assay with plant tissues (Koppen and Verschaeve 1996, Gichner and Plewa 1998, Angelis *et al.* 1999, Gichner *et al.* 1999, 2000a,b, Menke *et al.* 2001) significantly extends the utility of plants in basic and applied studies in environmental mutagenesis. In theory, the Comet assay can be applied to every type of eukaryotic plant cell. The objectives of this study were: *1*) to measure the diameter of the nuclei of the studied species and the degree of DNA denaturation in their nuclei under high alkaline conditions (pH > 13), *2*) to define the parameters for SCGE analysis using nuclei isolated from leaves of intact plants, and *3*) to generate concentration-response curves for DNA migration values from weed leaves treated with the monofunctional alkylating agent ethyl methanesulphonate (EMS). The data obtained could demonstrate the feasibility of using the Comet assay for detecting induced DNA damage in *in situ* studies with weeds growing on polluted soil.

Material and methods

Plants: For the experiments we used young leaves either of weeds collected from a recreational garden in Prague 6, Na Karlovce (*Convolvulus arvensis* L., *Bellis perennis* L., *Urtica dioica* L., *Lamium album* L., *Plantago media* L., *Poa annua* L., *Taraxacum officinale* Wiggers, and *Agropyron repens* (L.) P. Beauv. or of weeds that were cultivated in a greenhouse (*Arabidopsis thaliana* (L.) Heynhold, and *Chenopodium rubrum* L.).

Chemicals and mutagenic treatment conditions: Ethyl methanesulphonate (EMS, CAS No. 62-50-0), 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, USA. The lower parts of the leaves of the tested weeds were immersed in 2 cm^3 plastic microtubes containing 1 cm^3 of 0 to 10 mM EMS dissolved in distilled water for 18 h at 26 °C in the dark.

Isolation of nuclei and preparation of slides: After control (H_2O) or EMS treatments, excised leaves were placed in a 60 mm Petri dish kept on ice and spread with 0.2 cm^3 of cold 400 mM Tris buffer, pH 7.5. Using a fresh razor blade, the part of the leaf that was not immersed in the treatment solution, was gently sliced. The plate was kept tilted in the ice so that the isolated nuclei would collect in the buffer. All operations were conducted under dim or yellow light.

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 (0.05 cm^3) and 1 % LMP agarose (0.05 cm^3) 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 micropipet tip and a coverslip was placed on the mixture. The slide was placed on ice for a minimum of 5 min. Next, the coverslip was removed and a final layer of 0.5 % LMP agarose (0.1 cm^3) was placed on the slide. A coverslip was placed on the LMP agarose and the slide was kept at 4 °C for 5 min.

Measurement of the diameter of the nuclei: To measure the size of the nuclei of the studied weeds and the effect of high pH (> 13) on the relaxed nuclear DNA, the slides with nuclei imbedded in agarose were immersed in ice-cold electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH > 13) for 0, 20 and 45 min. The slides were then rinsed 3× with 400 mM Tris, pH 7.5, stained with 0.08 cm^3 ethidium bromide (20 μg cm^{-3}) for 5 min, dipped in ice cold water to remove the excess ethidium bromide and covered with a coverslip. Fifty randomly chosen nuclei per unwinding time 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 to measure the diameter of the nuclei. The objective of this study was to determine if there is a correlation between the level of DNA denaturation at high pH and the duration of unwinding and electrophoresis required to perform the Comet assay in the individual weed species. Keeping the slides with nuclei for 20 min in alkaline buffer (the same as used for unwinding and electrophoresis) corresponds to 5 min unwinding and 15 min electrophoresis, and 45 min in the alkaline buffer corresponds to 15 min unwinding and 30 min electrophoresis.

Comet assay: In order to analyze the EMS-induced DNA damage, the agarose slides with isolated nuclei from EMS-treated leaves were placed in a horizontal gel electrophoresis tank containing freshly prepared cold electrophoresis buffer (see above). Depending on the weed species the nuclei were incubated at pH > 13 for 5, 15 or 30 min to allow the DNA to unwind prior to electrophoresis at 0.72 V cm^{-1} (26 V; 300 mA) for 15 or 30 min at 4 °C. After electrophoresis the slides were rinsed, stained with ethidium bromide and analyzed using a fluorescence microscope and the same computerized image analysis system as described for nuclear size measurements (see above). For each slide, 25 randomly

chosen nuclei were analyzed. The tail moment (integrated value of tail DNA density multiplied by the migration distance) was used as the primary measure of DNA damage. Three slides were evaluated per treatment and each treatment was repeated at least twice. From the repeated experiments, the averaged median tail moment (TM) value was calculated for each treatment group from the median TM value of each slide (Lovell *et al.* 1999).

Statistical analysis: Data were analyzed using the statistical and graphical functions of *SigmaPlot 4.01* and *SigmaStat 2.03* (*SPSS, Inc.*, Chicago, USA). The median TM values were used in a one-way analysis of variance test. If a significant F-value of $P < 0.05$ was obtained, a Dunnett's multiple comparison versus the control group analysis was conducted. The diameter of nuclei and the TM values are given as means ± SE.

Results

Measurements of the diameter of the nuclei: Isolated nuclei of the weeds studied were subjected to treatments for 0, 20, and 45 min with alkaline buffer (pH >13). The alkaline buffer was the same as that used for the unwinding and electrophoresis in the Comet assay (Fig. 1, Table 1). The individual weeds are ordered according to the diameter of nuclei not subjected to treatments with alkaline buffer.

The diameter (mean ± SE) of leaf nuclei, not subjected to alkaline treatment, were in *A. thaliana*: 8.3 ± 0.2 µm, *C. arvensis*: 8.7 ± 0.3 µm, *B. perennis*: 9.0 ± 0.2 µm, *U. dioica*: 9.1 ± 0.2 µm, *L. album*: 11.1 ± 0.2 µm, *C. rubrum*: 12.8 ± 0.2 µm, *P. media*: 13.3 ± 0.3 µm, *P. annua*: 14.2 ± 0.2 µm, *T. officinale*: 14.9 ± 0.3 µm, and *A. repens*: 18.5 ± 0.4 µm. These values demonstrate great differences in the size of the nuclei, for example, the diameter of the nuclei of *A. thaliana* was half that of nuclei of *A. repens*. With 20 min treatment to alkaline buffer, the increase in the diameter (including the "halo" formed by relaxed DNA) was either very low, for example, in *T. officinale* (increase by 6 %) or between 37.6 to 45.6 % (*P. media, C. arvensis, B. perennis*). In species *L. album, P. annua* and *C. rubrum* the corresponding increase was between 57.7 and 60.9 %. The highest increase in the diameter of the nuclei after alkaline treatment for 20 min to alkaline buffer was observed in *A. repens* (by 87.6 %), *U. dioica* (by 90.1 %) and in *A. thaliana* (by 91.5 %).

Table 1. Diameter [means ± SE] of isolated nuclei, embedded in agarose on microscope slides, and treated for 0 to 45 min in alkaline buffer (pH > 13). Fifty nuclei were measured per treatment. The increase of the diameter [%] of nuclei after alkaline treatment was based on the diameter of nuclei not subjected to treatment with alkaline buffer. Individual species are ordered according to the diameter of their nuclei.

Plant species	0 min [µm]	20 min [µm]	[%]	45 min [µm]	[%]
Arabidopsis thaliana	8.3 ± 0.2	15.9 ± 0.4	91.5	19.8 ± 0.6	138.6
Convolvulus arvensis	8.7 ± 0.3	12.4 ± 0.2	42.5	14.2 ± 0.3	63.2
Bellis perennis	9.0 ± 0.2	13.1 ± 0.3	45.6	18.8 ± 0.4	108.9
Urtica dioica	9.1 ± 0.2	17.3 ± 0.3	90.1	26.7 ± 1.1	193.4
Lamium album	11.1 ± 0.2	17.5 ± 0,3	57.7	25.5 ± 0.6	129.7
Chenopodium rubrum	12.8 ± 0.2	20.6 ± 0.7	60.9	28.7 ± 1.1	124.2
Plantago media	13.3 ± 0.3	18.3 ± 0.4	37.6	23.5 ± 0.6	76.7
Poa annua	14.2 ± 0.2	22.7 ± 0.4	59.9	26.2 ± 0.5	84.5
Taraxacum oficinale	14.9 ± 0.3	15.8 ± 0.3	6.0	18.0 ± 0.3	12.1
Agropyron repens	18.5 ± 0.4	34.7 ± 0.5	87.6	38.2 ± 0.5	106.5

Comet assay: *Arabidopsis thaliana* (mouse ear cress, *Brassicaceae*): After a 18 h treatment period with 2 to 10 mM EMS, nuclei were isolated from leaves. With 5 min unwinding and 15 min electrophoresis, the average median TM ± SE significantly increased from 0.18 ± 0.05 (negative control) to 37.3 ± 2.1 µm (10 mM EMS) ($F_{5,34} = 141.1$; $P < 0.001$) (Fig. 2*A*). Induction of DNA damage in *A. thaliana* using another Comet assay protocol was reported by Menke *et al.* (2001).

Convolvulus arvensis (bind weed, *Convolvulaceae*): With shorter unwinding (5 min) and electrophoresis times (15 min), the DNA damage induced by EMS and expressed by the TM was comparatively low and reached after 10 mM EMS only 11.1 ± 1.7 µm. Thus the unwinding time was increased to 15 min and the electrophoresis to 30 min (Fig. 2*B*). These electrophoretic

conditions led to a significant increase of the TM values from 3.9 ± 0.3 μm (negative control) to 39.4 ± 2.2 μm (10 mM EMS) ($F_{5,29} = 67.7$; $P < 0.001$).

Bellis perennis (daisy, *Asteraceae*): With unwinding (5 min) and electrophoresis time (15 min), the DNA damage induced by EMS and expressed by the TM value was low and reached after 10 mM EMS only 8.3 ± 0.5 μm (Fig. 3*A*). Electophoretic conditions (15 min unwinding,

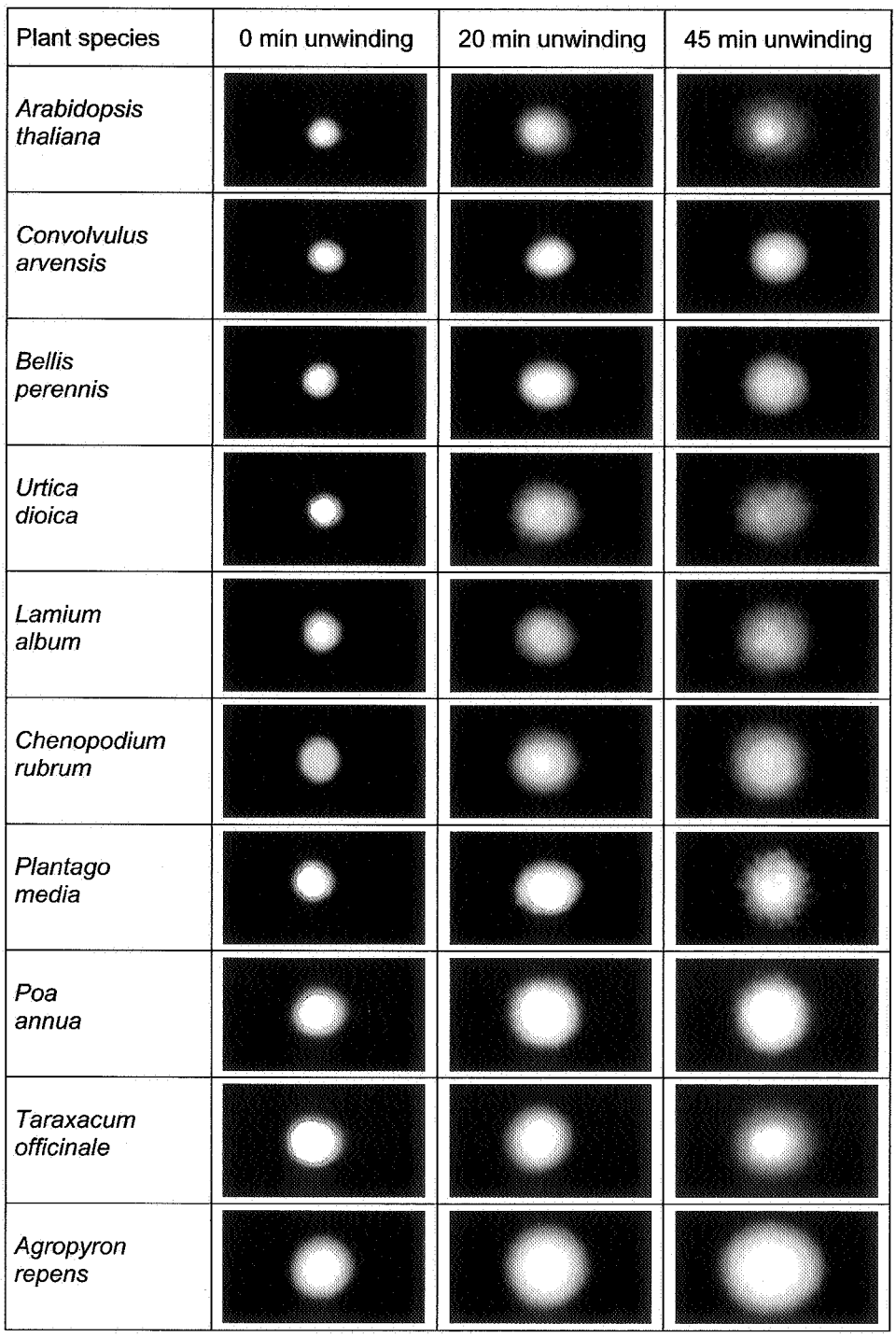

Fig. 1. Images of isolated nuclei, embedded in agarose on microscope slides and treated for 0 to 45 min in alkaline buffer (pH > 13).

30 min electrophoresis) resulted in a significant increaseof the TM value from 3.6 ± 0.4 μm (negative control) to 38.3 ± 1.8 μm (8 mM EMS) ($F_{5,30}$ = 125.9; $P < 0.001$).

Urtica dioica (burning nettle, *Amaryllidaceae*): Five min unwinding and 15 min electrophoresis resulted in a significant increase of the TM value from 3.8 ± 0.6 μm (negative control) to 40.1 ± 2.0 μm (8 mM EMS) ($F_{5,30}$ = 86.1; $P < 0.001$) (Fig. 3*B*).

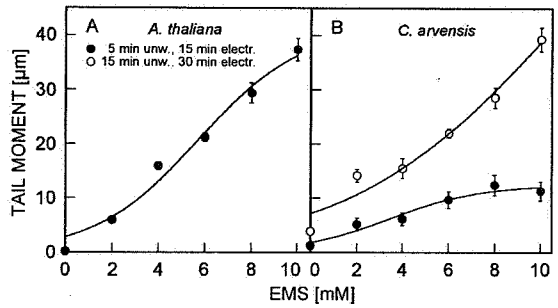

Fig. 2. Dose-response curves of the average medial tail moment values as a function of ethyl methanesulphonate (EMS) treatment of leaves of *Arabidopsis thaliana* (*A*) and *Convolvulus arvensis* (*B*) for 18 h at 26 °C in the dark. The *error bars* represent SE.

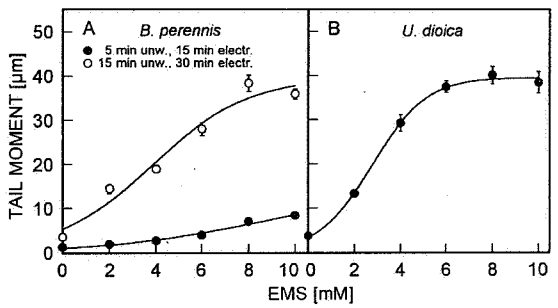

Fig. 3. Dose-response curves of the average medial tail moment values as a function of ethyl methanesulphonate (EMS) treatment of leaves of *Bellis perennis* (*A*) and *Urtica dioica* (*B*) for 18 h at 26 °C in the dark. The *error bars* represent SE.

Lamium album (white dead nettle, *Lamiaceae*): Five min unwinding and 15 min electrophoresis was sufficient to demonstrate a significant increase of EMS-induced DNA damage in nuclei of *L. album* (Figure 4*A*). The TM values increased from 1.2 ± 0.08 μm (negative control) to 42.0 ± 2.3 μm (10 mM EMS) ($F_{5,30}$ = 176.8; $P < 0.001$).

Chenopodium rubrum (red goosefoot, *Chenopodiaceae*): Five min unwinding and 15 min electrophoresis of the slides with EMS treated nuclei resulted in a significant increase of the TM values from 0.4 ± 0.07 μm (negative control) to 38.2 ± 1.2 μm (10 mM EMS) ($F_{5,30}$ = 353.8; $P < 0.001$) (Fig. 4*B*).

Plantago media (hoary plantain, *Plantaginaceae*): With shorter unwinding (5 min) and electrophoresis times (15 min), the DNA damage induced by EMS and expressed by the TM value in *P. media* was low and reached after 10 mM EMS only 17.9 ± 2.6 μm. Thus, the unwinding time was increased to 15 min and the electrophoresis to 30 min (Fig. 5*B*). These conditions led to a significant increase of the TM values from 8.7 ± 3.4 μm (negative control) to 50.9 ± 4.5 μm (10 mM EMS) ($F_{5,29}$ = 36.0; $P < 0.001$) (Fig. 5*A*).

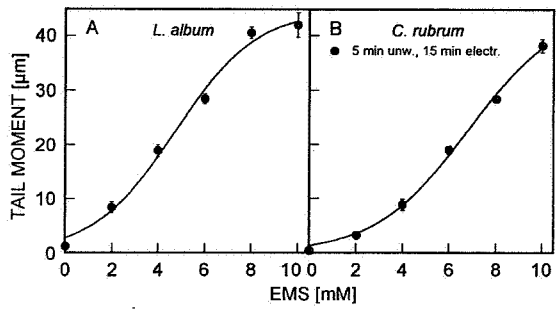

Fig. 4. Dose-response curves of the average medial tail moment values as a function of ethyl methanesulphonate (EMS) treatment of leaves of *Lamium album* (*A*) and *Chenopodium rubrum* (*B*) for 18 h at 26 °C in the dark. The *error bars* represent SE.

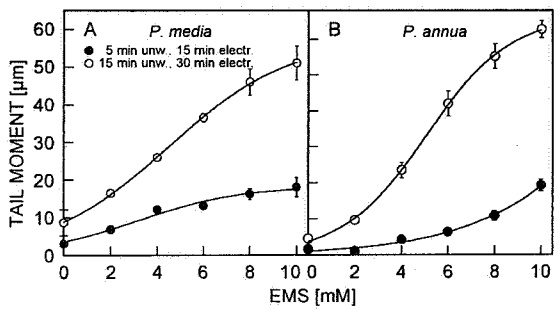

Fig. 5. Dose-response curves of the average medial tail moment values as a function of ethyl methanesulphonate (EMS) treatment of leaves of *Plantago media* (*A*) and *Poa annua* (*B*) for 18 h at 26 °C in the dark. The *error bars* represent SE.

Poa annua (annual meadow grass, *Poaceae*): With 5 min unwinding and 15 min electrophoresis time, the DNA damage induced by EMS and expressed by the TM was low and reached after 10 mM EMS only 19.2 ± 0.2 μm (Fig. 5*B*). Electrophoretic conditions extended to 15 min uwinding and 30 min electrophoresis resulted in a significant increase of the TM value from 4.5 ± 0.9 μm (negative control) to 62.8 ± 2.4 μm (10 mM EMS) ($F_{5,28}$ = 89.2; $P < 0.001$).

Taraxacum officinale (dandelion, *Asteraceae*): In *T. officinale*, the electrophoretic conditions (5 min unwinding and 15 min electrophoresis or 15 min unwinding and 30 min electrophoresis), used for other

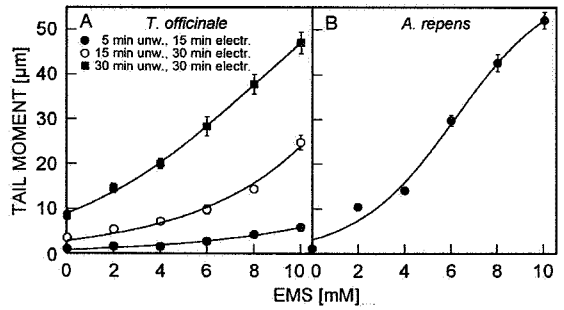

Fig. 6. Dose-response curves of the average medial tail moment values as a function of ethyl methanesulphonate (EMS) treatment of leaves of *Taraxacum officinale* (*A*) and *Agropyron repens* (*B*) for 18 h at 26 °C in the dark. The *error bars* represent SE.

weeds included in this study, resulted in comparatively low values of TM after the highest applied EMS concentration of 10 mM (5.8 ± 0.4 and 24.8 ± 1.6 μm, respectively). Thus, we have increased the unwinding time to 30 min followed by electrophoresis for 30 min. These conditions resulted in a significant increase of the TM values from 8.4 ± 1.1 μm (negative control) to 47.1 ± 2.4 μm (10 mM EMS) ($F_{5,30} = 68.1$; $P < 0.001$) (Fig. 6*A*).

Agropyron repens (quack grass, *Poaceae*): Electrophoretic conditions of 5 min unwinding and 15 min electrophoresis were sufficient to demonstrate a significant increase of EMS-induced DNA damage in nuclei of *A. repens*. The TM values increased from 1.1 ± 0.07 μm (negative control) to 52.2 ± 1.9 μm (10 mM EMS) ($F_{5,29} = 241.3$; $P < 0.001$) (Fig. 6*B*).

Discussion

Is there a relationship between the changes of the diameter of the nuclei treated with alkaline buffer, and the electrophoretic conditions required for measuring induced DNA damage by the Comet assay in individual weed species? Using the Head Extent parameter of the *Komet version 3.1*, we have measured the diameter of nuclei of the 10 weed species either immediately after the isolation of the nuclei or after 20 and 45 min of treatment of the nuclei with alkaline buffer (pH > 13) (Fig. 1, Table 1). The DNA damage, induced by the alkylating mutagen EMS, and measured by the Comet assay are presented in Figs. 2 to 6. In all weeds studied, a 5 min unwinding period followed by a 15 min electrophoresis period was applied. For some weeds (*A. thaliana, U. dioica, L. album, C. rubrum, A. repens*) these electrophoretic conditions resulted in a clear EMS - DNA damaging response with a control TM value not exceeding 5 μm and with 10 mM EMS treatment TM values exceeding 35 μm. For other weeds (*C. arvensis, B. perennis, P. annua and P. media*) the 5 min alkaline unwinding and 15 min electrophoresis treatment was not sufficient and times had to be increased to 15 min unwinding and 30 min electrophoresis. After the prolonged electrophoresis, clear EMS dose DNA damage responses were recorded. Exceptions were EMS treatments on *T. officinale* leaves, where even a 15 min unwinding and 30 elecrophoresis period resulted in a 10 mM EMS-induced TM value of only 24.8 ± 1.6 μm. First increasing the unwinding time to 30 min followed by a 30 min electrophoresis, led to a high and significant increase of the TM value from 8.4 ± 1.6 μm to 47.1 ± 2.4 μm.

By comparing the data of the diameter of nuclei after treatment in alkaline buffer (Table 1) and the results of the Comet assay (Fig. 2 to 6), the following conclusions can be drawn: *1*) if after 20 min in alkaline buffer the diameter of the nuclei increases by more than 60 %, a 5 min unwinding and a 15 min electrophoresis is sufficient for the Comet assay procedure (*A. thaliana, U. dioica, C. rubrum, A. repens*), *2*) if the increase is less than 50 %, a 15 min unwinding and 30 min electrophoresis Comet assay protocol has to be applied (*C. arvensis, B. perennis, P. media*), *3*) in *T. officinale*, where the treatment of nuclei for 45 min in alkaline buffer resulted in a 12 % increase in the diameter of the nuclei only, a prolonged unwinding of 30 min followed by a 30 min electrophoresis, had to be applied, and *4*) the effect of alkaline treatment on the diameter of nuclei of 2 weed species did not fit into this scheme. In *L. album*, where a 20 min alkaline treatment led to a 57.7 % increase in the nuclear diameter, a 5 min unwinding and a 15 min electrophoresis were sufficient to demonstrate a clear dose dependent EMS-induced DNA damage. By contrast, in *P. annua*, the alkaline treament for 20 min resulted in a similar increase in the diameter of the nuclei by 59.9 %, but a prolonged 15 min unwinding and a 30 min electrophoresis were required for obtaining an dose dependent EMS-induced DNA damage.

Is there a correlation between induced DNA damage measured by the Comet assay and the frequency of induced mutations? The Comet assay detects various types of DNA damage, however this damage is mostly not heritable. Induced mutations are a more reliable parameter of genetic changes, but for most plant species mutagenicity assays are not available. In previous papers (Gichner and Plewa 1998, Gichner *et al.* 1999) we reported a high correlation between DNA damage as measured by the Comet assay and somatic mutations in tobacco seedlings treated with the monofunctional

alkylating agents N-methyl-N-nitrosourea (MNU), N-ethyl-N-nitrosourea (ENU), methyl methanesulphonate (MMS) and EMS. The Pearson Product Moment Correlation (r) was higher than 0.96.

By contrast, after treatment of tobacco seedlings with the pesticide and plant growth regulator maleic hydrazide (MH), no correlation was observed (Gichner *et al.* 2000a). Although the yield of somatic mutations increased with the MH concentration, the DNA damage measured by the Comet assay was at the control level up to sublethal concentrations. Similarly, MH-induced chromosome aberrations in *Vicia faba*, but no DNA damage detectable by the Comet assay (Gichner *et al.* 2000a).

After irradiating tobacco seedlings with 1 to 10 Gy of γ-radiation, there is a high correlation (r = 0.996), between the SCGE measured DNA damage and the frequency of leaf somatic mutations (Ptáček *et al.* 2001). This is true, however, only if the DNA damage was analyzed immediately after the irradiation. When the DNA damage was analyzed 24 after irradiation, all DNA damage resolved by the used protocol of the Comet assay, was repaired. These data indicate that DNA strand breaks are rapidly repaired, however, other lesions such as oxidized bases may persist longer (Collins *et al.* 1997) and be misrepaired yielding somatic mutations. A repair of X-radiation induced DNA damage as measured by the Comet assay was also reported for *Vicia faba* roots (Koppen and Angelis 1998).

The data indicate that the results of the Comet assay do not always correlate with the frequency of induced mutations.

Is the Comet assay suitable for monitoring environmental mutagens in plants *in situ*? As mentioned above, γ-radiation induced DNA damage, measured by the Comet assay, is readily repaired within 24 h. Thus the Comet assay is not suitable for monitoring the late effects of acute ionizing radiations.

The data regarding DNA damage in leaf nuclei of EMS- or ENU-treated tobacco seedlings were strikingly different (Gichner *et al.* 2000b). Using the Comet assay, DNA damage induced by EMS and ENU persisted over 72 h after treatment without significant reduction. Even after 4 weeks the amount of DNA damage in nuclei isolated from mature leaves were significantly higher than compared to the controls. These data indicate that the Comet assay may detect DNA damage inflicted by some types of chemical genotoxic agents long after exposure.

The results given in this paper demonstrate that the DNA damage after the lowest applied concentration of EMS (2 mM) was, in all the weed species tested significantly ($P < 0.05$) higher than the DNA damage of the negative control. However, it has to be kept in mind that in polluted areas the possible concentrations of environmental DNA damaging agents will be usually comparatively low.

In conclusion, the Comet assay applied on weeds can be used for monitoring the DNA damaging effects of environmental pollutants *in situ*. However, not all types of induced DNA damage can be detected, as for instance rapidly repaired DNA damage after ionizing radiation or effects of some chemicals such as the pesticide maleic hydrazide.

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