Calibration of the Comet Assay for Potato Plants and Assessment of DNA Damage Induced by EMS, Cd^{2+} and γ-irradiation

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Aim

- Calibration of the comet assay for potato plants (*Solanum tuberosum* L.) using the monofunctional alkylating agent ethyl methanesulphonate (EMS).
- Assessment of DNA damage induced by the heavy metal cadmium and γ-irradiation.

The comet assay protocol

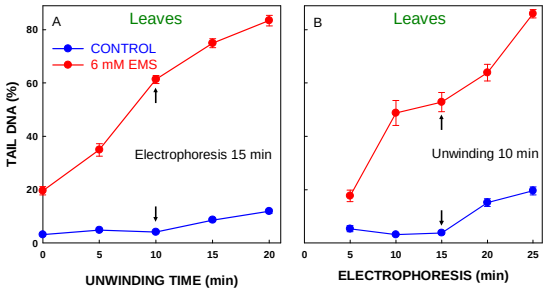
We used the comet assay protocol as described by Gichner et al. (2004).

Rooted potato cuttings cultivated at sterile conditions were transferred to vials with solutions of the tested agent. Then the nuclei were isolated from leaves and/or roots.

Two to three slides were evaluated per treatment and each treatment was repeated at least twice. At least 150 nuclei were evaluated for each treatment group. The percentage of DNA in the tail was measured in two groups of 25 nuclei for each slide. For each group of nuclei, the median percentage of DNA in the tail was determined. The mean of the medians was calculated from the repeated experiments (the error bars in the graphs represent the standard error of the mean).

Calibration of the comet assay for potato plants

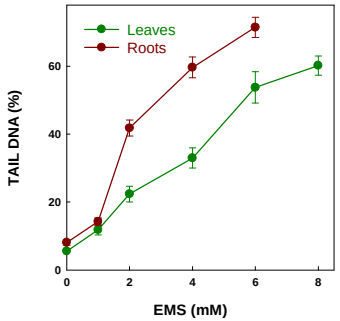
Our aim was to obtain a minimal migration of DNA in the control samples (plants in water for 24 h) and at the same time a maximal sensitivity of the assay to DNA damage in the treated plants (6 mM EMS for 24 h at 26 °C). The nuclei were isolated from leaves. Electrophoresis was performed at 0.74 V cm^{-1} and 300 mA.



For potato plants, the optimal unwinding time is 10 min (arrows in A) and the optimal electrophoresis time is 15 min (arrows in B).

Assessment of DNA damage induced by EMS

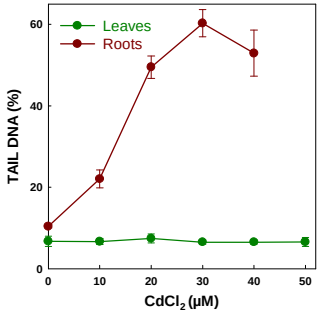
We tested the calibrated protocol using different concentrations of EMS. We treated the plants for 24 h at 26 °C.



EMS induced a dose-dependent increase of DNA damage both in potato leaves and roots.

Assessment of DNA damage induced by Cd^{2+}

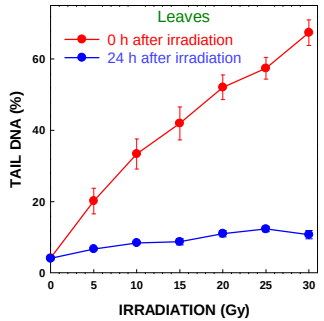
We treated the potato plants with cadmium chloride ($CdCl_2$) for 24 h at 26 °C.



Cd^{2+} induced a dose-dependent increase of DNA damage in potato roots, but no DNA damage in the leaves. At 50 μM Cd^{2+}, DNA damage in the roots was very high and comets were not suitable for analysis.

Assessment of DNA damage induced by γ-irradiation

We exposed the plants to γ-radiation and assessed the DNA damage immediately after treatment (0 h) or after 24 h. The nuclei were isolated from leaves.



Immediately after γ-irradiation, a dose-dependent increase of DNA damage in the leaf nuclei was observed. After 24 hours the DNA damage was mostly repaired.

Conclusion

Reference

Gichner T., Patková Z., Száková J. and Demnerová K. (2004). Mutation Research 559, 49-57.

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