Assessment of Genotoxicity with the Plant Comet Assay

Irena Žnidar, Barbara Vilhar

University of Ljubljana, Biotechnical Faculty, Department of Biology,
Večna pot 111, SI-1000 Ljubljana, SLOVENIA
Contact: irena.znidar@bf.uni-lj.si

Background

Ecosystem degradation caused by anthropogenic pollution can be evaluated with chemical and biological analyses. Chemical analysis detects the presence and quantity of a contaminant in the ecosystem. On the other hand, bioassays evaluate the toxicity of substances to organisms (biological effects), as well as bioavailability and biotransformation of toxic substances.

Although plants constitute the basis of ecosystems and many plant species are also economically important in agriculture and forestry, there is a lack of plant bioassays for environmental monitoring.

Contaminating substances can cause DNA damage, which can be assessed with the comet assay. However, the comet assay procedure has to be optimised for each type of plant material.

Aim

In this study, we used the comet assay to evaluate DNA damage in potato (*Solanum tuberosum* L. cultivar Desirée) roots and leaves. Plants were exposed to ethyl methanesulfonate (EMS), a direct acting alkylating compound, which is widely used in genotoxicity studies.

Reference

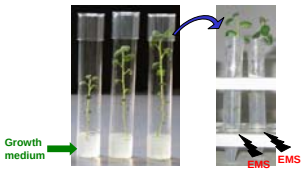
Gichner T., Patková Z., Száková J. and Demnerová K. (2004). Mutation Research 559, 49-57.

Acknowledgements

This work was supported by Slovenian Research Agency. We thank Dr. T. Gichner for valuable discussion.

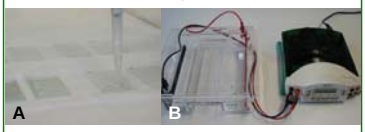
The comet assay

The comet assay procedure was based on protocol of Gichner et al. (2004).

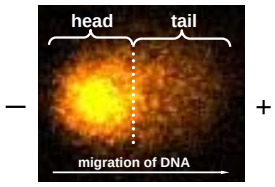


Genotoxic treatment. 6-week-old plants were transferred from growth medium to EMS-solution.

Isolation of nuclei. After short- (2 h) and long-term (24 h) treatment leaves or roots were gently sliced. Isolated nuclei were collected in the buffer.



Electrophoresis. Isolated nuclei were embedded in an agarose layer on a microscope slide (**A**). Slides were incubated in a electrophoresis buffer and electrophoresed (**B**).

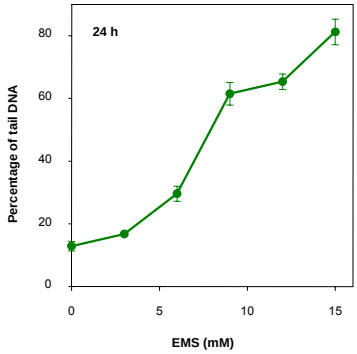


The principle of the comet assay. During electrophoresis, damaged DNA migrates from gel-embedded nuclei. Thus, nuclear DNA of individual cells acquires the appearance of a comet, with a *head* (undamaged DNA) and a *tail* (damaged DNA). The amount of DNA in the tail correlates with the number of DNA strand breaks and thus indicates the extent of DNA damage. DNA was stained with ethidium bromide and detected as fluorescence with green excitation.

Evaluation of results. We recorded images of the comets using an epifluorescence microscope linked to a monochrome CCD camera. The extent of DNA damage was assessed with the Komet 5 software package (Kinetic Imaging Ltd.).

DNA damage in EMS-treated plants

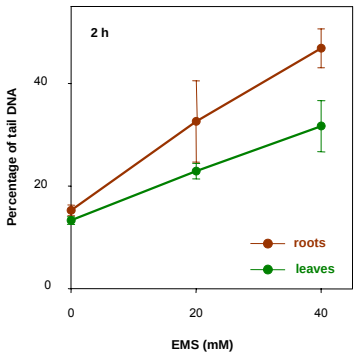
Nuclear DNA damage in potato leaves after 24 h treatment with ethyl methanesulfonate (EMS)



For each EMS concentration, the percentage of tail DNA was measured in two groups of 25 nuclei for each slide (two or three slides per treatment). For each group of nuclei, the median percentage of tail DNA was determined. The mean of the medians was calculated (the error bars represent the standard error of the mean). A higher percentage of tail DNA corresponds to a higher level of DNA damage.

The level of DNA damage in the leaf cells increased with increasing concentration of EMS.

Nuclear DNA damage in potato roots and leaves after 2 h treatment with EMS



The extent of DNA damage was higher in the roots than in the leaves. This organ-specific difference possibly arose because roots were in a direct contact with the genotoxic solution, whereas the genotoxic effect in leaves presumably depended on transport of the genotoxic compound within the plant.

Conclusion

The observed concentration-dependent response to a genotoxic substance demonstrates that potato plants of the cultivar Desirée are suitable for assessment of genotoxicity with the plant comet assay.