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Application of the SCGE Assay for Detecting Induced DNA Damage in Plant Leaves

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ABSTRACT

The possibility of using the alkaline protocol of the single cell gel electrophoresis (SCGE) assay as a method for detecting induced DNA damage has been studied for six major plants. The EMS was applied as a model genotoxic agent on young excised leaves of the tested crops for 18 h at 26 ℃ in the dark. With increasing concentrations of 0 to 10 mM EMS, the DNA damage, expressed by the averaged median tail moment values, significantly increased in nuclei of all plants studied. As the results, no correlation between the diameter of nuclei and sensitivity to EMS treatment was observed. The data obtained demonstrate the feasibility of using the SCGE assay for detecting induced DNA damage in plants.
Key words : single cell gel electrophoresis, EMS, DNA damage, plant

I. INTRODUCTION

The single cell gel electrophoresis assay, also called the comet assay, is a powerful genetic assay for the analysis of DNA damage in eukaryotic cells. 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 apyrimidine sites), incomplete excision repair sites and DNA crosslinks. Although this technique has been primarily applied to animal cells, the incorporation of the comet assay with plant tissues 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 to measure the diameter of the nuclei of the studied species and to generate concentration-response curves for DNA migration values from plant 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 plants growing on the polluted soil.

II. MATERIALS AND METHODS

Plants : Young leaves were used in the experiment from 3 wild plants and 3 agronomic plants, *Lamium album, Chenpodium rubrum, Poa_annua, Nicotiana tabacum, Zea mays,* and *Solanum tuberosum.*

Chemicals : 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, MO, USA).

Mutagenic treatment : For the experiments we used young leaves of plants. The lower parts of leaves of the tested plants were immersed in 2 ㎤ plastic microtubes containing 1 ㎤ of 0 to 10 mM EMS dissolved in distilled water for 18 h at 26℃ 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 ㎤ of cold 400 mM Tris buffer, pH 7.5. Using a fresh razor blade, the part of the leaf 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. In order to analyze the EMS-induced DNA

damage, the agarose slides with isolated nuclei from EMS-treated leaves were electrophoresed according to an alkaline protocol of the SCGE assay.

Measurement of the diameter of the nuclei : Isolated nuclei embedded in agarose, without additional unwinding and electrophoresis, were stained with EtBr as in the Comet assay. The diameter of the nuclei was measured using the Head Extent parameter of the software *Komet version 4.0*. Fifty nuclei were measured for each plants.

III. RESULTS

Lamium album : Five min unwinding and 15 min electrophoresis was sufficient to demonstrate a significant increase of EMS-induced DNA damage in nuclei of *L. album*. The TM value increased from 1.2±0.08 ㎛ (negative control) to 42.0±2.3 ㎛ (10mM EMS).

Chenopodium rubrum : Five min unwinding and 15 min electrophoresis of the sides with EMS treated nuclei resulted in a significant increase of the TM values from 0.4±0.07 ㎛ (negative control) to 38.2±1.2 ㎛ (10mM EMS).

Nicotiana tabacum : Five min unwinding and 15 min electrophoresis of the sides with EMS treated nuclei resulted in a significant increase of the TM values from 0.9±0.1 ㎛ (negative control) to 25.2±0.4 ㎛ (10mM EMS).

Poa annua : Five min unwinding and 15 min electrophoresis of the sides with EMS treated nuclei resulted in a significant increase of the TM values from 4.5±0.9 ㎛ (negative control) to 19.2±0.2 ㎛ (10mM EMS).

Zea mays : Five min unwinding and 15 min electrophoresis of the sides with EMS treated nuclei resulted in a significant increase of the TM values from 1.2±0.3 ㎛ (negative control) to 37.0±1.9 ㎛ (10mM EMS).

Solanum tuberosum : Five min unwinding and 15 min electrophoresis

of the sides with EMS treated nuclei resulted in a significant increase of the TM values from 4.0±0.2 ㎛ (negative control) to 28.1±1.6 ㎛ (10mM EMS).

The whole results are summarized in Table 1.

Table 1. The diameter of isolated nuclei and induced DNA damage as expressed by the average median tail moment value in the isolated nuclei after 5 min unwinding and 15 min electrophoresis

Species	Nuclei [㎛]	TM[㎛]	Species	Nuclei [㎛]	TM[㎛]
N. tabacum	13.9 ± 0.2	25.2 ± 0.4	*U. dioica*	9.1 ± 0.2	17.3 ± 0.3
Z. mays	14.3 ± 0.2	37.0 ± 1.9	*P. annua*	14.2 ± 0.2	22.7 ± 0.4
S. tuberosum	15.3 ± 0.2	28.1 ± 1.6	*T. oficinale*	14.9 ± 0.3	15.8 ± 0.3

** All the plant leaves were treated for 18h with 10mM EMS.

IV. DISCUSSION

The authors have compared the correlation between the nuclei diameter and the DNA damage induced by 10 mM EMS in the nuclei of leaves isolated from the studied plants. The TM values presented were obtained after 5 min unwinding and 15 min electrophoresis as these SCGE conditions were applied for all crops. The Pearson product moment correlation was only 0.64. This analysis indicated a very low correspondence between the diameter of the nuclei and their sensitivity to EMS. The earlier report by the authors demonstrated that the DNA damage after the lowest applied concentration of EMS (2 mM) was significantly higher ($P<0.05$) than the DNA damage of the negative control in all the plants tested. While gamma-radiation induced DNA damage, measured by the SCGE assay, is readily repaired within 24 h, DNA

damage induced by EMS 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 SCGE assay may detect DNA damage inflicted by some types of chemical genotoxic agents long periods after the initial exposure. In conclusion, the single cell gel electrophorecis assay can be applied for monitoring the DNA damaging effects of enviromental toxicants. 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. Also, the damage due to a mutation it does not measure with the comet assay.

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