Assessment of Genotoxicity of Ionizing radiation using *Tradescantia*-Comet assay

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1. Introduction

Over the last two decades, several new methodologies for the detection of DNA damage have been developed. The comet assay is currently used in different areas of biological sciences to detect DNA damage. The comet assay, also called the single cell gel electrophoresis (SCGE) was first introduced by Östling and Johanson as a microelectrophoretic technique for the direct visualization of DNA damage in individual cells [1]. The comet assay, due to its simplicity, sensitivity and need of a few cells, is ideal as a short-term genotoxicity test [2-4]. The comet assay can theoretically be applied to every type of eukaryotic cell, including plant cells.

Plants are very useful as monitors of genetic effects caused by pollution in the atmosphere, water and soil [5,6]. Although the genotoxic effects detected by *Tradescantia* tests cannot be associated with mutagenesis or even carcinogenesis in humans, these bioassays are very useful tools for screening the mutagenic potential in the environment [7]. Experiments were conducted to study the genotoxic effects of ionizing radiations on the genome integrity, particularly of *Tradescantia* [8, 9]. The increasingly frequent use of *Tradescantia* as a sensitive environmental bioindicator of genotoxic effects [10].

This study was designed to assess the genotoxicity of ionizing radiation using *Tradescnatia*-comet assay.

2. Materials and Methods

2.1 Leaf sampling and irradiation

Without any visible injury, 6 leaves of *Tradescantia* KU 9 were randomly collected from inner positions. Average leaf sizes of *Tradescantia* were ranged between 5-6 cm in length. Leaf samples packed in a conical tube were irradiated at 0.1, 10, 25, 50 Gy using a ^{60}Co irradiator at the Korea Atomic Energy Research Institute

2.2 Isolation of nucleoids

After irradiation *Tradescantia* leaf samples were briefly rinsed in buffer and placed on a plastic petridish to which a buffer is added, all on ice. Leaves are gently sliced with a fresh razor blade. The dish is kept tilted in the ice so that the nuclei would collect in the buffer. After centrifugation at 2000 rpm, for 5 min, the pellets were saved for assay.

2.3 Comet assay

Each slide was layerd with warm agarose solution, air-dried for 30 min, and stored in a dust proof box. 70 ㎕ of nucleoid suspension was mixed in 700 ㎕ of warm low melting point agarose solution. The mixture was then layered on coated slide and mounted with a cover glass, avoiding air bubbles. The slide was placed on ice for 30 min until a gel was formed and the cover glass removed. The prepared slide was put into a lysis solution (100 mM EDTA, 2.5 M NaCl, 10 mM Trizma base, 1 % Triton X-100, 10 % DMSO, pH 10.0) at 4 ℃ for 1 hr, and then slides were placed in the alkaline buffer (10 N NaOH, 200 mM EDTA, pH>13) for 20 min to allow unwinding of the DNA and the expression of alkali-labile damage. The electrophoresis apparatus was operated at 25V/cm with constant 300 mA for 10 min in an ice bath. Following electrophoresis, slides were rinsed 2 times for 5min in the distilled water and immersed in ethyl alcohol for 30 min and ari-dried. After drying well, each slide was stained with 20 ㎍/㎖ ethidium bromide and covered with a cover glass before viewing under the fluorescent microscope.

2.4 Comet analysis

The extent of DNA migration was determined using an image analysis system (Komet 4.0 from Kinetic Imaging Ltd., Liverpool). The DNA damage was evaluated by measuring tail length between comet head and tail and generally 50 cells were scored per dose.

3. Results

Leaf samples were exposed to 0.1 ~ 50 Gy and samples for comet assay were prepared immediately. A significant dose-dependent increase in the average median tail moment values over the negative control was observed (Fig. 1). As a rough estimate of DNA migration, the length of the comet tail was recorded versus its head diameter under different conditions. In the non-irradiated sample the tails can be seen, but the DNA comets are short. The comets from irradiated samples have longer tails that lengthen further as irradiation doses increase. The comet moment parameter which integrates both the morphometric parameter and the densitometric one may better reflect

the amount of DNA which has been displaced from the nucleus [11].

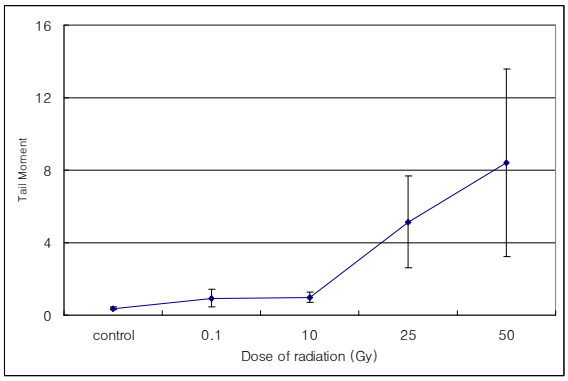

Fig. 1. The effects of dose of radiation on the average median tail moment values of nuclei isolated from leaves of *Tradescantia*. Tail Moment = Tail length x Tail % DNA/100.

The comet assay has demonstrated its sensitivity as a technique for the evaluation DNA damage among a variety of cell types, induced by a variety of physical and chemical agents [12]. The potential applications of the comet assay in such areas as genotoxicity, clinical, DNA repair, environmental biomonitoring and human monitoring are almost unlimited.

4. Conclusions

The development of comet assay has enabled investigators to detect DNA damage at the levels of cells. To adapt this assay to plant cells, nuclei were directly obtained from *Tradescantia* leaf samples. A significant dose-dependent increase in the average median tail moment values over the negative control was observed. Recently the adaptation of this technique to plant cells opens new possibilities for studies in variety area. The future applications of the comet assay could impact some other important areas, certainly, one of the limiting factors to its utility is the imagination of the investigator.

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