Repair of DNA Damage Induced by Radiation in Tobacco Plants

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1. Introduction

Ionizing radiation induces DNA damage through deposition of energy in cells or free radicals formation and oxidative damage. The main lesions in DNA molecules are strand breaks, cross-links, alkali labile sites and damage to bases [1]. All these types of DNA damage can be detected by the comet assay [2].

The tobacco and the other plant assay has been used for studies after exposure to ionizing radiation [3, 4] and of effects of various mutagens [5, 6].

The objective of this study was to study the kinetics of repair of DNA damage induced by gamma-rays as measured by the Comet assay in plant leaves and roots.

2. Materials and Methods

Sterilized seeds of *Nicotiana tabacum* var. *xanthi* were grown in a growth chamber at 26°C with a 16 h photoperiod to the 4 to 5 true leaf stage [5]. The intact seedlings at the stage of leaf 4 and 5 were irradiated in plastic containers at room temperature (about 20 - 22°C). The dose rate was 0.39 Gy/min. Alkaline protocol of the comet assay was applied to measure the radiation-induced DNA damage in isolated nuclei and in leaves of the tobacco seedlings

3. Results and Discussions

Intact seedlings in plastic containers were irradiated at room temperature and after irradiation nuclei were isolated from leaves and SCGE slides prepared.

Tail moment values which reflect the level of induced DNA damage in the leaf nuclei isolated from the irradiated seedlings at room temperature significantly increased from 1.08 ± 0.10 (negative control) to 20.26 ± 1.61 μm (10 Gy).

As shown in the Figure 1, differences in the TM values were significantly great between the irradiated intact seedlings and the irradiated nuclei isolated from leaves.

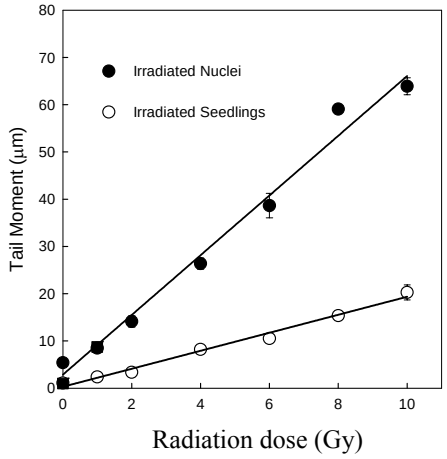

Figure 1. A comparsion of dose-response curves of the induction of DNA damage in nuclei isolated from tobacco leaves exposed to gamma-radiation. Tobacco seedlings irradiated at room temperature (●), and irradiated isolated nuclei on SCGE slides (○) in the dose range from 0 to 10 Gy.

The most sensitive to gamma-radiation were irradiated isolated nuclei, followed by nuclei isolated from excised leaves irradiated on ice, and nuclei isolated from leaves of irradiated intact seedlings. The very high response of isolated nuclei to gamma-radiation may be explained by lack of the cell cytosol where most of the repair enzymes and scavengers are located. The higher DNA damage after irradiation of leaves on ice compared to irradiation at room temperature was probably caused by the decreased rate of DNA repair at lower temperatures.

Kinetics of repair of damaged DNA caused by 30 Gy dose of gamma-radiation is presented in Figure 2. We measured DNA damage immediately and at intervals up to 240 minutes after irradiation at room temperature. Nuclei were isolated at given time from pieces (±1 cm^2) of tobacco leaves of irradiated seedlings. Simultaneously we isolated nuclei from leaves of control tobacco seedlings.

Immediately after irradiation the highest damage of DNA expressed by the TM (± SE) value was 40.50 ± 1.97 μm compared to the control (3.65 ± 0.34 μm).

After regression analysis of the DNA repair data the time to repair 50 % of the induced DNA damage ($t_{1/2}$) was 51.7 min. Four hours after irradiation, 80% DNA damage was repaired.

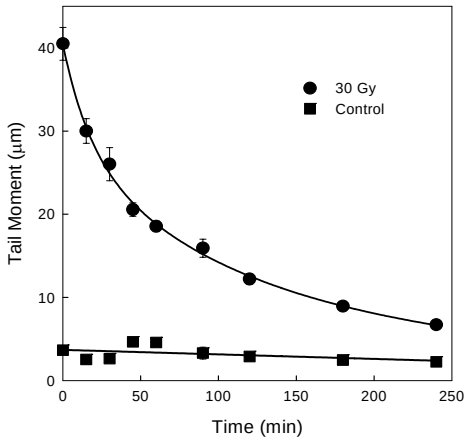

Figure 2. Kinetics of repair of damaged DNA caused by 30 Gy gamma-radiation on tobacco seedlings. The level of DNA damage measured by the Comet assay was expressed by the tail moment value (μm) in nuclei isolated from the non-irradiated control (■) and irradiated tobacco leaves (●). The error bars represent the standard error of the mean.

The DNA damage induced by 30 Gy gamma radiation that resulted in, or was converted to, double and single strand breaks was rapidly repaired with $t_{1/2}$ = 51.7 min (Figure 2). This process of DNA repair and misrepair is one mechanism of converting gamma ray induced lesions into heritable somatic mutations. These data indicate that DNA strand breaks are rapidly repaired, however, other lesions such as oxidized bases may persist longer [7] and be misrepaired to somatic mutations.

The reported data indicate that the standard alkaline SCGE protocol using nuclei from plant leaves may not be suitable alone for biomonitoring late effects of acute ionizing radiation, as the induced DNA damage is readily repaired.

4. Conclusions

Gamma-irradiation of tobacco seedlings induced a dose-dependent increase in somatic mutations from 0.5 (control) to 240 per leaf (10 Gy). With increased dose of gamma-radiation, the averaged median tail moment values (± SE) significantly increased from 1.08 ± 0.10 (control) to 20.26 ± 1.61 μm (10 Gy). Nuclei isolated from leaves 24 h after irradiation expressed tail moment values that were not significantly different from the control (2.08 ± 0.11). Thus a complete repair of DNA damage induced by gamma-irradiation and measurable by the Comet assay was observed. Data on the kinetics of DNA repair after gamma-radiation on intact seedlings are explained in this article.

Acknowledgements

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References

[1] R. Teoule, Radiation-induced DNA damage and its repair, Int. J. Radiat. Biol. 51, 573-589 (1987).

[2] R. R. Tice, E. Agurell, D. Andrerson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.-C. Ryu, and Y. F. Sasaki, Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35, 206-221 (2000).

[3] H. L. Dulieu, and M. A. Dalebroux, Spontaneous and induced reversion rates in a double heterozygous mutant of Nicotiana tabacum var. Xanthi n.c.-dose-response relationship, Mutation Res. 30, 63-70 (1975).

[4] O. Ptáček, D. A. Stavreva, J. K. Kim, and T. Gichner, Induction and repair of DNA damage as measured by the Comet assay and the yield of somatic mutations in gamma-irradiated tabacco seedlings, Mutation Res. 491, 17-23 (2001).

[5] T. Gichner, O. Ptáček, D. A. Stavreva, and M. J. Plewa, Comparison of DNA damage in plants as measured by single cell gel electrophoresis and somatic leaf mutations induced by monofunctional alkylating agents, Environ. Mol. Mutagen. 33, 279-286 (1999).

[6] T. Gichner, D.A. Stavreva, and J.K. Kim, DNA damage measured by the comet assay in eight agronomic plants, Biologia Plantarum, 47, 185-188 (2994).

[7] A. R. Collins, V. L. Dobson, M. Dušinská, G. Kennedy, and R. Štětina, The comet assay: what can it really tell us?, Mutation Res. 375, 183-193 (1997).