Applicability of Irradiation Combined with Antioxidant Enzyme Assays to Environmental Monitoring

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1. Introduction

Mixed air pollutants from industrial complexes are suspected as oxidative stress to living plants. As they grow in a fixed place, they can be a good indicator which reflects the level of pollution [1, 2]. It is inevitable for those plants to adapt themselves to the adverse environmental condition, or to develop a defense system against the oxidative stress from the pollution [3, 4]. It is assumable that adaptive plants are exposed to a second oxidative stress agent such as ionizing radiation, they may cope better with the second stress. If the plants are exposed to air pollutants prior to ionizing radiation, they already have developed the antioxidant defense system against oxidative stress from ionizing radiation [5]. To support the assumption, antioxidative capacity assay with DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging ability test and the content of superoxide dismutase (SOD) which catalyzes the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen can be assessed in conjunction with the biochemical response of the plants after irradiation. *Euonymus japonica* that is a well known road tree with high resistance to air pollution was used as experimental materials to check applicability of irradiation combined with plant bioassay to environmental monitoring.

2. Materials and Methods

2.1 Leaf sampling and irradiation

Leaves of *E. japonica*, road trees were collected from the Onsan industrial complex, one of the most polluted cities in Korea, and also from a Ki-jang area as a clean control group. The samples were irradiated with gamma rays from a cobalt-60 isotopic source at Korea Atomic Energy Research Institute, Advanced Radiation Technology Institute (Jeongeup, Korea). The dose rate was 50 Gy and 100 Gy per hour, as determined with a Frick dosimeter.

2.2 Extraction

One gram of ground powder was mixed with 10 ml of methanol (80%) and placed in a shaking incubator for 24h at 25℃. The macerated mixture was filtered through a Whatman No.2 filter paper. Extraction yields for each solvent were calculated by subtracting the dried weight of the plant material residues after extraction from the weight of the original plant material. The extracts were stored at -20℃ until further processing.

2.4 DPPH radical scavenging ability test

An aliquot (0.05 ml) of each extract was added to 2.95 ml of methanolic DPPH solution (0.1 mM) in a test tube and shaken vigorously. After incubation at 25℃ for 30 min in the dark, the absorbance of each solution was determined at 517 nm. The corresponding blank (control) reading was also recorded. The free radical-scavenging activity was expressed as percentage scavenging of the DPPH by the plant extracts and calculated as electron donating ability as follows.

(EDA, %) = [1-(absorbance of sample/absorbance of control)] X100

2.4 Antioxidant enzyme activities : SOD assay

The WST (water-soluble tetrazolium salt)-based SOD assay was performed using Dojindo's SOD Assay Kit-WST according to the protocol. For calculate SOD activity (% inhibition), using the following equation scheme with the result of absorbance having read at 450nm. SOD activity.

$$(\text{inhibition rate, \%}) = [(A_{\text{blank1}}-A_{\text{blank3}})-(A_{\text{sample}}-A_{\text{blank2}})]/(A_{\text{blank1}}-A_{\text{blank3}}) \times 100$$

3. Results

For the leaf samples from the clean control area, EDA values in the irradiated groups which had gone down to 53% and 50% 10 hours after irradiation rose back to 64% and 64%, respectively, which is similar to an original value, whereas that of the non-irradiated sample continued to decrease to 20%. In the meanwhile, the values of the leaf samples from the polluted area increased up to 60%, 80% and 83% three hours after irradiation and rose up to 84%, 83% and 82% twenty

four hours after irradiation, in the 0, 50 and 100 Gy irradiated groups, respectively.

Superoxide dismutase (SOD) is one of important antioxidant enzymes which catalyzes the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen. To compare the antioxidant enzyme activities between the samples from two areas, SOD was analyzed by means of SOD Assay Kit-WST. The values of the leaf samples from the clean area dropped from the original 70% down to 47% six hours after irradiation, and 57% ten hours after irradiation went back up to 54% and 63%, respectively, whereas that of the non-irradiated sample continued to decrease down to 38%. As for the samples from the polluted area, the values showed a gradual decrease of about 10% in the 50 and 100 Gy irradiated samples, and in the non-irradiated control, as well.

4. Conclusions

There were no significant changes in SOD activities and free radical scavenging abilities in the samples collected from a polluted area. In the meanwhile, SOD decreased in the samples from a clean area until 6 to 10 hours after irradiation, then showed a gradual recovery tendency until 24 hours after irradiation. The results indicate that the adaptive plants lived in polluted areas for a long time are not only less sensitive to the second oxidative stress such as gamma rays, but also have higher resistance to ionizing radiation compared to those from the clean area. It is suggested that the plant bioassay regarding antioxidative enzymes combined with gamma irradiation, if further elaborated, can be applied to bio-monitoring of the environmental pollution.

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REFERENCES

[1] J. M. Hellawell, Biological Indicators of Freshwater Pollution and Environmental Management. Elsevier, New York, pp.52-53, 1986.
[2] W. U. Müller, C. Streffer, Biological indicators for radiation damage. Int. J. Radiat. Biol.59:863-873, 1991.
[3] A. M. Edwards, M. Ruiz, E. Silva, E. Lissi, Lysozyme modification by the Fenton reaction and gamma radiation. Free Radic. Res. 36:277-284, 2002.
[4] M. A. Esnault, F. Legue, C. Chenal, Ionizing radiation. Advances in plant response. - Environ. Exp. Bot., 68:231-237, 2010.
[5] M. H. Baek, B. Y. Chung, J. S. Kim, I. S. Lee, Alleviation of salt stress by low dose gamma-irradiation in rice. Biol. Plant. 49:273-276, 2005.