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ORIGINAL ARTICLE

Electron Paramagnetic Resonance Investigation of Different Plant Organs after Gamma Irradiation

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Abstract Total reactive oxygen species (ROS) signals in irradiated Arabidopsis plants were examined by electron paramagnetic resonance (EPR) analysis. At 10 kGy, the EPR signal intensity was highest in the root, whereas relatively low intensity levels were observed in the leaf and stem. The relative unit (r.u.) of control plants was 0.38 in the leaf, which was gradually increased to 0.51, 0.71, and 0.95 r.u. at 1, 5, and 10 kGy, respectively. In the stem, the intensity in all irradiated samples was lowest compared with that in other plant organs such as the leaf and root. The r.u. in the root sharply increased from 0.13 r.u. in control samples to 1.58 r.u. at 10 kGy, with 0.30–0.42 r.u. observed in 1–5 kGy irradiated samples. Stem and leaf extracts showed remarkably high levels of radical scavenging activity at 89.12 and 71.45%, respectively, compared with the very low level of activity in the root at 10.75%. These findings were in good agreement with the extraction yield of each plant organ, which was 20.0, 14.8, and 10.0% in the stem, leaf, and root, respectively. Order of EPR signal intensity and radical scavenging activity was as follows: EPR signal intensity: 1) leaf > root > stem at 1 and 5 kGy, 2) root > leaf > stem at 10 kGy; radical scavenging activity: stem > leaf > root. Results showed high or low levels of EPR signal intensity in different plant organs could be caused by the ROS removal power of extracts from different plant organs.

Keywords Arabidopsis · 2,2-diphenyl-1-picryl-hydrazyl-hydrate · electron paramagnetic resonance · gamma irradiation

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Introduction

Recently, radiation techniques have begun to be widely used in various industrial areas such as food irradiation, plant mutation breeding, environmental science, and material science, among others. These applications are mainly based on the characteristic of radiation that it can produce various reactive oxygen species (ROS) in water or organic solvents (Paktaş and Sünnetçioğlu, 2007). Increased levels of ROS, referred to as oxidative stress, can damage DNA, proteins, and lipid membranes (Pedrajas et al., 2000). ROS can also induce uncontrolled cellular proliferation, aging, and programmed cell death (Cerutti, 1985).

Gamma radiolysis of water can produce various molecular species and free radicals, and ROS. In the first stage (10^{-16} s) of gamma radiolysis, water molecules (H_2O) are converted into water radical cations ($H_2O^{\cdot+}$) and excited water (H_2O^*) (Fig. 1). In the second stage (10^{-12} s), $H_2O^{\cdot+}$ produces hydronium ions (H_3O^+), hydroxyl radicals (·OH), and hydrogen ions (H^+), whereas H_2O^* produces hydrogen radicals (H·) and ·OH. In addition, electrons (e) originated from water molecules produce H· and aqueous or solvated electrons (e^-_{aq}). In the final stage (10^{-6} s), various molecules and radicals such as hydrogen peroxide (H_2O_2), hydroxyl ions (OH^-), superoxide radical anions ($O_2^{\cdot-}$), molecular hydrogen (H_2), H_2O, and H· are formed by the dissociation and condensation reactions of intermediates (Lee et al., 2009).

In spite of this understanding of the effects of radiation, no studies have examined ROS levels in different plant organs after

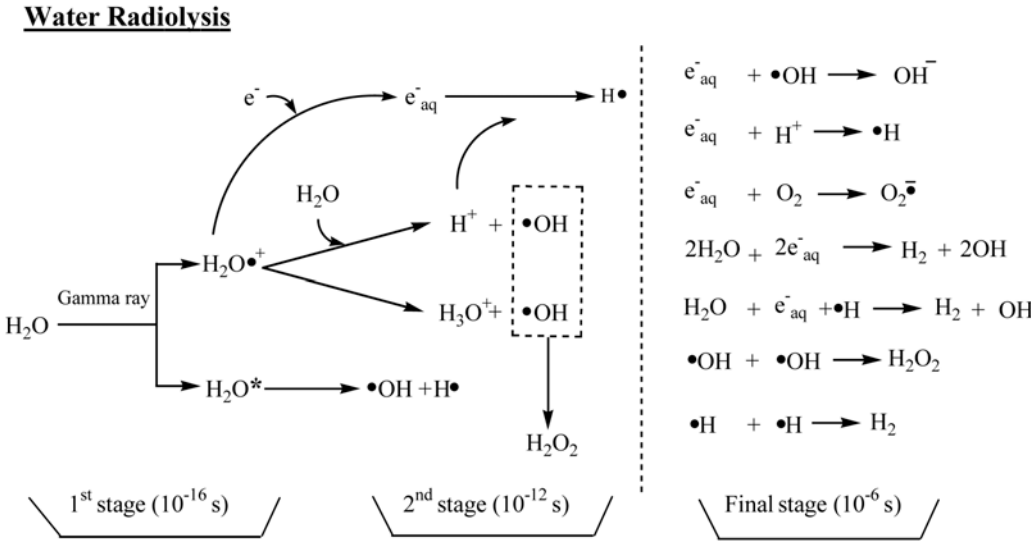

Fig. 1 The pathways involved in water radiolysis.

gamma irradiation. Arabidopsis, which was used in the present study, is popular as a model organism in plant biology and genetics. Arabidopsis, the first plant genome to be sequenced, is a popular tool for understanding the molecular biology of many plant traits, including flower development and light sensing (Leutwiler et al., 1984).

In the present study, ROS levels and scavenging capability against ROS were measured in different plant organs such as the root, stem, and leaf after gamma irradiation.

Materials and Methods

Plant materials and gamma irradiation. Wild-type plants of *Arabidopsis thaliana* (ecotype Columbia) were cultivated in a growth chamber with a 16-h photoperiod and a temperature regime of 22/18°C (day/night). Lighting was adjusted to a photosynthetic photon flux density of 130 μmol $m^{-2}s^{-1}$, supplied by six fluorescent lamps. Thirty-three days after sowing, seedlings were irradiated with gamma rays, at doses of 0.5, 2.5, and 5.0 kGy h^{-1} for 2 h. These rays were generated by a gamma irradiator (^{60}Co, ca. 150 TBq of capacity; Atomic Energy of Canada Limited, Ontario, Canada) at the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute. Subsequently, the plants were allowed to grow under the same growing conditions mentioned above.

Electron paramagnetic resonance (EPR) analysis. All samples were collected and immediately frozen in liquid nitrogen after gamma irradiation. For EPR analysis, samples were freeze-dried and ground. To study the different EPR spectra, a set of quartz sample tubes from the same batch with a calibrated diameter (inner diameter 4 mm and outer diameter 5 mm) was used, and the sample tubes were filled with samples (about 1 g) of a length (30 mm) longer than the whole tube cavity. Sample tubes were positioned to be symmetrical to the cavity center (Yordanova et al., 2005). EPR spectra were measured with an ESR spectrometer (JES-FA200; JEOL Ltd., Tokyo, Japan) working at the X band. Spectra were recorded at room temperature with 9.4-GHz microwave frequency, 10-mW microwave power, 1.0-mT modulation amplitude, 100-kHz modulation frequency, and 5.0×10 amplification. The amount of trapped ROS was given as the area of EPR absorption spectra (double integral of measured spectra).

2,2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity and extraction yield. The DPPH scavenging capacity was determined according to the procedure of Mensor et al. (2001), with slight modifications. Samples of 500 mg were collected and immediately frozen in liquid nitrogen. Extraction was performed twice on leaf, stem, and root samples with 10 mL of 70% EtOH for 12 h. The diluted extracts were mixed with 0.4 mL of 0.2 mM DPPH in MeOH and allowed to react at room temperature for 30 min. The absorbance values for DPPH were obtained at 517 nm and converted into the percentage antioxidant activity (AA) using the following formula: $AA\% = (1 - Abs_{sample}/Abs_{blank}) \times 100$.

Results and Discussion

Arabidopsis plants were exposed to various doses of ionizing radiation (1, 5, and 10 kGy) to observe ROS levels. In order to detect irradiation radicals, EPR analysis was performed. Although EPR spectroscopy is limited by the lifetime of radiologically produced free radicals, it is still a powerful and unique technique for the detection of paramagnetic species that are formed during the gamma radiation process (Figs. 2 and 3) (Polovka et al., 2006).

A dose-dependent increase in the peak intensity of EPR was

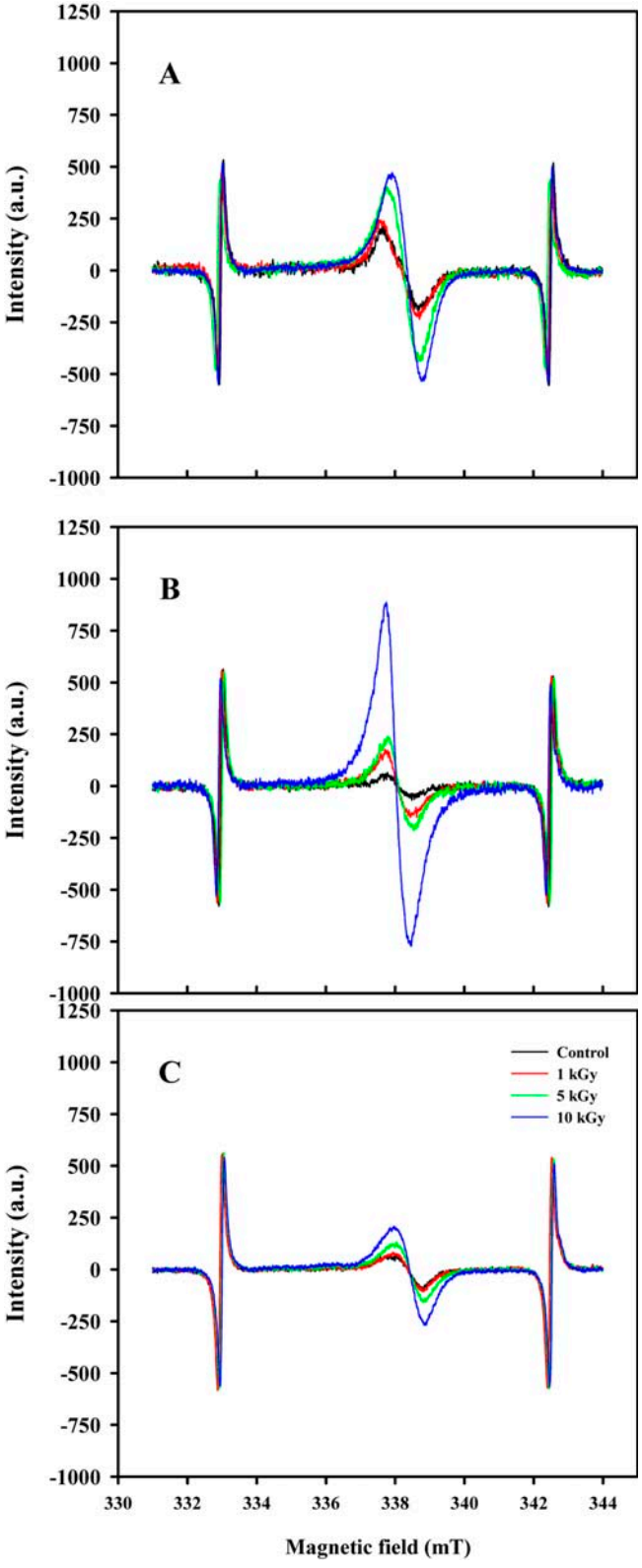

Fig. 2 Electron paramagnetic resonance spectra derived from Arabidopsis leaf, stem, and root after gamma irradiation. A, leaf; B, stem; and C, root.

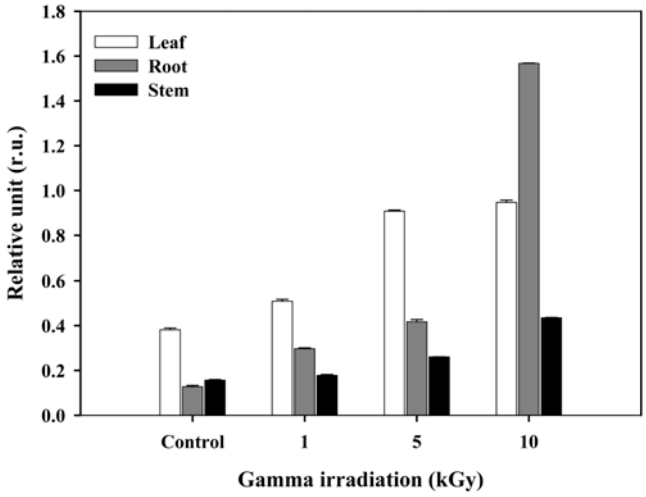

Fig. 3 Relative units (r.u.), expressed as a signal ratio of reactive oxygen species versus Mn, used as an internal standard. Bars indicate means ± SE (n=3).

observed in irradiated Arabidopsis plants, with the maximum value observed at 10 kGy in different plant organs including root, stem, and leaf (Fig. 3). At 10 kGy, the EPR signal intensity was the highest in the root, followed by the leaf and stem. The relative unit (r.u.), which is expressed as a signal ratio of ROS, of control samples was 0.38 in the leaf, and gradually increased to 0.51, 0.71, and 0.95 r.u. at 1, 5, and 10 kGy, respectively. In the stem, the intensity in all samples at different irradiation dose levels was lowest compared with those in the leaf and root. Signal intensity in the stem increased from 0.16 r.u. in control samples to 0.18–0.44 r.u. in irradiated samples. In contrast, the r.u. in the root sharply increased at 10 kGy to 1.58 r.u., whereas a relatively negligible increase was observed in all other irradiated samples, ranging from 0.13 r.u. in control samples to 0.30–0.42 r.u. in 1–5 kGy irradiated samples.

These results raise the question as to why the root showed the highest ROS signal after gamma irradiation. Therefore, a DPPH radical scavenging activity assay was carried out using extracts of different organs. The purpose of this assay was to determine the radical scavenging activity of the different extracts, which could be closely related to ROS removal power.

Stem and leaf extracts showed remarkably high levels of radical scavenging activity, at 89.12 and 71.45%, respectively, whereas very low level of activity was observed in the root, at 10.75% (Fig. 4A). The high levels of radical scavenging activity observed in the stem and leaf were in good agreement with the extraction yield of each plant organ, which was 20.0, 14.8, and 10.0% in the stem, leaf, and root, respectively (Fig. 4B). This result implies that the high yield of an extract may be associated with a high radical scavenging activity. Gamma irradiation generally increases phenylalanine ammaonia-lyase (PAL) activity in different fresh products, probably as a response to stress (Tomás-Barberán and Espín, 2001). In addition, Brown et al. (2003) reported glucosinolate

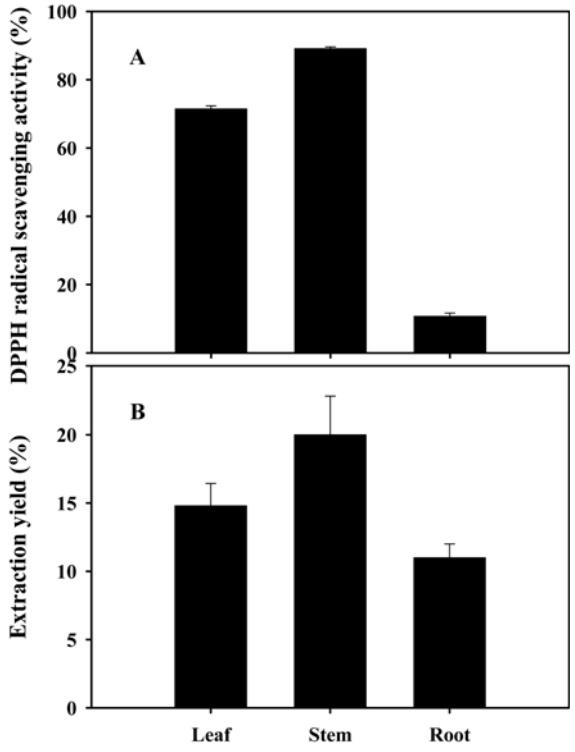

Fig. 4 DPPH radical scavenging activity (A) and extraction yield (B) in Arabidopsis leaf, stem, and root samples. Samples of 500 mg were extracted with 10 mL of 70% EtOH. Bars indicate means ± SE (n=3).

content of various organs of the model plant *A. thaliana* (L.). Upon comparison of the glucosinolate composition of different organs, seeds and fruit were found to have the highest diversity of individual compounds which may also reflect the need to maximize the defensive potential of these reproductive organs. Based on these results, high or low levels of EPR signal intensity in different plant organs appear to be affected by the ROS removal power of the extracts from different plant organs. That is, ROS produced from water radiolysis may be removed by different secondary metabolites in different plant organs, resulting in different levels of EPR signal intensity.

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