Spindle trees (*Euonymus japonica* Thunb.) growing in a polluted environment are less sensitive to gamma irradiation

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ABSTRACT

Background: Spindle trees (*Euonymus japonica* Thunb.) growing in an industrial complex area containing pollutants is chronically injured thus need to build up their resistance. Antioxidant enzymes and cell membrane stability have been widely used to differentiate stress tolerance. ***Materials and Methods***: Leaves of spindle trees from a clean control area (Kijang) and an industrial area (Onsan) where is one of the heavily polluted areas in Korea were subsequently irradiated with 0, 50 and 100 Gy of gamma rays from a ^{60}Co isotopic source, and evaluated for the level of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) and lipid peroxidation (LPO). ***Results***: Air pollution-stressed plants showed better adaptation to ionizing radiation (IR) stress and are better protected from the oxidative damage, and rapidly up-regulate their antioxidative systems. The adaptive plants growing in the polluted area are less sensitive to consecutive oxidative stresses such as gamma rays, having a higher capacity of resistance to ionizing radiation, compared to the samples taken from the clean area. ***Conclusion***: Air pollution-stressed plants showed lesser changes in the level of antioxidant enzymes after irradiation due to their enhanced antioxidant capacity. The enzymatic differences in plants irradiated with gamma rays can be used as biochemical end-points for environmental monitoring.

Keywords: *Spindle tree, oxidative stress, ionizing radiation, antioxidant enzymes, membrane stability, environmental monitoring.*

INTRODUCTION

Unlike animals higher plants are immobile. They can't escape from unfavourable conditions thus they have to cope with this adverse environment by their defence mechanisms. Even in optimal conditions ROS are regular constituents of plant metabolism. During adverse conditions overproduction of ROS participates in signalling and further triggers defence systems to prevent damage due to oxidative stress caused by ROS by modulating the activities of the detoxifying enzymes (e.g., superoxide dismutases, catalase and glutathione reductase) [1-3]. Superoxide dismutase is a major scavenger of superoxide ($O_2^{\bullet -}$) and its enzymatic action results in the formation of H_2O_2 and O_2. The hydrogen peroxide produced is then scavenged by catalase. Glutathione (GSH) appears to be one of the most important antioxidants that occur in biological systems [4]. Glutathione can regenerate dehydroascorbate to produce ascorbate and oxidized glutathione (GSSG) in a reaction catalyzed by dehydroascorbate reductase. In turn, GSSG is

reduced by glutathione reductase (GR), requiring the consumption of NADPH. Singlet oxygen and hydroxyl radical are eliminated in the glutathione pathway [5]. The ability of higher plants to scavenge the toxic active oxygen seems to be very important determinant of their tolerance to these stresses [6, 7].

Air pollutants including heavy metals present in high concentrations in the environment of the industrial area induce abiotic stress [8]. This leads to the overproduction of ROS in plants which is highly reactive and toxic. Under unstressed conditions, the formation and removal of ROS are in balance. However, a rise in ROS production can seriously disrupt normal metabolism through oxidative damage to lipids, proteins and nucleic acids [9, 10]. Lipid peroxidation leads to membrane damage. Thus, cell membrane stability has been widely used to differentiate stress tolerance [11] and in some cases higher membrane stability could be correlated with abiotic stress tolerance [12].

Gamma irradiation of plants increases the level of reactive free radicals with a short lifespan and increases the number of less active organic radicals with relatively long life-spans which are products of water radiolysis [13-15]. In cases of chronic exposure of irradiation, such as at the nuclear test site in Kazakhstan (a 40-year chronic exposure to caesium and strontium), the wild Poaceae, *Stipa capillata* L., elevated its radioresistance to a subsequent acute irradiation [16]. The progeny of *Arabidopsis thaliana* individuals irradiated at the Chernobyl site are able to resist a higher concentration of mutagens than the control plants, whether they are radiomimetic agents or free radical-producing agents [17] found that a first stress induced by IR could help plants respond to a secondary, different, stress [18]. Furthermore, the common response is set into action by the first stress. The secondary ROS are involved in cell signalling or that, after a first stress, a synthesis of secondary metabolites can help the plant to resist a new stress [2].

In very low doses gamma irradiation is ubiquitous from cosmic radiation and nuclear decay processes. It is highly energetic and can easily penetrate matter. When absorbed by living tissue it is highly dangerous and represents health hazard as it induces similar injury like X-rays that is oxidative damage, gene mutations, cancer, eventually cell death in doses exceeding natural background. This situation is especially dangerous and happens accidentally as atomic plant power disaster. As an ionizing radiation, it interacts with cellular components such as membranes, DNA and in plants also cell walls [19, 20]. Such action is both direct and indirect; the example of the latter is radiolysis of water when reactive oxygen species are produced.

In plants, gamma rays induced enhanced respiration as well as ethylene evolution [21]. Also metabolic responses were found as accumulation of sucrose [22], stimulation of enzyme activities of phenolic metabolism [23] and small heat-shock proteins are consequence of gamma irradiation [24]. Gene expression studies revealed that several genes of antioxidant enzymes as glutathione-S-transferase, peroxidase, superoxide dismutase, and catalase were up regulated, contrarily cytosolic and stromal ascorbate peroxidase were down regulated [25].

Naturally, several stresses frequently either follow or combine. In this study we focused on *Euonymus japonicus*, an evergreen woody plant that has the highest antioxidant capacity among the road trees in Ulsan area [26]. This means that this species can well tolerate continuous ambient stress factors at polluted areas. Leaves of this chronically stressed road trees were subsequently underwent with gamma irradiation treatment. We tested whether adapted or hardened plants to air pollutants might show a faster biochemical reaction or higher capacity of antioxidant against secondary oxidative stress of γ-rays, compared to plants in the clean area. This would signify the better tolerance against gamma irradiation.

MATERIALS AND METHODS

Growing area

The study area, Onsan, is located in the Ulsan Industrial Complex. It has been reported as one

of the most contaminated places with by-products from industrial plants in Korea. It has been designated as 'special countermeasure-area of air conservation' since 1985 (source: Pollutant Release and Transfer Registers, Ministry of Environment) [27]. Nonferrous metal industrial areas, assembled metal industrial areas, petrochemical industrial areas, waste treatment facilities and coal-fired facilities are heavily distributed in this complex. According to the Environmental Statistics Yearbook 21st published in 2008, the total number of factories mounts up to 127 [28]. The average oxides of nitrogen (NO_x), sulfur dioxide (SO_2), ozone (O_3), carbon monoxide (CO) and particulate matter (PM-10) concentration during Jan. 2006-Dec. 2008 were reported to be 0.023, 0.018, 0.023, 9 ppm and 50 μg/m^3, respectively,. Based on the data of PRTR (Pollutant Releases and Transfer Registers, Ministry of Environment), high concentrations of Cr, Pb, Ni and Zn which have been selected as 'restricted materials of air pollution' was contributed to the Onsan area [29].

Leaf sampling and irradiation

During June-October 2010, leaves of Spindle trees (*Euonymus japonica* Thunb.) which were planted as the road tree in the Onsan industrial complex (Ulju-gun, Ulsan, Kyeongsangnam-do, Korea) were collected. Twig samples were stored and transferred at 4 °C in the cooler after collection. Immediately after irradiation at room temperature, they were again stored at 4 °C. At each time point of sampling (0, 3, 6 and 24 hrs after irradiation), leaves were taken out and quickly immerged into liquid nitrogen, and then stored at -20 °C until further analysis. For comparison of the capacity of resistance of this group, same trees in the clean Kijang area, free of heavy industry (Kijang-gun, Busan, Korea) were selected as a control group. Sampling sites were showed on the map figure 1 and table 1 show the weather description of two investigated sites.

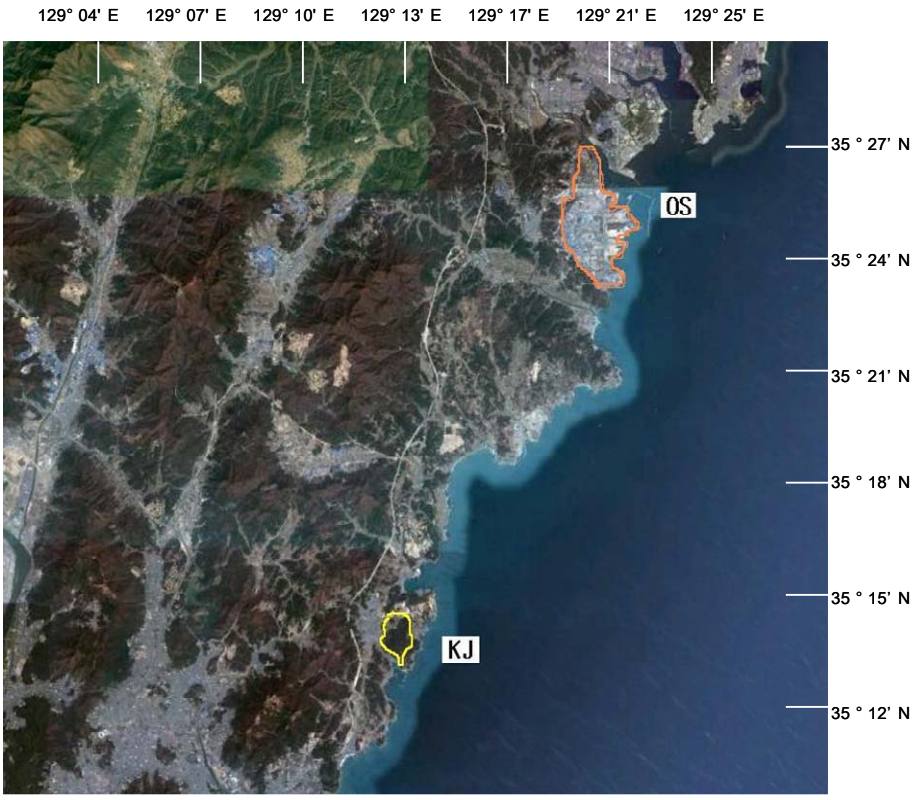


Figure 1. Sampling sites of the *Euonymus japonica.* (KJ: Kijang-Clean Area, OS: Onsan-Industrial Area).

Table 1. The weather descriptions of investigated sites (source: Monthly weather reports of May-Oct 2010, Korea meteorological administration) (S.L.P: See Level Pressure, Max: maximum Min: Minimum, D.P. Temp.: Dew Point Temperature, R.H.: Relative Humidity, W.S.: Wind Speed, Cloud Amt.: Cloud amount, Precip.: Precipitation).

2010 May-Oct/ month	S.L.P. (hPa)	Air Temp. (℃)			D.P. Temp. (℃)	R.H. (%)		W.S. (m/s)		Cloud Amt.	Precip. (mm)	Sunshine
	Mean	Mean	Max.	Min.	Mean	Mean	Min.	Mean	Max.	Mean		
OS	1012.1	22.1	26.9	18.1	16.8	74.3	54.7	2.1	8.6	5.9	129.9	189.0
KJ	1012.2	22.4	26.1	19.7	16.7	72.3	55.5	3.1	12.4	5.7	165.6	194.8

The harvested leaves of both groups were irradiated with 0, 50, and 100 Gy (1 Gy=1J kg^{-1}, dose rate 50 Gy h^{-1}, measured with a Fricke dosimeter) from a ^{60}Co γ-irradiator (150 TBq of capacity; AECL, Canada) at Korea Atomic Energy Research Institute, Advanced Radiation Technology Institute (Jeongeup 580-185, Korea). Leaves were detached and immediately frozen in liquid nitrogen at 0, 3, 6, 10 and 24 hours after the point of irradiation. We designated individual samples KJ for Kijang clean area and OS for Onsan, the polluted area. The Samples are further numbered 0, 50 and 100 according to irradiation dose (in Gy). Codes for representing investigated area and the absorbed doses are shown in table 2.

Preparation of leaf extracts

One g of ground leaf powder was mixed with 10 cm^3 of methanol (80%) and placed in a shaking incubator for 24 h at 25 °C. The macerated mixture was filtered through the Whatman No.2 filter paper. Extraction yields for each solvent were calculated by subtracting the dried weight of plant material residues after extraction form the weight of the original plant material. The extracts were stored at -20 °C until measurement of antioxidant capacity assay.

Antioxidant capacity

The assay for total radical scavenging ability with DPPH (1, 1-diphenyl-2-picrylhydrazyl) was employed [30]. 0.05 cm^3 of each extract was added to 2.95 cm^3 of DPPH methanolic solution (0.1 mM) in a test tube and shaken vigorously. After incubating at 25 °C for 30 min in the dark, the absorbance of each solution was determined at 517 nm. The results were expressed as percentage scavenging of the DPPH by the plant extracts and calculated as electron donating ability (EDA, %) = 100-[(absorbance of sample/ absorbance of the control without leaf extracts) × 100]. SOD, CAT and GR activities (table 2) assays were performed. Protein concentration was determined by the dye-binding method [31], using a bovine serum albumin (Sigma) as a standard.

SOD activity was assessed by utilizing Dojindo commercial kit with (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt), a water-soluble tetrazolium salt (WST-1) that produces a water-soluble formazan dye upon reduction with a superoxide radical according to protocol (Dojindo's SOD Assay Kit-WST, Dojindo, U.S.A.). The increase of the absorbance was monitored at 450 nm at 37°C and the results were expressed as U mg^{-1} protein. One unit of SOD was defined as the amount of the enzyme required to cause

Table 2. Codes for representing investigated area and the absorbed doses.

Area	Non-irradiated	Irradiated	
	0 Gy	50 Gy	100 Gy
The clean area (Kijang)	KJ-0	KJ-50	KJ-100
The industrial complex (Onsan)	OS-0	OS-50	OS-100

Table 3. The enzyme codes and reactions catalyzed.

Enzyme antioxidants	Enzyme code	Reactions catalyzed
Superoxide dismutase (SOD)	EC 1.15.1.1	$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$
Catalase (CAT)	EC 1.11.1.6	$H_2O_2 \rightarrow H_2O + ½O_2$
Glutathione reductase (GR)	EC 1.6.4.2	GSSG +NADPH + $H^+ \rightarrow$ 2GSH + $NADP^+$

50 % inhibition of the rate WST-1 reduction without a sample.

CAT activity was assayed at 20 °C in a 3 cm^3 reaction volume containing 2.8 cm^3 50 mM potassium phosphate buffer (0.1 M, pH 7 not containing EDTA), 120 mm^3 enzyme extract and 80 mm^3 of 0.5 M H_2O_2. Activity was determined as a decrease in absorbance at 240 nm for 30s [32]. The results were expressed as U mg^{-1} protein using a molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$. One unit was defined as the amount of the enzyme required for degradation of 1 µmol H_2O_2 per minute (table 3).

GR activity was assayed at 25°C in a 3 cm^3 reaction volume containing 1.5 cm^3 potassium phosphate buffer (0.1 M, pH 7), 150 mm^3 GSSG (20 mM), 200 mm^3 enzyme extract, 1 cm^3 bidistilled water and 150 mm^3 $NADPH_2$ (2 mM, dissolved in Tris-HCl buffer, pH 7). Activity was determined at 340 nm according to the method reported earlier [33]. The results were expressed as U mg^{-1} protein using a molar extinction coefficient of 6.22 $mM^{-1}cm^{-1}$. One unit was defined as the amount of the enzyme required to cause the oxidation of 1 µmol of NADPH to $NADP^+$.

Lipid peroxidation (LPO) was determined by measuring the amount of malondialdehyde (MDA) produced using thiobarbituric acid test as described by Heath and Packer (1968) [34]. MDA content was assayed in a 3 cm^3 reaction volume containing 1.2 cm^3 0.5 % (w/v) thiobarbituric acid solution and 0.8 cm^3 20 % (w/v) 2-thrichloroacetic acid mixed with the same volume of enzyme extract. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 3000 x g for 10 min. The absorbance of the supernatant was monitored at 532 and 600 nm and subtracted the nonspecific turbidity at 600nm. The MDA concentration was determined by its molar extinction coefficient of 155 mM^{-1} cm^{-1} and the results were expressed as µmol mg^{-1} protein. Every assay was repeated three times for each experimental group.

Statistical analysis

The significance of changes in responses was assessed by an analysis of variance with repeated measures, and after irradiation this was followed by a multiple comparison versus the control group analysis.

RESULTS

Comparison was done for the impact of gamma irradiation on Spindle tree leaves growing in two localities with contrasting air pollution. Adverse conditions evoked responses in plant cell tissue leading to oxidative stress which was associated with increased production of oxidized cellular components.

Oxidative stress

Evaluated were the oxidative injuries to membranes as a content of MDA, a product of lipid peroxidation using thiobarbituric test (figure 2. I, J). The results showed that the MDA content in the leaves of plants from the industrial area was slightly higher than that of the clean area at 0 Gy at 0 h. The irradiation caused in KJ samples a sharp increase in MDA content but only transiently after 6 h and from 0.806 µmol mg^{-1} protein (KJ-50, 0 h) and 0.725 (KJ-100, 0 h) up to 4.124 (KJ-50, 6 h) and 3.936 (KJ-100, 6 h) and rapidly decreased thereafter. On the other hand, in the samples from OS, the values decreased from 1.258 (OS-50, 0 h) and 1.260 (OS-100, 0 h) down to 0.856 (OS-50, 10 h) and 0.852 (OS-100, 10 h). Even though the values of the samples from OS showed changes, there was no significant impact on their MDA content in the plants irradiated with 50 Gy and 100 Gy.

Radical scavenging ability

The overall antioxidant capacity tests as electron donating ability (EDA) was determined by the DPPH assay according to Fayaz *et al.* (2005) [30] – figure 2 (A,B). EDA in KJ samples from clean area at of 0 h (KJ-0) was 52.44% i.e. lower compared to polluted one (OS-0), where it was 71.80%. These values continuously decreased to 31.63% and 48.25% until 24 hours in KJ and OS samples, respectively. After irradiation in KJ it increased to 67.99% (KJ-50, 6 h) and 68.83% (KJ-100, 6 h) from 59.98%

(KJ-50, 0 h) and 62.88% (KJ-100, 0 h), respectively. The EDA of the samples from OS continuously decreased to 71.11% (OS-50, 24 h) and 70.01% (OS-100, 24 h) from 75.99% (OS-50, 0 h) and 77.67%(OS-100, 0 h), respectively. There was only a slight change from 0 h to 24 h after irradiation of the samples from OS.

Antioxidant enzyme assays

Superoxide dismutase activity

Similarly, the SOD activity (figure 2 C, D) was higher in OS samples from polluted area - 2119 U mg^{-1} protein (OS-0, 0 h) and increased after irradiation. With time this activity declined. In KJ leaves SOD activity was lower at KJ-0 - 1360 U mg^{-1} protein. From 0 h to 6 h after irradiation, the curves of the two areas were totally different. In the samples from KJ, the values rapidly increased from 635 (KJ-50, 0 h) and 1012 (KJ-100, 0 h) up to 1394 (KJ-50, 6 h) and 1721 (KJ-100, 6 h). And, from 6 hours to 24 hours after irradiation, the value rapidly decreased down to 756 (KJ-50, 24 h) and 676 (KJ-100, 24 h). In the samples from OS, the values decreased from 2686 (OS-50, 0 h) and 2312 (OS-100, 0 h) down to 1518 (OS-50, 3 h) and 1702 (OS-100, 3 h). These values slightly went up until 6 h after irradiation. And from 6 h to 24 h after irradiation, the value decreased down to 1084 (OS-50, 24 h) and 1144 (OS-100, 24 h).

Catalase activity

CAT activity (figure 2 E,F), in samples from the industrial area OS-0 was 5.02 U mg^{-1} protein, higher than that in the samples from KJ, where it was 2.42 U mg^{-1} protein. The activity, however, declined with time. From 0 h to 6 h after irradiation, the curves of the samples were totally different according to the area. In KJ, the values rapidly increased from 2.20 (KJ-50, 0 h) and 1.43 (KJ-100, 0 h) up to 4.07 (KJ-50, 6 h) and 4.51 (KJ-100, 6 h). And, from 6 hours to 24 hours after irradiation, the value rapidly decreased down to 1.59 (KJ-50, 24 h) and 1.91 (KJ-100, 24 h). In the samples from OS, the values decreased from 6.81 (OS-50, 0 h) and 5.95 (OS-100, 0 h) down to 1.68 (OS-50, 10 h) and 1.40 (OS-100, 10 h). From 10 h to 24 h after irradiation, the value slightly increased up to 2.58 (OS-50, 24 h) and 2.26 (OS-100, 24 h).

Glutathione reductase activity

GR activity (figure 2 G, H) did not essentially differ between areas of leaf origin. It was 6.78 U

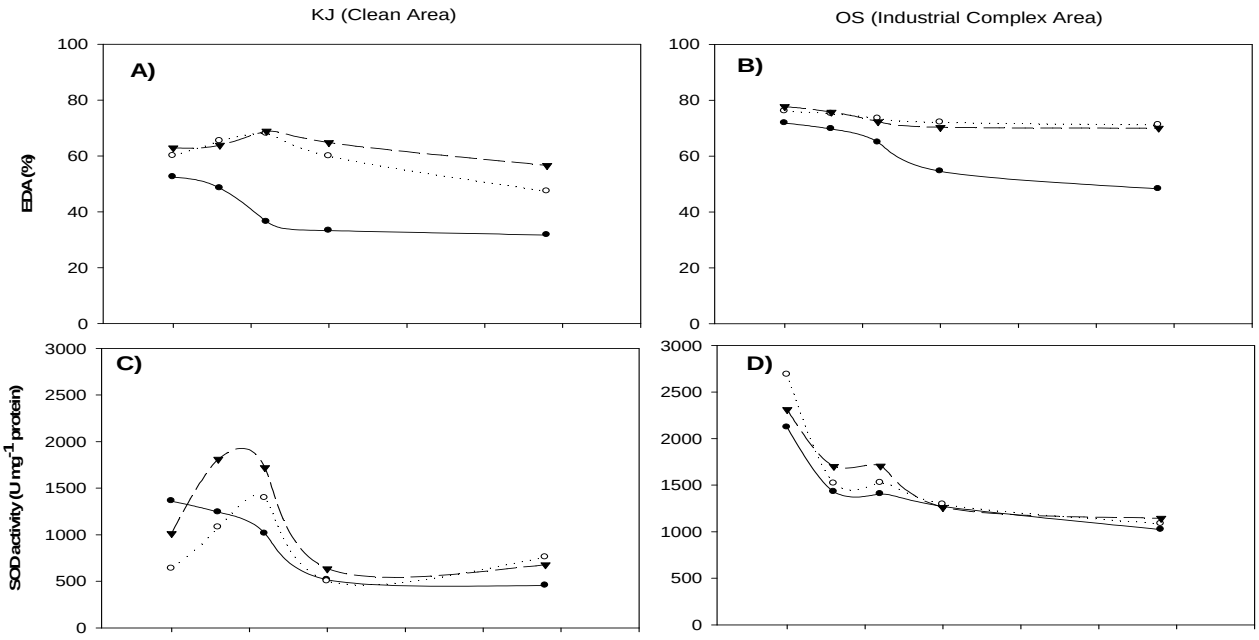
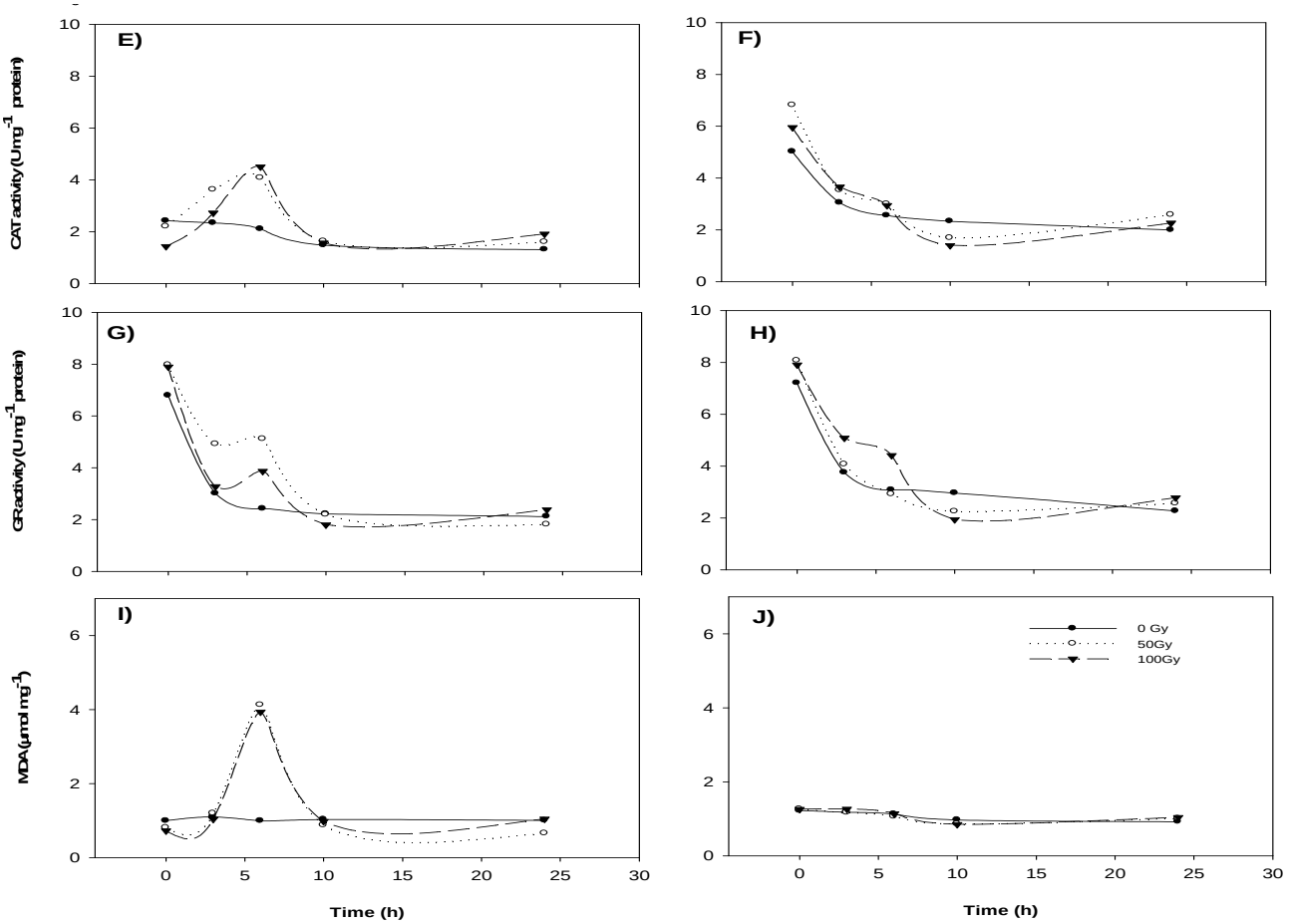


Figure 2 (Continued on next page). Curves of % EDA corresponding to DPPH scavenge ability (A,B), antioxidant enzymes activities of SOD (C,D), CAT (E,F) and GR (G,H) and MDA contents changes (I,J)



Continuation of figure 2. for doses during the post-irradiation period in KJ and OS. As the statistical errors were very small, average values were used to indicate the tendency of changes with time after irradiation in all panels.

mg^{-1}protein (KJ-0, 0 h) and 7.02 (OS-0, 0 h), respectively. From 0 h to 3 h after irradiation, the plots of the two areas were in a similarly decreasing pattern. In the sample from KJ, the values increased from 4.91 (KJ-50, 3 h) and 3.27 (KJ-100, 3 h) up to 5.11 (KJ-50, 6 h) and 3.87 (KJ-100, 6 h). And, from 6 hours to 24 hours after irradiation, the value decreased down to 1.80 (KJ -50, 24 h) and 2.38 (KJ-100, 24 h). In the samples from OS , the values decreased from 4.07 (OS-50, 3 h) and 5.08 (OS-100, 3 h) down to 2.24(OS-50, 10 h) and 1.94 (OS-100, 10 h). These values increased slightly after from 10 h to 24 h after irradiation with both 50 and 100 Gy. Non-irradiated plants from both areas had a similar starting point and the pattern of the plot from 0 h to 24 h was similar, as well. Plants from KJ of 6 h showed a slight increase compared to the plants from OS.

DISCUSSION

In spite of many investigations related to biomonitoring environmental impacts, the technique using γ radiation with biochemical changes of plant cells has not been introduced. Our results demonstrated that the radiation-induced differences in the activation of antioxidant enzymes, SOD, CAT and GR and lipid peroxidation in *E. japonica* could be used for the environmental monitoring purpose. In biochemistry, antioxidants are defined as enzymes or other organic substances that are capable of counteracting the damaging effects of oxidation [35]. ROS are produced continuously as byproducts of various metabolic pathways in plants. Under steady state conditions, the ROS are kept in balance by various antioxidative

defence mechanisms [36]. The disturbances in equilibrium between the production and the scavenging of ROS lead to a sudden increase in their intracellular levels which can cause significant damage to cell structures. ROS also act as a signalling factor and are able to initiate responses such as expression of new genes. Stress-induced ROS accumulation is counteracted by enzymatic antioxidant systems that include a variety of scavengers, such as SOD, APX, GPX, CAT and GR and non-enzymatic substances such as ASH, GSH, carotenoids and flavonoids [37-41]. The antioxidant capacity, ROS scavenging ability was examined by the DPPH assay. DPPH is a well-known stable free-radical molecule and a trap ("scavenger") for other radicals [42]. Having a higher EDA means that the plants may contains more antioxidant compounds. The components that influence the EDA are both enzymatic and non-enzymatic antioxidant components. The response of antioxidant enzymes activities to imposed stress depends on the duration, nature and strength of a stress imposed. The enzymes whose activities increase during stress treatment may play an important role in defence against a particular stress [43]. We investigated the responses of antioxidant enzymes including SOD, CAT and GR. To control the level of ROS and protect the cells upon exposure to the first stress of air pollutant and second stress of ionizing radiation, plants usually show changes of the concentration of enzymes such as SOD, CAT and GR.

Comparison of antioxidant capacity in KJ-0 and OS-0

When comparing the EDA of the 0 Gy plants between two areas, OS-0 was higher than KJ-0. Also activities of SOD and CAT of 0 Gy plants from the industrial area were higher so that plants which have been adapted themselves to the polluted air have higher ROS scavenging ability. These results suggest that the antioxidant capacity of the same plant species from two different environmental conditions differ due to different tolerance evolved from their air environment of habitat. In plants from OS higher antioxidant capacity was induced to overcome the frequent and strong ROS over-production induced by air pollutant than those in the clean area such as KJ. As the ROS and enzyme activities of the plant were affected by other environmental stresses such as temperature, irradiation intensity and water stress, the value of EDA and enzymes of the non-irradiated samples continuously changed. However, the result of LPO indicated that cell membrane stability was not depending on time lapse. MDA content for measuring LPO was not increased in plants from OS area due to their induced antioxidant defence.

Comparison of antioxidant capacity in non-irradiated (KJ-0) and irradiated (KJ-50 and KJ-100)

Gamma irradiation induced the increase in EDA of KJ after 6 h. The SOD and CAT activities were also stimulated with a peak 6 h after irradiation, also the GR activity showed increase at 6 h after irradiation but only a slight one. Increased MDA contents 6 h after irradiation indicated that the membrane lipids were more vulnerable to an attack from radicals induced by irradiation. They probably promoted subsequently transient increase in antioxidant enzyme activities as well as EDA.

Comparison of antioxidant capacity in non-irradiated (OS-0) and irradiated (OS-50 and OS-100)

It was not a peak in irradiated samples from OS area. Similarly, plant irradiation did not show marked maximum in activities of antioxidant enzymes 6 h after irradiation but they had higher activities right after irradiation. And there was no significant difference between the non-irradiated and irradiated plants except the activation just after irradiation. Such antioxidant defence was strong enough as MDA contents was not elevated after irradiation.

Comparison of antioxidant capacity in irradiated (50 and 100 Gy) plants from KJ and OS

The EDA of the irradiated plants from OS ranged from 70.01% to 77.67%, while the plants from KJ ranged from 47.26% to 68.83%. The

higher EDA of the OS samples meant higher antioxidant capacity which was activated by IR. A peak in the EDA of the KJ sample was observed between 6 h after irradiation, whereas there was no such tendency in the plants from OS. The plants from OS may be hardened in the adverse growing conditions and consequently they posses higher antioxidant defence in comparison with the plants from KJ.

The SOD and CAT activities in KJ plants were strongly stimulated when exposed to irradiation as they have maximum at 6 h in case of 50 Gy and 100 Gy. These activities in plants from the OS continuously decreased with time and the decreasing tendency was similar to that of the 0 Gy irradiated sample. Such stimulation of the activities in the samples from KJ after irradiation could indicate that ROS not removed by established antioxidant capacity react as signalling factor [41]. It is able to initiate responses of antioxidant enzymes, namely SOD and CAT. Over-expressing scavenging enzymes such as SOD and CAT have been engineered with the goal of increasing stress tolerance. On the contrary, SOD and CAT activities were increased only slightly but immediately after irradiation in the OS samples. This result indicated that, ROS generated by irradiation were removed by established antioxidant capacity of adapted plants so that they didn't activate the antioxidant system any more. Thus, chronic exposure to ROS stress generating agents such as air pollutants increased its resistance to a subsequent irradiation.

GR and GSH have been suggested to play a crucial role in determining the tolerance of a plant under various stresses [44]. Lowered GR activity in the irradiated plants and no prominent difference under low light seedling conditions have been reported, as well [45,46]. The difference of GR activities between the plants from KJ and OS without irradiation was not as significant as being able to classify. There was no correlation between GR activity and radiation dose in *E. japonica.* Only MDA content of the irradiated plants from KJ sharply increased whilst in samples from remained unaltered after irradiation.

CONCLUSION

As found in this study, air pollution-stressed plants showed better adaptation to ensuing ionizing radiation (IR) stress. The plant from Onsan didn't show any changes of antioxidant enzymes (SOD, CAT, and GR) and LPO after irradiation with 50 Gy and 100 Gy of IR due to their enhanced antioxidant capacity. Until 6 hours after irradiation, *Euonymus japonica* from Kijang showed an apparent increase in the enzymes activities and the level of LPO. These adaptive plants in the air polluted (Onsan) area are less sensitive to second oxidative stress such as gamma rays, and possess an increased resistance to IR, compared to the samples taken from the clean area. The enzymatic differences in plants irradiated with gamma rays can be used as biochemical end-points for environmental monitoring.

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