

Photosynthetic Capacity of *Arabidopsis* Plants at the Reproductive Stage Tolerates γ Irradiation

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The developmental stage has an influence on the overall responses of plants under biotic or abiotic stress conditions. However, there is a lack of data about the effects of ionizing radiation in plants at different developmental stages. We examined radiation sensitivity of *Arabidopsis* plants in terms of photosynthetic ability and oxidative stress resistance at two distinct vegetative and reproductive stages, which correspond to 23 and 43 d after seeding (DAS), respectively. When plants were exposed to γ rays at a dose rate 50 Gy h⁻¹ for 4 h, they were characterized as various common or differential cellular responses depending on the developmental stage. Radial expansion of leaves, inhibition of non-photochemical quenching, and production of $\cdot\text{O}_2^-$ and H_2O_2 under methyl viologen-induced photooxidative stress were commonly more conspicuous in the irradiated leaves of both plants than in the respective control. In contrast, the 23 and 43-DAS plants were explicitly discriminated in growth, chloroplast number & ultrastructure, photosynthetic pigment content & activity, and protein damage after γ irradiation. Natural leaf senescence was thereby enhanced in the irradiated leaves of the 23-DAS plants, while it was reversely alleviated in those of the 43-DAS ones. These results suggest that photosynthetic machineries of *Arabidopsis* plants at the reproductive stage can be relatively tolerant to γ rays of 200 Gy.

INTRODUCTION

Ionizing radiation causes various phenotypic aberrations in plants due to genetic alterations as well as biochemical and physiological disorders.^{1–6)} For instance, growth inhibition, chlorophyll degradation, anthocyanin accumulation, cell death, survival rate reduction and/or morphological anomaly have been observed in plants after treatment with γ rays.^{2,4,7–9)} However, whether even lethal doses of ionizing radiation accelerate or delay senescence (or programmed cell death) in plants may depend on plant species and developmental stage.^{6,10)}

Complexity is a hallmark of underlying mechanisms responsible for phenotypic aberrations of plants after exposure to ionizing radiation. Ionizing radiation induces genetic or epigenetic alterations through the error-prone repair mechanisms of DNA damages such as double strand breaks or the structural changes of chromatin such as DNA hypomethylation.^{11–13)} As well as these alterations in plant genomes, direct interactions between ionizing radiation and macromolecular structures and/or indirect actions of the reactive oxygen species (ROS) generated from water radiolysis contribute to unique phenotypes of plants.⁷⁾ Due to this complexity, different sets of the underlying mechanisms affected by ionizing radiation can be associated with the same or different phenotypes of plants, depending on plant species and developmental stage.^{3,5–7)} For instance, when *Arabidopsis* plants were exposed to γ rays at the reproductive stage, they showed various metabolic disturbances linked to delay in leaf senescence.⁶⁾ Transition of the vegetative to the reproductive stage seems to differentiate cellular responses of plants to ionizing radiation. However, common or differential cellular responses of plants to ionizing radiation have been rarely documented at least in terms of plant species and developmental stage, except for a few previous reports dealing with involvement of peroxidase in different sensitivity of two *Nicotiana* species to γ radiation²⁾ or radiation-induced

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trichome formation and gene expression pattern of different *Arabidopsis* strains.^{3,7)} Especially, there is quite a lack of data about effects of ionizing radiation in plants at different developmental stages.

A few recent studies about effects of γ rays on photosynthesis have shown that a photosystem defense mechanism, thermal energy dissipation of absorbed excess light in photosystems, which is called non-photochemical quenching (NPQ) of chlorophyll fluorescence, could be substantially suppressed in *Capsicum annuum*, *Brassica campestris*, *Cucumis sativus*, *Lycopersicon esculentum*, *Lactuca sativa* and *Arabidopsis thaliana* leaves after treatment with γ rays.^{5,6,14–16)} Moreover, photosynthetic pigments, e.g., chlorophyll *a*, *b*, and various carotenoids including xanthophyll cycle pigments responsible for the NPQ, were noticeably altered in their relative contents, suggesting a meaningful change in photosynthetic capacity.

Therefore, in the present study, we attempted to characterize γ -ray-induced cellular responses of *Arabidopsis* plants at the vegetative or reproductive stage by analyzing common or differential changes in photosynthetic ability and oxidative stress resistance. Here, we report that photosynthetic machineries of *Arabidopsis* plants at the late reproductive stage can be tolerated to γ rays of 200 Gy due to some changes in oxidative stress resistance.

MATERIALS AND METHODS

Plant material and γ irradiation

Arabidopsis thaliana ecotype Columbia (Col-0) plants were grown in a growth chamber with a pot-level photosynthetic photon flux density (PPFD) of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a 16-h photoperiod at 22/18°C (day/night) for 23 or 43 d after seeding (DAS) in a compound soil mixture (vermiculite:peat moss:perlite = 1:1:1). Irradiation of plants was carried out at a dose rate of 50 Gy h^{-1} for 4 h using a γ irradiator (⁶⁰Co, ca. 150 TBq of capacity; Atomic Energy of Canada Limited, Canada) at the Korea Atomic Energy Research Institute. Thereafter, the plants were placed under the growth condition. The whole rosette leaves except emerging ones were detached at 9, 13 or 16 d after the irradiation and three or four largest leaves in order of size were used for all analyses.

Microscopic analysis

Leaves were cut into 1-mm² segments and placed immediately in a freshly prepared mixture of 2% (w/v) glutaraldehyde and 4% (w/v) paraformaldehyde in 50 mM sodium cacodylate buffer (pH 7.4). Subsequent fixation and resin embedding were carried out as described previously.¹⁷⁾ For the light microscopy, semi-thin sections (1 μm) were stained with 1% toluidine blue and examined in a light microscope (TE300; Nikon, Japan). For the transmission electron microscopy, ultra-thin sections (70–90 nm) were obtained using an ultramicrotome (MT-7000; RMC, USA), mounted

on uncoated nickel grids (300 mesh), stained with uranyl acetate followed by lead citrate, and observed in a transmission electron microscope (J-1010; Jeol, Japan) at 80 kV.

Chlorophyll fluorescence analysis

Chlorophyll fluorescence was measured using the IMAGING-PAM fluorometer (Walz, Effeltrich, Germany) as described previously.¹⁴⁾ Readings were taken after 5-mm diameter leaf disks were dark-adapted for 15 min at room temperature. Chlorophyll fluorescence and quenching parameters, e.g., Fv/Fm, ETR, qP and NPQ, were calculated as described by Moon *et al.*¹⁶⁾ The maximal electron transport rate, ETR_{max}, was obtained from the relative ETR vs. PPFD curve as reported previously.¹⁸⁾

Chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant physiologists and ecophysicists.¹⁹⁾ The principle underlying chlorophyll fluorescence analysis is relatively straightforward. Light energy absorbed by chlorophyll molecules in a leaf can be used primarily to drive photochemistry, or it can be dissipated as heat or re-emitted as light - chlorophyll fluorescence. These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two. Therefore, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat (or thermal energy) dissipation can be gained. From the enormous biophysical and physiological data obtained for almost eighty years, the above parameters of chlorophyll fluorescence and quenching represent the physiological status of photosynthetic machineries as follows: Fv/Fm, maximal photochemical efficiency of photosystem II; qP, photochemical quenching (or photochemistry-dependent decrease) of chlorophyll fluorescence; NPQ, non-photochemical quenching (or non-photochemistry, mainly thermal dissipation,-dependent decrease of chlorophyll fluorescence), and ETR, apparent photosynthetic electron transport rate through photosystem II.

Pigment analysis

Separation of photosynthetic pigments was carried out in a HPLC system (Waters, Milford, MA) on a Spherisorb ODS-1 column (Alltech, Deerfield, IL), as previously described by Kim *et al.*¹⁴⁾ Concentrations of the pigments were estimated by using the conversion factors for the peak area to nanomoles, as determined by Gilmore and Yamamoto.²⁰⁾

Oxidative damage analysis of lipids and proteins

Lipid peroxidation was determined by the malondialdehyde (MDA) content from the thiobarbituric acid (TBA) method, according to Dhindsa and Matowe²¹⁾ and Borges *et al.*²²⁾ with some modifications. Leaves (about 250 mg) were ground to fine powder with liquid nitrogen using a Mixer Mill (MM301, Retsch GmbH, Haan, Germany). MDA

extraction was performed by vigorously vortexing the powder in 2 ml of 0.1% trichloroacetic acid (TCA) for 5 min and followed by centrifugation at 10,000 g for 5 min. The supernatant was collected and mixed at a ratio of 1:4 with a solution of 20% TCA and 0.5% thiobarbituric acid. The mixture was incubated at 95°C for 30 min and quickly cooled on ice. Then the sample was centrifuged at 10,000 g for 10 min. The absorbance of the supernatant was measured at 535 nm and corrected by subtracting the non-specific absorbance at 600 nm due to the limited specificity of the method. The concentration of the TBA reactive species (TBARS) was calculated by using the extinction coefficient

of $155 \text{ mM}^{-1} \text{ cm}^{-1}$, and results were expressed as nmol TBARS per gram FW.

Protein damage was estimated by measuring derivatization of protein carbonyls with 2,4-dinitrophenyl-hydrazine as described by Levine *et al.*²³⁾ and Matamoros *et al.*²⁴⁾ with some modifications. Leaf soluble proteins were extracted by vigorously vortexing the powder (as stated above) for 5 min with 2 ml of 100 mM potassium phosphate (pH 7.0), 0.1% (v/v) Triton X-100, 1 mM Na_2EDTA , and 2.5 μg each of aprotinin and leupeptin to prevent proteolysis of oxidized proteins during sample preparation. After precipitation of possible contaminating nucleic acids in the samples with 1%

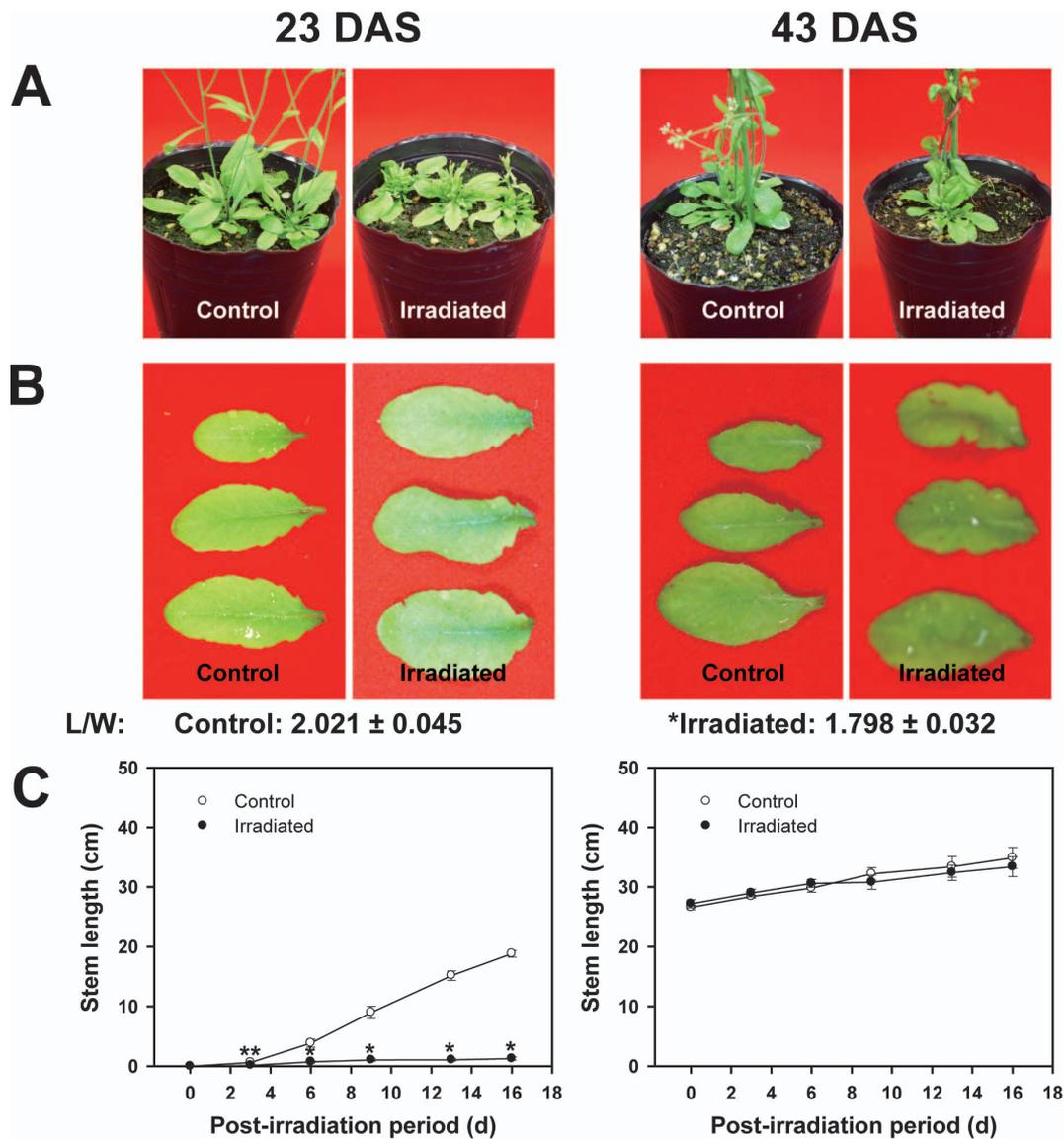


Fig. 1. Phenotypes of the control and irradiated plants. Irradiated plants were exposed to γ rays with a dose rate of 50 Gy h^{-1} for 4 h at 23 or 43 DAS. Pictures were taken at 9 (A) or 16 d (B) after γ irradiation. Digits in B represent means \pm SE ($n = 12$ from four different experiments) for the length/width (L/W) ratios of leaves from the 23–43-DAS plants. In C, error bars represent means \pm SE ($5 \leq n \leq 15$). Asterisks indicate statistically significant differences between the control and irradiated plants, as determined by the Student's *t*-test (*, $P < 0.01$; **, $P < 0.05$).

(w/v) streptomycin sulfate, an aliquot of 0.8 ml of the extracts was reacted with 0.2 ml of 20 mM dinitrophenylhydrazine (in 2 M HCl), and another aliquot (control) with 0.2 ml of 2 M HCl for 1 h, with vigorous shaking every 10 to 15 min. Proteins were then precipitated with 10% (w/v) TCA, and the pellet was washed four times with 1:1 (v/v) ethanol:ethyl acetate. Precipitated proteins were solubilized in 6 M guanidine-HCl (pH 4.5) by incubation for 30 min with shaking. The insoluble material was removed by centrifugation, and the absorbance of the hydrazones (derivatized carbonyls) was measured at 370 nm.²³⁾

Detection of reactive oxygen species

Production of superoxide ($\cdot\text{O}_2^-$) or hydrogen peroxide (H_2O_2) in leaves under a photooxidative stress condition was visualized by a dark blue insoluble formazan compound of nitroblue tetrazolium (NBT) or a deep brown polymerization product of 3,3'-diaminobenzidine (DAB) as previously reported by Fryer *et al.*²⁵⁾ Intact leaves or 1-cm leaf disks were floated on a solution of 50 μM methyl viologen with 6 mM NBT (pH 7.0) or 5 mM DAB (pH 3.8) at room temperature under vacuum and dim light for 2 h. Then, they were placed at 22°C for 3 h under a PPFD of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to induce photooxidative stress. Prior to imaging, photosynthetic pigments were removed from the leaves by boiling in a bleaching solution (lactic acid:glycerol:ethanol = 1:1:4) for 5 min. All images were presented without correction for the background.

RESULTS

Phenotypes of *Arabidopsis* plants after γ irradiation

Arabidopsis plants have a life cycle of about 6–8 weeks from germination to mature seeds. Their inflorescence stems for reproduction starts to emerge from 4 weeks after seeds were sown. In the present study, two different developmental stages, 23 and 43 d after seeding (DAS), represent a late vegetative and a late reproductive stages before and after emergence of inflorescence stems, respectively. When plants at 23 or 43 DAS were irradiated with γ rays at a dose rate 50 Gy h^{-1} for 4 h, they showed distinguishable phenotypic changes (Fig. 1). Irradiated plants of 23 DAS were the best characterized of stunted growth and pale green leaf color during the post-irradiation period of 16 d, while those of 43 DAS remained nearly unaffected in appearance. Inflorescence stems were rarely observed in the irradiated plants of 23 DAS, but almost normally developed in those of 43 DAS. These results may support the well-known inhibitory effect of γ rays on fast-growing tissues or dividing cells, which can be more conspicuous in the 23-DAS plants than in the 43-DAS ones. However, the length/width (L/W) ratios of leaves were significantly lower in the irradiated plants of 23–43 DAS than in the control, indicating γ -ray-induced radial expansion by the more inhibitory effect on

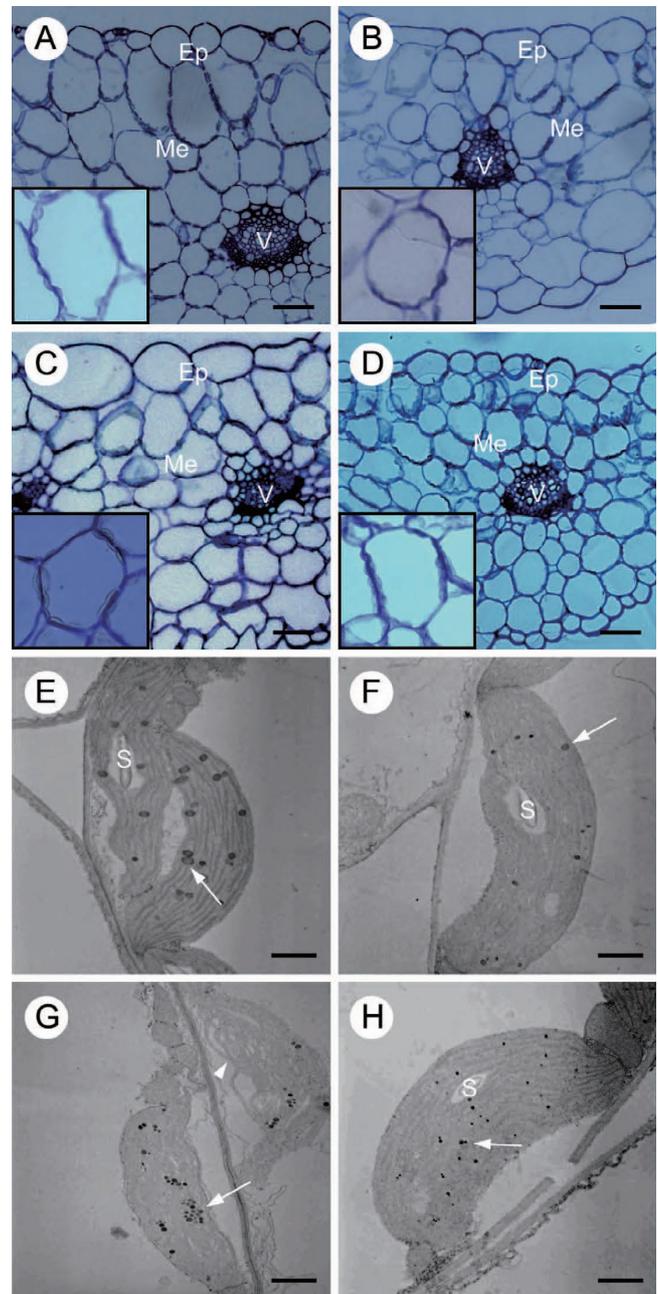


Fig. 2. Microscopic analysis of mesophyll cells and chloroplasts in the control (A, C, E and G) and irradiated (B, D, F and H) leaves. All leaves were harvested at 16 d after γ irradiation of 50 Gy h^{-1} for 4 h at 23 (A, B, E and F) or 43 DAS (C, D, G and H). A–D, Light microphotographs of transverse sections from leaves. Chloroplasts were placed along with cell membranes. Images in black boxes represent a magnification of the respective mesophyll cell. Ep, epidermis; Me, mesophyll; and V, vascular bundle. Scale bar = 100 μm . E–H, Electron micrographs showing chloroplast ultrastructures in mesophyll cells. Symbols (S) and arrows indicate starch granules and plastoglobuli, respectively. Scale bar = 1 μm .

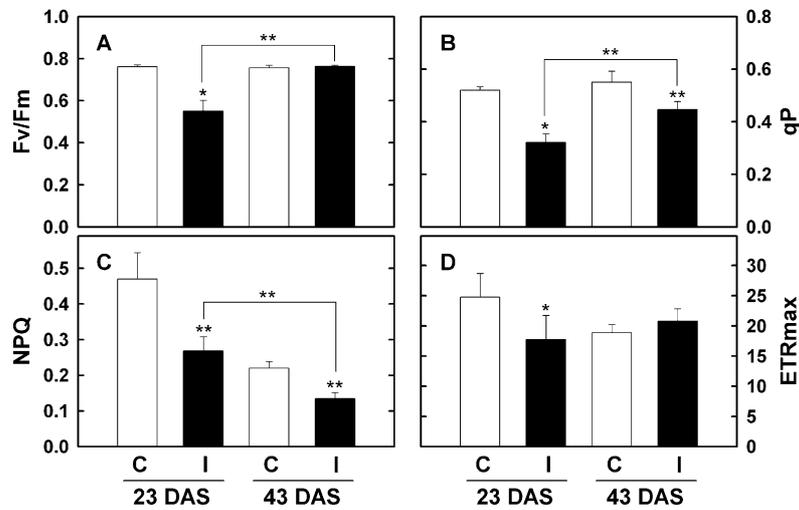


Fig. 3. Chlorophyll fluorescence parameters of the control and irradiated leaves. Fv/Fm, the maximal photochemical capacity of photosystem II; ETRmax, the maximal electron transport rate; and qP and NPQ, the parameters for photochemical quenching and non-photochemical quenching, respectively. C, control; and I, irradiated. More than five leaves excised from five to ten different plants were used for chlorophyll fluorescence analysis of each experimental group. All measurements were performed at 16 d after γ irradiation of 50 Gy h⁻¹ for 4 h at 23 or 43 DAS, except for ETRmax at both 13 and 16 d. Error bars represent means \pm SE (5 \leq n \leq 10 measurements from one or two different experiments). Asterisks indicate statistically significant differences between the control and irradiated leaves or between the 23 and 43-DAS irradiated leaves, as determined by the Student's *t*-test (*, $P < 0.01$; **, $P < 0.05$).

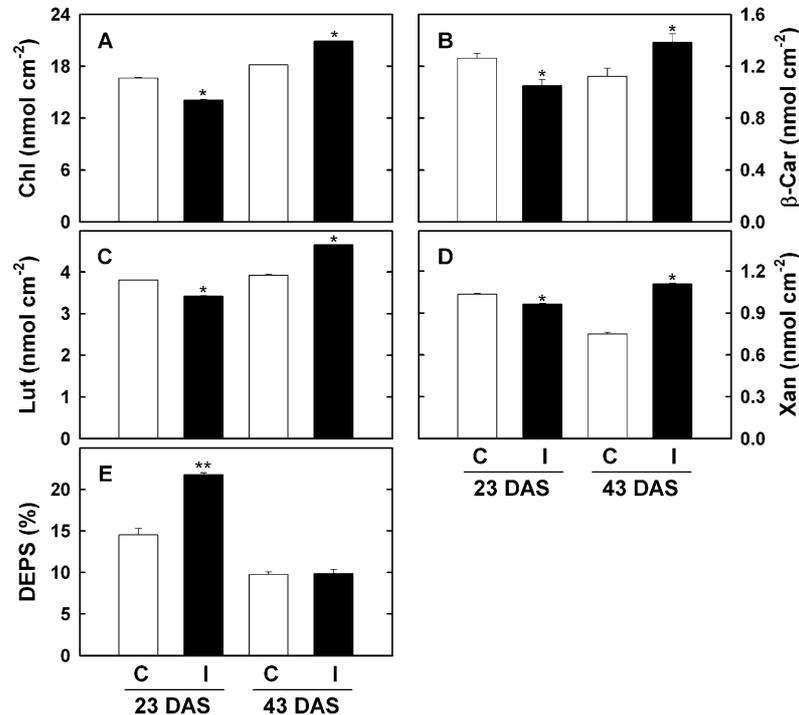


Fig. 4. Photosynthetic pigments of the control and irradiated leaves. All leaves were harvested at 16 d after γ irradiation of 50 Gy h⁻¹ for 4 h at 23 or 43 DAS. Chl, chlorophyll; β -Car, β -carotene; Lut, lutein; and Xan, violaxanthin (Vx) plus antheraxanthin (Ax) plus zeaxanthin (Zx). DEPS represents de-epoxidation state of xanthophyll cycle pigments, which was calculated as a percentage of (Zx + 0.5 Ax)/(Vx + Ax + Zx). C, control; and I, irradiated. Error bars represent means \pm SE (n = 3 measurements from three different experiments). Each measurement was performed using a total extract of five 5-mm diameter leaf disks from five different plants. Asterisks indicate statistically significant differences between the control and irradiated leaves, as determined by the Student's *t*-test (*, $P < 0.01$; **, $P < 0.05$).

the longitudinal growth of leaves.

Comparison of mesophyll cells and chloroplasts in leaves between the control and irradiated plants

Microscopic images of mesophyll cells and chloroplasts were obtained from rosette leaves of the control and irradiated plants (Fig. 2). Number of chloroplasts per cell was approximately 30% less in the irradiated leaves of the 23-DAS plants than in the control, partly contributing to the pale green leaf color (Fig. 2A and B). In contrast, those of the 43-DAS plants exhibited about 24% more chloroplasts per cell than did the control (Fig. 2C and D). Thylakoid membranes were also affected differently between the irradiated leaves of the 23 and 43-DAS plants. Compared to the respective control, the former showed less stacking of thylakoid membranes, while the latter maintained relatively intact structures of the membranes (Fig. 2E–H). In the control leaves of the 43-DAS plants, disintegration of thylakoid membranes was noticeably observed, indicating a progress of leaf senescence (Fig. 2G arrow head).

Change in photosynthetic capacity and pigment content of leaves after γ irradiation

To further delineate the relative functional contributions of the distinct structural changes in photosynthetic organelles in the control and irradiated leaves, we investigated photosynthetic activities in both leaves of the 23 and 43-DAS plants (Fig. 3). The maximal photochemical efficiency of photosystem II, F_v/F_m , decreased in the irradiated leaves of the 23-DAS plants, while it remained constant in those of the 43-DAS ones. Similarly, the maximal electron transport rate of photosynthesis, ETR_{max} , was about 28% lower in the irradiated leaves of the 23-DAS plants than in the control, indicating no significant difference in the 43-DAS plants. The parameter for photochemical quenching, qP , was less affected in the 43-DAS plants after γ irradiation than in the 23-DAS ones. In contrast, the parameter for non-photochemical quenching of chlorophyll fluorescence, NPQ, was significantly lower in the irradiated leaves of the 23 and 43-DAS plants than in the respective control. This decrease in NPQ after γ irradiation has been also observed in *Arabidopsis* plants at a different developmental stage,^{6,16} thereby representing a general response of irradiated plants.

A substantial change in contents of photosynthetic pigments can cause structural and functional defects in photosynthetic machineries.²⁶ Accordingly, as expected from the microscopic images and chlorophyll fluorescence parameters, the total content of chlorophyll, β -carotene, lutein, and xanthophyll cycle pigments such as violaxanthin, antheraxanthin, and zeaxanthin, was found to be substantially lower in the irradiated leaves of the 23-DAS plants than in the control, while the opposite was true for the 43-DAS plants (Fig. 4). NPQ, a photosystem defense mechanism, consists of a rapidly reversible qE and a slowly reversible qI ,

which are xanthophyll cycle-dependent and -independent components, respectively.²⁷ The former qE is the main portion of NPQ, which depends on de-epoxidation of xanthophyll cycle pigments.²⁸ However, the higher de-epoxidation state of xanthophyll cycle pigments in the irradiated leaves of the 23-DAS was not correlated with the lower NPQ values in them, suggesting that the xanthophyll cycle-independent NPQ would be mainly suppressed in the 23-DAS plants after γ irradiation (Fig. 4E).

Change in lipid peroxide and protein carbonyl contents of leaves after γ irradiation

Oxidative damage to lipids and/or proteins in plants has been reported to increase in response to various stress conditions, correlating with reduction in photosynthesis and disruption of thylakoid membranes.^{22,24,29,30} Therefore, we compared levels of lipid peroxides and protein carbonyls in the control and irradiated leaves of the 23 or 43-DAS plants, which revealed distinct differences in the chloroplast ultrastructures and photosynthetic activities. Although the level of lipid peroxidation was much higher in the 43-DAS plants than in the 23-DAS ones, it was only slightly affected in the irradiated leaves of both plants compared to the respective control (Fig. 5A). In contrast, the level of protein carbonyls

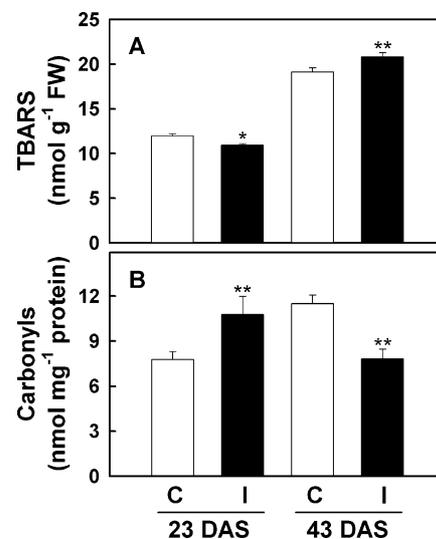


Fig. 5. Lipid peroxide and protein carbonyl contents in the control and irradiated leaves. All leaves were harvested at 9 and 16 d after γ irradiation of 50 Gy h⁻¹ for 4 h at 23 or 43 DAS. In A, lipid peroxide contents were expressed as a concentration of thiobarbituric acid-reactive species (TBARS) representing the malondialdehyde (MDA) content. In B, protein carbonyl (or oxidized protein) contents were expressed as a concentration of derivatized carbonyls formed from the reaction with 2,4-dinitrophenyl-hydrazine. C, control; and I, irradiated. Error bars represent means \pm SE ($n = 6$ measurements from two different experiments). Asterisks indicate statistically significant differences between the control and irradiated leaves, as determined by the Student's *t*-test (*, $P < 0.01$; **, $P < 0.05$).

representing oxidized proteins was significantly higher in the irradiated leaves of the 23-DAS plants than in the control, while the opposite was true for the 43-DAS plants (Fig. 5B). These results suggest that the degree of protein damage should be closely correlated with changes in the chloroplast ultrastructures and photosynthetic activities of the 23 or 43-DAS plants after γ irradiation.

Production of reactive oxygen species in leaves of the control and irradiated plants under photooxidative stress

Oxidative damage to lipids and proteins are generally induced by reactive oxygen species (ROS), e.g., $^1\text{O}_2$, $\cdot\text{O}_2^-$ or, H_2O_2 , and $\cdot\text{OH}$.³¹⁾ Methyl viologen (MV), which has been often used as an ideal chemical to study photooxidative stress, induces photoreduction of dioxygen (O_2) by accept-

ing electrons from the iron-sulfur clusters of photosystem I, and thereby accelerates production of $\cdot\text{O}_2^-$ and H_2O_2 .³²⁾ Therefore, production rates of $\cdot\text{O}_2^-$ and H_2O_2 during photooxidative treatment of MV were compared between the control and irradiated leaves of the 23 or 43-DAS plants (Fig. 6). These ROS were commonly more produced in the irradiated leaves of both plants than in the respective control, being much more distinguishable between the control and irradiated leaves of the 23-DAS plants. Considering that MV-mediated ROS production in chloroplasts depends on the photosynthetic activity,³²⁾ these results suggest that antioxidant systems to detoxify $\cdot\text{O}_2^-$ and H_2O_2 might be substantially or partially suppressed in the irradiated leaves of the 23 or 43-DAS plants, respectively.

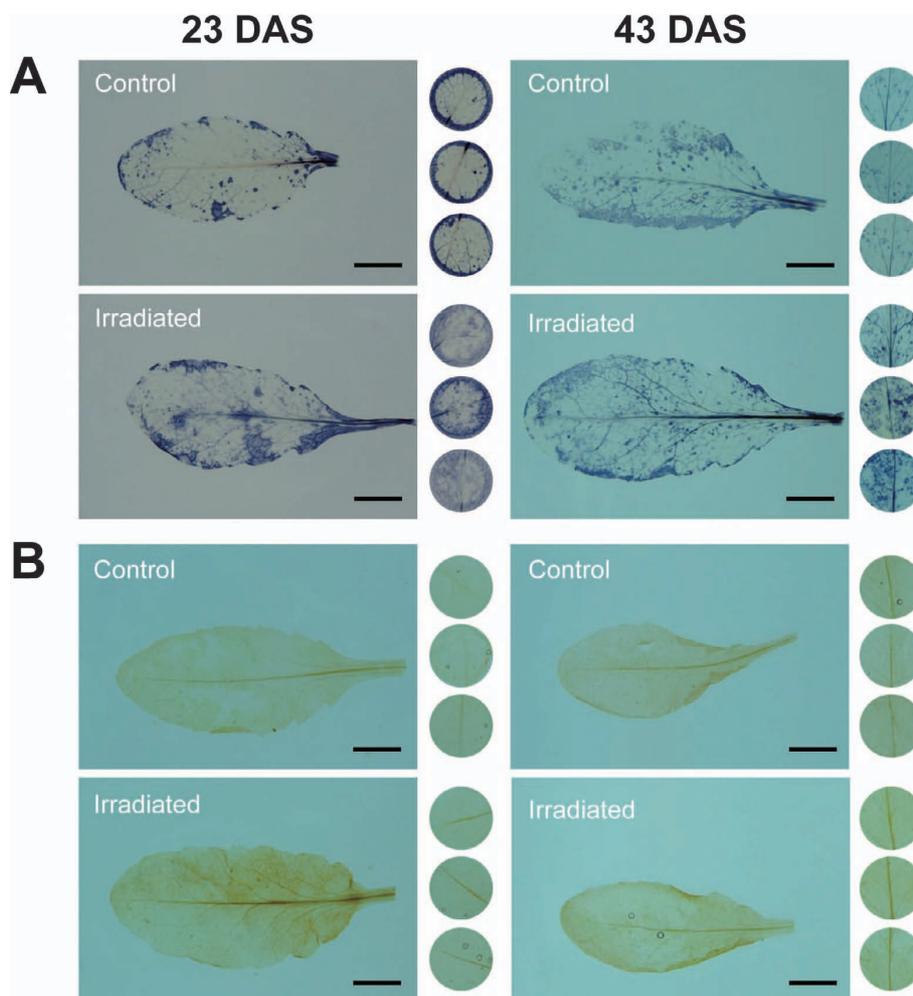


Fig. 6. $\cdot\text{O}_2^-$ and H_2O_2 production in the control and irradiated leaves under photooxidative stress. All leaves were harvested at 16 d after γ irradiation of 50 Gy h^{-1} for 4 h at 23 or 43 DAS. To induce photooxidative stress, intact leaves or 1-cm leaf disks were floated on a solution of $50 \mu\text{M}$ methyl viologen at 22°C for 3 h under a PPFD of $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$. In A or B, $\cdot\text{O}_2^-$ or H_2O_2 production was visualized by a dark blue insoluble formazan compound of nitroblue tetrazolium (NBT) or a deep brown polymerization product of 3,3'-diaminobenzidine (DAB), respectively. Scale bar = 0.5 cm.

DISCUSSION

Generally, massive doses (100–3,000 Gy) of γ rays have been found to cause growth inhibition, chlorophyll degradation, and/or morphological abnormality in various plant species, e.g., *Nicotiana* and *Arabidopsis*.^{2,6,7)} However, these phenotypic alterations and underlying cellular responses may differ in plants of different developmental stages. Following the conversion from the vegetative to the reproductive stage, *Arabidopsis* plants start to senesce and suffer from degradation of photosynthetic pigments, and finally death of their leaves as a functional unit is associated with completion of the reproductive stage.^{33,34)} In the present study, *Arabidopsis* plants at the vegetative or reproductive stages exhibited various common or differential phenotypic aberrations after γ irradiation of 50 Gy h⁻¹ for 4 h, having a great dependency on the developmental stage. They were explicitly discriminated in growth, chloroplast number & ultrastructure, photosynthetic pigment content & activity, and protein damage after γ irradiation (Figs. 1–5). Accordingly, the distinct γ -ray-induced traits of the 23 or 43-DAS plants may be partly determined by the developing or senescing state of rosette leaves before γ irradiation, respectively. However, some parameters such as radial expansion of leaves, MV-induced ROS production, and NPQ inhibition, were commonly more conspicuous in the irradiated plants of both stages than in the respective control (Figs. 1B, 3C, and 6). These results imply that the developmental stage may be crucial in dominating phenotypic aberrations of plants after γ irradiation.

Generally, photosynthetic capacity is closely linked to plant development and stress response.^{33,35)} It is also reported that the delay in the leaf senescence of P3845, a stay-green cultivar of maize, was correlated with a high photon-saturated photosynthetic rate as well as increased levels of chlorophyll and nitrogen.³⁶⁾ The current data demonstrated more noticeable changes of photosynthetic capacities in the 23-DAS plants after γ irradiation than in the 43-DAS ones (Figs. 2–4). As revealed by differences in the chloroplast ultrastructure, photosynthetic activity, and pigment content, the natural leaf senescence was enhanced in the irradiated leaves of the 23-DAS plants, while it was reversely alleviated in those of the 43-DAS ones. Similarly in the former case, chlorophyll degradation has been reported as a general phenomenon observable in several plants after γ or proton irradiation.^{2,37)} In contrast, the latter alleviation of leaf senescence in the irradiated leaves could be partly attributed to the increased or sustained levels of photosynthetic pigments, e.g., chlorophyll and carotenoid just as reported recently from *Arabidopsis* plants after γ irradiation at the early reproductive stages.⁶⁾ Therefore, although γ irradiation promotes leaf senescence and loss of photosynthetic activity in plants at the vegetative stage, its effects may be substantially toler-

ated or differentiated in those at the reproductive stage.

However, the production of ROS such as $\cdot\text{O}_2^-$ and H_2O_2 was greatly increased in the irradiated leaves of the 23 and 43-DAS plants under the MV-induced photooxidative stress, depending on the developmental stage subjected to γ irradiation (Fig. 6). Since the ROS at 16 d after γ irradiation are discriminated from the primary ROS produced by radiolysis,³⁸⁾ these data may reflect significant differences in antioxidant systems to detoxify $\cdot\text{O}_2^-$ and H_2O_2 between the irradiated leaves of the 23 and 43-DAS plants. This possibility is partly supported by the differences in the content of antioxidant pigments and oxidized proteins (Figs. 4 and 5). Therefore, increased or sustained levels of the antioxidant defense systems in chloroplasts, e.g., superoxide dismutase, ascorbate peroxidase, β -carotene, lutein, and xanthophyll cycle pigments, is suggested to be involved in the functional integrity of the thylakoid membranes in the irradiated leaves of the 43-DAS plants. In contrast, the more production of MV-induced ROS in the irradiated leaves of the 43-DAS plants than in the control should also be attributed partly to the slightly higher photochemistry and the significantly lower thermal dissipation of absorbed light (Fig. 3).

In summary, the present study revealed specific radiation-induced responses using *Arabidopsis* seedlings at the vegetative or reproductive stage. Although the mutagenic ability of ionizing radiation for a random mutagenesis has been generally accepted for decades,³⁹⁾ our results may support the possibility that some phenotypic aberrations in photosynthetic apparatus could be affected preferentially at a certain developmental stage by ionizing radiation. Therefore, a further study will be needed to elucidate genetic alterations or signaling factors responsible for the physiological phenotypes of *Arabidopsis* specified after γ irradiation in the present study.

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