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Effects of *in Planta* Gamma-Irradiation on Growth, Photosynthesis, and Antioxidative Capacity of Red Pepper (*Capsicum annuum* L.) Plants

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We investigated the effects of low-dose *in planta* irradiation on red pepper plants treated with gamma rays of 2, 4, 8, and 16 Gy. Growth was stimulated at 2 and 4 Gy but inhibited at 8 and 16 Gy. Photochemical quenching (qP) increased slightly in all treatment groups for 1 d after irradiation (DAI), whereas non-photochemical quenching (NPQ) decreased more noticeably. These changes in qP and NPQ were transient and had almost recovered to the control level by 2 DAI. Although carotenoid pigments also fluctuated during the experimental period, chlorophylls were almost entirely insensitive to the gamma rays. Irradiation also partially protected leaves from a decrease in photochemical efficiency (Fv/Fm) under conditions of UV-B (2.2 W m^{-2}) and high light intensity (800 μmol m^{-2} s^{-1}). This enhanced stress resistance could be partly explained by higher levels of SOD and APX activities, as well as ascorbate content. Our results demonstrate for the first time that the carotenoid pigments are the most radio-sensitive and fastest recovering compounds in plants, and that SOD, APX, and ascorbate are important inducible factors for improving stress resistance through the use of *in planta* gamma-irradiation.

Keywords: carotenoid, high light, *in planta* gamma-irradiation, non-photochemical quenching, reactive oxygen species, UV-B

It is a common misconception that ionizing radiation always has an adverse influence on living cells in a dose-dependent manner. Most of those beliefs stem from the genetic alterations induced by high-dose treatments that are widely used for mutation breeding, food sterilization, and medical healing. However, such radiation practices, albeit within a certain range of low doses, can also elevate the physiological activities of cells in plants and photosynthetic microorganisms, e.g., by accelerating cell proliferation (Conter et al., 1986; Planel et al., 1987; Taguchi et al., 1994; Okamoto and Tatara, 1995; Chakravarty and Sen, 2001), ameliorating germination and growth rates (Koepp and Kramer, 1981; Thiede et al., 1995; Al-Safadi and Simon, 1996; Korystov and Narimanov, 1997; Lee et al., 1998; Charbaji and Nabulsi, 1999; Kim et al., 1999, 2000), increasing stress resistance (Zaka et al., 2002; Lee et al., 2002a, b, 2003), and/or improving crop yields (Stan and Croitoru, 1970; Wiendl et al., 1995; Kim et al., 1998; Al-Safadi et al., 2000).

Although these phenomena of 'radiation hormesis' have long been analyzed (Luckey, 1980; Sagan, 1987; Calabrese, 2002), this concept has also been challenged by the small magnitude and transience of the reported hormetic effects of radiation (Spencer and Cabanillas, 1956; Marchi et al., 1962; Miller, 1987) and by frequent failures to reproduce those effects (Skok and Charney, 1962; Miller 1987; Zaka et al., 2004). In many cases, this may be due to differences in plant species/cultivars, developmental stages, or growing conditions (Kim et al., 2004a, b). Nevertheless, the lack of information available on the action mechanism for ionizing radiation at the cellular level has made it more difficult to create a unified hypothesis for the diverse and differential hormetic effects seen in various biological systems.

It has generally been supposed that the cellular responses to low-dose radiation occur via direct cellular-signaling participation of reactive oxygen species (ROS) produced by water radiolysis (Luckey, 1980; Miller, 1987). Another explanation has been the involvement of low-molecular-weight (LMW) signaling factors released from the reactions of ROS and neighboring cellular components (Luckey, 1991; Eidus, 2000). However, no LMW components have been characterized as signaling factors in plants. Because the effects of radiation are retained much longer in irradiated plants for the transience of direct ROS signaling, the participation of intermediates, e.g., LMW components, seems to be obligatory for these hormetic influences (Kim et al., 2004a, b). Therefore, to identify any candidate intermediates related to those long-term effects, researchers must continually investigate

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various cellular components after *in planta* radiation treatments. Such approaches would help elucidate the action mechanisms for radiation hormesis in plants.

In the present study, we used low-dose gamma rays to study the *in planta* effects of irradiation on red pepper plants. Our objective was to document the induced changes in their growth, photosynthesis, and antioxidative capacity. To do so, we measured and compared growth rates, chlorophyll fluorescence parameters, photosynthetic pigment contents, activities of antioxidant enzymes, and ascorbate content in control and treated plants.

MATERIALS AND METHODS

Plant Material and *in Planta* Gamma-Irradiation

Red pepper (*Capsicum annuum* L. cv. Taeyang) plants were irradiated with low doses of gamma-radiation (2, 4, 8, or 16 Gy) at 28 d after sowing (DAS). Radiation was generated by a gamma irradiator [^{60}Co, ca. 150 TBq of capacity; Atomic Energy of Canada Limited (AECL)] at the Korea Atomic Energy Research Institute. Plants were grown from late August to early October of 2003 in a greenhouse at Daejeon, Korea.

Growth Test

Plant growth was evaluated by measuring stem lengths and leaf areas (at 27 DAS), and stem diameters (at 33 DAS). The last two parameters were determined with a digital caliper (CD-6CS; Mitutoyo, Japan) and an area meter (LI-3100; LI-COR, USA). The second recording date, at 33 DAS, corresponded to 5 d after gamma-irradiation (DAI). For leaf areas, only fully expanded first or second leaves were harvested, and were kept in a vinyl bag with distilled water to prevent desiccation before measuring. Statistical analysis of the growth data was performed by Duncan's multiple range test ($P < 0.05$), using SPSS for Windows Release 11.0.1 (SPSS, USA).

UV-B and High-Light Treatments

To minimize the possible effects of positioning, leaves were detached at 7 DAI, then floated on water and acclimated at 25°C for 1 h to a photon flux density (PFD) of 200 μmol m^{-2} s^{-1}, as supplied by four fluorescent lamps and one tungsten lamp. For the UV-B treatment, 2-cm-diameter disks were excised from the detached leaves and exposed at 25°C for 6 h to the same PFD, but with supplementary UV-B from two ultraviolet lamps (XX-15B; Spectronics, USA). UV-B intensity was 2.2 W m^{-2}, as determined with a radiometer (DRC-100X; Spectronics) and a UV-B sensor (DIX-300A; Spectronics). For the high-light treatment, the leaf disks were held at 25°C for 5 h, but they were also placed closer to the light sources, thereby increasing the PFD to 800 mmol m^{-2} s^{-1}.

Chlorophyll Fluorescence Analysis

Chlorophyll (Chl) fluorescence was measured per manufacturer's instructions for the IMAGING-PAM Chl fluorometer (Walz, Germany). Readings were taken after the samples were dark-adapted for 10 min at room temperature (RT). Variable fluorescence (Fv) was calculated by subtracting initial Chl fluorescence (Fo) from maximum yield of fluorescence (Fm). The ratio of Fv/Fm is a measure of the maximal photochemical efficiency of Photosystem II (PSII) (Krause and Weis, 1991).

The parameters for photochemical (qP) and non-photochemical quenching (NPQ) were measured by analyzing Chl fluorescence quenching with the same fluorometer. Calculations were based on the equations of van Kooten and Snel (1990), as follows: qP = (Fm'-Ft)/(Fm'-o') and NPQ = (Fm-Fm')/Fm', where Fm' is the maximum yield of fluorescence at the steady-state level reached during application of a saturation pulse in light-acclimated leaves; Ft is the steady-state fluorescence level under continuous actinic illumination; and Fo' is Fo/(Fv/Fm + Fo/Fm'), as estimated by the approximation of Oxborough and Baker (1997).

Estimation of Total Soluble Protein and Chl Contents

Seedlings were ground to fine powder with a mortar and pestle after being frozen in liquid nitrogen. Proteins and Chls were extracted from this powder (0.5 and 0.1 g each) with ice-cold 50 mM potassium phosphate buffer (pH 7.8) and 80% (v/v) acetone, respectively. Protein contents were determined with the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, USA), based on the method of Bradford (1976) and using bovine serum albumin (BSA) as a standard. Chl contents were calculated according to the formula of Arnon (1949).

Pigment Analysis

Photosynthetic pigments were analyzed via the

method of Gilmore and Yamamoto (1991). After the leaves were frozen in liquid nitrogen and ground with a mortar and pestle, pigments were extracted with ice-cold 100% acetone from about 0.1 g of the powder. Cell debris was twice removed by centrifugation (4°C for 10 min) at 14,000 rpm (Micro 17R; Hanil, Korea). The extracts were then filtered through a 0.2-µm syringe filter. Pigment separation was performed on an HPLC 1100 Series System (Hewlett Packard, Germany) through a Spherisorb ODS-1 column (Alltech, USA), as described by Gilmore and Yamamoto (1991). Their concentrations were estimated by using the conversion factors for peak area (in nanomoles) produced by Gilmore and Yamamoto (1991).

Enzyme Assay

Seedlings at 7 DAI were ground with liquid nitrogen, and enzymes were extracted from the powder (about 1 g) with an optimized medium that included 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.5% (v/v) Triton X-100, and 1% (w/v) PVP. For the ascorbate peroxidase (APX) assay, however, the pH was adjusted to 7.0 and an additional 5 mM ascorbate was supplemented to prevent APX inactivation. All enzymatic activities were expressed on the basis of protein content.

SOD (superoxide dismutase; EC 1.15.1.1) activity was estimated by exploiting SOD-dependent inhibition of the reduction of nitroblue tetrazolium (NBT) to form purple formazan by superoxide, as described by Beyer and Fridovich (1987). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 9.9 mM L-methionine, 57 µM NBT, 0.025% (w/v) Triton X-100, 0.8 uM riboflavin, and the enzyme extract. After 7 min of illumination at RT, NBT photoreduction in the mixture was measured at 560 nm and an inhibition curve was made against different volumes of the extract. One unit of activity was defined as the amount of SOD causing half the maximum inhibition of NBT photoreduction.

APX (EC 1.11.1.11) activity was determined by the decrease in absorbance at 290 nm during ascorbate oxidation, using an absorbance coefficient of 2.8 mM^{-1} cm^{-1}, as described by Nakano and Asada (1981). The assay was carried out at RT in a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2, and the enzyme extract. The change in absorbance was recorded for 2 min after the addition of H_2O_2. A correction was made for the H_2O_2 oxidation of ascorbate and its autooxidation in the presence of the extraction medium, rather than the enzyme extract. One unit of activity was defined as the amount of APX that caused 1 µmole of ascorbate to oxidize for 1 min.

GR (glutathione reductase; EC 1.6.4.2) activity was assayed by a method modified from those of Schaedle and Bassham (1977) and Fryer et al. (1998), and was based on the decrease in absorbance at 340 nm due to the oxidation of NADPH to $NADP^+$ over 5 min. The reaction mixture was composed of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM β-NADPH, 0.5 mM oxidized glutathione (GSSG), and the enzyme extract. The reaction was initiated by the addition of NADPH at RT. A correction was made for the non-GR-dependent oxidation of NADPH by excluding GSSG from the reaction mixture. One unit of activity was defined as the amount of GR that caused 1 nmole of NADPH to oxidize for 1 min. We used an absorbance coefficient of 6.2 mM^{-1} cm^{-1} for NADPH at 340 nm.

Determination of Ascorbate Content in Seedlings

Pepper plants at 6 DAI were ground with liquid nitrogen and a pestle and mortar, and their powder (about 1 g) was extracted in 3 ml of 7% (v/v) $HClO_4$. After centrifugation at 10,000*g* for 5 min at 4°C, the seedling extract was immediately used to determine its ascorbate content, as described by Yoshimura et al. (2000). The extract first was adjusted to pH 6.0 with 1.25 M K_2CO_3 and incubated with 10 mM dithiothreitol in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-KOH buffer (pH 7.5) for 10 min at 20°C. Afterward, a 100-µl aliquot was mixed with 900 µl of 200 mM succinate buffer (pH 6.0). Changes in absorbance at 265 nm were recorded for 5 min after the addition of 5 units of ascorbate oxidase (Sigma, USA). Ascorbate content was determined by comparison with a linear calibration curve.

RESULTS

Effect of *in Planta* Gamma-Irradiation on Plant Growth

We studied the hormetic effects of ionizing radiation in red pepper plants after *in planta* gamma-irradiation. At 5 DAI, plants treated with 2 Gy at 28 DAS showed stimulated growth rates, while those in the 8-Gy and 16-Gy groups were inhibited in their development (Table 1). However, the LD50 for red pepper is 200 Gy (Luckey, 1980). Differences were more

Table 1. Effect of *in planta* gamma-irradiation on growth of red pepper seedlings.

	Dose (Gy)	Stem length (cm)	Stem diameter (mm)	Leaf area (cm^2)
Before γ-irradiation		8.5±0.1*	1.69±0.03*	10.5±0.4*
After γ-irradiation	0	12.8±0.2 c	2.61±0.06 b	10.8±0.7 ab
	2	13.5±0.3 c	2.79±0.05 c	12.7±0.9 b
	4	13.4±0.2 c	2.71±0.04 bc	12.3±0.7 ab
	8	12.0±0.2 b	2.41±0.04 a	10.8±0.7 ab
	16	10.9±0.3 a	2.34±0.06 a	10.2±0.6 a

All values are means ± S.E. of 15 replicate samples except for those indicated by '*' (n = 30). Values not followed by a common letter are significantly different according to Duncan's multiple range test ($P < 0.05$).

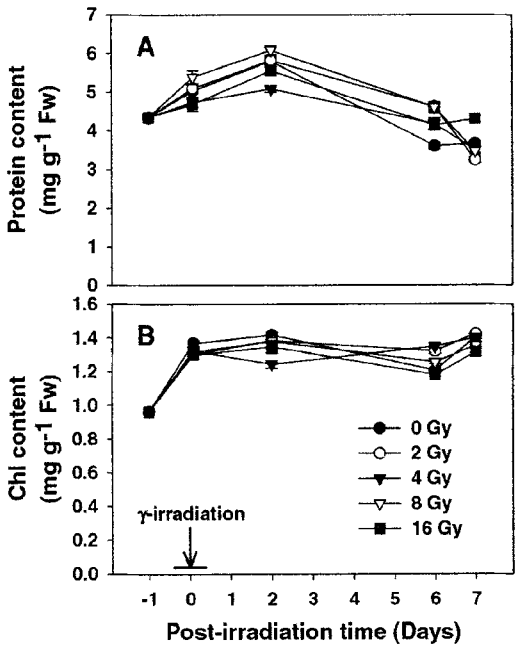

Figure 1. Change in protein and Chl contents in red pepper after *in planta* gamma-irradiation. Proteins and Chls were extracted from seedling powders (0.5 and 0.1 g) with ice-cold 50 mM potassium phosphate buffer (pH 7.8) and 80% acetone, respectively. Their respective contents were determined by methods of Arnon (1949) and Bradford (1976). **A**, protein; **B**, Chl. All values are means ± S.E. of three replicate experiments. Invisible error bars are smaller than their symbols.

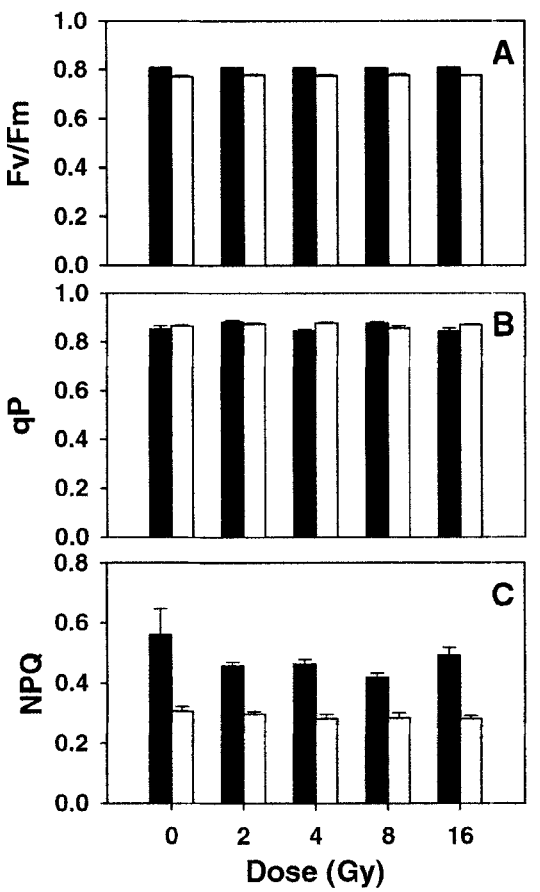

Figure 2. Effect of *in planta* gamma-irradiation on Chl fluorescence parameters. Disks (5 mm diam.) were excised from red pepper leaves and dark-adapted for 10 min at room temperature before measuring. **A**, maximal PSII photochemical efficiency (Fv/Fm); **B**, coefficient of photochemical quenching (qP); **C**, non-photochemical quenching (NPQ). Black and white bars are 1 and 2 d after gamma-irradiation, respectively. All values are means ± S.E. of six replicate samples.

remarkable for stem diameter data between the control and the irradiated groups. Nevertheless, these dosage levels that caused such stimulation or inhibition were much lower than those determined from gamma-irradiation trials with seeds (Lee et al., 1998; Kim et al., 2004a, b).

Because the modulation of plant growth by ionizing radiation is often accompanied by changes in Chl and protein contents (Lee et al., 2002a, b, 2003), we compared those parameters on a leaf fresh-weight basis among the control and irradiation groups (Fig. 1). Protein content increased for up to 2 DAI and decreased

afterward, while Chl content was maintained at a relatively constant level. No noticeable differences in protein or chlorophyll amounts were found between the control and the irradiation groups.

Effect of *in Planta* Gamma-Irradiation on Chl Fluorescence Parameters, Fv/Fm, qP, and NPQ

Maximal photochemical efficiency (Fv/Fm) and the parameters for photochemical (qP) and non-photochemical quenching (NPQ) were investigated to reveal modulations in photosynthetic activity by *in planta* gamma-irradiation. Compared with the control, all irradiation groups at 1 and 2 DAI showed no significant differences in their Fv/Fm and qP values (Fig. 2A and B). Instead, their NPQ values were generally lower in the irradiation groups than in the control at both 1 and 2 DAI (Fig. 2C). This difference became much smaller at 2 DAI, indicating that the decrease in NPQ after gamma-irradiation was recoverable.

Changes in Photosynthetic Pigment Composition after *in Planta* Gamma-Irradiation

The *in planta* gamma-irradiation also modulated photosynthetic pigment composition. Levels of Chl and various carotenoids remained constant among all groups for the first 2 h after gamma-irradiation (Fig. 3A). However, at 1 DAI, the total contents of neoxanthin (Neo), violaxanthin (V), anteraxanthin (A), zeaxanthin (Z), lutein (Lut), and β-carotene (β-Car) decreased in a dose-dependent manner, while the Chl content was not noticeably affected (Fig. 3B). However, the decreased amounts of carotenoids in the irradiated groups seen at 1 DAI had almost recovered to those of the control by 2 DAI (Fig. 3C). Interestingly, the 2-Gy group, with its significantly stimulated growth (Table 1), showed increases in both Chl and caro-

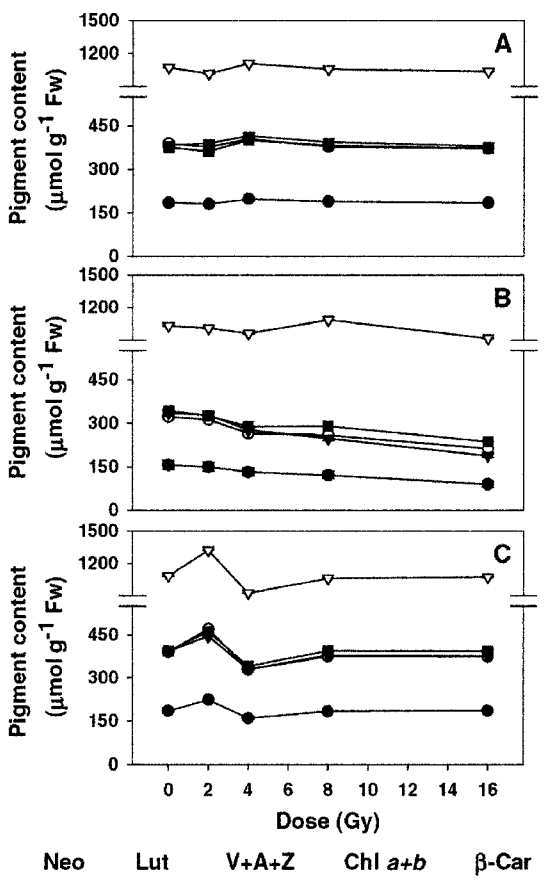

Figure 3. Change in photosynthetic pigment composition of red pepper leaves at **A**, 2 h; **B**, 1 d; **C**, 2 d after *in planta* gamma-irradiation. Neo, neoxanthin; Lut, lutein; V+A+Z, violaxanthin+anteraxanthin+zeaxanthin; β-Car, β-carotene. All values are means ± S.E. of three replicate experiments. Invisible error bars are smaller than their symbols.

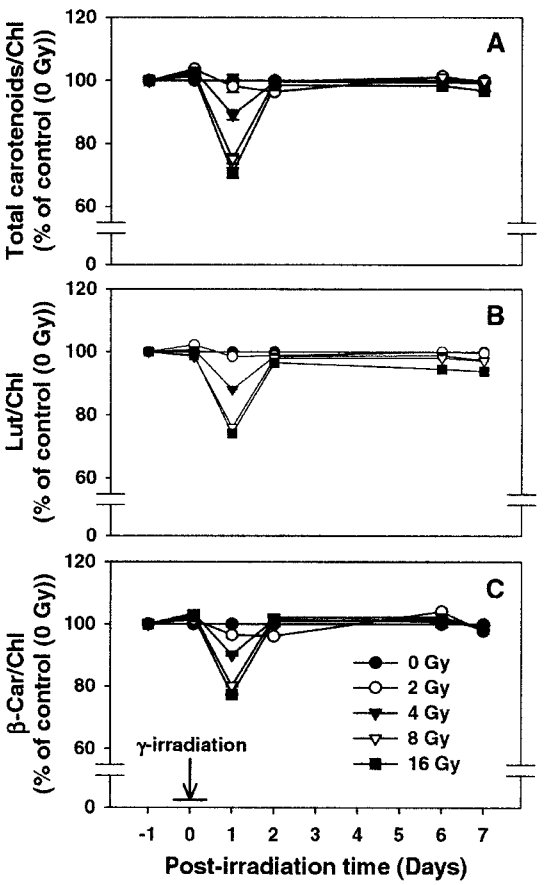

Figure 4. Change in ratios of carotenoids to Chl in irradiated leaves during 7-d post-irradiation period. **A**, total carotenoids/Chl; **B**, lutein, (Lut)/Chl; **C**, β-carotene, (β-Car)/Chl. All values are expressed as percentage of each control (0 Gy), and are means ± S.E. of three replicate experiments. Invisible error bars are smaller than their symbols.

tenoid contents that surpassed those of the control at 2 DAI.

Changes in relative contents were compared between the control and irradiation groups during the 7-d post-irradiation period. The ratio of total carotenoids to Chl declined in a stepwise manner with increasing irradiation doses at 1 DAI (Fig. 4A), but was almost fully recovered at 2 DAI and remained constant afterward. Similar changes were seen for the ratios of Lut to Chl and β-Car to Chl (Fig. 4B and C). The former, however, remained lower in the irradiation groups even after recovery at 2 DAI, and was noticeably smaller in the 16-Gy group at 6 and 7 DAI.

Response of Gamma-Irradiated Plant Leaves to UV-B and High-Light Stresses

We analyzed the response of plant leaves to UV-B exposure in terms of their Chl fluorescence. Generally, those parameters were similarly affected by UV-B and gamma-irradiation (Fig. 2 and 5). Values for NPQ, Fo, and Fm decreased after both treatments, but qP and the apparent rate of photosynthetic electron transport (ETR) increased slightly (data for Fo, Fm, and

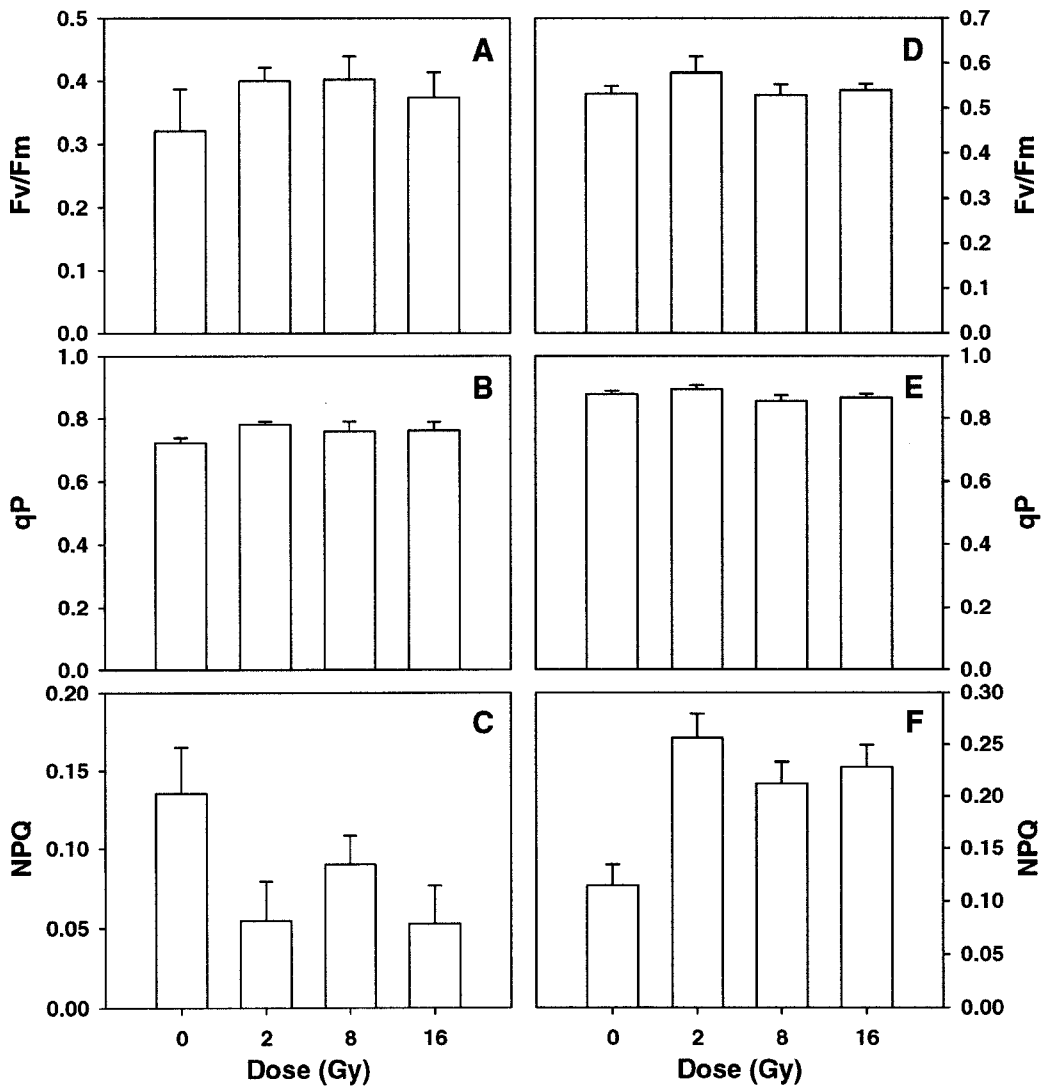

Figure 5. Differences in Chl fluorescence parameters between control and irradiated leaves after UV-B and high-light treatments. Disks (2 cm diam.) were excised from leaves, which were detached at 7 DAI and acclimated for 1 h under a photon flux density (PFD) of 200 μmol m^{-2} s^{-1} at 25°C. They were then exposed either to the same PFD with supplementary UV-B at 25°C for 6 h or to an increased PFD of 800 μmol m^{-2} s^{-1} at 25°C for 5 h. Measurements were made with 5-mm disks excised from the 2-cm leaf segments, as described in "MATERIALS AND METHODS" and Figure 2. **A-C**, UV-B; **D-F**, high light. All values are means ± S.E. of six replicate samples.

ETR not shown). Although Fv/Fm had been unaffected by *in planta* gamma-irradiation, it decreased in response to UV-B exposure, and was maintained at a significantly greater level in the irradiation groups (Fig. 2A and 5A). In contrast, after high-light treatment, NPQ, Fo, and Fm were higher in the irradiation groups than in the control, while qP, ETR, and Fv/Fm were significantly increased only in the 2-Gy group (Fig. 5D, E, and F; Fo, Fm, and ETR not presented). These results imply that, at least in the 2-Gy group, *in planta* gamma-irradiation imparted increased stress resistance against both UV-B and high-light stresses. Therefore, the significant growth stimulation observed in the 2-Gy group could have partly been attributed to this increased resistance.

Change in Antioxidative Capacity of Seedlings after *in Planta* Gamma-Irradiation

Modulation of antioxidative capacity by gamma-irradiation was investigated for irradiated leaves harvested at 7 DAI. As major ROS-scavenging machineries, SOD, APX, and GR activities, plus ascorbate content, were analyzed here (Fig. 6). SOD activity was 2 to 25% higher in the irradiation groups than in the control, while that of GR was 56 to 61% lower in the former. APX activity increased dose-dependently from 27 to 51% in the irradiation groups, while ascorbate content also increased in a similar manner, by 4 to 22%.

DISCUSSION

We have demonstrated here that low-dose *in planta* gamma-irradiation can positively influence both development and stress resistance in red pepper (cv. Taeyang) seedlings. In a previous study, we showed that when seeds of red pepper (cv. Yeomyung and Joheung) were first gamma-irradiated, their resultant plant growth was stimulated at 2 to 8 Gy but was hardly affected at 16 Gy (Kim et al., 2004a, b). In contrast, the present data indicated stimulation of plant growth at 2 to 4 Gy but inhibition above 8 Gy. Variations in cultivars between the two experiments did not make much difference. This implies that *in planta* irradiation increases plant sensitivity to gamma rays.

Plants that arise from irradiated seeds may have some characteristic changes in their Chl and protein contents that can be correlated with stimulated growth (Lee et al., 2002a, b, 2003). Similarly, the leaves from our 2-Gy group at 2DAI had higher amounts of chlorophyll than did those from the other treatments (Fig. 3C); this group also showed the most noticeable increase in growth rates (Table 1). Nevertheless, it is unlikely that such a correlation would hold true generally for the *in-planta*-irradiated plants,

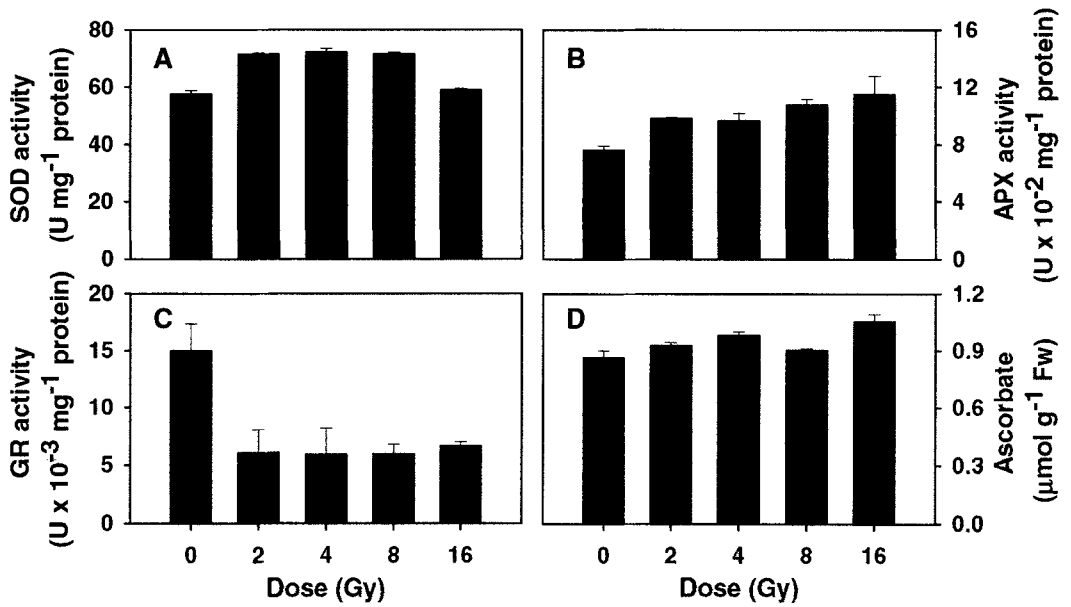

Figure 6. Effect of *in planta* gamma-irradiation on SOD, APX, and GR activities, and on ascorbate content, as extracted from seedlings harvested at 7 DAI (enzymes) and 6 DAI (ascorbate). Seedlings were ground to fine powder with liquid nitrogen before approx. 1-g samples were extracted. **A**, SOD; **B**, APX; **C**, GR; **D**, ascorbate. All values are means ± S.E. of three replicate experiments.

because Chl and protein contents were not noticeably different between the control and the irradiated seedlings during 7-d post-irradiation period (Fig. 1). Instead, the modulation in photosynthesis might partly have contributed to increased growth in the latter types, as was reported by Lee et al. (2002a, b, 2003). In the present study, the higher qP values (even if not significantly different) in the irradiated leaves demonstrated their increased use of absorbed light energy for photochemical reactions, together with their similarly higher ETR (data not shown). Moreover, the accompanying lower NPQ values in the irradiated leaves indicated a decrease in non-photochemical use of absorbed energy. However, our recent experiments also showed that low-temperature and high-light/high-temperature treatments partly shielded or reversed such changes in qP and NPQ (data not presented). Therefore, we believe that the effects of gamma-irradiation are not fixed but interactive, and depend on environmental factors.

NPQ quantifies the non-photochemical use of absorbed light energy through non-radiative dissipation by carotenoid pigments (Niyogi et al., 1997; Baroli and Niyogi, 2000; Müller et al., 2001). Although the total contents of various carotenoids, as well as the ratios of carotenoids to Chls, decreased in a dose-dependent manner at 1 DAI, they appeared to be similar to the control levels at 2 DAI (Fig. 3 and 4). In contrast, the Chl content remained relatively constant, except for the 2-Gy group. These results imply that carotenoids are more sensitive to gamma-radiation than are Chls, and that they are one of the most responsive cellular factors for *in planta* gamma-irradiation. Likewise, such radiation-induced changes in carotenoid contents are reversible and transient, a conclusion that is supported by the observed decline in NPQ differences at 2 DAI between our control and irradiated plants. However, the dose-dependent decrease in carotenoid contents at 1 DAI was not paralleled by NPQ values, and, inversely, the dose-dependency of NPQ decreases at 2 DAI was not found in the carotenoid values (Fig. 2 and 3). Therefore, the decrease in NPQ by low-dose *in planta* gamma-irradiation might have been affected by modifications in structural proteins and lumen energization, as well as by the reversible and transient decrease in carotenoid levels (Baroli and Niyogi, 2000; Müller et al., 2001; Ma et al., 2003).

Increased stress resistance of the plants from gamma-irradiated seeds is observable after UV-B or high-light treatments (Lee et al., 2002b, 2003). This phenomenon is considered a sort of acclimation, as has been reported from cold-stress experiments by Sane et al. (2003). However, the degree of stress resistance in gamma-irradiated plants may depend on the species/cultivars or stress conditions (Kim et al., 2004a, b). Because our irradiated plants had higher Fv/Fm values under UV-B and high-light stresses (Fig. 5), we believe our results show that *in planta* gamma-irradiation can increase stress resistance while also stimulating growth. However, values for qP and NPQ in the irradiated plants were oppositely affected by our stress treatments; this phenomenon was also made obvious by our values for ETR, Fo, and Fm. These results suggest that the manifested effects of gamma-irradiation *in planta* may differ according to environmental factors, such as UV-B and high light.

Although by 2 DAI the NPQ values and carotenoid contents had nearly recovered to those measured in the controls, the sustained effects of gamma-irradiation increased stress resistance for up to 7 DAI. This enhancement can be partly explained by higher levels of SOD and APX activities and ascorbate content (Fig. 6). Because limits on ascorbate, a substrate for APX and an antioxidant itself, can inhibit APX activity (Mano et al., 2001), its higher content might, to some extent, be involved in this increased APX activity, thereby contributing to improved stress resistance. Zaka et al. (2002) have also suggested the possibility of irradiation-induced SOD and APX activities at low doses. In contrast, less SOD activity and a lower Lut/Chl ratio might be a plausible explanation for the only slight improvement in resistance by the 16-Gy group. In fact, lutein, an α-carotene-derived xanthophyll that is a structural component of the subunits of the light-harvesting complexes (Bishop, 1996), contributes to the dissipation of excess absorbed light energy so that plants are protected from photo-oxidative damage (Niyogi et al., 1997). Unlike SOD, APX, and ascorbate, GR activities were significantly lower in the irradiated plants. Although these enzymes belong to the same metabolic (ascorbate-glutathione) pathway, they can vary in their effectiveness against gamma-radiation. Our results agree with those of Gupta et al. (1993a, b), who reported that resistance to oxidative stress in tobacco was due to the over-expression of SOD and APX activities, but not GR. Therefore, a physiological explanation for the decrease in GR activity in our irradiated plants remains to be elucidated.

In conclusion, we have demonstrated here that, first, *in planta* irradiation with gamma rays (i.e., 2 to 16 Gy) can either inhibit or stimulate growth in red pepper plants. This suggests that such treatment is more effective than seed irradiation in modifying

plant development. Second, although NPQ values and carotenoid contents are decreased by *in planta* gamma-irradiation, those declines are transient and reversible, and can be recovered to amounts measured in the controls. Third, Chls are virtually insensitive to low-dose irradiation, whereas the levels of carotenoids decline in a dose-dependent manner at the same degree of radiation. Fourth, irradiation increases resistance in red pepper plants to damage from both UV-B and high light intensities, but its mechanism of action for each may not be through a common pathway. Finally, modulations in the antioxidant capacity of irradiated plants partly contribute to their increased stress resistance. Therefore, we conclude that carotenoid pigments are the most radiosensitive and fastest recovering components in plants, and that SOD, APX, and ascorbate may be important inducible factors for improving stress resistance via *in planta* gamma-irradiation.

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