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Alleviation of Low-Temperature Photoinhibition in Gamma-Irradiated Red Pepper (*Capsicum annuum* L.) Plants

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We studied the radiation-induced stress resistance in red pepper leaves under conditions of low-temperature photoinhibition or artificially induced photo-oxidative stress. Plants irradiated with 4, 8, or 16-Gy gamma rays were more resistant to both stress factors than were the controls. However, exposure to a low temperature for 12 h with illumination or photo-oxidative treatment for 1 h differentially affected the irradiated leaves, although they had similar stress intensities as defined by their maximal photochemical efficiencies (Fv/Fm). Decreases in Fv/Fm induced by the two stress factors were instead alleviated, dose-dependently, by as much as 22 to 41% (low temperature) or 14 to 29% (photo-oxidation) in the irradiated groups. In contrast, non-photochemical quenching (NPQ) and the de-epoxidation state of xanthophyll cycle pigments could not be correlated with this enhanced stress resistance in the irradiated groups. These results suggest that the adaptive response of plants exposed to gamma radiation is more effective in protecting against low-temperature photoinhibition than against photo-oxidative stress. We also discuss here the involvement of antioxidative defense systems for increased resistance against low-temperature photoinhibition in irradiated red pepper.

Keywords: Capsicum annuum, chlorophyll fluorescence, gamma radiation, low-temperature photoinhibition, photo-oxidative stress

Low temperatures sensitize photosynthesis to photoinhibition, resulting from an over-excitation of the photochemical systems (Powles, 1984). This is thought to occur primarily through temperature-imposed thermodynamic constraints on carbon metabolism, which prevent excitation energy from being effectively utilized for CO_2 assimilation (Huner et al., 1993, 1998; Krause, 1994). There are species differences with respect to the mechanisms by which plants cope with low temperature-induced over-excitation (Huner et al., 1998). The increased resistance to photoinhibition has been ascribed to increases in photosynthesis (Huner et al., 1993; Xu et al., 1999), carotenoid contents and antioxidative enzyme activities (Boese and Huner, 1990; Krivosheeva et al., 1996), and to the increased capacity to dissipate excess light non-radiatively (Xu et al., 1999).

Within a certain range of low doses, ionizing radiation increases stress resistance in plants under various environmental conditions, e.g., high light, UV radiation (Kim et al., 2005a), and salt stress (Baek et al., 2006). Such effects can be regarded as an adaptation characterized by biphasic dose responses of generally similar quantitative features (Parsons, 2000; Calabrese and Baldwin, 2002). However, the adaptive responses induced by low-dose radiation are often controversial due to the limited magnitude of the response, such that heightened study design and replication requirements are necessary to ensure reliable judgments on causality (Calabrese and Baldwin, 2002). Therefore, the present study was aimed at gaining replicable documentation and confirmation of the radiation-induced adaptive responses in red pepper leaves under low-temperature photoinhibition or artificially induced photo-oxidative stress.

MATERIALS AND METHODS

Plant Material and Gamma-Irradiation

Red pepper (*Capsicum annuum* L. cv. Taeyang) plants were irradiated with low doses of gamma-radiation (4, 8, or 16 Gy) at 25 or 28 d after sowing (DAS). Radiation was generated by a gamma irradiator (^{60}Co, ca. 150 TBq of capacity; AECL, Canada) at the Korea Atomic Energy Research Institute. Plants were placed in a growth chamber with a pot-level photosynthetic photon flux density (PPFD) of 330 $\mu mol\ m^{-2}\ s^{-1}$ supplied by two natrium lamps in combination with six fluorescence lamps. The growth chamber was maintained at 28/20°C (day/night), with a 14-h photoperiod.

Low-Temperature and Photo-Oxidative Treatments

For our low-temperature treatments, 2-cm-diameter disks were excised from the leaves of red pepper plants that had been irradiated at 25 DAS. To induce photoinhibition, the disks were collected at 2, 24, or 72 h post-irradiation, and were then held abaxial-side down on water under a PPFD of 100 $\mu mol\ m^{-2}\ s^{-1}$ at 4°C for 3, 6, or 12 h, respectively. Photo-oxidative stress was induced by treating the plants with methyl viologen (MV). Afterward, leaf disks were prepared from the primary leaf pairs at 1 or 5 d after irradiation at 28 DAS. These disks were held abaxial side down for 2 h on a 10 μM MV solution in the dark at 28°C, and, subsequently, under a PPFD of 330 $\mu mol\ m^{-2}\ s^{-1}$ at 28°C for 1, 2, or 3 h.

Chlorophyll Fluorescence Analysis

Chlorophyll (Chl) fluorescence was measured using a Chl fluorometer (IMAGING-PAM; Walz, Germany) as described in the operation manual. Readings were taken after 5-mm-diameter leaf disks were dark-adapted for 15 min at room temperature (RT). Variable fluorescence (Fv) was calculated

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by subtracting initial Chl fluorescence (Fo) from maximum yield of fluorescence (Fm). The ratio of Fv/Fm represents the maximal photochemical efficiency of Photosystem II (PSII) (Krause and Weis, 1991). The parameter for non-photochemical quenching (NPQ) was measured by analyzing Chl fluorescence quenching with the same fluorometer. This calculation was based on the equation of van Kooten and Snel (1990), as follows: 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; and Ft is the steady-state fluorescence level under continuous actinic illumination. The actual quantum yield of PSII (Φ_{PSII}) and the apparent rate of photosynthetic electron transport (ETR) were calculated according to equations set by Genty et al. (1989), as follows: Φ_{PSII} = (Fm′-Ft)/Fm′ and ETR = (1 - Ft/Fm′) × 0.5 × PPFD × leaf absorptance, where 0.5 is a constant that assumes an equal distribution of the absorbed photons between PSII and PSI. Leaf absorptance was taken as 0.84.

Pigment Analysis

Photosynthetic pigments were analyzed via the method of Gilmore and Yamamoto (1991), as detailed by Kim et al. (2005a).

RESULTS AND DISCUSSION

We performed three separate photoinhibition experiments to examine radiation-induced adaptive responses in red pepper leaves exposed to a low temperature. The variables for these tests included leaf position, sampling time, and treatment time, while temperature and light intensity remained the same throughout. This approach was intended to ascertain a wide range of radiation-induced adaptive responses.

Low temperature coupled with illumination (here referred to as light-chilling) caused the maximal photochemical efficiency (Fv/Fm) to decrease by up to 37% in the control group (Fig. 1A). In contrast, irradiation conferred protection, so that the decline in efficiency was 22 to 41% less, depending upon dose, in the treated leaves. Moreover, experiments with different leaf positions, and sampling and treatment times, demonstrated that a distinct causality existed between the irradiation and the adaptive response to low-temperature photoinhibition (Fig. 1B). When combined with our previous results (Kim et al., 2005a), these data may imply that irradiated plants are more resistant to low temperatures than to other environmental stress factors, e.g., UV-B and high light intensities.

Our assumption described above was further supported by the fact that the irradiated groups were less protected from photo-oxidative stress than from low-temperature photoinhibition (Fig. 2). Three independent experiments using MV revealed that the decreases in Fv/Fm after photo-oxidative treatment for 1 h were moderated by as much as 14 to 29% in the irradiated groups (Fig. 2A). However, in terms of decreased Fv/Fm values, that level of protection was relatively low compared with the 22 to 41% calculated after a similar intensity of stress was applied with our 12-h chilling treatment. Overall, the irradiated groups showed relatively weak dose dependency and causality in their adaptive responses (Fig. 2B). Nevertheless, it seems certain that the irradiated leaves in all experiments were partially protected from both low-temperature photoinhibition and photo-oxidative stress (Fig. 1, 2). These results can be attributed to changes in their antioxidative defense systems, such as scavenging enzymes and antioxidants. Indeed, we have previously found that applications of gamma-irradiation from 2 to 16 Gy increases the activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) as well as ascorbate content in red pepper plants (Kim et al., 2005a). Because photo-oxidative damage to PSII, in particular, after MV treatment is caused mainly by H_2O_2 rather than O_2^- (Kim and Lee, 2005), we suggest that the greater ascorbate content and APX activity may have contributed to the higher resistance against photo-oxidative stress in our irradiated

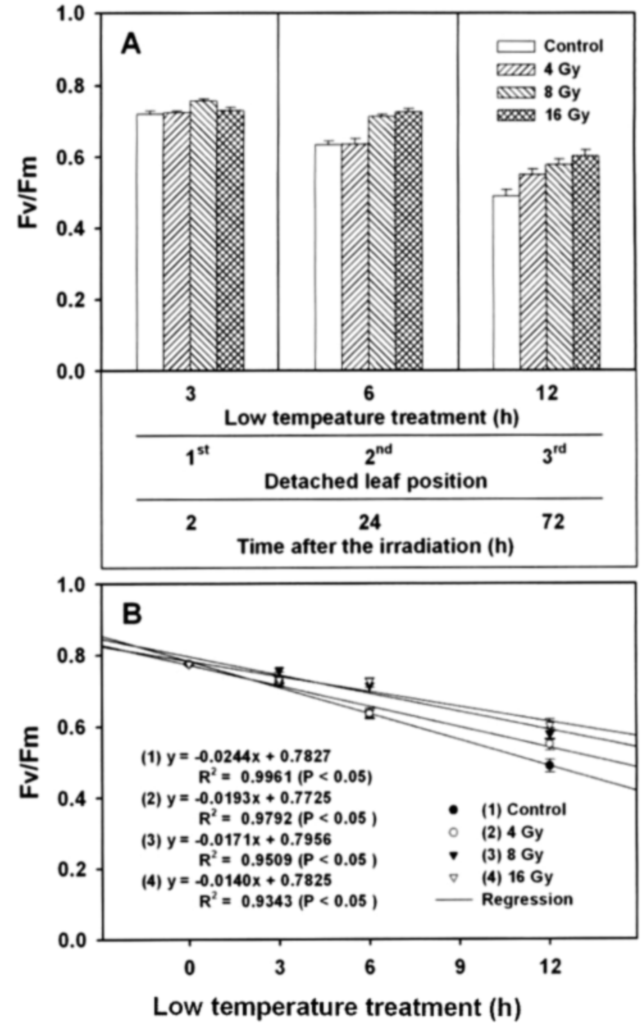

Figure 1. Maximal photochemical efficiency (Fv/Fm) in control and irradiated red pepper leaves after low-temperature treatment for 3, 6, or 12 h. **A**, Fv/Fm values from three different experiments, for which variables included leaf position, sampling time, and treatment time while constants included temperature and light intensity. **B**, Regression analysis of data from **A**, performed with SigmaPlot 2002 for Windows Version 8.02 (SPSS, USA). All values are means ± SE (n = 6).

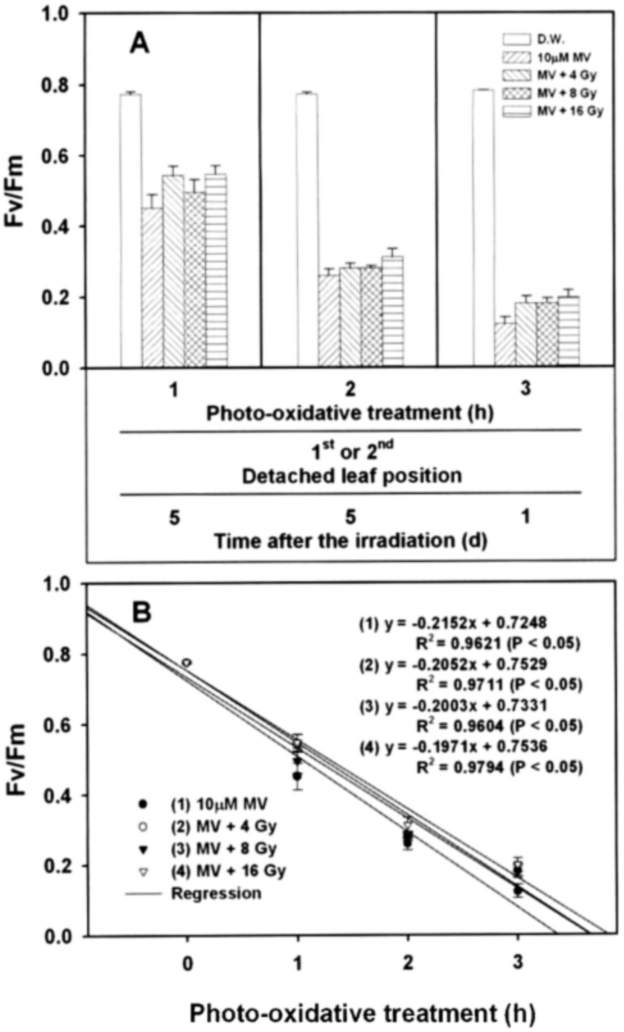

Figure 2. Maximal photochemical efficiency in control and irradiated red pepper leaves after photo-oxidative treatment for 1, 2, or 3 h. Details for **A** and **B** are included with Figure 1. All values are means ± SE (n = 6).

groups here.

The quantum yield of PSII (Φ_{PSII}) measures the proportion of the light absorbed by PSII-associated chlorophylls that is used in photochemistry (Maxwell and Johnson, 2000). This parameter represents the actual yield of PSII in light-acclimated leaves, in contrast to Fv/Fm, which instead measures the maximum yield of PSII. Overall values for Φ_{PSII} were 13 to 39% higher in the irradiated groups than in the control after the 12-h chilling treatment (Fig. 3A). A similar trend was observed in the apparent rate of photosynthetic electron transport (ETR) (Fig. 3B). In contrast, the parameter for non-photochemical quenching (NPQ) showed a different pattern, being decreased in the low-temperature-treated control, such that it was less affected, dose-dependently, in the irradiated groups (Fig. 4). Because our analysis of Chl fluorescence quenching was performed here using chilled leaf disks acclimated to RT for 15 min, these NPQ values may have involved the reversible recovery of PSII from, for example, the qE relaxation of low-temperature photoinhibition (Kim et al., 1997; Kim and Lee, 2003). Therefore, our present data cannot be compared with results reported for

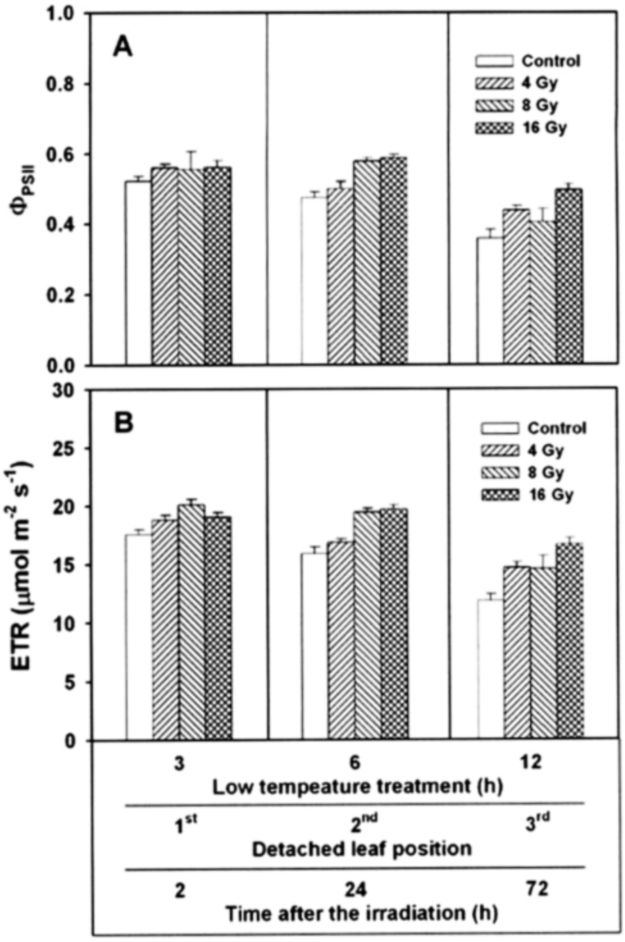

Figure 3. Quantum yield of PSII (Φ_{PSII}) (**A**) and apparent rate of photosynthetic electron transport (ETR) (**B**) in control and irradiated red pepper leaves after low-temperature treatment for 3, 6, or 12 h. Details are included with Figure 1A. All values are means ± SE ($3 \leq n \leq 6$).

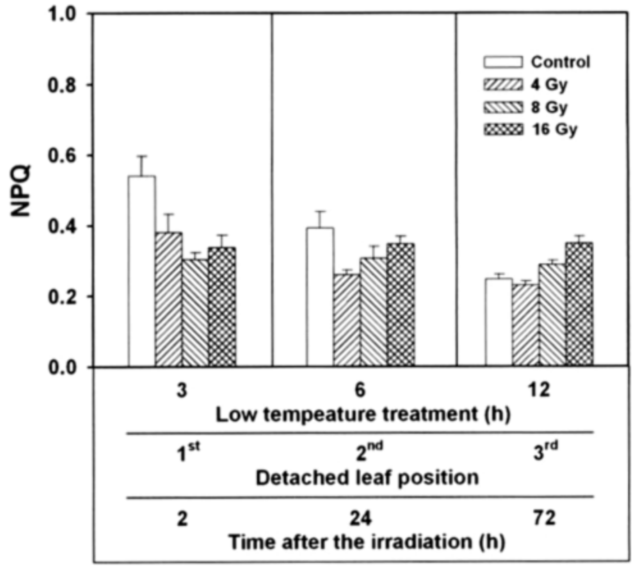

Figure 4. Non-photochemical quenching (NPQ) in control and irradiated red pepper leaves after low-temperature treatment for 3, 6, or 12 h. Details are included with Figure 1A. All values are means ± SE ($3 \leq n \leq 6$).

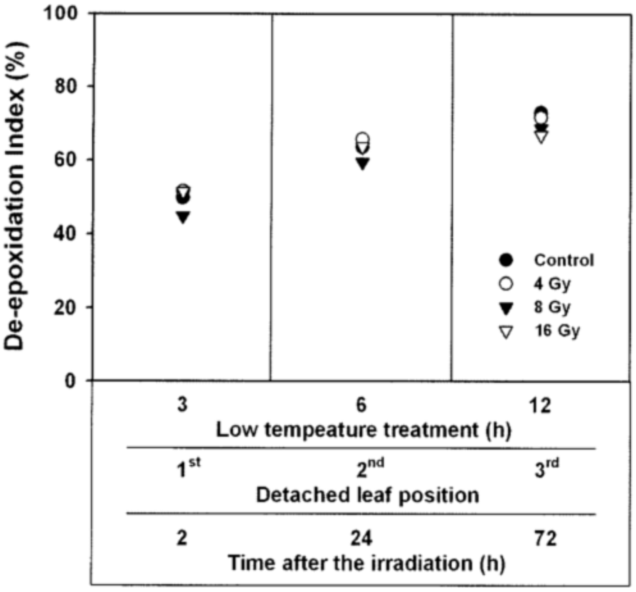

Figure 5. De-epoxidation state of xanthophyll cycle pigments in control and irradiated red pepper leaves after low-temperature treatment for 3, 6, or 12 h. Details are included with Figure 1A.

various plant species, in which NPQ increases during low-temperature photoinhibition (Xu et al., 1999; Oh et al., 2001; Kim and Lee, 2003). Although low NPQs in the irradiated groups could be partly related to a transient decrease in xanthophyll cycle pigments (Kim et al., 2005a), the NPQ changes in our irradiated groups were complicated more by chilling (Fig. 4). Accordingly, taken together with only a small difference noted in the de-epoxidation state among the control and irradiated groups during those treatments (Fig. 5), our results suggest that no changes in NPQ are necessarily required for conferring increased resistance by the irradiated groups to low-temperature photoinhibition.

Such photoinhibition can occur in both PSI and II. A significant portion within the latter is attributable to reversible down-regulation of PSII photochemistry and, therefore, is recoverable during subsequent incubation at RT (Kim et al., 1997; Kim and Lee, 2003). However, this recovery from irreversible damage in PSII requires de novo synthesis of the PSII reaction center D1 protein. In the case of PSI, photoinhibition may include its reversible photoinactivation due to the shortage of oxidizing equivalents on the PSI acceptor side (Kim et al., 2005b); irreversible damage in PSI, such as degradation of the PSI reaction center PsaB protein, has been reported in cucumber, a chilling-sensitive species (Sonoike, 1996). The latter case is correlated with the rapid inactivation of SOD activity in cucumber under light-chilling (Choi et al., 2002). Moreover, the inhibition of SOD activity by dimethyldithiocarbamate diminishes the content of iron-sulfur centers, F_X, F_A, and F_B, which serve as electron acceptors of PSI, in chilling-resistant spinach under the same treatment combination (Hwang et al., 2004). Because one can attribute the irreversible damage in PSII to the H_2O_2 produced through the SOD-mediated dismutation of O_2^- (Kim and Lee, 2005), ascorbate content and APX activity (to detoxify the H_2O_2) are also important factors in conferring resistance against low-temperature photoinhibition. Accordingly, this increased resistance in the irradiated groups can be reasonably explained by greater activities in the antioxidative defense systems (Kim et al., 2005a).

We have previously demonstrated that stress responses are generally limited in plants produced from irradiated seeds (Kim et al., 2004a, b), and that their antioxidative gene expressions are not significantly affected (Kim et al., 2004c). In a related experiment associated with our present study, leaves of pumpkin plants from irradiated seeds were exposed to a low temperature, and changes in their photochemistry were similar to those in the control (data not shown). Therefore, the increased stress resistance found in all these irradiated groups is thought to be a kind of adaptive response observable in individual plants exposed to ionizing radiation. Moreover, such adaptive responses seem to be more effective in protecting against low-temperature photoinhibition than photo-oxidative stress. Combining our previous data with current results, we can now suggest that gamma-irradiation increases resistance to low-temperature photoinhibition through elevated antioxidative defense systems.

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