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Thermal Dissipation of Excess Light in *Arabidopsis* Leaves is Inhibited after Gamma-irradiation

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To elucidate the effect of ionizing radiation on the non-photochemical quenching (NPQ) of chlorophyll fluorescence, we analyzed the buildup and release of NPQ in *Arabidopsis* wild-type (WT) and *npq1-2* mutant plants after gamma-irradiation. The *npq1-2* mutant cannot normally induce the buildup of NPQ by a mutation in the violaxanthin de-epoxidase gene. A dose of 50 Gy h^{-1} for 4 h significantly suppressed such buildup in the mutant and, more noticeably, in the WT. Both the initial rise and maximum level of NPQ were gradually inhibited after gamma-irradiation. In contrast, the release of NPQ and the maximum photochemical efficiency (Fv/Fm) of Photosystem II were largely unaffected in either genotype. This inhibition of NPQ buildup could be partly attributable to a significant decrease in the content of carotenoids, including xanthophyll pigments. Moreover, inhibition that was dependent on the xanthophyll cycle substantially enhanced the sensitivity of irradiated leaves to a photoinhibitory illumination of 800 μmol photons m^{-2} s^{-1}. The difference in Fv/Fm values between the WT and *npq1-2* under that photoinhibitory level of illumination was much smaller in the irradiated leaves than in the control. However, NPQ inhibition did not cause a significant difference in efficiency between WT and mutant when both were treated with UV-B irradiance of 2.4 W m^{-2}. Therefore, we suggest that a significant decrease in carotenoid content after gamma-irradiation should partially contribute to the enhanced sensitivity of irradiated plants, at least to high-light photoinhibition. This is accomplished by suppressing the thermal dissipation of excess light absorbed by photosynthetic pigments.

Keywords: gamma ray, high light, non-photochemical quenching (NPQ), ultraviolet (UV) ray, xanthophyll cycle

Non-photochemical quenching (NPQ) of chlorophyll fluorescence helps protect photosystems against photoinhibition by dissipating, as heat, the excess light absorbed by photosynthetic pigments (Horton et al., 1996). This mechanism minimizes photooxidative damage to the photosynthetic apparatus. The majority of NPQ is thought to occur in the Photosystem II (PSII) antenna pigment bed (Demmig-Adams and Adams, 1992; Horton et al., 1994) and, under most conditions, depends on the buildup of a trans-thylakoid ΔpH in excess light (Krause and Weis, 1991). Acidification of the thylakoid lumen leads to activation of an enzyme, violaxanthin de-epoxidase, that converts violaxanthin to zeaxanthin as part of the xanthophyll cycle (Niyogi et al., 2001). Binding of both H^+ and zeaxanthin to PSII proteins is thought to switch the antenna into a conformation that allows for efficient quenching of excitation energy instead of energy-transfer and trapping by the reaction center (Horton et al., 1996; Gilmore, 1997; Gilmore et al., 1998).

The *Arabidopsis npq1* mutant has a mutation in the violaxanthin de-epoxidase gene and, therefore, does not accumulate zeaxanthin under high light (Niyogi et al., 1998). Characterization of *npq1* plants has provided molecular genetic evidence that zeaxanthin is necessary for most of the qE component of NPQ. However, the *Chlamydomonas npq1* mutant, which cannot de-epoxidate violaxanthin to zeaxanthin, is only partially defective in NPQ. This suggests that not all NPQ in *Chlamydomonas* depends on the functioning of the xanthophyll cycle (Niyogi et al., 1997).

The xanthophyll cycle is affected by both ultraviolet radiation and high light intensities (Döhler et al., 1997; Bolink et al., 2001; Šprtová et al., 2003). Concentrations of pigments involving in that cycle can be increased by those environmental stresses, due to a higher need for energy dissipation (Demmig-Adams and Adams, 1992). Moreover, carotenoids, including xanthophyll-cycle pigments, are important antioxidants that protect cells from oxidative damage (Demmig-Adams and Adams, 2002). However, little research has been reported on the activity of the xanthophyll cycle when plants respond to ionizing radiation, e.g., gamma rays, beyond solar visible and ultraviolet radiation. Because of the possible roles for carotenoids as antioxidants, any changes in the contents of β-carotene, lutein, and xanthophyll-cycle pigments should be under consideration when using irradiation to sterilize and protect fresh fruits and vegetables.

Here, we analyzed fluctuations in the thermal dissipation of excess light by the xanthophyll cycle after applying gamma-radiation to wild-type and *npq1* leaves of *Arabidopsis*. The contents of various carotenoids, including the xanthophyll-cycle pigments, were investigated to explain alterations in that dissipation as well as the antioxidative abilities of irradiated leaves.

MATERIALS AND METHODS

Plant Materials

Wild-type (WT) and *npq1-2* mutant plants of *Arabidopsis thaliana* (ecotype Columbia) were cultivated in a growth chamber with a 16-h photoperiod, a temperature regime of 22/18°C (day/night), and a compound soil mixture (1:1:1 vermiculite:peat moss:perlite). Lighting was adjusted to a photosynthetic photon flux density (PPFD) of 130 μmol m^{-2}

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s^{-1} supplied from six fluorescent lamps. The *npq1-2* seeds were obtained from the Arabidopsis Research Center (Ohio State University, Columbus, OH, USA).

Gamma-irradiation and Treatment with UV-B or High Light

At 29 days after sowing (DAS), seedlings were irradiated with gamma rays, at a dose rate of 50 Gy h^{-1} for 4 h. These rays were generated by a gamma irradiator (^{60}Co, *ca.* 150 TBq of capacity; Atomic Energy of Canada Limited, Canada) at the Korea Atomic Energy Research Institute. Afterward, they were returned to the same growing conditions. For the UV-B treatment, disks (2-cm diam.) were excised from the detached leaves of 31-DAS seedlings and exposed at 22°C for 1, 3, or 5 h to the same PPFD, but with supplementary UV-B of 2.4 W m^{-2} from two ultraviolet lamps (XX-15B; Spectronics, Westbury, NY, USA). For our high-light treatment, leaf disks were prepared from 30-DAS seedlings and placed under a PPFD of 800 µmol m^{-2} s^{-1}, which was provided by six fluorescent and two tungsten lamps, at 22°C for 1, 3, or 5 h. All disks were floated on distilled water, abaxial side down, throughout these treatments.

Chlorophyll Fluorescence Analysis

Chlorophyll (Chl) fluorescence was measured with a Chl fluorometer (IMAGING-PAM; Walz, Effeltrich, Germany). Readings were taken after leaf disks (5-mm diam.) were dark-adapted on water-soaked filter paper for 15 min at room temperature. Variable fluorescence (Fv) was calculated by subtracting the initial Chl fluorescence (Fo) from the maximum yield of fluorescence (Fm). The ratio of Fv/Fm represented the maximum 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 reached during application of a saturation pulse (2400 µmol quanta m^{-2} s^{-1}) in light-acclimated leaves under continuous actinic illumination (135 or 1210 µmol quanta m^{-2} s^{-1}).

Pigment Analysis

To analyze their photosynthetic pigments, we froze five 5-mm disks from different leaves in liquid nitrogen and ground the tissues in a micro-centrifuge tube with a plastic pestle. The pigments were then extracted with ice-cold 100% acetone by vigorous agitation at 4°C for 1 h. Cell debris was removed twice by centrifugation at 4°C and 15,000g for 15 min. The extracts were filtered through a 0.2-µm syringe filter. Pigment separation was performed in an HPLC system (Waters, Milford, MA, USA) on a Spherisorb ODS-1 column (Alltech, Deerfield, IL, USA), as described by Gilmore and Yamamoto (1991). Concentrations of these pigments were estimated by using the conversion factors for the peak area to nanomoles, as determined by Gilmore and Yamamoto (1991).

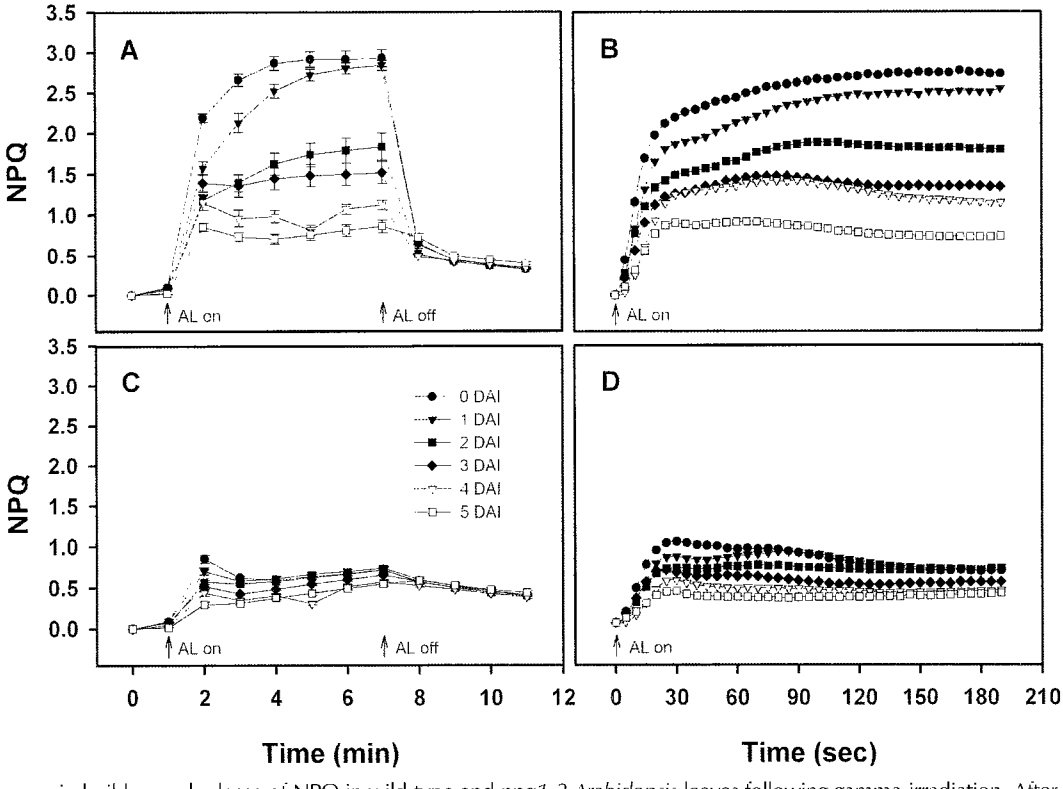

Figure 1. Changes in buildup and release of NPQ in wild-type and *npq1-2 Arabidopsis* leaves following gamma-irradiation. After dark-adaptation for 15 min, disks (5-mm diam.) were exposed to continuous actinic illumination of 1210 µmol quanta m^{-2} s^{-1} during NPQ induction, as indicated by up and down arrows; buildup and release of NPQ were measured at 5-s intervals. **A** and **B**, wild type; **C** and **D**, *npq1-2*. DAI, days after gamma-irradiation. AL, actinic light. Bars represent means ± SE (n=5).

RESULTS AND DISCUSSION

Inhibition of Thermal Dissipation of Excess Light after Gamma-irradiation

Xanthophyll pigments that are involved in the thermal dissipation of excess light in photosynthetic organisms are sensitive to ionizing radiation. However, the decrease in pigment contents that is induced by exposure to approximately 10-Gy gamma rays is fully recoverable within 48 h (Kim et al., 2005). Here, we investigated the effect of gamma-irradiation (50 Gy h^{-1} for 4 h) on *Arabidopsis* leaves. The efficiency of thermal dissipation of excess light was evaluated using non-photochemical quenching of chlorophyll fluorescence.

Irradiation significantly inhibited the buildup of NPQ in wild-type leaves under continuous actinic illumination of 1210 μmol quanta m^{-2} s^{-1} (Fig. 1). This inhibition gradually became more prominent from 1 to 5 d after irradiation. However, this change was much smaller in the leaves from *npq1-2* plants, which were defective in NPQ buildup due to a mutation in the violaxanthin de-epoxidase gene (Niyogi et al., 1998). The small but significant amount of reversible NPQ in those leaves seemed to be independent of the xanthophyll cycle (Niyogi et al., 1998). Unlike the initial rise and maximum level of NPQ, the release of NPQ was mostly unaffected in both the WT and *npq1-2*.

Although the maximum level of NPQ buildup was significantly decreased after gamma-irradiation, the photochemical efficiency of PSII, Fv/Fm, was almost constant in both the

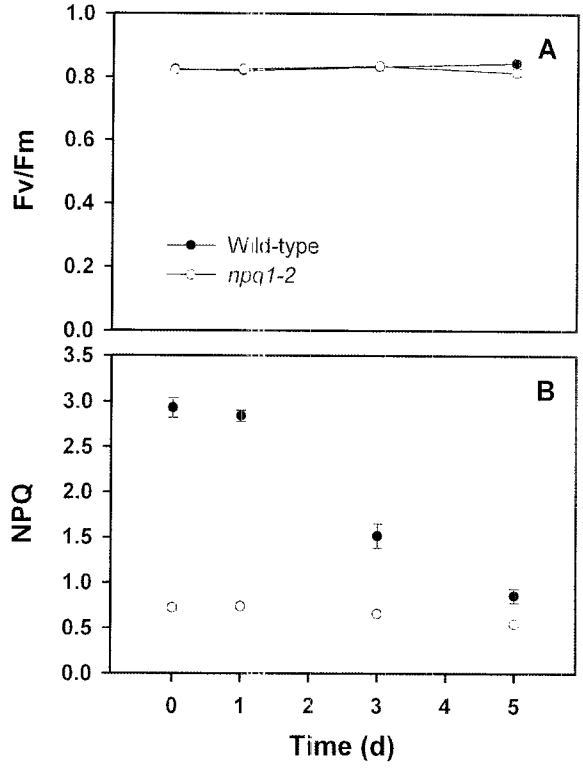

Figure 2. Changes in maximum photochemical efficiency of PSII and maximum NPQ in wild-type and *npq1-2 Arabidopsis* leaves after gamma-irradiation. Maximum NPQ implies steady-state level of NPQ induction or buildup, as described in Fig. 1 legend. Bars represent means ± SE (n=5).

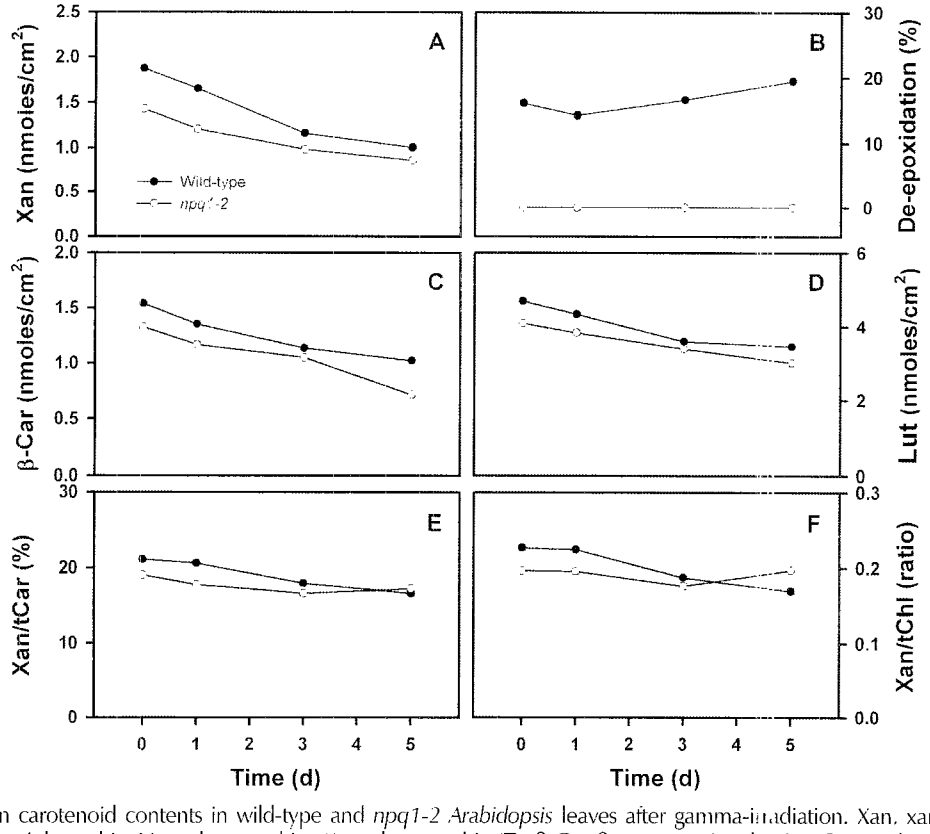

Figure 3. Changes in carotenoid contents in wild-type and *npq1-2 Arabidopsis* leaves after gamma-irradiation. Xan, xanthophyll pigments, including neoxanthin, violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z); β-Car, β-carotene; Lut, lutein; tCar, total carotenoids, including xanthophyll pigments, β-carotene, and lutein; tChl, total chlorophylls. De-epoxidation state of xanthophyll-cycle pigments was calculated as (A × 0.5 + Z) × 100 / (V + A + Z). All values are means of 3 replicate measurements; their errors are smaller than symbols.

WT and the mutant (Fig. 2). This is in good agreement with a previous report that, despite the inhibition of thermal energy dissipation in PSII, the absence of the violaxanthin-to-zeaxanthin cycle in *npq1* has relatively minor effects on efficiency (Havaux and Niyogi, 1999). Our results suggest that the NPQ buildup dependent on the xanthophyll cycle is gradually inhibited after gamma-irradiation whereas the photochemical efficiency of PSII remains relatively constant. Moreover, the minor portion of NPQ independent of the cycle is also diminished by gamma-irradiation. However, without additional stress factors, such as high-light and UV-B irradiance, wild-type irradiated leaves could maintain their normal PSII photochemistry under non-stressed growing conditions. This was illustrated by our *npq1-2* control leaves that lacked NPQ buildup.

Decrease in the Content of Carotenoids after Gamma-irradiation

With regard to the pronounced inhibition of NPQ after gamma-irradiation, we measured the content of carotenoids, including xanthophyll pigments, in WT and *npq1-2* plants. The total content of neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin was significantly decreased after gamma-irradiation (Fig. 3A), especially in the wild type. However, the de-epoxidation states in both genotypes under our growing conditions remained nearly unaffected after gamma-irradiation, minimizing the possibility for radiation-induced alterations in de-epoxidase activity (Fig. 3B). Moreover, β-carotene and lutein contents were decreased similarly in both the WT and *npq1-2* plants (Fig. 3C, D). Such a post-irradiation decline in carotenoid contents, e.g., xanthophyll-cycle pigments, β-carotene, and lutein, has also been reported in red pepper leaves (Kim et al., 2005). Our results suggest that the decrease in xanthophyll-cycle pigments that was more pronounced than for other carotenoids (as well as the chlorophyll in the WT leaves, as shown in Figure 3E and F) may have partly contributed to the inhibition of NPQ buildup after gamma-irradiation.

Increase in Sensitivity to Photooxidative Stress after Gamma-irradiation

The inhibition of thermal energy dissipation in the *npq1* mutant causes a notable decrease in the photochemical efficiency of PSII when plants are treated with short-term photoinhibitory strong light or chilling stress in the light (Havaux and Niyogi, 1999). Because we might infer from this that the wild type plants would be similarly sensitized to photoinhibition after gamma-irradiation, we investigated the photochemical efficiency of PSII in the control and irradiated leaves of the WT and *npq1* under either a high PPFD of 800 μmol m^{-2} s^{-1} or UV-B radiation at 2.4 W m^{-2}.

Upon high-light illumination, Fv/Fm values were markedly decreased, with this response being more pronounced in the mutant (Fig. 4A). Although the difference in efficiency between the wild type and *npq1-2* became much smaller after gamma-irradiation, the irradiated leaves from both types had higher Fv/Fm values than did the control under high-light illumination. Possible reasons for this can be inferred from our previous studies, in which gamma-irradiation of red pepper plants at 0.5~4 Gy h^{-1} for 4 h induced an enhancement of oxidative stress-resistance and/or antioxidative defense systems, e.g., superoxide dismutase, ascorbate peroxidase, and ascorbate contents (Lee et al., 2002; Kim et al., 2005, 2006). Moreover, a few genome-wide analyses of transcripts from *Arabidopsis* after gamma-irradiation with 200~3000 Gy have shown that many antioxidative defense systems are substantially affected by ionizing radiation (Nagata et al., 2005; Kim et al., 2007). Therefore, the lower magnitude of radiation-induced enhancement of oxidative stress-resistance in our WT leaves under high-light illumination, compared with the *npq1-2* ones, could be partly attributed to the inhibition of thermal energy dissipation after gamma-irradiation.

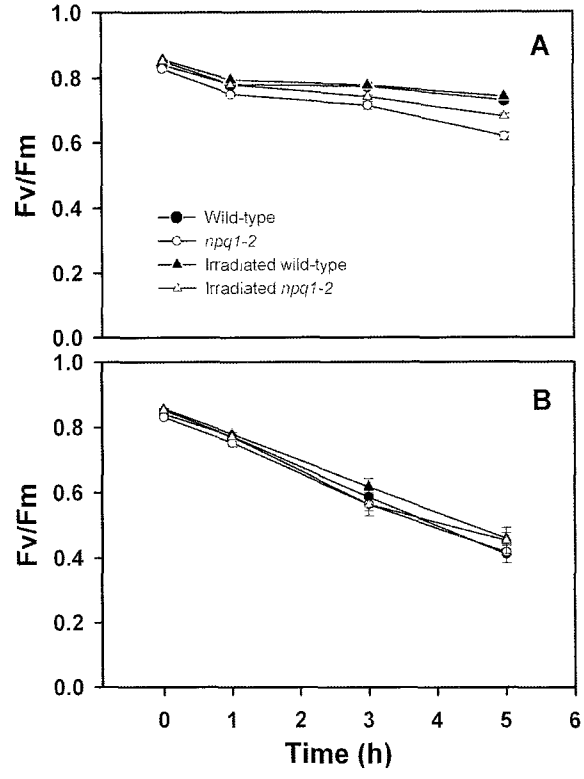

Figure 4. Decrease in maximum photochemical efficiency of PSII in control and irradiated leaves under treatment with high light (**A**) or UV-B (**B**). Bars represent means ± SE (n=5).

Unlike with high-light irradiance, treatment with UV-B did not cause a significant difference in the PSII photochemistry between WT and *npq1-2* or between control and irradiated leaves (Fig. 4B). That is, the xanthophyll cycle was less affected by UV-B radiation than by intense light. One possible explanation is that, in the case of higher plants, UV-B radiation inhibits photosynthetic electron transport, with PSII as the major site of UV-B damage, thereby resulting in a significant drop in its photochemical efficiency (Bornman, 1989; Olsson et al., 2000). Alternatively, the degree of stress-resistance in irradiated plants depends on the species or cultivar, developmental stage, or irradiation/stress conditions (Kim et al., 2004a, b, 2006).

Finally, we investigated the relationship between the xanthophyll cycle and NPQ buildup while the WT leaves were being treated with high light or UV-B radiation after

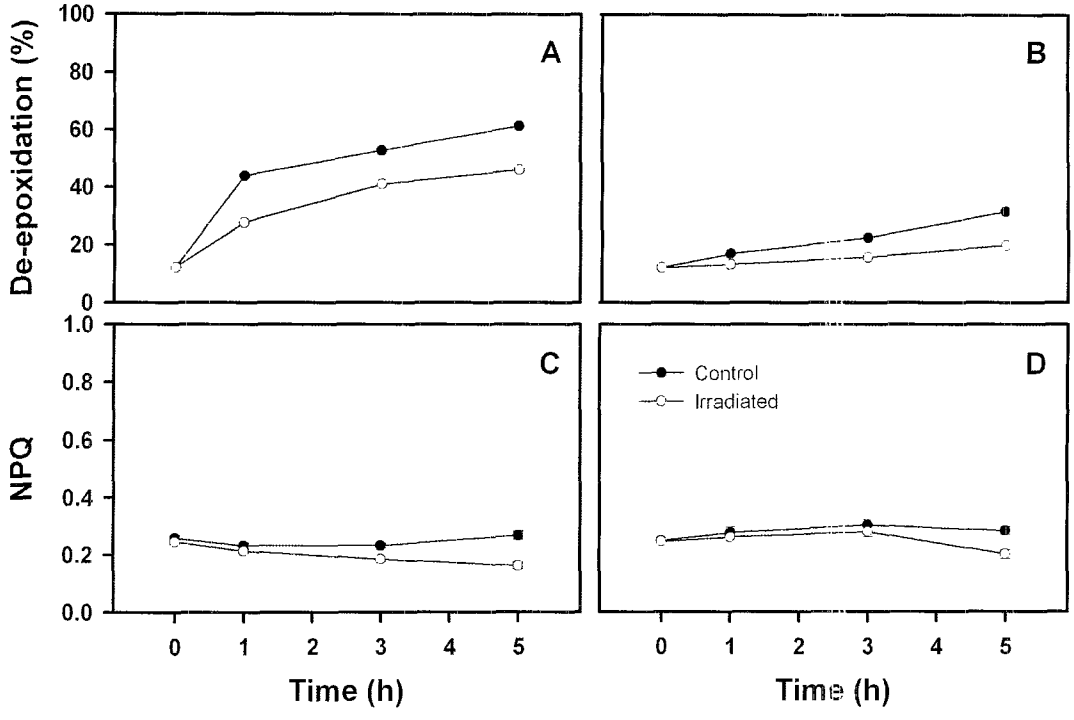

Figure 5. Changes in NPQ and de-epoxidation state of xanthophyll cycle in control and irradiated leaves under high-light (**A**, **C**) or UV-B (**B**, **D**) treatment. NPQ analysis was carried out with 15-min-dark-adapted leaf disks; actinic illumination was 135 μmol quanta m^{-2} s^{-1}, which is almost equivalent to standard intensity of growth lights. Therefore, present NPQ values are actual levels of thermal dissipation of excess light in control and irradiated leaves after treatment. All values in **A** and **B** are means of 3 replicate measurements, their errors are smaller than symbols. Bars in **C** and **D** represent means ± SE (n=5).

gamma-irradiation. Here, de-epoxidation was significantly increased by either, although more markedly by high light (Fig. 5A, B). After either type of treatment, NPQ levels were significantly lower in the irradiated leaves than in the control (Fig. 5C, D). These results suggest that inhibition of thermal dissipation of excess light that is dependent on the xanthophyll cycle should be correlated with the increased sensitivity of irradiated leaves to, at least, high-light photoinhibition.

CONCLUSIONS

These present data demonstrate that the thermal dissipation of excess light in *Arabidopsis* leaves can be significantly inhibited after gamma-irradiation. Moreover, the increased sensitivity of irradiated leaves to high-light photoinhibition could be partly attributable to the inhibition of NPQ buildup that is dependent on the xanthophyll cycle. Accordingly, we suggest that a significant decrease in the content of carotenoids, including the xanthophyll-cycle pigments, after gamma-irradiation would substantially enhance the sensitivity of plants to, at least, photoinhibition by exposure to high light intensities.

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