

Influence of Gamma Irradiation on Greening of Mung Bean Seedlings

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Abstract – Ionizing radiation causes many alterations in photosynthetic machineries. However, there is no information about effects of ionizing radiation on the development of photosynthetic machineries in plants. We investigated the greening of etiolated mung bean seedlings after gamma-irradiation of 50 to 300 Gy. The irradiation inhibited seedling growth with great dependence on the radiation dose. In particular, growth of stems was more affected than that of hypocotyls. Irradiated leaves showed inhibition in growth, aberration in morphology, and yellowing in color depending on the radiation dose. Contents of photosynthetic pigments such as chlorophylls and carotenoids were significantly decreased in the irradiated leaves. The apparent electron transport rate for photosynthesis, ETR, was similarly changed depending on the radiation dose. However, the maximal photochemical efficiency of Photosystem II (PSII), Fv/Fm, was little affected by the irradiation. Moreover, the 50-Gy seedlings maintained the control level of light saturating for photosynthesis and showed slightly higher Fv/Fm values in spite of significant decreases in the photosynthetic pigment content and ETR. These results suggest that the inhibition of the overall photosynthetic capacity couldn't be causally related with the repression in the initial development of irradiated seedlings and that the overall photosynthetic machineries can develop and work to some extent as a concerted system for photosynthesis even after exposure to acute doses of ionizing radiation.

Key words : gamma irradiation, mung bean, photosynthesis, pigment

INTRODUCTION

Ionizing radiation influences on pigment metabolism and photosynthesis in plants. A high-dose irradiation to plants generally accelerates senescence and cell death with a significant loss of photosynthetic pigments necessary for photosynthesis (Wada *et al.* 1998). However, pigment contents in *Arabidopsis* plants may also be increased by a high-dose gamma radiation, depending on the developmental stage and radiation dose (Kim *et al.* 2007; unpublished data). Photosynthetic pigments are not only necessary for photo-

synthesis but also crucial for plant development. Carotenoid pigments also behave as antioxidants against oxidative stress (Frank and Cogdell 1993; Edge *et al.* 1997; Demmig-Adams and Adams III 2002). These effects of ionizing radiation on pigment metabolism in plants rely on the plant species and irradiation condition (Wada *et al.* 1998; Kim *et al.* 2007). Therefore, radiation-induced phenomena in plants are very confusing and difficult to be generalized.

Photosynthesis in plants is an essential cellular mechanism for development and sustained survival. Generally, ionizing radiation within several grays has been known to improve early growth and photosynthetic capacity of various plant species (Koepp and Kramer 1981; Thiede *et al.* 1995; Lee *et al.* 2002a, b; Kim *et al.* 2004). However, these

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beneficial effects of ionizing radiation have also been challenged by the weak reproducibility and the small magnitude (Calabrese 2002; Calabrese and Baldwin 2002). In fact, our preliminary experiments showed that many of the major proteins constituting photosynthetic machineries were kept constant even after exposure to a high-dose gamma radiation of 1 kGy. Accordingly, the influence of ionizing radiation on photosynthesis is somewhat attributable to changes in the photosynthetic pigment content. Although the photosynthetic pigment content was significantly affected by either a low-dose or high-dose gamma radiation, the effects were more sustainable in the latter case (Kim *et al.* 2005, 2007; unpublished data).

Despite several noticeable evidences showing effects of ionizing radiation on plant pigment metabolism (Kim *et al.* 2004, 2005), there were no detailed studies about synthesis and degradation of photosynthetic pigments relating to ionizing radiation. Especially, effects of ionizing radiation on *de novo* synthesis of photosynthetic pigments and organization of photosynthetic machineries, which are essential for plant development, remain to be elucidated. Such studies will provide critical information to reduce negative aspects of plant irradiation or to establish appropriate criteria for irradiation of plant materials in radiation industry.

Mung bean is one of fast growing plant species, which are appropriate for study of greening and development of plants. Especially, their organs and tissues are clearly distinguishable enough to be separately analyzed for a photosynthesis or developmental study (Song *et al.* 2005). Accordingly, the present study aims to elucidate the influence of ionizing radiation on the synthesis of photosynthetic pigments and the functional organization of photosynthetic machineries by investigating dose-dependent effects of gamma radiation on greening, photosynthesis, and development of mung bean seedlings. Experimental details include analyses of organ development, photosynthetic pigment content, and photosynthetic ability during the greening or de-etiolation stage after gamma-irradiation of dark-grown and etiolated mung bean seedlings.

MATERIALS AND METHODS

1. Plant materials and gamma-irradiation

Mung bean (*Vigna radiata* L.) seedlings were germinated

and cultivated in darkness at 26°C for 3 d after seeds were sown. The etiolated seedlings were exposed to gamma-rays at a dose rate of 12.5, 25, 50, or 75 Gy/h for 4 h in darkness at room temperature, which were generated by a gamma irradiator (⁶⁰Co, ca. 111 TBq of capacity, MDS Nordion, Ottawa, Canada) at the Advanced Radiation Technology Institute in Jeongeup. Then, they were incubated in darkness at 26°C for 2 d to induce radiation-inducible responses in cells. Finally, greening of the irradiated and etiolated seedlings was induced at 26°C for 6 d under a photosynthetic photon flux density (PPFD) of 80 μmol m⁻² s⁻¹ supplied by fluorescence lamps.

2. Microscopic analysis

Adaxial and abaxial surfaces of leaves were examined at room temperature by a stereomicroscope (Stemi 2000, Carl Zeiss, Oberkochen, Germany) equipped with a digital camera (Mega-Pixel Digital CCD, Pamtech Co., Camarillo, CA).

3. HPLC analysis

For analysis of photosynthetic pigments, five 5-mm leaf disks from different leaves were frozen with liquid nitrogen and ground in a micro-centrifuge tube with a plastic pestle. Then, the pigments were 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 15,000 × g for 15 min. The extracts were filtered through a 0.2-μm syringe filter. Pigment separation was performed in a HPLC system (Waters, Milford, MA) on a Spherisorb ODS-1 column (Alltech, Deerfield, IL) and as previously described by Gilmore and Yamamoto (1991). Concentrations of the pigments were estimated by using the conversion factors for the peak area to nanomoles as determined by Gilmore and Yamamoto (1991).

4. Chlorophyll fluorescence analysis

Chlorophyll fluorescence was measured using a chlorophyll fluorometer, IMAGING-PAM (Walz, Effeltrich, Germany). Readings were taken after 5-mm-diameter leaf disks were dark-adapted for 15 min at room temperature. Variable fluorescence (Fv) was calculated by subtracting the initial chlorophyll fluorescence (Fo) from the maximum yield of fluorescence (Fm). The ratio of Fv/Fm represents the maximal photochemical efficiency of Photosystem II (PSII)

(Krause and Weis 1991).

Subsequently, photosynthesis vs. PPFD curves were recorded with the same fluorometer as follows: Leaf disks were irradiated with increasing PPFD of actinic red light (650 nm; PPFD=4~1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Every 20 s a saturating pulse (2,400 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was applied to measure effective quantum yield of PSII before actinic light was further increased. The effective quantum yield of PSII is defined as $1 - F_t/F_m'$ according to Genty *et al.* (1989), where F_m' is the maximum yield of fluorescence at the steady-state level reached during an application of a saturation pulse in light-acclimated leaves; F_t is the steady-state fluorescence level under continuous actinic illumination. The apparent electron transport rate (ETR) of photosynthesis was calculated as follows: $\text{ETR} = (1 - F_t/F_m') \times 0.5 \times \text{PPFD} \times \text{leaf absorptivity}$, where 0.5 is a constant that assumes an equal distribution of the absorbed photons between PSII and PSI; and leaf absorptivity was taken as 0.84. Relative ETRs were plotted against the PPFD of actinic light.

RESULTS AND DISCUSSION

1. Effect of gamma radiation on growth of mung bean seedlings

Overall growth inhibition of mung bean seedlings were observed after gamma-irradiation. When dark-grown etiolated seedlings were irradiated with gamma radiation of 50, 100, 200, and 300 Gy, their further developments of leaves, stems, hypocotyls, and roots under the greening condition were significantly but differentially inhibited, showing great dependence on the radiation dose (Fig. 1). Gamma radiation of 100 Gy or over induced noticeable alteration of leaf color and morphology, while 50-Gy radiation was enough to repress emergence of root hair. Moreover, growth of stems and hypocotyls was markedly inhibited from 50-Gy and 200-Gy irradiation, respectively (Fig. 2). Without two-day darkness after gamma-irradiation, the radiation effects on development of leaves, stems, hypocotyls, and roots under the greening condition were much less manifested (data not shown). As shown in leaf color of Fig. 1, synthesis of photosynthetic pigments can be also inhibited. The entire results are in good agreement with previous papers, which dealt with growth inhibition and chlorophyll degradation by a high-dose gamma radiation (Wada *et al.*

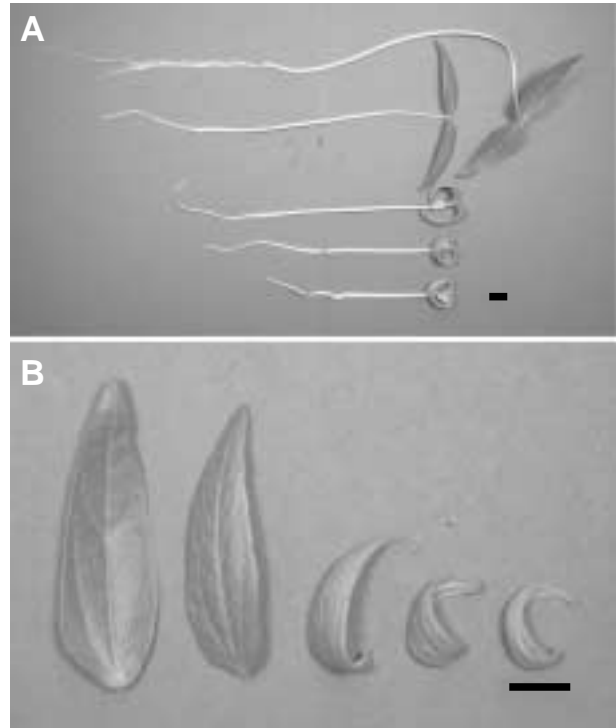


Fig. 1. Growth of mung bean seedlings after gamma-irradiation. A, 0, 50, 100, 200, 300-Gy seedlings from the top; B, 0, 50, 100, 200, 300-Gy leaves from the left. Bar=2 cm.

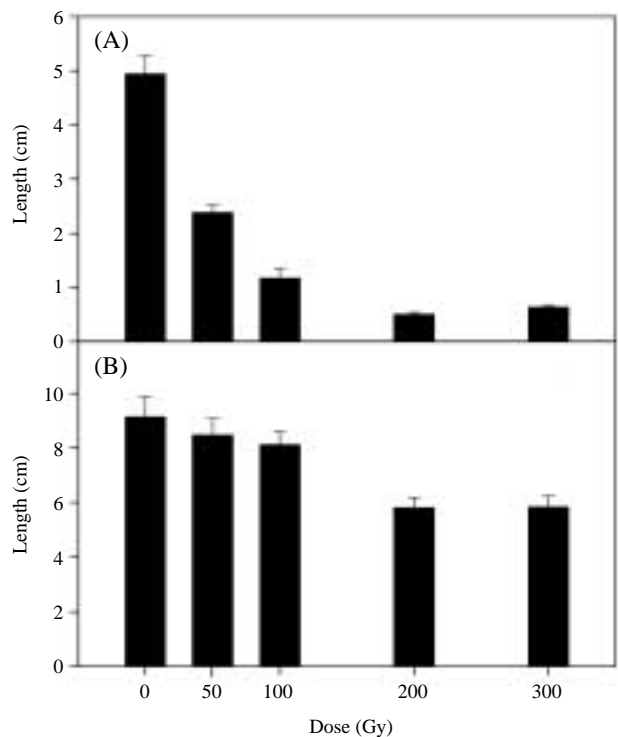


Fig. 2. Differential effect of gamma radiation on the growth of mung bean stems and hypocotyls. Bars represent means \pm SE ($14 \leq n \leq 20$). A, stem; B, hypocotyl.

1998). However, the current data demonstrate that gamma radiation suppressed development of leaves, root hairs, and stems more than that of hypocotyls.

2. Effect of gamma radiation on leaf surface and photosynthetic pigment of mung bean leaves

Morphological changes of the irradiated leaves were investigated in detail under a stereomicroscope. When compared to the control, the 50-Gy-irradiated leaves showed

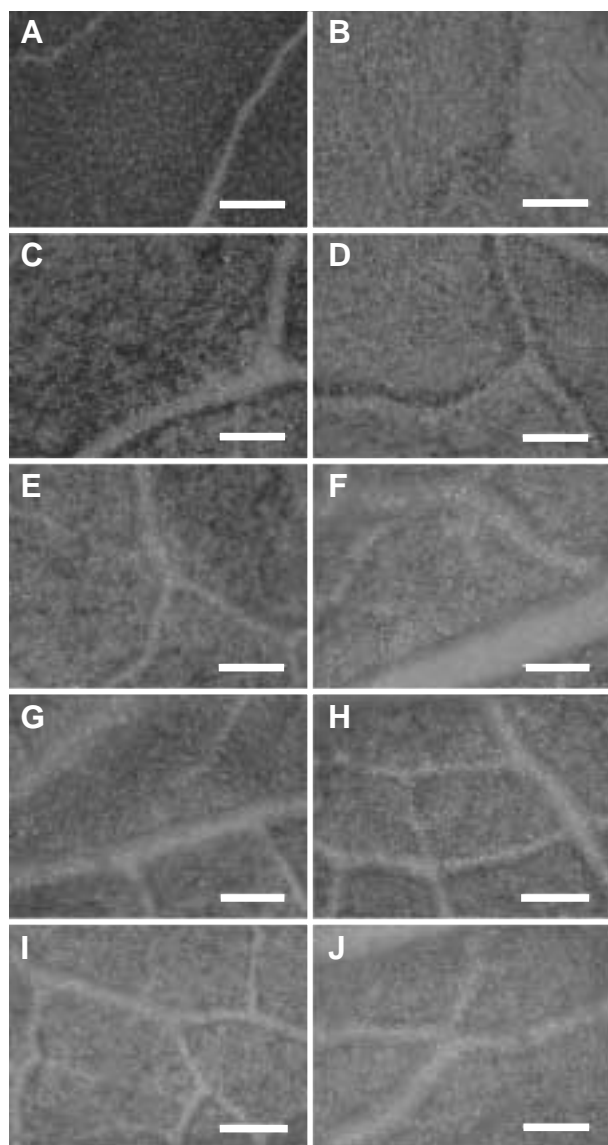


Fig. 3. Change in the adaxial and abaxial surface of mung bean leaves after gamma-irradiation. A and B, control; C and D, 50 Gy; E and F, 100 Gy; G and H, 200 Gy; I and J, 300 Gy. A, C, E, G and I, adaxial side; B, D, F, H and J, abaxial side. Bar=0.5 mm.

notably lumpy and dark green surface of adaxial and abaxial sides (Fig. 3). In contrast, the lumpy surface of leaves became less noticeable from the 100-Gy-irradiated seedlings and their leaf color turned into pale green. Pigment analysis demonstrated that all gamma-irradiations including the 50 Gy significantly decreased contents of chlorophylls and carotenoids such as β -carotene, lutein, and xanthophylls in leaves (Fig. 4A, B, C, and D). This decrease was dose-dependently observed in the 50-Gy and 100-Gy-irradiated leaves but less pronounced in the other ones. Interestingly, β -carotene, lutein, and xanthophylls, which serve as both a photosynthetic pigment and an antioxidant, were different in the relative pattern of change. The relative content of β -carotene to total carotenoids increased with the increasing radiation doses, while that of lutein decreased reversely (Fig. 4E and F). Moreover, the relative content of xanthophylls to total carotenoids increased in the 50-Gy and 100-Gy-irradiated leaves but decreased in the other ones (Fig. 4G). These results are in good agreement with the relative importance of β -carotene, lutein, and xanthophylls as antioxidants (Niyogi *et al.* 1998, 2001; Demmig-Adams and Adams III 2002). The ratio of total carotenoids to chlorophylls was slightly higher in the irradiated leaves than in the control ones (Fig. 4H). Our previous paper revealed that carotenoids in red pepper were more sensitive to gamma radiation of 2 to 16 Gy than chlorophylls (Kim *et al.* 2005). However, the present data may imply that gamma radiation of 50 Gy or more should inhibit synthesis of chlorophylls more than carotenoids, especially with the initiation of greening of etiolated seedlings. This discrepancy can be attributed in the first place to differences in the radiation dose and plant developmental stage (Kim *et al.* 2007).

3. Effect of gamma radiation on photosynthetic activity of mung bean leaves

Inhibitory effects of gamma radiation on synthesis of photosynthetic pigments during the greening of etiolated seedlings can be resulted in a significant decrease of photosynthetic ability. Although the maximum yield of fluorescence, F_m , increased with the radiation doses, the maximal photochemical efficiency of PSII, F_v/F_m , remained almost constant in the irradiated leaves with the exception of the slightly high F_v/F_m in the 50-Gy leaves due to the low F_o (Fig. 5). These results may imply that most structural pro-

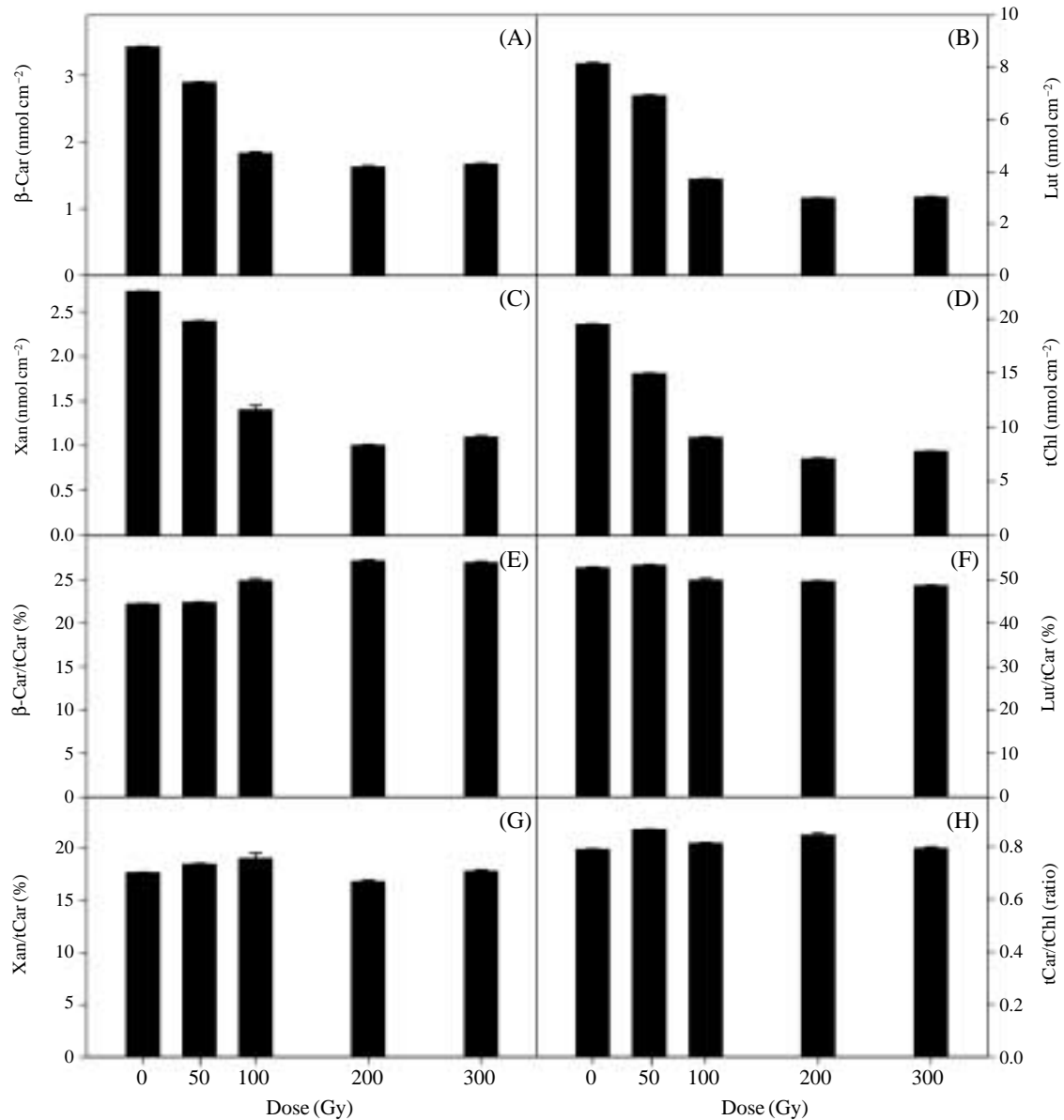


Fig. 4. Change in the photosynthetic pigment content of mung bean leaves after gamma-irradiation. Xan, xanthophyll cycle pigments including violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z); β -Car, β -carotene; Lut, lutein; tCar, total carotenoids including xanthophyll cycle pigments, β -carotene, and lutein; tChl, total chlorophylls. All values are means of three replicate measurements and their errors are smaller than symbols.

teins constituting photosystems and photosynthetic electron carriers could play a functional role for photosynthesis during the greening of etiolated seedlings after gamma-irradiation (Kim *et al.* 2007; unpublished data). Actually, thylakoid proteins in isolated chloroplasts remained in relatively stable forms even after exposure to a high-dose gamma radiation of 1 kGy (data not shown). However, the apparent electron transport rate for photosynthesis, ETR, decreased dose-dependently in the irradiated leaves and the saturating

light intensity for photosynthetic electron transport showed a similar pattern except for the 50-Gy leaves (Fig. 6). Therefore, it seems likely that both the photosynthetic pigment metabolism and photosynthetic electron transport are substantially inhibited by gamma radiation, at least of 100 Gy or more. Nevertheless, this inhibition of the overall photosynthetic ability didn't affect causally the actual photosynthetic efficiency and the development of seedlings during the greening of etiolated seedlings under the growth light of

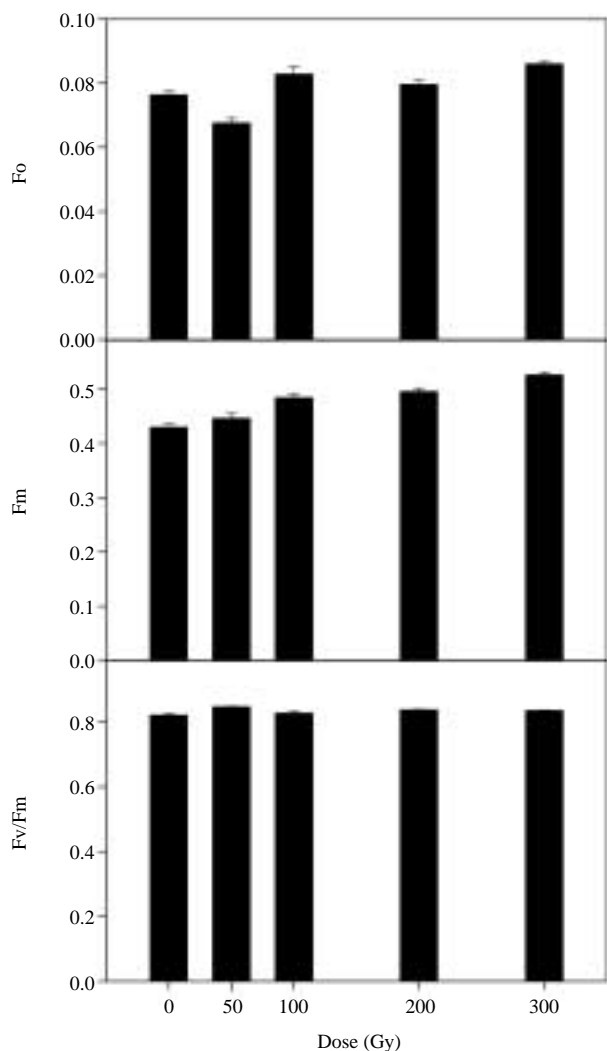


Fig. 5. Change in the maximum PSII photochemical efficiency of mung bean leaves after gamma-irradiation. Bars represent means \pm SE (n=5).

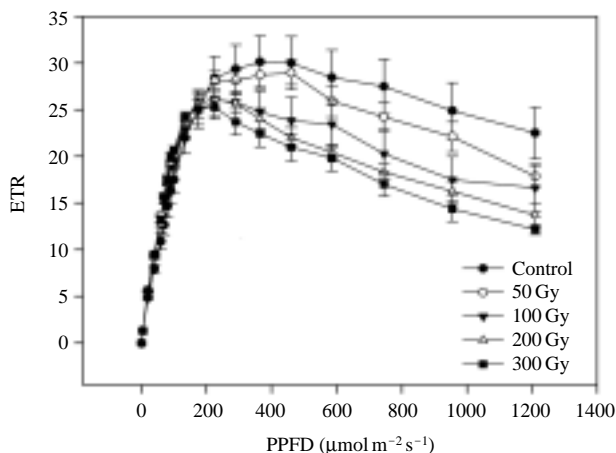


Fig. 6. Change in the relative ETR of mung bean leaves after gamma-irradiation. Bars represent means \pm SE (3 \leq n \leq 5).

80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ after gamma-irradiation (Figs. 5 and 6).

CONCLUSION

The present study revealed that the greening and initial development of etiolated mung bean seedlings after gamma-irradiation was inhibited dose-dependently to some extent with substantial decreases in the photosynthetic pigment content and ETR. However, it seems that such inhibition of the overall photosynthetic capacity didn't causally repress the initial development of mung bean seedlings at least under the growth light condition for induction of the greening. Moreover, the 50-Gy seedlings maintained the control level of light saturating for photosynthesis and showed slightly higher Fv/Fm values in spite of significant decreases in the pigment content and ETR. Therefore, alterations in the initial development of plants after gamma-irradiation might rely on changes in the general growth regulators such as phytohormones more than those in photosynthetic machineries.

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