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# Effect of a Serial Irradiation of Low Dose Gamma Rays on the Growth and Photosynthesis of Red Pepper (*Capsicum annuum* L.) Plants

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**Abstract - To reveal the relationship between the changes in the growth and photosynthesis induced by low dose radiation, red pepper (*Capsicum annuum* L.) plants were serially irradiated three times with gamma rays of 0.5, 1, 2, 3, and 4 Gy. The plant growth was monitored by the fresh weight, the stem length, and the leaf length & width. All the irradiation groups (0.5-4 Gy) were stimulated in growth at 1 day after the 1st irradiation (DA1I), but rather inhibited at 3 days after the 3rd irradiation (DA3I). The maximum photochemical efficiency (Fv/Fm), the photochemical quenching (qP), the non-photochemical quenching (NPQ) and the apparent rate of the photosynthetic electron transport (ETR) were used to represent the changes in the photosynthesis by the serial irradiation. The irradiation groups except 0.5 Gy had higher Fv/Fm values at 3 DA3I than the control one. After the 3rd irradiation, the qP values appeared to be a little lower in the 1-4 Gy groups than in the control and 0.5 Gy ones. In contrast, the NPQ values were rather higher in the irradiation groups except 0.5 Gy. During the whole experimental period, the ETRs decreased in the control group but remained relatively constant in the 4-Gy one. In conclusion, the results obtained indicate that the stimulatory effect of ionizing radiation on the plant growth was determined by the incident dose of the single irradiation rather than by the cumulative one of the serial irradiation. They also demonstrate that the growth stimulation induced by a low dose radiation could not be positively correlated with an alteration in the photosynthesis. Additionally, we discuss in text that an ionizing radiation may partly protect the leaf senescence by delaying the development of the plants.**

**Key words : low dose radiation, chlorophyll fluorescence, photosynthesis, red pepper**

## INTRODUCTION

Ionizing radiation at several grays can induce growth stimulation in plants (Thiede *et al.* 1995; Charbaji and Nabulsi 1999; Lee *et al.* 2002a; Kim *et al.* 2004a). This phenomenon has been called 'radiation hormesis' (Luckey 1980). Ionizing radiation can produce free radicals from water in cells (Luckey 1980; Miller 1987) and cause the release of biologically important low-molecular weight (LMW) compounds through various reactions of the radicals with neighboring cellular components (Luckey 1991; Eidus 2000). However, no LMW compounds have been characterized as a signaling factor to induce such 'radiation hormesis' at least in plant

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cells. Recently, it has been reported that an ionizing radiation at low doses induced an increase in the actual quantum yield of the photosynthetic electron transport and the net photosynthesis rate (Lee *et al.* 2002b), and that it caused an alteration in the photosynthetic pigment contents (Kim *et al.* 2004b). These results imply that a low dose radiation modulated the photosynthesis. Accordingly, the photosynthetic components might be considered as signaling mediators, and an alteration in the photosynthesis be thought to be involved in the growth stimulation of irradiated plants. However, few reports are available to clarify the possibility of a growth stimulation through a modulation in photosynthesis. In the present study, we attempted to reveal a clear relationship between them by comparing the effects of a serial gamma-irradiation on the growth and photosynthesis of red pepper. The results obtained indicate that the overall changes in the growth and photosynthesis during the post-irradiation period did not allow for a causal relation between them. Furthermore, something beyond the dose effect of an ionizing radiation is discussed by this new experimental approach (serial gamma-irradiation).

## MATERIALS AND METHODS

### 1. Plant materials and *in planta* gamma-irradiation

Red pepper (*Capsicum annuum* L. cv. Taeyang) plants were irradiated with low doses (0.5, 1, 2, 3, and 4 Gy) of gamma-radiation at 28, 31, and 34 days after sowing (DAS). The gamma-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 (KAERI). Before and after the irradiation, plants were grown in a growth chamber with a photosynthetic photon flux density (PPFD) at a pot level of 330 μmol $m^{-2}$ $s^{-1}$ supplied by two natrium and six fluorescence lamps. The growth chamber was maintained at 28/20°C (day/night) with a 14 h photoperiod.

### 2. Growth test

Plant growth was evaluated by the fresh weight, stem length, and leaf length & width measured at 27, 29, 32, 35, and 39 DAS which corresponded to 1 day after the 1st irradiation (DA1I), 1 day after the 2nd irradiation (DA2I), 1 day after the 3rd irradiation (DA3I), and 3 DA3I, respectively. Seedlings were harvested at the level of the soil. Five randomly selected seedlings were used to measure the stem length and fresh weight, while ten leaves from the seedlings were used to measure the leaf length & width. As the plants developed, the leaf length & width were measured using the first & second leaves at 1 DA1I, the second & third ones at 1 DA2I, and the third & fourth ones at 1 DA3I and 3 DA3I.

### 3. Chlorophyll fluorescence analysis

Chlorophyll (Chl) fluorescence was measured using a Chl fluorometer (IMAGING-PAM, Walz, Germany) as described in the operation manual. Leaf samples were excised at 0.5 cm in diameter from the leaves used for the growth test. Readings were taken after the samples were dark-adapted for 15 min at room temperature. The variable fluorescence (Fv) was obtained by subtracting the initial Chl fluorescence (Fo) from the maximum yield of the fluorescence (Fm). The ratio of Fv/Fm served as a measure of the maximal photochemical efficiency of photosystem II (PSII) (Krause and Weis 1991).

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

In the light response curve, the apparent rate of the photosynthetic electron transport (ETR) was calculated as $(1 - Ft/Fm') \times 0.5 \times PPFD \times$ leaf absorptance, according to Genty *et al.* (1989), where 0.5 is a constant assuming an equal distribution of the absorbed photons

between PSII and PSI. Leaf absorptance was taken as 0.84.

### 4. Data analysis

Data from the growth test were analyzed by the polynomial fit using a Microcal Origin Version 7.0 software package (Microcal Software Inc., USA).

## RESULTS AND DISCUSSION

### 1. Effect of a serial gamma-irradiation on the growth of red pepper seedlings

As shown by the solid lines of Fig. 1, the gamma-irradiation could stimulate the early growth of plants at low doses up to 4 Gy. This result is consistent with the previous reports on red pepper (Lee *et al.* 1998; Kim *et al.* 2004a), although the irradiation was applied to seeds in these reports, not to plants. However, the growth in all the irradiation groups was rather inhibited at 3 DA3I. Therefore, the stimulatory effect of an ionizing radiation on the plant growth could not be directly related to the cumulative dose of the incident radiation. For instance, the total incident dose of three irradiations at 1 Gy was 3 Gy, but this cumulative dose inhibited an increase in the fresh weight, which is opposite to the result from the same dose of single irradiation (1 DA1I and 3 DA3I in Fig. 1A).

Stems seemed to be more resistant to the serial irradiation than leaves, because the stem length was rather higher in the irradiation groups at 1 DA3I when the growth of the leaf length & width started to be inhibited (Figs. 1B, C, D). Moreover, the similar growth inhibition in the leaf length & width of the irradiation groups (1 DA3I and 3DA3I in Figs. 1C, D) may imply that the serial irradiation did not cause any noticeable changes in the leaf morphology.

Accordingly, it is suggested that unlike the single irradiation, the serial irradiation might be harmful for the plant growth even at low (cumulative) incident doses of an ionizing radiation. Additionally, the irradiation interval might be a crucial factor that brings about the deleterious effects of the serial irradiation to the irradiation groups.

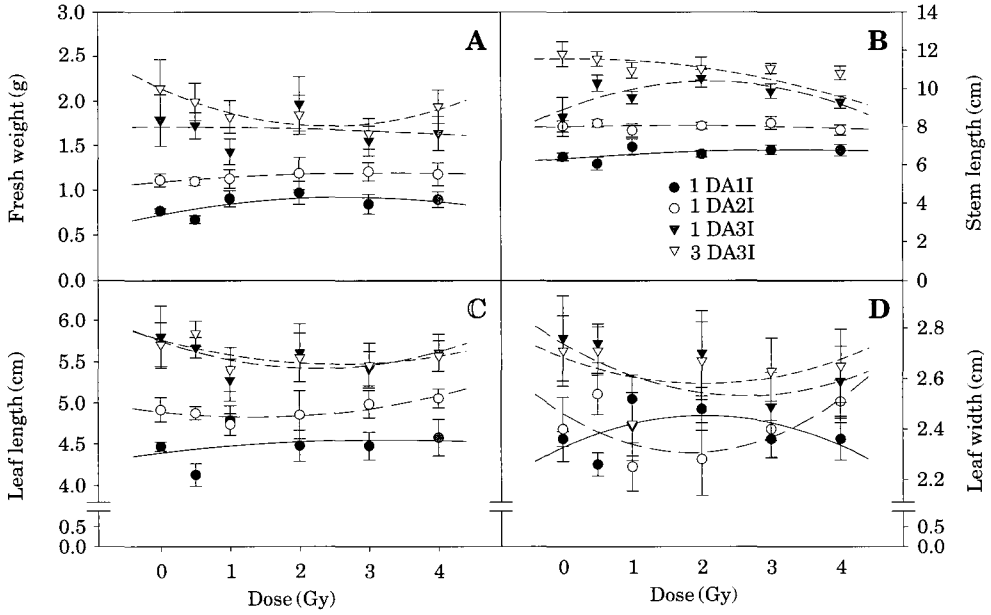


**Fig. 1.** Changes in the fresh weight, stem length, and leaf length & width of red pepper seedlings by a serial irradiation of different doses of gamma rays. DA1I, DA2I, and DA3I represent day after 1st, 2nd, and 3rd irradiation, respectively. All the line plots were produced by a polynomial regression of the original data with a Microcal Origin Version 7.0 software package (Microcal Software Inc., USA). Error bars represent the S.E. (n = 5 in the fresh weight and stem length, and n = 10 in the leaf length & width).

## 2. Alteration in the photosynthetic activity of the red pepper leaves by a serial gamma-irradiation

The values of Fv/Fm, the maximum photochemical efficiency of PSII, were not significantly different between the control and irradiation groups until 3 DA3I (Fig. 2A). At 3 DA3I, the irradiation groups except 0.5 Gy had higher Fv/Fm values than the control one. However, the growth of the seedlings was inhibited in all the irradiation groups at 3 DA3I (Fig. 1). Taken together, these data indicate that the overall changes in the growth rates observed in the irradiated plants cannot be causally related to an alteration in the photosynthesis. The decrease in the Fv/Fm during the post-irradiation period is thought to be caused by a leaf senescence. One of the most notable and important changes observed during the whole senescence of a monocarpic plant such as red pepper is leaf senescence (Leshem *et al.* 1986; Noodén 1988). Although a leaf senescence occurs in an age-dependent manner (Batt and Woolhouse 1975; Hensel *et al.* 1993; Jiang *et al.* 1993), the initiation of a leaf senescence can be induced by a variety of external factors such as temperature, mineral deficiency, drought and pathogen infection, and by many internal factors such as plant growth regulators, reproduction, shading, and physical constraints (Thomas and Stoddart 1980; Smart 1994). Especially, a sequential leaf senescence seems to result from competition for resources between the lower leaves and younger foliage. Accordingly, the limited physical space and nutrient deficiency in a pot might have brought about the decrease in the photochemical efficiency for the control and 0.5 Gy leaves at 3 DA3I. In contrast, the higher Fv/Fm values in the other irradiation groups are possibly due to the delayed development as shown in Fig. 1.

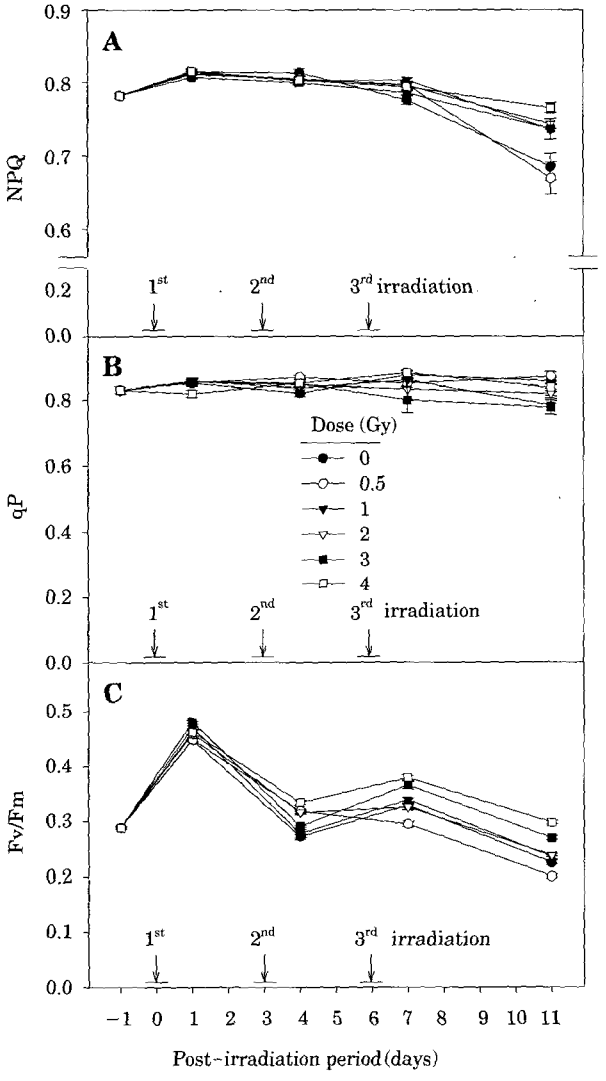


**Fig. 2.** Changes of the Chl fluorescence parameters in the red pepper leaves by a serial irradiation of different doses of gamma rays. Fv/Fm, the maximum photochemical efficiency of PSII; qP and NPQ, the photochemical and non-photochemical quenching. Error bars represent the S.E. (n = 6).

After the 3rd irradiation, the photochemical use of the absorbed light energy, qP, appeared to be a little lower in the 1-4 Gy groups than in the control and 0.5 Gy ones (Fig. 2B). In contrast, the non-photochemical dissipation of the light energy, NPQ, was rather higher in the irradiation groups except 0.5 Gy (Fig. 2C). Generally, the NPQ occurs in almost all the photosynthetic eukaryotes and it helps to regulate and protect the photosynthesis in environments in which the light energy absorption exceeds the capacity for light utilization (Horton *et al.* 1994; Müller *et al.* 2001). Therefore, the amount of NPQ is an indicator of the stress severity relating to the excess of the absorbed light. Taken together with the qP data, the higher NPQ values in the 3 and 4 Gy groups at 1 and 3 DA3I suggest that the serial irradiation could behave as a stress factor at least in these groups after the 3rd irradiation. Furthermore, this possibility implies that at least the growth inhibition in the irradiation groups at 3 DA3I might be partly due to an alteration in the photosynthesis, a reduction in the photochemical use of the absorbed light energy. Never-

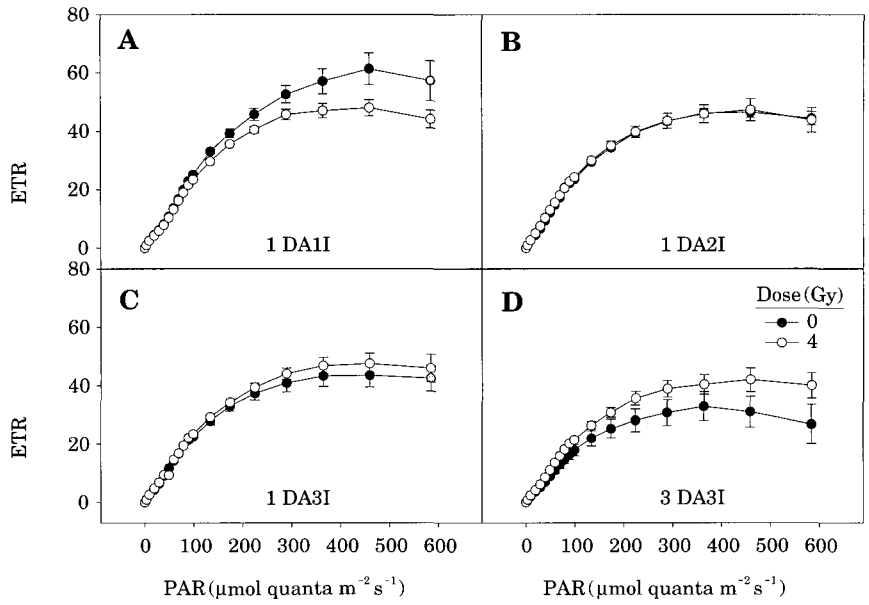


**Fig. 3.** Difference in the light response pattern between the control and irradiated red pepper leaves during a serial gamma-irradiation. ETR, the apparent rate of the photosynthetic electron transport; PAR, photosynthetically active radation. DA1I, DA2I, and DA3I represent day after 1st, 2nd, and 3rd irradiation, respectively. Error bars represent the S.E. (n = 6).

theless, as stated in Fig. 2A, the overall changes in the growth and photosynthesis during the post-irradiation period did not allow for a causal relation between them.

The light responses of the irradiation groups were distinguishable from that of the control one. The apparent rates of the photosynthetic electron transport, ETRs, decreased in the control group during the post-irradiation period, depending on the photosynthetically active radiation (PAR), while they remained relatively constant in the 4 Gy one (Fig. 3). Accordingly, all the irradiation groups had lower ETRs at 1 DA1I and higher ETRs at 3 DA3I than the control one (data not shown for the irradiation groups except 4 Gy). These results overall are in good agreement with the higher Fv/Fm values in the 1-4 Gy groups at 3 DA3I (Fig. 2A). Therefore, it's suggested that possibly a serial gamma-irradiation could protect against a reduction in the photosynthetic activity by a leaf senescence.

In conclusion, our data indicate that the stimulatory effect of an ionizing radiation on the plant growth could be determined by the incident dose of single irradiation rather than by the cumulative one of a serial irradiation. However, the growth stimulation induced by a low dose radiation was not positively correlated with an alteration in the photosynthetic activity. Instead, we propose the possibility that an ionizing radiation may partly protect against a leaf senescence by delaying the development of the plants.

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