Growth and Mineral Contents of Spinach (*Spinacia oleracea* L.) and Radish (*Raphanus sativus* L.) as Related with a Low Dose Gamma Irradiation

Jun-Kwon Hwangbo*, Jae-Sung Kim, Ji Hyeok Lim, Myung Hwa Baek, Byung Yeoup Chung and Jin-Hong Kim

Korea Atomic Energy Research Institute (KAERI), Yusong, Daejeon 305-303, Korea

Abstract - This study was to evaluate the effects of gamma irradiation on the germination, nutrient concentrations and growth of spinach and radish. Both the spinach and radish seeds exhibited relatively higher germination rates in response to the low doses of gamma irradiation compared to the non-irradiated control. Leaf DW of the radish did not respond to gamma irradiation but that of the spinach increased significantly in response to a gamma radiation of 4 Gy ($P < 0.05$). Leaf growth parameters of the spinach including the leaf area and SLA (leaf area/leaf dry weight) also demonstrated increased responses to gamma irradiation. R/S (root dry weight/shoot dry weight), root DW and root length of the spinach exhibited a positive response to gamma irradiation while those of the radish did not. In contrast, SRL (root length/root dry weight) significantly decreased with gamma irradiation at 8 Gy for the spinach, but not for the radish. The tissue nitrogen concentrations of the spinach showed an increased response to gamma irradiation while that of the radish did not. Furthermore, higher concentrations of phosphorus, potassium, calcium and magnesium were found in the irradiated spinach, but not in the irradiated radish. It seems that the non-specific physiological and/or biochemical activities of spinach might be accelerated by gamma irradiation, possibly accounting for the stimulation of nutrient uptake from the root media and early biomass accumulation in the current study.

Key words : gamma irradiation, germination, growth, biomass allocation, spinach, radish

INTRODUCTION

The growth and survival of native and crop plants could be affected by radioactive environments with the materials released during weapon production or testing, industrial waste disposal and nuclear power production (Thiede *et al.* 1995). Whether or not plants are influenced by the radiation positively or negatively with regard to growth and survival is dependent on the species, developmental stages, environmental conditions and irradiation doses (Luckey 1980). For several decades, the negative impacts of a high dose of radioactive radiation on plants have been well documented and established while the beneficial effects of a low dose ionizing radiation are not, mainly due to the large variations in the plant responses (Gaur 1985; Sheppard and Regitnig 1987; Sato and Matsui 1995; Yoon *et al.* 2000).

The stimulating effect induced by low doses of agents that are otherwise harmful at high doses on biological organisms was termed as hormesis by Southam and Ehrlich (1943); high concentrations of bark extract re-

* Corresponding author: Jun-Kwon Hwangbo, Tel. 042-868-8772, E-mail. hwangbo24@lycos.co.kr

tarded fungi growth, but a low concentration of this agent stimulated fungi growth. A low dose of ionizing radiation is known to be one of those agents producing beneficial effects and there are numerous reports that ionizing radiation, irrespective of its sources, has stimulating effects on the germination and subsequent growth of various plants at low doses (Koep and Kramer 1981; Sheppard and Evenden 1986; Lee *et al.* 1998; Charbaji and Nabulsi 1999; Chakravarty and Sen 2001), despite the fact that a high dose of ionizing radiation invariably produced detrimental impacts on the organisms (Sato and Matsui 1995; Wang and You 2000).

For instance, irradiation doses between 5 and 10 Gy significantly increased the fruit yield of two tomato varieties (Sidrak and Suess 1973) and the weight and volume growth of the root and bulb were stimulated in radish and onion, respectively, after 5-25 Gy gamma irradiation (Gaur 1985). Debates are still ensuing on the occurrence of radiation hormesis and its mechanism in plants, but free radical-mediated biochemical and physiological effects of ionizing radiation, e.g. causing alterations in the growth rate and/or biomass allocation (Thiede *et al.* 1995), are proposed as a possible candidate for the hormesis mechanisms (Luckey 1980; Miller and Miller 1987).

In this preliminary study, we examined the alterations in the growth parameters and biomass allocation of spinach and radish such as specific leaf area (SLA), root/shoot ratio (R/S), specific root length (SRL) as well as dry weight accumulation after a low dose of gamma irradiation, through which a better understanding might be possible for the plant growth and survival under radioactive environments.

MATERIALS AND METHODS

Nong-Woo Bio Company provided the seeds of spinach and radish in this study. Both seeds were stored at room temperature after harvest in 2002 and their moisture contents were 4.40±0.23 and 4.79±0.35% on a dry weight basis, respectively. Seed moisture content was measured with an Infrared Moisture Analyzer (SMO 01, Scaltec, Germany). Dry seeds of both species were subjected to gamma radiation with doses of 4 and 8 Gy generated by a gamma irradiator (^{60}Co, *ca.* 150 TBq of capacity, AECL) in the Korea Atomic Energy Research Institute (KAERI). Dose rate was 2 Gy hr^{-1}. Radiation doses used in this study were low enough that both seedlings germinated from the irradiated seeds did not exhibited the stunted growth or visible damages (data not shown). Irradiated seeds were sown in the pot (Ø 10 cm) with Horticulture Nursery Media® (BioMedia) and germinated in a greenhouse, Chung-Nam National University, in May 2003.

Seedlings were harvested 2 weeks after sowing. Leaf area was measured with a leaf area meter (LICOR, Model LI-3100, USA) and root length was evaluated with the method of Tennant (1975). Seedling samples were separated into root and shoot, oven-dried at 70°C for 48 hr and weighed. Growth parameters including SLA (leaf area/ leaf dry weight), SRL (root length/root dry weight) and R/S (root dry weight/shoot dry weight) were calculated. Root and shoot samples of each irradiation treatment were pooled in order to obtain the sample weight appropriate for the nutrient analysis, which led to only two replicates per irradiation treatment. The tissue nitrogen (N) concentration was determined with a Kjeldahl digestion and distillation system (BDD unit B-324, Japan). Phosphorus (P) concentration was measured with the method of Chapman and Pratt (1961). The tissue mineral concentrations of potassium (K), calcium (Ca) and magnesium (Mg) were determined with an atomic absorption/flame emission spectrophotometer (AA-680, Shimadzu, Japan). The data was analyzed by Tukey's HSD test (SPSS 10.0, USA).

RESULTS AND DISCUSSION

1. Germination and growth parameters with gamma irradiation

Both the spinach and radish seeds exhibited relatively higher germination rates in response to low doses of gamma irradiation compared to the non-irradiated control (Table 1), although this effect of pre-sowing irradiation was not statistically significant, possibly due to the large variations within each treatment. However, this increased germination rate is not a rare phenomenon when the seeds are irradiated with a low dose gam-

Table 1. Germination and growth parameters of spinach and radish with gamma irradiation. Values with same letters are not significantly different within each row and species at the level of 0.05 (Tukey test)

Species / Dose (Gy)	Spinach			Radish		
	0	4	8	0	4	8
Germination (%)	67.0a	70.5a	75.5a	77.0a	84.0a	82.0a
Leaf DW (mg/leaf)	16.1a	24.2b	20.5a	–	–	–
Leaf area (cm^2/leaf)	4.02a	6.09a	5.84a	16.6a	15.4a	19.0a
SLA	251.4a	286.2a	299.3a	–	–	–
Root DW (mg/plant)	15.2a	28.6a	28.5a	49.3a	53.0a	44.3a
Root length (cm/plant)	62.5a	97.6a	80.6a	9.18a	8.65a	7.40a
Shoot DW (mg/plant)	74.4a	116.2a	115.5a	626.3a	533.3a	614.0a
R/S	0.20a	0.22a	0.23a	0.08a	0.10a	0.07a
Total DW (mg/plant)	89.6a	144.8a	144.1a	675.7a	586.3a	658.3a

ma radiation (Sheppard and Evenden 1986; Lee *et al.* 1998; Lee *et al.* 2002; Hwangbo *et al.* 2003).

Leaf DW of the radish did not respond to gamma irradiation but that of the spinach increased significantly in response to a gamma radiation of 4 Gy (Table 1, $P < 0.05$), which was also shown in a study with sunflowers (Thiede *et al.* 1995). According to Gaur (1985), it is suggested that the increased leaf growth of the lettuce could be due to the improved leaf area and/or photosynthetic efficiency after x-ray irradiation. Furthermore, more photosynthates resulting from an increased photosynthesis are allocated into the leaf, strengthening the leaf growth, when maize was irradiated with a low dose gamma radiation (Koep and Kramer 1981). Other leaf growth parameters of the spinach including leaf area and SLA also demonstrated the same responses although the extent was not enough to be statistically significant (Table 1).

The plant growth is highly likely to gain benefits from both the increased leaf area leading to an increased assimilation of the atmospheric carbon in a leaf and the higher SLA resulting from an increased efficiency of utilizing and/or allocating more carbon assimilates in a leaf (Poorter 1990; Poorter and Remkes 1990), which was also reflected in an enhanced leaf DW, shoot DW and ultimately total DW in this study (Table 1). These positive effects of low dose gamma irradiation on the aboveground biomass accumulation and/or biomass allocation pattern were commonly found in other studies (Koep and Kramer 1981; Sheppard and Evenden 1986; Thiede *et al.* 1995).

Root to Shoot ratio (R/S), root DW and root length of the spinach exhibited a positive response to gamma irradiation while those of the radish did not (Table 1), suggesting that the plants differ in their sensitivity to gamma irradiation (Gaur 1985). It also seems that the response of the plant root growth to a low dose gamma irradiation varied with the doses irradiated, growth condition as well as the developmental stages (Miller and Miller 1987; Al-Safadi and Simon 1996). For example, it was observed that the positive effects of gamma irradiation on the root growth were more evident when the plants were grown in the growth chamber than either the greenhouse or the field (Yoon *et al.* 2001).

Active shoot growth found in this study could be partly supported by these improved root growth parameters since increased root biomass and length facilitate soil exploitation for water and nutrient uptake (Atkinson 1991). Although the biochemical and physiological mechanisms by which gamma irradiation induces a positive root growth has not been well defined as yet, it is suggested that a low dose gamma irradiation increases the mitotic index of the root tip cells (Okamoto and Tatara 1995), probably leading to an improved root growth of the irradiated plants.

In contrast to the other root growth parameters mentioned above, SRL (specific root length) significantly decreased with gamma irradiation at 8 Gy for the spinach, but not for the radish (Fig. 1). Al-Safadi and Simon (1996) observed that doses below 50 Gy increased the root weight of carrot by about 35% compared to the control (non-irradiated), but above 50 Gy it decreased the root weight significantly. Therefore, it is possible that a decrease in the SRL of the spinach at 8 Gy of gamma

Table 2. Tissue nutrient concentrations in spinach and radish with gamma irradiation

Species / Dose (Gy)	Spinach			Radish		
	0	4	8	0	4	8
N (%)	1.93*	2.29	2.40	–	–	–
K (%)	1.06	1.31	1.15	0.58	0.32	0.30
P (ppm)	2406	6825	7451	4199	3982	3945
Ca (ppm)	1222	1203	1061	873.4	692.1	742.4
Mg (ppm)	1045	1335	1603	610.0	490.0	475.0

*The values are the means of two replicates per irradiation treatment due to the pooling of samples for mineral analysis.

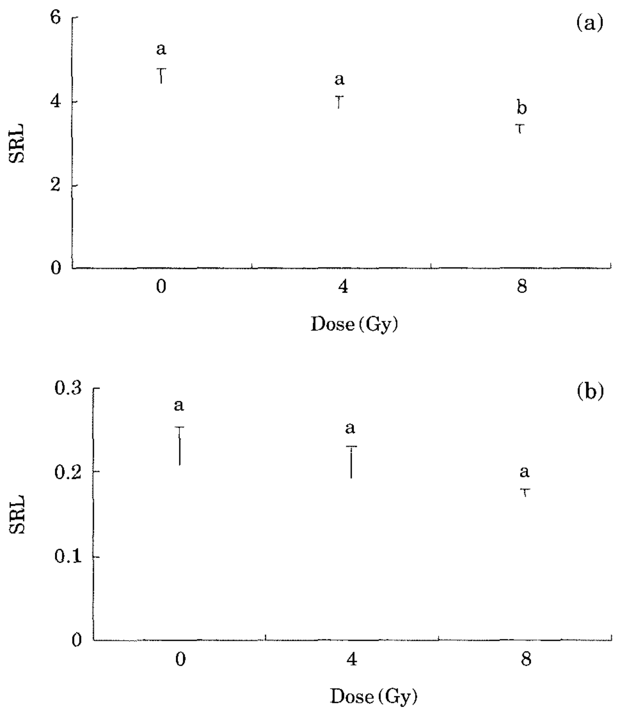

Fig. 1. Specific root length (SRL) of (a) spinach and (b) radish as a function of gamma irradiation. Bars represent means±standard error. Bars with same letters are not significantly different at the level of 0.05 (Tukey test).

irradiation was partly accounted for by the relatively higher increase in the root dry weight rather than the root length (Table 1), and the root dry weight could be a more sensitive biological parameter than the root length when the potential effects of a low dose gamma irradiation are evaluated for the plant growth, particularly for the belowground parts.

2. Nutrient contents with gamma irradiation

The tissue nitrogen concentration of the spinach showed an increased response to gamma irradiation (Table 2). The tissue nitrogen concentration of the radish, unfortunately, was not determined because the dry weight of the radish sample was not enough to be analyzed. It is well established that nitrogen is the most essential element in determining the potential of plant growth (Mae 1997), which may be accounted for by the fact that the tissue nitrogen concentration typically showed a positive relationship with the mass-based maximum photosynthesis (Evans *et al.* 2001). Accordingly it is not surprising that the biomass accumulation of the spinach, particularly the leaf dry weight, increased after gamma irradiation in this study (Table 1). Furthermore, higher concentrations of phosphorus, potassium and magnesium were found in the irradiated spinach with exception of calcium, but not in the irradiated radish (Table 2). According to Korosi and Krakkai (1983), an increased phosphorus uptake by plants grown from irradiated seeds occurred, which was reflected in the increased foliar phosphorus concentration. It seems that the non-specific physiological and/or biochemical activities of spinach might be accelerated by gamma irradiation (Sheppard and Evenden 1986), possibly accounting for the stimulation of nutrient uptake from the root media and early biomass accumulation in the current study.

ACKNOWLEDGMENTS

This research was carried out under the Nuclear R & D Program of the Ministry of Science and Technology, Korea.

REFERENCES

Al-Safadi B and PW Simon. 1996. Gamma irradiation-induced variation in carrots (*Daucus carota* L.). J. Amer. Soc. Hort. Sci. 121:599-603.

Atkinson D. 1991. Plant root growth: An ecological perspective. Blackwell Scientific Publications, Oxford, UK.

Chapman HD and PF Pratt. 1961. Method of analysis for soil, plants and water. Univ. of Calif. Div. Agr. Sci., Berkeley, CA.

Charbaji T and I Nabulsi. 1999. Effect of low doses of gamma irradiation on *in vitro* growth of grapevine. Plant Cell Tissue and Organ Culture 57:129-132

Chakravarty B and E Sen. 2001. Enhancement of regeneration potential and variability by gamma irradiation in cultured cells of *Scilla indica*. Biol. Planta 44:189-193.

Evans CA, EK Miller and AJ Friedland. 2001. Effect of nitrogen and light on nutrient concentrations and associated physiological responses in birch and fir seedlings. Plant and Soil 236:197-207.

Gaur BK. 1985. Physiological responses of plants to ionizing radiation. Indian J. Plant Physiol. 28:1-13.

Hwangbo J-K, J-S Kim, JH Lim, MH Baek and BY Chung. 2003. Alterations in seed vigour and viability of soybean (*Glycine max* L.) as related with accelerated seed ageing and low dose gamma irradiation. Korean J. Crop Sci. 48:334-338.

Koepp R and M Kramer. 1981. Photosynthetic activity and distribution of photoassimilated ^{14}C in seedlings of *Zea mays* grown from gamma-irradiated seeds. Photosynthetica 15:484-489.

Korosi F and I Krakkai. 1983. Effect of gamma irradiation of *Phaseolus vulgaris* L seeds on $^{32}PO_4^{3-}$ uptake of seedlings and its translocation pattern. Environ. Exp. Bot. 23:149-153.

Lee EK, J-S Kim, YK Lee and YB Lee. 1998. Effect of low dose gamma ray irradiation on the germination and growth in red pepper (*Capsicum annuum* L.). J. Kor. Soc. Hort. Sci. 39:670-675.

Lee H-Y, J-S Kim, MH Baek, YK Lee and DS Im. 2002. Effect of low dose gamma radiation on the growth, activities of enzymes and photosynthetic activities of gourd (*Lagenaria siceraria*). Korean J. Environ. Biol. 20:197-204.

Luckey TD. 1980. Hormesis with ionizing radiation. pp. 1-10. CRC Press, Boca Raton Publisher, In Japanese Soft Science Inc., Tokyo.

Mae T. 1997. Physiological nitrogen efficiency in rice: nitrogen utilization, photosynthesis and yield potential. Plant and Soil 196:201-210.

Miller MW and WM Miller. 1987. Radiation hormesis in plants. Health Physics 52:607-616.

Okamoto H and A Tatara. 1995. Effects of low dose gamma irradiation on the cell cycle duration of barley roots. Environ. Exp. Bot. 35:379-388.

Poorter H. 1990. Interspecific variation in relative growth rate: On ecological causes and physiological consequences. In: Causes and consequences of variation in growth rates and productivity in higher plants. Eds. Lamberts H, ML Cambridge, H Konings and TL Pons. SPA Academic Publishing, The Hague, The Netherlands.

Poorter H and C Remkes. 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83:553-559.

Sato M and T Matsui. 1995. Differential radiosensitivity between seeds and seedlings of *Nicotiana debneyi* in respect of chlorogenic acid content. Plant Sci. 109:139-144.

Sidrak GH and A Suess. 1973. Effects of low doses of gamma radiation on the growth and yield of two varieties of tomato. Radiation Bot. 13:309-314.

Sheppard SC and WG Evenden. 1986. Factors controlling the response of field crops to very low doses of gamma irradiation of the seed. Can. J. Plant Sci. 66:431-441.

Sheppard SC and PJ Regitnig. 1987. Factors controlling the hormesis response in irradiated seed. Health Physics 52:599-605.

Southam CM and J Ehrlich. 1943. Effect of extract of western red-cider heartwood on certain wood decaying fungi in culture. Phytochemistry 33: 517-522.

Tennant D. 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63:995-1001.

Thiede ME, SO Link, RJ Fellows and PA Beedlow. 1995. Effects of gamma radiation on stem diameter growth, carbon gain and biomass partitioning in *Helianthus annuus*. Environ. Exp. Bot. 35:33-41.

Wang Z and R You. 2000. Changes in wheat germination following gamma ray irradiation: an *in vivo* electronic paramagnetic resonance spin-probe study. Environ. Exp. Bot. 43:219-225.

Yoon YM, HI Cho, SH Chang, NB Kim, J-S Kim and J-G Kim. 2000. Effects of low dose gamma radiation on the root growth of soybean cultivars. Korean J. Environ. Agr. 19:394-400.

Manuscript Received: October 23, 2003
Revision Accepted: November 14, 2003
Responsible Editorial Member: Jin Kyu Kim (KAERI)