

The effect of gamma radiation on seed germination and seedling growth of *Lathyrus chrysanthus* Boiss. under *in vitro* conditions



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ABSTRACT

The effects of radiation at different doses (0, 50, 100, 150, 200 and 250 Gy) of radioactive cobalt (⁶⁰Co) γ rays on seed germination and seedling growth of *Lathyrus chrysanthus* were investigated under *in vitro* conditions. The results showed that irradiated seeds had increased seed germination percentage, seedling and root lengths, seedling fresh weight, seedling dry matter content and total chlorophyll content in the leaves of seedlings. However, at higher doses stress was evident and significant decreases in all parameters were observed. The highest seed germination percentage was recorded as 62.4%, 7 days after study initiation when seeds were irradiated with 150 Gy gamma dose, while 100 Gy gamma dose was ranked in second order. Fourteen days after culture initiation, the best shoot growth initiation was again obtained from 150 Gy gamma dose as 75.7% and this was followed by 100 Gy gamma radiation as 74.6%. Gamma doses over 150 Gy resulted in sharp decreases in all parameters examined. On the 14th day, the highest shoot and root lengths were recorded from 150 Gy gamma dose as 1.2 and 2.9 cm, respectively. Twenty eight days after study initiation, the highest values of seedling and root lengths, seedling fresh weight, seedling dry matter content and total chlorophyll content were noted from 50 Gy gamma radiation as 9.7 and 6.3 cm, 0.39, 0.09 g (23.08%) and 471.6 $\mu\text{g/g}$ fresh tissue, respectively. This study is important from the aspect of showing that stimulatory effect of low gamma doses for germination and seedling growth may not be the same.

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1. Introduction

Gamma rays as an ionizing radiation affect plant growth and development by inducing cytological, biochemical, physiological and morphological changes in cells and tissues via producing free radicals in cells (Gunckel and Sparrow, 1961; Kim et al., 2004; Wi et al., 2005). The higher doses of gamma radiation were reported to be inhibitory (Radhadevi and Nayar, 1996; Kumari and Singh, 1996), whereas lower doses may be stimulatory. Low doses of gamma rays have been reported to increase cell proliferation, germination, cell growth, enzyme activity, stress resistance, and

crop yields (Charbaji and Nabulsi, 1999; Baek et al., 2005; Chakravarty and Sen, 2001; Kim et al., 2000, 2005).

Using aseptic seedlings as source of explants is highly recommended in tissue culture studies (Yildiz et al., 1997). The genus *Lathyrus*, from the family *Fabaceae*, consists of 187 taxa (Allkin et al., 1983) which are found in the Mediterranean region, Asia Minor, East Africa, North and South America (Kupicha, 1977; Simola, 1986). *Lathyrus chrysanthus* is being evaluated as an ornamental plant with their big, attractive colored and fragrant flowers (Davis, 1970). Because of the fact that *in vitro* seed germination frequency is low due to dormancy in *L. chrysanthus*, obtaining high frequency healthy seedlings which will be used as explant sources for further studies such as shoot regeneration and transformation, is difficult. Various methods such as scarification of seed coat, temperature and light treatments, growth regulators and chemicals have been widely used to break the dormancy of seed. It has been shown that sodium hypochlorite solutions can also be used successfully as a

Abbreviations: ⁶⁰Co, cobalt 60; Gy, gray; kGy, kilogray; MS, Murashige and Skoog; γ , gamma.

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dormancy-breaking agent in seeds of *L. chrysanthus* Boiss. (Telci et al., 2011).

This study was aimed to determine the effects of different gamma doses on seed germination and seedling growth of *L. chrysanthus* seeds and dormancy breaking under *in vitro* conditions.

2. Materials and method

2.1. Plant material, seed irradiation and germination

L. chrysanthus seeds of an ecotype (Diyarbakir) found in south-east of Turkey were used in the study. Seeds were irradiated with different doses (0-control, 50, 100, 150, 200 and 250 Gy) of ^{60}Co γ rays at 0.8 kGy h^{-1} at the Turkish Atomic Energy Authority, Sarayköy Nuclear Research and Training Center, Sarayköy, Ankara. For each gamma dose, two sets for two parallel experiments each with 100 seeds were irradiated separately. Fricke and alanine dosimeters were used for dose mapping and determination of dose rates of gamma source. Seeds were irradiated along with a dosimeter for each dose to be sure that ionization was uniform.

Prior to germination, the seeds were surface-sterilized with a 3.75% sodium hypochlorite solution at $35 \text{ }^\circ\text{C}$ temperature for 15 min as reported by Telci et al. (2011) to obtain healthy uninfected seedlings in large quantities under *in vitro* conditions.

Three replicates of 30 sterilized seeds were placed between filter papers in Petri dishes containing each 6 mL of distilled water. Petri dishes were incubated at $15 \pm 1 \text{ }^\circ\text{C}$ in the dark for 7 days for seed germination. At 14 days after study initiation, germinated seeds were transferred to Magenta vessels ($12 \times 12 \text{ cm}$). These vessels contained an autoclaved basal medium of Murashige and Skoog's (MS) mineral salts and vitamins (Murashige and Skoog, 1962), 3% sucrose, and 0.7% agar. The pH of the medium was adjusted to 5.8 prior to autoclaving. Then, all cultures were transferred to growth chamber for incubation at $25 \pm 1 \text{ }^\circ\text{C}$ under cool white fluorescent light ($27 \mu\text{mol m}^{-2} \text{ s}^{-1}$) with a 16 h light/8 h dark photoperiod.

2.2. Observations

For each gamma dose, three replicates were tested, and there were 30 seeds per replication. All experiments were repeated twice. That means two parallel experiments were carried out at the same time, each with 3 replicates of 30 seeds to guarantee the accuracy of the study. Seed germination percentage was determined at the end of 7th day while shoot growth initiation, shoot and root lengths were recorded 14 days after culture initiation (ISTA, 2003). On 28 days after study initiation, seedling and root lengths, seedling fresh weight, seedling dry matter content and total chlorophyll content in the leaves of seedlings were recorded. A seed was considered germinated when the emerged radicle reached to 2 mm.

2.3. Determination of chlorophyll contents

Total chlorophyll content was calculated in leaves of seedlings according to the protocol described by Curtis and Shetty (1996). Fresh leaf tissue of 50 mg was put in 3 mL methanol and kept in total darkness at $23 \pm 1 \text{ }^\circ\text{C}$ for 2 h. By this way, chlorophyll in fresh tissue passed through into the methanol. After 2 h, absorbancies were determined at 665 and 650 nm. Total chlorophyll content was calculated as $\mu\text{g/g}$ fresh tissue.

2.4. Statistical analysis

Data were statistically analyzed by Duncan's multiple range test using SPSS for Windows. Data given in percentages were subjected to arcsine (\sqrt{X}) transformation before statistical analysis (Snedecor and Cochran, 1967).

3. Results and discussion

Effect of different gamma doses on seed germination and shoot growth initiation percentages, shoot and root lengths, seedling fresh weight, seedling dry matter content and total chlorophyll content in the leaves of 28-day-old seedlings is shown in Table 1. Reported stimulatory effects of low gamma doses were also observed in our study at 150 Gy gamma irradiation, where the best results were obtained for seed germination percentage at the end of the 7th day, and shoot growth initiation percentage, shoot and root lengths at the 14th day (Charbaji and Nabulsi, 1999; Kim et al., 2000, 2005; Chakravarty and Sen, 2001; Baek et al., 2005). However, the inhibitory effect of gamma radiation on seed germination percentage was observed in the doses over 150 Gy as reported formerly (Radhadevi and Nayar, 1996; Kumari and Singh, 1996; Chaudhuri, 2002). Seed germination percentage increased gradually as gamma doses increased up to 150 Gy at the end of 7th day. The highest seed germination percentage was 62.4% from the 150 Gy gamma treatment. Stimulatory effect of low dose of gamma irradiation on seed germination could be attributed to the activation of RNA or protein synthesis (Abdel-Hady et al., 2008).

Fourteen days after study initiation, similar results were obtained for shoot growth initiation percentage, shoot and root lengths. The highest shoot growth initiation percentage, shoot and root lengths were recorded again from 150 Gy gamma dose as 75.7%, 1.2 cm and 2.9 cm, respectively. The root length obtained from seeds irradiated with 150 Gy gamma was significantly increased as 63.2 percent from 1.8 cm in control application (0 Gy) to 2.9 cm which was confirmed by Melki and Marouani (2010). When the gamma doses increased over 150 Gy, all parameters decreased significantly (Fig. 1A, Table 1). Our findings were parallel to that of Chaomei and Yanlin (1993) who reported that higher doses of gamma irradiation decreased seed germination and plant growth.

At the end of the study (at the 28th day), the highest results were obtained from 50 Gy treatment in the parameters of seedling and root lengths, seedling fresh weight, seedling dry matter content and total chlorophyll content. In control and in the doses over 50 Gy, results were lower than the ones of 50 Gy. Seedlings grown from seeds irradiated with 50 Gy gamma dose were observed to grow faster than that of irradiated with other doses (Fig. 1B). The highest scores regarding seedling and root lengths were 9.7 cm and 6.3 cm, respectively. The lowest results recorded from 250 Gy gamma radiation in all cases could be attributed to inhibitory effect of higher gamma ray (Table 1). Our findings were parallel to the ones who have reported that seed irradiation with high doses of gamma rays disturb protein synthesis (Xiuzher, 1994), water exchange and enzyme activity (Rabie et al., 1996), production of growth hormones and indole acetic acid (IAA) (Chandorkar and Clark 1986), leaf gas-exchange (Stoeva and Bineva, 2001), water exchange and enzyme activity (Stoeva et al., 2001).

The highest result was recorded as 0.39 g in seedling fresh weight in 50 Gy gamma irradiation. The highest seedling dry matter content was again recorded as 0.09 in g and 23.08% of seedling fresh weight when seeds were irradiated with 50 Gy gamma. Seedling dry matter content in percentage decreased gradually by increasing gamma irradiation dose. The difference between fresh and dry weights gives the tissue water content. From

Table 1Effects of different gamma doses on *in vitro* seed germination, seedling growth, seedling and root lengths, seedling fresh weight, seedling dry matter content and total chlorophyll content in leaves of *L. chrysanthus* Boiss.

Gamma doses (Gy)	Day 7	Day 14			Day 28					
	Seed germination (%) ^a	Shoot growth initiation (%) ^b	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Root length (cm)	Seedling fresh weight (g)	Seedling dry matter content (g %)	Seedling water content (g %)	Total chlorophyll content (µg/g fresh tissue)
0	35.0 ± 5.0c	40.0 ± 2.0 b	0.5 ± 0.05d	1.8 ± 0.10cd	8.0 ± 0.76b	4.0 ± 0.14b	0.28 ± 0.02bc	0.05 ± 0.005bc – 17.86	0.23 ± 0.01b – 82.14	460.8 ± 33.6a
50	26.7 ± 2.9c	45.0 ± 3.2b	0.7 ± 0.03c	2.1 ± 0.14bc	9.7 ± 0.29a	6.3 ± 0.34a	0.39 ± 0.02a	0.09 ± 0.004a – 23.08	0.30 ± 0.02a – 76.92	471.6 ± 41.8a
100	48.6 ± 3.5b	74.6 ± 4.7a	0.9 ± 0.047b	2.4 ± 0.08b	6.0 ± 0.38c	3.5 ± 0.16cd	0.30 ± 0.01b	0.06 ± 0.003b – 20.00	0.24 ± 0.01b – 80.00	321.2 ± 39.4b
150	62.4 ± 2.5a	75.7 ± 3.3a	1.2 ± 0.08a	2.9 ± 0.15a	4.1 ± 0.13d	4.5 ± 0.32b	0.27 ± 0.02bc	0.05 ± 0.005bc – 18.52	0.22 ± 0.01bc – 81.48	241.0 ± 63.0bc
200	35.4 ± 1.7c	68.8 ± 2.8a	0.6 ± 0.05cd	1.6 ± 0.11d	4.0 ± 0.25d	4.5 ± 0.20b	0.25 ± 0.02cd	0.04 ± 0.009cd – 16.00	0.21 ± 0.01bc – 84.00	153.2 ± 13.7cd
250	14.3 ± 1.5d	37.5 ± 2.0b	0.5 ± 0.01cd	1.3 ± 0.04e	2.0 ± 0.23e	3.0 ± 0.18d	0.22 ± 0.01d	0.03 ± 0.005d – 13.64	0.19 ± 0.01c – 86.36	105.0 ± 11.6d

The values represent the mean of 3 replications.

Values within a column followed by different letters are significantly different at the 0.01 level.

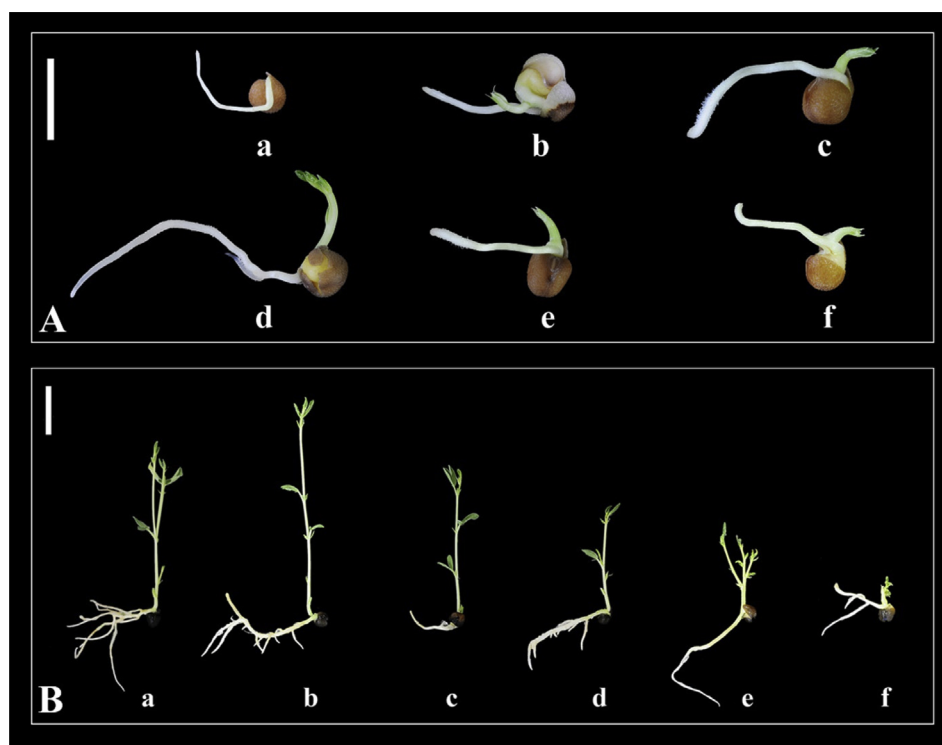
^a Seed germination means germinated seeds out of total seed number.^b Shoot growth initiation means developed shoots out of total seed number.

Fig. 1. **A.** *In vitro* seed germination and shoot growth from *L. chrysanthus* seeds irradiated with (a) 0 Gy, (b) 50 Gy, (c) 100 Gy, (d) 150 Gy, (e) 200 Gy and (f) 250 Gy gamma doses at the 14th day. (Bar = 1 cm). **B.** *In vitro* seedling growth from *L. chrysanthus* seeds irradiated with (a) 0 Gy, (b) 50 Gy, (c) 100 Gy, (d) 150 Gy, (e) 200 Gy and (f) 250 Gy gamma doses at the 28th day. (Bar = 2 cm).

the results, tissue water content was calculated as 0.30 g (0.39–0.09) which was 76.92% of seedling fresh weight. It was observed that seedling water content in percentage was increased by increasing gamma doses. This was due to the fact that seedling fresh weight and seedling dry matter content were both decreased by increasing gamma doses over 50 Gy. In our study, higher results regarding seedling fresh weight and seedling dry matter content in 50 Gy gamma irradiation were confirmed by Dale (1988) who noticed that cell enlargement by water absorption, cell vacuolation, and turgor-driven wall expansion is the main reason of fresh weight

increase. The increase in dry weight is closely related to the cell division and new material synthesis (Sunderland, 1960). In the current study, low dose of gamma irradiation may have softened the seed coat as reported by Kovács and Keresztes (2002). A softened seed coat may have increased the permeability which caused to high tissue metabolic activity by increasing water and hormone uptake from the medium. Thus, higher seedling fresh weight, seedling dry matter content in g and in percentage and total chlorophyll content obtained from 50 Gy irradiation could be attributed to an increase in the absorption of water and other

components from the basal medium as reported by Melki and Marouani (2010). In our case, root elongation of plants grown from irradiated seeds by 50 Gy gamma allowed plants to maintain a higher water level and stable membrane structure compared to plants from non-irradiated seeds as reported by Melki and Sallami (2008).

From our findings, it could be that the lower levels of all parameters obtained from 250 Gy gamma irradiation were due to a decreased water uptake from the environment as reported by Rabie et al. (1996) and Stoeva and Bineva (2001), and consequently a reduced mobilization of solutes.

The highest results regarding total chlorophyll content at 50 Gy was recorded as 472 µg/g fresh tissue (Table 1). At this dose, seedlings were well grown and higher results indicated high metabolic activity of seedlings (Fig. 1B). Chlorophyll content of the leaf, which is accepted as an indicator of photosynthetic capacity of tissues (Pal and Laloraya, 1972; Wright et al., 1994; Nageswara et al., 2001), affects photosynthesis directly (Rensburg and Kruger, 1994; Kyparissis et al., 1995; Jagtap et al., 1998). Kim et al. (2004) reported that chlorophyll content of irradiated red pepper plant increased significantly. Our findings were confirmed by Koeppe and Kramer (1981) who reported that plants grown from irradiated seeds had higher photosynthetic activity than control.

Sheppard and Evenden (1986) reported that higher gamma irradiation has a deleterious effect on the growth of an organism while low doses stimulate growth which is known as 'hormesis'. The hormesis phenomenon is described as the stimulating effect of low levels of any stress factor on the growth of an organism (Szarek, 2005), is achieved by low gamma irradiation in the current study. Stimulatory effects of low doses of gamma were reported in many plants such as *Vitis vinifera* (Charbaji and Nabulsi, 1999) and *Pisum sativum* (Zaka et al., 2004). And also these positive effects of low gamma irradiation were reported in the characters of fruit weight increase and early ripening in tomato (Sidark and Suess, 1973), an increase in seed germination in *Avena fatua* L. (Maherchandani, 1975), plant growth increase in *Cucumis sativus* (Kuzin et al., 1986) and an increase in seed germination and root weight in carrot (Al-Safadi and Simon, 1995). From the results, it could be concluded that low doses of gamma radiation could simply be used for overcoming dormancy and for obtaining healthy seedlings in *L. chrysanthus* under *in vitro* conditions. Although the highest results in germination percentage at the end of 7th day, shoot growth initiation, shoot and root lengths at the end of 14th day were obtained from 150 Gy, the highest values in seedling and root lengths, seedling fresh weight, seedling dry matter content in g and in percentage and total chlorophyll content were recorded from 50 Gy gamma radiation 28 days after study initiation. Higher results obtained from 150 Gy gamma radiation at the end of the 14th day in seed germination and shoot growth initiations, shoot and root lengths could be attributed to an increase of enzyme activity, cell growth, water absorption and photosynthetic activity as reported by Charbaji and Nabulsi (1999), Baek et al. (2005), Chakravarty and Sen (2001), Kim et al., (2000, 2005). However, in the following days, from the parameters examined, it was seen that 150 Gy inhibited seedling growth significantly. The success of *in vitro* studies based firstly on the viability of seedling from which explant was excised. In our study, 50 Gy gamma radiation gave rise to the healthiest and well grown seedlings.

References

Abdel-Hady, M.S., Okasha, E.M., Soliman, S.S.A., Talaat, M., 2008. Effect of gamma radiation and gibberellic acid on germination and alkaloid production in *Atropa belladonna*. *Aust. J. Basic Appl. Sci.* 2 (3), 401–405.
 Al-Safadi, B., Simon, B.W., 1995. Gamma irradiation-induced variation in carrots (*Daucus carota* L.). *J. Am. Soc. Hortic. Sci.* 121, 599–603.

Allkin, R., Goyder, D.J., Bisby, F.A., White, R.J., 1983. List of species and subspecies in the Viciaeae. *Viciaeae Database Proj.* 1, 4–11.
 Baek, M.H., Kim, J.H., Chung, B.Y., Kim, J.S., Lee, I.S., 2005. Alleviation of salt stress by low dose gamma irradiation in rice. *Biol. Plant.* 49 (2), 273–276.
 Chakravarty, B., Sen, S., 2001. Enhancement of regeneration potential and variability by gamma irradiation in cultured cells of *Scilla Indica*. *Biol. Plant.* 44, 189–193.
 Chandorkar, K.R., Clark, G.M., 1986. Physiological and morphological responses of *Pinus strobus* L., and *Pinus sylestris* L. seedlings, subjected to low-level continuous gamma irradiation at a radioactive waste disposal area. *Environ. Exp. Bot.* 26, 259–270.
 Charbaji, T., Nabulsi, I., 1999. Effect of low doses of gamma irradiation on *in vitro* growth of grapevine. *Plant Cell Tissue Organ Cult.* 57, 129–132.
 Chaomei, Z., Yanlin, M., 1993. Irradiation induced changes in enzymes of wheat during seed germination and seedling growth. *Acta Agric. Nucleatae Sin.* 7, 93–97.
 Chaudhuri, K.S., 2002. A simple and reliable method to detect gamma irradiated lentil (*Lens culinaris* Medik.) seeds by germination efficiency and seedling growth test. *Radiat. Phys. Chem.* 64, 131–136.
 Curtis, O.F., Shetty, K., 1996. Growth medium effects on vitrification, total phenolics, chlorophyll, and water content of *in vitro* propagated oregano clones. *Acta Hortic.* 426, 498–503.
 Dale, J.E., 1988. The control of leaf expansion. *Annu. Rev. Plant Physiol Plant Mol. Biol.* 39, 267–295.
 Davis, P.H., 1970. Flora of Turkey and the East Aegan Island. In: Davis, P.H. (Ed.), *Lathyrus* L. Edinburgh University Press, Edinburgh, pp. 328–369.
 Gunckel, J.E., Sparrow, A.H., 1961. *Encycl. Plant Physiology*. In: Ruhland, W. (Ed.), *Ionizing Radiation: Biochemical, Physiological and Morphological Aspects of Their Effects on Plants*. Springer-Verlag, Berlin, pp. 555–611.
 ISTA, 2003. *International Rules for Seed Testing*. International Seed Testing Association, Basserdorf.
 Jagtap, V., Bhargava, S., Sterb, P., Feierabend, J., 1998. Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.). *Moench. J. Exp. Bot.* 49, 1715–1721.
 Kim, J.S., Lee, Y.K., Park, H.S., Back, M.H., Kim, D.H., 2000. Influence of low dose gamma radiation on the growth of maize (*Zea mays* L.) varieties. *Korean J. Environ. Agric.* 19, 328–331.
 Kim, J.H., Baek, M.H., Chung, B.Y., Wi, S.G., Kim, J.S., 2004. Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. *J. Plant Biol.* 47, 314–321.
 Kim, J.H., Chung, B.Y., Kim, J.S., Wi, S.G., 2005. Effects of *in vitro* gamma irradiation on growth, photosynthesis, and antioxidative capacity of red pepper (*Capsicum annuum* L.) plants. *J. Plant Biol.* 48, 47–56.
 Koeppe, R., Kramer, M., 1981. Photosynthetic activity and distribution of photo-assimilated ¹⁴C in seedlings of *Zea mays* grown from gamma-irradiated seeds. *Photosynthetica* 15, 484–489.
 Kovács, E., Keresztes, A., 2002. Effect of gamma and UV-B/C radiation on plant cells. *Micron* 33, 199–210.
 Kumari, R., Singh, Y., 1996. Effect of gamma rays and EMS on seed germination and plant survival of *Pisum sativum* L. and *Lens culinaris* Medic. *Neo Bot.* 4, 25–29.
 Kupicha, F.K., 1977. The delimitation of the tribe Viciaeae and the relationship of *Cicer* L. *Botanical J. Linn. Soc.* 74, 131–162.
 Kuzin, A.M., Vagabova, M.E., Vilenchik, M.M., Gogvadze, V.G., 1986. Stimulation of plant growth by exposure to low-level gamma radiation and magnetic field, and their possible mechanism of action. *Environ. Exp. Bot.* 26, 163–167.
 Kyparissis, A., Petropoulou, Y., Manetas, Y., 1995. Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiatae) under Mediterranean field conditions: avoidance of photoinhibitory damage through decreased chlorophyll contents. *J. Exp. Bot.* 46, 1825–1831.
 Maherchandani, N., 1975. Effects of gamma radiation the dormant seeds of *Avena Fatua*. *Radiat. Bot.* 15, 439–443.
 Melki, M., Sallami, D., 2008. Studies the effects of low dose of gamma rays on the behaviours of chickpea under various conditions. *Pak. J. Biol. Sci.* 11 (19), 2326–2330.
 Melki, M., Marouani, A., 2010. Effects of gamma rays irradiation on seed germination and growth of hard wheat. *Environ. Chem. Lett.* 8, 307–310.
 Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15, 473–479.
 Nageswara, R.R.C., Talwar, H.S., Wright, G.C., 2001. Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using chlorophyll meter. *J. Agron. Crop Sci.* 189, 175–182.
 Pal, R.N., Laloraya, M.M., 1972. Effect of calcium levels on chlorophyll synthesis in peanut and linseed plants. *Biochem. Physiol. Pflanze* 163, 443–449.
 Rabie, K., Shenata, S., Bondok, M., 1996. Hormone imbalance, germination, growth and pod shedding of Faba beans as affected by gamma irradiation. *Ann. Agric. Sci.* 41, 551–556.
 Radhadevi, D.S., Nayar, N.K., 1996. Gamma rays induced fruit character variations in Nendran, a varieties of banana (*Musa paradisiaca* L.). *Geobios* 23, 88–93.
 Rensburg, L.V., Kruger, G.H.J., 1994. Evaluation of components of oxidative stress metabolism for use in selection of drought tolerant cultivars of *Nicotiana tabacum* L. *J. Plant Physiology* 143, 730–737.
 Sheppard, S.C., Evenden, W.G., 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.
 Sidark, S.D., Suess, A., 1973. Effects of low doses of gamma irradiation on the growth and yield of two cultivars of tomato. *Radiat. Bot.* 3, 54–63.

- Simola, L.K., 1986. Structural and chemical aspects of evolution of Lathyrus species. In: Kaul, A.K., Combes, D. (Eds.), *Lathyrus and Lathyrism: Proceedings of the International Symposium of IBEAS*, pp. 225–239. Paris (France).
- Snedecor, G.W., Cochran, W.G., 1967. *Statistical Methods*, sixth ed. Iowa State University Press, Ames, IA.
- Stoeva, N., Bineva, Z., 2001. Physiological response of beans (*Phaseolus vulgaris* L.) to gamma-radiation contamination, I. Growth, photosynthesis rate and contents of plastid pigments. *J. Environ. Prot. Ecol.* 2, 299–303.
- Stoeva, N., Zlatev, Z., Bineva, Z., 2001. Physiological response of beans (*Phaseolus vulgaris* L.) to gamma-radiation contamination, II. Water-exchange, respiration and peroxidase activity. *J. Environ. Prot. Ecol.* 2, 304–308.
- Sunderland, N., 1960. Cell division and expansion in the growth of the leaf. *J. Exp. Bot.* 11, 68–80. Physiological response of beans (*Phaseolus vulgaris* L.) to gamma-radiation contamination, II. Water-exchange, respiration and peroxidase activity. *Journal of Environmental Protection and Ecology* 2, 304–308.
- Szarek, S., 2005. Use of concept of hormesis phenomenon to explain the law of diminishing returns Part II. *Electron. J. Pol. Agric. Univ. Ser. Econ.* 8 (4), #61.
- Telci, C., Yildiz, M., Pelit, S., Onol, B., Erkilic, E.G., Kendir, H., 2011. The effect of surface-disinfection process on dormancy-breaking, seed germination, and seedling growth of *Lathyrus chrysanthus* Boiss. under in vitro conditions. *Propag. Ornam. Plants* 11, 10–16.
- Wi, S.G., Chung, B.Y., Kim, J.H., Baek, M.H., Yang, D.H., Lee, J.W., Kim, J.S., 2005. Ultrastructural changes of cell organelles in Arabidopsis stem after gamma irradiation. *J. Plant Biol.* 48, 195–200.
- Wright, G.C., Nageswara, R.R.C., Farquhar, G.D., 1994. Water use efficiency and carbon isotope discrimination in peanut under water deficit conditions. *Crop Sci.* 34, 92–97.
- Xiuzher, L., 1994. Effects of irradiation on protein content of wheat crop. *J. Nucl. Agric. Sci. China* 15, 53–55.
- Yildiz, M., Avci, M., Ozgen, M., 1997. Studies on sterilization and medium preparation techniques in sugarbeet (*Beta vulgaris* L.) regeneration. In: *Turkish–German Agricultural Research Symposium V*, pp. 125–130. Antalya (Turkey).
- Zaka, R., Chenal, C., Misset, M.T., 2004. Effect of low doses of short-term gamma irradiation on growth and development through two generations of *Pisum sativum*. *Sci. Total Environ.* 320, 121–129.