Journal of Environmental Radioactivity 162-163 (2016) 129–133

Contents lists available at ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

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

Ramazan Beyaz [a, *], Cansu Telci Kahramanogullari [b], Cigdem Yildiz [c], E. Selcen Darcin [d], Mustafa Yildiz [b]

[a] Ahi Evran University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Bağbaşı, Kırşehir, Turkey
[b] Ankara University, Faculty of Agriculture, Department of Field Crops, 06110, Diskapi, Ankara, Turkey
[c] University of Ankara, Biotechnology Institute Golbasi, Ankara, Turkey
[d] Bilecik Şeyh Edebali University, Faculty of Agriculture and Natural Sciences, Department of Field Crops, Bilecik, Turkey

ARTICLE INFO

Article history:
Received 6 February 2016
Received in revised form
9 May 2016
Accepted 11 May 2016

Keywords:
Gamma radiation
In vitro seed germination
Lathyrus chrysanthus
Seedling growth

ABSTRACT

The effects of radiation at different doses (0, 50, 100, 150, 200 and 250 Gy) of radioactive cobalt (^{60}Co) 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 μg/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.

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

* Corresponding author.
E-mail address: ramazanbeyaz@gmail.com (R. Beyaz).

http://dx.doi.org/10.1016/j.jenvrad.2016.05.006
0265-931X/

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 °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 ± 1 °C in the dark for 7 days for seed germination. At 14 days after study initiation, germinated seeds were transferred to Magenta vessels (12 × 12 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 ± 1 °C under cool white fluorescent light (27 μmol m^{-2} 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 ± 1 °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 μg/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