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IRRADIATION AND POLYAMINES IMPACT ON GROWTH TRAITS OF GRECIAN FOXGLOVE (*DIGITALIS LANATA* L.) CULTIVATED IN VITRO ROOTING

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SUMMARY

Grecian foxglove (*Digitalis lanata* L.) is an ornamental plant rich in cardiac glycosides, which stimulates heart activities and achieves greater attention for propagation through various traditional methods and modern tissue culture using gamma irradiation alone and in combination with polyamines. The recent study aimed to screen and select the effective doses and concentrations of irradiation and polyamines, respectively, for better growth traits of *Digitalis lanata*. The *D. lanata* seeds were irradiated with gamma-ray doses (0, 25, 50 grays [Gy]), and the spermidine (SPD) was used with various concentrations (0.0, 0.5, 1.0, 1.5, and 2.0 mg L-1). The results showed the superiority of gamma radiation with a dosage of 50 Gy by achieving the highest average germination rate of 100% (7.10 seeds day^{-1}). In the rooting experiment, results further indicated that the radiation treatment (50 Gy) also excelled over other treatments, giving the highest percentage of rooting and root number, and root length, as well as, fresh and dried weights of root total, with values of 65.80%, 41.18 roots plant^{-1}, 3.83 cm, 1.90 mg, and 0.90 mg, respectively compared with neutral (control) treatment. Concerning the effect of polyamine, the concentration of 1.5 mg L^{-1} proved to lead by producing the highest percentage of rooting and root number, roots length, as well as, fresh and dried weights of root total, amounting to 73%, 54.18 roots plant^{-1}, 4.21 cm, 2.25 mg, and 1.19 mg, respectively compared with control. Low-dose gamma irradiation affected the seed germination and growth traits of *D. lanata*. The individual use of spermidine (polyamine) also enhanced the root number and length.

Keywords: Grecian foxglove (*Digitalis lanata* L.), gamma rays, polyamine spermidine, plant tissue culture, germination, rooting, fresh and dry root weight

Key findings: Results indicated that *Digitalis lanata* seeds, with gamma radiation of 50 Gy, excelled all other treatments by producing the highest percentage of rooting, root numbers, root length, and fresh and dried root weights.

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INTRODUCTION

Many ornamental plants have been distinguished by their aesthetic gratification and medicinal importance and have achieved greater attention for both purposes. The *Digitalis lanata* plant is one of these plants, having the highest proportion of cardiac glycosides that stimulate the heart. The genus *Digitalis*, which belongs to the family

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Plantaginaceae, includes approximately 20 vegetarian types of perennial, herbaceous, and shrubs. *D. lanata* is the most widely used plant due to its therapeutic activity as it includes the highest percentage of cardiac glycosides (Kuate, 2008).

This herbaceous flowering perennial and biennial grow naturally in Eastern and Western Europe, Western and Central Asia, and Northwest Africa. It is grown as an ornamental plant in gardens for the beauty of its leaves and flowers in the first and second seasons, respectively. In the first year, the plant has long, basal, dense leaves forming a green bouquet, arranged in a spiral shape with a light green color, which dries and falls in the winter and regenerates in the spring. Shamrock flowering appears in the second year. The florets are bell-shaped, drooping, creamy-white, with a purplish-brown reticulate vein. The flowers have beautiful-looking woolly hairs (Jograna *et al*., 2020). Therefore, its propagation consists of plant tissue culture technology, aside from the well-known traditional methods. Remarkable developments happened in the aspect of multiplication with advanced technologies to enhance crop production with insights and new ways for availability, nutritional values, and increase the ability to produce substances of high medicinal value (Harris, 2000; Karuppusamy, 2009). In plant biotechnology, the large-scale production of cardenolides using *in vitro* techniques has become the need of the day. The reasons are twofold: first, to reduce the excessive use of natural Digitalis population, and second to improve the plant quality, as well as, preserve the superior seed stock for future use (Verma *et al*., 2016).

The rooting process plays a vital role in the success of plant tissue culture. As this stage aims to form the adventitious roots on the vegetative branches previously formed in stages of multiplication *in vitro*. This happens by transferring the vegetative branches from the medium of multiplication, which often contains one of the cytokinins, to the medium forming the roots, which often contains one of the auxins (Trigiano and Gray, 2005). Many researchers have studied the factors affecting the rooting of plants and found that it depends upon the concentrations of auxins and their types that were added to the nutrient medium because of their control on the emergence and development of roots (Hartmann *et al*., 2002).

Polyamines have a larger effect on the induction of roots *in vitro* because of their crucial role in forming transverse roots on the branches resulting from tissue culture (Jessica *et al*., 2015). Also, the stimulating effect of radiation on plant growth and detection has been recognized a long time ago. The results of most studies showed that the stimulus often appears at the beginning of growth and emergence. Several studies validated an increase in the speed of the germination process and branching of many plants when taking a low dosage of irradiation resulting from the division of fetal cells (Chandrashekar *et al*., 2013; Katerova *et al*., 2013; Patial *et al*., 2014; Gupta *et al*., 2016; Sood *et al*., 2016; Sholihin *et al*., 2019; Khaled *et al*., 2022). The research aims to determine the effects of gamma irradiation properties and spermidine (polyamine), and their interaction to improve the rooting of vegetative branches of the *D. lanata* plant *in vitro*. Polyamines help seeds to germinate faster by increasing water absorption and metabolic activity in the embryonic stage, which accelerate the appearance of seedlings (Alcázar *et al*., 2020).

MATERIALS AND METHODS

Plant material and procedure

Digitalis lanata seeds with 10% humidity were exposed to gamma rays at the doses of 25 and 50 Gy; seeds were irradiated according to the method of Bodnar and Cheban (2022). The radiated and non-radiated (control) seeds were disinfected in 3% sodium hypochlorite solution for 20 min then rinse three successive times with sterile distilled water to eliminate the remnants of the sterile substance. Then, the 50 seeds were sown on free media (Murashige and Skooge, 1962) in glass bottles of 50 ml capacity, with 10 repetitions for each dose of gamma rays, apart from the control treatment (Figure 1). The cultures were placed in a growth room at 25°C and with illumination intensity of 1000 lux for 16 h day^{-1}. The rate and speed of seed germination were calculated after four weeks of planting using the following equations (ISTA, 2017):

$$\text{Percentage of germination} = \frac{\text{number of germinating seed}}{\text{number of total seed}} \times 100$$

$$\text{average of germination speed} = \frac{\text{first count per germinating seed number}}{\text{first count to days number}} + \frac{\text{second count in germinated seed number}}{\text{second count to days number}} + \cdots Etc$$

According to the calculations, the dose coefficients exceeded 50 Gy. The plants of this dose were selected in the execution of the posterior experiments. The tips of shoots, with a length of 2 cm, were taken from the initiation stage of the plant at the age of four weeks and grown on MS media prepared with the concentrations of 0.0, 0.5, 1.0, 1.5, and 2.0 mg L^{-1} of polyamine Spermidine (SPD) and with a fixed concentration of 1 mg L^{-1} IBA and 0.2 mg L^{-1} BA. Plants were planted according to a fully randomized design and factorial arrangement, with 10 replications for each concentration, then placed within the same previous condition to stimulate rooting (Figure 2).

Using a statistical program (SAS, 2004) analyzed the results, and a comparison of averages used the least significant difference (LSD) test with a probability level of 0.05. Data on rooting percentage, root number, length of roots, and fresh and dry weights of rooting system got recorded in the next five weeks of farming. The roots were cleaned with distilled water to rid remnants of the nutrient media, which is a good source for the growth etiology. Then, dipped in a concentrated fungicide solution of 0.1% for 15 min to decrease the danger of infection with fungi. Further, placing the roots in a liquid media with a 1/4 concentration of MS salt for one week to harden the plants. Afterward, transfer the samples to plants in anvils containing sterile mixed soil mixed with peat moss in a ratio of 1:2, irrigated, then, capped by vitreous covers to keep them at a suitable humidity. Humidity was raised progressively until the level of natural atmosphere, and the recording of observations took place on the success rate of adapted plantlets in the next 14 days of farming (Figure 2).

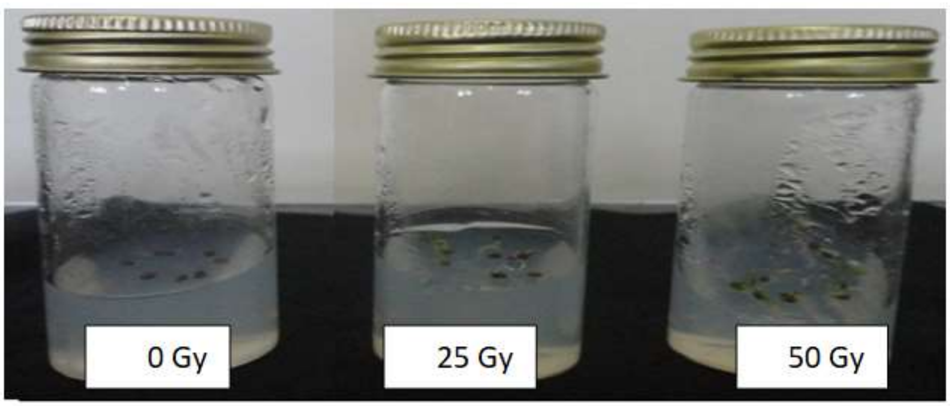

Figure 1. Germination of *Digitalis lanata* seeds after seven days of sowing.

Figure 2. Effect of a) gamma rays (50 Gy) and SPD (1.5 mg L^{-1}), and b) gamma rays (0 Gy) and SPD (1.5 mg L^{-1}) on the rooting of *Digitalis lanata.*

RESULTS

Irradiation effects on seed germination percentage

The results revealed significant differences among the irradiation treatments for the *Digitalis lanata* seed germination percentage rate (Table 1). The irradiation treatment with a dose of 50 Gy gave the highest germination rate, reaching 100% after 14 days of planting, followed by the irradiation treatment with a dose of 25 Gy, which achieved an average percentage of germination (88%). Germination of seeds reached 88% after 21 days of sowing, while the percentage rate of germination reached 25% in the control treatment after 28 days of planting.

Table 1. Effect of irradiation on the seeds germination percentage rate of *Digitalis lanata* after 30 days of culturing.

Gamma-rays	Germination rate (%)
0 Gy	25
25 Gy	88
50 Gy	100
$LSD_{0.05}$	10.25

Irradiation effects on germination speed rate

The results showed that *D. lanata* seed, irradiated with 50 Gy, gave the highest seed germination rate (7.10 seeds day^{-1}) after seven days of sowing (Table 2). Seeds with the irradiation treatment dose of 25 Gy followed, which achieved the seed germination rate of 4.30 seeds day^{-1} after seven days of sowing. However, the control treatment revealed the lowest average germination speed of 0.89 seeds day^{-1} after 14 days of planting.

Table 2. Effect of irradiation on average seed germination speed of *Digitalis lanata* after 30 days of culturing.

Gamma-rays	Speed of germination (seeds day^{-1})
0 Gy	0.89
25 Gy	4.30
50 Gy	7.10
$LSD_{0.05}$	2.15

Irradiation and SPD concentrations, and their interaction effects on rooting percentage

The results indicated that irradiated seeds of *D. lanata,* with SPD concentrations, showed a positive impact on the rooting percentage (Table 3). The irradiation treatment with a dose of 50 Gy produced the highest rooting percentage rate (65.80%) compared with the lowest percentage obtained in the control treatment (45.60%). Results also indicated that the SPD, with the concentration of 1.5 mg L^{-1}, provided the highest rooting percentage rate (73.00%), compared with the control treatment having the lowest rooting percentage (37.50%). However, it did not differ significantly with the concentration of 2 mg L^{-1} (70.50%). The interaction between gamma rays and SPD concentrations prepared for the medium caused a significant effect on the rate of rooting percentage . The irradiation treatment with a dose of 50 Gy and the medium prepared with SPD at the concentration of 1.5 mg L^{-1} achieved the highest rate of rooting percentage (90%) compared with the control (45%). However, the non-irradiated treatment interacting with SPD concentration of 2 mg L^{-1} enunciated the highest rooting percentage rate (61%) compared with the control treatment alone (30%).

Table 3. Effect of irradiation and spermidine (SPD) concentrations on the rooting percentage rate of *Digitalis lanata* after five weeks of cultivation on MS media.

Spermidine (SPD) concentrations (mg L^{-1})	Gamma-rays		Means (%)
	0 Gy	50 Gy	
0.0	30	45	37.50
0.5	38	50	44.00
1.0	43	64	53.50
1.5	56	90	73.00
2.0	61	80	70.50
Means (%)	45.60	65.80	

$LSD_{0.05}$ Gamma-rays: 6.22, $LSD_{0.05}$ SPD Concentrations: 8.30, $LSD_{0.05}$ GR × SPD Interactions: 12.14

Irradiation and SPD concentrations and their interaction effects on the number of roots

The *D. lanata* seed irradiated with 50 Gy showed significant superiority by producing the highest average number of roots (41.18 roots plant^{-1}), compared with the control treatment having the lowest average (30.67 roots plant^{-1}) (Table 4). The results also showed the superiority of SPD at a concentration of 1.5 mg L^{-1} by achieving the highest number of roots (54.18 roots plant^{-1}) compared with the control treatment (16.78 roots plant^{-1}). However, the interaction between the irradiation treatments and SPD concentrations gave differing values for the said trait. The irradiation, with 50 Gy and SPD at the concentration of 1.5 mg L^{-1}, produced the highest average number of roots (60.33 roots plant^{-1}) compared with the least values obtained by the treatment having irradiation and no SPD (20.18 roots plant^{-1}), and control with no irradiation and no SPD (13.37 roots plant^{-1}).

Table 4. Effect of irradiation and spermidine (SPD) concentrations on the average number of rooting of *Digitalis lanata* after five weeks of cultivation on MS media.

Spermidine (SPD) concentrations (mg L^{-1})	Gamma-rays		Means (#)
	0 Gy	50 Gy	
0.0	13.37	20.18	16.78
0.5	28.15	35.08	31.61
1.0	30.22	43.16	36.69
1.5	48.03	60.33	54.18
2.0	33.60	47.17	40.38
Means (#)	30.67	41.18	

$LSD_{0.05}$ Gamma-rays: 6.01, $LSD_{0.05}$ SPD Concentrations: 8.23, $LSD_{0.05}$ GR × SPD Interactions: 12.20

Irradiation and SPD concentrations and their interaction effects on root length

The *D. lanata* irradiated seeds with SPD concentrations show a significant effect on the average root length on the tips of the branches in the medium (Table 5). The results showed that irradiation with a dosage of 50 Gy showed significant superiority in the average root length (3.83 cm) compared with the control (2.49 cm). The SPD concentration of 1.5 mg L^{-1} gave significant superiority on average root length (4.21 cm) compared with the control (2.02 cm). The interaction between the radiation treatments and SPD concentrations also gave significant differences in the average root length. The irradiation treatment with 50 Gy and SPD concentration of 1.5 mg L^{-1} excelled all the interactions by achieving a maximum root length (5.08 cm) compared with the lowest values shown by the control having irradiated seed and no SPD (2.44 cm), and control with non-irradiated and no SPD seed (1.60 cm).

Irradiation and SPD concentrations and their interaction effects on fresh and dry root weight

The irradiated seeds of *D. lanata* at 50 Gy in addition to SPD concentrations caused a significant impact on the average fresh and dry weights of roots (Tables 6 and 7). The seed irradiated with 50 Gy produced the highest

Table 5. Effect of irradiation and spermidine (SPD) concentrations on the average root length of *Digitalis lanata* after five weeks of cultivation on MS media.

Spermidine (SPD) concentrations (mg L^{-1})	Gamma-rays		Means (cm)
	0 Gy	50 Gy	
0.0	1.60	2.44	2.02
0.5	2.40	3.17	2.78
1.0	3.27	4.22	3.74
1.5	3.35	5.08	4.21
2.0	1.83	4.28	3.05
Means (cm)	2.49	3.83	

$LSD_{0.05}$ Gamma-rays: 0.12, $LSD_{0.05}$ SPD Concentrations: 0.12, $LSD_{0.05}$ GR × SPD Interactions: 0.18

Table 6. Effect of irradiation and spermidine (SPD) concentrations on the fresh weight of roots of *Digitalis lanata* after five weeks of cultivation on MS media.

Spermidine (SPD) concentrations (mg L^{-1})	Gamma-rays		Means (mg)
	0 Gy	50 Gy	
0.0	0.40	1.02	0.71
0.5	1.02	1.78	1.40
1.0	1.50	2.01	1.75
1.5	1.83	2.66	2.25
2.0	0.64	2.07	1.35
Means (mg)	1.08	1.90	

$LSD_{0.05}$ Gamma-rays: 0.01, $LSD_{0.05}$ SPD Concentrations: 0.02, $LSD_{0.05}$ GR × SPD Interactions: 0.03

Table 7. Effect of irradiation and spermidine (SPD) concentrations on the dry weight of roots of *Digitalis lanata* after five weeks of cultivation on MS media.

Spermidine (SPD) concentrations (mg L^{-1})	Gamma-rays		Means (mg)
	0 Gy	50 Gy	
0.0	0.01	0.22	0.12
0.5	0.22	0.40	0.31
1.0	0.36	1.08	0.72
1.5	0.68	1.70	1.19
2.0	0.06	1.12	0.59
Means (mg)	0.26	0.90	

$LSD_{0.05}$ Gamma-rays: 0.01, $LSD_{0.05}$ SPD Concentrations: 0.02, $LSD_{0.05}$ GR × SPD Interactions: 0.02

fresh (1.90 mg) and dry (0.90 mg) root weights, compared with the lowest values obtained from control treatments (1.08 and 0.26 mg), respectively. Results also indicated that the SPD with the concentration of 1.5 mg L^{-1} produced the highest fresh (2.25 mg) and dry (1.19 mg) root weights compared with control treatments (0.71 and 0.12 mg), respectively. Among the interactions of irradiation and SPD concentrations, the irradiated seed with SPD concentration of 1.5 mg L^{-1} showed the highest values for fresh (2.66 mg) and dry (1.70 mg) root weights compared with the lowest values shown by control having irradiated seed and no SPD (1.02 and 0.22 mg), and control with non-irradiated seed and no SPD (0.40 and 0.01 mg).

Plant acclimatization

The process of acclimatization was carried out for the *D. lanata* plants that were rooted in the laboratory, after sterilizing the soil and peat moss in the sterilizer, blended with a ratio of 1:2, and then planted in anvils (Figure 3). Afterward, the plants were watered with tap water.

Figure 3. *Digitalis lanata* plants at the beginning of the acclimatization stage.

DISCUSSION

From the foregoing results, the study concluded that the radiation dose of 50 Gy, which falls approximately in the low ranges of irradiation dosage, had a stimulating influence on the growth and unveiling of *Digitalis lanata* seeds, differing significantly from the rest of the radiation dose treatments. Such type of stimulation can be attributed to the enhancement in the efficiency of RNA and protein synthesis during the first stage of growth (Aly, 2010). The stimulation is further attributed to the abundance in the division rate of fetal cells (Sumira *et al*., 2012), and the energy is a basic requirement in the germination of seeds, and the low dosage of gamma rays probably raised the enzymatic activity energizing the division of cells (Andreu *et al*., 2010). Moisture affects the life reactions in cells, causing an increase in the cell's response to radiation.

Irradiation causes the mutation, which is one of the chromosomal aberrations in many crop seeds. The irradiation, studied with different percentages of moisture, revealed that the seeds are more resistant to radiation when containing 11% moisture (Kainsa *et al.,* 2012; Almukhtar *et al*, 2019). Such humidity is suitable for seed storage under normal conditions, where the cells of seeds show very few chromosomal deviations compared with other treatments. Past studies also indicated that when the seeds are soaked in water, the mitotic process of cells begins, and seeds become more sensitive to the radiation, and the increased radio sensitivity is mitosis stage than dormancy (Yücesan *et al*, 2018; Mohammed *et al*, 2015).

It is inferred from the study results that in general, the irradiation treatments gave significant superiority in the studied root growth traits compared with the non-irradiated treatment (control). Gamma rays may have worked to increase the enzymatic activity that stimulates cell division continuously throughout the vegetative growth period (Singh and Datta, 2010). Seeds irradiated with low doses are rapidly characterized in cell divisions, especially in meristematic cells at the top of the roots, which increase the penetration property and the growth of roots in the soil (Boonsua *et al*., 2020). This result also agrees with the previous studies, which found an increase in activity of the developing tops on the tips of roots in plants with seeds irradiated with low doses of gamma rays. Then, it causes interaction in the root system, which leads to increased efficiency of fixing plants and absorbing various elements (Yasemin *et al*., 2007; Lixiang *et al*., 2010). In contrast, the irradiation with high levels of gamma rays weakens the root system, and sometimes the loss of the developing tops on the tips of roots, and thus the inability of the plant to establish itself in the soil.

The study noted the superiority of spermidine (polyamine) at a concentration of 1.5 mg L^{-1} in the growth-related traits. Hence, it plays a vital role in the formation of roots as it stimulates the cell growth, replication, vitality, and stability of biological molecules and proteins (Taiz and Zeiger, 2010). It bears life stresses, and the reason may be that polyamine plays a considerable function in the emergence and formation of roots in different crop plants (Ganeshan *et al*., 2011;

Chandrasekaran *et al.*, 2019). It is possible due to its ability to bind with aggregates of phospholipids and some ionic sites in cell membranes (Hussain *et al*., 2011). Polyamines also control the formation of transverse and lateral radicals by increasing the activity of the peroxidase enzyme in prokaryotes and eukaryotes (Igarashi and Kashiwagi, 2010).

Polyamines stimulate the formation of radicals during cell division and differentiation, and their effect in inducing radical formation and elongation may be because it inhibits the biosynthesis of ethylene (Pegg, 2009). The increase in the rate of rooting percentage might be due to the role of SPD in protecting the plasma membrane forbidding the fashioning of auxins free radicals that lead to spoiling the cell membranes and nucleic acids, as well as, proteins of the plant cells (Ghisalberti *et al*., 2013). In general, polyamine has a similar role to auxins in the elasticity of cell walls, causing their elongation and enlargement by increasing the activity of some enzymes responsible for the softness of cell walls and increasing their permeability in plant tissue culture (Zhu and Chen, 2005; Amiri and Shahsavar, 2010; Rakesh *et al*., 2021).

CONCLUSIONS

The study concludes that irradiation of *Digitalis lanata* seeds with low concentrations of gamma rays had a significant effect on the germination and growth traits, and the impact reached the maximum with the dose of 50 Gy, which revealed the highest rooting rate compared with lower rooting in control. The use of spermidine (polyamine) alone caused an enhancing role in the root number and length, especially the SPD concentration of 1.5 mg L^{-1}, compared with the control treatment, which gave the lower average values. However, the interaction between irradiation (50 Gy) and spermidine concentration (1.5 mg L^{-1}) performed better by achieving the best characteristics of the root system.

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