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Hormetic growth of *Pinus pseudostrobus* seedlings exposed to low-dose gamma and X-ray irradiation

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Abstract: Mexico ranks among the countries with the highest deforestation rates, increasing the demand for high-quality forest seedlings of valuable species such as *Pinus pseudostrobus* Lindl., which face germination limitations due to seed dormancy and low viability. Ionising radiation has emerged as an alternative pregermination treatment capable of inducing adaptive responses in plants through hormesis. This study evaluated the effects of different doses of gamma radiation (^{60}Co) and high-energy X-rays (linear accelerator, 6 MeV) on the germination, growth, and quality of *P. pseudostrobus* seedlings. A total of 1 440 seeds were irradiated per radiation source with 12 doses (0–25 Gy) and sown under nursery conditions in a completely randomised design. Germination parameters, morphological traits, photosynthetic pigment content, and quality indices were analysed. With both radiation sources, low doses (0.5–1.5 Gy) significantly enhanced germination, chlorophyll content, and seedling height and diameter, while doses above 15 Gy inhibited these responses. The LD_{50} (median lethal dose) was estimated at 20 Gy for gamma rays and 12 Gy for X-rays, whereas GR_{50} (median growth reduction dose) exceeded 45 Gy in both treatments. These findings demonstrate that low radiation doses elicit a beneficial hormetic effect in *P. pseudostrobus*, representing a viable biotechnological approach to improve seedling production and ecological reforestation efficiency.

Keywords: forest biotechnology; ionising radiation; hormesis; lethal dose 50 (LD_{50}); growth reduction 50 (GR_{50}); low-dose radiation

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Mexico is known for its high diversity of *Pinus* species (Perry 1991; Gernandt, Pérez-de la Rosa 2014). However, it faces a serious forestry problem, as nearly 45% of the country's territory is degraded to some degree, primarily as a result of land use changes due to agricultural activities (SEMARNAT 2014). This situation has led the country to rank fifth in the world in terms of deforestation. For this reason, the production of forest seedlings has gained great relevance as a key strategy in reforestation and ecological restoration programs (Benítez et al. 2002; Rueda-Sánchez et al. 2014).

However, the propagation of large quantities of forest plants has certain limitations due to the low germination rate of their seeds. This is largely due to the dormancy of their seeds, which have a thick, hard, and impermeable seed coat, which limits the entry of oxygen and water for proper imbibition and embryonic growth (Ruiz et al. 2007; González et al. 2009). Furthermore, conifers have been found to exhibit great variation in reproductive attributes among populations of the same species, due to genetic and environmental factors (Castellanos-Acuña 2013).

Recent studies suggest that these effects could be linked to radiation-induced epigenetic alterations (Cedergreen 2008; Vergara et al. 2018). Therefore, it is essential to determine two key parameters: the median lethal dose (LD_{50}), which indicates the amount of radiation that causes the death of 50% of the seeds, and the median growth reduction dose (GR_{50}), which represents the dose at which growth is reduced by 50% (Ángeles-Espino et al. 2013). Both indicators are specific to each species, genotype, and even plant tissue (Hernández-Muñoz et al. 2017).

In this context, conventional pre-sowing treatments, such as physical and chemical scarification, are commonly used to improve germination; however, they often exhibit variable efficacy, limited reproducibility, and potential negative effects on seed integrity or environmental sustainability (Bewley et al. 2012). In contrast, seed irradiation with low doses of ionising radiation is a promising alternative for uniformly irradiating seed lots with low ionising radiation (Kovács, Keresztes 2002; Wi et al. 2007). Furthermore, low-dose irradiation induces hormetic responses, which improve germination, seedling vigour, and early seedling growth by activating cell repair mechanisms and gene regulation processes (Calabrese, Blain 2011). Therefore, seed radiotherapy is considered suitable for use in forest species where germination is required, allowing a uniform establishment of seedlings that contributes to better nursery production and the success of reforestation programs (Rueda-Sánchez et al. 2014).

Considering the background and the limited information available on the effects of ionising radiation on forest species, particularly on *Pinus pseudostrobus*, this study aimed to determine the LD_{50} and GR_{50} in seeds and seedlings irradiated with different doses of ^{60}Co and a linear accelerator, and to evaluate their germinative, morphological, and physiological responses as criteria for their potential application in forest nurseries.

MATERIAL AND METHODS

The germplasm used in this study consisted of *P. pseudostrobus* seeds collected in 2018 from a natural stand located in Tenextepec, Perote, Veracruz, Mexico. These seeds were obtained from a mixture obtained from the selection of ten plus trees and were intended for sowing under *ex vitro* conditions.

A total of 2 880 seeds (1 440 per irradiation type) were selected to evaluate the effect of low doses of ionising radiation. The applied doses were 0, 0.25, 0.5, 1.5, 3, 5, 7.5, 10, 12, 15, 20, and 25 Gy, with 120 seeds per dose, distributed into four replicates of 30 seeds each. For the Cobalt-60 irradiation, a GAMMACELL 220 irradiator (MDS Nordion, Canada) was used at a dose rate of 15.229402 Gy·h^{-1}. For the linear accelerator (Elekta Synergy Platform, Sweden) irradiation, photons with an energy of 6 MeV were applied. The seeds were placed at a depth of 1.5 cm within a 30 × 30 cm field.

Germination assessment

Germination was assessed using a completely randomised design with four replicates of 30 seeds per treatment (including the control), sown in TB-310 tubes with peat moss, vermiculite, and agrolite substrate (3 : 1 : 1) under greenhouse conditions. Two seeds were placed per tube at a depth of 0.5–1 cm. Monitoring was performed daily for 15 days from emergence, and four germination indices were calculated: germination capacity (*GC*), as the percentage of germinated embryos (Tan et al. 2017); germination index (*GI*), which assesses the speed of the process (Scott et al. 1984);

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germination energy (*GE*), calculated as the days required to reach 50% of *GC* (Farooq et al. 2005); and peak value (*PV*), which represents the highest cumulative germination percentage over time (Kolotelo et al. 2001).

Growth assessment

A total of 10 seedlings were randomly selected for each dose and radiation source to evaluate their growth in the nursery for 6 months. Stem height without needles (cm), stem height with needles (cm), total height (cm), and basal diameter (root collar) (mm) were evaluated using a digital vernier calliper (Truper, Mexico). Finally, the root length of the seedlings was measured. Finally, to analyse the similarity between irradiation treatments on the growth of *P. pseudostrobus* seedlings, a hierarchical cluster analysis based on these morphological variables was used.

Photosynthetic pigment content

The methodology described by Porra et al. (1989) was used to determine the photosynthetic pigment content. Chlorophyll (*Chl*) A, B, A + B, and carotenoids (*Car*) were obtained from plant material. To do this, 0.25 g of each seedling was weighed and macerated in liquid nitrogen (N_2) with 5 mL of acetone (80%). They were then centrifuged at 6 000 rpm for 12 min in 15 mL polypropylene tubes. The supernatant was finally transferred to new polypropylene tubes for pigment readings on a spectrophotometer (Jenway, United Kingdom). For the determination of chlorophyll A, the reading was taken at 663.6 nm, for chlorophyll B at 646.6 nm, and for carotenoids at 440.5 nm. The following Equations (1–4) were used to calculate the photosynthetic pigment content.

$$Chl\ A = \left(12.25 \times A_{663} - 2.25 \times A_{645}\right) \times \frac{V}{100} \times W \quad (1)$$

$$Chl\ B = \left(20.30 \times A_{645} - 4.91 \times A_{663}\right) \times \frac{V}{100} \times W \quad (2)$$

$$Chl\ A + B = \left(7.34 \times A_{663} + 17.76 \times A_{645}\right) \times \frac{V}{100} \times W \quad (3)$$

$$Car = \left(4.46 \times A_{441} - Chl\ A + Chl\ B\right) \times \frac{V}{100} \times W \quad (4)$$

where:

Chl A – chlorophyll A;
Chl B – chlorophyll B;
V – total volume of acetone extract (mL);
W – fresh weight (g) of the sample;
Car – carotenoids.

Seedling quality. Twelve months after planting, destructive sampling was performed on three plants per dose. Leaves, branches, roots, and stems were separated using pruning shears. Height (*AT*, cm) was recorded from the root collar to the apex of the plant; root collar diameter (*DC*, mm) was obtained with a digital calliper (Truper, Mexico), with an accuracy of ±0.1 mm. Fresh and dry biomass (g) of the root and shoots was assessed by separating leaves, branches, and stems. To determine dry weights, the various organs of each plant were placed separately in paper bags and transferred to an electric convection oven (Memmer, Germany) at a constant temperature of 70 °C for 72 h, until a constant weight was obtained (Reyes-Reyes et al. 2005). After this time, the dry weights were determined using a digital scale (Model H-2716, Humboldt Mfg. Co., USA).

Plant quality indexes were obtained according to Rodríguez (2008) using the following Equations (5–8):

$$IE = \frac{AT}{DC} \quad (5)$$

$$ID = \frac{PST}{\frac{AT}{DC} + \frac{PSA}{PSR}} \quad (6)$$

$$RAS = \frac{PSA}{PSR} \quad (7)$$

$$RAR = \frac{AT}{LR} \quad (8)$$

where:

AT – total height (cm);
DC – root collar diameter (mm);
ID – Dickson quality index;
IE – slenderness index;
LR – root length (cm);
PSA – shoot dry weight (g);
PSR – root dry weight (g);

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PST – total dry weight (g);
RAR – shoot/root ratio;
RAS – aboveground biomass to belowground biomass ratio.

Statistical analysis

All experiments were conducted using a completely randomised design with twelve treatments (radiation doses) and a control, each with four replicates of 30 seeds per treatment, totalling 1 440 seeds per radiation source. Germination capacity data were transformed using the arcsine square root of per cent to fit a normal distribution for regression analysis. Based on the regression equation resulting from the germination percentage, the mean lethal dose (LD_{50}) was estimated, and then the mean growth reduction (GR_{50}) of the evaluated variables was estimated. The data were analysed using a simple classification analysis of variance (ANOVA), and the means were compared using the Tukey test (P < 0.05), for which the Statistica for Windows software (Version 10.0, 1998) was used. Additionally, a hierarchical cluster analysis (Ward's method and Euclidean distance) was performed to assess the morphological similarity among treatments. The similarity index threshold was established at 50% to define the groups.

RESULTS AND DISCUSSION

Effect of irradiation with ^{60}Cobalt and a linear accelerator on germination. Seed germination of *P. pseudostrobus* began approximately 30 days after sowing, from which point onward daily monitoring was conducted. In the gamma-ray (^{60}Co) treatment, seeds irradiated with 0.5, 3.0, 5.0, and 25.0 Gy showed early emergence on the second day after the start of monitoring, while the remaining doses, including the non-irradiated, germinated starting on the third day (Figure 1A). In contrast, with the linear accelerator, the first seeds to germinate were those treated with 0.25 and 0.5 Gy (Figure 1B). The cumulative germination curves clearly show that low doses in both treatments accelerated and increased germination, while high doses had a negative effect.

The 5.0 and 7.5 Gy gamma ray doses resulted in the highest germination capacity (25.0%), with a germination value (*GV*) of 3.0 and a peak value (*PV*) of 2.08%·day^{-1}. The linear accelerator, on the other hand, showed its optimal point at 0.5 Gy, reaching the maximum germination capacity (31.25%), a *PV* of 2.60%·day^{-1} and a *GV* of 2.87, these being the highest values observed throughout the experiment (Figure 2). However, starting at 12.0 Gy, both treatments showed a progressive decline in all germination parameters, with the greatest decline in the linear accelerator. For example, at 25.0 Gy, seeds irradiated with ^{60}Co maintained a 12.5% germination rate, similar to the linear accelerator (11.25%), but with a lower relative decline compared to its optimal dose.

These observations reflect a clearly defined hormetic effect: low doses of ionising radiation stimulate germination, while high doses inhibit it (Calabrese, Baldwin 2003; Deshmukh et al. 2016). The greater efficiency of the linear accelerator at ultra-low doses (0.25–1.5 Gy) could be attributed to its higher energy (6 MeV), which causes a more

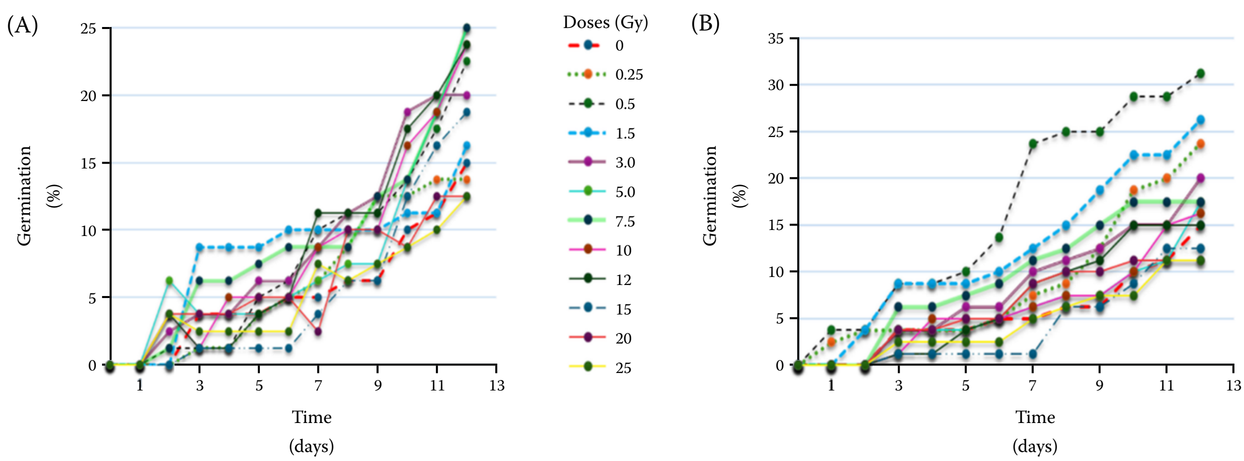

Figure 1. Cumulative germination percentage (%) of *P. pseudostrobus* seeds irradiated with different radiation doses: (A) gamma ray treatment (^{60}Co), (B) linear accelerator treatment (6 MeV photons)

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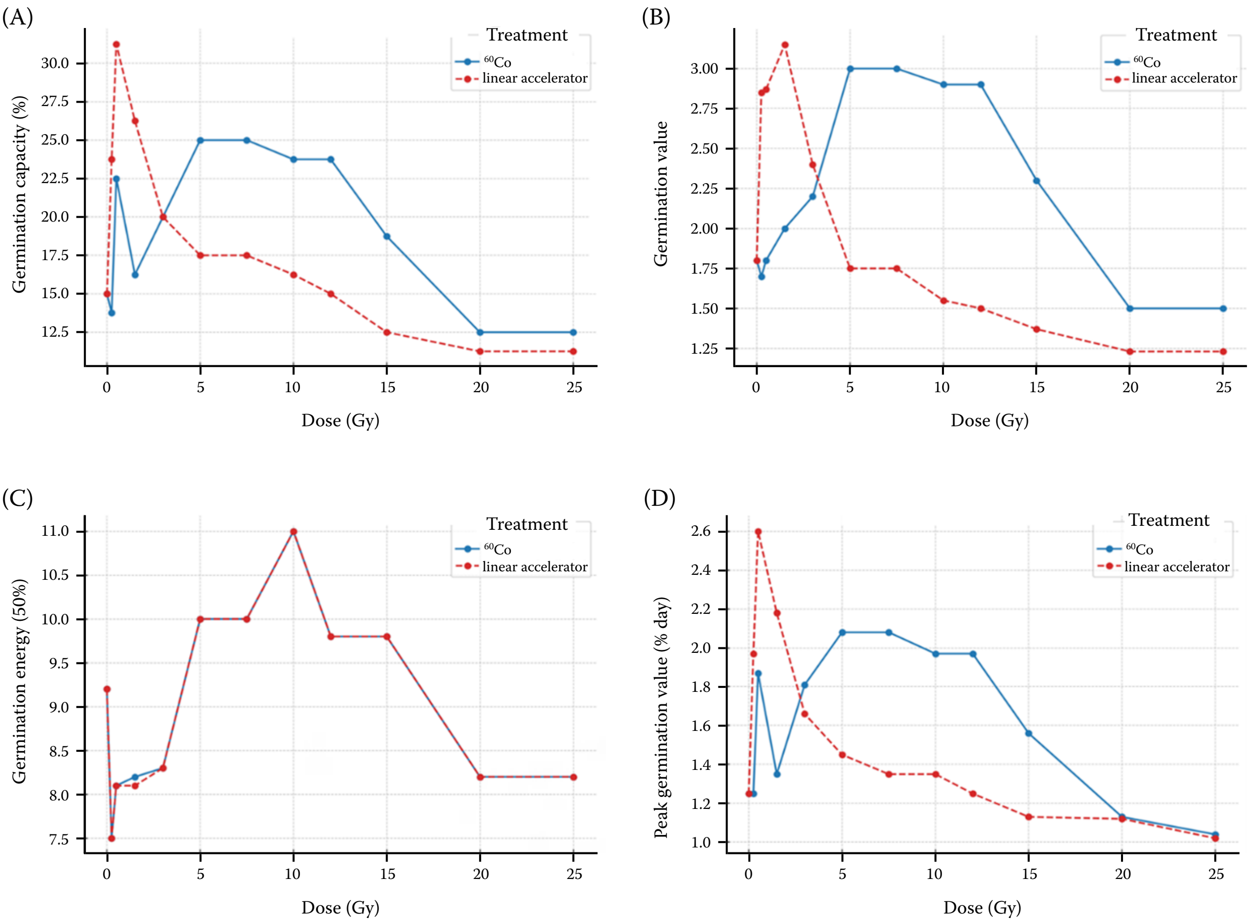

Figure 2. Comparison of germination parameters of *P. pseudostrobus* grown after seed irradiation with ^{60}Co and linear accelerator (6 MeV)

intense interaction with DNA and cellular structures, possibly inducing early activation of genes related to germinative metabolism, as reported in other conifers (Arena et al. 2014). However, this same energy can cause irreversible cell damage at high doses, decreasing viability (Agathokleous, Calabrese 2019).

In contrast, gamma radiation (1.25 MeV) has more uniform penetration and a lower delivery rate, which may explain its broader hormetic window (up to 7.5 Gy) and greater stability in the germinative response. Other studies in *Pinus* spp. (Zhang et al. 2007; Flores-López et al. 2022) agree that doses between 0.5 Gy and 5 Gy of gamma rays significantly improve germination, depending on the genotype and physiological condition of the seeds.

Effect of ^{60}Co and linear accelerator irradiation on seedling morphology. Height and diameter are extremely important morphological variables in forest production. The height variable helps predict future plant growth in the field, while seedling diameter determines its survival capacity (Romero-Arenas et al. 2019). Therefore, in this study, growth variables were evaluated monthly, considering height with and without needles, as well as stem diameter. The results showed that seedlings irradiated with doses of 0.5 Gy, 1.5 Gy, and 3.0 Gy, both ^{60}Co and linear accelerator, had greater total height (22.30–25.45 cm) and more developed stems. Specifically, with 0.5 Gy of linear accelerator, the highest height (25.45 cm) and one of the largest diameters (3.17 mm) were observed, even exceeding the ranges reported by Aguilera Rodríguez et al. (2016), who recorded values between 22 cm and 24.5 cm at 10 months for *P. pseudostrobus*. On the other hand, in the gamma ray treatment, although good heights were also reached with 0.5 Gy and 1.5 Gy (22.60–22.61 cm), the diameter values were slightly lower (2.88–2.92 mm).

The dendrogram generated from the growth data (Figure 3), computed with the Jaccard similarity

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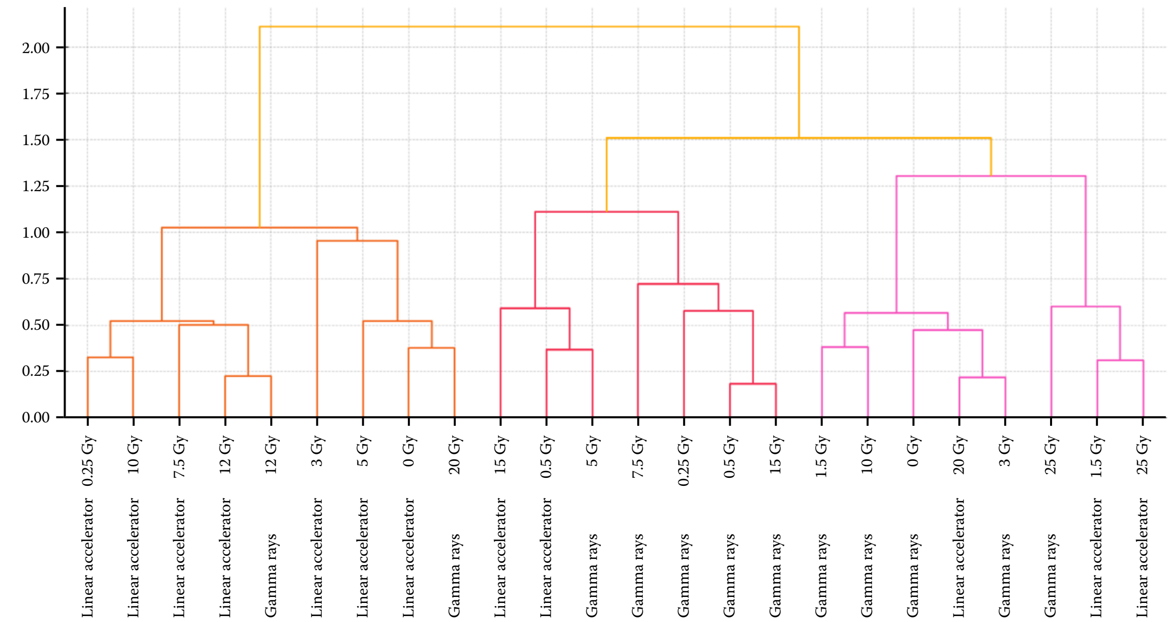

Figure 3. Dendrogram of the cluster analysis performed (Ward method and Euclidean distance) showing the grouping of irradiation treatments (gamma rays and linear accelerator) applied to *P. pseudostrobus* seeds, based on morphological variables evaluated in M_1 seedlings (first-generation seedlings derived from irradiated seeds)

index and clustered by UPGMA (unweighted pair group method with arithmetic mean), grouped irradiated treatments by morphological similarity. Using a 62% similarity threshold, a clear separation emerged between treatments with positive growth responses (0.5–3 Gy) and those with lower responses (20–25 Gy and controls). In particular, low linear accelerator doses (0.5 and 1.5 Gy) formed a distinct cluster (Group A), reflecting their outstanding performance in height and diameter. Conversely, high gamma-ray doses (20 Gy and 25 Gy) clustered distantly (Group C), consistent with reduced morphological development.

These results support the proposal by Fonseca et al. (2012), who suggested that low doses of radiation induce more efficient metabolic pathways through controlled free radicals, resulting in superior growth. They also agree with Romero-Rangel et al. (2017), who reported lower growth (height of 14.9 cm, diameter of 2.1 mm) in *P. pseudostrobus* without pregermination treatments. Finally, the dendrogram provides a comprehensive view of the relationships between treatments, confirming that the linear accelerator at low doses generates more consistent and grouped morphological responses than gamma rays, positioning it as an effective alternative for the physiological improvement of this forest species.

Median lethal dose (LD_{50}) and growth reduction dose (GR_{50}). The median lethal dose is the dose applied at which 50% of irradiated individuals die (LD_{50}). This dose may be more likely to produce mutations (Golubinova, Gecheff 2011; Ángeles-Espino et al. 2013). Other researchers point out that other doses with a high probability of producing effective mutations are those where growth is reduced by 50% (GR_{50}) (Akgün, Tosun 2004; Khalil et al. 2014). This helps to determine that low doses of radiation generate minimal impacts on the genome; therefore, phenotypic changes will also be scarce. On the other hand, at high doses, the genome suffers multiple impacts that regularly produce aberrations or negative changes (Songsri et al. 2011; Thole et al. 2012).

As the irradiation dose increases, the germination percentage showed a negative linear trend ($P < 0.0001$). However, the only doses that showed differences ($P < 0.01$) with respect to the control treatment (control) were 12 Gy, 15 Gy, 20 Gy, and 25 Gy for the linear accelerator and 20 Gy and 25 Gy for ^{60}Co. In general terms, the germination percentage tended to decrease as the irradiation dose increased. This result is consistent with those of several authors (Golubinova, Gecheff 2011; Rajarajan et al. 2016), who found that the germination of different species tends

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to decrease as the radiation dose increases. This is because ionising radiation can depress or inhibit vital cell functions, causing the death of some cells and even the death of the embryo. This phenomenon tends to increase with increasing irradiation dose, resulting in decreased germination (Olasupo et al. 2016). The regression equation indicates that the LD_{50} occurred at 20 Gy for ^{60}Co and 12 Gy for the linear accelerator.

The GR_{50} was determined through linear regression. It was found that no radiation dose from either source resulted in a lower height than the control. The model that best fit (highest R^2) simulated the effect of radiation as it increased. A projection was made from the equations generated, and the GR_{50} was determined. For the linear accelerator, the dose was 45 Gy, and for ^{60}Co, it was 55 Gy (Table 1). The variation between radiation sources may be due to the energy emitted by each source.

Photosynthetic pigment content. ^{60}Co irradiation had a positive effect on chlorophyll and carotenoid concentrations, showing statistically significant differences (Tukey's test; $P < 0.05$) compared to the control (Figure 4). In particular, all doses induced an increase in total chlorophyll A (*Chl A*) content in the range of 0.40–0.50 mg·g^{-1} and carotenoids (0.18–0.19 mg·g^{-1}) (Figure 4). On the other hand, the 0 Gy dose showed higher chlorophyll B (*Chl B*) (0.78 mg·g^{-1}) and chlorophyll A + B (*Chl A* + *Chl B*) (0.82 mg·g^{-1}) contents. The phenomenon of high doses of certain factors leading to increased chlorophyll concentrations but reduced growth is observed in various plants and may be related to the physiological stress experienced by plant organisms when exposed to extreme conditions. This phenomenon could be due to the effects of stress, growth inhibition, and/or metabolic imbalance.

Table 1. Regression models, coefficients of determination (R^2), and estimated values of the median lethal dose (LD_{50}) and growth reduction dose (GR_{50}) in *P. pseudostrobus* seeds and seedlings irradiated with ^{60}Co and linear accelerator

Radiation source	Parameter	Regression model	R^2	Estimated dose (Gy)
^{60}Co	DL_{50}	$y = -0.8801x + 32.7168$	0.85	20
Linear accelerator		$y = -0.3368x + 18.7735$	0.82	12
^{60}Co	GR_{50}	$y = -0.041x^2 + 1.1812x + 25.976$	0.87	55
Linear accelerator		$y = -0.0534x^2 + 1.328x + 27.412$	0.84	45

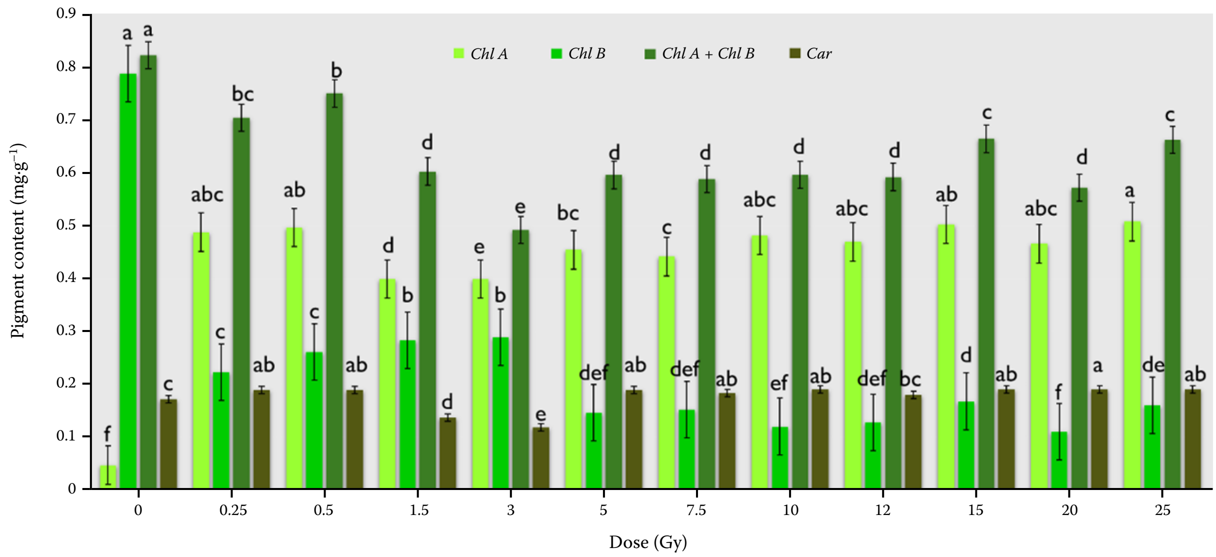

Figure 4. Effect of different ^{60}Co irradiation doses evaluated on the photosynthetic pigment content of *P. pseudostrobus* seedlings

Chl A – chlorophyll A; *Chl B* – chlorophyll B; *Chl A* + *Chl B* – chlorophyll A + chlorophyll B; *Car* – carotenoids; values with different letters in the column are statistically different according to the Tukey test ($P \leq 0.05$)

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On the other hand, statistically significant differences ($P \leq 0.05$) were found between the different doses for the effect on the photosynthetic pigment content of seeds irradiated with a linear accelerator. The lowest values for the variables evaluated (chlorophyll A, chlorophyll B, chlorophyll A + B, and carotenoids) were obtained at doses of 3.0 Gy. On the other hand, the dose of 25 Gy did not present significant differences ($P \leq 0.05$) in chlorophyll B content with respect to the control (Figure 5).

Effect of ^{60}Co on plant quality. The results showed significant differences ($P \leq 0.05$) for the root height-to-length ratio (*RHLR*) and the shoot-to-root biomass ratio (*SAR*), with the highest average values for plants from 1.5 to 5.0 Gy: 1.82 and 5.65, respectively. According to Rodríguez-Ortiz et al. (2020), *RHLR* values < 2 indicate high seedling quality. This ratio will help improve their survival.

The slenderness index (*SI*) is an indicator of a plant's resistance to wind desiccation, survival, and growth in dry sites (Rodríguez-Ortiz et al. 2020). For example, values equal to or less than 6 were found, indicating high-quality seedlings. However, higher values indicate that the plant has a thin stem relative to its height (Prieto et al. 2009). The dose with the thinnest stem relative to height is 10.0 Gy to 25.0 Gy. These results are similar to those found by Rodríguez-Ortiz et al. (2020), with values ranging from 5.5 to 6.1 for *P. pseudostrobus*.

The Dickson quality index (*DI*) and the *RAR* are indicators that predict planting success (Rodríguez-Ortiz et al. 2021). The doses with the highest *DI* were 0.5 Gy, 1.5 Gy, and 3.0 Gy (0.24 and 0.25). These values refer to average seedling quality. However, doses of 0 Gy, 3.0 Gy, 5.0 Gy, and 7.5 Gy showed values below 0.2. Therefore, it is considered of poor quality according to the *Pinus* classification of Rodríguez-Ortiz et al. (2020). Prieto et al. (2009) indicated that a certain balance must exist between the shoot and root systems of seedlings for their survival. The lignification index showed lower values (18.54%) at doses of 12.0 Gy than in the rest of the seedlings, and the doses of 0.5 Gy and 1.5 Gy presented the highest value (28.66%) (Table 2).

Effect of the linear accelerator on plant quality. The results showed significant differences ($P \leq 0.05$) for the root height-to-length ratio (*RHLR*) and the shoot-to-root biomass ratio (*SAR*), with the highest averages for plants from 1.5 Gy to 5.0 Gy, at 1.82 and 5.65, respectively. According to Rodríguez-Ortiz et al. (2020), *RHLR* values < 2 indicate high seedling quality. This ratio will help improve seedling survival.

The slenderness index (*SI*) is an indicator of plant resistance to wind desiccation, survival, and growth in dry sites (Rodríguez-Ortiz et al. 2020). For example, values equal to or less than 6 were found, indicating high-quality seedlings. However, higher

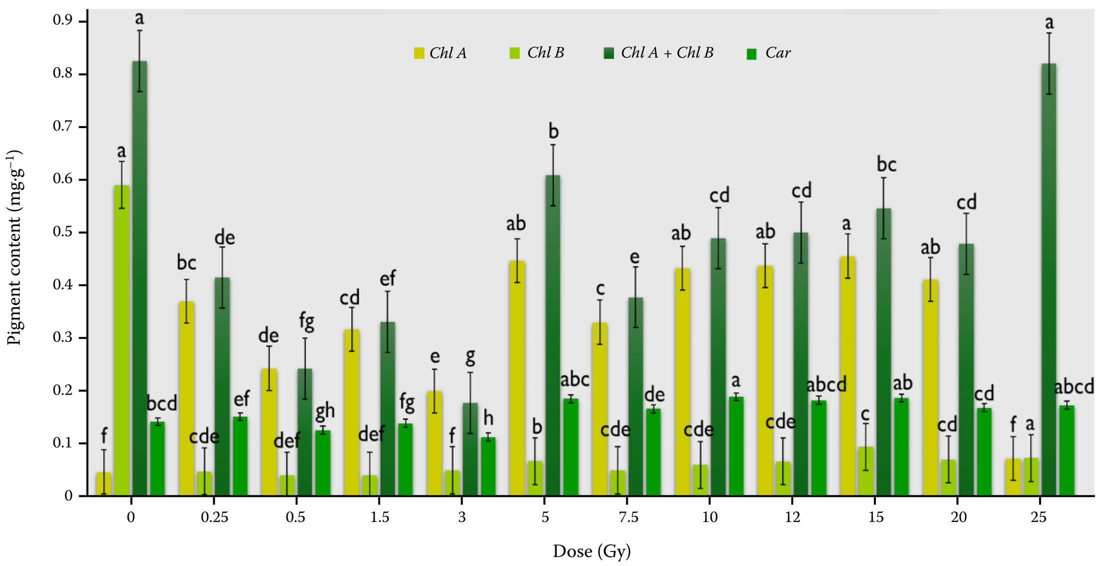

Figure 5. Effect of different irradiation doses with a linear accelerator on the photosynthetic pigment content of *P. pseudostrobus* seedlings

Chl A – chlorophyll A; *Chl B* – chlorophyll B; *Chl A* + *Chl B* – chlorophyll A + chlorophyll B; *Car* – carotenes; values with different letters in the column are statistically different according to the Tukey test ($P \leq 0.05$)

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Table 2. Quality indices of *P. pseudostrobus* seedlings from seeds irradiated with different doses of a linear accelerator

Doses (Gy)	*RAS*	*RAR*	Slenderness index (*IE*)	Dickson index (*ID*)	Lignification index (*IL*)(%)
0.0	4.45[c]	1.73[bc]	5.67[ab]	0.14[d]	22.30[d]
0.5	4.30[cd]	1.82[a]	5.71[ab]	0.24[ab]	25.86[c]
1.5	5.45[a]	1.82[a]	5.80[bc]	0.25[a]	28.66[a]
3.0	5.45[a]	1.82[a]	5.95[bc]	0.25[a]	28.66[a]
5.0	5.45[a]	1.82[a]	5.95[bc]	0.25[a]	27.77[b]
7.5	5.45[a]	1.78[b]	5.80[bc]	0.25[a]	27.77[b]
10.0	4.90[b]	1.78[b]	5.55[a]	0.16[d]	27.77[b]
12.0	4.90[b]	1.78[b]	5.55[a]	0.16[d]	18.54[e]
15.0	3.98[e]	1.57[f]	6.03[c]	0.15[cd]	18.54[e]
20.0	3.57[ef]	1.62[e]	6.03[c]	0.14[d]	18.54[e]
25.0	3.57[ef]	1.62[e]	6.03[c]	0.14[d]	18.54[e]
Standard error	0.65	0.05	0.38	0.01	1.30

RAS – aboveground biomass to belowground biomass ratio; *RAR* – shoot/root ratio; values with different letters in the column are statistically different according to the Tukey test ($P \leq 0.05$)

values indicate that the plant has a thin stem relative to its height (Prieto et al. 2009). The dose that presents the thinnest stem relative to height is 10.0 Gy to 25.0 Gy. These results are similar to those found by Rodríguez-Ortiz et al. (2020), with values ranging from 5.5 to 6.1 for *P. pseudostrobus*.

The Dickson quality index (*DI*) and the *RAR* are indicators that predict plantation success (Rodríguez-Ortiz et al. 2021). The doses that presented the highest *DI* were 0.5 Gy, 1.5 Gy, and 3.0 Gy (0.24 and 0.25). These values refer to the average quality of the seedlings. However, doses of 0 Gy, 3.0 Gy, 5.0 Gy, and 7.5 Gy presented values lower than 0.2. Therefore, it is considered of poor quality according to the *Pinus* classification of Rodríguez-Ortiz et al. (2020). Prieto et al. (2009) have indicated that there must be a certain balance between the aerial part and the root system of the seedlings for their survival. The lignification index showed lower values (18.54%) at the 12.0 Gy doses than in the other seedlings, with the 0.5 Gy and 1.5 Gy doses showing the highest values (28.66%) (Table 3).

Table 3. Quality indexes of *P. pseudostrobus* seedlings from seeds irradiated with different doses of a linear accelerator

Doses (Gy)	*RAS*	*RAR*	Slenderness index (*IE*)	Dickson index (*ID*)	Lignification index (*IL*) (%)
0.0	4.45[c]	1.73[bc]	5.67[ab]	0.14[d]	22.30[d]
0.5	4.30[cd]	1.68[d]	5.71[ab]	0.24[ab]	25.86[c]
1.5	5.65[a]	1.82[a]	5.80[bc]	0.25[a]	28.66[a]
3.0	5.65[a]	1.82[a]	5.95[bc]	0.25[a]	28.66[a]
5.0	5.65[a]	1.82[a]	5.95[bc]	0.15[cd]	27.77[b]
7.5	3.47[a]	1.62[e]	5.80[bc]	0.15[cd]	27.77[b]
10.0	4.70[b]	1.78[b]	5.55[a]	0.16[d]	27.77[b]
12.0	4.7[b]	1.78[b]	5.55[a]	0.16[d]	18.54[e]
15.0	3.88[e]	1.57[f]	6.03[c]	0.15[cd]	18.54[e]
20.0	3.47[ef]	1.62[e]	6.03[c]	0.14[d]	18.54[e]
25.0	3.47[ef]	1.62[e]	6.03[c]	0.14[d]	18.54[e]
Standard error	0.67	0.06	0.36	0.01	1.3

RAS – aboveground biomass to belowground biomass ratio; *RAR* – shoot/root ratio; values with different letters in the column are statistically different according to the Tukey test ($P \leq 0.05$)

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CONCLUSION

The results showed that the application of doses lower than 20 Gy (LD_{50}) with the gamma-ray source resulted in a hormetic effect on germination capacity. On the other hand, doses lower than 12.0 Gy (LD_{50}) with the linear accelerator radiation source are required to generate the same effect. Based on these results, it is concluded that the radiation doses used generated a hormetic effect on the height and diameter of the irradiated seedlings. However, the 0.5Gy dose applied with both radiation sources was the one that presented the greatest hormetic effect on the expression of these variables in the M_1 seedlings evaluated.

Finally, it is confirmed that the effect of radiation on a species will depend on the genotype, storage time, applied dose, and energy emitted by the radiation source. Both sources of ionising radiation proved useful in increasing germination capacity, germination rates, diameter, and height of *P. pseudostrobus* seedlings, making them an important biotechnological tool for *ex vitro* propagation and for contributing to reforestation and restoration. Taken together, these results indicate that both radiation sources are capable of inducing hormetic responses in *P. pseudostrobus*, although with significant differences in their effective range and the level of stimulation achieved. This information is relevant for biotechnological applications aimed at improving plant production in nurseries, provided that the dosage is precise and appropriate for the type of radiation used.

Although the use of ionising radiation carries potential risks for humans and the environment, these risks are minimal when low doses of ionising radiation are applied in authorised facilities and under strict radiation protection protocols. Therefore, we believe that these conditions ensure its safe application for use in plant production.

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