Annals of Silvicultural Research
47(2), 2022: 78-84
https://creajournals.crea.gov.it/asr/

Research paper

Hormetic effect of Gamma rays on *Pinus pseudostrobus* Lindl. seed

Laura Yasmin Flores López[1], Lourdes Georgina Iglesias Andreu[1*], Lourdes Palafox-Chávez[2], Juan Carlos Noa Carrazana[1]

Received: 8/04/2022 *Accepted: 24/06/2022* *Available online: 24/08/2022*

ABSTRACT *Pinus pseudostrobus* Lindl. is one of the most used conifer species in reforestation projects. However, it presents some problems with the viability of its seeds. Although the usefulness of the radiostimulant effect of gamma rays in processes such as germination and growth of forest species has been demonstrated, there is no information available on this species. For this reason, it was proposed to evaluate the "hormetic effects" of low doses (0.5, 1.5, 3.0, 5.0, and 7.5 Gy) of gamma rays (60Co Theratron 780E Unit) on germination capacity, seedling growth, and chlorophyll content in this species. The results showed that all doses (0.5, 1.5, 3.0, 5.0, and 7.5 Gy) improved germination percentage, but not seedling growth. The highest germination percentage was recorded at 61.53 percent with the 0.5 Gy dose, while the gamma doses of 1.5 and 3.0 Gy ranked second with 53.84%. The height and diameter of seedlings from 0, 0.5, 1.5, and 3.0 Gy irradiated seeds did not show significant differences. However, as the dose increases, the stress generates a decrease in growth. The photosynthetic pigment content was lower at doses of 0.5 and 1.5 Gy (Ch a= 0.35, Ch b = 0.15, Ch a+b = 0.50, Car = 0.18). This study is important because it shows that the stimulatory effects of low gamma doses on germination and seedling growth may not be the same.

KEYWORDS: gamma ray, conifers, photosynthetic pigment, hormesis.

Introduction

Hormesis is a dose-response phenomenon in which at low doses it induces a stimulatory effect and at high doses it induces an inhibitory effect (Vargas-Hernandez et al. 2017, Agathokleous and Calabrese 2019). The "hormetic effect" provides the first quantitative description of biological plasticity at all levels of plant and animal organization through adaptive responses to stressful conditions (Calabrese and Mattson 2017, Agathokleous and Calabrese 2019). These adaptive responses can be triggered by exposing plants to low levels of biotic or abiotic stresses that protect them through processes that stimulate cellular defense mechanisms (Berry III and Lopez-Martinez 2020).

Among physical and chemical methods to improve germination effectiveness, stimulation with radiation has shown positive effects on seed germination and seedling growth of several species (Podleśny et al. 2012). In plants, radiation stimulation is a physical phenomenon based on the ability of cells to absorb and store energy (Prośba-Bialczyk et al. 2013). The application of low doses of gamma radiation can induce the phenomenon of "hormesis," which contributes to improved seed germination and initial plant growth. Although little is known about the basic mechanism of this phenomenon, it has been suggested that there is a link between hormesis and epigenetic alterations in irradiated species that show an adaptive response (Cedergreen 2008, Vergara et al. 2018, Agathokleous and Calabrese 2019). In addition, other studies have concluded that the biological effect of gamma radiation is mainly due to the formation of free radicals by hydrolysis of water, which may result in the modulation of an antioxidant system, accumulation of phenolic compounds, and chlorophyll pigments (Wi et al. 2007, Ashraf 2009).

Forest harvesting in Mexico is mainly based on the use of natural forests with a great diversity of *Pinus* and *Quercus* species (Sánchez-González 2016). Among the forest species of greatest economic interest is *P. pseudostrobus* (Perry 1991, Ramírez-Herrera et al. 2005). It is used for lumber, sleepers, particleboard, packing cases, veneer and plywood, pulp and paper, resin, handicrafts, joinery, and furniture (Perry 1991, Sánchez-González 2016).

Pinus pseudostrobus is widely distributed at an altitude of 1,500–3,200 m a.s.l. and receives rainfall of 1,000–1,500 mm, so this species is adapted to temperate and semi-warm sub-humid climate conditions (Gernandt and Pérez-de la Rosa 2014), which has helped it to have an ecological association with various forest species (Sandoval-García 2020). Because of this, *P. pseudostrobus* is one of the most propagated species in Mexico for reforestation programs, agroforestry, and commercial timber plantations due to the quality of its wood and its rapid growth (Sígala-Rodríguez et al. 2016, Flores García et al. 2019), with an annual production of approximately

1 - Universidad Veracruzana Instituto de Biotecnologia y Ecologia Aplicada – INBIOTECA, Xalapa, Veracruz - Mexico
2 - Centro Estatal de Cancerología Servicios de Salud de Veracruz, Xalapa, Veracruz - Mexico
* Corresponding author: liglesias@uv.mx

http://dx.doi.org/10.12899/asr-2401

19 million plants, cultivated in traditional (polyethylene bags) and technological (polystyrene trays) systems (CONAFOR 2011). However, seedlings used for this purpose can have a high mortality rate, apparently due to poor seed handling in the nursery (Bustamante-García et al. 2012).

Low doses of gamma radiation have shown stimulatory effects in several forest and agricultural species without causing genetic variation (Ramírez et al. 2008, Iglesias-Andreu et al. 2010, González and Nakayama 2015). Although the molecular mechanisms underlying the observed "radio-hormetic effect", especially in the germination processes of forest species, have not been clearly elucidated, its use in the development of reforestation and restoration programs of forest species is of great interest. For these reasons, the purpose of this research was to produce a "hormetic effect" on the germination capacity and growth of *P. pseudostrobus* seedlings for reforestation applications using low doses of gamma rays.

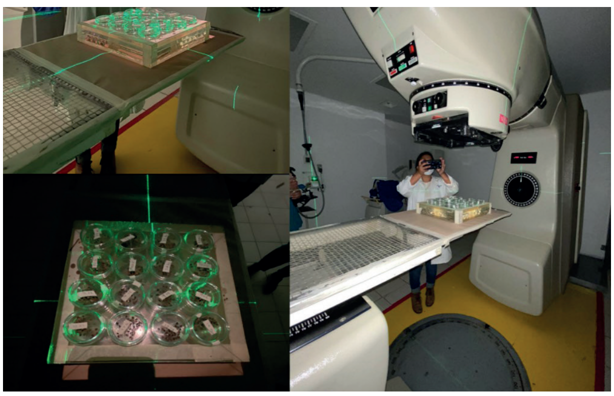
Materials and methods

For the development of the present study, 15-20 cones were collected from 20 "plus-trees" in a natural stand of *P. pseudostrobus*, which develops at 2,700 m a.s.l. in the locality of "El Aguaje" belonging to the municipality of "Las Vigas de Ramírez", in Veracruz, Mexico (19° 38' north latitude and 97° 06' west longitude).

Forest owners and wood processing companies

To perform the low dose irradiation with gamma rays, a total of 720 seeds were taken to be irradiated with doses of 0, 0.5, 1.5, 3, 5, and 7.5 Gy, for 0, 0.81, 2.43, 4.86, 8.09, and 12.14 minutes, in the Theratron 780E Cobalt 60 Unit (photons with an energy of 1.25 MV) of the "Centro Estatal de Cancerología" in Xalapa, Veracruz, Mexico. The samples were placed at a depth of 0.5 cm in a 30 x 30 cm field (Fig. 1). For each dose, four replicates of 30 seeds each were used.

Figure 1 - Irradiation of *P. pseudostrobus* seeds with gamma rays in the Theratron 780E Cobalt 60 Unit. (Flores-López 2021).



Germination and seedling emergence

The irradiated seeds and the control treatment were sown under greenhouse conditions at the Agricultural Sciences Forest Nursery of the Universidad Veracruzana. Irradiated seeds were sown in PVC tubes 10 cm in diameter and 16 cm long (TB-310), with a 3:1:1 substrate mixture of peat moss, vermiculite, and agrolite (Green Forest Mexico) in a completely randomized design with four replications. A total of 720 seeds were sown (120 seeds per treatment).

Germinated seeds were recorded daily for 25 days. For each dose, germination capacity (GC) was determined as the germination percentage at the end of the trial. According to Kolotelo et al. (2001), the maximum germination value was also determined as the maximum value of the sum of the germination percentage divided by the number of days. According to Juarez-Agis et al. (2006), germination energy (GE) was also determined as the number of days on which 50% germination is achieved.

Survivance and growth of seedlingsWood quality

From each of the doses, 20 seedlings were randomly selected to evaluate their growth in the nursery for 6 months. A randomized complete block design was used, with six treatments, four replications (blocks), and five plants per experimental unit. A Truper® digital vernier (mm) was used to measure monthly survival (percent), total height (cm), and basal diameter (root collar).

Content of photosynthetic pigments

To determine the photosynthetic pigment content, the methodology described by Porra et al. (1989) was followed. Plant material was used to extract chlorophyll (Chl) a, b, a + b, and carotenes (Car). For this purpose, 0.25 g of each seedling was weighed and macerated in liquid nitrogen (N2) with 5 mL of acetone (80%). They were then centrifuged for 12 minutes at 6,000 rpm in 15 mL polypropylene tubes. The supernatant was finally transferred to new polypropylene tubes for pigment readings in a spectrophotometer (JENWAY, Staffs, UK). For chlorophyll a (Chl a), the reading was taken at 663.6 nm, for chlorophyll b (Chl b) at 646.6 nm, and for carotenes (Car) at 440.5 nm. Photosynthetic pigment content was calculated using the following formulas:

$$Chl\ a = [(12.25 \times A_{663} - 2.25 \times A_{645})] \times V/100 \times W \quad (eq. 1)$$

$$Chl\ b = [(20.30 \times A_{645} - 4.91 \times A_{663})] \times V/100 \times W \quad (eq.2)$$

$$Chl\ a + b = [(7.34 \times A_{663} + 17.76 \times A_{645})] \times V/100 \times \quad (eq. 3)$$

$$Car = [(4.46 \times A_{441} - Chl\ a + Chl\ b)] \times V/100 \times W \quad (eq. 4)$$

Where: V is the total volume of acetone extract (mL) and W is the fresh weight (g) of the sample.

Statistical analysis

Statistical analysis of the data was performed using STATGRAPHICS (version 16.17). To normalize the distribution, germination capacity data were previously transformed with the arcsine function of the square root of p (= arcsine p) (Fienberg et al. 1970). Finally, mean comparisons were performed with Tukey's test ($p \leq 0.05$) for all variables evaluated. The data was analyzed with regression analysis to estimate the mean growth reduction (GR50) of the variable hypocotyl length of *P. pseudostrobus* seedlings. In addition, a linear correlation analysis ($p \leq 0.01$) between radiation doses as an independent variable (X) and hypocotyl length as a dependent variable (Y) was performed to determine the mean lethal dose (LD50) or mean reductive dose (GR50) (Ángeles-Espino et al. 2013).1

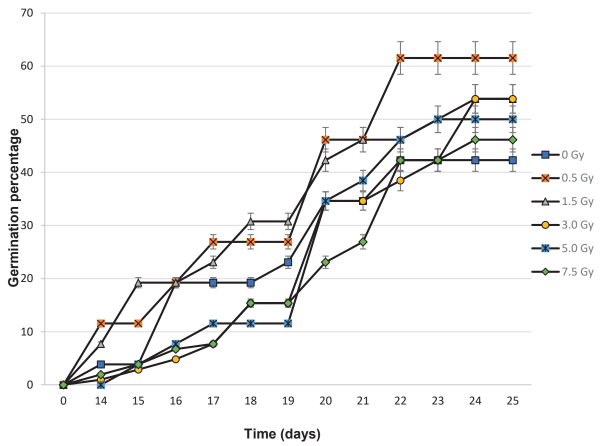
Results

Gamma ray effect on germination

The effects of different doses of gamma radiation on the germination capacity (GC), germination energy (GE), peak value (PV), and germination value (GV) of seeds can be seen in Table 1. The 0.5 Gy dose presented the highest percentage of germination capacity (61.53%), and as the irradiation dose increased, the germination capacity decreased. However, no dose showed a lower percentage than non-irradiated seeds.

The seeds irradiated with the doses of 0.5, 1.5, 3.0, and 7.5 Gy emerged 14 days after sowing at the same time as the non-irradiated seeds. Those with 5.0 Gy initiated germination at 15 days. It can be observed in the graph (Fig. 2) that the doses of 0.5 and 1.5 Gy presented a higher percentage of germinated seeds as the days passed. In all the doses applied (0.5, 1.5, 3.0, 5.0, 7.5), a steep curve was maintained and stabilized until 22, 22, 24, 23, and 24 days, respectively, resulting in the final germination percentage. An increase in the percentage of germinated seeds irradiated with a dose of 0.5 Gy. This increase ranged from 26.92% on day 19 to 46.15% of germinated seeds on day 20.

Figure 2 - Cumulative germination percentage curve of *P. pseudostrobus* seeds irradiated with various doses of gamma rays (0.0.5, 1.5, 1.5, 1.5, 3.0, 5.0, and 7.5 Gy).



Survival and growth of seedlings

The percentage of seedling survival was evaluated for each dose used, and it was found that there was no death of the 0.5 Gy and 5.0 Gy seedlings. The opposite was found with doses of 0, 1.5, 3.0, and 7.0 Gy. However, this percentage was not less than 90%. (Tab. 2).

Regarding growth, it was observed throughout the evaluation period that the hypocotyl length of

Table 1 - Mean germination values were obtained from the use of different doses of gamma irradiation for *P. pseudostrobus* seeds.

Dose (Gy)	(%) Germination Capacity (GC)	Germination Energy (GE)	Peak value (% day^{-1})	Germination Value (GV)
0	42.30±1.60^{a}	18.45±0.87^{b}	1.92±0.26^{b}	3.25±0.012^{a}
0.5	61.53±2.4^{e}	17.00±0.87^{a}	2.79±0.26^{c}	6.88±0.012^{e}
1.5	53.84±1.41^{d}	18.00±0.87^{b}	2.24±0.26^{a}	4.83±0.012^{d}
3	53.84±2.1^{d}	19.92±0.87^{c}	2.24±0.26^{a}	4.83±0.012^{d}
5	50.00±2.1^{c}	19.90±0.87^{c}	2.17±0.26b^{c}	4.34±0.012^{c}
7.5	46.15±1.52^{b}	19.15±0.87b^{c}	1.92±0.26^{b}	3.55±0.012^{b}
Calabria	69,370	14	35	30

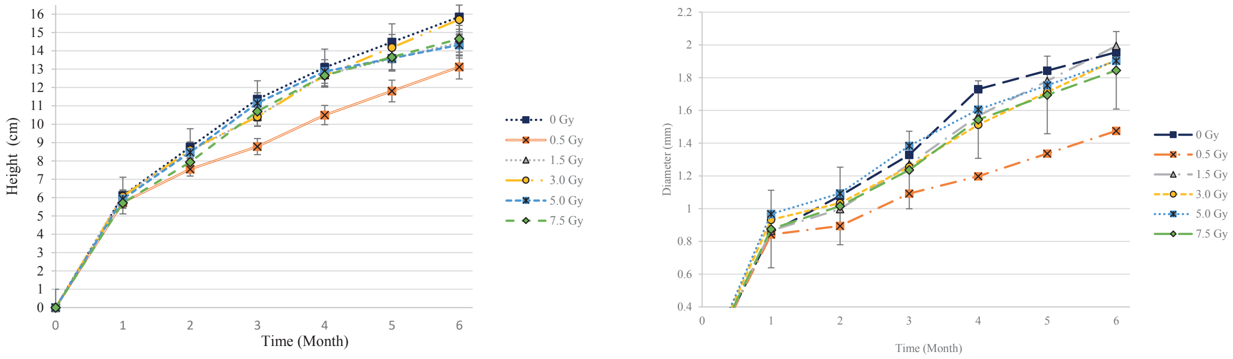
Values represent the mean ± SE (Standard Error). Means with different letters represent different significances. (Tukey, $p \leq 0.05$).

Table 2 - Effect of gamma ray doses on survival and growth variables of *P. pseudostrobus* seedlings.

Dose (Gy)	Hypocotyl length (cm)	Stem diameter (mm)	Survival (%)
0	15.85 ± 0.54^{c}	1.95 ± 0.63^{b}	90
0.5	15.76 ± 0.54^{bc}	1.47± 0.63^{a}	100
1.5	15.73 ± 0.54^{bc}	1.99± 0.63^{b}	90
3	15.71 ± 0.54^{bc}	1.91± 0.63^{b}	95
5	14.66 ± 0.54^{ab}	1.90 ± 0.63^{b}	100
7.5	14.33 ± 0.54^{ab}	1.84 ± 0.63^{b}	90

Values represent the mean ± SE (Standard Error). Means with different letters represent different significances. (Tukey, $p \leq 0.05$).

Figure 3 - Effect of gamma ray doses in P. pseudostrobus seedlings. a) Effect on hypocotyl length. b) Effect on the hypocotyl diameter.

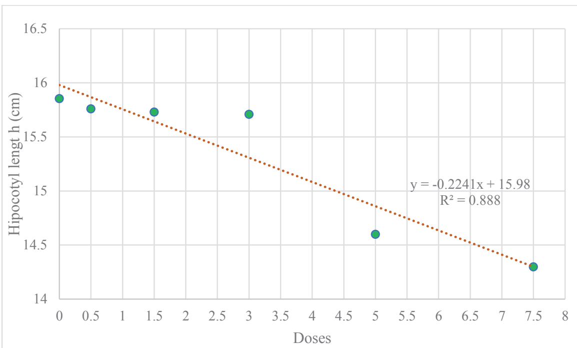


seedlings of non-irradiated seeds was greater than that of irradiated seeds. However, there was a greater significant difference with the 5.0 y 7.0 Gy dose because these seedlings presented a smaller height (Fig. 3a), and the 0.5 Gy dose had the smallest mean stem diameter (Fig. 3b).

Determination of GR_{50}

The linear regression model showed the best fit to explain the effect of doses on the hypocotyl length data of *P. pseudostrobus* seedlings. The mean growth reduction dose (GR_{50}) for the hypocotyl length of seedlings was 2.9 Gy (Fig. 4).

Figure 4 - Effect of gamma radiation on the hypocotyl length of *P.pseudostrobus* seedlings.

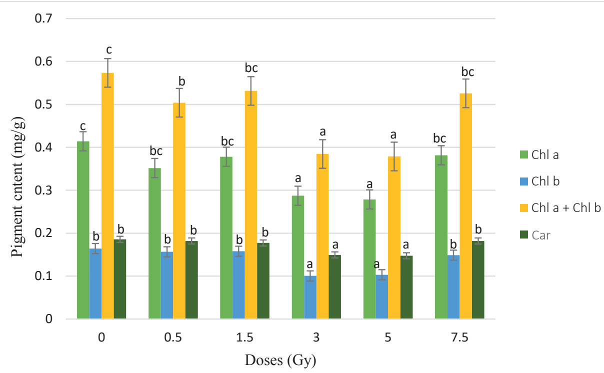


In addition, a negative (-0.94232) and highly significant (p<0.0001) correlation was obtained between Co_{60} doses and hypocotyl length, indicating that radiation altered normal seedling development as the dose increased.

Content of photosynthetic pigments

Statistically significant differences ($p \leq 0.05$) were found between the different doses of gamma rays evaluated. At the 3.0 and 5.0 Gy doses, the lowest values were obtained for the variables evaluated (chlorophyll a, chlorophyll b, chlorophyll a + b, and carotenoids). On the other hand, between the doses of 0.5, 1.5, 7.5, and 0 Gy, there were no significant differences ($p \leq 0.05$), the seedlings from non-irradiated seeds presenting the highest values in all the evaluated variables (chlorophyll a, chlorophyll b, chlorophyll a + b, and carotenoids) (Fig. 5).

Figure 5 - Effect of different gamma ray doses on the photosynthetic pigment content of *P. pseudostrobus* seedlings. Bars represent the average value of pigmentation content per dose; corresponding error bars (+/-ES) are shown. Bars with different letters represent different mean values according to Tukey ($p \leq 0.05$).



Discussion

Radiation accelerates the germination of different types of seeds when they are dormant or under stressful conditions that delay or prevent their germination (Ramírez et al. 2006). In recent years, it has been shown that the physiological effects caused by ionizing radiation on plants were first observed in germination and seedling growth (Ramírez et al. 2006, Antúnez-Ocampo et al. 2017). In the present study, it was determined that irradiation doses applied to seeds (0.5, 1.5, 3.0, 5.0, 5.0, and 7.5 Gy) were effective in increasing the germination percentage with respect to non-irradiated seeds (0 Gy). However, as the doses increased, the germination percentage decreased. These results agree with those described by Gutiérrez-Caro et al. (2021), who mention that applying doses higher than 10 Gy to *Eucalyptus nitens* H. Deane & Maiden does not show significant differences in the germination percentage with respect to the non-irradiated control. In another study by Zanzibar and Sudrajat (2016) in which gamma radiation was used on *Magnolia champaca* L. with doses of 0. 5, 10, 15, 20, 40, 60, 80, and 100 Gy, it was concluded that the dose that had a greater effect on germination was 10 Gy (34.1% increase to the control), and for the mean germination time and germination value, an increase was observed up to the dose of 15 Gy, and then as the dose increased, these decreased. On the other hand, Avendaño-Arrazate et al. (2021) irradiated seeds of *Coffea arabica* L. with Co_{60} at doses of 0, 10, 50, 100, 200, and 300 Gy, showing that all the doses used generated a reduction in the germination percentage. Akshatha and Chandrashekar (2014) mention that low doses of radiation activate metabolic processes that accelerate seed germination. This is because radiation causes de-differentiation, which affects protein synthesis, hormone balance, gas exchange, and enzyme activity (Akshatha and Chandrashekar 2014, Alvarez-Holguìn 2018). However, several authors mention that even when the theory explains the effect of irradiation on the speed of emergence and growth, there are cases in which the opposite effect is present because the biological effect of ionizing radiation depends on the type of radiation, the absorbed dose, and the genotype (De Micco et al. 2010, Antúnez-Ocampo 2017).

Regarding our results on height and diameter growth, it was found that all the doses applied generate a growth reduction effect, since if the dose increases, these variables decrease. This agrees with the results obtained by Thapa (2004) in *Pinus kesiya* D. Don and *Pinus wallichiana* and by Iglesias-Andreu et al. (2010) on *Abies religiosa* (Kunth) Schltd. et Cham., who showed that if the radiation dose is increased, the height variable decreases. Beyaz et al. (2016) irradiated seeds of *Lathyrus chrysanthus* Boiss. at different doses (0, 50, 100, 150, 200, and 250 Gy) of gamma rays, showing that seeds irradiated with the dose of 150 Gy had increased seedling and root length. However, at higher doses of 150 Gy, stress was evident and significant decreases were observed in all parameters evaluated. On the other hand, Akshatha et al. (2013) observed in *Terminalia arjuna* Roxb. that the dose of 25 Gy generated a greater stimulatory effect on the height and roots of their seedlings with respect to the control. Fonseca et al. (2012) mentioned that low-dose radiation applications favor various species where free radicals, ions, and excited molecules formed by their effects contribute to greater efficiency in the utilization of biochemical-metabolic pathways, which is reflected in plant growth and development.

The survival of seedlings from irradiated seeds is an important factor to consider when determining the use of a dose in an organism or part of it. In the present investigation, GR_{50} was determined with an average of 2.9 Gy. A linear regression analysis was performed on the height variable, which was the best fit (highest R_2 value). According to Jahan et al. (2020), GR_{50} allows breeders to evaluate mutagenic efficacy in relation to mutagen-induced biological damage. However, there are few studies in which doses lower than 10 Gy have been used, so a comparison with other species cannot be made.

Finally, the results of photosynthetic pigment content (Chlorophyll a, Chlorophyll b, Chlorophyll a + b, carotenes) showed that the highest values of photosynthetic content were obtained in the control. However, there were no significant differences with the 0.5 Gy dose, which was the dose that caused the greatest stimulation of germination.

Conclusions

The above results corroborate that plant radiosensitivity varies as a function of the absorbed irradiation doses. The absorbed radiation doses (0.5, 1.5, 3.0, 5.0, and 7.5) had a “hormetic effect” on germination capacity, higher than that of non-irradiated seeds. However, as the irradiation dose increases, the germination capacity decreases.

The doses evaluated did not generate a “hormetic effect” on the height and diameter of seedlings of irradiated seeds with respect to non-irradiated seeds. However, if the objective is to increase germination capacity, these doses do not have an inhibitory effect on seedling growth. On the other hand, pigment content is lower at low doses and higher at high doses.

The use of gamma ray irradiation technology at low doses could be used to improve the germination and quality of *P.pseudostrobus* seeds and produce healthy seedlings.

Acknowledgments

LYFL expresses gratitude to the “Consejo Nacional de Ciencia y Tecnologa” (CONACyT) for grant No. 1088083, as well as the “Centro Estatal de Cancerología Servicios de Salud de Veracruz Xalapa Veracruz” and the “Vivero Forestal Universitario de Ciencias Agrícolas” of the “Universidad Veracruzana”.

References

Agathokleous E., Calabrese E.J. 2019 - *Hormesis can enhance agricultural sustainability in a changing world*. Global Food Security 20: 150-155. https://doi.org/10.1016/j.gfs.2019.02.005

Akshatha, Chandrashekar K.R., Somashekarappa H.M., Souframanien J. 2013 - *Effect of gamma irradiation on germination, growth, and biochemical parameters of* Terminalia arjuna *Roxb*. Radiation Protection and Environment 36(1): 38. https://doi.org/10.4103/0972-0464.121826

Akshatha, Chandrashekar K.R. 2014 - *Gamma sensitivity of forest plants of Western Ghats*. Journal of Environmental Radioactivity 132: 100-107. https://doi.org/10.1016/j.jenvrad.2014.02.006

Álvarez Holguín A., Morales Nieto C.R., Avendaño Arrazate C.H., Santellano Estrada E., Melgoza Castillo A., Burrola Barraza M.E., Corrales Lerma R. 2018 - *Dosis letal media y reducción media del crecimiento por radiación gamma en pasto africano* (Eragrostis lehmanniana *Ness*). Ecosistemas Y Recursos Agropecuarios 5(13): 81-88. https://doi.org/10.19136/era.a5n13.1268

Ángeles-Espino A., Valencia-Botín A.J., Virgen-Calleros G., Ramírez-Serrano C., Paredes-Gutiérrez L., Hurtado-De la Peña S. 2013 - *Determinación de la dosis letal (DL_{50}) con Co_{60} en vitroplántulas de* Agave tequilana *var.* Azul. Revista Fitotecnia Mexicana 36(4): 381-386. [Online]. Available: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-73802013000400003&lng=es. [2022]

Antúnez-Ocampo O.M., Cruz-Izquierdo S., Sandoval-Villa M., Santacruz-Varela A., Mendoza-Onofre L.E., De la Cruz-Torres E., Peña-Lomelí A. 2017 - *Variabilidad inducida en caracteres fisiológicos de* Physalis peruviana *L. mediante rayos gamma $_{60}$CO aplicados a la semilla*. Revista Fitotecnia Mexicana 40(2): 211-218. https://doi.org/10.35196/rfm.2017.2.211-218

Ashraf M. 2009 - *Biotechnological approach of improving plant salt tolerance using antioxidants as markers*. Biotechnology advances 27(1): 84-93. https://doi.org/10.1016/j.biotechadv.2008.09.003

Avendaño-Arrazate C.H., Gómez-Simuta Y., Martínez-Bolaños M., Méndez-López I., Ortíz-Curiel S., Oriza-Flores R., Reyes-López D. 2021 - LA *Radiación gamma de 60Co en características morfológicas y reproductivas de plantas M1 en* Coffea arabica *L.: Radiación gamma de 60Co en Café*. Ecosistemas y Recursos Agropecuarios 8(1): e2730. https://doi.org/10.19136/era.a8n1.2730

Beyaz R., Kahramanogullari C. T., Yildiz C., Darcin E. S., Yildiz M. 2016 - *The effect of gamma radiation on seed germination and seedling growth of* Lathyrus chrysanthus *Boiss. under in vitro condition*s. Journal of Environmental Radioactivity 162: 129-133. https://doi.org/10.1016/j.jenvrad.2016.05.006

Berry III R., López-Martínez G. 2020 - A *dose of experimental hormesis: when mild stress protects and improves animal performance*. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 242: 110658. https://doi.org/10.1016/j.cbpa.2020.110658

Bustamante-García V., Prieto-Ruíz JÁ., Merlín-Bermudes E., Álvarez-Zagoya R., CONAFOR (Comisión Nacional Forestal, MX) 2011 - *Indicadores de gestión de desempeño del programa PROCOREF durante el ejercicio fiscal 2010*. Universidad Autónoma Chapingo (UACH). [Online]. Available: http://www.cnf.gob.mx:8080/snif/portal/evaluaciones/documentos-y-alcances-de-las-evaluaciones [2022]

Calabrese E.J., Mattson M.P. 2017 - *How does hormesis impact biology, toxicology, and medicine?* NPJ Aging and Mechanisms of Disease 3(1):1-8. https://doi.org/10.1038/s41514-017-0013-z

Cedergreen N. 2008 - *Herbicides can stimulate plant growth*. Weed Research 48(5): 429-438. https://doi.org/10.1111/j.1365-3180.2008.00646.x

De Micco V., Arena C., Pignalosa D., Durante M. 2010 - *Effects of sparsely and densely ionizing radiation on plants*. Radiation and Environmental Biophysics 50(1):1-19. https://doi.org/10.1007/s00411-010-0343-8

Fienberg S.E., Sokal R.R., Rohlf F.J., Rohlf F.J., Sokal R.R. 1970 - *Biometry. the principles and practice of statistics in biological research*. Biometrics 26(2): 351-355. https://doi.org/10.2307/2529087

Flores García A., Ojeda T.P., Ayala E.F. 2019 - *Potencial de reforestación de seis especies de pino para la restauración de zonas degradadas*. Revista Mexicana De Ciencias Forestales 10(55): 171-179. https://doi.org/10.29298/rmcf.v10i55.604

Fonseca A.Á., Suárez L.C., Fernández R.R., Brizuela R.P., Prado W.E. 2012 - *Indicadores fisiológicos en plántulas de* Solanum lycopersicum *L., procedentes de semillas irradiadas con rayos X*. Biotecnología Vegetal 12 (3):173-177. [Online]. Available: https://revista.ibp.co.cu/index.php/BV/article/view/172 [2022]

Gernandt D.S., Pérez-de la Rosa J.A. 2014 - *Biodiversidad de* Pinophyta *(coníferas) en México*. Revista Mexicana De Biodiversidad 85: 126-33. https://doi.org/10.7550/rmb.32195

González M.C., Nakayama H.D. 2015 - *Radioestimulación de la germinación en* Stevia rebaudiana *cultivar KH-IAN VC-142 (Eireté), mediante el empleo de rayos gamma 60Co*. Cultivos Tropicales 36(4): 117-119. [Online]. Available: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362015000400015&lng=es&tlng=pt. [2022]

Gutiérrez-Caro B., Koch Z., Villegas D., González -Campos J.M., Doris L., Molina- Brand M.P., Velásquez E. 2021 - *Análisis de germinación de semillas de* Eucalyptus nitens *tratadas con radiación gamma: indicios de efecto hormético*. Ciencia & Investigación Forestal 27(3): 7-16. https://doi.org/10.52904/0718-4646.2021.554

Iglesias-Andreu L.G., Sánchez-Velásquez L.R., Tivo-Fernández Y., Luna-Rodríguez M., Flores-Estévez N., Noa-Carrazana J.C., Ruiz-Bello C., Moreno-Martínez J.L. 2010 - *Effect of gamma radiation on* Abies religiosa *(Kunth) Schltd.* et Cham. Revista Chapingo Serie Ciencias Forestales y del Ambiente 16(1): 5-12. https://doi.org/10.5154/r.rchscfa.2009.06.021

Jahan R., Malik S., Ansari S.B., Khan S. 2020 - *Evaluation of Optimal Doses for Gamma Rays and Sodium Azide in Linseed Genotypes*. Agricultural Science Digest. A Research Journal (OF). https://doi.org/10.18805/ag.d-5165

Juárez-Agis A., López-Upton J., Vargas-Hernández J.J., Sáenz-Romero C. 2006 - *Variación geográfica en la germinación y crecimiento inicial de plántulas de* Pseudotsuga menziesii *de México*. Agrociencia 40: 783-792. [Online]. Available: https://agrociencia-colpos.mx/index.php/agrociencia/article/view/509 [2022]

Kolotelo D., Steenis E. V., Peterson M., Bennett R., Trotter D., Dennis J. 2001 - *Seed handling guidebook*. British Columbia Ministry of Forests, Tree Seed Centre Press. 46 p. [Online]. Available: https://rngr.net/publications/seed-handling-guidebook/complete/at_download/file ISBN: 0772646589. [2022]

Perry J.P. 1991 - *The pines of Mexico and Central America*. Portland, Or: Timber Press. 231 p.

Porra R.J., Thompson W.A., Kriedemann P.E. 1989 - *Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy*. Biochimica Et Biophysica Acta (BBA) - Bioenergetics 975(3): 384-94. https://doi.org/10.1016/s0005-2728(89)80347-0

Podleśny J., Stochmal A., Podleśna A., Misiak L. E. 2012 - *Effect of laser light treatment on some biochemical and physiological processes in seeds and seedlings of white lupine and faba bean*. Plant Growth Regulation 67: 227-233. https://doi.org/10.1007/s10725-012-9681-7

Prośba-Bialczyk U., Szajsner H., Grzys E., Demczuk A., Sacala E., Bąk K. 2013 - *Effect of seed stimulation on germination and sugar beet yield*. International Agrophysics 27: 195-201. https://doi.org/10.2478/v10247-012-0085-8

Ramírez-Herrera C., Vargas-Hernández J.J., López-Upton J. 2005 - *Distribución y conservación de las poblaciones naturales de* Pinus greggii. Acta Botánica Mexicana (72):1-16. https://doi.org/10.21829/abm72.2005.997

Ramírez R., González L.M., Camejo Y. Z., Nircia F.Y. 2006 - *Estudio de radiosensibilidad y selección del rango de dosis estimulantes de rayos x en cuatro variedades de tomate (*Lycopersicon esculentum *Mill)*. Cultivos Tropicales 27(1): 63-67. [Online]. Available: https://www.redalyc.org/articulo.oa?id=193215885012 [2022]

Ramírez R., González L.M., Chávez L., Camejo Y., González M.C., Fernández A. 2008 - *Estudio bioquímico-molecular de la estabilidad genética en plantas de tomate procedentes de semillas tratadas con bajas dosis de rayos X*. Cultivos Tropicales 29(3): 39-46. [Online]. Available: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362008000300006&lng=es&tlng=en. [2022]

Sánchez-González A. 2016 - *Una visión actual de la diversidad y distribución de los pinos de México*. Madera y Bosques 14(1): 107-120. https://doi.org/10.21829/myb.2008.1411222

Sandoval-García R., González-Cubas R., Bautista-Cruz A. 2020 - *Asociación ecológica de* Pinus pseudostrobus (Pinaceae) *como respuesta de las variaciones biogeográficas en el Centro-Sur de México*. Acta Botanica Mexicana (127): e1627 https://doi.org/10.21829/abm127.2020.1627

Sígala-Rodríguez J. Á., González Tagle M. A., Prieto Ruíz J. Á., Basave Villalobos E., Statgraphics Centurion 2009 - X. V. I. Version, 16. 17 Statpoint Technologies. INC. Thapa C.B. 2004 - *Effect of acute exposure of gamma rays on seed germination and seedling growth of* Pinus kesiya *gord and* Pinus wallichiana *A.B. Jacks*. Our Nature 2(1):13-17. https://doi.org/10.3126/on.v2i1.318

Vargas-Hernández M., Macias-Bobadilla I., Guevara-Gonzalez R.G., Romero-Gomez S.D., Rico-Garcia E., Ocampo-Velazquez R.V., Alvarez-Arquieta L.D., Torres-Pacheco I. 2017 - *Plant hormesis management with biostimulants of biotic origin in agriculture*. Frontiers in Plant Science 8: 1762. https://doi.org/10.3389/fpls.2017.01762

Vergara R.P., Aguirre P., Durán O., Nario A. 2018 - *Aplicaciones de tecnologías nucleares en conservación y mejoramiento genético forestal bajo un escenario de cambio climático*. Ciencia & Investigación Forestal 24(3): 116-127. https://doi.org/10.52904/0718-4646.2018.506

Wi S.G., Chung B.Y., Kim J.S., Kim J.H., Baek M. H., Lee J.W., Kim Y.S. 2007 - *Effects of gamma irradiation on morphological changes and biological responses in plants*. Micron 38(6): 553-564. https://doi.org/10.1016/j.micron.2006.11.002

Zanzibar M., Sudrajat D. J. 2016 - *Effect of Gamma Irradiation on Seed Germination, Storage, and Seedling Growth of* Magnolia Champaca L. Indonesian Journal of Forestry Research 3(2): 95-106. https://doi.org/10.20886/ijfr.2016.3.2.95-106