Radiohormetic effect on the germination of Pinus pseudostrobus Lindl. seeds irradiated with linear accelerator

Laura Yasmin Flores-López
Instituto de Biotecnología y Ecología Aplicada, Universidad Veracruzana
Lourdes Georgina Iglesias-Andreu (liglesias@uv.mx)
Instituto de Biotecnología y Ecología Aplicada, Universidad Veracruzana
María de Lourdes Palafox-Chávez
Centro Estatal de Cancerología Servicios de Salud de Veracruz

Research Article

Keywords: Hormesis, germinating capacity, growth, chlorophyll, biomass

Posted Date: September 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2002227/v1

Abstract

Pinus pseudostrobus Lindl. is one of the most widely propagated species in Mexico for reforestation, agroforestry, and commercial timber plantations because of the quality of its wood and its rapid growth. Among the physical and chemical methods to improve the effectiveness of germination, stimulation with radiation has shown positive effects on seed germination and seedling growth in several species. However, there are not many studies that have explored the radiohormetic effect on germination, survival, and seedling quality in conifers. Therefore, the present study was carried out with the objective of evaluating the radiohormetic effects of the use of low doses of linear accelerator on the germination capacity, growth, and quality of seedlings as well as the chlorophyll content of *P. pseudostrobus* for the purpose of reforestation and the establishment of forest plantations. A total of 720 seeds were taken to be irradiated with doses of 0, 0.5, 1.5, 1.5, 3, 5, and 7.5 Gy, in the linear accelerator Elekta Synergy Platform. For each dose, four replicates of 30 seeds each were used. It was determined that all the doses used increased the number of germinated seeds and showed an increase in seedling growth with respect to the control. The plant quality indexes showed that the doses of 0.5, 1.5, and 3.0 Gy contributed most to obtaining plants with larger aerial and root parts, but with a balanced conformation and a great capacity for the formation of new roots, which will help to present better levels of survival.

Introduction

Mexico is one of the countries with the greatest diversity of pine species (Pausas, 2015), which are of great ecological, economic, and social importance (Ramírez-Herrera et al. 2005; Sánchez-González, 2008). According to the Ministry of Environment and Natural Resources (SEMARNAT), 45% of the national territory presents some type of degradation, mainly due to changes in land use by agricultural activities; Mexico currently ranks fifth in the world for deforestation (SEMARNAT, 2014). Therefore, one of the activities that has regained great importance in recent decades is the production of forest seedlings due to the need to reestablish vegetation cover through reforestation and restoration activities (García et al. 2001; Pulido et al. 2002).

For the propagation of large quantities of forest plants, there are limitations due to seed dormancy caused by the presence of a thick, hard, and impermeable testa, which does not allow the entry of oxygen and water for imbibition to occur and embryo growth to begin, so the percentage of seeds that germinate is low (González et al. 2009; Ruiz et al. 2007).

Pinus pseudostrobus Lindl. is one of the most widely propagated species in Mexico for reforestation, agroforestry, and commercial timber plantations due to the quality of its wood and its rapid growth (Sígala-Rodríguez et al. 2016), with an annual production of approximately 19 million plants, cultivated in both traditional (polyethylene bags) and technological (polystyrene trays) systems (CONAFOR 2011). It is a species with high genetic variation and a wide altitudinal distribution between 1600 and 3250 m.a.s.l. It thrives in temperate to warm temperate climates with temperatures ranging from – 9.0 to 40°C (Viveros-Viveros et al. 2005; Delgado et al. 2007).

Studies of this species have focused mainly on its structure and natural regeneration (Sáenz-Romero et al. 2012). It is therefore important to carry out studies on the factors that influence seed quality and seedling growth, since this would increase the probability of success in regional planting and reforestation programs.

Among physical and chemical methods to improve germination effectiveness, radiation stimulation has shown positive effects on seed germination and seedling growth of several species (Podleśny et al. 2012; Jamil et al. 2013). 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 literature data affirms that irradiation as a method of pre-germinative seed stimulation has a positive effect on the growth and metabolism of many species. As a result, the current study aims to assess the "hormetic" effects of *P. pseudostrobus* seedlings irradiated in a linear accelerator on seed germination and growth.

Materials And Methods

Plant material and site characteristics

The germplasm (seeds) was obtained by collecting 15-20 cones from 20 plus trees in a natural stand of *P. pseudostrobus*, located at 2700 m.a.s.l. in the locality "El Aguaje" belonging to the municipality of "Las Vigas de Ramírez", Veracruz, Mexico (19°38' north latitude and 97°06' west longitude) (Fig. 1).

Irradiation with low doses of linear accelerator

To perform low-dose irradiation with the linear accelerator, 720 seeds were taken and subjected to various doses (0, 1.5, 3, 5, 5, 7.5 Gy) in the Elekta Synergy Platform linear accelerator (photons with an energy of 6 MV) of the State Cancer Center in Xalapa, Veracruz. The samples were placed at a depth of 1.5 cm (30 x 30 cm field) (Fig. 2). For each dose, four replicates of 30 seeds each were used.

$$T = \frac{(Prescribed\ dose)}{(FC)(PDD)(Scp)}$$

Where: T is the exposure time of the seeds to irradiation (UM), FC is the calibration factor of 1.0 cGy/UM, PDD, which determines the depth at which the sample is placed, in this case is 1.002, and Scp represents the radiation due to the dispersion of the linear accelerator head in a field of 30 x 30 cm and is equal to 1.076.

Germination and seedling emergence

Under greenhouse conditions in the Agricultural Sciences Forest Nursery of the "Universidad Veracruzana", the irradiated seeds and the control treatment were sown in plastic tubes (TB-310) 16 cm long, with a mixture of peat moss, vermiculite, and agrolite, in a 3:1:1 ratio. A completely randomized design with four replications was used to sow a total of 720 seeds (120 seeds per treatment). Germinated seeds were counted daily for 25 days. For each dose, germination capacity (GC) was determined as the germination percentage at the end of the test. The peak value, which is the maximum value of the sum of the germination percentage divided by the number of days, represents the germination speed. The germination value is calculated by multiplying the peak value by the average germination (Kolotelo et al. 2001); and germination energy (GE) is calculated as the number of days required to achieve 50% germination (higher values indicate lower EG) (Juárez-Agis et al. 2006).

Seedling growth

Twenty seedlings were randomly selected for each of the doses applied for growth in the nursery for 6 months. Finally, after 6 months, root measurements were taken. Monthly evaluations of survival (%), total height (cm), and basal diameter (root collar) (mm) were performed with the assistance of a Truper® digital vernier.

Plant quality

Eight months after planting, a destructive sampling of three plants per dose was carried out, from which leaves, branches, roots, and stems were separated with the help of pruning shears. The height (AT, cm) from the root collar to the apex of the plant was recorded; the diameter of the root collar (DC, mm) was obtained with a Truper® digital vernier with an accuracy of ± 0.1 mm; and biomass (g) in fresh and dry weight of the root and aerial parts separating leaves, branches, and stem was also recorded. To determine the dry weights, the various organs of each plant were placed separately in paper bags and placed until a constant weight was obtained in a Memmer electric convection oven at a constant temperature of 70 ºC for 72 hours (Reyes-Reyes et al. 2005). After this time, the weights were determined with a digital scale (Model H-2716, Germany).

Plant quality indexes were obtained according to Rodriguez (2008) with the following formulas:

$$IE = \frac{AT}{DC};\ ID = \frac{PST}{\left(\frac{AT}{DC}\right)+\left(\frac{PSA}{PSR}\right)};\ RAS = \frac{PSA}{PSR};\ RAR = \frac{AT}{LR}$$

Where: *IE* = slenderness index, *ID* = Dickson quality index, *RAS* = aboveground biomass to belowground biomass ratio, *RAR* = aboveground part to root ratio, *AT* stands for total height (cm), *DC* stands for diameter at root collar (mm), *PST* stands for total dry weight (g), *PSA* stands for aboveground part dry weight (g), *PSR* stands for root dry weight, and LR stands for root length (cm).

Photosynthetic pigment content

To determine the photosynthetic pigment content, the methodology described by Porra et al. (1989) was followed. The content of chlorophyll (Chl) a, b, a + b, and carotens (Car) was obtained from plant material. For this purpose, 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 6000 rpm for 12 min 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, the reading was taken at 663.6 nm, for chlorophyll b at 646.6 nm and for carotens at 440.5 nm. The following formulas were used to calculate photosynthetic pigment content:

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

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

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

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

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 information was performed using the statistical program STATISTICA (version 7) (Statsoft, Inc. 1998). Germination capacity data were previously transformed with the arcsine function of the square root of p (= arcsine p) to normalize the distribution (Sokal and Rohlf, 1981). Finally, mean comparisons were performed with Tukey's test ($P \leq 0.05$) for all variables evaluated.

Results And Discussion

Effect of gamma rays on germination.

Irradiated seeds germinated on day 12 after sowing at the same time as non-irradiated seeds. This can be observed in the graph (Fig. 3). As the days passed, the doses of 0.5, 1.5, and 3.0 Gy presented a higher percentage of germinated seeds. In all the doses applied (0.5, 1.5, 3.0, 5.0, and 7.5 Gy), a steep curve was maintained and stabilized until 21, 20, 19, 21, and 20 days, respectively, resulting in the final germination percentage. The greatest increase in the percentage of germinated seeds was obtained with the 1.5 Gy dose from day 16 to 17, increasing from 19.23% to 34.05%.

Germination capacity and germination energy are the variables that define seed quality for plant production (Bonner et al. 1994; Trujillo, 1996). Kolotelo et al. 2001). The faster and more uniform the germination rate (Kolotelo et al. 2001); the higher the germination capacity and the greater the uniformity of plant production (Bonner et al. 1994; Trujillo, 1996). Therefore, the effect of different doses of the linear accelerator on germination capacity (GC), germination energy (GE), peak value (PV), and germination value (GV) of seeds can be observed in Table 1. The 0.5 Gy dose presented the highest percentage of germination capacity (50%), and as the irradiation dose increased, the germination capacity decreased. However, no dose presented a lower percentage than non-irradiated seeds. The germination energy (50%) was lowest at the 3.0 Gy dose (12.8 days), followed by the 0.5 Gy dose (13.2 days), and the highest peak value was 2.38 % on day [1]. The highest germination values were observed with the 0.5 and 3.0 Gy doses (4.76 and 4.75, respectively).

Table 1 Average values of the means of the effect of different doses of irradiation on seed germination parameters of *P. pseudostrobus*

Dose (Gy)	Germination Capacity (%)	Germination Energy (EG) (50%)	Maximum value (% day1)	Germination value (% day1)
0	38.46^{a}	15.00^{e}	2.13^{ab}	3.28^{a}
0.5	50.03^{e}	13.25^{b}	2.38^{cd}	4.76^{e}
1.5	46.46^{f}	16.5^{d}	2.32^{c}	4.31^{d}
3	47.50^{d}	12.8^{a}	2.5^{d}	4.75^{e}
5	45.15^{c}	14.5^{c}	2.15^{b}	3.88^{c}
7.5	42.31^{b}	13.2^{b}	2.11^{a}	3.57^{b}
Standard Error ±	1.8	0.5	0.32	0.65

Values with different letters in the column are statistically different. Tukey's ($P \leq 0.05$).

When these results were compared to those obtained by Rangel et al. (2017), lower data was obtained in terms of germination capacity for *P. pseudostrobus*, but this could be attributed to the germination method, which is carried out in a germination chamber with controlled environmental conditions. On the other hand, this same author mentions that it is common in the propagation practices of different *Pinus* species to resort to direct sowing in soil with the objective of ensuring higher survival percentages, since not having to manipulate the seedlings to establish them in substrate once they have already germinated avoids damaging the radicle during this procedure (Rangel et al. 2017).

Seedling growth

Regarding growth, in the height variable, it was observed that during the first 3 months of evaluation there were no significant differences between treatments (Tukey; $P \leq 0.05$). However, in the fourth month, a greater increase was noted in seedlings of doses of 0.5 and 3.0 Gy for both diameter and height. As the dose increases, this effect on growth decreases (Fig. 4 and Fig. 5).

The percentage of seedling survival was evaluated for each dose used, and it was found that seedlings of 0.5, 1.5, 3.0, 5.0, and 7.0 Gy had no more than 10% mortality, while seedlings from non-irradiated seeds had a 15% mortality rate (Table 2).

According to Mexal and Landis (1990), plant height is a good predictor of future height in the field, although not for survival; on the other hand, Romero-Arenas et al. (2019) mention that the diameter of a forest seedling is perhaps the most important variable to evaluate since it is directly related to the survival capacity of the plant and defines the robustness of the stem and is therefore associated with the vigor and survival of the plantation. In the last month of evaluation, values with statistically significant differences were obtained with respect to the non-irradiated treatment. The 0.5 Gy dose showed greater height and diameter (26.4 cm and 4.63 mm) (Table 2). The results obtained for seedling height and diameter are higher than those referred to by Rangel et al. (2017), who at six months reported mean height values of 14.90 cm and a diameter of 2.10 mm for *P. pseudostrobus*. It has been indicated (Fonseca et al. 2012) that low-dose radiation applications lead to the formation of free radicals, ions, and molecules that contribute to greater efficiency in biochemical-metabolic pathways, which is reflected in improved plant growth and development.

For root length, it was found that the 1.5 Gy dose presented a greater length (16.20 cm) than the 0 Gy dose. Córdoba-Rodríguez et al. (2011) mentioned that an important characteristic for the successful establishment and survival of plants is the growth and development of the root since the absorption of water and essential nutrients for various physiological processes depends to a great extent on it.

Table 2 Survival and growth variables of *P. pseudostrobus* seedlings irradiated with linear accelerator

Dose	Total average height (cm)	Stem diameter (mm)	Root length (cm)	Survival (%)
0 Gy	22.515 ± 1.2^{a}	3.97 ± 0.63^{a}	13.00± 0.71^{a}	85
0.5 Gy	26.442 ± 1.2^{b}	4.63 ± 0.63^{c}	15.66 ± 0.71^{c}	95
1.5 Gy	26.262 ± 1.2^{b}	4.53± 0.63^{bc}	16.20± 0.71^{c}	90
3.0 Gy	26.073 ± 1.2^{b}	4.38± 0.63^{abc}	14.26± 0.71^{abc}	90
5.0 Gy	24.27 ± 1.2^{ab}	4.37 ± 0.63^{abc}	13.56 ± 0.71^{ab}	95
7.5 Gy	24.13 ± 1.2^{ab}	4.00 ± 0.63^{ab}	15.33 ± 0.71^{bc}	90

Values with different letter in the column are statistically different Tukey ($P \leq 0.05$).

Effect of linear accelerator on plant quality.

In the present work, Tukey's test showed significant differences ($P \leq 0.05$) for root height/length ratio (RAR) and aerial biomass/root biomass ratio (RAS), obtaining the highest average for 3.0 Gy plants of 1.82 and 5.65, respectively. According to Rodriguez-Ortiz et al. (2020), values < 2 in the RAR refer to high seedling quality. This ratio will help to 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, which indicates that the seedlings are of high quality; however, higher values indicate that the plant has a thin stem in relation to its height (Prieto et al. 2009). The dose that presents the thinnest stem in relation to height is 7.0 Gy. These results are like those found by Rodriguez-Ortiz et al. (2020) values in a range of 5.5 to 6.1 for *P. pseudostrobus*.

The Dickson quality index (DI) and RAR are indicators that predict the success of the plantation (Ortiz et al. 2021). The doses that presented a higher ID were those of 0.5 and 1.5 Gy (0.24 and 0.25). These values refer to the medium quality of the seedlings. However, the doses of 0, 3.0, 5.0, and 7.5 Gy presented values lower than 2. Therefore, it is considered of poor quality according to the classification for *Pinus* made by Rodriguez-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 them to survive. The lignification index showed lower values (18.54%) in doses of 5.0 Gy than in the rest of the seedlings, and the dose of 1.5 Gy presented the highest value (28.66%) (Table 3).

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

Doses	RAS	RAR	Slenderness Index (SI)	Dickson Index (DI)	Index of Lignification (IL%)
0 Gy	5.45^{c}	1.73^{bcd}	5.67^{ab}	0.14^{a}	28.3^{e}
0.5 Gy	$4.3b^{c}$	1.68^{abc}	5.71^{ab}	0.24^{c}	25.86^{c}
1.5 Gy	3.47^{a}	1.62^{ab}	5.8^{bc}	0.25^{c}	28.66^{e}
3.0 Gy	5.65 cd	1.82^{d}	5.95^{bc}	0.15^{ab}	27.77^{de}
5.0 Gy	4.7^{bc}	1.78^{cd}	5.55^{a}	0.16^{ab}	18.54^{a}
7.5 Gy	3.88^{ab}	1.57^{a}	6.03^{c}	0.15^{ab}	22.09^{b}
Standard Error ±	0.67	0.06	0.36	0.01	1.3

RAS = aboveground biomass to belowground biomass ratio; RAR = height to root ratio; values in the column with different letters are statistically different. Tukey's ($P \leq 0.05$).

Photosynthetic pigment content

Statistically significant differences ($P \leq 0.05$) were found between the different linear accelerator doses. 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 carotens). On the other hand, the 1.5 Gy dose did not present significant differences ($P \leq 0.05$) with respect to seedlings from unirradiated seeds in all the variables evaluated (chlorophyll a, chlorophyll b, chlorophyll a + b, and carotens) (Fig. 6).

Conclusions

The above results corroborate that radiosensitivity in plants varies according to the absorbed irradiation doses, among other factors. The radiation doses absorbed (0.5, 1.5, 3.0, 5.0, and 7.5) had a “hormetic” effect on germination capacity, which was higher than non-irradiated seeds. However, as the irradiation dose increases, this germination capacity decreases.

The doses used generated a “hormetic” effect on the height and diameter of irradiated seedlings with respect to non-irradiated seeds. However, the dose that presented a greater effect on these variables was the dose of 0.5 Gy.

Plant quality indexes in *P. pseudostrobus* from linear accelerator-irradiated seeds revealed that doses of 0.5, 1.5, and 3.0 Gy contributed more to obtaining plants with larger aerial and root parts, but with a balanced conformation and a high capacity for the formation of new roots, which will help to present better survival levels. The dose that did not show significant differences in photosynthetic pigment content with respect to the non-irradiated treatment was the 1.5 Gy dose.

Declarations

Manuscript title: “Radiohormetic effect on the germination of *Pinus pseudostrobus* Lindl. seeds irradiated with linear accelerator”

Authors: Laura Yasmin Flores-López, Lourdes Georgina Iglesias-Andreu and María de Lourdes Palafox-Chávez.

Intended for publication in the journal: New Forest journal.

The corresponding author of above stated manuscript on behalf of the listed co-authors, declares that:

- all authors were actively involved in obtaining the results;
- all authors are acquainted with the manuscript;
- all authors agree to the publication of the submitted manuscript in the above-stated journal after amendments arising from the peer review;

- all authors agree to the posting of the full text of this work on the journal web page and to the inclusion of references in databases accessible on the Internet;
- submitted manuscript is original, has not been published previously, and is not currently being considered for publication elsewhere
- no results of other researchers were used in the submitted manuscript without their consent, proper citation, or acknowledgement of their cooperation or material provided;
- submission of the manuscript for publication was completed in accordance with the publishing regulations pertaining to their workplace;
- performed experiments comply with the current laws and the written consent of the Research Ethics Committee / National Animal Care Authority (as is mentioned in the manuscript submitted) (where applicable);
- all sources of financial support are declared in the manuscript;
- any conflict of interest is declared in the manuscript;
- The corresponding author is aware of his duty to inform co-authors and (where applicable) project co-ordinators of the contents of referees‘ assessments and amendments made to the manuscript.
- All authors agree that the editorial correspondence will be sent to the corresponding author. Nevertheless, all authors are responsible for the content of the submitted and revised manuscript.

References

1. Bonner FT, Vozzo JA, Elam WW, Land SB (1994) Tree Seed Technology Training Course. Instructor’s Manual USDA, Forest Service. General Technical Report SO-106 New Orleans, Louisiana. 160 p
2. CONAFOR Comisión Nacional Forestal, MX (2011) Indicadores de gestión de desempeño del programa PROCOREF durante el ejercicio fiscal 2010. Universidad Autónoma Chapingo (UACH). Available: http://www.cnf.gob.mx:8080/snif/portal/evaluaciones/documentos-y-alcances-de-las-evaluaciones. Accessed 25 January 2022
3. Córdoba-Rodríguez D, Vargas-Hernández JJ, López-Upton J et al (2011) Crecimiento de la raíz en plantas jóvenes de *Pinus pinceana* Gordon en respuesta a la humedad del suelo. Agrociencia 45: 493-506. Available: https://www.scielo.org.mx/pdf/agro/v45n4/v45n4a8.pdf . Accessed 23 February 2022
4. Delgado P, Salas-Lizana R, Vázquez-Lobo A et al (2007) Introgresive hybridization in *Pinus montezumae* Lamb. and *Pinus pesudostrobus* Lindl. (Pinaceae): morphological and molecular (cpSSR). evidence Int. J. Plant Sci 168: 861-876. https://doi.org/1058-5893/2007/16806-0007
5. Fonseca AÁ, Suárez LC, Fernández RR et al (2012) Indicadores fisiológicos en plántulas de *Solanum lycopersicum* L., procedentes de semillas irradiadas con rayos X. Biotecnología Vegetal 12(3): 1-15. Available: https://revista.ibp.co.cu/index.php/BV/article/view/172. Accessed 20 January 2022
6. García OC, Alcantar GG, Cabrera RI et al (2001) Evaluación de sustratos para la producción de *Epipremnum aureum* y *Spathiphyllum wallisii* cultivadas en maceta. Terra 19(3): 249-258. Available:

https://www.redalyc.org/articulo.oa?id=57319306. Accessed 26 February 2022

7. González Y, Reino J, Machado R (2009) Dormancia y tratamientos pregerminativos en las semillas de *Leucaena* spp. cosechadas en suelo ácido. Pastos y forrajes 32(4): 1-1. Available: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-03942009000400005&lng=es&nrm=iso. Accessed 20 November 2021
8. Jamil Y, Perveen R, Ashraf M et al (2013). He-Ne laser induced changes in germination, thermodynamic parameters, internal energy, enzyme activities and physiological attributes of wheat during germination and early growth. Laser Physics Letter 10: 1-8. https://doi.org/10.1088/1612-2011/10/4/045606
9. Juárez-Agis A, López-Upton J, Vargas-Hernández JJ et al (2006). Variación geográfica en la germinación y crecimiento inicial de plántulas de *Pseudotsuga menziesii* de México. Agrociencia 40(6): 783-792. Available: https://agrociencia-colpos.mx/index.php/agrociencia/article/view/509. Accessed 27 January 2022
10. Kolotelo D, Steenis EV, Peterson M et al (2001) Seed handling guidebook. British Columbia Ministry of Forests, Tree Seed Centre 46 p. Available: https://rngr.net/publications/seed-handling-guidebook/complete/at_download/file. Accessed 28 January 2022
11. Mexal JG, Landis TD (1990) Target seedling concepts: height and diameter. Target seedlings symposium. Department of Agriculture, Forest Service, Rocky Mountain Forest, and Range Experiment Station 105-119 Pp. https://doi.org/10.2737/RMRS-GTR-200
12. Ortiz GR, Hernández YJ, del Valle JRE et al (2021) Calidad de plántula de árboles seleccionados de *Leucaena esculenta* en sistema agroforestal. CIENCIA ergo-sum 28(1): 80-90. https://doi.org/10.30878/ces.v28n1a7
13. Pausas J.G (2015) Evolutionary fire ecology: lessons learned from pines. Trends in Plant Science 20(5): 318-324. https://doi.org/10.1016/j.tplants.2015.03.001
14. Podleśny J, Stochmal A, Podleśna A et al (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
15. Porra RJ, Thompson WA, Kriedelman PE (1989) Determination of accurate extraction and simultaneously equation for assaying chlorophyll a and b extracted with different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta 975:384–94
16. Prieto RJA, García RJL, Mejía BJM et al (2009) Producción de Plántula del Género *Pinusen* Vivero del Clima Templado Frío. Publicación Especial N° 28 Campo Experimental Valle del Guadiana, INIFAP-SAGARPA. México 48 p.
17. Prośba-Bialczyk U, Szajsner H, Grzys E et al (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
18. Pulido MT, Equihua M, Benítez G (2002) Diagnóstico de la situación de los viveros oficiales de Veracruz y su papel para apoyar programas de reforestación y restauración. Revista Chapingo Serie

Ciencias Forestales y del Ambiente 8 (1): 5-12. Available: https://www.redalyc.org/articulo.oa?id=62980101. Accessed 10 January 2022

19. Rangel SR, Licona LER, Serrano LC et al (2017) Comportamiento germinativo y crecimiento temprano de *Pinus devoniana* y *Pinus pseudostrobus* (Pinaceae). Biocyt: Biología, Ciencia y Tecnología, 10(37): 749-756. http://dx.doi.org/10.22201/fesi.20072082.2017.10.61719
20. Ramírez-Herrera C, Vargas-Hernández JJ, López-Upton J (2005) Distribución y conservación de las poblaciones naturales de *Pinus greggii*. Acta Botánica Mexicana (72), 1-16. Available: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-71512005000300001&lng=es&tlng=es. Accessed 25 January 2022
21. Ramírez CA, Rodríguez TDA (2004) Efecto de la calidad de planta, exposición y micrositio en una plantación de *Quercus rugosa*. Revista Chapingo Serie Ciencias Forestales y del Ambiente 10(1): 5-11. Available: https://www.redalyc.org/articulo.oa?id=62910101. Accessed 23 February 2022
22. Reyes-Reyes J, Aldrete A, Cetina-Alcalá VM (2005) Producción de plántulas de *Pinus pseudostrobus* var. apulcensis en sustratos a base de aserrín. Revista Chapingo Serie Ciencias Forestales y del Ambiente 11(2): 105-110. Available https://www.redalyc.org/articulo.oa?id=62911205 Accesssed: 22 april 2021
23. Rodríguez TDA (2008). Indicadores de calidad de planta forestal. México. Mundi Prensa.
24. Rodríguez-Ortiz G, Aragón-Peralta RD, Enríquez-del Valle JR et al (2020) Calidad de plántula de progenies selectas de *Pinus pseudostrobus* Lindl. var. oaxacana del sur de México. Interciencia 45(2): 96-101. Available: https://www.redalyc.org/journal/339/33962521007/33962521007.pdf Accessed 23 November 2021
25. Sáenz-Romero C, Rehfeldt GE, Soto-Correa JC et al (2012). Altitudinal genetic variation among *Pinus pseudostrobus* populations from Michoacán, México: Two location shadehouse test results. Revista Fitotecnia Mexicana, 35(2): 111-121. Available: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-73802012000200003&lng=es&tlng=en. Accessed: 11 de noviembre de 2021
26. Sánchez-González A (2008) Una visión actual de la diversidad y distribución de los pinos de México. Madera y Bosques 14(1): 107-120. http://dx.doi.org/10.21829/myb.2008.1411222
27. Sígala-Rodríguez JÁ, González-Tagle MA, Prieto-Ruíz JÁ et al (2016) Relaciones alométricas para predecir biomasa en plantas de *Pinus pseudostrobus* cultivadas en diferentes sistemas de producción en vivero. Bosque (Valdivia) 37(2): 369-378. http://dx.doi.org/10.4067/S0717-92002016000200015
28. Sokal RR, Rohlf. FJ (1981) Biometry: The Principles of Practice of Statistics in Biological Research. W.H. Freeman and Company. San Francisco, California, USA. 776 p. https://doi.org/10.2307/1378519
29. Romero-Arenas O, Flores-Flores ADP, Rivera-Tapia JA et al (2019) Producción de plántulas de *Pinus pseudostrobus* en composta a base de residuos de Shiitake en vivero. Madera y Bosques 25(1): e2511675. https://doi.org/10.21829/myb.2019.2511675

30. Ruiz TE, Feble G, Castillo E et al (2007). La experiencia cubana en la agronomía y manejo de *Leucaena leucocephala*. La Habana: Instituto de Ciencia Animal. Available: http//www.cipav.org.co/redagrofor/memorias99/RuizTE2.htm. Accessed 15 December 2021
31. SEMARNAT. Secretaría de Medio Ambiente y Recursos Naturales (2014) Evaluación de degradación de los suelos causada por el hombre en la República Mexicana. Escala 1:250,000
32. Trujillo NE 1996 Análisis y pruebas rápidas de la calidad de la semilla. In: Memorias de curso para profesores; Mejoramiento Genético, Selección y Manejo de Fuentes Semilleras y de Semillas Forestales. PROSEFOR, CATIE. Turrialba, Costa Rica 86-101 Pp.
33. Viveros-Viveros H, Sáenz-Romero C, López-Upton et al (2005). Variación genética altitudinal en el crecimiento de plantas de *Pinus pseudostrobus* Lindl. en campo. Agrociencia, 39: 575-587. Available: https://www.redalyc.org/articulo.oa?id=30239511. Accessed 24 January 2022

Figures

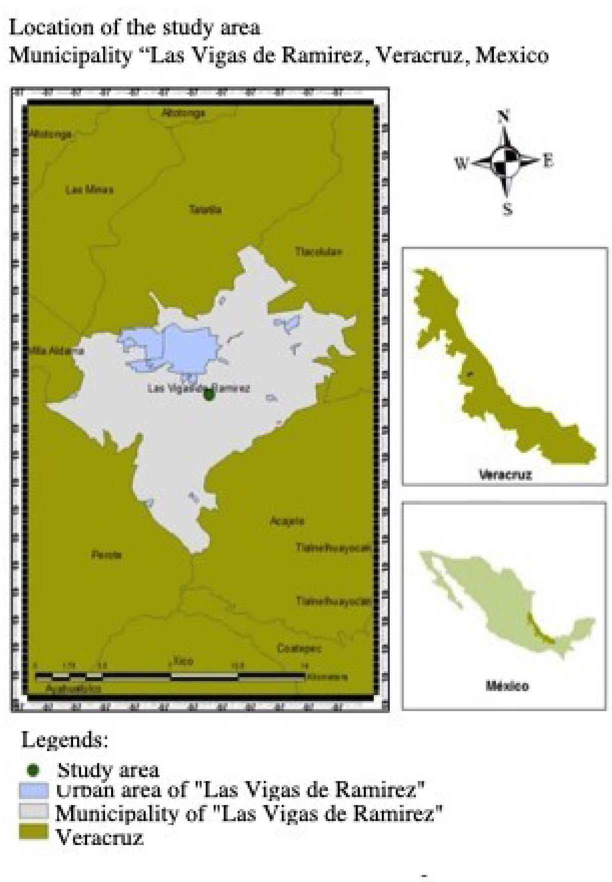

Figure 1

Location of the study area. The Municipality of “Las Vigas de Ramírez”, Veracruz, Mexico. Source: Flores-López (2021)

Figure 2

Irradiation of *P. pseudostrobus* seeds with an Elekta Synergy Platform linear accelerator. Source: Flores-López (2021).

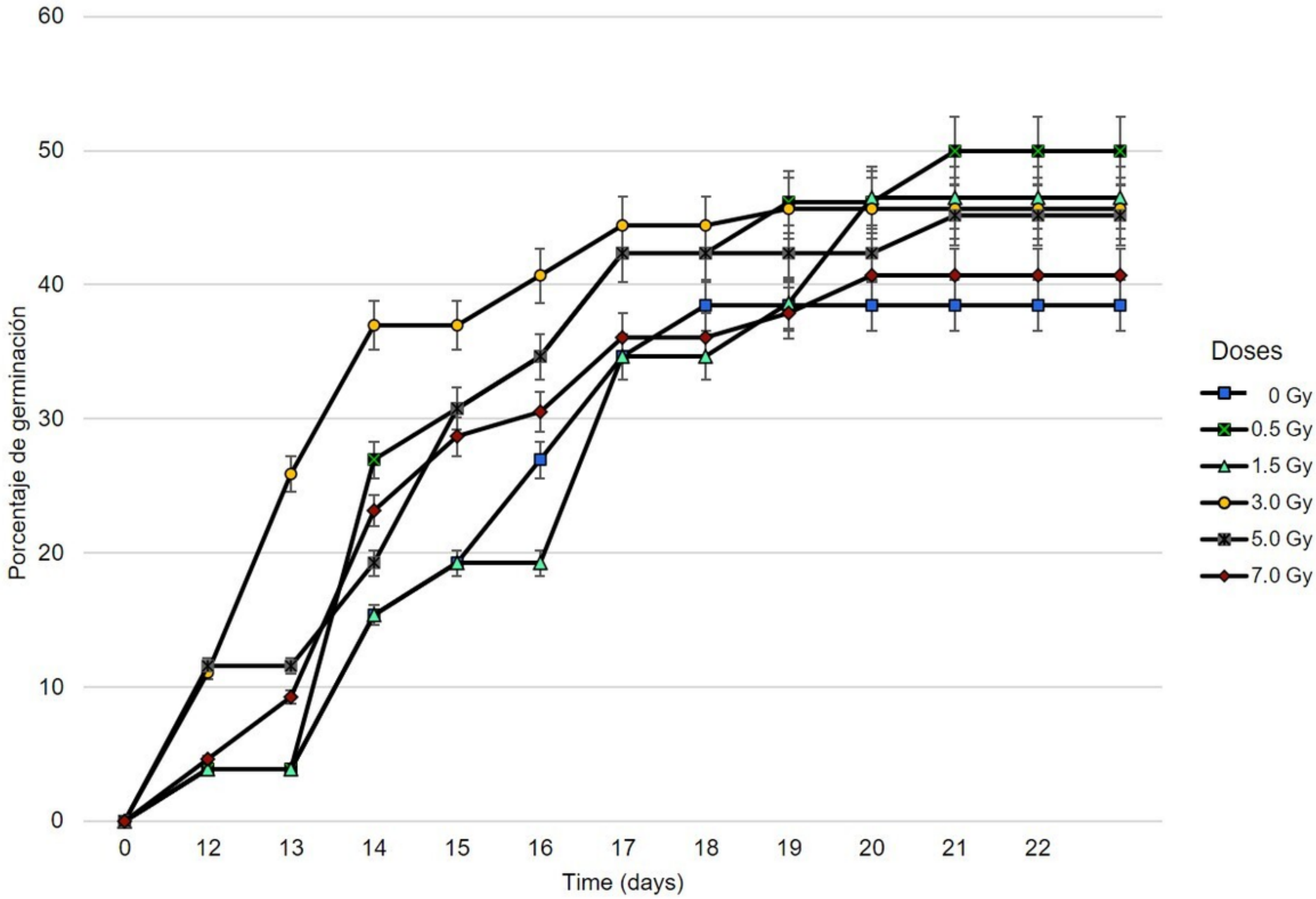

Figure 3

Cumulative germination curve of *P. pseudostrobus* seeds irradiated (0, 0.5, 1.5, 1.5, 3.0, 5.0, and 7.5 Gy) with linear accelerator

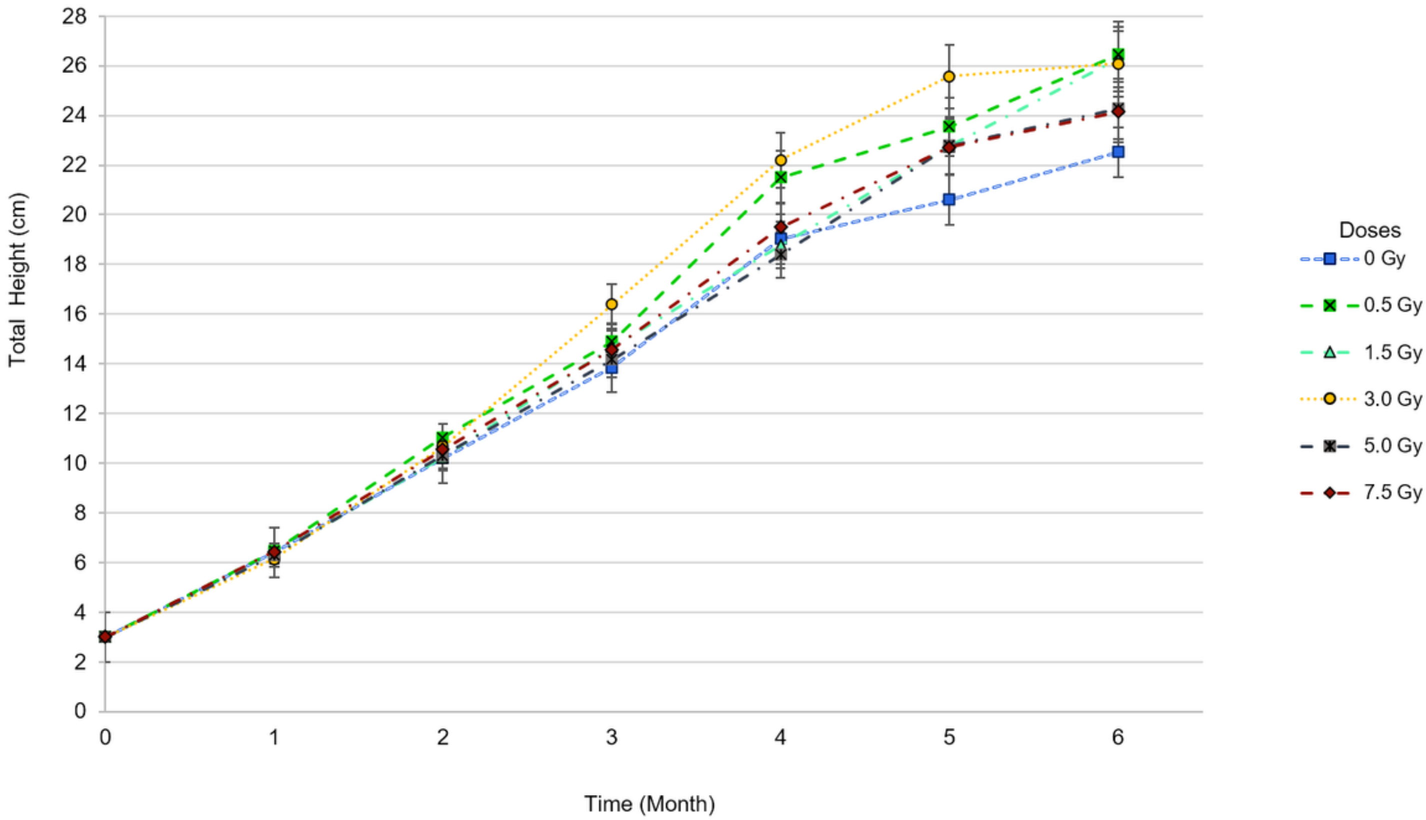

Figure 4

Effect of irradiation doses on the height of *P. pseudostrobus* seedlings

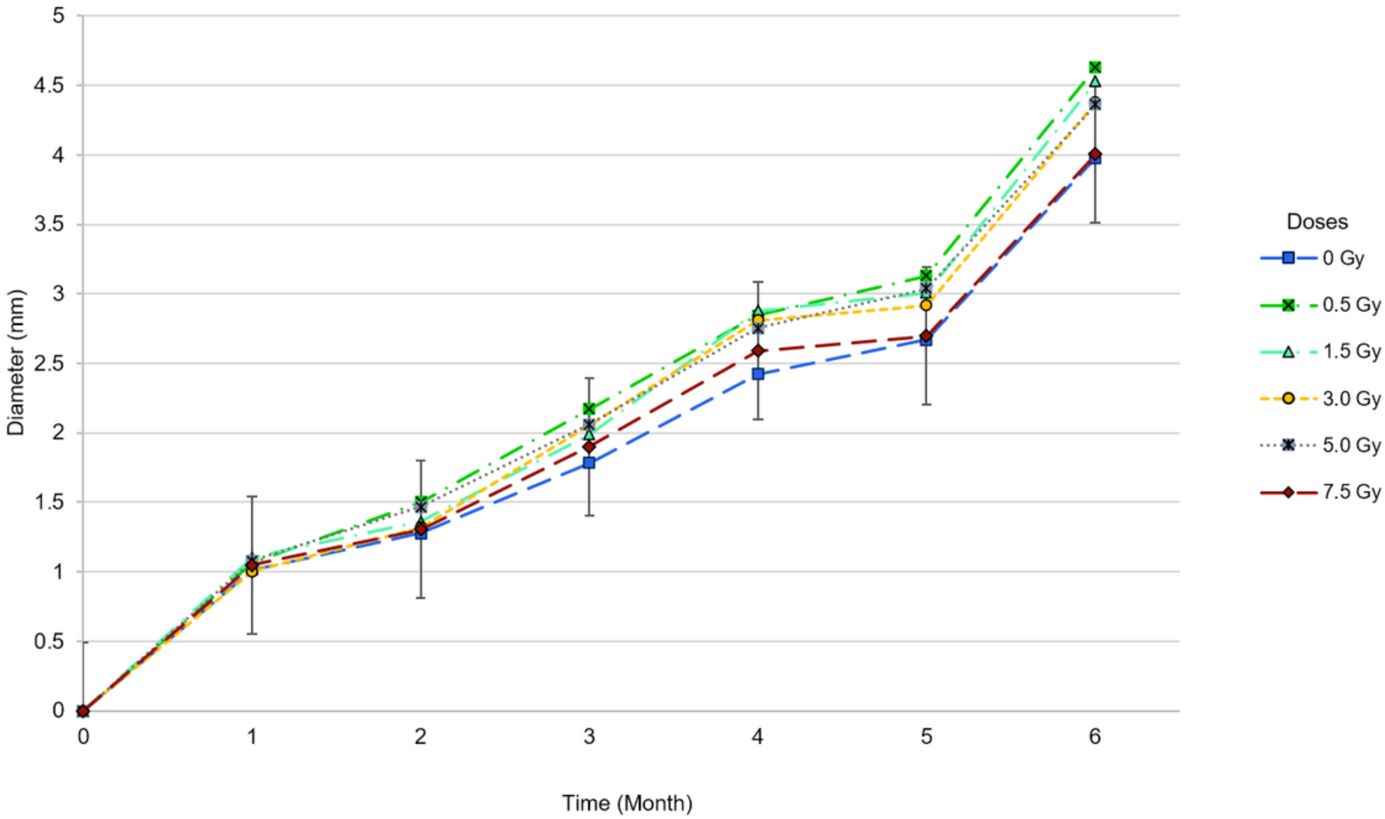

Figure 5

Effect of irradiation doses on the diameter of *P. pseudostrobus* seedlings

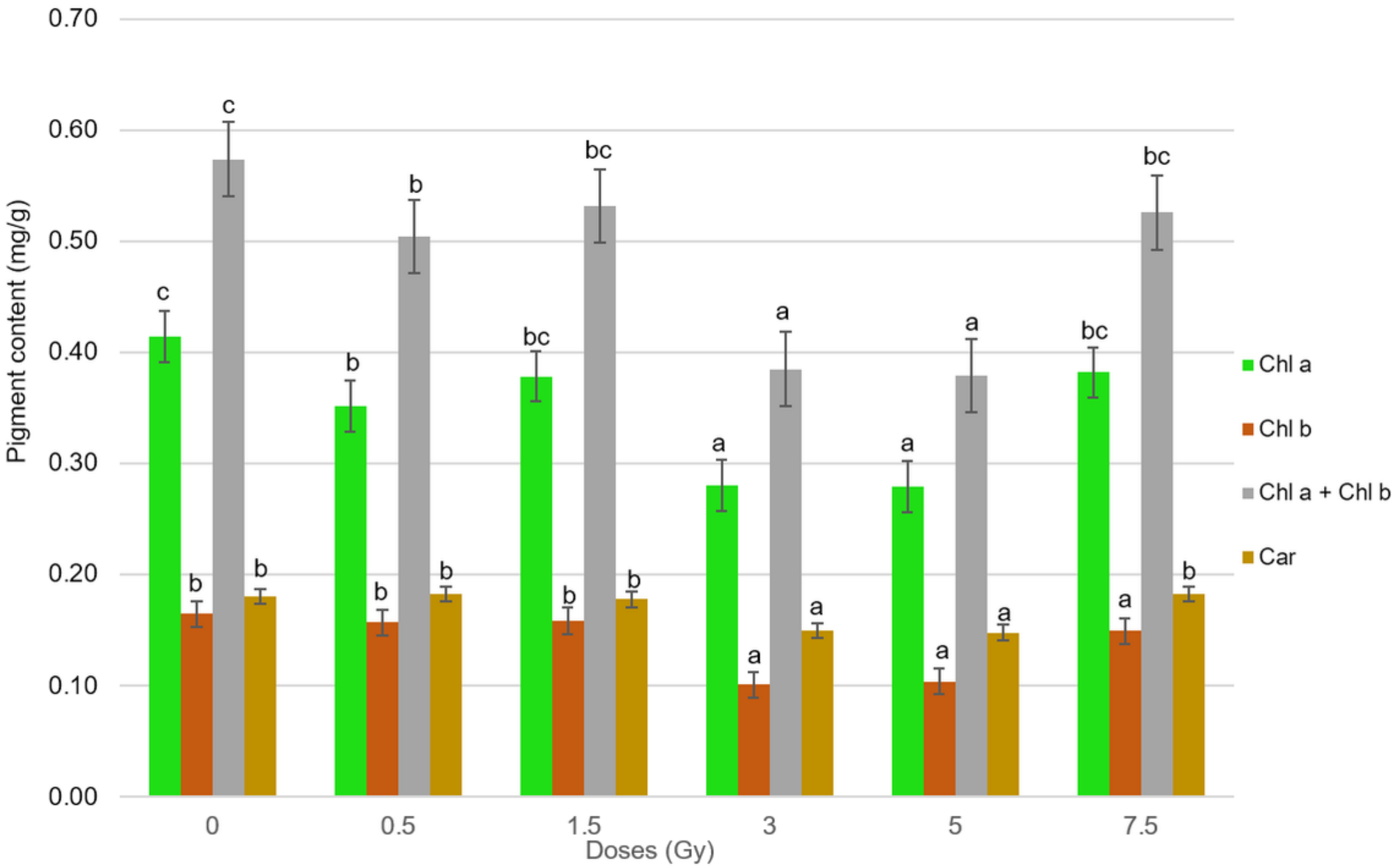

Figure 6

Effect on photosynthetic pigment content (Chl a = Chlorophyll a; Chl b = Chlorophyll b; Chl a + Chl b = Chlorophyll a + Chlorophyll b; Car =Carotene) in irradiated *P. pseudostrobus* seedlings; values with different letters in the column are statistically different. Tukey's ($P \leq 0.05$)