Article

Genetic variability in sunflower root by gamma of ^{60}Co

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Abstract

Sunflower is a crop that belongs to the family asteraceae. This plant has multiple uses within which can be cited: oil extraction from its seeds, ornamental and due to the large volume of the radical system as a bio-remediator plant. This is caused by the extraction capacity of heavy metals such as: Pb, Mg and Ca, which affect agricultural soils. The objective of this research was to: induce genetic variability in the radical sunflower system, by using ^{60}Co gamma irradiation. For this, achenes were planted under greenhouse conditions in 4 kg pots during the summer of 2019 at the National Institute for Nuclear Research located in the Marquesa, Mexico, which were evaluated under a completely random design. Treatments consisted of eleven ^{60}Co gamma irradiation levels and four repetitions (11 x 5)= 55 experimental units. The results indicate that the largest volume, length and dry root biomass, were obtained with the application of 200 Gy of gamma irradiation of ^{60}Co. LD_{50} for germination was reached at 207 Gy, while for sprouting with 183 Gy. It is concluded from this work that the application of 200 Gy of ^{60}Co, can induce genetic variability in desirable attributes in sunflower, when it is intended for the bioremediation of agricultural soils.

Keywords: bioremediation, phenotypic plasticity, radical volume, Tehuacán Valley.

Reception date: February 2021
Acceptance date: April 2021

Introduction

Sunflower (*Helianthus annuus* L.) is a crop that for many years has been considered oily, due to the fact that high quality fatty acids are extracted from its seeds, for human consumption. However, it is currently being used as a soil bioremediation plant (Escalante *et al*., 2017), due to the great development of its radical system, as well as its ability to absorb soil cations such as: Ca^{++}, Mg^{++} and Na^{+}, including heavy metals such as lead and cadmium (Chico *et al*., 2012; Suaña, 2018).

This crop has a wide phenotypic plasticity to adapt to agricultural soils, where there are problems of alkalinity, due to the irrigation of them with hard water, such as in the Tehuacán Valley, where these practices have caused the deterioration of some chemical and physical parameters of the soil, such as: alkaline pH, high electrical conductivity and defloc culated soils. Cardoso *et al*. (2018), found that sunflower plants grown under limited water conditions significantly adjusted the osmotic potential in the leaf, which was related to a prolongation of the stomatal opening as the soil dried and a lower sensitivity of photosynthesis to damage caused by water stress.

Recent studies focusing on morphological issues have shown that the radical system of this asteraceae can reach up to 1.5 m in length and have a high radical volume, allowing great absorption of water and nutrients, being considered as a plant par excellence, for studies of root physiology.

In this regard Díaz *et al*. (2017), studied the pH dynamics and electrical conductivity, of a soil irrigated with hard water when it is planted with sunflower and mention that it can support up to a pH of 10.5 and 6.5 dS m^{-1} conductivity, in addition to absorbing 160 mg L^{-1} of calcium, considering it as a plant with potential to lower the pH. For its part Escalante *et al*. (2017), mention that sunflower seedlings have the ability to support high osmotic potentials and concentrations of up to 62.8 mg L^{-1} of $CaCO_3$, consequence of the presence of Ca^{++} salts in the soil solution, which other species would not support.

Therefore, the general objective of this research was: to induce genetic variability in the length and volume parameters of the sunflower root system, through the use of ^{60}Co gamma radiation. The hypothesis was: the gamma irradiation of ^{60}Co, will induce genetic variability in low doses, when the achenes are subjected to it.

Materials and methods

This research was conducted at the National Institute of Nuclear Research (ININ), located in Ocoyoacac, Mexico, in a Mini-Green greenhouse. The germplasm consisted of sunflower achenes, *cv* Periquero (Morales *et al*., 2015), which corresponds to a free pollination material, with three masal selection cycles.

Treatments consisted of 11 gamma radiation doses of ^{60}Co, 0; 100; 200; 300; 400; 500; 600; 700; 800; 900 and 1 000 Gy, which were irradiated in LGI-01 transelektro model, on 6 June 2019 at a current dose rate of 697.85 Gy h^{-1}, according to the irradiator department of the ININ.

The experimental unit was constituted, by a pot of 4 kg capacity and six sunflower seedlings, with three seedlings being the useful plot. The substrate used consisted of site soil from the site and organic matter at a ratio (50:50) v/v, the physical and chemical properties of the site are presented in Table 1.

Table 1. Main physical and chemical properties of sunflower evaluation substrate (*Helianthus annuus* L.) *cv* Periquero. National Institute of Nuclear Research, 2019.

Physical properties		Chemical properties	
Apparent density	1.15 g cm^{-3}	pH	5.5
Texture	Silty clay loam	CE	1.3 dS m^{-1}
Percolation speed	1.1 cm s^{-1}	MO	3.5%
Color	Dry 5YR 4/2 Wet 5YR 3/2	Nitrogen	13.5 mg kg^{-1}

pH= hydrogen potential; CE= electrical conductivity; MO= organic material.

The experimental design was completely randomized (DCA) and five repetitions (11x5)= 55 experimental units, which were evaluated under the mathematical model $Yij = \mu + Ti + \varepsilon ij$. Where: Yij, is the variable response of the i-th dose of irradiation in the j-th repetition; μ, is the true general mean; Ti, is the effect of the i-th dose of gamma irradiation of ^{60}Co and εij, is the experimental error of the i-th dose of irradiation in the j-th repetition (Steel and Torrie, 1996; Infante and Zárate, 2012).

The response variables were: germination percentage, for this, ten achenes were sown in plastic Petri boxes of 10 cm in diameter, previously disinfected in a solution of calcium hypochlorite at 1%, for 5 min.

For the moisture retention in the bottom of the Petri box, a filter paper bottom was placed with distilled water. Once the experimental unit was constituted, they were placed on a stove at 20 °C, until the radicle broke the pericarp (Taiz and Zeiger, 2000).

The germination percentage was determined by the equation PG = [ss/sg] x 100. Where: PG= is the germination percentage; SS= sown seeds and SG= germinated seeds (Loeza *et al*., 2013). of Sprouting percentage, determined by the relation PB= [PB/SS] x 100. Where: PB= percentage of sprouting; PB= sprouted plants and SS= sown seeds. Root volume, this was calculated by the principle of Archimedes, placing the radical system of the seedling, in a 20 ml measuring cylinder with a known volume of distilled water (10 ml), the radical volume being the volume of water displaced in cm^3.

Root length, measuring from the axonomorphic root cap, up to hypocotyl start with a stainless steel vernier with an accuracy of +/- 0.03 mm, to express the result in cm. For the previous two variables, destructive sampling was required.

The phenological variables days to sprouting and germination, were determined by accounting the days on which said phenological states occurred. For this study, it was necessary to perform the radiosensitivity curve and by interpolation, determine the average lethal dose of radiation (LD_{50})

for germination and sprouting, for this a quadratic regression model was performed by least squares. The survival percentage was calculated with equation PS= [PB, PV] x 100. Where: PS= survival percentage; PB= sprouted plants and PV= live seedlings, for this variable in the same way LD_{50} was determined.

Another variable evaluated was, total dry biomass, determined for root, stem and nomofilos, subjecting these to drying on a model 6M stove, for 72 h, until reaching the constant weight (Escalante, 2017), SPAD units, measured with a SPAD Minolta-502 units meter, taking readings directly from the foliar sheet of five nomofilos at 45 dds. When the response variables were significant, was applied the Tukey multiple comparison test, at a significance level of 5% error probability.

Results and discussion

Radiosensitivity curve for germination, sprouting and LD50

Figure 1 shows the dynamics of the radiosensitivity curve of sunflower achenes cv. Periquero, subjected to gamma irradiation of ^{60}Co. In the curve can be seen that germination had a tendency to decrease, as the irradiation dose increased, thus adjusting to a quadratic model, with a highly significant coefficient of determination, which indicates, that 96% of germination is due to the increase in gamma irradiation. From this curve, drastic decay began from 700 Gy with a germination percentage of 4%. The quadratic interpolation of the model shows that the LD50 occurs with the application of 207 Gy.

These results differ from those reported by Díaz *et al*. (2017), who mention that the fit model when sunflower achenes cv. Victoria, are subjected to gamma irradiation of ^{60}Co, is polynomial of degree three, with a determination coefficient of 0.99 proving to be highly significant.

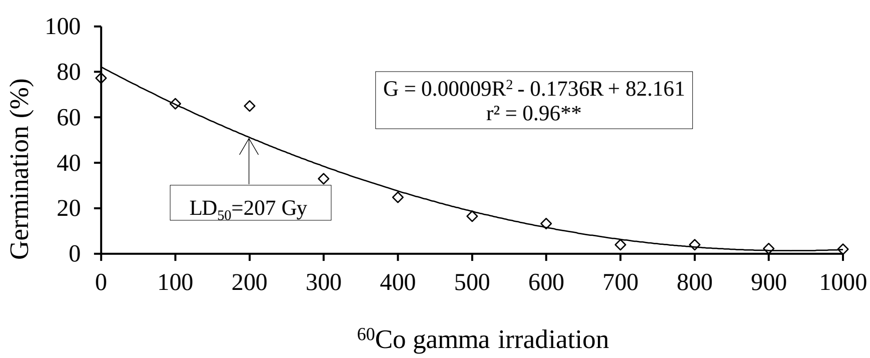

Figure 1. Germination dynamics, in sunflower achenes (*Helianthus annuus* L.), subjected to 11 levels of gamma irradiation of ^{60}Co. National Institute of Nuclear Research. Ocoyoacác, Mexico, 2019. B= sprouting; and R= radiation; **; *; ns= significant to 0.01; 0.05 and not significant.

These differences are attributed to the different cultivars used, as well as to the contrasting climatic conditions of both study sites. In addition, the proposed mathematical model for irradiation vs germination was only used to describe germination dynamics and not for the determination of the LD50.

In relation to the average lethal dose obtained in this study, difference was found with what was reported by Alvarez *et al*. (2018), who mention an LD50 for *Eragrostis lehmanniana* is 2076 Gy, very discrepant differences, which are attributed to the difference in species and the water content, among other variables

Sprouting had a similar tendency to germination, adjusting to a quadratic model, for the same irradiation interval (0-1 000 Gy), decreasing the sprouting drastically to 700 Gy, thus the interpolation to determine the LD50 in sprouting, was reached at 183 Gy, 11.59% less than germination, indicating that the sprouting in sunflower cv. Periquero turns out to be more sensitive to gamma irradiation of ^{60}Co than germination (Figure 2).

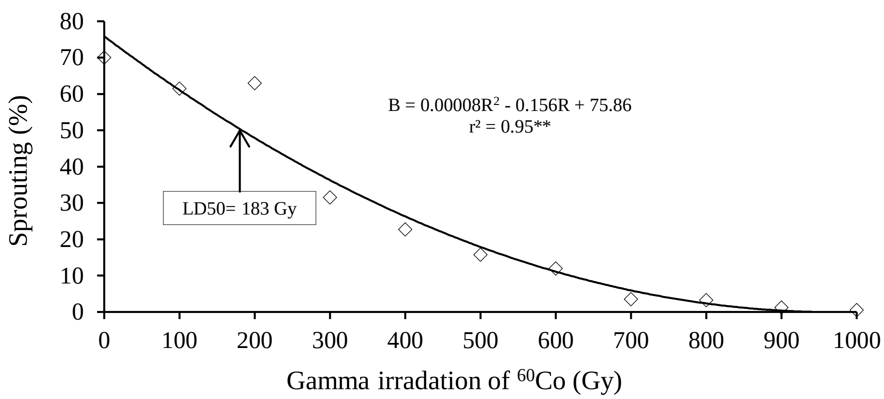

Figure 2. Sprouting dynamics, in sunflower achenes (*Helianthus annuus* L.), subjected to 11 levels of gamma irradiation of ^{60}Co. National Institute of Nuclear Research. Ocoyoacác, Mexico, 2019 B= sprouting; R= radiation; **; *; ns= significant to 0.01; 0.05 and not significant.

Correction index between germination *vs* sprouting

When performing the linear regression model with intercept, between germination vs sprouting, it had a linear adjustment B= 0.9307G, with a highly significant coefficient of determination, thus the slope of the model, 0.9307 can be used as a coefficient for the estimation of germination, by sprouting, Figure 3 (De la Cruz *et al*., 2019).

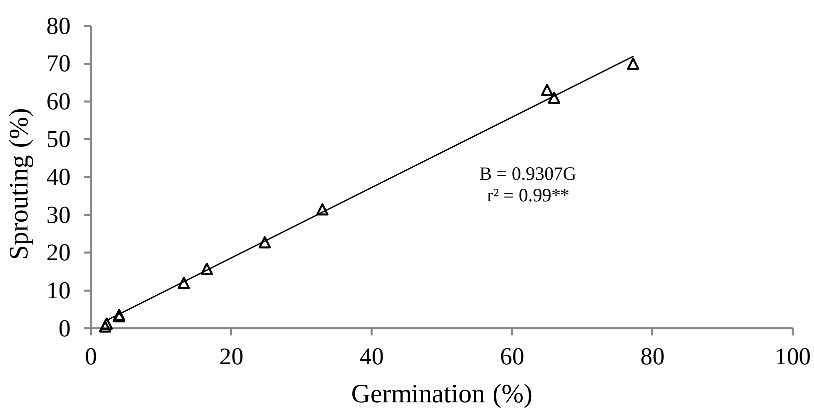

Figure 3. Correction coefficient for germination *vs* sprouting, of achenes of (*Helianthus anuuus* L.), *cv* Periqueros subjected to eleven gamma radiation levels of ^{60}Co. National Institute of Nuclear Research, 2019. G= germination; and B= sprouting.

Percentage of germination, sprouting and days of germination and sprouting

Table 2 presents the variance analysis and multiple comparison test for germination and sprouting percentage, as well as for germination and sprouting days. The variance analysis indicated that there were highly significant differences for the variables under study, so the coefficient of determination for all variables ranged from 3.35 to 5.4 for days of sprouting, germination and percentage of sprouting, thus showing that the data were very reliable, while for germination percentage the variability coefficient 27.65%, shows that the data are reliable.

Table 2. Variance analysis and multiple comparison test, for four parameters in sunflower achenes (*Helianthus annuus* L.), subjected to eleven gamma irradiation levels of ^{60}Co. National Institute of Nuclear Research, 2019.

Irradiation	PG	PB	DG	DB
Gy	(%)		Days	
0	77.25 a	70 a	7.75 c	11.75 c
100	66 a	61.5 b	7.25 d	11.5 c
200	65 a	63 b	6.25 e	9.75 d
300	33 b	31.5 c	8 c	11.75 c
400	24.75 b	22.75 d	9 ab	12 bc
500	16.5 b	15.75 e	9.5 ab	12.25 b
600	13.25 bc	12 f	10 a	12.75 ab
700	4 bc	3.5 g	9.75 a	12.75 ab
800	4 bc	3.25 g	10 a	13 a
900	2.25 bc	1.25 gh	10 a	13 a
1 000	2 bc	0.5 gh	10 a	13 a
DSH	19.25^{**}	2.02^{**}	0.5^{**}	0.588^{**}
CV %	27.65	5.4	3.92	3.35

Means within column with the same letter, are statistically equal according to Tukey to $p \leq 0.05$; PG= germination percentage; PB= percentage of sprouting; DG= days to germination; DB= days of sprouting; DSH= honest significant difference; CV= coefficient of variability; **; *; n.s.= significant to 0.01; 0.05 and not significant.

Regarding the multiple comparison test, it indicates that the highest germination percentage occurred with the application of 100 and 200 Gy, including the witness with 66, 65 and 77.25%. The biggest sprouting was in the witness with 70%. In relation to the days of germination and sprouting, the highest values were for high doses, 800, 900 and 1 000 Gy with 10 and 13 days respectively, this shows that the application of high doses of gamma irradiation of ^{60}Co, delay both germination and sprouting, as Antúnez *et al*. (2017) have shown, who, when applying 300 Gy of ^{60}Co gamma irradiation, reached an emergence of seedlings 11 days after planting, in *Physalis peruviana* L.

Survival percentage

The survival percentage was a reflection of the germination and sprouting percentage, since this in the same way, had a tendency to decrease as the irradiation increased. The fit model was quadratic (Figure 4), presenting a drastic drop to 700 Gy. For this case, the LD50, was to 374 Gy where the highest survival percentage, occurred in the witness treatment with 90% being this the intercept, as the model shows.

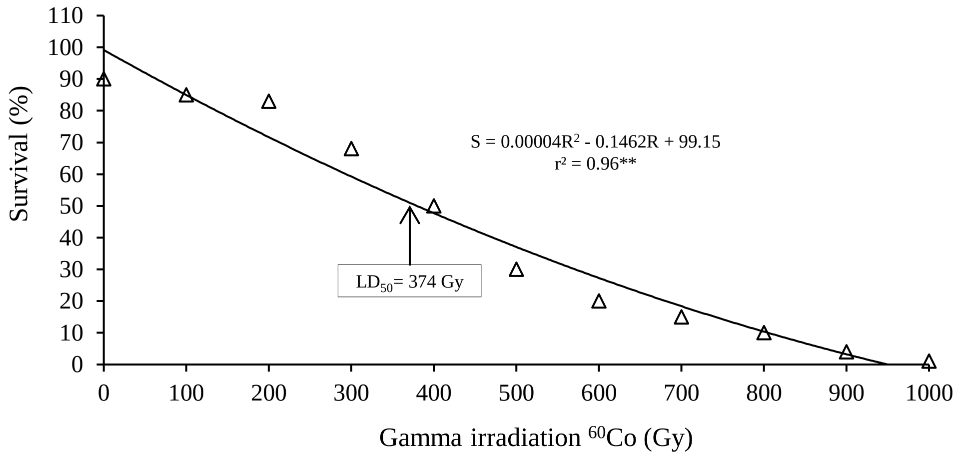

Figure 4. Survival dynamics and LD_{50} in sunflower seedlings *cv* Periquero (*Helianthus annuus* L.), from achenes irradiated with gamma of ^{60}Co, at 30 dds. National Institute of Nuclear Research, 2019.

Genetic variability in root length and volume

The variance analysis, as well as the multiple comparison test in genetic variability, for root length and volume in sunflower seedlings, is presented in (Table3), it can be seen that there were highly significant differences for both variables. For root length, the coefficient of variation shows that the data turned out to be very reliable 4.23%, while, for volume, they were reliable with 19.96%.

Table 3. Analysis of variance and multiple comparison test in sunflower (*Helianthus annuus* L.), *cv* Periquero, subjected to eleven gamma irradiation levels of ^{60}Co. National Institute of Nuclear Research, 2019.

Irradiation ^{60}Co	LR	VR
Gy	(cm)	(cm^3)
0	6.2 b	2.1b
100	5 c	1.6 c
200	8 a	2.5 a
300	3.4 d	1.2 d
400	2.6 e	0.8 e
500	2.3 f	0.5 f
600	2.1 g	0.5 f

Irradiation ^{60}Co	LR	VR
700	1.6 h	0.3 f
800	1.4 i	0.3 f
900	0 j	0 g
1 000	0 j	0 g
DSH	0.187**	0.26**
CV%	4.23	19.96

Means within columns with the same letter, are statistically equal according to Tukey to $p \leq 0.05$; LR= root length; VR= root volume; DSH= honest significant difference; CV= coefficient of variation; **; *; ns= significant to 0.01; 0.05 and not significant.

The application of 200 Gy for both cases was positive, inducing a longer root length and volume, with 8 cm and 2.5 cm^3 respectively, surpassing all treatments including the witness. The lowest values occurred with the application of 900 and 1 kGy, because the radical system did not achieve optimal development compared to treatments where a lower dose of irradiation was applied.

The application of 200 Gy in sunflower crop, can induce morphological changes, increasing the length of the axonomorfa root and in the same way, increases the volume of the radical system, which can result, in a greater anchoring of the plant to the soil, as well as greater absorption of water and nutrients.

In a study with gamma irradiation in seeds of *Leucaena leucocephala* Cepero *et al*. (2001), mention that one of the organs of the plant most sensitive to the effect of irradiation is the root, decreasing the length by up to 10%, when 100 Gy is applied. For their part Ramírez *et al*. (2006), by increasing the dose of gamma rays in four varieties of *Solanum lycopersicum* L. they observed that 5 and 20 Gy, stimulate the root length in this species, contrasting with this research.

Correlation analysis

The correlation analysis for morphological variables, plant height, length root and volume is presented in Table 4. It can be observed that the variables: length and volume root, plant height, were positively correlated, also being highly significant. For their part, the variables: root length and volume, were only significant, in the same way positively correlating. These results are consistent with those reported by Barrios *et al*. (2014), who report that the volume and length of root are highly and significantly correlated, despite being different species.

Table 4 Correlation analysis for three morphological variables in sunflower (*Helianthus annuus* L.), *cv* Periquero. Based on eleven gamma irradiation levels of ^{60}Co. National Institute of Nuclear Research, 2019.

Variables	AP	LR	VR
AP	1	0.949**	0.952**
LR	0.949**	1	0.98**
VR	0.952**	0.99**	1
BT	0.622	0.778*	0.756*

AP= plant height; LR= root length; VR= root volume; **; *; ns= significant to 0.01; 0.05 and not significant.

Total biomass dynamics in sunflower seedlings

The dynamics of aerial dry biomass in sunflower seedlings are presented in Figure 4. It can be seen that this had a tendency to increase from 0 to 45 dds, for all irradiation levels under study, so the maximum values were reached in the period of 30 to 45 dds. The largest biomass, was reached in the witness 0.7 g plant^{-1}, followed by treatments 100 and 200 Gy, which accumulated a dry biomass of 0.45 and 0.4 g plant^{-1} respectively. In contrast, the smallest accumulation of biomass by effect of gamma radiation, was presented in the high doses 900 and 1 000 Gy, thus accumulating only 0.40 and 0.35 g plant^{-1}.

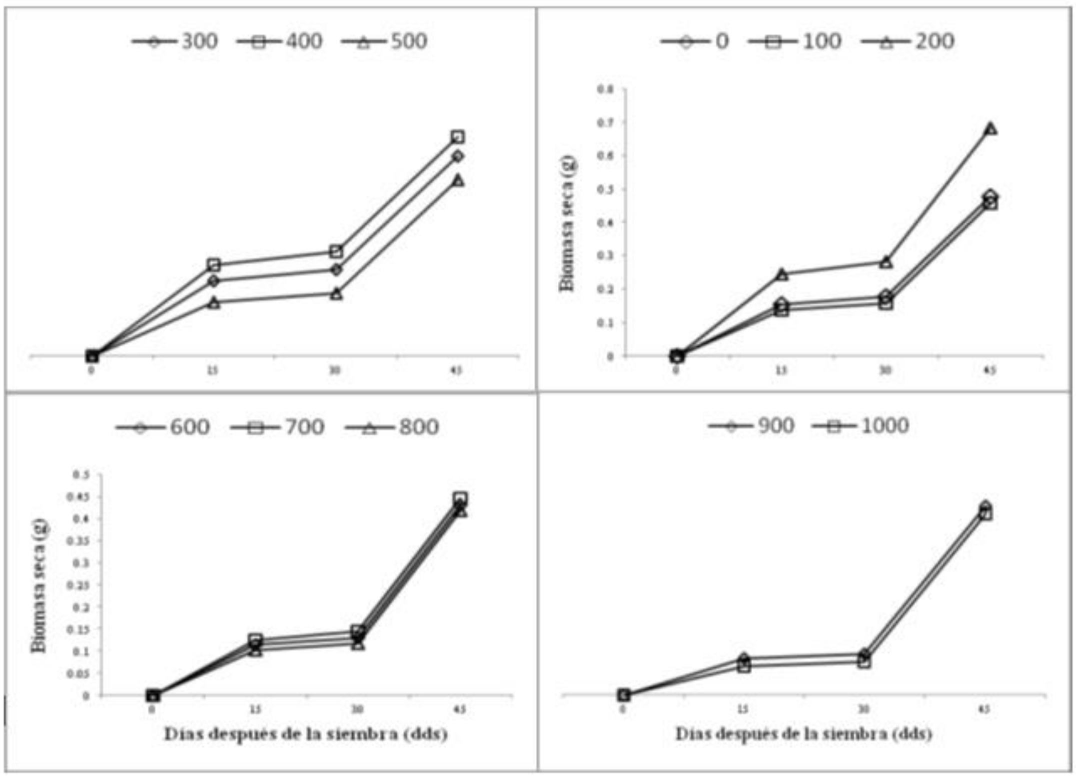

Figure 4. Total dry biomass dynamics in sunflower seedlings (*Helianthus annuus* L.) cv. Periquero. Subjected to eleven levels of irradiation 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1 000 Gy with gamma of ^{60}Co, to 15, 30 and 45 days after planting. National Institute of Nuclear Research, 2019.dds, days after planting.

The smallest accumulation of dry biomass in seedlings by irradiation effect, has been studied by Yoon *et al*. (2014), who mention that the cells that make up the tissues of the seedling are damaged by increasing the dose of gamma radiation, preventing the cellular DNA repair mechanism from doing its job, repairing the damage caused. Pavan *et al*. (2013), mention that the decrease in biomass in seedlings subjected to high doses of radiation is due to the production of free radicals in protoplast, which cause metabolic disorders in the cell, thus causing alterations in the pattern of gene expression, which regulate metabolic pathways and defense systems.

SPAD units

SPAD units showed a tendency to decrease, as the irradiation dose increased, adjusting the data to a linear model, turning out to be highly significant. Thus, the coefficient of determination was 96%, indicating that 96% of the SPAD Units were due to the increase in gamma radiation of ^{60}Co (Figure 5).

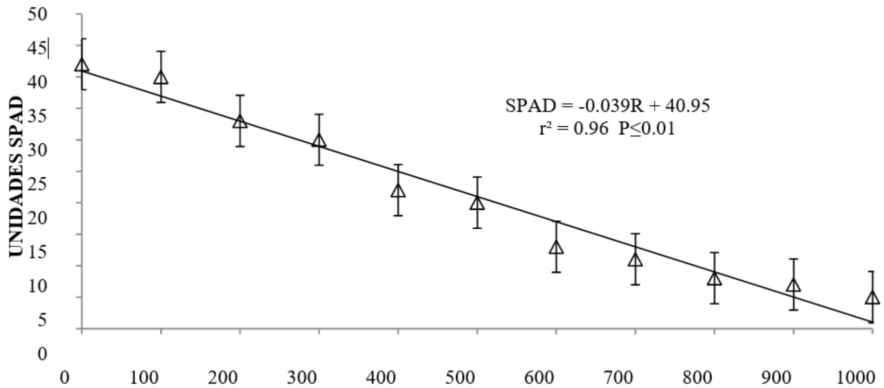

Figure 5. Gamma irradiation of ^{60}Co. SPAD units in sunflower seedlings (*Helianthus annuus* L.) cv. Periquero, at 45 dds, subjected to eleven levels of irradiation 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1 000 Gy with gamma of ^{60}Co. National Institute of Nuclear Research, 2019.

The slope of the model, -0.039 indicates that, for each Gy of irradiation applied to sunflower achenes, SPAD units decrease by 0.039, thus presenting chlorosis. The data presented here match those reported by Antúnez *et al*. (2017), who report SPAD unit values of 39.48, for witness treatment. Physiologically this chlorotic effect was due to the arrest of the cell cycle in the G2/M phase during the mitotic division of somatic cells in the nomofilos (Patil *et al*., 2015).

This behavior has also been reported by Estrada *et al*. (2011), who mention that pale (chlorotic) leaves, in nard (*Polianthes tuberosa* L.), occur when the bulbs of this geophyte are subjected to 25 and 30 Gy, gamma irradiation with ^{60}Co.

Conclusions

The mathematical models for germination, sprouting and survival, in sunflower seedlings, subjected to different levels of gamma irradiation of ^{60}Co, were adjusted to second-order models. The maximum $LD_{50}$was presented in decreasing order for survival, germination and sprouting percentage. Gamma radiation of ^{60}Co can induce genetic variability in sunflower seedlings, in doses of 100 and 200 Gy, stimulating both the volume and length of root. Total dry biomass decreased as irradiation increased, with doses of 900 and 1 000 Gy, where the seedling accumulated less biomass. The application of irradiation of 700 to 1 000 Gy caused the SPAD units to decrease due to the effect of chlorosis. To stimulate genetic variability in the radical sunflower *cv* Periquero system, gamma irradiation of ^{60}Co should be applied in doses of 100 and 200 Gy.

Acknowledgments

The authors thank the technical staff of the Gamma irradiator of the National Institute of Nuclear Research (ININ), for their support for the irradiation of genetic material.

Cited literature

Álvarez, H. A.; Morales, N. C. R.; Avendaño, A. C. H.; Santellano, E. E.; Melgoza, C. A.; Burrola, B. M. E. y Corrales, L. 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. Doi: https://doi.org/10.19136/era.a5n13.1268.

Antúnez, O. O. M.; Cruz, I. S.; Sandoval, V. M.; Santacruz, V. A.; Mendoza, O. L. E.; De la Cruz, T. E. y Peña, L. A. 2017. Variabilidad inducida en caracteres fisiológicos de *Physalis peruviana* L. mediante rayos gamma ^{60}Co aplicados a la semilla. Rev. Fitotec. Mex. 40(2):211-218. Doi: https://doi.org/10.35196/rfm.2017.2.211-218.

Barrios, M. B.; Buján, A.; Debelis, S. P.; Sokolowski, A. C.; Blasón, A. D.; Rodríguez, H. A.; López, S. C.; De Gracia, J.; Mazo, C. R. y Gagey, M. C. 2014. Relación biomasa de raíz/biomasa total de soja (*Glicine max* L.) en dos sistemas de labranza. Terra Latinoam. 32(3):221-230.

Cardoso, A.; Crodribb, T.; Lucani, C. DaMatta, F. and McAdam, S. 2018. Coordinated plasticity maintains hydraulic safety in sunflower leaves. Plant, Cell Environ. 41(11): 2567-2576. Doi: https://doi.org/10.1111/pce.13335.

Cepero, L.; Mesa, A. R.; Lajonchere, G. y Prieto, M. 2001. Estimulación del crecimiento de *Leucaena leucocephala cv* Cunninham con rayos gamma de ^{60}Co. Pastos y Forrajes. 24(3):235-240.

Chico, R. J.; Cerna, R. L.; Rodríguez, E. M. y Guerrero, P. M. 2012. Capacidad remediadora de la raíz de girasol, *Helianthus annuus* L. cuando es sometida a diferentes concentraciones de plomo. REBIOL. 32(2):13-19.

De la Cruz, T. E.; García, A. J. M. and Díaz, L. E. 2019. Slopes comparison of germination and seedling emergence curves VS ^{60}Co gamma radiation dose sunflower achenes. Inter. J. Current Microbiol. Appl. Sci. 8(7):1606-1611. Doi: 10.20546/ijcmas.2019.807.191.

Díaz-López, E.; Báez, I.; Hernández, P. and Loeza-Corte, J. 2017. Potential absorption of calcium in sunflower (*Helianthus annuus* L.) and its effect on pH and electrical conductivity. Inter. J. Current Microbiol. Appl. Sci. 6(6):3299-3304. Doi: 10.20546/ijcmas.2017.606.387.

Escalante, E. J. A.; Morales, R. A.; Loeza, C. J. M.; Olivar, H. A.; Rosales, M. B. E.; Vargas, R. E. J. and Díaz, L. E. 2017. Lethal concentration of carbonate of Ca as a function of the osmotic potential of the solution in sunflower (*Heliantus annuus* L.). Inter. J. Adv. Eng. Res. Sci. 4(2):103-108. Doi: 10.22161/ijaers.4.2.22.

Estrada, B. J. A.; Pedraza, S. M. E.; De la Cruz, T. E.; Martínez, P. A.; Sáenz, R. C. y Morales, G. J. L. 2011. Efecto de rayos gamma de ^{60}Co en nardo (*Polianthes tuberosa* L.). Rev. Mex. Cienc. Agríc. 3(3):445-458.

Infante, G. S. y Zárate, G. P. 2012. Métodos estadísticos: un enfoque multidisciplinario. Mundi-Prensa. 3ª. 643 p.

Loeza, C. J. M.; Díaz, L. E.; Campos, P. J. M. y Orlando, G. J. I. 2013. Efecto de lignificación de estacas sobre enraizamiento de *Bursera morelensis* Ram. y *Bursera galeottiana* Engl. en la Universidad de la Cañada en Teotitlán de Flores Magón, Oaxaca, México. Ciencia Ergo sum. 20(3):222-226.

Morales, M. E. J.; Morales, R. E. J.; Díaz, L. E.; Cruz, L. A. J.; Medina, A. N. y Guerrero, D. M. 2015. Tasa de asimilación neta y rendimiento de girasol en función de urea y liberación lenta. Agrociencia. 49(2):163-176.

Patil, U. H.; Deshmukh, G. N. and Kazi, N. A. 2015. Mutation breeding in chrysanthemum (*Dendranthema grandiflora* T.) Asian J. Multidisciplinary Studies. 6(6):230-232.

Pavan, K.; Chaturvedi, A.; Sreedjar, M.; Aparna, M.; Venuvadu, P. and Singhal, R. K. 2013. Gamma radiosensitivity study on rice (*Oryza sativa* L.). Asian J. Plant Sci. Res. 3(1):54-68.

Ramírez, R.; González, L. M.; Camejo, Y.; Zaldivar, N. y Fernández, Y. 2006. Estudio de radiosensibilidad y selección del rango de dosis estimulantes de rayos X en cuatro variedades de Tomate (*Lycopersicum esculentum* Mill.). Cultivos Tropicales. 27(1):63-67.

Steel, D. R. G. y Torrie, J. H. 1996. Bioestadística: principios y procedimientos. Mc Graw Hill. 2ª (Ed.). 640 p.

Suaña, Q. M. E. 2018. Capacidad del girasol (*Helianthus annuus* L.) para absorber cadmio de suelos contaminados en ambiente controlado, Puno. Rev. de Investigaciones de la escuela de Postgrado, Universidad Nacional del Altiplano. 7(1):393-401.

Taiz, L. and Zeiger, E. 2000. Plant physiology. McGrall-Hill. 3ª (Ed.). 675 p.

Yoon, A.; Kamaludin, A.; Tajudin, K.; Hazmi, Z.; Bakar, D.; Nezhadahmadi, A. and Golam, F. 2014. The contribution of muslim scientist in botanical science: studies on the using of gamma rays for ginger plants (*Zingiber officinale* L.). Stem Cell. 5(4):88-94.