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# Effect of gamma radiation of $^{60}\text{Co}$ on sunflower plants (*Helianthus annuus* L.) (Asteraceae), from irradiated achenes

Díaz, L.E.<sup>1</sup>; García, S.A.L.<sup>2</sup>; Morales, R.A.<sup>1,\*</sup>; Báez, R.I.<sup>2</sup>; Pérez, V.E.<sup>2</sup>; Olivar, H.A.<sup>1</sup>; Vargas, R.E.J.<sup>2</sup>; Hernández, H.P.<sup>2</sup>; De la Cruz, T.E.<sup>3</sup>; García, A.J.M.<sup>3</sup>; Loeza, C.J.M.<sup>4</sup>

<sup>1</sup> *Cuerpo académico Ecofisiología aplicada a cultivos en zonas áridas. Universidad Tecnológica de Tehuacán. Prolongación de la 1 sur 1101, San Pablo Tepetzingo Tehuacán, Puebla. C. P. 75859.*

<sup>2</sup> *Ingeniería en Agricultura Sustentable y Protegida. Universidad Tecnológica de Tehuacán. Prolongación de la 1 sur 1101, San Pablo Tepetzingo Tehuacán, Puebla. C. P. 75859.*

<sup>3</sup> *Instituto Nacional de Investigaciones Nucleares. Laboratorio de radiobiología. Carretera México-Toluca, La Marquesa S/N Ocoyoacac México. C. P. 52750.*

<sup>4</sup> *Ingeniería en Agroindustrias. Universidad de la Cañada. Carretera Teotitlán-San Antonio Nanahuatipam. Km 1.7 Teotitlán de Flores Magón Oaxaca, México.*

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### Abstract

In order to know the effect of  $^{60}\text{Co}$  gamma irradiation, in the sunflower crop, were irradiated achenes in the Transelektro LGI-01 in the Instituto Nacional de Investigaciones Nucleares. The data was evaluated under a completely randomized design, where the treatments were 0, 100, 200, 300, 400, 500, 600, 700, 800 and 900 Gy and four repetitions (10x4) = 40 experimental units. The response variables were: plant height, root length and volume, dry biomass. The results indicated that germination and sprouting decreased as the radiation increased, adjusting these to a quadratic model. Plant height, length, root volume and dry biomass decreased at high doses. From this investigation it was concluded, that doses of 100 and 200 Gy, have a stimulating effect on plant height and root length, being an important agent, to induce genetic variability in sunflower.

**Keywords:** genetic variability; mutation; phenotype.

### 1. Introduction

Sunflower (*Helianthus annuus* L.), it is an oleaginous crop that for many years was considered to be of European origin, particularly from the old Russia, although its center of origin is northeast of Mexico and southeast of the United States of America (Torretta *et al.*, 2010; Bye *et al.*, 2009), where there are the ancestors of this, such as the genera *Helianthus* and *Tithonia*, with the latter has genetic compatibility and can be crossed due to their equal chromosome number  $2n = 34$  (Luévanos *et al.*, 2010). Among the uses of this crop has been: ornamental, fodder and bioremediator plant of soils affected by hard water, due to its great capacity to absorb cations, such as  $\text{Ca}^{++}$  (Díaz *et al.*, 2017). Regarding gamma irradiation, it is a type of ionizing radiation with great penetration power, due to its short wavelength. This is emitted by some radioactive elements such as  $^{60}\text{Co}$ , which is the most used in breeding programs such

as radioinduced mutagenesis, to induce genetic variability that almost does not exist in nature (Iglesias *et al.*, 2010). The prior art, has its foundation in the great penetration power of gamma radiation 7.5 MeV ( $1.2 \times 10^{-12}$  J) (OIEA, 2017), which causes direct damage to the DNA molecule, since it breaks the hydrogen bonds that join the purine and pyrimidic bases, including causing changes in the mating of the same, causing in the soma mutations known as molecular mutations, which can be heritable (Lyaz and Naz, 2014). These changes, in most cases, can cause unwanted changes in the phenotype of the mutant, but in some cases, those mutations may be of interest to the breeder, thus, radio-induced changes can be: high yield, resistance to some adverse factor for the crop, new color shades, etc. (Gómez *et al.*, 2017). Regarding the application of  $^{60}\text{Co}$  gamma radiation in crops, Díaz *et al.*, (2003), mention that  $^{60}\text{Co}$  gamma rays, induce genetic variability in bulbs of

\* Corresponding author  
E-mail: [alejandro.morales@uttehuacan.edu.mx](mailto:alejandro.morales@uttehuacan.edu.mx) (A. Morales).

*Tigridia pavonia* (L. f.) DC, in the M<sub>1</sub>V<sub>1</sub> generation, when *Tigridia* is destined to gardening. On the other hand, Fe *et al.* (2000), mention that the application of 350 Gy of radiation, inhibit the production of pods in the cultivation of soybean (*Glicine max* L.). In horticultural crops such as potatoes (*Solanum tuberosum* L.), Salomon *et al.* (2017), they indicate that dose of 20 Gy of gamma irradiation of Co <sup>60</sup>Co, stimulate the germination of the botanical seed Atzimba x TPS-13 whose germination is low. As you can see, in the sunflower crop, Work on this tendency to induce genetic variability is limited. Therefore, the general objective of the present study was: to evaluate the effect of <sup>60</sup>Co gamma radiation, from seedlings from irradiated achenes.

## 2. Materials and methods

### Study area

The present investigation was carried out in the experimental field of the Universidad Tecnológica de Tehuacan, located at 18°24'51" north latitude, 97°20'00" west longitude and 1409 meters of altitude.

### Germplasm and radiation dose

The genetic material consisted of achenes of sunflower cv. Victoria, which were donated by the germplasm bank, of "Ecofisiología de Cultivos" Colegio de Postgraduados Campus Montecillos, México. The achenes were irradiated, in the Transelektro LGI-01, of Instituto Nacional de Investigaciones Nucleares (ININ), located in Marquesa Ocoyoacac, Mexico.

### Experimental design and treatments

The experimental design was completely randomized (DCR) with four repetitions (10x4) = 40 experimental units, following the mathematical model  $Y_{ij} = \mu + T_i + \varepsilon_{ij}$  where:  $Y_{ij}$ , is the response variable of the  $i$ -th radiation dose in the  $j$ -th repetition;  $\mu$ , is the true general average;  $T_i$ , is the effect of the  $i$ -th radiation dose and  $\varepsilon_{ij}$ , is the experimental error of the  $i$ -th radiation dose in the  $j$ -th repetition (Cochran and Cox, 2010; Infante and Zarate, 2005; Steel y Torrie, 1985). The treatments consisted of radiation doses: 0, 100, 200, 300, 400, 500, 600, 700, 800 and 900 Gy, which were previously determined for sunflower, as established by Díaz *et al.* (2017).

### Sowing and handling of the experiment

The irradiated achenes were planted in 200-well polystyrene trays, using Peat moss as substrate, depositing an achene for each

cavity. In order to obtain a good germination, the substrate during sowing was maintained at field capacity. To avoid damage by solar radiation, the tray with the genetic material, was placed in the incubation area of a saw-type greenhouse. To avoid damage by pathogens, when the seedlings emerged, they were treated with streptomycin sulfate at a rate of 0.60 g L<sup>-1</sup>, using running water as a vehicle.

### Response variables

#### Germination percentage

It was determined for each dose of radiation, by means of the relationship  $GP = \frac{GS}{SS} \times 100$  where: GP, is the percentage of germination; GS, germinated seeds and SS, seeds sowing.

#### Sprouting percentage

It was determined for each dose of radiation, by means of the relationship  $SP = \frac{SP}{SS} \times 100$  where: SP, is the percentage of sprouting; SP, sprouted plants and SS, seeds sowing.

#### Plant height

This variable was measured, starting from the epicotyl of the plant, up to the apical bud, using a ruler three meters high and reporting the result in centimeters.

#### Length and root

A representative sample of five seedlings was taken, to later measure, from the cap of the root, to the beginning of the epicotyl. To achieve the above, a digital vernier was used and the results were reported in centimeters.

#### Root volume

The root volume was calculated using the Archimedes principle. Using a 100 mL test tube, a volume of water known in this case was added, 50 mL. The volume of water displaced, when introducing the sunflower root, will be the root volume in cm<sup>3</sup>.

#### Dry biomass

Three seedlings were taken, which were introduced in paper bags, to be introduced in a foamed air oven, for 72 hours, until reaching the constant weight and measuring its weight, in a model analytical balance USS-DBS3-3 (Barrios *et al.*, 2014). The variables were subjected to an analysis of variance (ANOVA), in order to determine significant differences between them.

When the response variables were significant, they were applied Tukey's multiple comparison test, at a level of significance of 5% probability of error.

### 3. Results and discussion

Percentage of germination and sprouting  
Germination as well as sprouting, were adjusted to a third-degree polynomial model, with a highly significant coefficient of determination. The highest percentages of these variables occurred with doses of 100 and 200 Gy, whose values were 95 and 93% respectively, to decrease progressively as the radiation dose was increased (Figure 1). These mathematical models, differ with those presented by Loeza *et al.* (2016), who mention that the germination in seeds of *Hibiscus sabdariffa* L., subjected to gamma irradiation of  $^{60}\text{Co}$ , decreases as the radiation dose increases from 5 Gy to 50 Gy and whose model had a linear fit. Similarly, Antúnez *et al.* (2017), mentions that the application of 100, 200 and 300 Gy of gamma radiation, does not affect the germination percentage of irradiated seeds of *Physalis peruviana* L., which differs with the present study. This is mainly due to the different species used in both studies, as well as the irradiation ranges used.

#### Morphological variables

The highest plant height, as well as root length and dry biomass, were induced with 100 and 200 Gy, while in the range of 400 to 700 Gy, plant height and root length were

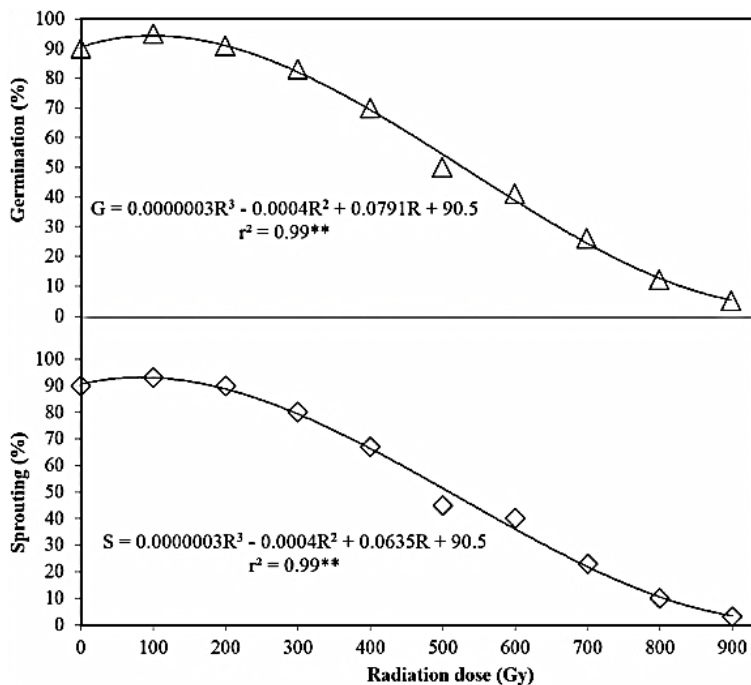
statistically equal. The lowest cumulative biomass was found in the high doses of radiation, 800 and 900 Gy, with 3.6 and 3.3 g respectively (Table 1). This response was due to a lower height of the plant, which caused the plant to accumulate a lower dry biomass. This same trend was observed in *Leucaena leucocephala* plants from irradiated seeds, where doses of 20, 80 and 100 Gy of irradiation, inhibit root growth, by 5 and 10% in relation to the control individual (Cepero *et al.*, 2001).

**Table 1**

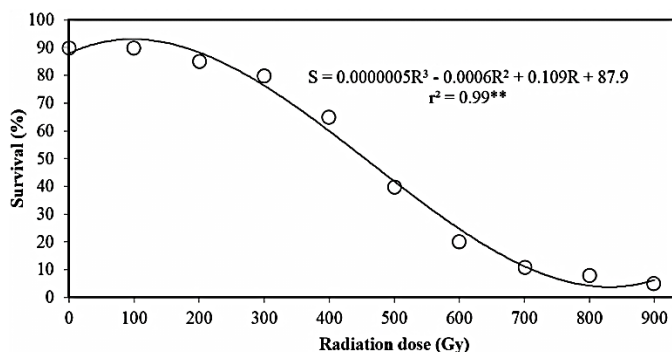
Analysis of variance and multiple comparison test, for four response variables in sunflower (*Helianthus annuus* L.), subjected to ten levels of gamma radiation of  $^{60}\text{Co}$

Treatment Gy	PH cm	RL	RV cm <sup>3</sup>	DB g
0	5.5 b	3.0 ab	2.0 a	4.0 b <sup>†</sup>
100	8.0 a	3.9 a	3.0 a	5.3 a
200	7.0 a	3.4 a	2.0 a	5.0 a
300	6.5 b	3.2 ab	2.0 a	4.6 b
400	6.0 b	3.0 b	2.0 a	4.5 ab
500	5.6 b	3.0 b	2.0 a	4.5 ab
600	4.8 b	2.9 b	2.0 a	4.2 b
700	4.3 b	2.6 b	2.0 a	4.0 b
800	3.6 c	2.0 c	1.0 ab	3.6 bc
900	2.5 c	1.3 d	1.0 ab	3.3 bc
HSD	1.20**	0.4**	1.50*	0.50**
CV%	8.50	5.10	7.90	10.70

Values within column with the same literal, statistic mind are equal to  $P \geq 0.05$  probability of error, according to Tukey; PH, plant height; RL, root length; RV, root volume; DB, dry biomass; HSD, honest significant difference; CV, coefficient of variation; \*, \*\*, n.s., significant to 0.05; 0.01 y not significant.



**Figure 1.** Percentage of germination and sprouting, of achenes of sunflower (*Helianthus annuus* L.), irradiated with gamma of  $^{60}\text{Co}$ . Universidad Tecnológica de Tehuacan. 2017. G, germination; S, sprouting; \*, \*\*, n.s., significant to 0.05; 0.01 and not significant.



**Figure 2.** Survival percentage of sunflower seedlings (*Helianthus annuus* L.), subjected to ten levels of  $^{60}\text{Co}$  gamma radiation. Universidad Tecnológica de Tehuacán. 2017. S, survival; R, radiation; \*\*,\*, n.s, significant at 0.01; 0.05 y not significant.

### Percentage of survival

Survival percentage was a consequence of germination and sprouting together, having a third order polynomial behavior (Figure 2), and whose coefficient of determination was high 0.99 and highly significant. As the radiation dose increased from 200 to 900 Gy, the percentage of survival decreased, indicating that the sunflower is a sensitive species at high levels of radiation, thus in this way the application of 500 Gy decreases survival by 54.9% (Figure 3). This same response was found in Polianthes, where the percentage of survival, decreases, by increasing the radiation dose from 10 to 30 Gy, despite being a tuberous plant (Estrada *et al.*, 2011). In contrast Lemus *et al.* (2002), worked with two varieties of vigna bean (*Vigna unguiculata* L.), in similar ranges of irradiation, found that the application of 600 Gy, causes a drastic fall in the percentage of survival. This response to gamma irradiation was also studied by Ramírez *et al.* (2006), who when working with tomato seeds (*Lycopersicon esculentum* Mill.), mention that the first effects of gamma radiation, affect physiological processes such as germination and seedling growth, submitted to these agents and report, then in tomato, the germination begins to decrease in 500 Gy, checking what was found in this investigation.



**Figure 3.** Effect of  $^{60}\text{Co}$  gamma irradiation on survival in sunflower seedlings (*Helianthus annuus* L.). Universidad Tecnológica de Tehuacán. 2017.

### 4. Conclusions

The following conclusions were derived from the present investigation. Germination, sprouting as well as the percentage of survival in sunflower, are affected as the gamma radiation of  $^{60}\text{Co}$  is increased from 300 to 900 Gy. Doses of 100 and 200 Gy have a stimulating effect on plant height and root length. The sunflower, is a species sensitive to gamma irradiation of  $^{60}\text{Co}$ , for this reason this physical agent can be used to induce genetic variability in this crop and in future studies of genetic improvement. for future work, It is recommended not to use radiation greater than 500 Gy, because 50% of the population dies, therefore, superior radiations are not necessary for a work of genetic variability.

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