Gamma Irradiation Effect of ^{60}Co on the Germination of two subtropical species in the Tehuacán-Cuicatlán Valley

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Abstract—*In order to know the influence of ^{60}Co gamma radiation, on the germination of two semitropical species, roselle seeds and sunflower were irradiated at the Transelektro LGI-01 of the InstitutoNacional de InvestigacionesNucleares, At doses 0, 5, 10, 15, 20, 25, 30 and 35 Gy, to determine the radiosensitivity curve and to determine the LD_{50} of both species, under a randomized complete block design with factorial arrangement, Where the study factors were: radiation doses and species. The results indicate that sunflower is more sensitive to gamma radiation than roselle, so the LD_{50} for sunflower and roselle was not reached, because the doses of radiation used, did not achieve 0% germination. Thus, the radiosensitivity curves were fitted to a linear model, with a high coefficient of determination. From the present investigation, It can be concluded that to determine the LD_{50} in the species in question, It is necessary to increase the dose of irradiation, perhaps up to 1000 Gy.*

Keywords— **LD_{50}, mutation, ionizing radiation, mutagen, mosaic.**

I. INTRODUCTION

Sunflower (*Helianthus annuus* L.) and roselle (*Hibiscus sabdariffa* L.), are considered crops of semi-arid zones,and although these present a wide range of adaptability due to their rusticity and phenotypic plasticity,which have been little used to be introduced in tropical areas of the world including Mexico.Sunflower is a plant that originates in northeastern Mexico and southeastern United States (Povereneet *al*., 2002). This species for many years was used as oilseed,since their seeds are obtained fatty acids with high nutritional value (Gallegos *et al*., 2003),but nowadays it has gained importance from the ornamental point of view,due to the great size of its foliage and to the beauty of its chapter which has favored,that is used in flower arrangements and as a cut flower (Melgares, 2001).With regard to roselle,is a plant that belongs to the family malvaceae and has its center of origin in Africa specifically in Sudan,although some authors like Vavilov, mention that its origin is the Abyssinian center (López, 2002).This crop for many years has been used as medicinal because of its diuretic properties,although of its stems also is extracted fiber for the elaboration of ropes which are resistant to the salinity and its seeds,a very fine oil is obtained as the one extracted from *Luffa cylindrical* L.which can be used for the preparation of cosmetics, in addition to possessing antimicrobial properties (Amaya *et al*., 2000).On the other hand, the genetic improvement of plant species has gone through multiple stages,from selection, introgression, crossing, to the use of potent physical and chemical mutagens, a process called Mutagenesis,to obtain cultivars that meet the characteristics

https://dx.doi.org/10.22161/ijaers.4.8.10 *ISSN: 2349-6495(P) | 2456-1908(O)*

desired by the breeder.This technique is based on the application of physical agents such as gamma radiation emitted by certain radioactive elements such as ^{60}Co (Aros*et al*., 2012).This type of radiation has a very short wavelength of 10^{-12} m, which allows a very high penetration power and is used by geneticists, Causing damage to the DNA molecule causing mutations at the molecular level (Almeida *et al*., 2004), Which will manifest in the phenotype of organisms in the form of somatic mutations known as "Mosaic".This advantage of generating mutations in the plant, can be of great utility since in the same way, resistance to some adverse environmental conditions such as drought,which is of great help in selecting materials that may present the resistance genes to drought,Or even induce this quality to genotypes with the help of gamma radiations (González *et al*., 2007).To achieve this, when irradiating seeds or propagules, It is necessary to do studies on the effect of ionizing radiation on these structures, which is known as radiosensitivity curve,which allows us to determine even the average lethal dose known as LD_{50}.For this reason the main objective of the present study was:determine the radiosensitivity and LD_{50} curve in roselle seeds and sunflower caryopsids under eight ^{60}Co gamma ray levels.

II. MATERIALS AND METHODS.

2.1 Location of the experiment.

The present study was carried out in the laboratory of soils of the Universidad Tecnologica de Tehuacan, during the spring of 2015, located in San Pablo TepetzingoTehuacan Puebla, Mexicoat 18º 24 'north latitude, 97º 20' longitude west and 1409 altitude.

2.2 Genetic material.

The germplasm of roselle (Hibiscus sabdariffa L.) was collected from an accession on the coast of Oaxaca, Mexico in PinotepaNacional at a location of 16º 21 'north, 98º 02' west and 28 altitude,whose characteristics are: average postage 0.50 to 1.50 m high and red chalices,the sunflower cariopsides (*Helianthus annuus* L.) cv. Victoria, were donated by the germplasm bank of Ecofisiología de Cultivos of Colegio de Postgraduados,which were irradiated in the Transelektro LGI-01 of the InstitutoNacional de InvestigacionesNucleares (ININ) in Ocoyoacac, Mexico.

2.3 Determination of radiosensitivity curve.

This was calculated for both irradiated materials, performing a test of germination,Disinfecting seeds and caryopsids with a solution of 1% sodium hypochlorite and placed in petri dishes with filter paper, depositing 10 seeds per box and equally 10 cariopsides per box, separately for later incubation in a room of growth at constant temperature at 25ºC,And the percentage of germination was calculated using the following expression PG = (Gs / Ss) * 100 where:PG, Percentage of germination; Gs, Germinated seeds; Ss, seed sown(Gil & Miranda, 2008), After determining the percentage of germination, the rediosensitivity curve was calculated by means of a linear regression plotting the percentage of germination vs radiation dose and obtaining the model of adjustment by least squares.From the obtained model an interpolation was made, taking the dependent variable the value of 50% of germination, and clearing the independent variable to determine the LD_{50} of both species under study (Infante and Zarate, 1990).

2.4 Experimental design.

The design used was complete random blocks with three replicates and factorial arrangement following the mathematical model:$Y_{ijk} = \mu + A_j + B_k + \beta_i + (AB)_{jk} + \varepsilon_{ijk}$where: Y_{ijk} is the response variable of the i-th radiation dose in the j-th species of the k-th block;μ, is the true overall mean;A_i, is the effect of the i-th radiation dose;B_j, is the effect of the j-th species under study; B_k, is the effect of the k-th block; $(AB)_{ij}$, is the effect of the interactiondosis of radiation i on the species j under study and ε_{ijk}, is the experimental error of the i-th radiation dose on the j-th species in the k-th block,thus the study factors were integrated by: the radiation dose and the species under study.The experimental unit consisted of a roselle seed and a sunflower.

2.5 Response variable.

This was only constituted by the percentage of germination in both study factors and when it was significant, the Tukey test was applied to a level of significance of 5% of probability of error.

III. RESULTS.

The analysis of variance and the comparison test of means for dose and species, showed highly significant differences for: treatments, both study factors as well as repetitions.Radiation x species interaction was only significant.Regarding the coefficient of variation, this oscillated between 6.34% for radiation dose, whereas for the species it was 10.50%,indicating that the experimental data were very reliable during the development of the experiment (Table 1). For the radiation factor, the highest percentage of germination occurred in the control and the dose of 5 Gy with 96.50 and 92.02%, respectively,being also statistically equal.Levels 10, 15, 20 and 25 Gy of radiation were found to be statistically equal but differ numerically,In this way the high levels of gamma radiation 30 and 35 Gy presented the lowest values of germination with 81.90 and 79.50%.Regarding the species factor, the highest germination was in roselle with 89.31%, surpassing sunflower that only obtained 80.43%.The radiosensitivity curve for germination with respect to the radiation in roselle, is presented in figure 1.In it is observed that the mathematical model was linearly adjusted in a decreasing way, whose coefficient of determination turned out to be highly significant 0.98 **, and whose slope was -0.37 indicating that for each Gy of radiation, supplied to roselleseeds, there was a decrease of 0.37% germination.On the other hand the germination of sunflower caryopsids, submitted to different levels of radiation, maintained a tendency similar to roselle, keeping the linear model decreasing. For this case the coefficient of determination was 0.92* and slope -0.50, for each unit of radiation the germination of sunflower caryopsids is reduced by 0.50% (Figure 2).

Table.1: Analysis of variance and multiple comparison test, for two study factors based on ^{60}Co gamma radiation, in the Tehuacán-Cuicatlán Valley. 2016.

Radiation factor	G (%)	Species factor	G (%)
T_0	96.50 a	Roselle	89.31 a¶
T_5	92.02 a	Sunflower	80.43 b
T_{10}	89.60 ab		
T_{15}	87.17 ab		
T_{20}	86.75 ab		
T_{25}	83.82 ab		
T_{30}	81.90 b		
T_{35}	79.50 b		
HSD	6.40		5.69
CV %	6.34		10.50
	ANOVA		
Treatments		**	
Repetitions		**	
Radiation (R)		**	
Species (S)		**	
R x S		*	

¶Means within columns with the same literal are statistically the same according to Tukey at (*P≤0.05*). G, germination; HSD, honestly significant difference; CV, coefficient of variation.; *; n.s, significant at 0.01; 0.05 and not significant.**

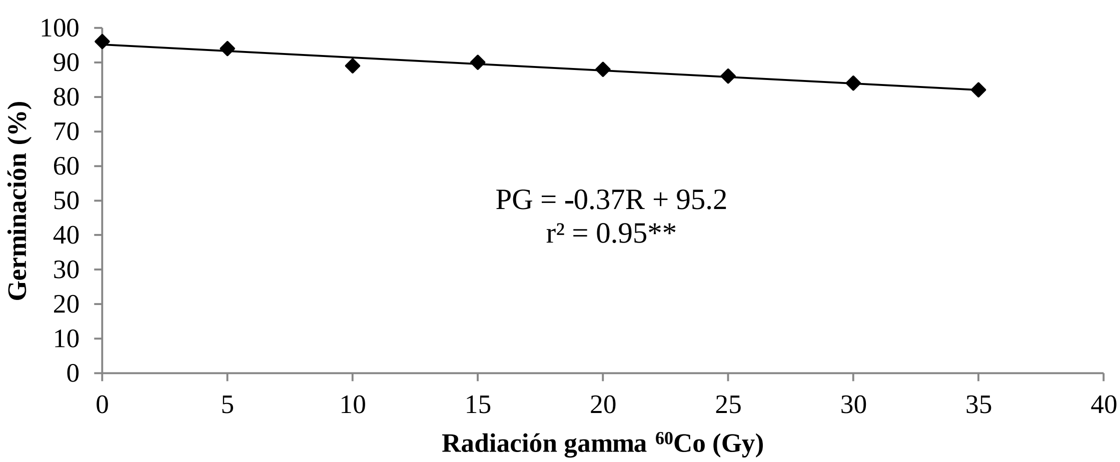

Fig.1: Radiosensitivity curve in Jamaican seeds (Hibiscus sabdariffa L.), subjected to eight levels of ^{60}Co gamma irradiation in the Tehuacán-Cuicatlan Valley. 2016. PG, Percentage of germination; R, radiation.

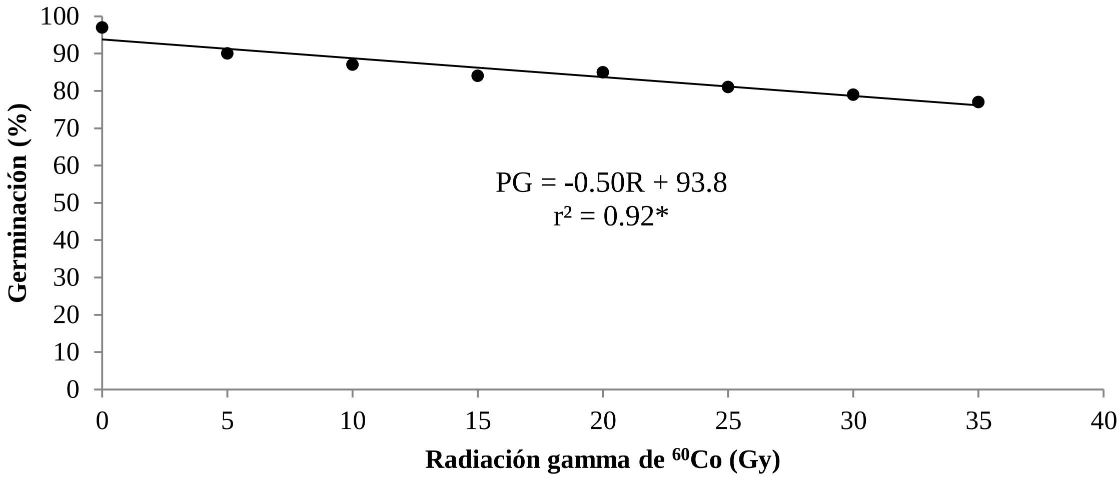

Fig.2: Radiosensitivity curve in sunflower achenes (Helianthus annuus L.), submitted to eight levels of gamma irradiation of ^{60}Co, in the Tehuacán-Cuicatlan valley. 2016.PG, Percentage of germination; R, radiation.

The mean lethal dose,to be derived from the mathematical models of adjustment for both species under study,was not reached,due to the fact that in none of the species did germination reach 0.0%, as can be seen in Figures 1 and 2.Thus in this way,the lowest percentages of germination occurred with 35 Gy,for the species in question with 81.00% germination for roselle and 72.00% for sunflower.This indicates,that roselle has a lower sensitivity to gamma radiation than sunflower,that is to say,11.11% less sensitivity to gamma radiation than 60Co than sunflower (Table 2).

Table.2: Mathematical models of fit, coefficient of determination and mean lethal dose, for roselle (Hibiscus sabdariffa L.) and sunflower (Helianthus annuus L.), subjected to ^{60}Co gamma irradiation.Tehuacan-Cuicatlan Valley. 2016.

Especie	**Modelo**	**r^2**	**LD_{50} (Gy)**
Jamaica	PG=-0.37R+95.2	0.95**	-------------
Girasol	PG=-0.50R+93.8	0.92*	-------------

PG, percentage of germination; R, radiation dose; LD, mean lethal dose.

In the interaction radiation x species, we can see that both species interacted in a decreasing way with respect to radiation,in the range of 0 to 25 Gy,that is to say, as the radiation increased the germination of both decreased,to then decrease drastically from 25 to 30 Gy in sunflower,while roselle does not experience a change of slope keeping the same trend. From 30 at 35 Gy,roselle experienced a steady slope,indicating that in this radiation range,the species presents insensitivity to gamma rays (Figure 3).

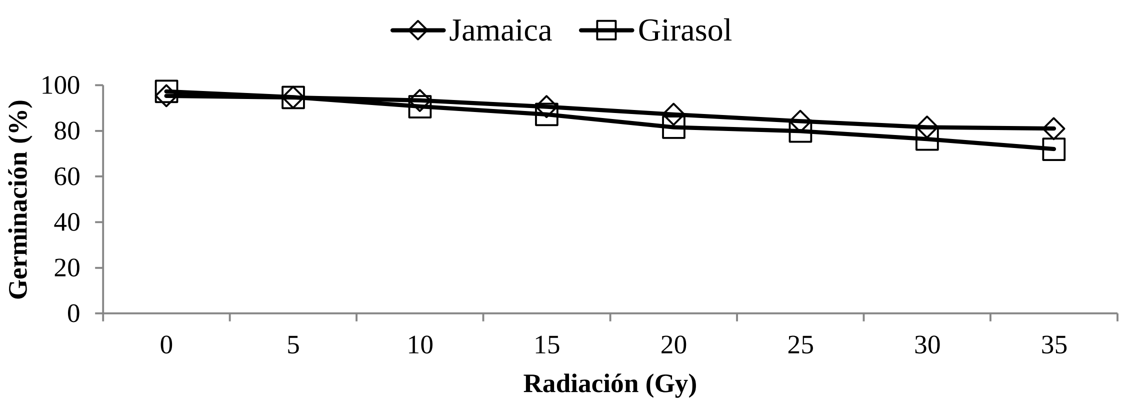

Fig.3: Radiation x species interaction, in a factorial experiment at the Universidad Tecnologica de Tehuacan. San Pablo Tepetzingo, Tehuacán Puebla. Spring 2014.

IV. DISCUSSION

Germination decreased as the radiation dose increased,both in Jamaican seeds and in sunflower caryopsids,the latter presenting a reduction of 11.11% in germination compared to roselle.This response coincides with that observed and reported by Díaz*et al*. (2003),who worked with *Tigridiapavonia* (L.f) D.C. And mention a progressive reduction in the budding of the bulbs of this species,when subjected to gamma rays of ^{60}Co in a range of 5 to 30 Gy despite being different species.On the other hand, Ramírez*et al*. (2006), mention that dose 500 Gy,result in a significant decrease in the germination of tomato hybrids of up to 23%,data that differ from those reported in this present study,the above may be due to the differences between species in which both studies were carried out and the high germination percentage of the tomato hybrids,as well as the different doses of radiation used.The results presented in this study,indicate that both structures are sensitive to gamma radiation of ^{60}Co,and are of great importance for being the basis for establishing doses of radiation,when it is desired to improvement genetic using the mutagenesis technique and thus eliminate the doses that are lethal when killing more than 50% of the study population,which was not reached in this investigation.For this reason the establishment of radiosensitivity curves is of great importance in this type of studies.

V. CONCLUSIONS

From the present investigation carried out, under the conditions of the valley Tehuacan-Cuicatlan, the following conclusions were derived:

- In the low doses of gamma irradiation of ^{60}Co, both species presented an equal germination, statistically speaking.
- Sunflower presented the lowest germination than Jamaican, when applying high dose of gamma rays.
- Sunflower prove be more susceptible to gamma radiation, as it decreased its germination, compared to roselle.
- The germination vs radiation adjustment models were linear with high determination coefficients for both species.
- The LD_{50} could not be determined, because in both species the zero germination percentage was never reached.
- For further studies, where it is intended to establish the LD_{50} of the species in question, it will be necessary to irradiate up to 1 kGy to obtain a positive response.

ACKNOWLEDGEMENTS

The authors of the present study are grateful for the financial support received from PRODEP for the project, "**Mejoramiento genético de atributos agronomicos en jamaica (*Hibiscus sabdariffa* L.) para generar metabolitos secundarios en zonas áridas",** Developed by the academic body, "**Ecofisiología aplicada a cultivos en zonas áridas",** With registration **UTTEH-04**,as well as the staff of the radiobiology laboratory of theInstituto Nacional de Investigaciones Nucleares (ININ), **Dr. Eulogio de la Cruz Torres and M. en C. Juan Manuel García Andrade,**for the support received during the irradiation of the genetic material.

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