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Radio-Sensitivity on Huasteca-100 Soybean Seeds Variety with ^{60}Co Gamma Radiation

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Abstract

In the current study, radio-sensitivity on soybean seeds from the Huasteca-100 variety with ^{60}Co gamma rays was evaluated. Fifteen doses of radiation (1-1250 Gy) and control (without irradiation) were utilized. The trial was carried out in nursery polybags under randomized block design with four repetitions. Germination, survival, height, and the number of seeds per plant were evaluated so LD_{50} and GR_{50} calculation could be done through the linear and quadratic regression. Survival, height and number of seeds per plant were modeled, and at what dose was significant in these variables. LD_{50} for survival was calculated at 438 Gy, whereas GR_{50} for height and number of seeds was calculated at 252-298 Gy, respectively. No radio-stimulation regions were observed, yet, transition regions were achieved amongst 1 and 440 Gy, but height and number of seeds are more radio-sensitive than survival (1-240 Gy). The inhibition region was obtained from 240 Gy for plant and number of seeds, but from 440 Gy survival was obtained. Based on the GR_{50} for plant height, a 252 Gy ± 5% dosage could be considered appropriate for genetic variability inducement on Huasteca-100 soybeans.

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Keywords

Glycine max, radio-sensitivity, gamma rays, LD50, and GR50.

Introduction

Due to its high protein and oil content on the seed, soybean is considered to be a very important functional food on a man's diet. Only in Mexico, the area sown it's of 281.978 hectare with an annual production of 426.743 t (SIAP, 2016), nevertheless; seed production in the country is insufficient to meet the domestic demand since only a 3.5% of the annual requirements are covered. The seed production is being affected the lack of high-yielding varieties and the lack of edafoclimatic conditions adaptation from soybean producing regions.

In order to address all these problems, improvement strategies on soybean are being mainly focused on classical breeding improvement as well as on (in a low frequency) induced mutagenesis, in Mexico. Induced mutagenesis, as opposed to other methods, allows to generate random mutations in the entire genome (either in a locus or in a gene), creates a highly variability (Foster y Shu, 2012); and therefore, it permits the development of new mutating lines or varieties.

Induced mutagenesis is carried throughout chemical and physical agents (Mba and Shu, 2012; Leitão, 2012). Gamma radiation, which is a physical mutagen, has permitted, to a greater extent, the creation of high-yielding mutating lines that are resistant to drought, diseases, precocity, among others. These have contributed to food security (Kharkwal y Shu, 2009).

So as to initiate an improvement genetic program through mutagenesis, a mutagen dosage must be determined for it can permit an optimal mutagenic frequency in the targeted population. On that subject, Mba *et al.*, (2010) report that the median lethal dose ± 5 unit is the ideal for genetic variability inducement. Kodyn *et al.*, (2012), on the other hand, report that GR_{50} could be considered as the optimal dosage for genetic variability inducement.

The determination of these parameters increases the chances of succeeding in mutants' generation provided that radio-sensitivity's respond is properly differentiating from genotype, DNA amount, ploidy level, water content, and time of exposure (Horn and Shimelis, 2013; Kahrizi *et al*., 2012; Raghuvanshi and Singh, 1976). Hence, the aim of this work was to study radio-sensitivity on Huasteca-100 soybean seeds variety with ^{60}Co gamma rays.

Materials and Methods

Irradiation and genetic materials

Soybean seeds from the Huasteca-100 variety owned by INIFAP, were utilized and subjected to 15 doses of gamma radiation (1, 5, 10, 20, 50, 100, 150, 200, 240, 320, 360, 400, 440, 620, and 1250 Gy). Doses were determined using the Gafchromic dosimetry system and an RADCAL ionization chamber model Accudose.

The irradiator was a Gamma Beam 127 MDS Nordion panoramic equipment, with a storage source of 50 g cobalt 60 (^{60}Co) in dry (owned by Moscafruit Experimental Station). One control without irradiation was also used. Sixty seeds with 10.72% of humidity were employed on the control and in every dosage.

Experiment setup

The experiment was carried out at Rosario Izapa Experimental Station of the INIFAP which is located at 14º 40' 16.1´´ north, 92º 42´ 59.1´´ west, and at 435 m-altitude in Tuxtla Chico, Chiapas, México. From June to November 2015, the average temperature was of 26.3 °C, precipitation was of 2538 mm, and relative humidity was of 86%. Trials were conducted on 20-cm-dia x 40-cm-depth nursery polybags. From every nursery polybag, 10 kg of soil substrate was taken (5.0% of organic matter, 6.86 of pH and a loamy-sand texture).

Experimental design and variable responses

A four-repetition randomized block design was applied. Each repetition consisted in three nursery polybags whereby five seeds have been sown. The germination percentage has been evaluated after 9 days of the sown (A9DS), plants' survival at the start of their flowering (R1) based on the number of emerging plants A9DS (covariate), plant's height at the start of its physiological maturity (R8) that was measured at ground level up to the apex from the main stem (3 plants per repetition), and the number of seeds produced by plant (3 plants per repetition).

Data analysis

LD_{50} for germination and survival plus GR_{50} for height and number of seeds per plant were determined based on the parameters of the linear and quadratic regression models. The selection of the model was done in accordance to the following criteria: i) simple or adjusted coefficient of determination higher value (that's one or two independent variables), ii) mean squared error lower value, and iii) Mallow´s Cp test lower value.

Analysis of variance and covariance are performed in accordance with the model's assumptions. By applying the Dunnett test, a comparison between medians was carried out. Data analysis was, executed by using a SAS 9.0, but random assignment of treatment to experimental units was through R version 3.3.2.

The determination of LD_{50} was performed through exploration by utilizing the parameters from study models, whose initial number was of 12 plants; Excel software 2013 was used for this. While the calculation of GR_{50} was for height, and the number of seeds per plant was based on the following equations (i.e. plant's height): Simple linear regression model, GR_{50}=

(Vmt*0.5)- β_0/ β_1, and Quadratic regression model GR_{50}=Square root [(Vmt*0.5)- β_0/ β_1)], where: GR_{50}= median dose reduction for plant height; Vmt=Highest value of the controller's height plant (without irradiation); β_0= model parameter, origin of ordinate, and β_1= model parameter, slope of a line. Furthermore, radio-sensitive regions were established based on what Ramírez *et al.*, (2006) suggested.

Results and Discussion

Germination

Germination percentage is the first variable within radio-sensitivity trials. Díaz-López *et al.*, (2016) and Rajarajan *et al.,* (2016) report a decrease in the germination by increasing gamma rays dosage on Hibiscus flower and rice Var ADT (R) 47, however; Ramírez *et al*., (2006) report that lower doses causes stimulation, but higher doses inhibits germination in four varieties of tomato. The germination percentage on Huasteca-100 variety did not present, after 9 days of the sown, any significant reduction even in doses of 1250 Gy (Table 1 & Figure 1A). Similar results were given by Aminah *et al.*, (2015) who irradiated soybean seeds from the Anjasmoro variety at ten doses of gamma radiation (100-1000 Gy, more control) and found no significant difference in the germination after being radiated with gamma treatment. Nonetheless, when Khan and Tyagi (2010) irradiated Pusa-16 and PK-1042 soybean varieties and Fe *et al.*, (2000) irradiated the Cubasoy-23 variety, they reported a decrease in the germination by increasing the gamma radiation dosage. Various authors mention that the grade of sensitivity at gamma radiation is due to genotype, radiation dosage, time of exposure, DNA amount, ploidy level, and humidity content (Horn and Shimelis, 2013; Kahrizi *et al*., 2012; Raghuvanshi and Singh, 1977.)

Both the simple linear regression analysis and the quadratic one, permitted to define that the used doses were not significant in the germination, thus LD_{50} cannot be estimated.

Plant's survival

Maluszynski *et al.,* (2009) published the methods for mutation isolation and inducement for grain legumes' genetic improvement and indicated that M2 generation must be of 50.000 plants minimum. Due to sown methodology in M2 generation is by individual furrows per plant offspring M1, survival percentage and seed production become variables of interest that allow seed calculation M_0 to be implemented within an improvement programme assisted by induced mutation.

Plants survival M1 on Huasteca-100 variety had a decreasing trend when gamma radiation doses are being increased, this result is due to aberrations in mitosis as a result of the irradiation (Murugan *et al*., 2015). In this regard, Ramesh *et al.,* (2013) reported in Mulberry genotypes a reduction of survival by increasing the dosage of gamma radiation.

Based on the selection criteria, linear as well as quadratic regression models are being adjusted to observed data, however; due to the mean squared error's value and Mallows Cp' value, the quadratic model had to have a higher adjustment (Table 2). According to the quadratic model parameters, LD_{50} for plants survival M1 was calculated at 438.49 Gy. Likewise, from 360 Gy, a significant reduction in plants survival M1 (31.49% with regard to the control) could be determined, whilst in doses of 620 and 1250 Gy, a 100% of plants mortality could be observed (Table 1) since they did not give any unfolded leaves to carry out photosynthesis.

Figure 1B presents the survival behaviour and its simulation throughout the quadratic model. Three series are shown: simulation through the corresponding model that has an initial population of twelve plants (grey-coloured line), observed data whose populations had a starting number different from twelve individuals (green-coloured marker), and those populations whose initial number was of twelve individuals (black-coloured square marker). It's visible that the observed data from which the initial plant number was of twelve individuals (20, 50, 320, and 360 Gy), divert from the simulation line just a little but, at the same time, confirms that the adjusted correlation coefficient value that's indicated in Table 2, it is acceptable.

Plant height

Soybean Huasteca-100 variety plant height was lower dependant on the ^{60}Co gamma radiation dosage increased. These results coincide with Aminah *et al.*, (2015) and Kadhimi *et al.*, (2016) works, they observed a decrease on soybean and rice plant height because of an increase on the gamma radiation dosage. Throughout the linear regression analysis, this dosage shown to be highly significant for plant height (Pr>F=<.0001). The simple linear regression model was the best model expressed by the coefficient of determination (R^2= 71.96%), mean squared error's low value (184.80), and Mallow´s Cp low value (1.17) (Table 3 and Figure 1C). Based on these parameters, GR_{50} for plant height was calculated at 252.73 Gy, likewise, a significant reduction was determined from 200 Gy (33.12%) with regard to the control). But at 440 Gy doses, a 71.89 % decrease could be calculated (Table 1).

Number of seeds per plant

Higher doses of gamma radiation on soybean cause a greater rate of infertility on their pollen (Khan y Tyagi, 2010) which has an effect in seed amount. In the current study, the average values of seed per plant presented a negative trend by increasing the radiation dosage. Such decreasing trends were also reported by Mudibu *et al.*, (2012) and Fe *et al.*, (2000). The quadratic model was the one with a greater adjust to the observed data since it displayed an adjusted coefficient of determination of 81.57%, and a Mallow's Cp lower value (2.4319) (Table 4 and Figure 1D). By the model's parameters, GR_{50} for number of seeds per plant was estimated at 298 Gy. From 320 Gy, a significant decrease in accordance to the control (50.94%) was presented, while the plants that were exposed to a dosage of 400 Gy were extremely significant by an average of 19.75 seeds per plant^{-1} (Table 4).

Even though some plants M1 that were obtained from seeds and treated at 440 Gy survived, they presented flowers with no pods formation. Aminah *et al.*, (2015) exposed similar morphological results in the generation M1 of Anjasmaro soybean variety in doses at 200 and 300 Gy.

Radio-sensitivity regions

Table 5 exhibits the intervals of the radio-biological regions from Huasteca-100, LD_{50} and GR_{50} were obtained from the models of higher adjustment than those from observed data. To sum up, the interval dosage that causes a median minimization or a median fatality for the three determined variables in Huasteca-100, are found between 252 Gy and 438 Gy. Additionally, transition regions are placed between 1 and 440 Gy, being height and number of grains per plant more radio-sensitive than survival (1-240 Gy.) Finally, the inhibition region was found from 240 Gy for height and number of seeds, but survival was at 440 Gy.

Based on GR_{50} value for plant height which, was obtained in this current study, a gamma radiation dosage of 252 Gy ± 5% in Huasteca-100 soybean seeds could be considered suitable for genetic variability inducement with an acceptable seed production per plant (a calculation of 164 seeds per plant^{-1}) and a lower frequency of unwanted effects.

Table.1 Gamma radiation effects on germination, survival, height, and number of seeds per plant from the Huasteca-100 variety

Dose	Germination	Survival	Plant height	Seeds per plant
0	85.00	10.9 (12.7)[1]	96.0	224.0
1	83.33	12.1 (12.5)	95.8	212.9
5	83.33	11.1 (12.5)	86.3	201.1
10	85.00	10.4 (12.7)	74.2	228.0
20	81.67	11.0 (12.2)	68.8	194.6
50	80.00	10.1 (12.0)	73.2	202.4
100	81.67	10.7 (12.2)	74.4	234.7
150	76.67	11.6 (11.5)	73.7	187.2
200	75.00	11.3 (11.2)	64.2*	227.0
240	85.00	11.2 (12.7)	51.0***	180.9
320	73.33	8.4 (11.0)	31.2***	109.9*
360	78.33	7.5* (11.7)	34.1***	65.3***
400	68.33	3.9*** (10.2)	29.3***	19.7***
440	80.00	3.1*** (12.0)	27.0***	0.00***
620	75.00	0.0*** (11.2)	-	-
1250	86.67	0.0*** (13.0)	-	-

Table.2 As a result of ^{60}Co gamma radiation dosage, linear regression models were utilized for carrying out LD50 determination in Huasteca-100 soybean plants survival phase R1

Model	R^2	R Adjusted	Parameters	MSE	Mallows´s CP	DL_{50}
Multiple linear	75.67	74.80	β_0=3.53052 β_1=-0.01513 Pi=0.71697	3.97	3.000	405.43
Quadratic	83.01	82.41	β_0=1.41889 β_1=-2.738X10^{-5} Pi=0.82047	2.77	3.000	438.49

R^2 and R^2 adjusted, expressed by %; MSE= Mean squared error; DL_{50}, expressed by Gy.

Table.3 As a result of ^{60}Co gamma radiation dosage, linear regression models were applied for GR50 determination in Huasteca-100 soybean plant height phase R8

Model	R^2	R^2 Adjusted	Parameters	MSE	Mallows´s CP	GR_{50}
Simple linear	71.96	71.41	β_0=85.31146 β_1=-0.13972	184.80	1.1726	252.73
Quadratic	68.23	67.61	β_0=78.96462 β_1=-3.3012x10^{-4}	209.36	7.8405	296.20

R^2 and R^2 adjusted, expressed by %; MSE= Mean squared error; GR_{50}, expressed by Gy.

Table.4 As a result of gamma radiation dosage, linear regression models were used for GR50 determination on number of seeds per plant in Huasteca-100

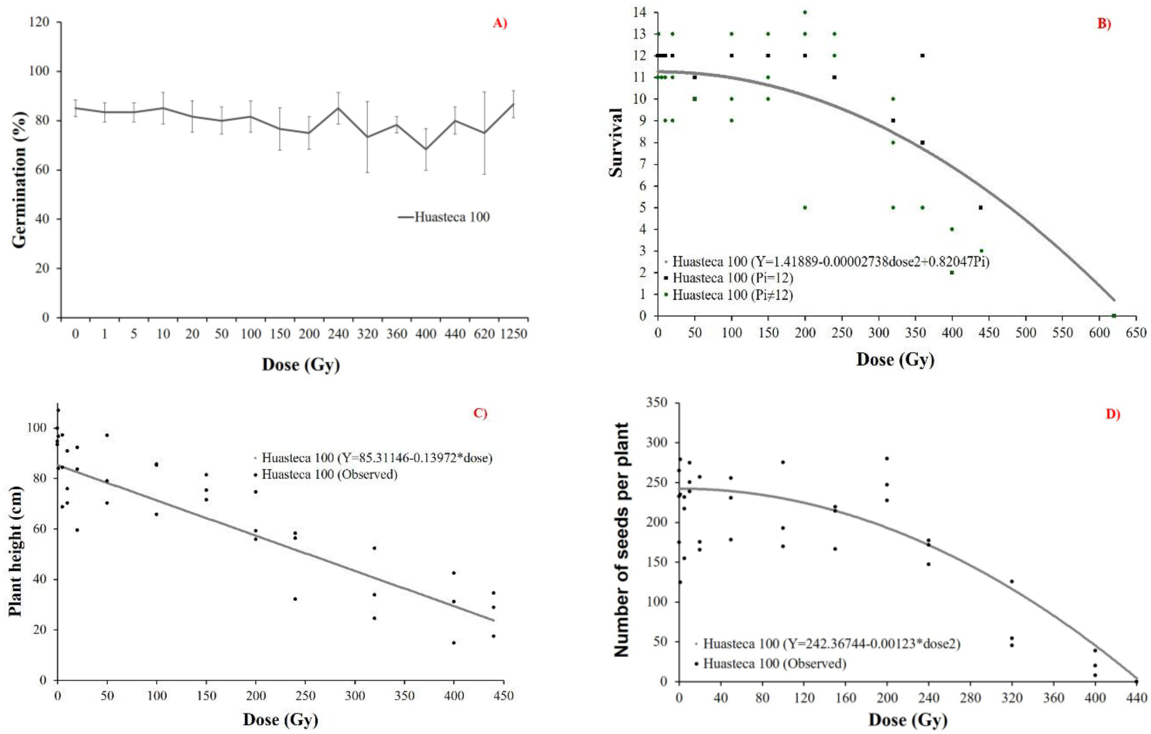
Model	R^2	R^2 Adjusted	Parameters	MSE	Mallows´s CP	GR_{50}
Simple linear	72.06	70.89	β_0=260.39178 β_1=-0.48722	2403.90	16.5799	262.63
Quadratic	82.31	81.57	β_0=242.36744 β_1=-0.00123	1522.35	2.4319	298.96

R^2 and R^2 adjusted, expressed by %; MSE= Mean squared error; GR_{50}, expressed by Gy.

Table.5 Intervals of radio-biological regions from Huasteca-100 variety were obtained as a result of LD50 and GR50 (Gy) gamma radiation

Variable	DL_{50}	GR_{50}	Radio-biological regions		
			E stimulation	Transition	Inhibiticion
Survival	438	-	-	1-440	440-650
Plant height	-	252	-	1-240	240-440
Seeds per plant	-	298	-	1-240	240-440

Fig.1 Effect of ^{60}Co gamma radiation within the variables from studying Huasteca-100 variety and its stimulation by regression models of higher adjustment: A) Germination, B) Survival, C) Plant height, and D) Number of seeds per plant



Conclusion

An increase in ^{60}Co gamma radiation dosage caused a decrease on survival, height, and number of seeds per plant in Huasteca-100 soybean. Even though a dosage of 1250 Gy was used, germination did not show any significant reduction. LD_{50} for survival was calculated at 438 Gy, but GR_{50} for height and number of seeds per plant was calculated at 252 Gy and 298 Gy, respectively. Based on the foregoing, a dosage of gamma radiation at 252 Gy ± 5% can be used for this variety and be seen as appropriate for a big scale genetic variability inducement in Huasteca-100 soybean variety.

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