Colegio de Postgraduados

LD_{50} and GR_{50} estimation with gamma rays (^{60}Co) in *Arachis pintoi* Var. amarillo

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ABSTRACT

Objective: To estimate the median lethal dose (LD_{50}) and mean reductive dose (GR_{50}) due to gamma radiation in *Arachis pintoi* var. Amarillo seeds.

Design/methodology/approach: Ten doses were used (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy) plus a control (without radiation). The experimental design was completely random with three replications and 50 seeds per repetition. Seed germination was evaluated 29 days after sowing (das) and plant survival, plant height, root length and leaf area at 60 das. The LD_{50} and GR_{50} for survival and plants height were estimated by linear regression.

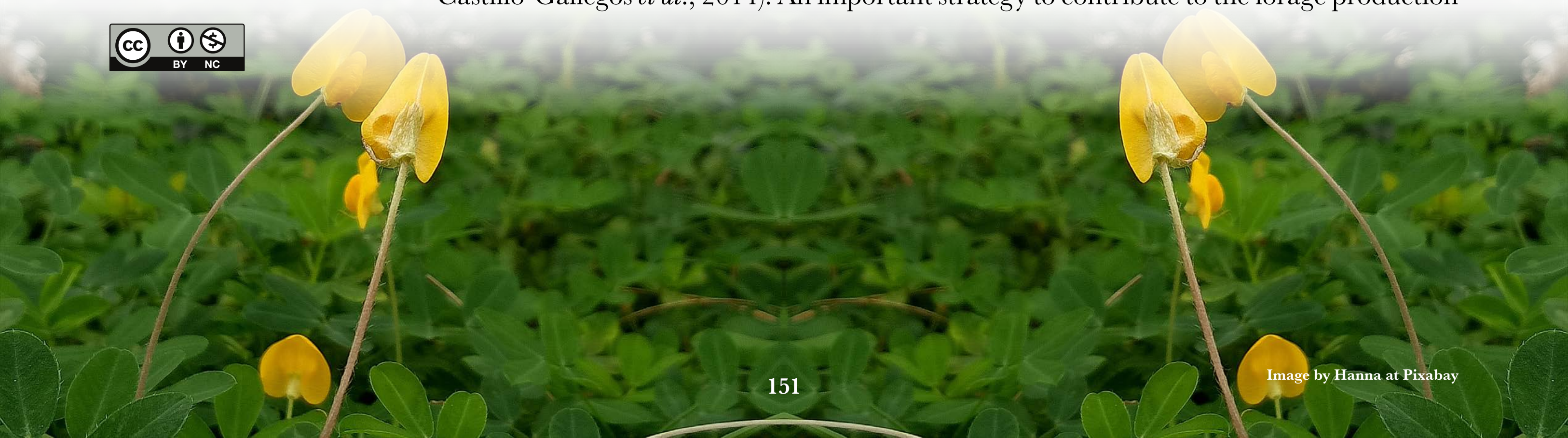
Results: There was a significant reduction of seed germination and plant survival from 300 and 200 Gy doses compared to the control (61.64 and 49.15% each); for the plants height the dose was of 100 Gy (35.22%). There were no differences in the root length and leaf area with 100 and 200 Gy regard to the control. The LD_{50} was estimated at 212.54 Gy and the GR_{50} at 162.16 Gy.

Findings/conclusions: The gamma radiation doses to induce genetic variation in *A. pintoi* var. Amarillo seeds were between 162 and 212 Gy.

Keywords: Arachis pintoi, LD_{50}, GR_{50}, gamma radiation.

INTRODUCTION

Forage production for cattle feeding in the Mexican tropics is mainly carried out in medium to low fertility soils, during seasonal rainfall and four to six months dry season (Ramos and Peralta, 1988). To maintain good forage quality production throughout the year, various legume species are used, including Arachis pintoi with an average protein content of 15.1%, and whose forage production is 6.44 t ha^{-1} $year^{-1}$, (Cab *et al.*, 2008; Castillo-Gallegos *et al.*, 2014). An important strategy to contribute to the forage production



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with great persistence in the dry season is the development of genetic variation to obtain new *A. pintoi* genotypes. With this purpose, different methods including induced mutagenesis have been used (Suprassana *et al.*, 2015).

Before starting an assisted improvement program by induced mutagenesis, it is necessary to know the median lethal dose or the mean reductive dose, which relates to the effective dose to produce genetic variation (Kodym *et al.*, 2012). The results on the determination of the median lethal doses (LD_{50}) and mean reductive doses (GR_{50}) indicates that it is species, variety, and plant tissue specific. Plant genotypes with less genetic redundancy show higher sensitivity to radiation compared with those of greater genetic redundancy (Ukay, 1981).

A. pintoi varieties are generated by selection and hybridization methods. However, other improvement methods such as induced mutagenesis have not been explored. This method has allowed the development of mutant variety lines in cereals, flowering plants and legumes (FAO/IAEA, 2016) in short time, compared to conventional methods. Induced mutagenesis is carried out with chemical and physical agents. Still, gamma radiations (physical mutagenic agent) are often used because of several advantages such as the easiness to handle materials after radiation exposure, their availability, reproducibility, uniformity, and ability to penetrate tissues (Mba and Shu, 2012).

Therefore, the objective of this study was to estimate the LD_{50} and GR_{50} of gamma rays (^{60}Co) in *A. pintoi* seeds var. Amarillo and its effect on seed germination, plant survival, plant height, root length and leaf area.

MATERIALS AND METHODS

The irradiation of *A. pintoi* var. Amarillo seeds was carried out at the Moscafrut irradiation plant, SADER, located at Metapa de Domínguez, Chiapas, Mexico. An MDS Nordion Gamma Beam 127 panoramic equipment was used, with a 50 g ^{60}Co dry storage source and 0.029 Gy s^{-1} ratio dose. Seeds were exposed to gamma radiation doses of 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy, using 150 seeds per dose with 7.8% humidity.

The seed germination, plant survival, plant height, root length and foliar area evaluation of *A. pintoi* var. Amarillo was done in greenhouse conditions at the Rosario Izapa Experimental Field located at Tuxtla Chico, Chiapas (14° 40' 16.1" N, 92° 42' 59.1" W, and 435 m altitude). The day after the seed's irradiation, seeding was carried out using 200 cavities (1×1×4 cm) germination trays, with peat moss® as substrate. The experimental design was completely random with three repetitions; each consisted of 50 cavities where a single *A. pintoi* seed was placed.

Total seed germination percentage was evaluated 29 days after sowing (das), while the plant survival percentage, plant height, root length and leaf area were evaluated at 60 das; the last of them recorded with a leaf area integrator LI-COR, LI 3100 (three measurements per plant). The variable's record of seed germination and plant survival were done relative to the number of existing plants by repetition; the plant survival percentage was determined with the equation: (number of live plants per repetition/number of plants at 29 das) X 100.

Plant height, root length, and leaf area were assessed on five plants per repetition, on a total of 15 plants per treatment.

The data were analyzed by ANOVA, and the means comparison to the control was tested using the Dunnett test at 95% confidence interval. For plant height, root length and leaf area, only doses of 100 and 200 Gy were analyzed, given the fact that other treatments had less than five plants by repetition. The LD_{50} and GR_{50} for plant survival and plant height were estimated using parameters of simple linear regression models, for both analyzes data normality assumptions were checked in each statistical model. All statistical analyses were performed with the SAS statistical software version 9.0 (SAS Institute, 2002).

RESULTS AND DISCUSSION

Seed germination

Gamma radiation influenced seed germination of *A. pintoi* var. Amarillo ($P<0.01$). Doses of 100 and 200 Gy did not affect seed germination compared to the control treatment ($P>0.05$). The gradual doses increase, in the transition from 200 to 300 Gy, showed the greatest decrease in germination, close to 50%; the 300 to 1000 Gy range showed germination between 16 and 21.33%. These radiation doses provoked reductions from 56.16 to 64.38%, compared to the control (Table 1). High radiation doses affected seed germination in rice (*Oryza sativa* L.) varieties (Pavan-Kumar *et al.*, 2013), *Vigna unguiculata* L. Walp Nakare and Shindimda varieties (Horn and ShimelIs, 2013) and *Arachis hypogaea* L. Narayani variety (Aparna *et al.*, 2013). While doses between 5-30 Gy promoted significant increase rate and seed germination percentage in TSh variety corn seeds (Marcu *et al.*, 2014). However, there are also reports that gamma radiation does not significantly affect total germination of soybean Huasteca 100 variety in doses from 100 up to 1250 Gy (Gálvez-Marroquín *et al.*, 2017). Olasupo *et al.* (2016) also argue that the different sensitivity responses to gamma radiation in *Vigna unguiculata* accessions are due to genetic background.

Lokesha *et al.* (1992) reported that the inhibition of seed germination due to radiation effects is attributed to numerous histological and cytological changes, disruption and the tunic's disorganization and cellular division cease at meristematic tissues during germination.

Plant survival

The *A. pintoi* var. Amarillo plants survival was negatively affected by gamma radiation ($P<0.01$). At the 100 Gy dose plant survival was similar to that of the control treatment (92.78 and 98.33%), while at doses from 200 to 400 Gy there was a survival reduction of up to 50%; 100% plants mortality was observed at doses higher than 400 Gy (Table 1). High gamma radiation doses have affected *Laelia autumnalis* protocorms survival (Hernández-Muñoz *et al.*, 2017) and rice (*Oryza sativa* L.) var ADT (R) 47 (Rajarajan *et al.*, 2016). The plant's mortality can be attributed to abnormalities in chromosomes with an increment in the radiation dose. Murugan *et al.* (2015) report an increase, dependent of radiation dose, in mitotic aberrations of *Catharanthus roseus*, they also observed a dose-dependent reduction in seed germination, plant survival, and plant height. Another factor that contributes to the survival decrease is the gamma photons interaction, particularly with water, which produces free radicals and in turn significant damage to vegetable cells (Kovacs and Keresztes, 2002).

Furthermore, in non-lethal cases, cells have a repair DNA mechanism that can fix different types of damage to a certain accuracy degree (Dexheimer, 2013). Based on this, it will give rise to mutations present more frequently in the second generation, since these are from recessive characters (Foster and Shu, 2012). The main mutations that are present in the second generation are simple base substitutions and insertions/deficiencies, as reported on six M2 rice plants (*Oryza sativa* L.) var. Nipponbare (Li *et al.*, 2016).

Plant height

Gamma radiation significantly affected plant height of *A. pintoi* var. Amarillo ($P<0.05$). The control treatment showed the highest average plant height with 5.28 cm, while the 100 and 200 Gy doses show average values of 3.42 and 2.15 cm each; these correspond to reductions of 35.22 and 59.34% (Table 1). These results concur with those obtained by Taheri *et al.* (2016); they determined a decrease in plant height of *Curcuma alismatifolia* var. Chiang Mai Red, Doi Tung 554, Sweet Pink and Kimono Pink using gamma radiation doses from 14.6 to 87.4 Gy. Hanafiah *et al.* (2016) found a bidirectional response in plant height at the M1 generation of *Hibiscus sabdariffa* L. var. Roselindo 2 with the increase of gamma radiation doses (from 150 to 600 Gy). They did not observe significant effects per dose. However, the maximum value was at doses of 450 Gy. The reduction in plant height has been related to auxins destruction by radiation (Momiyama *et al.*, 1999). Although, Ali *et al.* (2016) indicate that gamma rays decrease the growth rate through mutations in the DNA that synthesizes DNA

Table 1. Percentage of seed germination and plant survival, plant height, root length and leaf area of *Arachis pintoi* var. Amarillo in function of ^{60}Co gamma radiation dose.

Dose	Germination (%)	Plant survival (%)	Plant height (cm)	Root length (cm)	Leaf area (cm^2)
Control	48.67	98.33	5.28	10.16	28.75
100	44.00	92.79	3.42**	9.51	33.48
200	36.64	50.00**	2.15**	8.79	21.00
300	18.67**	4.44**	-	-	-
400	18.00**	2.56**	-	-	-
500	21.33**	0.00**	-	-	-
600	19.33**	0.00**	-	-	-
700	16.00**	0.00**	-	-	-
800	18.67**	0.00**	-	-	-
900	17.33**	0.00**	-	-	-
1000	17.33**	0.00**	-	-	-

** Significant difference respect to control with 95 % confidence interval.

at the interface, which provoke the interruption of the plant bud and the consequent interruption of cell differentiation.

Root length

Gamma radiation caused no effects on *A. pintoi* var. Amarillo root length ($P>0.05$). Plants in control treatment showed an average root length of 10.16 cm, while in the 100 and 200 Gy doses it was 9.58 and 8.78 cm, each (Table 1). However, in 100 and 200 Gy doses it showed a reduction of 5.7 and 13.5% compared to the control. Verma *et al.* (2017) found a reduction of root length in *Foeniculum vulgare* Mill. in doses of 150 Gy up to 250 Gy, with the lowest value at the maximum dose reduction of 77.21% compared to control. This relates with a reduction in mitotic activity at the roots tips, as reported for *Vigna unguiculata* L. varieties Kaha 1, Azmerly, Cream 7 and Giza 6 at 200 and 300 Gy (Badr *et al.*, 2014).

Leaf area

Gamma radiation did not affect *A. pintoi* var. Amarillo leaf area ($P>0.05$); however, 100 Gy dose increased 16.4% leaf area related to control; while with 200 Gy, the lowest leaf area average value was obtained (20.99 cm^2), which was 26.9% less than the control (Table 1). Ramesh *et al.* (2013) reported that the mulberry genotype (Morus) Kosen had a 9% leaf area increase with a dose of 100 Gy, compared to control.

LD_{50} and GR_{50} estimation

The parameters to determine an optimal dose to induce genetic variation in a genotype of interest are the median lethal and mean reductive doses. In *Arachis hypogaea*, the most economically important species from the *Arachis* genus the median lethal and mean reductive doses to induce genetic variation with gamma radiation are established, as well the particular mix of gamma radiation with EMS for each genotype. In reports from *A. hypogaea* L. var. VRI-2 the estimated LD_{50} was of 500 Gy + 0.5% of EMS (Gunasekaran and Pavadai, 2015); and the GR_{50} for local peanuts (*Arachis hypogea*) type Virginia with 12% humidity required doses of 700 Gy (Brito-Damián and Ángeles-Espino, 2016). In the present study the LD_{50} for *A. pintoi* var. Amarillo plants survival was 212.54 Gy, while the GR_{50} for height plants was 162.16 Gy (Table 2).

Therefore, gamma radiation doses from 162.16 to 212.54 Gy can be useful to induce genetic variation in *Arachis pintoi* var. Amarillo.

Table 2. LD_{50} and GR_{50} for plant survival and plant height of *Arachis pintoi* var. Amarillo of ^{60}Co gamma radiation, estimated by linear regression.

Variable	Pr > F	R^2	Equation	LD_{50}	GR_{50}
Plant survival	<0.0001	0.87	Y=99.06130−0.23083x	212.54	-
Plant height	0.0068	0.67	Y=5.18222−0.01567x	-	162.16

CONCLUSIONS

The increase in ^{60}Co gamma radiation dose provokes a tendency in germination decrease, plant survival, plant height, root length and leaf area of *Arachis pintoi* var. Amarillo plants. Radiation doses of 100 and 200 Gy have no significant influence in these variables, except for plant survival and plant height. ^{60}Co gamma radiation doses from 162.16 to 212.54 Gy can be useful to induce genetic variation in *Arachis pintoi* var. Amarillo.

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