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Original

Medium Lethal Dose of Gamma Radiation to Induce Mutations in Caribgrass (*Eriochloa polystachya* Kunth)

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ABSTRACT

Background: Gamma radiation can be used in plant genetic breeding to produce useful mutations. This research was performed in order to determine the medium lethal dose (LD_{50}) of gamma radiation in Caribgrass (*Eriochloa polystachya* Kunth).
Methods: Overall, 8 600 stolons measuring 8 cm long, bearing a node, were cut from mature plants (over six month old), and were radiated with doses of 0, 25, 50, 75, and 100 Gray of gamma rays Co^{60}. The establishment percentage, plant height, and stolon mortality were evaluated. The data were analyzed by probabilistic linear regression analysis.
Results: According to variable percentage of establishment, LD_{50} was equal to 52.60 Gy, in the genotype studied, with an R^2 of 57.73.
Conclusions: The medium lethal dose to induce mutations of Caribgrass (*Eriochloa polystachya* Kunth) was achieved with 52.60 Gy.

Key words: establishment, plant breeding, graminaceae, *in vitro*, gamma radiation (Source: *AIMS)*

INTRODUCTION

Caribgrass (*Eriochloa polystachya* Kunth) is a graminaceae native to tropical South America, Central America, and the Caribbean. This is a perennial, creeping, stoloniferous species which grows up to 1.20 m high, producing plenty of lanceolate leaves, and has hollow stems. It grows perfectly well in humid or low areas, which are flooded throughout the rainy season, but can hardly withstand dry conditions, and it can recover well after being burned (Bishop *et al.*, 1989).

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The plant's root system is deep, producing abundant rhizomes that originate thick and compact layers of organic matter that generate a firm ground in swamps and marshes. This species produces little fertile seeds with low viability, so its propagation is made from stems and rhizomes (Enríquez, Hernández, Quero, and Martínez, 2015).

MATERIALS AND METHODS

The collection and sowing of grass was made on San Pablo Experimental Farm, from the Technical University of Babahoyo (UTB), located on km 7.5, Babahoyo-Montalvo Road. The UTM geographical coordinates were X, 1.7723946; Y: 79.7102593. The local climate is humid tropical, with mean temperature values of 24-26 °C, relative humidity of 88%, mean annual precipitation 1 262 mm, and 8 meters above sea level. The annual sunlight exposure time is 990 h.

Selected plant material

A total of 8 600 Caribgrass stolons (Eriochloa polystachya Kunth) were collected in the premises of the Faculty of Agricultural Sciences of UTB. They were selected from mature plants with inflorescence, 8 cm long samples with nodes from which the roots emerged, were cut.

Methodology

The Caribgrass plants (Eriochloa polystachya Kunth) from which the material (stolons) was collected, were in adequate condition. The samples were cut with gardening shears previously washed with sterile distilled water (three rinses). Then they were dipped in azoxystrobin + difenoconazole solution, (fungicide) for 15 minutes. A number of 430 stolons were stored in plastic bags with bores. After labeling with with adhesive tape and sorting, they were carried in an ice box until radiation (Ortega, 2010).

Gamma radiation of the Caribgrass stolons with a node each was performed in the 11 500 Curies isotope radiator Co^{60}, 109-68 model, from the National Office of Nuclear Research and Applications, at the Ministry of Electricity and Renewable Energy, in Aloag, Pichincha, Ecuador. Four radiation doses were used: 25, 50, 75, 100 Gray (Gy). The control (0 Gy), was given the same treatment. The duration of phase 1 was 28 h, considering the transportation periods from and to the experimental radiation facility.

Then the cultivation bed was readied with the following materials: 50 kg of rice hulls, 50 kg of sawdust, 50 kg of sand, and 150 kg of loose, fresh soil, which were mixed to produce a homogeneous blend (Acosta-Durán, Gallardo, Kämpf, and Bezerra, 2008).

Overall, 430 stolons were planted per experimental unit (EU), including the control, in a cultivation bed watered until the optimum field moisture was achieved. A completely randomized block design with a factorial arrangement (AXB), including five treatments and four repetitions, was used.

Variables evaluated

Every 7-8 days, the stolons' rooting percentage was evaluated for a period of 30 days per EU. The mortality percentage was recorded (considering mortality as the stolons that did not root). Finally, the plant's length was calculated from the ground to the base of the top leaf, following the frequency and evaluation mentioned.

Statistical processing

An data analysis of variance that comprised rooting, mortality, and plant's length was performed, using InfoStat. The LSD Fisher test (0.05% significance) was applied to determine the statistical significance between the treatment means. Regression analysis with the probit linear model (Statgraphics Centurion XVI) was performed to analyze the ratio between the radiation dose and rooting, mortality, and plant's length. II. (LD_{50}) was determined in the preset doses from 0 to 100 Gy.

RESULTS

The analyses of variance indicated that the radiation dose underwent significant differences ($P \leq 0.01$) as to rooting, mortality, and plant's length.

An analysis of the radiation dose absorbed (radio sensitivity) in rooting (Table 1) showed that this variable differed significantly ($P<0.05$) between the stolons irradiated at 50, 75, and 100 Gy and the control (0 Gy). But it did not differ significantly ($P < 0.01$) between the stolons irradiated with 25 Gy. According to these results, the number of stolons that rooted decreased as the dose was raised.

Concerning mortality, highly significant differences were observed between treatments ($P<0.01$); treatments 0 Gy and 25 Gy showed the lowest mortality, averaging 0.15 and 0.12, respectively. The highest mortality was observed in the 75 Gy and 100 Gy treatments, averaging 0.94 and 0.97, respectively. Therefore, it can be said that lethality rises with the increased dose of radiation.

There was a significant difference between treatments regarding plant's length ($P<0.05$). An analysis of the results from the multiple comparison showed that the effect of the radiation dose absorbed (radiosensitivity) using the 25 and 50 Gy dose treatments differed significantly ($P<0.05$), whereas neither of them differed from the 0 Gy. Moreover, no statistical differences were observed between the 75 and 100 Gy doses. It was also found that plant length received a negative impact as the radiation level increased.

Table 1. Average of rooting, mortality, and plant length.

	Dose Gy				
Indicator	**0**	**25**	**50**	**75**	**100**
Rooting	0.85^{a}	0.88^{a}	0.65^{b}	0.06^{c}	0.03^{c}
Root length (cm)	16.00^{ab}	17.85^{a}	14.19^{b}	7.84^{c}	7.27^{c}
Mortality	0.15^{c}	0.12^{c}	0.35^{b}	0.94^{a}	0.97^{a}

Means with equal letters are not significantly different ($p > 0.05$)

Table 2 shows the results of the analysis of the effect on the evaluation time, four days after the radiation of Caribgrass stolons with gamma rays. Regarding rooting, there was a significant difference ($P<0.05$) between the evaluation times, the greatest rooting percentage was observed on days 16 and 22, whereas this percentage varied on days 8 and 30, from 0.45 to 0.42, respectively.

Significant differences were observed in mortality ($P<0.05$) on the days evaluated, with a higher mortality index at 8 and 30 days, with 0.55 and 0.58, respectively.

Highly significant differences ($P<0.05$) were observed in plant length on the days evaluated, so it was inferred that plant length increased in time after gamma radiation of the stolons.

Table 2. Evaluation days of rooting average, mortality, and plant length

	Days after planting			
Indicator	**8**	**16**	**22**	**30**
Rooting	0.45^{b}	0.55^{a}	0.56^{a}	0.42^{b}
Plant length (cm)	5.1^{a}	9.63^{b}	15.4^{bc}	20.3^{c}
Mortality	0.55^{a}	0.45^{b}	0.44^{b}	0.58^{a}

Means with equal letters are not significantly different ($p > 0.05$)

LD_{50} was determined in the preset doses from 0 to 100 Gy (Table 3). A highly significant negative correlation was achieved between the doses of gamma rays and the variables studied, which indicated that this model was efficient to determine LD_{50}. However, the rooting variable showed the best adjustment according to probit analysis (greater R^2), averaging 52.60 Gy.

Table 3. Probit regression correlation of rooting percentage, plant height, and stolon mortality vs. Radiation dose.

	Establishment (%)	**Plant length (cm)**	**Mortality (%)**
Correlation “r”	- 0. 86**	- 0. 85**	- 0. 73**
Critical value	P = 0.000	P = 0.000	P = 0.000
R^2	57.73	28.90	55. 75
LD_{50} Gy	52.60 Gy	82.33 Gy	48.89 Gy
** Highly significant differences from $P \leq 0.05$			

DISCUSSION

The characters generally evaluated in plant species are germination percentage, plant height, root length, sterility, etc. (Gómez-Pando and Eguiluz-De La Barra, 2013).

This study showed that the lowest mortality was observed in treatments with low radiation doses (0, 25, and 50 Gy). Accordingly, increased mortality is directly proportional to increases in the radiation doses. These results coincide with the reports of Songsri *et al.* (2011) Thole *et al.* (2012) Olasupo, Ilori, Forster and Bado (2016), and Corrales (2017); who exposed an organism to low doses of ionizing radiation. The effects on the cellular structure or DNA were minimal, whereas higher doses caused multiple impacts on the genome, and might cause death. Therefore, the first step to induce effective mutagenesis with ionizing radiations is to determine the optimum radiation dose through radiosensitivity curves. Fuchs *et al.* (2002) and Corrales (2017) said that in this proportion there is a greater frequency of useful mutations for genetic breeding programs.

According to the results, the radiation dose that should be applied to induce variability in Caribgrass stolons directly propagated in the field, are between 42 and 50 Gy. These data coincide with Fuchs *et al*. (2002) who said that doses between 40 and 50 Gy increase the likelihood of inducing favorable mutations for selection and genetic breeding of grass.

In this study, a LD_{50} was determined, using an average of 52.60 Gy. To achieve that, a probit analysis was performed to variable establishment, the one with the best adjustment (greater R^2). These data coincide with Valarezo (2015), who determined a LD_{50} in the 55-60 Gy range, in a gamma radiation study of apical meristems in banana (*Musa spp*), cultivar “Williams”. Similar results were reported by Reyes-Borja (2007), who exposed varieties of banana (*Musa* spp) to gamma radiation, with Co^{60}, and found LD_{50} of 77.9 Gy for dwarf Cavendish, 83.9 Gy for Williams, 65 Gy for Orito, and 77.7 Gy for FHIA-01. Moreover, the IAEA reports LD_{50} a 90 Gy dose for Tifway II, and 70 Gy for Tifgreen II. Additionally, Gonzales (2007), applied 10-50 Gy doses to apical shoots of micropropagated henequen, and reported a medium lethal dose of 30 Gy to generate new axillary shoots, and 20 Gy to diminish the fresh weight of callouses.

In species propagated by seeds, the doses of gamma radiation that produce the LD_{50} are different (Gómez-Pando and Eguiluz-De La Barra, 2013), in quinoa seeds (*Chenopodium quinoa* Willd.), with the best results found using the LD_{50} dose of 150 Gy. It differs from the current study bacause the LD_{50} is placed between 42 and 50 Gy.

Furthermore, plant length varied depending on the radiation level, with significant numerical differences observed as the dose was raised. This result coincides with the findings of Ángeles-Espino (2013), who determined that the dose of radiation absorbed had a direct effect on the development of plantlets and callouses, in vitroplantlets of *Agave tequilana var*. Azul, since both variables underwent high reduction indexes with doses over 30 Gy. The LD_{50} was between 20 and 25 Gy for induction of adventitious shoots and plantlet height, and 15-25 Gy for callouses.

Therefore, the radiation doses to induce variability in *in vitro* propagated agave plantlets must be between 15 and 25 Gy to favor the occurrence of favorable mutations.

Similar results were found by Valdez *et al.* (2004), who pointed out that growth of sugar cane (*Saccharum officinarum* L.) calluses is affected by an increase in the radiation dose, whereas the greatest growth of calluses was observed with the 10 Gy dose. However, it was significantly lower than the non-irradiated stems, and growth was reduced while the radiation dose was raised to 30 Gy. Just as in the Caribgrass used in this study, the establishment trend decreased as the radiation dose was raised to 75 Gy; a 100 Gy dose produced 8% rooting.

Plant length at different evaluation dates (8-30 days), underwent significant differences; hence it can be assumed that it rose as time elapsed. This coincides with the findings of Otahola-Gomez and Aray (2001), who said that radiation of chrysanthemum explants (*Dendrathema grandiflorum* Ramat) with 5-20 Gy doses produced no significant differences in growth in the 5-15 Gy doses compared to the control, when growth was evaluated at 7, 14, 21, and 28 days after. Meanwhile, the 20 Gy dose caused a reduction in growth on all the evaluation days. These results coincide with the reports of Ángeles-Espino (2013), in which doses higher than 20 Gy led to decreased growth, between 43-71%, whereas the 10 Gy did not differ from the non-irradiated treatment.

When determining the best dose of gamma radiation to seeds of pinkgrass, Corrales (2017) suggested 304 Gy as the optimum dose to induce effective mutations, which differs from the LD_{50} in most articles reviewed on plant materials.

CONCLUSIONS

The radiation dose absorbed had a direct effect on the establishment of stolons and plant height, since both variables underwent high reduction indexes with doses over 75 Gy. The LD_{50} was between 42 and 52 Gy.

REFERENCES

Acosta-Durán, C. M., Gallardo, C. S., Kämpf, A. N., & Bezerra, F. C. (2008). Materiales regionales utilizados en Latinoamérica para la preparación de sustratos. *Investigación Agropecuaria*, *5*(2), 93-106. https://www.academia.edu/3244556/MATERIALES_REGIONALES_UTILIZADOS_EN_LATINOAM%C3%89RICA_PARA_LA_PREPARACI%C3%93N_DE_SUSTRATOS

Ángeles-Espino, A., Valencia-Botín, A. J., Virgen-Calleros, G., Ramírez-Serrano, C., & Paredes-Gutiérrez, L. (2013). Determinación de la dosis letal (DL50) con Co60 en vitroplántulas de Agave tequilana var. Azul. *Revista fitotecnia mexicana*, *36*(4), 381-386. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-73802013000400003

Avendaño-Arrazate, C. H., Cadena-Iñiguez, J., Arévalo-Galarza, M. L., Cisneros-Solano, V. M., Aguirre-Medina, J. F., Moreno-Pérez, E. D. C., ... & Ramírez-Vallejo, P. (2012). Variación genética en el complejo infraespecífico de chayote evaluada mediante sistemas isoenzimáticos. *Pesquisa Agropecuária Brasileira*, *47*(2), 244-252. http://dx.doi.org/10.1590/S0100-204X2012000200013

Bishop, J., Betancourt, R., Carrión, F., Reyes, F., Zambrano, J., Ronquillo Solórzano, S., ... & Berrezueta, L. (1989). Manual de pastos tropicales. Quito, Ecuador: Programa de Pastos y Ganadería. https://scholar.google.com/scholar?cluster=4639553725260272755&hl=es&assdt=0,5

Corrales Lerma, Raúl. (2017) Mutagénesis con radiación gamma para mejoramiento genético de pasto rosado (melinis repens (willd) zizka). Mexico: Doctorado tesis, Universidad Autónomade Chihuahua. http://repositorio.uach.mx/162/

Enríquez, Q.J.F.A. Hernández, G., Quero, C.Y.D., & Martínez, M. (2015) Producción y Manejo de Gramíneas Tropicales para Pastoreo en Zonas Inundables. Folleto Técnico, Mexico: INIFAP -Colegio de Postgraduados, 60 p. https://docplayer.es/66580748-Produccion-y-manejo-de-gramineas-tropicales-para-pastoreo-en-zonas-inundables.html

Fuchs, M., González, V., Castroni, S., Díaz, E., & Castro, L. (2002). Efecto de la radiación Gamma sobre la diferenciación de plantas de caña de azúcar a partir de callos. *Agron. Trop*, *52*(3), 311-323. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0002-192X2002000300004

Gómez-Pando, L. R., & Eguiluz-de la Barra, A. (2013). Developing genetic variability of quinoa (Chenopodium quinoa Willd.) with gamma radiation for use in breeding programs. American Journal of Plant Sciences 4(02):349-355. DOI: 10.4236/ajps.2013.42046

Gonzales, G. (2007). Radiosensitivity to gamma rays (60Co) in shoot tips of henequen. Biotecnología Vegetal, 7(2), 115-117. https://biblat.unam.mx/hevila/Biotecnologiavegetal/2007/vol7/no2/7.pdf

Krishna, G., Shivashankar, G., & Nath, J. (1984). Mutagenic response of rhodes grass (Chloris gayana Kunth.) to gamma rays. *Environmental and experimental botany*, *24*(2), 197-205. https://link.springer.com/article/10.1007/BF00021152

Nobel, P. S. (1999). *Physicochemical & environmental plant physiology*. Academic press. https://www.amazon.es/Physicochemical-Environmental-Plant-Physiology-Nobel/dp/0-123741432

Olasupo, F. O., Ilori, C. O., Forster, B. P., & Bado, S. (2016). Mutagenic effects of gamma radiation on eight accessions of Cowpea (Vigna unguiculata [L.] Walp.). *American Journal of Plant Sciences*, *7*(2), 339-351. DOI: 10.4236/ajps.2016.72034

Ortega Perez, N.V. (2010) « Obtención de Multimeristemos y Callos de diferentes variedades de Banano y Plátano (Musa spp.) a partir de 'Meristemos Apicales'y'Scalps'. http://www.dspace.espol.edu.ec/handle/123456789/10918

Otahola-Gomez, V., & Aray, M. (2001). Inducción de mutantes para el color de la flor en crisantemos (Dendranthema grandiflora (Ram.) Tzvelev) mediante radiaciones gamma. Revista UDO Agrícola, 1(1), 56-63. https://tspace.library.u-toronto.ca/bitstream/1807/5215/1/cg01009.pdf

Reyes-Borja, W. O. (2007). *Gamma rays and carbon ion-beams irradiation for mutation induction to breed banana (Musa spp.), especially on response to black Sigatoka disease* (Doctoral dissertation, 筑波大学). https://www.researchgate.net/publication/50983844_-Gamma_rays_and_carbon_ionbeams_irradiation_for_mutation_induction_to_breed_banana_Musa_spp_especially_on_response_to_Black_Sigatoka_Disease

Rosenberg, S. M., Shee, C., Frisch, R. L., & Hastings, P. J. (2012). Stress-induced mutation via DNA breaks in Escherichia coli: a molecular mechanism with implications for evolution and medicine. *Bioessays*, *34*(10), 885-892. https://www.ncbi.nlm.nih.gov/pubmed/22911060

Songsri, P., Suriharn, B., Sanitchon, J., Srisawangwong, S., & Kesmala, T. (2011). Effects of gamma radiation on germination and growth characteristics of physic nut (Jatropha curcas L.). *Journal of Biological Sciences*, *11*(3), 268-274. https://scialert.net/abstract/?doi=jbs.2011.268.274

Thole, V., Peraldi, A., Worland, B., Nicholson, P., Doonan, J. H., & Vain, P. (2012). T-DNA mutagenesis in Brachypodium distachyon. *Journal of experimental botany*, *63*(2), 567-576. https://doi.org/10.1093/jxb/err333

Valarezo Pacheco, A. V. (2015). *Detección temprana de mutantes de banano tolerantes o resistentes a Sigatoka negra (Mycosphaerella fijiensis, Morelet) en condiciones de vivero* (Bachelor's thesis, Babahoyo: UTB. 2015). http://dspace.utb.edu.ec/handle/49000/1073

Valdez, A., Pedro, A., Pérez, O., Veitía, N., & Torres, D. (2004). Crecimiento, regeneración y radiosensibilidad de callos de caña de azúcar (Saccharum spp. híbrido var. "SP 70-1284") tratados con radiación gamma fuente 60Co. Biotecnologia Vegetal, 4, 165-169. https://revista.ibp.co.cu/index.php/BV/article/view/419

AUTHOR CONTRIBUTION

Conception and design of research: JCGV, LAT, LGP WRB; data analysis and interpretation: JCGV, LAT, WRB, JRA; redaction of the manuscript: JCGV, LAT, LGP, WRB, JRA, LAV.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.