Colegio de Postgraduados

Genetic Improvement of Miahuateco Chili (*Capsicum annuum* L.) (Solanaceae) through Gamma Radiation of ^{60}Co

Bravo-Delgado, Humberto R.[1]; **Báez-Rodríguez, Iván**[1]; **López-Sánchez, Isidro**[1]; **Morales-Ruiz, Alejando**[1*]

[1] Universidad Tecnológica de Tehuacán. San Pablo Tepetzingo, Tehuacán, Puebla, México. C.P. 75859.
* Correspondence: Alejandro.morales@uttehuacan.edu.mx

ABSTRACT
Objective: To improve genetic attributes in Miahuateco chili (*Capsicum annuum* L.) through gamma irradiation with ^{60}Co.
Design/Methodology/Approach: Seeds were irradiated at doses of 0, 100, 200, and 300 Gy at the National Institute of Nuclear Research. The evaluated variables were LD_{50}, agronomic yield, plant height, polar and equatorial diameters, percentage of aborted flowers, number of branches, and plant color, which were assessed under a completely randomized design (4×4)=16 experimental units.
Results: The results indicate that the application of 200 Gy induces higher agronomic yield. The LD_{50} is reached at 145 Gy. High doses of gamma irradiation caused a high percentage of aborted flowers, as well as chlorosis in the plant.
Study Limitations/Implications: The seeds used in this project were landraces, which exhibited significant heterogeneity that could have affected flowering and fruiting. However, this was not a substantial factor for this project.
Findings/Conclusions: It is concluded that 200 Gy of gamma radiation from ^{60}Co induces genetic variability in the species, improving certain agronomic attributes of interest.

Keywords: LD_{50}, agronomic yield, flower abortion, color.

Citation: Bravo-Delgado, H. R., Báez-Rodríguez, I., López-Sánchez, I., & Morales-Ruiz, A. (2024). Genetic Improvement of Miahuateco Chili (*Capsicum annuum* L.) (Solanaceae) through Gamma Radiation of ^{60}Co. *Agro Productividad*. https://doi.org/10.32854/agrop.v17i11.2938

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: June 18, 2024.
Accepted: October 26, 2024.
Published on-line: December XX, 2024.

Agro Productividad, 17(11). November. 2024. pp: 203-209.

INTRODUCTION

Mexico, according to Vavilov, is the center of origin for many cultivated plants, among which chili (*Capsicum annuum* L.) can be cited (Toledo *et al.*, 2011). This plant is a vegetable whose fruit is consumed, and after the red tomato, it is the most cultivated in Mexico (Acosta and Chávez, 2003). Moreover, it has been known since pre-Columbian times and was cultivated by ancient pre-Hispanic cultures in Mesoamerica (Castellón *et al.*, 2012). There are multiple varieties of this fruit, varying in shape and color (yellow, green, red, and brown), as well as in their pungency and flavors (Aguirre and Muñoz, 2015). Some are endemic to certain areas in the central region of the country because they are adapted to the ecological characteristics of the places where they are cultivated, such as habanero chili, which is adapted to the soil conditions, precipitation,

and temperature specific to the Yucatán Peninsula (Ruíz *et al.*, 2011). Miahuateco chili is an example of the above, as it is endemic to the Santiago Miahuatlán area in Puebla. The species is highly valued by the local inhabitants, as it is used in the preparation of traditional mole Poblano, typical of the region (Pérez *et al.*, 2017). Currently, this species has been losing its typical fruit characteristics, such as shape, color, and flavor, due to genetic erosion, which threatens the loss of agricultural plant species due to cross-hybridization with other chiles, such as Poblano (Secretariat of the Convention on Biological Diversity, 2008). A morphological characteristic of the fruit that has been observed in recent years in Miahuateco chili is the presence of a "pedicel cavity," commonly referred to as "Cajete" by the local producers, which is typical of Poblano chili. This demonstrates the hybridization between the two species. This characteristic causes water accumulation at the base of the fruit, leading to rot, and as the pathogen translocates, it also damages the root due to the interaction between water and the pathogens *Fusarium* and *Pythium*, which reduce agronomic yield and quality (Pérez *et al.*, 2017; Velásquez *et al.*, 2001). Gamma rays from certain radioactive elements, such as ^{60}Co, have proven to be an important tool in genetic improvement for both vegetable species and staple and ornamental crops like Polianthes. Additionally, it is free from the restrictions and regulations imposed on genetically modified organisms (Álvarez *et al.*, 2017; Estrada *et al.*, 2011), as it induces somatic mutations through irradiation of the DNA molecule, resulting in molecular mutations that can be observed in the short term directly in the phenotype (Caro *et al.*, 2012), saving time compared to other techniques such as crossing. This genetic improvement technique has been termed radiation-induced mutagenesis. Therefore, the main objective of the present research was to improve certain agronomic attributes in the fruit of Miahuateco chili through four levels of gamma irradiation with ^{60}Co. The proposed hypothesis was that the application of 300 Gy of gamma irradiation from ^{60}Co would induce morphological changes in the V1 generation of Miahuateco chili fruits.

MATERIALS AND METHODS

Location of the Study Area

The research was conducted under greenhouse conditions at the Technological University of Tehuacán, located at 18° 24' 51" north latitude, 97° 20' 00" west longitude, and at an altitude of 1,409 meters.

Germplasm, Seed Irradiation, and Planting

The germplasm consisted of open-pollinated Miahuateco chili seeds, derived from a stratified mass selection, which were irradiated at the National Institute of Nuclear Research (ININ, its acronym in Spanish) using the Transelektro LGI-01. The irradiated seeds were sown in polystyrene trays with 200 cavities, using peat moss as the substrate. When the plants reached a height of 12 cm, they were transplanted into a Mini greenhouse under a topological arrangement of (0.80×0.30 m), resulting in a population density of 4.16 plants per m^2.

Fertilization and Crop Management

The fertilization used was 150-100-200 of (NPK), using urea (46% N), single superphosphate (21% P_2O_5), and potassium chloride (60% K_2O) as sources of these nutrients, which were applied 14 days after transplanting. Weed management was done manually. Copper oxychloride was applied at a rate of 1 g L^{-1} as protection against fungal diseases. To control *Bemisia tabaci* (HEMIPTERA), imidacloprid was applied at 0.3 L ha^{-1}.

Treatments and Experimental Design

The treatments consisted of gamma irradiation doses of ^{60}Co: 0, 100, 200, and 300 Gy, with four replications (4×4)=16 experimental units, which were evaluated under a completely randomized design using the mathematical model

$$Y_{ij} = \mu + \mathrm{T}_i + \varepsilon_{ij}$$

where: Y_{ij} is the response variable of the *i*-th gamma irradiation level of ^{60}Co in the *j*-th repetition; μ is the true general mean; T_i is the effect of the *i*-th gamma irradiation dose of ^{60}Co, and ε_{ij} is the experimental error of the *i*-th dose of irradiation in the *j*-th repetition (Cochran and Cox, 2005).

Response Variables

Median lethal dose (LD_{50}) was determined based on survival as a function of irradiation, using a quadratic regression to interpolate the dependent variable at 50% survival. The result was the LD_{50}. Agronomic yield was determined by harvesting the total yield, weighing the total fresh fruits per plant using an analytical balance model USS-DBS-3 (Barrios *et al.*, 2014). Plant height was measured from the base of the epicotyl to the apical bud of the last branch using a measuring tape model 30088. Polar and equatorial diameters were determined using a digital caliper model 500-195-30. For the polar diameter, measurements were taken from the base of the fruit to the apex, and for the equatorial diameter, the measurement was taken at the center of each fruit. Pedicel length was measured from the base of the fruit to the point where the pedicel connects to the plant branch. The number of branches was calculated by counting the total number of primary and secondary branches per plant. Aborted flowers were counted as the total number of pollinated flowers that were aborted per plant. SPAD units were measured by taking five readings on the leaf petiole using a SPAD-502 chlorophyll meter. The fresh fruit color was measured with the Munsell color chart for plant tissues, comparing the fruit color to the different shades on the chart. When the response variables were found to be significant, a Tukey multiple comparison test was performed at a significance level of 5% ($P \leq 0.05$).

RESULTS AND DISCUSSION

LD_{50}

The median lethal dose (LD_{50}) was fitted to a descending quadratic model, with a coefficient of determination of 0.89, which is significant. This indicates that 89% of the survival is influenced by the increase in irradiation. Upon interpolation in the quadratic model, it indicated that the LD_{50} is reached at 145 Gy. Regarding maximum survival, it was achieved in the control group with 79.45%, being this value the intercept. In relation to high doses, they had a negative effect on survival as it drastically decreased to a value of 4.45%. This study differs from that reported by Ramírez *et al.* (2006), who reported a survival rate of 60% with the application of 300 Gy of X-rays and concluded that there is a negative correlation between survival and the increase in irradiation dose. These differences are primarily attributed to the different species used in both studies, as well as the different sources of irradiation utilized in each study (Figure 1).

Agronomic Yield

The yield per cut and total yield is presented in Figures 2A and 2B, where the maximum yield per cut was 680.9 g plant^{-1} for the second cut, achieved with 200 Gy. The maximum total yield resulted from the application of 200 Gy of ^{60}Co, with 1470.25 g plant^{-1}. This indicates that this parameter is stimulated by the application of 200 Gy; thus, applications above this level of irradiation cause a decrease in agronomic yield, reaching only 235.99 g plant^{-1}. These results are consistent with those reported by Gómez *et al.* (2017), who worked with irradiated seeds of wheat (*Triticum aestivum* L.) var. T-89 and reported that the yield components, including the number of grains and spike length, are stimulated by the application of 200 Gy. They mention that the application of gamma irradiation in seeds of any species produces new genetic variability with advantages such as higher yield, pest resistance, and improved nutritional quality.

Effect of gamma irradiation on morphological parameters

The morphological parameters of plant height, polar diameter, and equatorial diameter were affected by irradiation. Thus, the greatest plant height was at 200 Gy with 1.24 m, followed by T_0 and T_{200} with 1.04 and 0.95 m, respectively, which were statistically similar. This behavior indicates that plant height is affected by radiation; as it increases, height

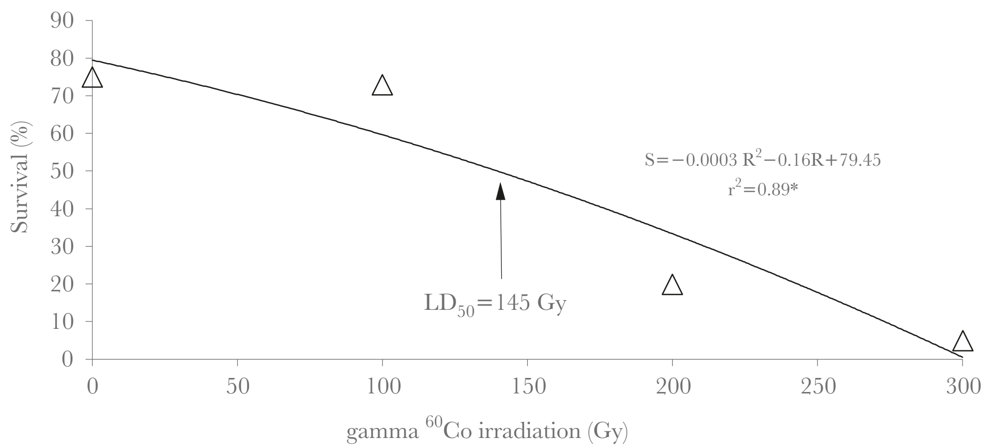

Figure 1. The median lethal dose (LD_{50}) in Miahuateco chili seedlings (*Capsicum annuum* L.) under four levels of gamma irradiation from ^{60}Co. Technological University of Tehuacán. Spring-Summer cycle, 2020.

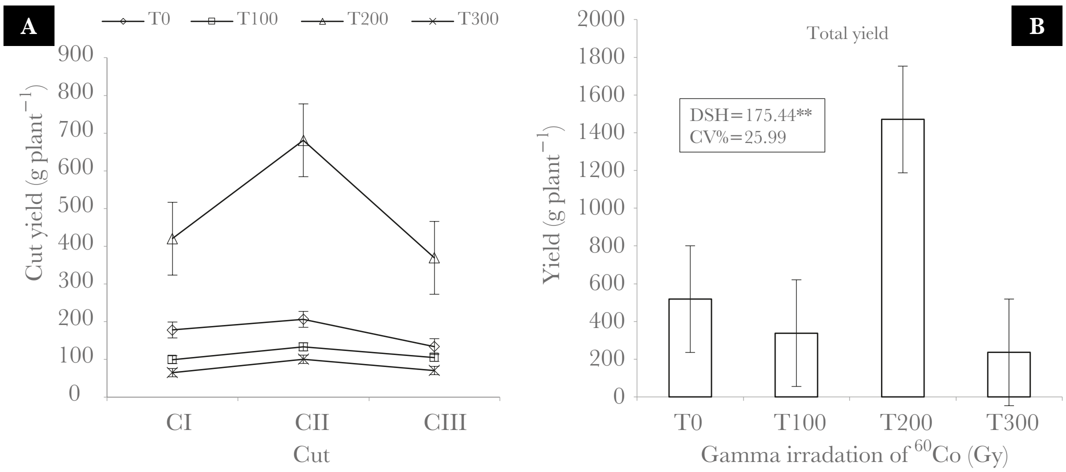

Figure 2. A: Dynamics of yield per cut and B: total yield from three cuts in Miahuateco Chile (*Capsicum annuum* L.) (SOLANACEAE), in relation to four levels of gamma irradiation of ^{60}Co. Spring-Summer cycle, 2020. CI, CII, CIII: cuts; DSH: Honest Significant Difference; CV: coefficient of variation; **; *; n.s: significant at 0.01; 0.05, and not significant.

tends to decrease, as noted by Álvarez *et al.* (2017), who, when working with Navajita, Banderita, Buffel, and Llorón grasses, observed a decrease in seedling height when subjected to gamma irradiation of ^{60}Co. Regarding the polar and equatorial diameter of the fruit, the maximum values were recorded at 200 Gy, measuring 8.83 and 4.31 cm, respectively (see Table 1).

Aborted Flowers, Number of Branches, SPAD Units, and Color

The highest number of aborted flowers was a consequence of the high irradiation dose; thus, the application of 300 Gy induced a 29% abortion rate in the flowers, while in the control treatment, as well as at 100 and 200 Gy, the percentage of aborted flowers was 12.75%, 13.00%, and 19.00%, respectively. The highest number of branches, 6.25 and 9.75, was observed for the control treatments and T_{100}, which were statistically similar

Table 1. Analysis of variance and multiple comparison test for four response variables in Miahuateco chile (*Capsicum annuum* L.), under four levels of gamma irradiation with ^{60}Co. Technological University of Tehuacán. Spring-Summer cycle, 2020.

Treatment Gy	PH	PD	ED	PL
	cm			
T_0	1.04 b	7.29 bc	3.62 b	0.436 a[¶]
T_{100}	1.24 a	7.56 b	2.84 c	0.356 a
T_{200}	0.95 b	8.83 a	4.31 a	0.445 a
T_{300}	0.60 c	8.12 b	3.56 b	0.398 a
HSD	0.12**	0.60**	0.50**	0.090$^{n.s}$
CV%	20.12	15.33	16.37	22.14

[¶] Means within columns with the same letter are statistically equal according to Tukey at P≤0.05; AP, plant height; DP, polar diameter; DE, equatorial diameter; LP, pedicel length; T_0, T_{100}, T_{200}, and T_{300}, treatments; DSH, honest significant difference; CV, coefficient of variation; *; ** n.s, significant at 0.01; 0.05 and not significant.

despite numerical differences (Table 2). Regarding the SPAD units, the minimum values were recorded in T_{200} and T_{300}, with 59.6 and 48.9, respectively, which was reflected in a decrease in color tone, primarily in T300, showing a reduction in tone as indicated by the fresh color 2.5 GY 6/8. This response was reported by Hernández *et al.* (2017), who stated that the color of photosynthetic protocorms of *Loelia autumnalis* tends to decrease with the increase in irradiation under *in vitro* conditions. On the other hand, Martínea *et al.* (2018) mention that seedlings from seeds irradiated with gamma rays of ^{60}Co showed chlorosis at doses of 60 Gy and attribute this response to the fact that high doses of gamma irradiation cause an imbalance in growth regulators, which manifests as chlorosis.

Table 2. Analysis of variance and multiple comparison test for four parameters of Miahuateco Chile (*Capsicum annuum* L.) under four levels of gamma irradiation from ^{60}Co. Spring-Summer Cycle, 2020.

Treatment Gy	AF	NB	SPAD	Color in fresh
	%			
T_0	12.75 bc	6.25 a	65.4 a[¶]	5.0 GY 3/4
T_{100}	13.00 b	9.75 a	62.5 a	5.0 GY 3/4
T_{200}	19.50 b	4.74 ab	59.6 b	5.0 GY 3/4
T_{300}	29.00 a	3.00 b	48.9 c	2.5 GY 6/8
HSD	7.14**	3.55**	5.3**	---------------
CV%	15.99	28.33	14.33	---------------

[¶] Means within columns with the same letter are statistically equal according to Tukey at $P \leq 0.05$; FA, aborted flowers; NR, number of branches; SPAD, SPAD units; T_0, T_{100}, T_{200}, and T_{300}, treatments; DSH, honest significant difference; CV, coefficient of variation; *; ** n.s, significant at 0.01; 0.05 and not significant.

CONCLUSIONS

The median lethal dose for Miahuateco chili is reached with the application of 145 Gy of ^{60}Co. The maximum yield, as well as the number of fruit cuts, is stimulated by 200 Gy. Applications of 300 Gy lead to an increase in flower abortion, as well as a decrease in plant color, which manifests as chlorosis. Gamma irradiation of ^{60}Co is an important source that induces genetic variability in Miahuateco chili, thus it can be used in future improvement programs involving the species.

REFERENCES

Acosta, R. G. F.; Chávez, S. N. 2003. Arreglo topológico y su efecto en rendimiento y calidad de la semilla de chile jalapeño. *Agricultura Técnica en México* 29: 49-60. Disponible en: http://www.redalyc.org/articulo.oa?id=60829105

Aguirre, H. E.; Muñoz, O. V. 2015. El Chile como alimento. *Ciencia. 66*(3): 16-23. Disponible en: https://www.revistaciencia.amc.edu.mx/images/revista/66_3/PDF/Chile.pdf

Álvarez, H. A., Corrales, L. R., Morales, N. C. R., Avendaño, A. C. H.; Villareal, G. F. 2017. Dosis óptima de irradiación gamma con ^{60}Co para inducción de mutagénesis en pastos. *Nova Scientia. 9*(19): 65-82. https://doi.org/10.21640/ns.v9i19.886

Barrios, B.M.; Buján, A.; Debelis, S.P.; Sokolowski, A.C.; Blasón, A.D.; Rodríguez, H.A.; López, S.C.; Grazia, J.; Mazo, C.R.; Gagey, M.C. 2014. Root Biomass/Total Ratio in Soybean (*Glycine max*) Under Two Tillage Systems. *Terra Latinoamericana*. 32: 221-230. Recuperado en 17 de junio de 2024, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S01875779201400030 0221&lng=es&tlng=. Recuperado en 17 de junio de 2024, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S 01875779201400030 0221&lng=es&tlng=.

Caro, M. D. P., Estupiñán, R. S. Y., Rache, C. L. Y., Pacheco, M. J. C. 2012. Efecto de rayos gamma sobre yemas vegetativas de *Physalis peruviana* L. *Acta Agronómica. 61*(4): 305-314. Recuperado en 13 de enero de 2024, de: https://www.redalyc.org/articulo.oa?id=169926831002

Castellón, M. E., Chávez, S. J. L., carrillo, R. J. C. y Vera, G. A. M. 2012. Preferencias de consumo de chiles (*Capsicum annuum* L.) nativos en los valles centrales de Oaxaca, México. *Revista Fitotecnia Mexicana. 35*(5): 27-35. Recuperado en 20 de junio de 2023, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-73802012000500007&lng=es&tlng=es.

Cochran, G.W.; Cox, M.G. 2010. Experimental designs. Ed. Trillas. Mexico, D. F. 661 pp.

Estrada, B. J. A., Pedraza, S. M. E., De la cruz, T. E., Martínez, P. A., Sáenz, R. C.; Morales, G. J. L. 2011. Efecto de rayos gamma ^{60}Co en Nardo (*Polianthes* spp.). *Revista Mexicana de Ciencias Agrícolas*. 3: 445-447. Recuperado en 17 de junio de 2024, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-09342011000900004&lng=es&tlng=es.

Hernández, M. S., Pedraza, S. M. E., López, P. A., De la Cruz, T. E., Fernández, P. S. P., Martínez, P. A. y Martínez, T. M. 2017. Determinación de la LD_{50} y GR_{50} con rayos gamma de ^{60}Co en protocormos de *Loelia autumnalis in vitro*. *Agrociencia*. 51: 507-524. Recuperado en 01 de febrero de 2024, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-31952017000500507&lng=es&tlng=es.

Martinea, R. A., Veitía, N., García, L. R., Collado, R., Torres, D., Rivero. L. y Ramírez, L. M. 2028. Dosis óptimas de radiaciones gamma para la regeneración de plantas *in vitro* de *Phaseolus vulgaris* L. cultivar-Bat-93. *Biotecnología Vegetal. 18*(1): 21-29.

Pérez, C. L. J., Tornero, C. M. A., Escobedo, G. J. S. y Sandoval, C. E. 2017. El chile poblano criollo en la cultura alimentaria del Alto Atoyac. *Estudios Sociales*. 49: 49-66.

Pérez, A. C. E., Carrillo, R. J. C., Chávez, S. J. L., Perales, S. C., Enríquez, D. R. y Villegas, A. Y. 2017. Diagnóstico de síntomas y patógenos asociados con marchitez del chile en valles centrales de Oaxaca. *Revista Mexicana de Ciencias Agrícolas. 8*(2): 281-293.

Ramírez, R., González, M. L., Camejo, Y., Zaldivar, N. y Fernández, Y. 2006. Estudio de radiosensibilidad y selección del rango de dosis estimulantes de rayos X en cuatro variedades de tomate (*Lycopersicon esculentum* Mill.). *Cultivos tropicales. 27*(1): 63-67.

Ruíz, L. N., Medina, L. F. y Martínez, E. M. 2011. *El chile habanero: su origen y usos. 62*(3): 70-77.

Secretaría del Convenio Sobre la Biodiversidad Biológica. 2008. La biodiversidad y la Agricultura. Salvaguardando la biodiversidad y asegurando alimentación para el mundo. Montreal. 56 p.

Toledo, A. R., López, S. H., Antonio, L. P., Guerrero, R. J., Santacruz, V. A. y Huerta, D. A. 2011. Características Vegetativas, reproductivas y de rendimiento de fruto de variedades nativas de chile "Poblano". *Revista Chapingo Serie Horticultura. 17*(3): 139-150.

Velásquez, V. R., Medina, A. M.M., Luna, R. J. J. 2001. Sintomatología y géneros de patógenos asociados con las pudriciones de la raíz del chile (*Capsicum annuum* L.) en el norte-centro de México. *Revista Mexicana de Fitopatología. 19*(2): 175-181.