Chapter 4 Morphological evaluation of *Dioscorea sparsiflora* and *D. alata* minitubers irradiated by gamma rays

Capítulo 4 Evaluación morfológica de minitubérculos de *Dioscorea sparsiflora* y *D. alata* irradiados con rayos gamma

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Abstract

Dioscorea genus comprises underground tubers with economic-nutritional importance. Plagues affect its production, and as in Mexico, there is no established farming system, so its utilization is limited. Using biotechnological tools, like *in vitro* culture and genetic improvement, has led to the development of varieties with important agronomic characteristics. *In vitro* propagated plants of *D. sparsiflora* and *D. alata*, acclimatized to greenhouse conditions, were radiated with different gamma ray doses (0, 10, 20, 30, 40, 50, and 60 Gy) to improve their genetic characteristics. Minitubers were measured and weighed to identify differences between treatments. *D. sparsiflora* plants resisted all doses those with the biggest sizes were obtained when radiated with 30 Gy and higher radiation treatments. *D. alata* plants radiated with 50 and 60 Gy did not survive, and the highest number of tubers was obtained when radiating with 10 Gy. The biggest sizes were obtained when radiating with 20 Gy. These morphological changes in the minitubers could be considered variations, allowing for greater crop utilization.

Dioscorea, Minituberization, Hill sweet potato

El género *Dioscorea co*mprende tubérculos subterráneos con importancia económico-nutricional. Las plagas afectan su producción y, como en México, no existe un sistema de cultivo establecido, por lo que su aprovechamiento es limitado. El uso de herramientas biotecnológicas, como el cultivo in vitro y el mejoramiento genético, ha permitido el desarrollo de variedades con características agronómicas importantes. Plantas propagadas in vitro de *D. sparsiflora* y *D. alata*, aclimatadas a condiciones de invernadero, fueron irradiadas con diferentes dosis de rayos gamma (0, 10, 20, 30, 40, 50 y 60 Gy) para mejorar sus características genéticas. Se midieron y pesaron los minitubérculos para identificar las diferencias entre tratamientos. Las plantas de *D. sparsiflora* resistieron todas las dosis, obteniéndose los mayores tamaños cuando fueron irradiadas con 30 Gy y tratamientos de radiación superiores. Las plantas de *D. alata* irradiadas con 50 y 60 Gy no sobrevivieron, y el mayor número de tubérculos se obtuvo al irradiar con 10 Gy. Los mayores tamaños se obtuvieron al irradiar con 20 Gy. Estos cambios morfológicos en los minitubérculos podrían considerarse variaciones, permitiendo un mayor aprovechamiento del cultivo.

Dioscorea, Minituberización, Camote De cerro

4.1 Introduction

Population increases and food demand have started the search for new crops and improvement of existing products. The *Dioscorea* genus comprises species generally cultivated in developing countries, and the aim is to produce an eatable tuber rich in starch, fiber, vitamins, and minerals (Rodríguez et al., 2008; Muimba-Kankolongo, 2018).

Production systems established in some productive and commercial areas in Asia, South America, and Africa consist mainly of tuber harvest and storage until the subsequent sprouting and planting season. This can cause losses due to putrefaction and nematode infestation during the long storage periods, besides needing long periods of labor to prepare the planting field, increasing production costs (Amusa et al., 2003; Xiao et al., 2023). Of despite having defined production systems, Mexico has no *Dioscorea* or *camote de cerro* established production, even when the tuber is consumed in significant quantities, diminishing wild populations due to annual exploitation.

Implementing biotechnological tools, such as the use of *in vitro* culture techniques, represents an alternative for optimizing production and improving crop characteristics, increasing production and diminishing grow time and loss probability (Wheatley et al., 2003; Borges et al., 2004; Díaz-Godínez, 2022).

Other biotechnological techniques, such as mutagenic physical and chemical agents to help genetic variant production with improved characteristics, have been implemented (Ángeles-Espino et al., 2013). Among the physical mutagenic agents, the most used is gamma radiation, generally coming from Co60, a radioactive isotope with high ionizing capacity used in vegetable cells research and food technology to obtain resistant specimens to different types of biotic and abiotic stress, improve yield and response to disease or chemical agents and even enhance ornamental characteristics (Corrales-Lerma et al., 2019; Balvoa-Caguana et al., 2021).

Based on what has been discussed, the objective of this work was to evaluate morphologically the minitubers produced by plants radiated with gamma rays at different intensities.

4.2 Method

4.2.1 Obtaining plants

Plants of *D. alata* and *D. sparsiflora* were propagated at Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, in *in vitro* conditions in Murashige and Skoog medium (1962) (MS) supplemented with 30 g/L sucrose, 2 mg/L kinetin (KIN) and 8 g/L agar as gelling agent. pH was adjusted to 5.8.

4.2.2 Plant irradiation

Radiation was carried out at Instituto Nacional de Investigaciones Nucleares. Plants of both species were placed in Petri boxes, in MS medium two days before gamma radiation. Ten *D. sparsiflora* plants and five *D. alata* plants were used. Seven treatments with three repetitions were performed at radiations: 0, 10, 20, 30, 40, 50, and 60 Gy. Plants then were transferred to MS medium with 30 g/L sucrose, 2 mg/L KIN, and 8 g/L agar added to stimulate sprouting.

4.2.3 Vegetable material propagation

Radiated specimens were propagated by axillary buds in MS medium supplemented with 0.5 mg/L paclobutrazol (PBZ), 30 g/L sucrose, and 8 g/L de agar as gelling agent; pH 5.8. Plants were incubated at 16 h light / 8 h darkness at 25 ± 2°C until acclimatization.

4.2.4 Acclimatization and greenhouse conditions

Plant acclimatation for the different treatments was carried out in 50 cavities germination trays using as substrate 60 % peat and 40 % perlite. Plants were then placed in 2 L pots with the same substrate in greenhouse conditions, watering three times a week, and fertilized with Peter® (20-20-20) twice weekly. Plants were kept in these conditions until the end of the growing cycle and the complete development of the minitubers.

4.2.5 Harvest and minituber evaluation

Minitubers were harvested during October and November 2022; once the plant aerial part was completely dried. They were weighed individually and in groups according to treatment. Also, length and diameter were measured for each minituber on the same day they were harvested.

4.2.6 Statistical analysis

Statistical analysis was carried out with Statgraphics software. Data was tested for normality. ANOVA Analysis was used for normal data and the Kruskal-Wallis test for other data.

4.3 Results

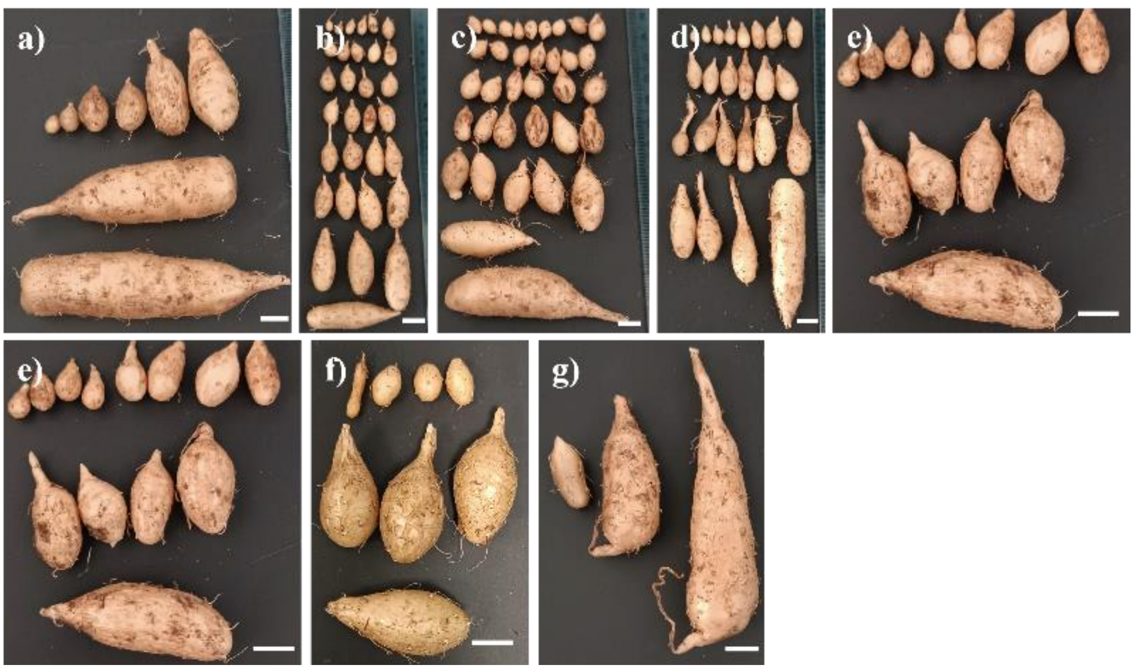
Radiation initial results showed that *D. sparsiflora* plants survived all radiation intensities. *D. alata,* plants only survived up to 40 Gy radiation (Table 4.1), contrasting with Imeh et al. (2012), who reported that 30 % of *D. alata* sprouting plants survived 80 Gy radiation and 10 % when radiated with 100 Gy. Nevertheless, they saw inhibition of plant sprouting when radiating with 100-140 Gy. All these findings differ from another work reporting a lethality percentage up to 26 % in *D. alata* plants radiated with 30 Gy (Yalindua et al., 2014).

Table 4.1 Survival and plant counting after gamma radiating. *Plants with low propagation percentage. Minitubers were harvested between November and December after aerial parts had wilted and dried. All minitubers were stored for the next sprouting period (Fig. 1 and 2)

Specie	Treatment	Plant survival	Total plant
D. alata var. Púrpura	*Control*	YES	80
D. alata var. Púrpura	*10Gy*	YES	103
D. alata var. Púrpura	*20Gy*	YES	64
D. alata var. Púrpura	*30Gy*	YES	53
D. alata var. Púrpura	*40Gy*	YES	111
D. alata var. Púrpura	*50Gy*	NO	-
D. alata var. Púrpura	*60Gy*	NO	-
D. sparsiflora	*Control*	YES	75
D. sparsiflora	*10Gy*	YES	81
D. sparsiflora	*20Gy*	YES	25
D. sparsiflora	*30Gy*	YES	28
D. sparsiflora	*40Gy*	YES	56
D. sparsiflora	*50Gy*	YES	10*
D. sparsiflora	*60Gy*	YES	42

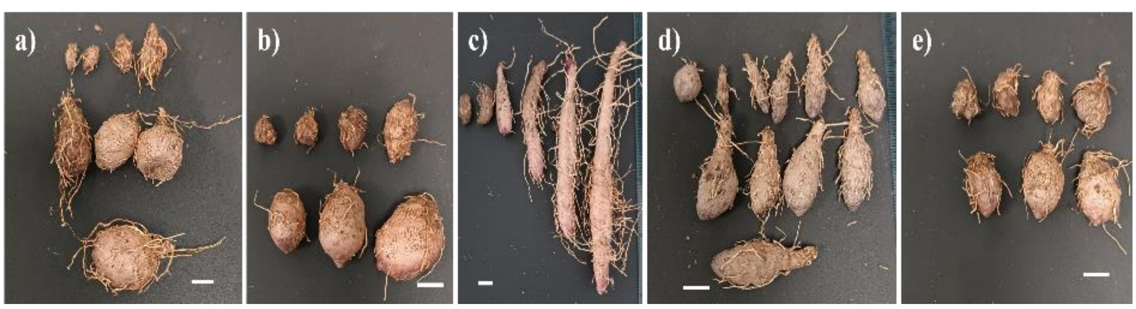
Source: Own elaboration

Figure 4.1 Minitubers obtained from *D. sparsiflora* plants under different doses of gamma rays. a) Control; b) 10 Gy; c) 20 Gy; d) 30 Gy; e) 40 Gy; f) 50 Gy; g) 60 Gy. Bar: 1 cm.



Source: Own elaboration

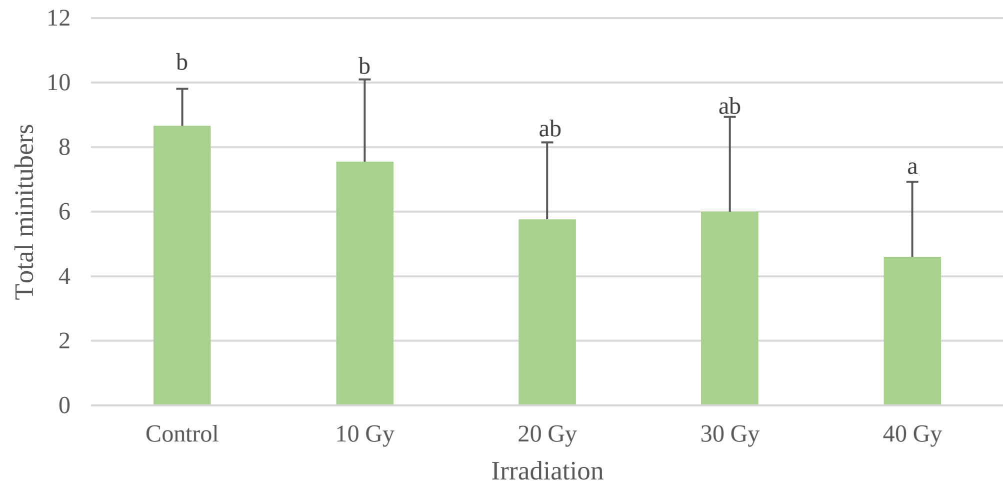
Figure 4.2 Minitubers obtained from *D. alata* plants treated with different doses of gamma rays. a) Control; b) 10 Gy; c) 20 Gy; d) 30 Gy; e) 40 Gy. Bar: 1 cm.



Source: Own elaboration

Analysis for *D. sparsiflora* number of minitubers did not show significant differences (*P*=0.4968); but for *D. alata* var. Purpurea, the total of minitubers showed significant differences (*P*=0.0069). The total number of minitubers obtained in the control group and radiation of 10 Gy was eight minitubers on average, while for the rest of the radiations, the quantity was smaller than six (Graphic 4.1). Rodríguez (2000) mentions that early tuberization and production of more than one tuber per plant are desirable characteristics in yam plants, which could also bring an increase in yield. In potatoes, an increase of 38% has been reported in the number of microtubers produced *in vitro*, after plants were radiated with 2.5 Gy gamma rays (Al-Safadi et al., 2000).

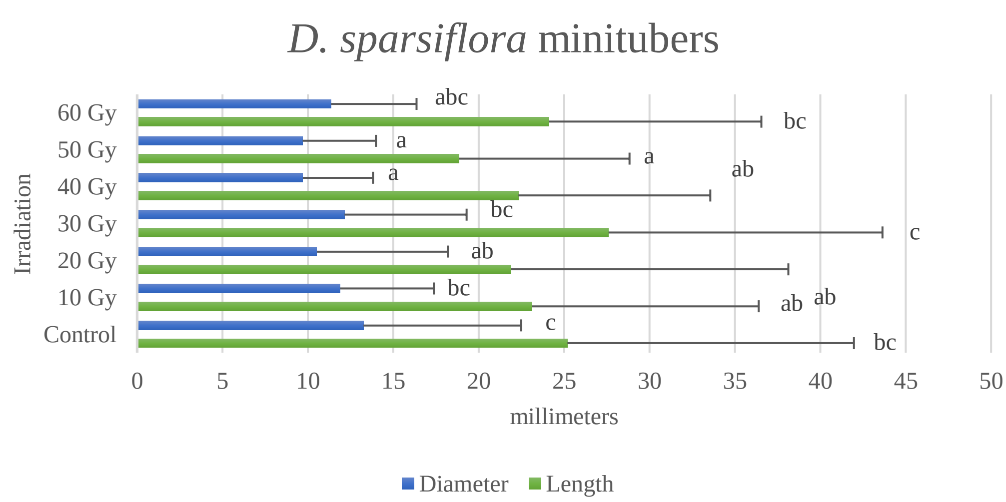
Graphic 4.1 Total of *D. alata* minitubers obtained according to different gamma radiation intensities. Different letters mean significantly different at treatments



Source: Own elaboration

D. sparsiflora minitubers length showed significant differences (*P*= 0.0338) and diameter (*P*=0.0007) (Graphic 4.2). Minitubers of radiated plants with 30 Gy were longer, compared with other radiations, while for minitubers diameter, only slight differences between the control and the rest of the treatments were found. Nevertheless, fresh minitubers weight did not show significant differences (*P*=0.6396).

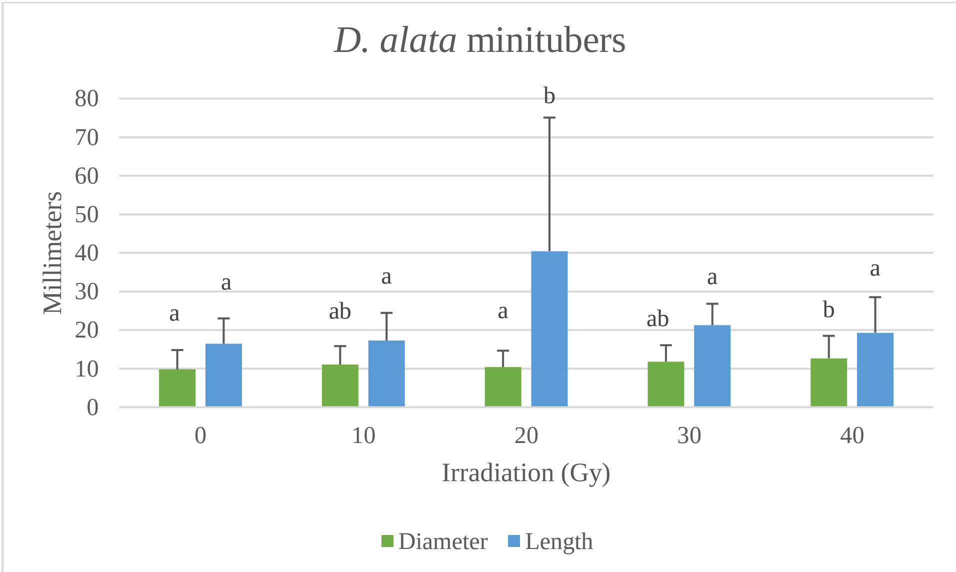
Graphic 4.2 *D. sparsiflora* minituber length and diameter under different gamma radiation doses. Different letters mean significantly different at treatments



Source: Own elaboration

In the case of *D. alata*, minituber length was longer for those obtained from plants radiated with 20 Gy (Graphic 4.3), reaching up to 4 cm. Minituber diameter was lower than 1.5 cm for most minitubers, compared with other tuber roots which reach 5 cm in diameter like in *Ipomoea batata* at low level of radiation (15 Gy) for first generation; in the second generation, the majority of tuber roots reach diameters of 5 cm approximately, even in radiated plants at 75 and 90 Gy (Kalal et al., 2022).

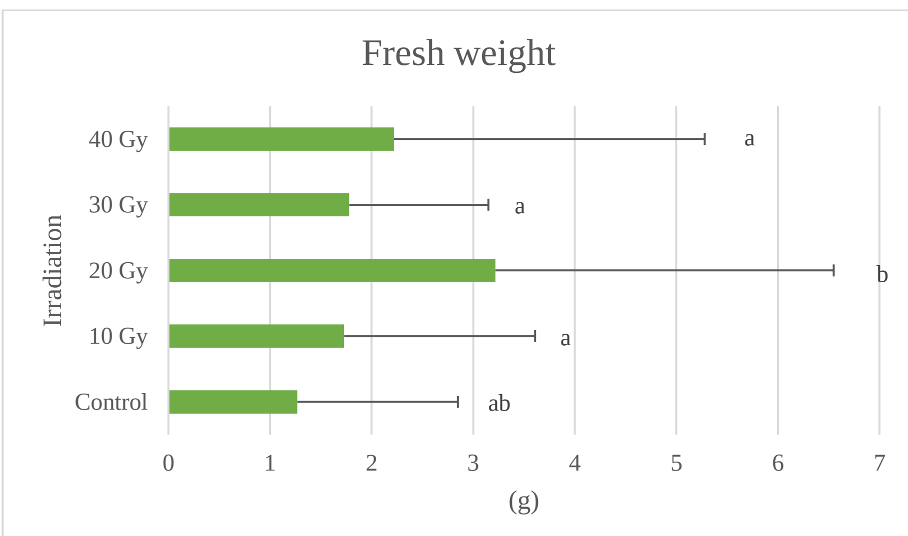
Graphic 4.3 *D. alata* minituber length and diameter obtained after using different doses of gamma rays. Different letters mean significantly different at treatments



Source: Own elaboration

The fresh weight of minitubers from plants radiated with 20 Gy was greater (Graphic 4). Nevertheless, weight decreases in potato minitubers when incrementing gamma ray doses have been reported, where control weighted 119 mg approximately; while those obtained with greater radiation (20 Gy) did not surpass 1 mg (Bado et al., 2016). On the other hand, Mahfouze et al. (2012) reported better weights in potato microtubers radiated with 5-10 Gy doses.

Graphic 4. Fresh weight of *D. alata* minitubers produced from plants radiated with different gamma ray doses. Different letters mean significantly different at treatments



Source: Own elaboration

4.4 Acknowledgment

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4.5 Financing

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4.6Conclusions

Gamma ray radiation is a tool contributing to generating specific changes in different species. These genetic variations could result in new cultivars with superior characteristics than those of conventional species, incrementing tuber size and weight, increasing agronomic yield, and probably increasing important compounds in the food and pharmaceutical industries, among others. Analyzing chemical characteristics in the development of new plants is a fundamental part of studying and implementing new food sources and it is necessary to include them in future research.

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