

Gamma radiosensitivity of coffee (*Coffea arabica* L. var. *typica*)

Estudio de la radiosensibilidad gamma del café (*Coffea arabica* L. var. *typica*)

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Abstract

The effects of gamma radiation on the germination, survival, and morphological damage in characteristics of the M1 generation of coffee (*Coffea arabica* L. var. *typica*) plants was evaluated using seeds collected from Santa Teresa and Chaupimayo, Peru. Under net house, the percentage of germination was 68%, 35%, 10%, and 0% for the Santa Teresa seeds and 75%, 49%, 17% and 0% for the Chaupimayo seeds with irradiation treatments of 0, 50, 100 and 150 Gy, respectively, whereas under laboratory conditions, germination levels were between 70% and 94% across all treatments. The survival rate also decreased with increasing radiation levels, with values of 45%, 32%, 28%, and 10% in the laboratory and 29%, 9%, 6%, and 0% in the net house for the Santa Teresa seeds and 58%, 45%, 38%, and 8% in the laboratory and 42%, 15%, 7%, and 0% in the net house for the Chaupimayo seeds with irradiation treatments of 0, 50, 100, and 150 Gy, respectively. Morphological changes were observed in the plants that survived irradiation at a dose of 100 Gy in terms of the leaf shape, leaf apex shape, young leaf color, plant height, stem diameter, number of leaves per plant, leaf length, leaf width, and distance from the cotyledon to the first node.

Key words: *Coffee, radiosensitivity, gamma ray, germination, survival, morphology.*

Resumen

Se evaluó los efectos de la radiación gamma sobre la germinación, la supervivencia y características morfológico de plantas de café de generación M1 (*Coffea arabica* L. var. *typica*), utilizando semillas colectadas de Santa Teresa y Chaupimayo, Perú. En casa malla, el porcentaje de germinación fue del 68%, 35%, 10% y 0% para las semillas de Santa Teresa y 75%, 49%, 17% y 0% para las semillas de Chaupimayo con tratamientos de irradiación de 0, 50, 100 y 150 Gy, respectivamente, mientras que en condiciones de laboratorio, los niveles de germinación fueron entre 70% y 94% en todos los tratamientos. El porcentaje de supervivencia también disminuyó con el aumento de los niveles de radiación, con valores de 45%, 32%, 28% y 10% en el laboratorio y 29%, 9%, 6% y 0% en la casa malla para las semillas de Santa Teresa y 58 %, 45%, 38% y 8% en el laboratorio y 42%, 15%, 7% y 0% en la casa malla para las semillas de Chaupimayo con tratamientos de irradiación de 0, 50, 100 y 150 Gy, respectivamente. Se observaron cambios morfológicos en las plantas que sobrevivieron a la irradiación a una dosis de 100 Gy en características como forma y ápice de la hoja, color de la hoja joven, altura de la planta, diámetro del tallo, número de hojas por planta, longitud de la hoja, ancho de la hoja y distancia del cotiledón al primer nudo.

Palabras clave: Café, radiosensibilidad, radiación gamma, germinación, supervivencia, morfología.

Introduction

Coffee (*Coffea* spp.) is reported to support the livelihoods of millions of small-scale farmers around the world (Bacon, 2004), and is an important economic crop for many tropical countries. In Peru, more than 100 000 families that have settled in the jungle depend on activities associated with coffee production. However, the recent epidemic of coffee

rust (*Hemileia vastatrix*) from Mexico to Peru has been particularly severe, with some reports anticipating a reduction in yield (40%–50%) over the entire region, which will potentially affect many coffee production nations in Latin America (Cressey, 2013). For example, Vandermeer, Jackson, and Perfecto (2014) reported that over 60% of the plants in an experimental plot in Mexico experienced >80% defoliation and nearly 9% died, and similar patterns have been reported

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anecdotally from across the region (Cressey, 2013).

Despite the success of rust breeding programs for coffee in Colombia and Brazil (Van der Vossen, 2001), there is evidence that some improved commercial varieties derived from the Timor Hybrid (*C. canephora* × *C. arabica*) have lost their resistance, possibly due to the emergence of new virulent races of the pathogen (Alvarado, 2005; Várzea & Marques, 2005). Variability in the virulence of *H. vastatrix* appears to be due to natural mutation processes, but could also arise from other mechanisms, such as cryptic sex, the hidden sexual reproduction of the pathogen (Carvalho *et al.*, 2011). Therefore, breeding programs have focused on efforts to broaden the genetic base of commercial coffee varieties (Castro-Caicedo, Cortina-Guerrero, Roux & Wingfield, 2013).

Mutation induction using different mutagenic agents has been acknowledged reliable method for breeding plants with improved characteristics in many crops [International Atomic Energy Agency (IAEA) database, <https://www.iaea.org>]. Therefore, the objectives of this study were to determine the germination, survival and morphological characteristics of coffee seedlings derived from irradiated seeds.

The sensitivity of plant materials to various mutagens has been investigated and summarized for many plant species. However, these should be considered as a guide, only rather than being treated as fixed numbers, particularly for those plant species that have not been extensively studied, as there is significant variation in the sensitivity of plants to mutagenic treatments both at the species level and within genotypes (Shu, 2009). The radiosensitivity of the irradiated genotypes is determined by exposing the material to a range of radiation intensities and selecting those doses that allow visible effects of the radiation, to be observed while maintaining the survival of the tissues (Tullmann-Neto, 1997). Most of the effects observed in the M₁ generation will be physiological, with the lesions that appear on the M₁ plants being indicative of the degree of mutagenic effects in the plants. These effects can be quantified in a number of ways and can be used to establish dose threshold values of mutagens that cause the mutation required.

Materials and methods

The effect of gamma radiation was examined in coffee (*C. arabica* L. var. *typica*) seeds that had been collected from two locations: Santa Teresa and Chaupimayo, Peru. Dry seeds of coffee with endocarps (parchments) were irradiated with gamma rays at doses of 50, 100, 150, 200 and 300 Gy at the Peruvian Energy Nuclear Institute using a Gammacell 220.

Germination and Survival Tests

In the laboratory, the irradiated seeds from all the treatment groups were germinated in a Pol-Eko-Aparatura thermostatic cabinet (Poland) that was set to 30°C and a photoperiod of 12 h of illumination and 12 h of darkness. All of the seeds with endocarps, were first disinfected with HOMAI® WP fungicide (thiophanate-methyl + thiram) washed 2% detergent, followed by 2% bleach, and rinsed with plenty of distilled water. The seeds were then hydrated by immersing them under water in glass containers for a period of 24 hours at room temperature, after which they were placed on paper towel in the growth chamber. After sowing, the fungicide benomyl was applied to the paper towel with a spray.

The net house had the same environmental conditions as the laboratory, i.e., a temperature of 30° C and a photoperiod of 12 h of light and 12 h of darkness. Irradiated seeds from each treatment group had the endocarp removed. Then, these seeds were disinfected, laid on top of sand in a tray, and kept hydrated with distilled water the germination process.

The seedlings that survived, in each treatment group were transplanted in the nursery at “Genova Farm” at the Regional Development Institute (IRD) of the National Agrarian University La Molina (UNALM) in San Ramon District, Chanchamayo Province, Department of Junín, Peru, for further plant growth and development.

Recorded Data. The following data were recorded during the study: percentage of germination, percentage of survival and a morphological description of the seedlings, which included the seedling height, number of true leaves, stem diameter, distance from the cotyledon to the first node, leaf shape, leaf color, stipule shape, leaf apex shape, leaf length and width leaf).

Results and discussion

Germination

According to Rosa *et al.* (2010), the germination stage *sensu stricto* begins approximately 7 days, after sowing in coffee plants and is completed when the radicle penetrates the outer layer of the endosperm. In the laboratory experiment, the percentage of *sensu strictu* was evaluated 62 days after sowing. Seeds from Santa Teresa, that were irradiated with 0 Gy and 50 Gy exhibited 90% germination, whereas those that were irradiated with 100 Gy and 150 Gy had 84% and 70% germination, respectively (Figure 1). By contrast, seeds from Chaupimayo exhibited 94%, 88%, and 78% germination following irradiation with 0, 50, 100, 150 Gy, respectively. Thus, the percentage of germination was higher than 70% for both types of seeds across all treatment groups, but the seeds from Chaupimayo obtained higher values than those from Santa Teresa. Since the optimum

temperature range for coffee seed germination is between 28°C and 30°C (Wellman, 1961; Huxley, 1964) and the growth chamber had a temperature of 30°C, this difference in the germination percentage may have resulted from differences in the quality of the seeds.

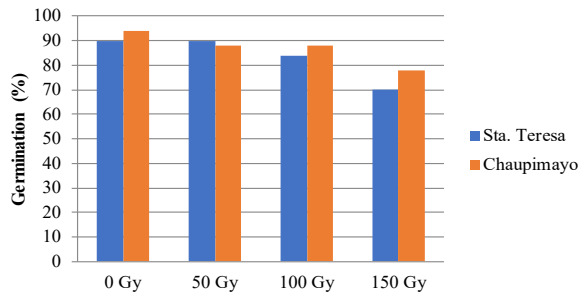


Figure 1. Percentage of germination of coffee (*Coffea arabica* L. var. typica) seeds originating from Santa Teresa and Chaupimayo under laboratory conditions at 62 days after sowing following irradiation with gamma rays at 0 (control), 50, 100, and 150 Gy.

In the net house, experiment the percentage of germination was evaluated at 75 days after sowing. The seeds from Santa exhibited germination levels of 68%, 35%, 10%, and 0%, while those from Chaupimayo, had germination levels of 75%, 49%, 17%, and 0%, following irradiation with 0, 50, 100 and 150 Gy, respectively (Figure 2). Similarly, Kumar, Nepolean & Gopalan (2003) found that the survival and germination, of *Phaseolus lunatus* seeds decreased with increasing gamma radiation over a range of 200–1000 Gy, and Cheema and Atta (2003) showed that seed germination in the M1 generation of three different varieties of rice (*Oryza sativa*) decreased with an increased radiation dose under field conditions.

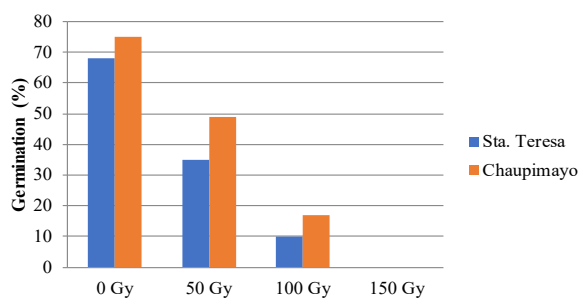


Figure 2. Percentage of germination of coffee (*Coffea arabica* L. var. typica) seeds originating from Santa Teresa and Chaupimayo under net house conditions at 75 days after sowing following irradiation with gamma rays at 0 (control), 50, 100, and 150 Gy.

Survival

In the laboratory, seedlings that germinated from Santa Teresa seeds exhibited survival rates of 45%, 32%, 28%, and 10% at 22 days after germination, while those that germinated from the Chaupimayo seeds had survival rates of 58%, 45%, 38%, and 8% in the 0, 50, 100, and 150 Gy treatment groups, respectively (Figure 3). No published information is available on the effects of gamma radiation

on the survival rates of coffee, but it has been shown that increasing doses of gamma radiation to the seeds, decreases the number of plants surviving for *Jatropha curcas* (Songsri *et al.*, 2011) and rye (*Secale cereale* L.) “Sorom” (Soraluz, 2015).

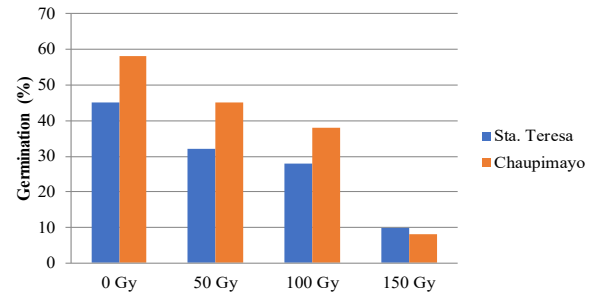


Figure 3. Percentage of germination of coffee (*Coffea arabica* L. var. typica) seeds originating from Santa Teresa and Chaupimayo under laboratory conditions at 84 days after sowing following irradiation with gamma rays at 0 (control), 50, 100, and 150 Gy.

In the net house, seedlings that germinated from Santa Teresa seeds had survival rates of 29%, 9%, 6%, and 0% at 112 days after germination, while those that germinated from the Chaupimayo seeds had survival rates of 42%, 15%, 7%, and 0% in the 0, 50, 100 and 150 Gy treatment groups, respectively (Figure 4).

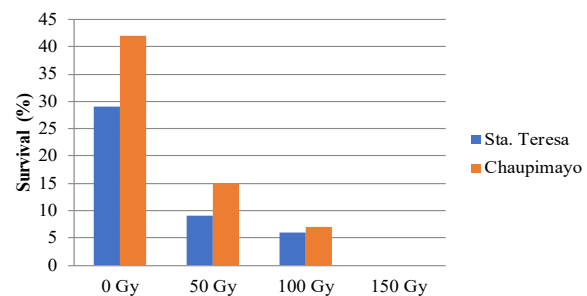


Figure 4. Percentage of survival of germinated coffee (*Coffea arabica* L. var. typica) seeds originating from Santa Teresa and Chaupimayo under net house conditions at 112 days after sowing following irradiation with gamma rays at 0 (control), 50, 100, and 150 Gy.

Rate of Plant Growth and Development

Based on the germination and survival results, only the M1 generation of plants in the 100 Gy treatment group were transplanted to the nursery for comparison of growth and development.

In general, the M1 plants that originated from the irradiated seeds had slower development than the control (0 Gy treatment) plants. In the control group, 3% of plants were in the matchstick (initial) stage, 6% were in the butterfly stage, and 91% were in the true leaf formation (Figure 5). Similarly, Kiong, Lai, Hussein, and Harun (2008) found that mutagenic treatments resulted in a lower germination rate and slower development, and Vargas (2016), found that

increased sodium azide dose increased the time to true leaf generation in coffee plants. Furthermore, Al-Salhi *et al.* (2004) and Hameed *et al.* (2008), reported that exposure to high doses of gamma radiation affect protein synthesis, the hormonal equilibrium, the foliar interchange of gases, and enzymatic activity in a range of crop, all of which are related to plant growth.

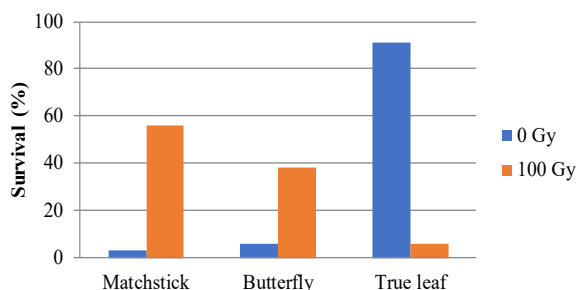


Figure 5. Effect of 100 Gy gamma irradiation on the grade of development of M1 coffee (*Coffea arabica* L. var. *typica*) plants grown under nursery conditions “Genova Farm” San Ramon District, Department of Junín, Peru.

Morphological Characteristics

Stem: The plant height, stem diameter, and length of the first internode, had mean values of 22 cm, 2.7 cm, and 1.01 cm; respectively, in control plants and 12 cm, 2.2 cm, and 0.15 cm, respectively, in M1 plants in the 100 Gy treatment group (Figure 6). Canul *et al.* (2012) similarly reported irradiated *Euphorbia pulcherrima* plants had a reduced plant height, while Vargas (2016) showed that increased doses of sodium azide reduced the height of coffee plants.

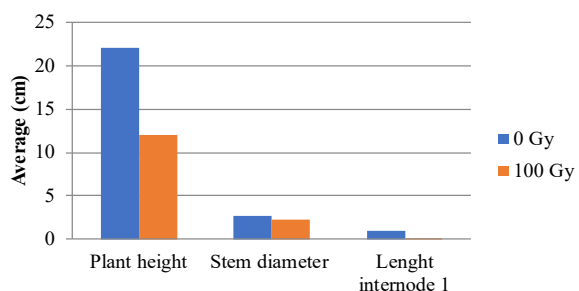


Figure 6. Effects of 100 Gy gamma irradiation on the stem characteristics of the M1 generation of coffee (*Coffea arabica* L. var. *typica*) plants grown under nursery conditions on “Genova Farm” San Ramon District, Department of Junín, Peru.

Leaves: The leaf length, leaf width, and number of leaves had mean values of 6.7 cm, 3.2 cm and 10 leaves, respectively, in the control plants and 5.5 cm, 2.2 cm, and 7 leaves, respectively, in M1 plants in the 100 Gy treatment group (Figure 7).

Thus, M1 plants irradiated with 100 Gy showed lower values for all characters than the unirradiated control plants.

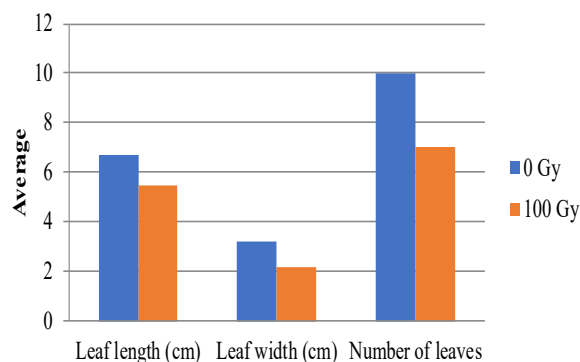


Figure 7. Effects of 100 Gy gamma irradiation on the leaf characteristics of the M1 generation of coffee (*Coffea arabica* L. var. *typica*) plants grown under nursery conditions on “Genova Farm,” San Ramon District, Department of Junín, Peru.

Leaf shape: All of the control plants had a lanceolate leaf shape, compared with only 54% of the M1 plants in the 100 Gy treatment group (Figure 8). Among the remaining M1 plants, 18% exhibited an ovate leaf shape and 28% had irregular modifications (Figure 9). Similarly, Moh and Orbegozo (1960) and Moh (1961) reported that the irradiation of coffee seeds with X rays induced a high frequency of morphological variation in the leaves, of M1 plants, particularly the formation of narrow leaves, which, according to Mendes (1955), could be the result of aneuploidy.

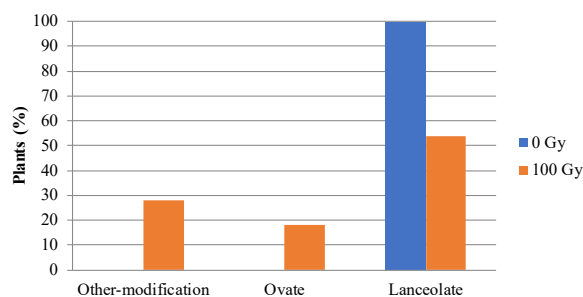


Figure 8. Effects of 100 Gy irradiation on the leaf morphology of the M1 generation of coffee (*Coffea arabica* L. var. *typica*) plants grown under nursery conditions on “Genova Farm,” San Ramon District, Department of Junín, Peru.

There was also some modification of the leaf apex, which was acuminate shaped in all of the control plants but only 66% of the M1 plants from the 100 Gy treatment group, with the remainder being apiculate shaped (6%) or having other modifications (28%) (Figure 10). The stipule shape was ovate in all control plants. However, only 76% of the M1 plants exhibited this shape, with the 24% showing modification of the stipule without a defined form.

Leaf color: All of the control plants had green leaves, whereas 2% of the M1 plants exhibited some other color (Figure 11), ranging from yellow-green, through to white with yellow stripes or spots.

May - August 2019

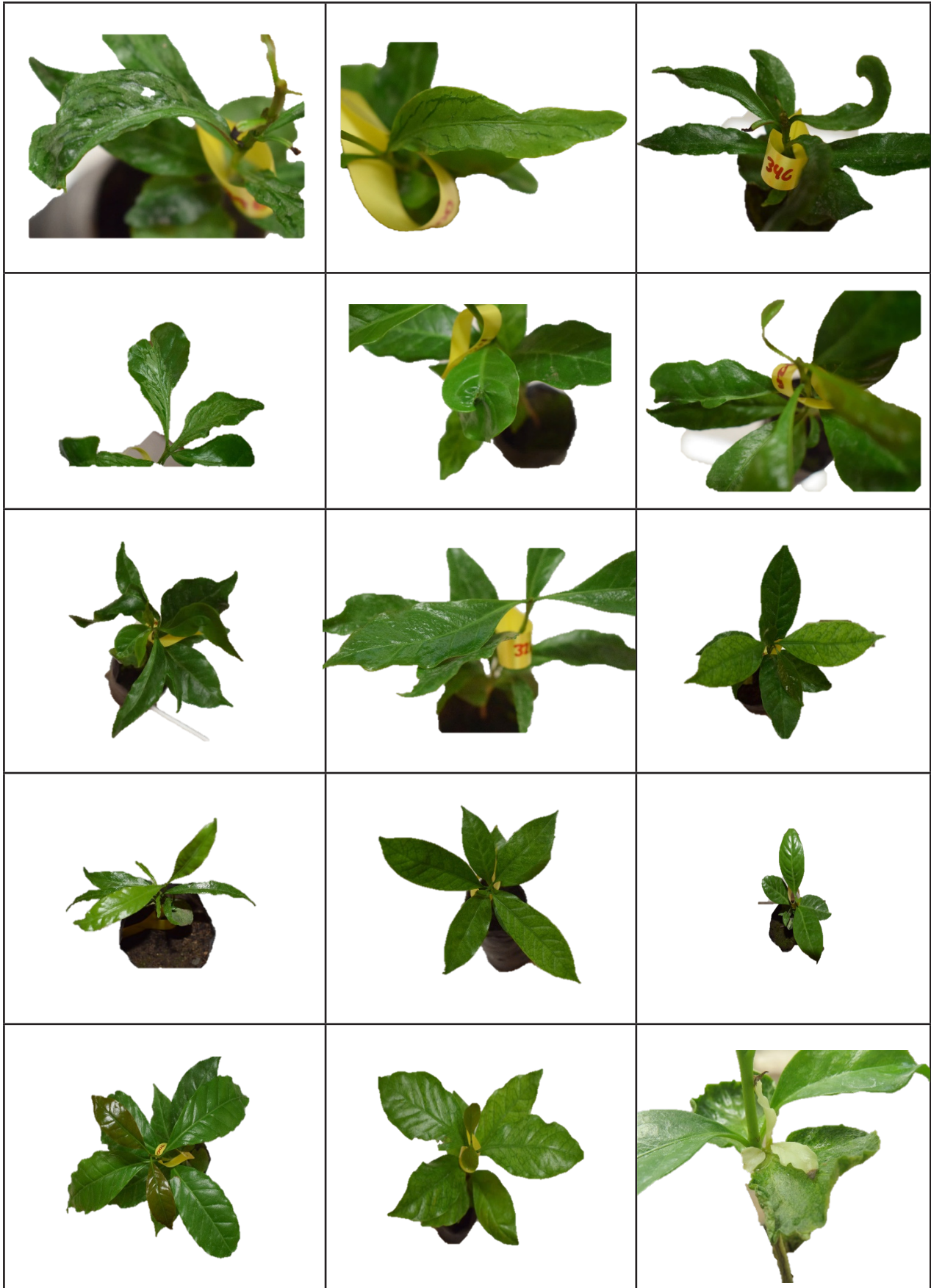


Figure 9. Morphological modifications in the leaves of the M1 generation of coffee (*Coffea arabica* L. var. *typica*) plants grown under nursery conditions on "Genova Farm," San Ramon District, Department of Junín, Peru.

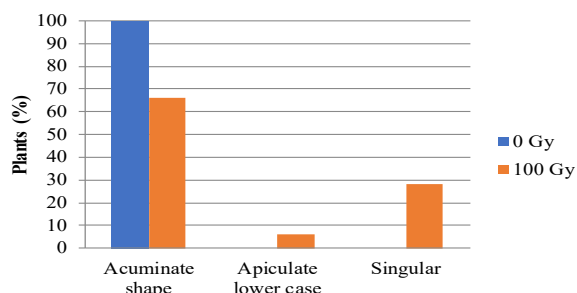


Figure 10. Effect of 100 Gy gamma irradiation on the leaf apex shape of the M1 generation of coffee (*Coffea arabica* L. var. *typica*) plants grown under nursery conditions on “Genova Farm,” San Ramon District, Department of Junín, Peru.

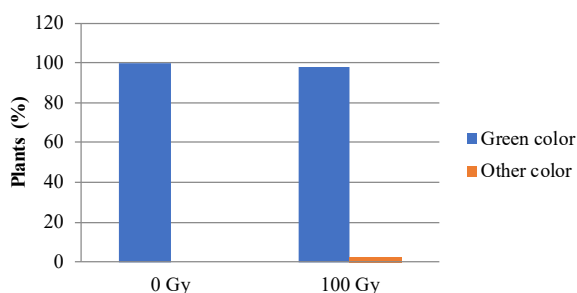


Figure 11. Effect of 100 Gy gamma irradiation on the leaf color of the M1 generation of coffee (*Coffea arabica* L. var. *typica*) plants grown under nursery conditions on “Genova Farm,” San Ramon District, Department of Junín, Peru.

It was observed in 2 % of the M1 plants (Figure 11). Some plants had yellow-green color and white and yellow stripes or spots.

Conclusions

Gamma radiation affected the percentage of seed germination, the survival rate, the growth, and the duration of the phenological phases in the *typica* variety of coffee, with these negative effects increasing with an increased dose; under laboratory, growth chamber, and nursery conditions.

Among the doses used in the present study, a fairly high survival of seedlings was observed with 100 Gy. However, 100 Gy of gamma radiation caused somatic changes in the M1 population, with modifications in the shape, apex type, and color of the leaves.

Acknowledgments

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