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Research Article

Growth dynamics of morphological and reproductive traits of *Physalis peruviana* L. M_1 plants obtained from seeds irradiated with Gamma rays

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Abstract

There is an increasing interest in the development of uchuva (*Physalis peruviana* L.) cultivars adapted to greenhouse farming. Sexual behavior makes it difficult to obtain uniform commercial uchuva cultivars by conventional breeding methods. Mutations induced by gamma rays is an alternative approach. M_1 plants derived from 14 irradiation ^{60}Co doses, from 0 to 275 Gy, that were applied to uchuva seeds were evaluated. Recorded data included days to first flower and growth dynamics (four to seven samplings) of morphological traits (plant height, stem diameter, basal stems) and reproductive traits (floral buds, flowers and green fruits). Treatments were distributed in a completely randomized blocks experimental design with six replications, in a greenhouse. The experimental unit was a single M_1 plant. Statistical differences were found for irradiation doses, growth samplings, and its interaction. Growth dynamics results indicate that all traits showed a linear increase with plant age (R^2 = 0.92* to 0.98**), but the effect of the irradiation doses on morphological and reproductive traits was no linear. Irradiation reduced plant height by 79%. M_1 plants developed from irradiated seeds at doses of 125, 175 and 200 Gy showed greater stem diameter, with more basal stems, floral buds, flowers and green fruits than the control. It is concluded that intermediate irradiation doses had a stimulating effect on vegetative growth and fruiting traits of M_1 uchuva plants.

Keywords: crop breeding; genetic variability; horticultural crops; mutagenesis; uchuva

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Introduction

Uchuva (*Physalis peruviana* L.) also known as golden berry, is a perennial species that grows wild in tropical highlands (1500 to 3000 m, altitude) of Chile and Colombia (Rodrigues *et al*., 2009, Fischer *et al*., 2011). Mature fruits are small (2.5 cm diameter), resembling mini-tomatoes (*Solanum lycopersicum* L.), with colors varying from yellow to orange and a bitter-sweet flavor. Fruits are consumed fresh and are high in fiber, provitamins A and C, iron, phosphorus and antioxidants (Fischer, 2000).

Uchuva can be sexually reproduced although vegetative propagation techniques (cutting) can also be used. Sexual behavior makes it difficult to obtain uniform commercial uchuva varieties by conventional breeding methods because half of the flowers are open-pollinated, favoring allogamy (Santana and Angarita, 1997) while the rest are self-pollinated, as an autogamous species (Lagos *et al*., 2008).

Natural mutations are random changes in DNA that occur in low frequency and spontaneously. The induction of mutations is used in plant breeding to increase genetic variability, which allows the subsequent application of selection methods of individuals with outstanding characteristics (Fuchs *et al*., 2002, Honda *et al*., 2006). Artificial mutations may be induced by chemical and physical mutagens. The latter group refers to the application of X-rays, gamma irradiation (Mohan Jain, 2006; Yamaguchi *et al*., 2008), ultraviolet rays (Ahloowalia and Maluszynski, 2001), and carbon ion-beam irradiation (Wu *et al*., 2009, Matsumura *et al*., 2010). Mutagenic agents produce structural, phenotypic and developmental alterations in cells, tissues and organs (Wi *et al*., 2005). Drastic alterations are usually lethal, while slight changes might be favorable for some traits related to the growth, development and reproduction of the plant.

In particular, gamma rays irradiation influences the growth and development of the plants, causing genome instability of cells and tissues, which produces cytological, biochemical, physiological and morphological changes through the production of free radicals in the cells (Kim *et al*., 2004; Wi *et al*., 2005). The use of high doses of this type of radiation inhibits plant growth (Aladjadjiyan, 2007; Canul-Ku *et al*., 2012), while low and intermediate doses can have a positive effect, by increasing cell proliferation, improving seed germination, cell growth, enzymatic activity, resistance to stress and yield (Chakravarty and Sen, 2001; Baek *et al*., 2005; Kim *et al*., 2005). When radiation induces mutations in a cell, there is a risk that a favorable mutation will be accompanied by undesirable genetic changes (Otahola-Gómez *et al*., 2001).

Results regarding the response of *P. peruviana* to gamma irradiation are scarce. Caro-Melgarejo *et al*. (2012) analyzed the effects of irradiation doses from 50 to 300 Gy applied to vegetative buds of this species on morphological and cytogenetics traits of the regenerated plants. They observed that doses between 100 and 200 Gy produced the largest phenotypic variability while doses higher than 200 Gy had negative effects. As for *P. peruviana* and *P. angulata* (L.) when doses of 200, 400 and 500 Gy applied to seeds were compared, it was observed that the dose of 200 Gy increased the growth of the M_1 plants while doses greater than 200 Gy inhibited it (Raghava and Raghava, 1989). Literature references regarding uchuva traits measured throughout the biological cycle of the plant in order to monitor the effect of the application of artificial mutagens were not found.

There is an increasing interest in the development of uchuva cultivars adapted to greenhouse farming, as other *Solanaceae* species do. Therefore, early maturity, high yield and plant uniformity should be some of the agronomic traits of interest involved in a breeding program in this species. The aim of the present research was to determine the growth dynamics of morphological and reproductive traits of M_1 plants of *Physalis peruviana* L. originated from seeds irradiated with gamma rays in order to analyse the relationship between irradiation doses and plant growth samplings as well as to identify the best radiation doses for each trait.

Materials and Methods

Biological material and applied treatments

Uchuva seeds from 'Ecotipo Colombia' were irradiated with ^{60}Co gamma rays with a Transelektro irradiator (Model LGI-01, Hungary) at the Instituto Nacional de Investigaciones Nucleares, located at Ocoyoacac, Mexico. The irradiation treatments applied to the seed were 14 doses of ^{60}Co gamma rays (0, 5, 10, 20, 50, 75, 100, 125, 150, 175, 200, 225, 250 and 275 Gy).

Experimental unit

In August 2015, 100 seeds of each dose were sown in expanded polystyrene trays with peat as a substrate, and irrigated with potable water (pH 7.6). In October 2015, the best six M_1 seedlings were selected in each treatment. After that, M_1 plants were distributed in a completely randomized experimental design, with six replications. The experimental unit was an M_1 plant, placed in a black polyethylene bag of 9 L size. Tezontle (volcanic rock) was the main substrate support; other substrate characteristics were: granulometry, 1 to 10 mm; average apparent density, 0.82 g cm^{-3}; total porosity, 50%; aeration porosity, 45%; readily available water, 5.42%; cation exchange capacity, none; electrical conductivity, close to zero (Gutiérrez-Castorena *et al*., 2011). A tunnel-type greenhouse with UVII-720 polyethylene cover and galvanized steel structure, with lateral ventilation, located in Montecillo, State of Mexico was used.

The M_1 plants were held upright by tutoring. The Steiner solution was used at 50% of its original ionic strength; pH of the solution was adjusted to 6.0 (Gastelum-Osorio *et al*., 2013). Average monthly environmental conditions that prevailed from October 2015 to February 2016 were: light intensity, 652.21 μmol m^{-2} s^{-1}; maximum temperature, 37 °C; and minimum temperature, 8 °C.

Morphological and reproductive traits

In each M_1 plant, the first record of plant height (PH, cm; from the substrate level to the apex of the longest branch) and stem diameter (SD, mm; at 2 cm from the base of the stem), was registered 25 days after transplant (dat). Corresponding initial records for the number of basal stems (NBS) were at 31 dat, 55 dat for floral buds (NFB) and 70 dat for flowers (NF) and green fruits (NGF). Afterwards, all traits were registered every 15 days. Therefore, data from 24 to 42 individual M_1 plants were involved in each growth sampling average. In addition, days to the first flower were recorded.

Statistical analysis

A combined analysis of variance was applied for each trait (except for days to the first flower). Sources of variation were: irradiation doses, growth samplings and irradiation doses × growth samplings interaction. Tukey test ($p \leq 0.05$) was used for means comparisons. Analyses were carried out with the statistical program SAS, version 9.1 (SAS Institute, 2002). In addition, linear regression was applied to growth samplings data for each trait, while polynomial regression was performed over irradiation doses data.

Results and Discussion

Significant differences ($p \leq 0.05$) for irradiation doses, growth samplings and the doses × growth sampling interaction were found in the combined analysis of variance for all traits (Table 1).

Growth dynamics

The expression of all traits related to the growth dynamics of vegetative and reproductive traits, of *P. peruviana* increased according to a linear model (R^2 between 0.92* and 0.98**) as the age of the M_1 plants increased (Figure 1). This means that averaged over the 14 irradiation doses, the growth rate of these traits was constant throughout the time. The highest expression occurred in the last growth sampling, which is attributed to the indeterminate and perennial habit of this species (Fischer *et al.*, 2011).

Table 1. Statistical significance of the sources of variation for morphological and reproductive traits of M_1 plants of *Physalis peruviana* L.

Source	Morphological and reproductive traits					
	PH	SD	NBS	NFB	NF	NGF
Irradiation doses (D)	*	*	*	*	*	*
Growth samplings (S)	*	*	*	*	*	*
D × S	*	*	*	*	*	*
V. C. (%)	17	16	20	35	39	52

Note: V. C. = Variation coefficient; PH = Plant height; SD = Stem diameter; NBS = Number of basal stems; NFB = Number of floral buds; NF = Number of flowers; NGF = Number of green fruits. * = Significant F test ($p \le 0.05$).

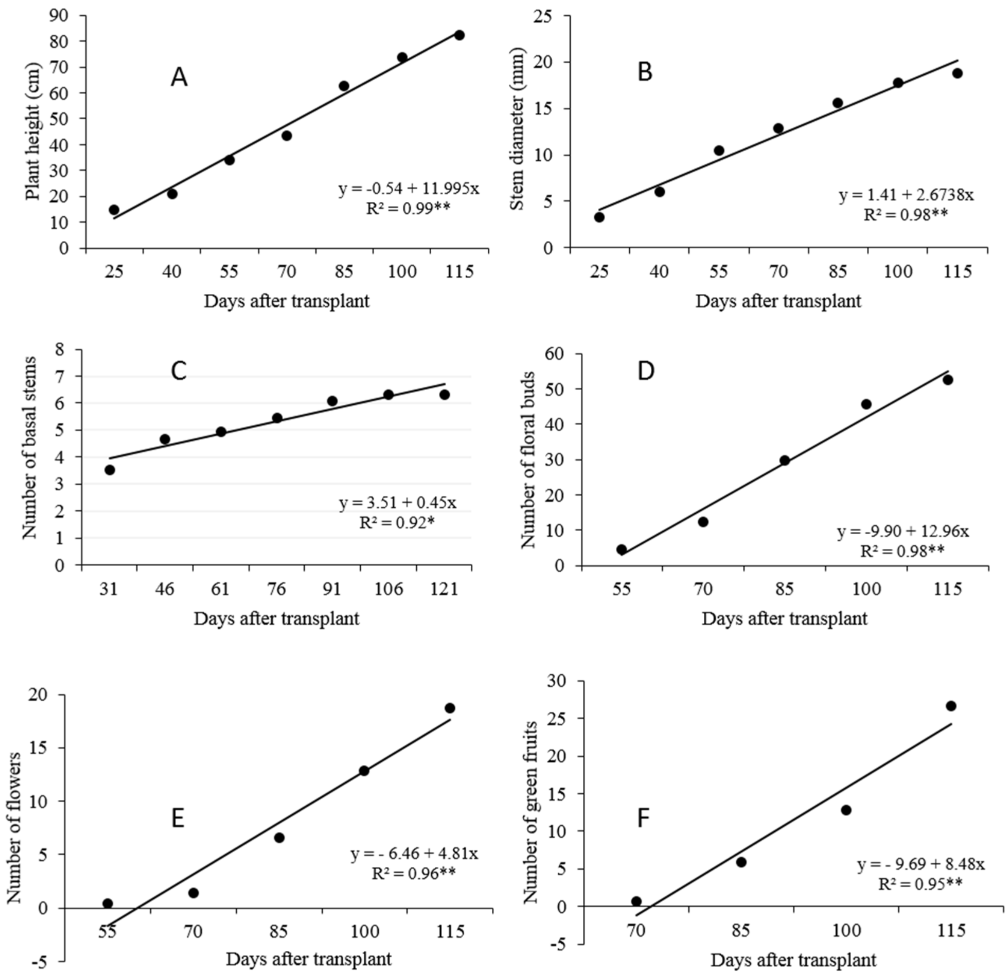

Figure 1. Growth dynamics of morphological and reproductive M_1 uchuva traits. Plant height (A), stem diameter (B), and number of basal stems (C), floral buds (D, flowers (E) and green fruits (F) through plant age (days after transplant). Each point corresponds to the average of 14 irradiation doses applied to the seed

To our knowledge, this is the first report in which plant traits are measured throughout most part of the crop cycle to evaluate the averaged effect of the application of artificial mutagens. Generally, as for Solanaceae species are concerned, data is recorded in a single phenological stage, most of the times at flowering (López-Mendoza *et al*., 2012) or at harvest (Álvarez *et al.*, 2013).

Irradiation doses effects

The effect of irradiation doses did not follow a linear response for any of the uchuva traits (R^2 from 0.50* to 0.80*) (Figure 2). M_1 plants whose seeds were exposed to low or high doses of radiation generally showed lower values than M_1 plants from seeds irradiated with intermediate doses. For breeding purposes, the most agronomical favorable doses were 125, 150, 175 and 200 Gy, since they produced M_1 plants of smaller plant height, with greater numbers of basal stems, floral buds, flowers and green fruits than plants from non-irradiated seeds (Figure 2). The earliest M_1 plants flowered at 53 dat (dose of 200 Gy) almost one week earlier than the control. In terms of yield components, M_1 plants from seeds irradiated at intermediate doses produced twice the amount of floral buds, flowers and green fruits than those from the non-irradiated seeds (Figure 2). These results are encouraging since one of the purposes of our uchuva breeding program is to select for early flowering and high yielding genotypes.

When a wide range of irradiation doses are applied, the response of plants to irradiation doses is not always linear (Yamaguchi *et al*., 2008; Canul-Ku *et al*., 2012); in addition, diploid organisms are more susceptible than polyploid organisms (Chopra, 2005). Regarding results in uchuva studies, Raghava and Raghava (1989) and Caro-Melgarejo *et al*. (2012) also observed that intermediate doses (100 to 200 Gy) favored the growth of M_1 plants of uchuva while doses higher than 200 Gy negatively affected plant growth. In other *Solanaceaea* species, Aladjadjiyan (2007) mentioned that M_1 plants of tomato (*Solanum lycopersicum* L.) from seeds irradiated with X-rays (10 Gy) increased by 25% the stem thickness, and he demonstrated that the radiation stimulus depends on the wavelength, source of irradiation and exposure time. López-Mendoza *et al*. (2012) indicated that the flowering and fructification of the M_1 plants from irradiated seeds of *C. annuum* L. at doses of 0 to 120 Gy, occurred in a period similar to the control. They also mentioned that at the dose of 60 Gy the M_1 plants showed more fruits than the control. On the other hand, doses between 5 and 20 Gy increased by 66 and 72% the number of fruits per plant of *C. annuum* L., but doses higher than 130 Gy decreased it (Álvarez *et al*., 2013).

Irradiation doses × Growth samplings interaction

Several factors influence crop responses to irradiation treatments. These factors include: the source of radiation, irradiation dose and exposure time (De Souza *et al*., 2006); the irradiated organ (Otahola-Gómez *et al*., 2001; Caro-Melgarejo *et al*., 2012; Álvarez *et al*., 2013); the water content of the irradiated material (Ramírez *et al*., 2006); and the agronomic trait as well as the phenological stage in which measurements are taken. In the present study, the irradiation doses × growth samplings interaction was significant for all traits (Table 1). This means that the effect of the radiation is expressed in a particular way according to the plant age (*i.e*. the phenological stage represented by each sampling date) and the irradiation dose. Therefore, there is an optimal irradiation dose for each morphological and reproductive trait.

In the present study, this interaction is illustrated with the traits most closely related to the fruit yield of uchuva: *i.e*. the number of basal stems and that of reproductive traits (flowers and green fruits) at contrasting phenological growth stages (Figure 3). On average of the 14-irradiation doses there were 4.7 basal stems per plant at 46 dat and 6.3 at 121 dat (Figure 1). However, the interaction growth sampling × irradiation doses indicates that at 46 dat, plants from irradiated seeds with doses of 0 and 50 Gy had four basal stems while those from doses of 125, 175 and 200 Gy had six basal stems. In contrast, at 121 dat, plants from 200 Gy produced nine basal stems, significantly higher than those obtained in all irradiated plants at any other dose (Figure 3A).

The advantage on the number of flowers and green fruits produced by the M_1 plants from seeds irradiated with 200 Gy was more evident in the last sampling than in previous samplings (Figure 3B, 3C).

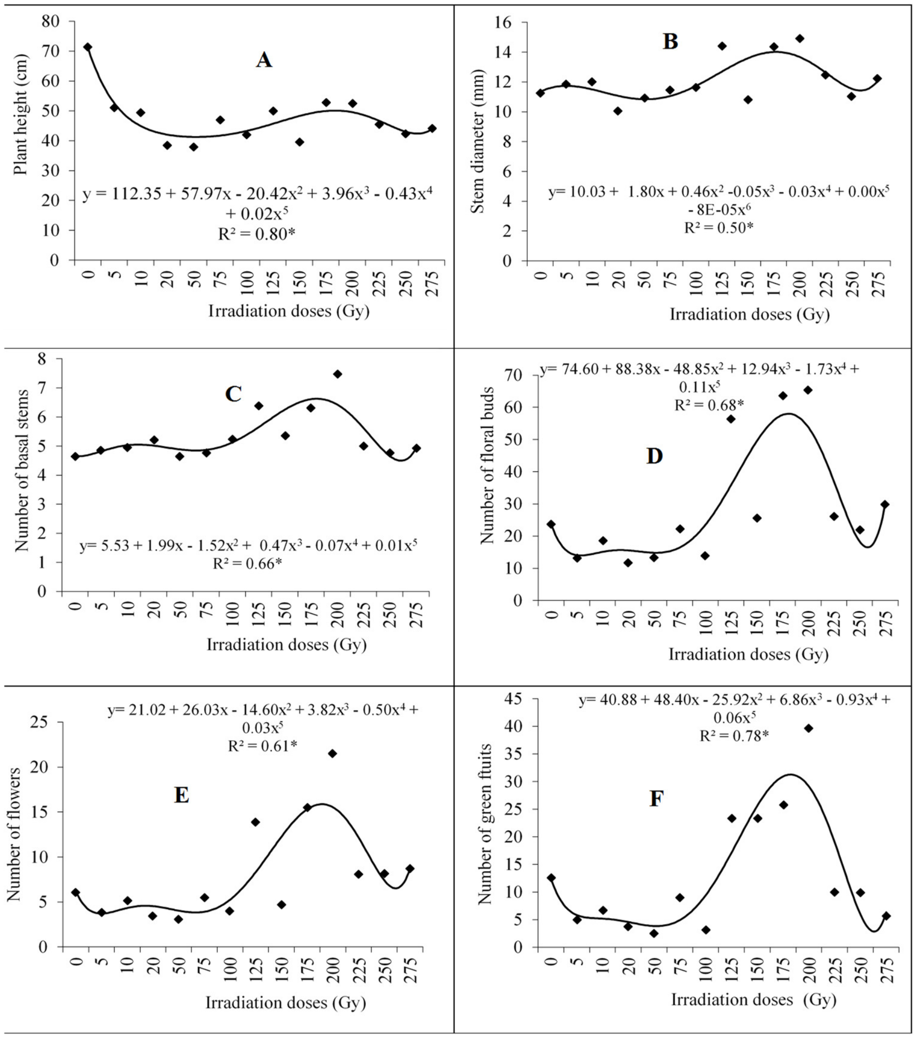

Figure 2. Irradiation doses response on morphological and reproductive of M_1 uchuva traits. Plant height (A; n = 7), stem diameter (B; n = 7), number of basal stems (C; n = 7), floral buds (D; n = 5), flowers (E; n = 5) and green fruits (F; n = 4). Each point represents the average of n growth samplings

Finally, the application of ^{60}Co gamma rays to seeds induces random changes in the DNA, which in most cases are recessive (Prina *et al.*, 2011), so the expression of the induced mutations should be detected in the second generation (M_2), when recessive mutations are in homozygous condition. However, phenotypic changes can be detected in M_1 individuals as result of physiological effects of radiation (Kodym *et al.*, 2011), although in a low frequency. In order to observe these specific changes for genetic improvement purposes, it can be appropriate to evaluate each M_1 individual of the irradiated population (Maluszynski *et al.*, 2009), particularly when the crop, as the uchuva species, is suitable for vegetative propagation by crafting techniques. Therefore, although selection of early-maturity and high-yielding uchuva mutants will be performed in M_2 plants, vegetative propagation of outstanding M_1 plants is underway.

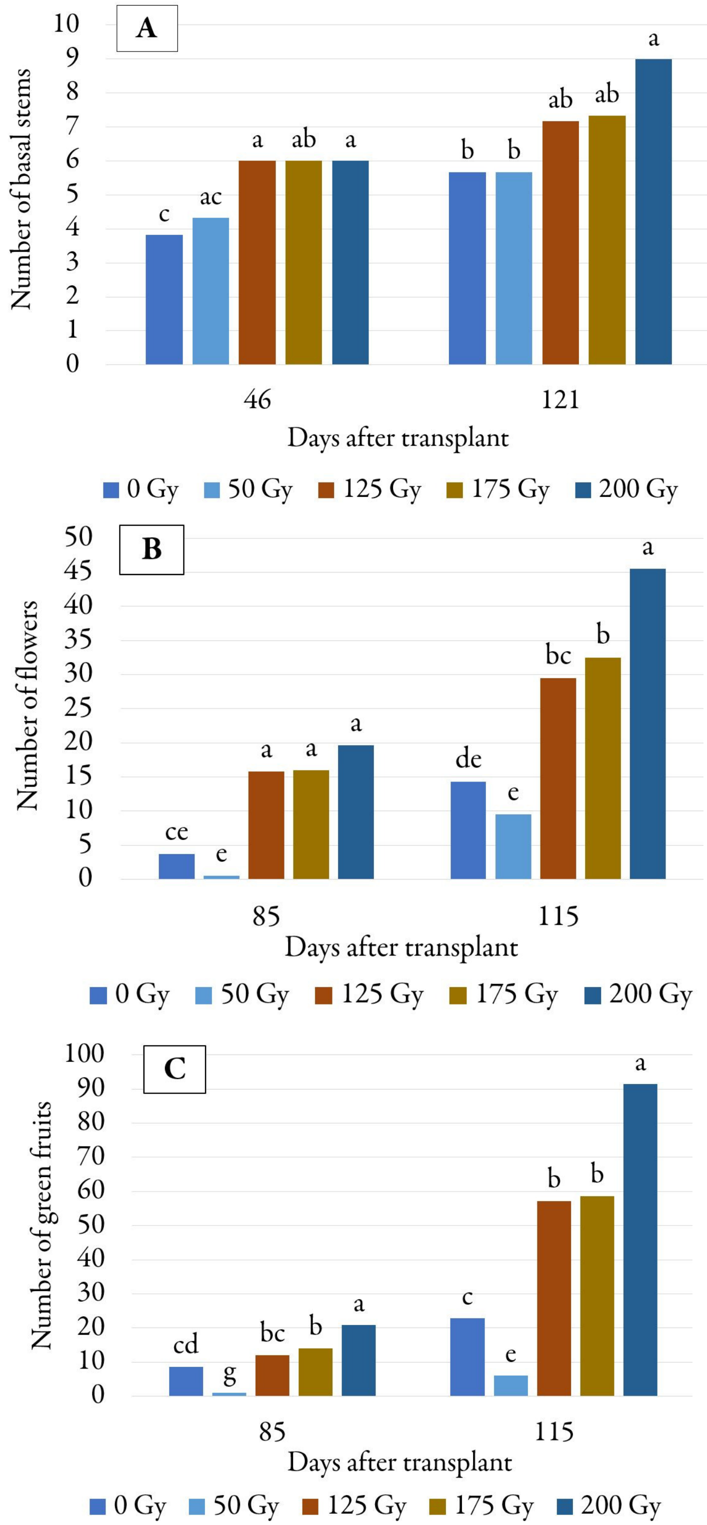

Figure 3. Examples of growth samplings × gamma-ray dose interaction for the number of basal stems (A), flowers (B) and green fruits (C) of M_1 uchuva plants. Bars with different small letter for each growth sampling (days after transplant) denote significant differences (Tukey, $p \leq 0.05$)

Conclusions

The application of ^{60}Co gamma rays (doses from 5 to 275 Gy) to seeds of *Physalis peruviana* L. reduce M_1 plants size. The stem diameter and the number of basal stems, floral buds, flowers and green fruits per plant increase during the growth cycle. The application of intermediate doses of gamma rays (125, 150, 175 and 200 Gy) stimulate vegetative growth and fruiting traits on M_1 uchuva plants, particularly at the dose of 200 Gy.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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