Effect of gamma rays on vegetative buds of *Physalis peruviana* L.

Efecto de rayos gamma sobre yemas vegetativas de Physalis peruviana L.

Diana Patricia Caro-Melgarejo[1]†, Sandra Yaneth Estupián-Rincón[1]‡, Leidy Yanira Rache-Cardenal[2]††, and José Constantino Pacheco-Maldonado [3][*]

[1]Biologist, Research GroupBIOPLASMA-UPTC. School of Biological Sciences. Faculty of Basic Sciences. Universidad Pedagógica y Tecnológica de Colombia. Tunja, Boyacá, Colombia. [2]Biologist, M.Sc., Professor Department of Biology and Microbiology, Universidad de Boyacá. Tunja, Boyacá, Colombia. [3]PhD in Biologyist, Senior School of Biological Sciences, Director Group BIOPLASMA-UPTC, Universidad Pedagógica y Tecnológica de Colombia. Tunja, Boyacá, Colombia. *Corresponding author: jocpach@gmail.com; †dianapcaro@yahoo.com; ‡sandraer22@hotmail.com ; ††leidyrache@yahoo.com

Abstract

In Colombia, studies carried out to establish modern methods for propagation and selection of cape gooseberry (*Physalis peruviana*) plant material are scarce, therefore it is necessary to explore alternative techniques such as the application of ionizing radiation, which enables greater genetic variability and to obtain seedlings with phenotypic characteristics that could be beneficial to contribute to improve productivity. To induce chromosomal alterations, axillary buds of *P. peruviana* with 50, 100, 200 and 300 Gy (Grays) of gamma radiation were irradiated and were cultured in MS medium supplemented with BA 0.1 mgL^{-1} plus IBA 0.05 mgL^{-1}. The experiment was conducted using a complete random design, five treatments each with thirty experimental units. After nine subcultures (9 multiplication cycles, 30 days each), regenerated shoots from irradiated buds presented smaller viability than the ones regenerated from non-irradiated buds. The regenerated plantlets from irradiated buds with 100 Gy had higher rooting percentage, shoot length and number of leaves; the dose of 300 Gy inhibited the development of shoots and roots, although the explants remained viable. In cells taken from radical tips of regenerated plantlets of irradiated buds it was observed bridges in anaphase and telophase, laggards and isolated chromosomes, being higher the frequency of laggard chromosomes. The higher percentage of cells with chromosomal alterations was quantified in radical tips of regenerated plantlets of buds irradiated with 200 Gy. In greenhouse, 86-93.8% of regenerated plantlets of irradiated or not irradiated buds were viable.

Key words: Cape gooseberry, chromosomal alterations, gamma radiation, in vitro culture, ionizing radiation, *Physalis*, plantlets.

Resumen

En Colombia los estudios para establecer métodos modernos de propagación y selección de materiales vegetativos de uchuva (*Physalis peruviana*) son escasos; por tanto, es necesario explorar técnicas alternativas como la aplicación de radiación ionizante, la cual permite ampliar la variabilidad genética, obtener plántulas con características fenotípicas favorables y contribuir al mejoramiento de la productividad. Con el objeto de inducir alteraciones cromosómicas, yemas axilares de *P. peruviana* cultivadas en medio MS suplementado con 0.1 mg/lt de BA y 0.05 mg/lt de AIB fueron irradiadas con 50, 100, 200 y 300 Grays (Gy) de radiación gamma. El experimento se llevó a cabo utilizando un diseo completamente al azar, con cinco tratamientos, cada uno con 30 unidades experimentales. Después de nueve subcultivos (ciclos de multiplicación de 30 días cada uno), los microtallos regenerados de yemas irradiadas presentaron menor viabilidad que los regenerados de yemas no irradiadas. Las plántulas

regeneradas de yemas irradiadas con 100 Gy presentaron mayor porcentaje de enraizamiento, longitud del tallo y número de hojas; la dosis de 300 Gy inhibió el desarrollo de tallos y primordios radicales, aunque los explantes permanecieron viables. En células de ápices radicales tomados de plántulas regeneradas de yemas irradiadas se observaron puentes en anafase y telofase, cromosomas rezagados y cromosomas aislados, siendo mayor la frecuencia de los cromosomas rezagados. El mayor porcentaje de células con alteraciones cromosómicas se cuantificó en ápices radicales de plántulas regeneradas de yemas irradiadas con 200 Gy. En invernadero, 86 93.8% de plántulas regeneradas de yemas irradiadas y no irradiadas fueron viables.

Palabras claves: Alteraciones cromosómicas, cultivo in vitro, plántulas, *Physalis*, radiación gamma, radiación ionizante, uchuva.

Introduction

Cape gooseberry (*Physalis peruviana* L.) has the second place on the Colombian exported fruits (Flórez *et al.,* 2000). Application of alternative propagation and selection methods is an option to improve fruit quality and reach a good acceptance by the consumers. In the last decades new techniques to study and manipulate plant material have emerged, making possible the genetic breeding of phenotypes favoring fruit production with better quality.

Cape gooseberry is propagated by sexual and asexual methods, being the first one the most used; however, for production of usable seedlings for commercial crop establishment this is not the most advised method, since the genetic variability of sexually originated plants can negatively affect important characters such as productivity (Almanza, 2000).

In vitro techniques are used in production programs and had advantages to obtain mutations. In these programs, meristematic cells or tissues and actively mitotic cells can be propagated in *in vitro* conditions establishing proliferative chains, meaning that axillar microshoots produced from meristematic cells or buds culture are subdivided on apical parts and node segments that serve as secondary explants to continue the propagation, this is in order to prepare enough material that can be subjected to mutagenic treatments (Suprasanna *et al.,* 2008). Induction of mutants by ionizing radiation is widely proposed for breeding of productive plants (Lu *et al.,* 2007). This radiation produces alterations in structure, phenotype and behavior of cells, tissues, organs and complete plants (Cervantes *et al.,* 1996). Such mutations allow increments on genetic variability and help the selection of plants with improved characteristics, that can favor the production of more acceptable fruits.

Growth and productivity patterns of plants can be easily modified by mutagenic agents (Raghava and Raghava, 1989). Additionally to the discovery of X Rays and the use of ionizing radiations such the gamma rays and neutrons, methodologies to induce useful variations on plant breeding have been established (Fuchs *et al.,* 2002).

Radiation can induce genomic instability on cells that is transmitted to their progeny by generations of cellular division with genetic effects on the following generations such as mutations and chromosomic aberrations (Little, 2006). In this sense it should be taken into account that exposition to high doses of radiation can induce several mutational events per cell, with the growing risk that a favorable mutation would be accompanied by non-desirable genetic changes (Otahola *et al.,* 2001). Fuchs *et al.* (2002), Lu *et al.* (2007), Hung ND Johnson (2008), Suprasanna *et al.* (2008), Yamaguchi *et al.* (2009) and Rodríguez (2004) performed cytogenetic studies focused on the karyotypic characterization and induction of numerical chromosome changes in *P. peruviana* and other species.

The objective of the present study was to induce morphological and cytogenetic changes using gamma rays on axillary buds of microshoots of cape gooseberry (*P. peruviana*) and to evaluate characters of interest that can favor production of better quality fruits.

Materials and methods

The research was performed in the Plant Ti-

ssue Culture Lab, Bioplasma, of the Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja.

Plant material selection

In commercial orchards of the town Arcabuco (Boyacá) (5°42'20" N, 73°25'59" W; 2739 MASL; 15 °C average temperature and, 1781.3 mm average rainfall), were selected healthy cape gooseberry plants with good fruit production, from which basal sprouts of 10 cm length were taken and wrapped on humid paper to be transported to the lab in plastic bags.

Superficial asepsis of sprouts

Sprouts were divided in nodal segments, washed with distilled water with Tween 20 (Merck), (0.25 ml/100 ml) for 5 min under constant agitation. Then they were submerged on 70% ethanol for 15 s and in 1.05% p/v sodium hypochlorite (NaOCl) for 30 min, then were washed three times with distilled water.

From the aseptic nodal segments were separated the axillary buds and cultured for 30 days in MS medium (Murashige and Skoog, 1962) supplemented with agar (oxoid) 6500 mg/lt, BA (6-benzylaminopurine) 0.1 mg/lt and IBA (indole-3-butyric acid) 0.05 mg/lt. Media pH was adjusted to 5.6 with NaOH or 1N HCl and were sterilized on autoclave at 1 kg/cm^2 pressure at 121 °C for 20 min. Cultures were incubated on growth chamber at 23 ± 1 °C under continuous light (70 - 80 µmol/m^2) with 75 Watt Sylvania fluorescent tubes. Once the microshoots were developed, they were divided on segmental nodes that were used to establish propagation chains.

Axillary buds exposition to gamma rays

In total it was taken 150 cauline nodal segments with an axillary bud that were originated in microshoots kept on propagation chains, were subjected to different doses of gamma rays produced by a ^{60}Co source located at the UPTC. Radiation doses applied to the explants were 50, 100, 200 and 300 Gy according to the times and distances required, that appeared in Table 1. To each radiation dose were exposed 30 axillary buds. During

Table 1. Time and distances used to irradiate axillary buds of *Physalis peruviana* with gamma rays.

Dose (Gy)	Time (h:min)	Distance (cm)
50	16:00	10
100	64:20	10
200	88:22	10
300	66:38	8

the radiation process the explants were kept on Petri dishes with MS medium.

Sprouting of seedlings from irradiated buds

Nodal segments with irradiated buds were cultured on MS media for nine subcultures of 30 days each, at the end the developed microshoots were used to establish propagation chains. After the first and ninth subculture macromorphological observations were made related to viability, rooting, stem length, leaf number and shape in regenerated plants from the propagation chains of irradiated and non-irradiated materials, additionally, roots were taken for observation and chromosomal analysis.

Seedlings hardening

In this phase, regenerated seedlings of the propagation chain of irradiated and non-irradiated materials, obtained after the ninth subculture, were transferred to greenhouse after being washed with tap water to remove agar in the roots. Then the seedlings were transferred to plastic cups with substrate composed of soil, sand and rice husk in 2:1:1 proportion, respectively.

Chromosomic alterations in root tip cells

Chromosome observations were done following the protocol proposed by Rodríguez (2004) and modified by Díaz *et al.* (2008). Verification of the presence of chromosomal alterations was performed on root tips of seedlings originated on irradiated and non-irradiated buds of the ninth subculture (270 days after bud irradiation). For each observation in each treatment were collected between 2 and 4 roots of 1 to 3 cm, between 14:00 and 16:00 h and from each one of them the 4 – 5 mm tip

was separated.

Isolated root tips were washed with distilled water and submerged on a 0.5% colchicine solution for 5 h at 4 °C. Next, they were washed with distilled water and submerged in Farmer solution (96% ethanol:glacial acetic acid, 3:1) for 24 h at 4 °C. Root tips that were not immediately use were stored for 30 days in 70% ethanol at 4 °C.

Next, the root tips were subjected to the action of an enzymatic mix composed of cellulose (3%), pectinase (1.5%) and macerozyme (1.0%) kept in the darkness, at 37 °C for 25 min in the water bath. To stop the enzymatic reaction, tips were washed with distilled water before isolating the meristematic zone (1 – 2 mm) that was placed on a microscope slide adding 2 drops of 2% acetic orcein for 25 min.

Stained meristematic tips were slightly flamed, a cover slip was placed on top of them and a snap with a rubber bar on the tissues was performed; then, with the help of paper towel, the slide was taken between the thumb and index to press without moving the cover slip and finally it was sealed with transparent nail polish. To select the slides with better cellular dispersion, observation were done at 25X and the analysis was done at 50X and 100X. To take pictures it was used a Carl Zeiss microscope, Jena Jenamed, with 50X and 100X objectives, 16X and 20X projectives and a Kodak easy c533 digital camera of 5.0 megapixels.

Statistical analysis

In total five treatments were performed (0, 50, 100, 200, 300 Gy), each one with 30 experimental units. *In vitro* rooting data are expressed as percentages of rooting explants after the first and ninth subcultures. Leaves/explant and stem length data were analyzed by Anova with a confidence level of 95%. For the factors that were statistically significant a DHS (honest significant difference) test was done. Data were processed with the statistical software Statgraphics Plus v. 2.0 (1996). Stem length was measured over sterile millimetric paper. To identify the presence of chromosomal alterations 100 slides were observed; each one contained one root where 10 fields were observed. Data were expressed as total percentage of cells with alterations and of cells revealing a specific alteration in relation to the number of cells in division per treatment.

Results and discussion

Superficial asepsis of sprouts

The protocol used for asepsis of the nodal segments coming from field seedlings sprouts allowed the recovery of 73% of aseptic explants with elimination of superficial contaminants, allowing in vitro propagation of the nodal segments and later obteinment of buds for irradiation. Similar asepsis protocols to establish *in vitro* culture were used in species of the genus *Physalis* using NaOCl in *P. peruviana* (Díaz *et al.*, 2008) and in *P. ixocarpa* (Ramírez and Ochoa, 1991; Contreras and Almeida, 2003) and mercuric chloride ($HgCl_2$) in *P. pubescens* (Rao *et al.*, 2004) as disinfectant agent.

Axillary buds cultured on MS media that were taken from nodal segments of plant sprouts in the field, facilitated the development of microshoots used to establish the propagation chains with subcultures each 30 days. Díaz *et al.* (2008) found a good development of *P. peruviana* buds cultured on MS media with BA and IBA.

Seedling regeneration from irradiated buds

Despite of the precautions kept during culture, transfer and exposition of the explants to the ^{60}Co source, some irradiated buds were contaminated with fungi and/or bacteria; besides contamination, radiation exposition contributed to the death of some buds. Buds irradiated with 300 Gy did not develop stems neither root primordias and after three subcultures they died. According to Fuchs *et al.* (2002) gamma rays, being a ionizing radiation, have high penetration capacity and their lethal action in cells is measured as loss of mitotic activity caused by chromosomal aberrations. In this sense, one of the most important and difficult aspects of radio-protection is to establish the limits between low and high doses (Zaka *et al.*, 2002). Certainly, the establishment of a limit is a controversial topic since it highly depends on the sensibility of the species, individual and even on tissue or cell. Raghava and Raghava (1989) considered

that high doses of radiation inhibit growth regulator synthesis. Reduction in explants survival when increasing radiation doses was in the following way: with 50 Gy viability was 60%, with 100 Gy 73%, with 200 Gy 10% and with 300 Gy 0%, noticing that in low (50 Gy) and high (200 and 300 Gy) doses viability was reduced. Low responses of regeneration to high radiation doses can be attributed to the toxic effect of the gamma radiation on embriogenic or organogenic cells and to the loss of competitiveness between these cells and their progeny (Suprasanna *et al.*, 2008). The obtained results agree in part with observations in *Narcissus tazetta* var. *chinensis* (Lu *et al.*, 2007), *Wasabia japonica* (Hung and Johnson, 2008) and *Chrysanthemum morifolium* (Yamaguchi *et al.*, 2009), in which it was found that high radiation doses can cause high mutation frequencies but low proportions of regenerating material in *in vitro* culture. Additionally, the authors established that the probability of survival reduction is due to the fact that in vitro callus and tissues are more sensitive to radiation treatments and thus, require very low doses (5-15 Gy) compared to high doses used for seeds and bulbs (Lu *et al.*, 2007).

Regenerated seedlings from irradiated and non-irradiated buds showed different rooting responses. During the first subculture, a rhizogenic response was found in two microshoots treatments from non-irradiated and irradiated buds with 50 Gy found 65% and 3.3% of explants with roots, respectively. In the ninth subculture, rhizogenic responses were observed in all the treatments being

higher on regenerated seedlings from exposed buds to 50 Gy and non-irradiated buds (100 to 96%, respectively) (Table 2). In the same subculture differences were observed ($P < 0.05$) in the number of leaves grown on seedlings from irradiated and non-irradiated with an average of 3.7 leaves/explant in the 100 Gy treatment (Table 2).

In all the subcultures, the regenerated seedlings from irradiated buds showed shorter stems (1.8 – 2.2 cm) in comparison to non-irradiated seedlings, however, in the ninth subculture seedlings showed stems of different length on a range of 2.99 to 7.8 cm (Table 2). Similar results to the ones found in this study were found in cicer (Toker *et al.*, 2005), *Wasabia japonica* (Hung and Johnson, 2008) and sugar cane (Suprasanna *et al.*, 2008); however, they are contrasting with the ones of Rekha and Langer (2007) in *Artemisia pallens* who registered shorter stems in the control plants than in the irradiated ones. It is possible that the increase in microshoots length of the seedlings induced by low gamma rays doses (50 and 100 Gy) could be the result of the promoting effect of these doses, that induce auxin synthesis and other regulators and consequently, activate dormant growth points (Otahola, 1999; Wi *et al.*, 2007).

Differences in rooting percentages between treatments, together with average of leaves/explant and stem length at the end of the ninth subculture, are due to the inhibition or stimulus of the physiological and biochemical processes by the ionizing radiation that

Table 2. Macromorphological observations in microplants of *Physalis peruviana* regenerated from irradiated and non-irradiated buds.

Dosis (Gy)	Explants rooted in subculture (%)		Average of leaves (no.)		Average stem length (cm)	
	first	ninth	first	ninth	first	ninth
50	3.3	100	2.0±0.5	2.9±1.7[ab†]	0.3±0.3	4.2±1.7[a]
100	0	95	3.5±1.3	3.7±1.3[a]	0.7±0.2	5.8±1.8[ab]
200	0	70	1.4±0.3	2.4±0.5[b]	0.3±0.2	2.99±1.4[a]
300	0	0	0	0	0	0
Control	65	96	3.3±1.5	3.6±1.7[a]	2.5±0.9	7.8±:.1[b]

[†] Values in the same column followed by different letters are significantly different ($P < 0.05$).

depends on the applied dose (Raghava and Raghava, 1990).

Regenerated seedlings from irradiated and non-irradiated buds presented leaves with different shapes: oval, elongated, deformed and fused heart shapes, and smooth and wavy edges (Picture 1). The highest percentage of seedlings with abnormal leaves was observed in the treatment with 200 Gy (47%), whereas in the 50 and 100 Gy treatments and without irradiation, this percentage was 29%, 15% and 12%, respectively (Table 3). Hung and Johnson (2008) found similar variations in shape and size of irradiated leaves of *Wasabia japonica.*

Hardening of seedlings

After 90 days from the beginning of the experiment, hardening of seedlings from irradiated buds was 86.18% and 92.3% and from non-irradiated buds was 93.8% (Table 3). These values are similar with the ones obtained by Hung and Johnson (2008) in *W. japonica* under greenhouse conditions, which were 72% and 92% of seedlings hardened after gamma radiation.

## Chromosomal alterations on root tip cells

Some cells from meristematic tips showed chromosomal alterations in different cycle phases, mainly in anaphase, bridges and laggard chromosomes (Picture 2a and Picture 2c); in telophase, bridges (Picture 2b) and in metaphase and anaphase, isolated chromosomes (Picture 2d). Chromosomal alterations during anaphase indicate that radiation specially affects the achromatic spindle function. Bridges are generally due to formation of dicentric chromosomes originated for chromo-

Table 3. Percentage of *Physalis peruviana* seedlings with leaves with abnormalities and of hardened plants regenerated from irradiated and non-irradiated buds on the ninth subculture.

Dose (Gy)	Seedlings with abnormal leaves (%)	Hardened plants (%)
50	29.4	86.1
100	15.0	92.7
200	46.7	92.3
Control	12.0	93.8

some interchange after breaking of the double helix of DNA (Zaka *et al.,* 2002). Accoding to Little (2006) the breaking of that DNA strand is considered as a characteristic lesion responsible of the biological effects of the ionizing radiation. In the studies of Zaka *et al.* (2002) with *P. sativum* were found bridges and lagging chromosomes during anaphase and telophase.

In Table 4 are included the percentages of cells showing alterations in relation to the number of dividing cells, the highest percentage (4.5%) of altered cells was found with the 200 Gy treatment and, the smallest (2.7%) was in the one with100 Gy. This demonstrated that as the radiation doses increase the presence of altered cells increases till certain dose in which a lethal effect on cells is observed; in this study this lethal effect was shown with the 300 Gy dose. Similar results were obtained by Zaka *et al.* (2002) who established a lineal relation for the dose-effect variables that shows an increment in the presence of chromosomic aberrations according to the increase in radiation dose. The low percentages (2.7-4.5) of altered cells that were observed in this work with respect to the ones
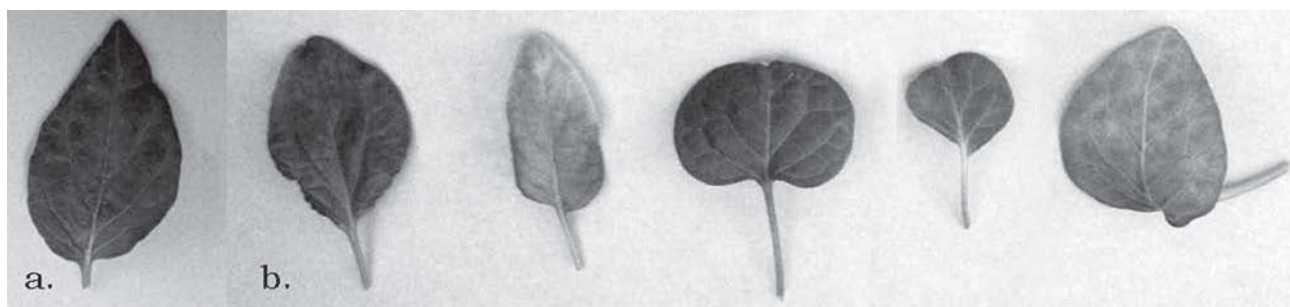
Picture 1. Developed leaves on *Physalis peruviana* seedlings regenerated from irradiated buds **(a)** normal leaf; **(b)** abnormal leaves.
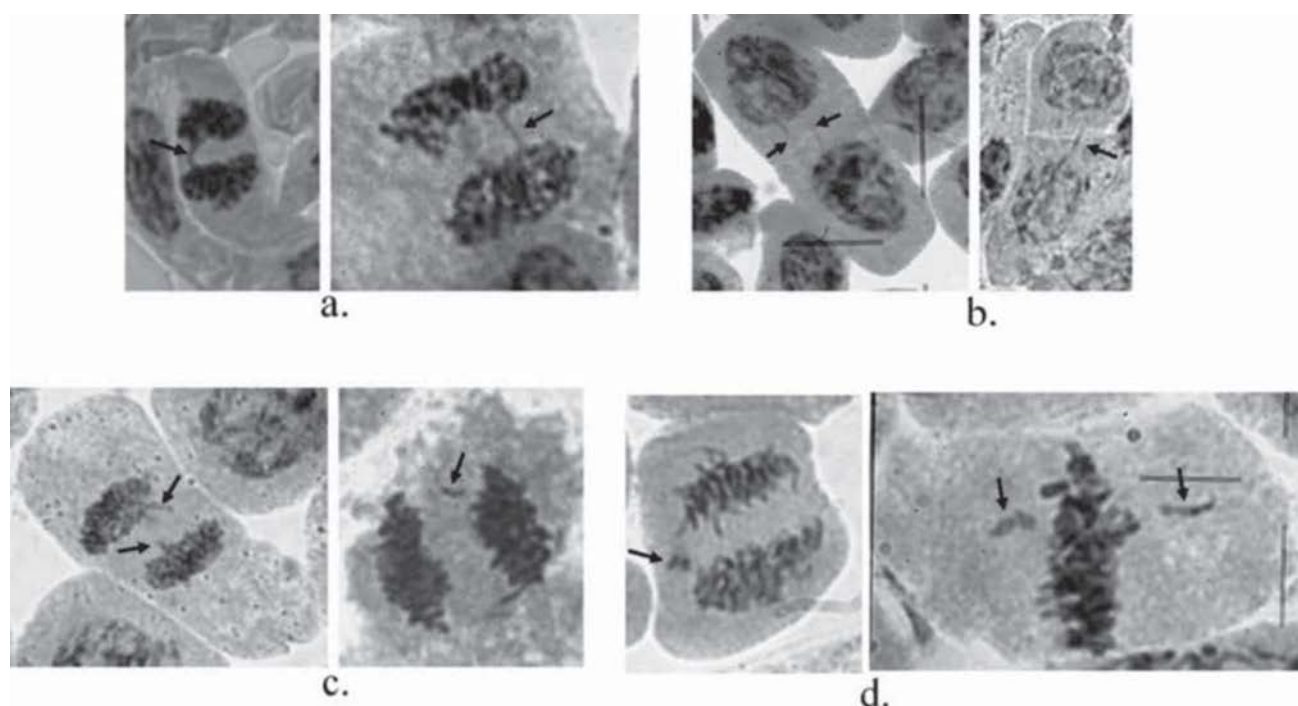
Table 4. Percentage of root tip cells taken from *Physalis peruviana* seedlings regenerated from irradiated and non-irradiated buds that presented chromosomic alterations.

Dose (Gy)	Cells in division (no)	Altered cells (%)
50	1658	3.0
100	2046	2.7
200	1844	4.5
Control	1950	1.1

obtained by Zaka *et al.* (2002) (15% in 2 Gy dose and 20% in 3-10 Gy doses), could be due to differences in radiation doses used, to the sensibility of each species, to the tissue type and/or cells in each tissue subjected to radiation. Additionally, it is possible that only one small percentage of chromosomal aberrations during the first mitosis have appeared on the irradiated cells able to divide, while the other chromosomal aberrations are integrated in the genome and is gradually expressed in the following cell divisions (Zaka *et al.,* 2002).

Among the treatments, the most frequent chromosomal alterations (1.0, 1.5 and 0.2%) (Table 5) were quantified in cells of buds treated with 200 Gy showing bridges, both in anaphase and telophase, and isolated chromosomes, respectively. The highest frequency of lagging chromosomes (1.9%) was found in cells of buds treated with 100 Gy. On the other hand, similar frequencies of chromosomal alterations were found on non-irradiated buds and 100 Gy dose for anaphase bridges (0.4%), in 50 Gy and telophase bridges (0.2%) and in 50 and 100 Gy for isolated chromosomes (0.1%). Fuchs *et al.* (2002) consider that when physical or chemical mutagenic agents are applied in material for asexual or sexual propagation, there is a direct relation between dose and cellular damage. This agrees with the results of this experiment since a similar or directly proportional relation was observed, thus, at higher irradiation doses higher cellular damage, including the 300 Gy dose caused cell death. In this sense, Otahola *et al.* (2001) indicate that in increasing expositions to gamma radiation the mutational frequency increases and survival decreases. According to Kovács and Keresztes (2002) when ionizing radiation is absorbed by

Picture 2. Chromosome alterations on root tips of *Physalis peruviana* seedlings regenerated from irradiated and non-irradiated buds. **(a)** bridge in anaphase; **(b)** bridge in telophase; **(c)** lagging chromosomes; **(d)** isolated chromosomes.

Table 5. Chromosomic alterations and number of quantified cells in root tips taken from *Physalis peruviana* seedlings regenerated from irradiated and non-irradiated buds.

Alterations	50 Gy		100 Gy		200 Gy		Control	
	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)
Bridges in anaphase	16	1.0	8	0.4	19	1.0	8	0.4
Bridges in telophase	4	0.2	9	0.4	:8	1.5	3	0.2
Laggard chromosomes	29	1.8	M8	1.9	31	1.7	10	0.5
Isolated chromosomes	1	0.1	1	0.1	4	0.2	1	0.1

biological materials, there is a possibility that it acts directly on specific point in the cell, particularly water, producing free radicals that can be diffused and damaging important components for the same cell.

In general, on cells of non-irradiated buds the alteration frequency was low compared to the ones found on cells from irradiated buds (Table 5 and Picture 2). Presence of alterations in cells of non-irradiated materials could be considered as an effect of spontaneous mutations. According to Poehlman and Allen (2003) there is not a clear difference between spontaneous and induced mutations; what seems to be a spontaneous mutation could be an induced one, because all the plants in nature are subjected to low doses of natural radiation.

As general observation, the results obtained in this study indicate that lagging chromosomes was the most frequent alteration in all the treatments, whereas isolated chromosomes was the less frequent alteration (Table 5).

Conclusions

- With the 100 Gy radiation dose was obtained the highest percentage of bud viability, microshoot rooting, number of leaves per explant, stem length, hardening percentage and lagging chromosomes on *Physalis peruviana.*

- Radiation doses between 100 and 200 Gy are suitable to be evaluated in future investigations, since at increasing radiations there is an increment in alteration frequency on the exposed cells but it also re-duce viability, rooting, number of leaves per explant, stem length and hardening of regenerated plants.

- In cells of root tips of irradiated buds regenerants, were observed chromosome alterations such as bridges in anaphase and telophase, isolated chromosomes, and lagging chromosomes, being the last ones the most frequent.

Acknowledgments

References

Almanza, P. J. 2000. Propagación. In: Flórez, R. V.; Fischer, G. y Sora R., A. (eds.). Producción, poscosecha y exportación de la uchuva (*Physalis peruviana* L.). First edition. Universidad Nacional de Colombia, Unibiblos, Bogotá. p. 27 - 40.

Cervantes, L.; Báez, J.; and Fernández, R. 1996. Efectos de radiación gamma en plantas de *Diantus* sp. propagadas por cultivo in vitro. Rev Cien. Des. 2 (3):46 - 54.

Contreras, I. and Almeida, J. 2003. Micropropagación del tomatillo (*Physalis ixocarpa* L.). Rev. Fac. Farm. 45(1):61 - 64.

Díaz, D. E.; González, D. C.; Rache, L. Y.; and Pacheco, J. 2008. Efecto citogenético de la colchicina sobre yemas vegetativas de *Physalis peruviana* L. Prospec Cient. 4:27 - 40.

Flórez, V. J.; Fischer, G.; and Sora, A. D. (eds.). 2000. Producción, poscosecha y exportación de la Uchuva (*Physalis peruviana* L.). Bogotá. First edition. Universidad Nacional de Colombia, Unibiblos. 175 p.

Fuchs, M.; González, V.; Castroni, S.; Díaz, E.; and 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.

Hung, C. D. and Johnson, K. 2008. Effects of ionizing radiation on the growth and allyl isothiocyanate accumulation of *Wasabia japonica in vitro* and *ex vitro*. In: Vitro Cell Develop. Biol. - Plant. 44:51 - 58.

Kovács, E. and Keresztes, A. 2002. Effect of gamma and UV-B/C radiation on plant cells. Micron. 33:199 - 210.

Little, J. 2006. Cellular radiation effects and the bystander response. Mut. Res. 597:113 - 118.

Lu, G.; Zhang, X.; Zou, Y.; Zou, Q.; Xiang, X.; and Cao, J. 2007. Effect of radiation on regeneration of *Chinese narcissus* and analysis of genetic variation with AFLP and RAPD markers. Plant Cell Tiss Organ. Cult. 88:319 - 327.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 15:473 - 493.

Otahola, G. V. 1999. Radiosesibilidad de las semillas y diferentes tipos de explantes en cultivo *in vitro* de parchita (*Passiflora edulis* f. *flavicarpa* Deg.) a radiaciones gamma. Master thesis. Centro de Estudios de Posgrado del núcleo Monagas, Venezuela. 155 p.

Otahola, G. V.; Aray, M.; and Antoima, Y. 2001. Inducción de mutantes para el color de la flor en crisantemos (*Dendranthema grandiflora* (Ram.) Tzvelev) mediante radiaciones gamma. Rev UDO Agríc. 1(1):56 - 63.

Poehlman, J. and Allen, D. 2003. Mejoramiento genético de las cosechas. México, D.F. Segunda Edición. Editorial Limusa S.A. 511 p.

Raghava, R. P. and Raghava, N. 1989. Effects of gamma irradiation on fresh and dry weights of plant parts in *Physalis* L. Geobios. 16:261 - 264.

Raghava, R. P. and Raghava, N. 1990. Carotenoid content of husk tomato under the influence or growth regulators and gamma rays. Indian J. Plant Physiol. 33(1):87 - 89.

Ramírez, M. R. and Ochoa A. N. 1991. Adventitious shoot formation and plant regeneration from tissues of tomatillo (Physalis ixocarpa Brot.). Plant Cell Tiss. Organ. Cult. 25:185 - 188.

Rao, Y. V.; Ravi, A.; Lakshmi, T. V. R.; and Raja, K. G. 2004. Plant regeneration in Physalis pubescens L. and its induced mutant. Plant Tiss. Cult. 14 (1):9 - 15.

Rekha, K. and Langer, A. 2007. Induction and assessment of morpho-biochemical mutants in *Artemisia pallens* Bess. Genet. Resour. Crop Evol. 54:437 - 443.

Rodríguez, N. 2004. Estudio citogenético en *Physalis peruviana* L.: Uchuva (Solanaceae). Bogotá. Undergraduat thesis (Biology). Universidad Nacional de Colombia-Bogotá. Bogotá, Colombia. 78 p.

Statgraphics Plus for windows. 1996. Manugistics, Inc, Rockville, MD. Version 2

Suprasanna, P.; Rupali, C.; Desai, N. S.; and Bapat, V. A. 2008. Partial desiccation augments plant regeneration from irradiated embryogenic cultures of sugarcane. Plant Cell Tiss. Organ Cult. 92:101 - 105.

Toker, C.; Uzun, B.; Canci, H.; and Ceylan, F. O. 2005. Effects of gamma irradiation on the shoot length of Cicer seeds. Radiat. Phys. Chem. 73:365 - 367.

Wi, S. G.; Chung, B. Y.; Kim, J. S.; Kim, J. H.; Baek, M. H.; Lee, J. W.; and Kim, Y. S. 2007. Effects of gamma irradiation on morphological changes and biological responses in plants. Micron. 38:553 - 564.

Yamaguchi, H.; Shimizu, A.; Hase, Y.; Degi, K.; Tanaka, A.; and Morishita, T. 2009. Mutation induction with ion beam irradiation of lateral buds of chrysanthemum and analysis of chimeric structure of induced mutants. Euphytica 165:97 - 103.

Zaka, R.; Chenal, C.; and Misset, M. T. 2002. Study of external low irradiation dose effects on induction of chromosome aberrations in *Pisum sativum* root tip meristem. Mutat. Res. 517:87 - 99.