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Effects of ionizing radiation on *Capsicum baccatum* var. *pendulum* (Solanaceae)

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HIGHLIGHTS

- Cytogenetic and somatic effects of x-rays treatments in *Capsicum* were evaluated.
- Frequencies of chromosome aberrations correlated with radiation doses.
- Highest frequency of chromosome aberrations occurred with 20 Gy+soaking seeds.
- In TUNEL test, the nuclei with DNA fragmentation were higher than in the control.
- The strongest effects were observed with doses of 300 Gy or 20 Gy after soaking.

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ABSTRACT

Cytogenetic and somatic effects of various x-ray treatments were evaluated in pepper, *Capsicum baccatum* var. *pendulum* cv. "Cayenne", with the aim to assess optimal conditions for obtaining viable lines. The cytogenetic effects were quantified by counting chromosome aberrations. The level of DNA fragmentation was estimated with TUNEL test (terminal transferase mediated dUTP-fluorescein nick end labeling). Irradiation to 20 Gy with 16-h presoaking can be a suitable treatment of the selected pepper cultivar for a mutagenesis program.

1. Introduction

Capsicum L. (tribe Solaneae, subtribe Capsicinae) is an important American genus, which grows in tropical and temperate regions from south Mexico to the center of Argentina. It comprises of approximately 32 species with a few varieties, including five cultivated by humans and consumed as vegetables and spices ("pepper", "chili"). Induced mutations in plants have wide applications, not only in basic genetic research, but also in plant breeding programs. Recent studies of experimentally induced mutations (Ahloowalia and Maluszynski, 2001) were successful in major crops (e. g., wheat, rice, barley, cotton, peanuts, or beans). Many authors have been studying the effect of ionizing radiation and chemical mutagens in order to estimate the sensitivity of the *Capsicum* cultivars to mutagens, to select mutants for breeding programs, to increase the allele gene bank, and to enrich knowledge of linkage groups (Saccardo and Sree Ramulu, 1977; Daskalov, 1986). Most contributions regarding induced mutagenesis in chili pepper refer to gene mutations. There are only few studies of the somatic and cytogenetic effects in this species (Katiyar, 1977, 1978; Indira and Abraham, 1977; Kumar and Raja Rao, 2003). Data on the effects of x-rays on the *C. baccatum* var. *pendulum* cultivar "Cayenne" ($2n=24$) are also lacking.

The cultivar "Cayenne" is commercially used as human food in northwestern Argentina (Scaldaferro et al., 2004). Ionizing radiation is a very effective, widely accepted way to induce structural chromosome rearrangements (Gaul, 1977).

This paper describes the cytological and somatic effects in the selected cultivar of pepper induced by various doses of x-rays. The objective of this work was to find the optimal dose of ionizing radiation by the cytogenetic and TUNEL test techniques in order to assess the sensitivity of the *Capsicum* cultivar "Cayenne" to the

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x-ray mutagen. This study is important for improvement of breeding programs.

2. Materials and methods

2.1. Plant material and treatment

Dry seeds of *Capsicum baccatum* var. *pendulum* (Willd.) Eshbaugh cultivar "Cayenne" ($2n=2x=24$) were treated with different acute doses of x-rays in the dose range reported for other peppers in previous mutation experiments, namely, 100, 200, and 300 Gy (Daskalov, 1986). Additionally, one sample of seeds was treated with 20 Gy after being soaked in water for 16 h.

2.2. Analysis of cytogenetic and somatic effects

In order to estimate the cytological effects of x-rays, 50 seeds per treatment (100 Gy, 200 Gy, 300 Gy, and 20 Gy+16-h soaking in water), including a control, were germinated on filter paper in Petri dishes at 24 °C. The seedling roots of about 3–4 mm in length were fixed in ethanol–glacial acetic acid (3:1) and stained using the Feulgen method (Gaul, 1977; Prina, 1989; Jong, 1997). In each preparation, the frequency of cells with aberrations (bridges and fragments) in anaphase/telophase was analyzed (2000–4000 cells were scored for each x-ray treatment). Chromosomes were observed and photographed in transmitted light using a Leica DMLB microscope equipped with Leica DC250 digital camera and Leica IM1000 image management system.

Additionally, with the purpose to assess the somatic effects induced by various doses of x-rays in the first generation (M_1), 50 seeds per each experimental group were germinated under controlled conditions in a growth chamber at 27/18 °C (day/night). Various somatic parameters were measured in order to evaluate the somatic effects of x-rays, namely, frequency of germinated seeds, frequency of growing plants, stem length (cm) 20 days after germination, frequency of plants with flowers, number of fruits per plant, number of seeds per fruit, and fruit length.

2.3. TUNEL test

In order to visualize the DNA damage induced by x-rays (100 Gy, 200 Gy, 300 Gy, and 20 Gy+16 h soaking in water) in *C. baccatum* var. *pendulum* cv. "Cayenne" cells, the frequency of nuclei with DNA fragmentation was estimated in M_1 embryos and seedling root cells using TUNEL test (terminal transferase mediated dUTP-fluorescein nick end labeling). The embryos from seeds that had been pre-soaked for 1 h and roots of different lengths (12, 17, 22 mm) were used for TUNEL test.

The procedure for TUNEL test was adapted and used according to Juchimiuk and Maluszynska (2003, 2005). Isolated embryos and 10-mm root tips were fixed with freshly prepared 4% paraformaldehyde (Fluka) for 1 h at room temperature. The fixed material was washed 3×5 min in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.8 mM KH_2PO_4). Slides were prepared by squashing the root meristems or embryos in a PBS buffer without enzymatic digestion. Three roots or one embryo were used to make one slide. The slides were frozen at −70 °C and stored at 4 °C for several days. Cell permeabilization was done by incubating the slides in 0.1% Triton X-100 (Sigma) in 0.1% sodium citrate for 2 min on ice (4 °C). Slides were then rinsed with PBS. DNA fragment labeling was carried out using the TUNEL reaction mixture (*in situ* Cell Death Detection Kit, Fluorescein, Roche). A 50 μL TUNEL reaction mixture (enzyme solution, terminal transferase: label solution, 1: 9, v/v) was applied to the slides, which were then incubated for 1 h at 37 °C in a humid chamber in the dark. The positive control was treated with a 50 μL of a DNAse solution (1 U), which was applied to one slide of the control sample and incubated for 30 min at 37 °C in the humid chamber. The slides were rinsed with PBS twice, and DNA fragment labeling was carried out. As a negative control of the TUNEL reaction, a mixture without terminal transferase was used. After the TUNEL reaction, slides were rinsed 3 times with

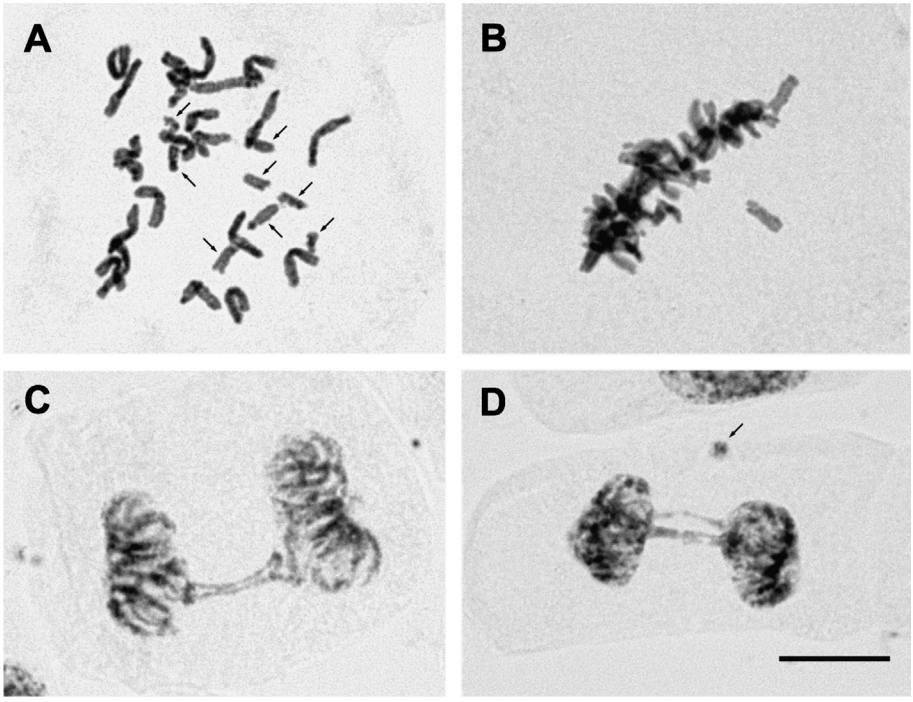

Fig. 1. Mitotic chromosome aberrations in *Capsicum baccatum* var. *pendulum* cv. "Cayenne" ($2n=24$) root meristematic cells after x-ray irradiation of seeds. (A): Metaphase with eight chromosome fragments. (B): Metaphase with one chromosome outside plate. (C–D): Late anaphases with bridges; (C): double bridge; and (D): three bridges and an acentric fragment. Arrows indicate fragments. The bar represents 10 μm.

PBS, stained with DAPI (2 μg/mL), air-dried, and mounted in Citifluor. The slides were evaluated using an Olympus fluorescence microscope with an FITC filter (with a 495 nm excitation filter and a 525 nm barrier filter) and a DAPI filter (with a 355 nm excitation filter and a 450 nm barrier filter). Labeled nuclei were counted, and the total number of analyzed nuclei was determined. The frequency of the labeled cells was calculated based on 2000 cells, which were analyzed on two slides for each treatment.

2.4. Statistics

The experimental data were analyzed with the INFOSTAT program, Version 1.1 (Grupo Infostat, 2002). The Pearson correlation coefficient (Sokal and Rohlf, 1995) was used to estimate simple correlations between the frequency of abnormal anaphases and the number of germinated seeds or surviving plants at various x-ray doses.

3. Results

Fig. 1 and Table 1 show cytogenetic effects of x-rays on root meristematic cells of *C. baccatum* var. *pendulum* cv. "Cayenne". After treatment with x-rays, we identified chromosome fragments (Fig. 1A) and laggards (Fig. 1B) in the metaphase, bridges (Fig. 1C, D), and fragments (Fig. 1D) in the anaphase/telophase. The frequency of cells with chromosome aberrations was the highest, 46.9%, when a 16-h presoaking of seeds was followed by irradiation to 20 Gy; it was also high, 45.8%, when dry seeds were irradiated to 300 Gy. The lowest x-ray dose, 100 Gy, induced chromosome aberrations in 21.9% of the anaphase/telophase cells. The frequency of chromosome aberrations found in the anaphase correlated with the radiation dose ($r=0.99$; $p=0.01$) (Fig. 4A). In the control cells, the frequency of chromosome aberrations was only 1.1%. In this work, only one sample of seeds was irradiated to 20 Gy after being soaked in water for 16 h, because radiation susceptibility of a biological system increases with an increase of water content (Stadler, 1928). It is widely accepted that irradiation of soaked seeds results in a much greater chromosome damage than irradiation of dry ones. Irradiated water is very reactive due to radiation-induced free radicals. That results in enhanced indirect effects in soaked seeds..

Our data clearly show a positive linear correlation between the cytological effects and the radiation dose. The frequency of chromosome bridges in the anaphase increased linearly with the radiation dose also in other species, e. g., barley (Caldecott et al., 1952, 1954).

TUNEL test was used to analyze DNA fragmentation in the embryo and meristematic cells of the 12-, 17-, and 22-mm-long roots. The results revealed that the used doses of x-rays induced DNA breakage (Fig. 2, Table 2). The green-labeled nuclei indicate the presence of DNA fragmentation. In general, the intensity of the fluorescence of the nuclei after the irradiation was low, which indicated a low level of DNA fragmentation.

Table 1
Cytogenetic effects in the M_1 generation of *Capsicum baccatum* var. *pendulum* cv. "Cayenne" after irradiation of seeds to different x-ray doses *.

Radiation dose, (Gy)	Percentage of cells with bridges	Percentage of cells with fragments	Total percentage of cells with chromosome aberrations
0 (Control)	0.5	0.7	1.1
100	14.5	7.4	21.9
200	13.5	18.0	31.5
300	18.9	26.9	45.7
20 (with 16h presoaking)	24.6	22.4	46.9

* 50 seedlings were analyzed per treatment.

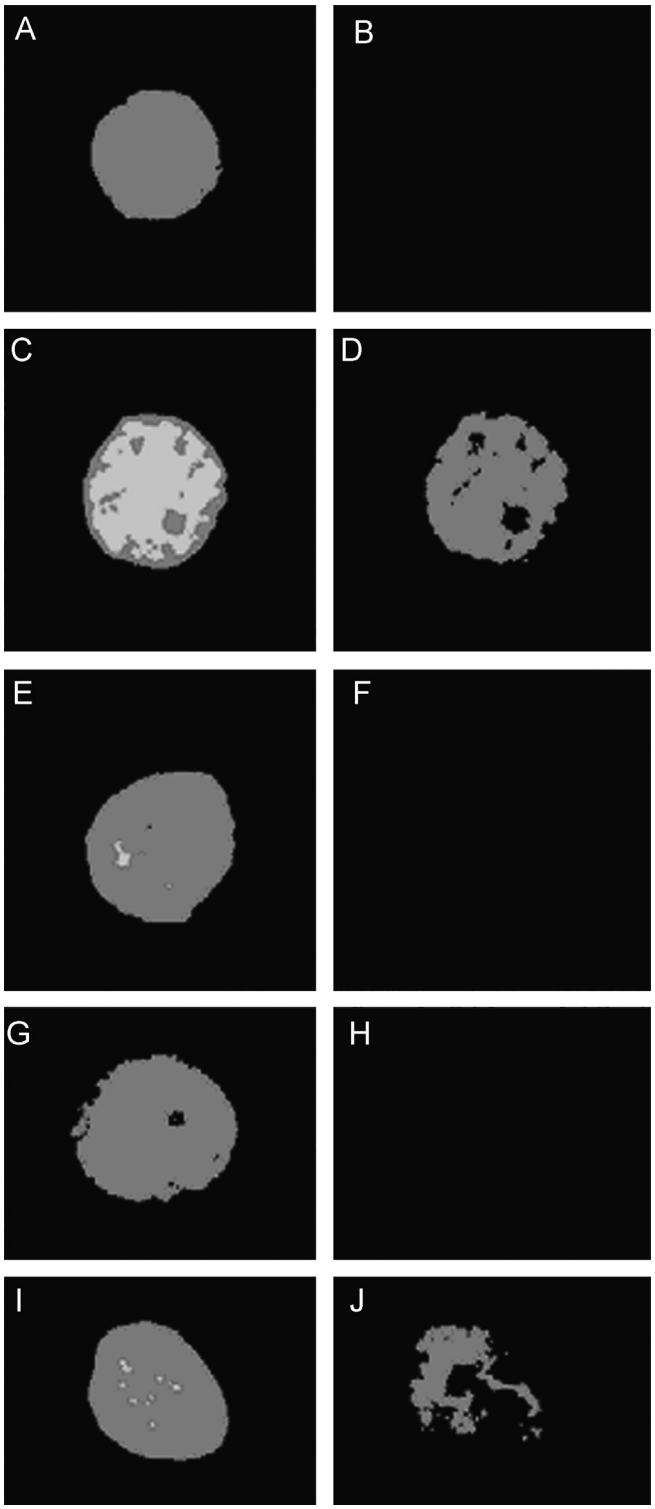

Fig. 2. Results of TUNEL test in *Capsicum baccatum* var. *pendulum* cv. "Cayenne" root interphase nuclei after irradiation to various x-ray doses. A, C, E, G, I: DAPI staining, all nuclei are stained. B, D, F, H, J: Nuclei with or without green fluorescence, which corresponds to-FITC-labeled DNA, as a result of TUNEL reaction. (A–B): Negative in an unirradiated line plant control (nucleotide solution without terminal transferase was used in the TUNEL reaction). (C–D): Positive control (DNA solution used to induce DNA strand breaks) in an unirradiated line plant. (E–F): Unirradiated control line plant. (G–H): After irradiation to 100 Gy. I: After irradiation to 200 Gy. J: After irradiation to 20 Gy with presoaking.

Fig. 3A shows the frequencies of embryo labeled nuclei in TUNEL test, while Fig. 3B provides the frequencies of labeled nuclei of root meristematic cells. The dose 20 Gy to the presoaked seeds induced DNA fragmentation in 56.3% of the embryo nuclei, but the dose 100 Gy had the effect only in 66% of them.

Table 2
TUNEL test for the embryo and primary root cells from irradiated *Capsicum baccatum* var. *pendulum* cv. "Cayenne" seeds.

Tissue	Frequency of labeled nuclei (%)				
	Control line (no irradiation)	20 Gy with 16-h pre-soaking	100 Gy	200 Gy	300 Gy
Embryo	11.4	56.3	66.0	74.7	76.4
Root					
12-mm long	4.3	29.8	14.1	16.3	60.6
17-mm long	6.1	16.4	12.2	16.1	29.7
22-mm long	5.8	10.3	8.7	9.6	14.7

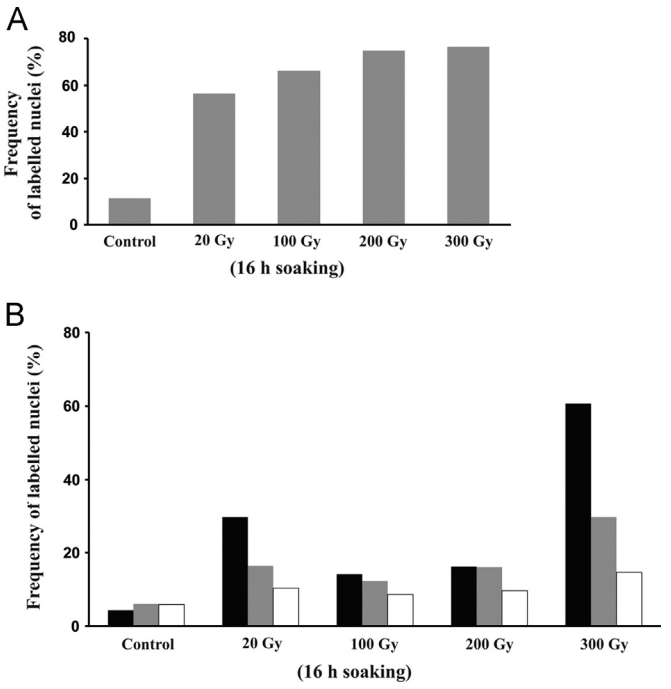

Fig. 3. Frequency of labeled nuclei in TUNEL test in *Capsicum baccatum* var. *pendulum* cv. "Cayenne" embryo (A) and primary root meristematic cells (B) after irradiation of seeds to various doses. The control represents unirradiated line plants. In (B), different root lengths are represented in the graph by black (12 mm), gray (17 mm) and white (22 mm) color.

The frequencies of FITC-labeled embryo nuclei after irradiation to 200 and 300 Gy were similar, namely, 74.7% and 76.4%, respectively.

When dry seeds were irradiated to a 5-fold higher dose of x-rays than the presoaked ones, the frequency of labeled nuclei was only 10% higher. Identical x-ray doses resulted in lower frequencies of labeled nuclei in the root meristematic cells than in the embryo cells. After an irradiation of presoaked seeds to 20 Gy, the frequency of labeled nuclei was 56.3% in embryo cells, but only 29.8% in 12-mm-long roots. However, after an irradiation to 300 Gy, the frequency of labeled nuclei decreased only from 76.4% in the embryo cells to 60.6% in roots. A comparison of the frequencies of the labeled nuclei in roots of different lengths showed the highest frequency of DNA-damaged nuclei in 12-cm-long roots. The frequency of labeled nuclei was the highest after an irradiation to 300 Gy (60.6%) and almost half as high after an irradiation to 20 Gy that followed presoaking (29.8%). After a treatment to 20 Gy preceded by soaking, the frequency of labeled nuclei was three times lower in 22 mm-long roots than in 12-mm-long ones. After an irradiation to 300 Gy, the frequency of damaged nuclei was 4 times lower in the 22 mm-long roots than in the 12 mm-long ones. TUNEL experiments included negative and positive controls. There was no TUNEL-specific fluorescence in the negative control, where the nucleotide solution used in the reaction did not contain terminal transferase (Fig. 2A, B). However, almost all nuclei showed strong green fluorescence in the positive control, where a DNAse solution was used to induce DNA strand breaks.

In order to assess the somatic effects of x-rays on the M_1 generation, various parameters were evaluated, namely, frequency of germinated seeds and growing plants, frequency of plants with reproductive organs, stem and fruit lengths, number of fruits per plant, and number of seeds per fruit. Table 3 lists results of an analysis of the somatic effects in the M_1 generation. Radiation dose 300 Gy drastically reduced the frequency of surviving plants to 3.3%. An irradiation to 20 Gy (after 16-h soaking) did not substantially affect the survival of M_1 plants as compared with the result of irradiation to 200 Gy, which turned out to be practically semilethal. Fig. 4B shows a very strong negative relationship ($r=-0.93$; $p=0.07$) between the frequency of surviving plants and the radiation dose. Radiation doses 200 and 300 Gy affected seed germination. Fig. 4C shows a strong negative correlation between the percentage of germinated seeds and the radiation dose ($r=-0.87$; $p=0.13$).

The radiation doses 200 and 300 Gy reduced the number of seeds per fruit significantly, but the number of fruits per plant was not affected. Stem and fruit lengths were also lower after irradiation to these doses.. Irradiation to 20 Gy with presoaking did not significantly alter the number of seeds per fruit as compared with the control.

Table 3
Somatic effects in the M_1 generation of *Capsicum baccatum* var. *pendulum* cv. "Cayenne" after irradiation.

Dose, (Gy)	Percentage of germinated seeds	Percentage of plants growing in pots	Stem length (cm) from cotyledons to 1st node*	Percentage of growing plants with flowers	No. of fruits per plant*	No. of seeds per fruit*	Fruit length, (cm) *
100	100	93.3	2.0 ± 0.3	96.4	7.9 ± 5.07	36.4 ± 18.5	3.5 ± 0.7
200	86.7	56.7	1.2 ± 0.2	88.2	7.0 ± 6.6	22.3 ± 17.3	2.8 ± 0.8
300	83.3	3.3	0.70	100	16	8.2 ± 5.5	2.3 ± 0.6
20 (with 16-h pre-soaking)	96.7	90.0	2.3 ± 0.5	81.5	5.6 ± 5.5	34.3 ± 17.5	3.4 ± 0.7
Control (no irradiation)	96.7	93.3	2.2 ± 0.3	100	7.5 ± 4.3	40.5 ± 20.9	3.3 ± 0.7

Test with 50 seeds per treatment.

* Mean value ± SD.

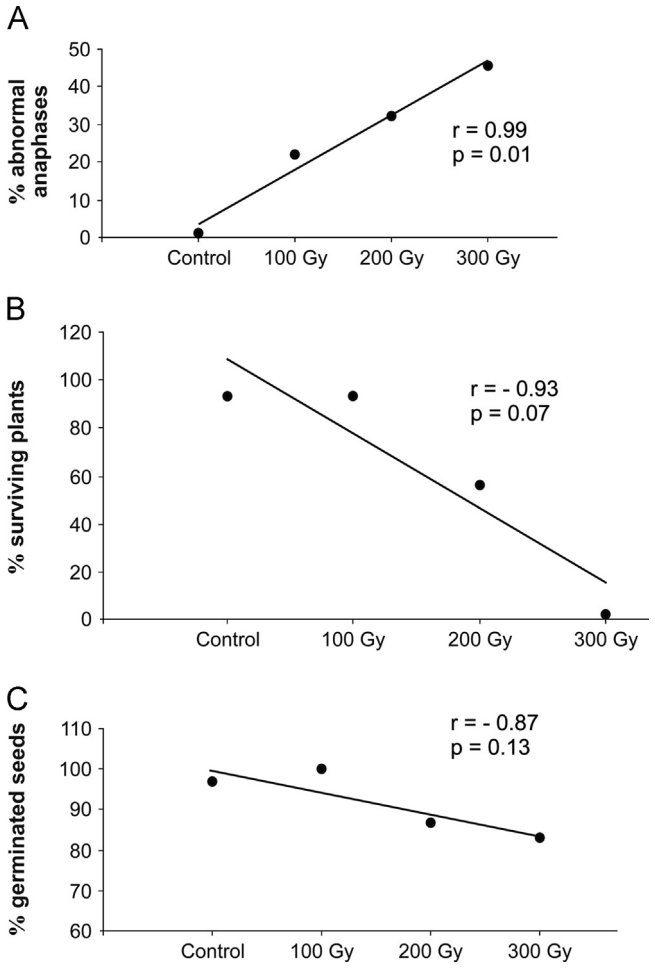

Fig. 4. Correlations between: frequency of abnormal anaphases and radiation dose (A); frequency of surviving plants and radiation dose (B); frequency of germinated seeds and radiation dose (C).

4. Discussion

Cytogenetic effects are a very effective indicator in determining optimum treatment conditions that would produce viable lines with structural changes on chromosomes. Two types of chromosome changes induced by ionizing radiation are commonly recognized, namely, classical unstable aberrations (including dicentric chromosomes, acentric fragments, and rings) and stable ones (such as translocations and insertions) (Darroudi and Natarajan, 2000). In this study, conventional staining methods were used, which found only unstable chromosome aberrations. The results of this work indicate that soaking the seeds before irradiation strongly amplified the cytogenetic effects induced by x-rays, as previously reported for other species, e.g., barley (Prina et al., 1986).

Several studies have shown a correlation between the radiation dose and the frequency of mutations, but only for highest doses (Russel, 1956; Bond, 1981). It was shown in *Tradescantia* (Santos et al., 2005) that DNA repair occurred after three/four weeks of exposure to low levels of radiation (25.0 μR min^{-1}). Likewise, in triticale, an increase in the frequency of chromosome aberrations correlated with the increase in radiation dose (0, 20, 50 and 100 Gy with mean aberrations per cell 0.02, 1.92, 7.66, and 11.07, respectively) (Ahmad et al., 2000). Dhamayanthi and Reddy (2000) reported a dose-dependent increase in meiotic anomalies in *Capsicum annuum* when γ-rays and ethyl methane sulfonate (EMS) were used as mutagens. A following study conducted with EMS in *C. annuum* (Kumar and Gupta, 2009) showed co-linearity between the time of treatment and the frequency of chromosomal abnormalities and found a linear relationship between low-energy radiation dose and the induction of single strand breaks. Double strand breaks were detected at high doses; they are believed to arise from two independent shocks, each produced in the vicinity of each single strand of DNA. Such curves have been interpreted as simple quadratic relationships (Hagen, 1989, 1994).

TUNEL test is a very useful technique, in which labeling the 3-OH ends of DNA with fluorescein-conjugated dUTP using terminal deoxynucleotidyltransferase (TdT) shows the nuclear DNA fragmentation. This technique had been used mainly for apoptosis studies, but it was successfully adapted to detect DNA damage in mutagenesis and genotoxicity studies (Juchimiuk and Maluszynska, 2003, 2005). In our studies, the TUNEL test was used to visualize the DNA damage induced by x-rays in *C. baccatum* var. *pendulum* cv. "Cayenne" cells. The results proved that the embryo cells of presoaked seeds are more sensitive to x-rays than the embryo cells of dry seeds. Although the interaction between the effects of radiation and hydration is well known, there are no published data regarding the influence of a presoaking of seeds on DNA fragmentation. It is widely accepted that the radiation susceptibility of a biological system increases with the increase of water content (Stadler, 1928). According to Konzak (1957), sensitivity of barley seeds to x-rays can change 20-fold with a change in their moisture content. Ehrenberg and Nybom (1954) found that presoaking of seeds (except of presoaking for very short periods of time) resulted in a manifold increase of the x-ray sensitivity. This finding was confirmed in the latest studies of onion seeds (Amjad and Anjum, 2002). However, little information is available to assess the mechanism. Hydration of seeds may increase mobility of the radicals and facilitate their recombination into harmless products (Kyumagai et al., 2000). Water regulates the degree to which oxygen interacts with radiation in the induction of biological effects. It is not known if presoaking could activate any enzymes related to repair processes.

A soaking of seeds prior to irradiation increased the susceptibility of the pepper cells. They became more sensitive to radiation than the cells of the rest tissue of dry seeds. Generally, cells are most radiosensitive in Phases M and G2 and most radioresistant in Phase S. The observed enhancement of the cytological effects of presoaked seeds in our studies suggests that, during the irradiation, the cells were probably in Phase M or G2. The irradiation of dry seeds, where most cells were in Phase G1, was not so effective.

The frequencies of labeled nuclei in the root meristematic and in the embryo cells were different. This can be also explained by the differences in the cell populations in root and embryo at particular stages of the cell cycle. With presoaked seeds, the difference was bigger after irradiation to 20 Gy than to 300 Gy. The analysis of DNA breakage in irradiated seeds revealed lower frequencies of labeled nuclei in longer roots than in shorter ones. The decrease in the frequency of labeled nuclei during growth indicates that an effective DNA repair processes occurred in the root meristematic cells after irradiation. Also, elimination of a cell after its death can decrease the frequencies of labeled nuclei.

The evaluation of somatic effects revealed that 300 Gy of x-rays was almost lethal, and, thus, should not be used to obtain viable lines of the selected cultivar of pepper. Besides, high doses of radiation significantly reduced seed germination. In contrast to our results, it was previously observed that germination was independent of γ-ray doses in pea cultivars (ÇiftÇi et al., 2006). It is noteworthy that an irradiation to 20 Gy with presoaking did not significantly alter the number of seeds per fruit as compared with the control. In order to ensure proper propagation, factors such as sterile or semisterile fruits in segregants should be sought in next generations.

Effects of various doses of radiation can be predicted from measured primary injury criteria (Brunner, 1995). For example, in the case of monocotyledons, such criteria can be the relative height of the primary leaves in the seedlings of irradiated seeds in comparison with non-treated controls or the length of epicotyls in dicotyledonous species. It has been found that these criteria for primary injury in M_1 are well correlated with viability, survival, and/or sterility. Moreover, correlations between the injury parameters in M_1 with the frequency of mutations in the M_2 generation, e.g., chlorophyll mutation indicators, make it possible to find the most useful radiation doses for various breeding objectives. In this context, Konzak (1984) proposed to use doses that generate an optimal mutation frequency rather than the maximal one. Kawai (1969) defined efficient mutagenesis as production of desirable changes free of generally associated undesirable ones, such as aberrations, sterility and lethality. The x-ray dose 20 Gy proved to be an efficient dose to induce desirable chromosome translocations in wheat-rye (Ahmad et al., 2000).

5. Conclusions

To conclude, the cytogenetic and TUNEL test techniques showed that irradiation to 20 Gy of seeds presoaked for 16 h may be the most efficient way to induce chromosome mutations in *Capsicum baccatum* var. *pendulum* cv. “Cayenne”, which can be implemented in breeding programs. Thus, these treatment conditions should be taken into consideration in pepper mutagenesis programs. They proved to be more efficient in the induction of chromosome aberrations, while some minor somatic effects were observed. We propose a program to follow the subsequent generations (M_2, M_3) with the aim to find plants that carry structural rearrangements in their chromosomes.

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References

Ahloowalia, B.S., Maluszynski, M., 2001. Induced mutations—a new paradigm in plant breeding. Euphytica 118, 167–173.
Ahmad, F., Comeau, A., Chen, Q., Collin, J., St-Pierre, C.A., 2000. Radiation induced wheat-rye chromosomal translocations in triticale: Optimizing the dose using fluorescence in situ hybridization. Cytologia 65, 1–6.
Amjad, M., Anjum, M.A., 2002. Radiosensitivity of onion seeds with different moisture contents. Int. J. Agric. Biol 4, 468–473.
Bond, V., 1981. Radiobiological input to radiation protection standards. Health Phys. 41 (6), 799–806.
Brunner, H., 1995. Radiation induced mutations for plant selection. Appl. Radiat. Isot. 46 (6/7), 589–594.
Caldecott, R., Frolik, E., Morris, R., 1952. A comparison of the effects of x-rays and thermal neutrons on dormant seeds of barley. Proc. Natl. Acad. Sci. USA 38, 804–809.
Caldecott, R., Beard, B., Gardner, C., 1954. Cytogenetics effects of x-ray and thermal neutron irradiation on seeds of barley. Genetics 39, 240–259.
ÇiftÇi, C.Y., Divanli Türkan, A., Khawar, K.M., Atak, M., Özcan, S., 2006. Use of gamma rays to induce mutations in four pea (*Pisum sativum* L.) cultivars. Turk. J. Biol. 30, 29–37.
Darroudi, F., Natarajan, A., 2000. Application of Fish chromosome painting assay for dose reconstruction: state of the art and current views. Radiat. Prot. Dosim. 88 (1), 51–58.
Daskalov, S., 1986. Mutation breeding in pepper. In: FAO/IAEA, United Nations (Ed.), Mutation Breeding Review no 4. International Atomic Energy Agency, Vienna, pp. 1–26.
Dhamayanthi, K.P.M., Reddy, V.R.K., 2000. Cytogenetic effects of gamma rays and ethyl methane sulphonate in chilli pepper (*Capsicum annuum* L.). Cytologia 65 (2), 129–133.
Ehrenberg, I., Nybom, N., 1954. Ion density and biological effectiveness of radiations. Acta Agric. Scand. 4, 396–418.
Gaul, H., 1977. Mutagen effects in the first generation after seed treatment: cytological effectsManual on Mutation Breeding2nd ed. International Atomic Energy Agency, Vienna, ISBN: 9201150776 pp. 91–96.
Grupo Infostat, 2002. *INFOSTAT.* Version 1.1. Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba. Editorial Brujas, Argentina.
Hagen, U., 1989. Biochemical aspects of radiation biology. Experientia 45, 7–12.
Hagen, U., 1994. Mechanisms of induction and repair of DNA double-strand breaks by ionizing radiation: some contradictions. Radiat. Environ. Biophys. 33, 45–61.
Indira, C., Abraham, S., 1977. Morphological and cytological studies on a radiation induced polyploid in *Capsicum annuum* Linn. Cytologia 42, 371–375.
Jong, K., 1997. Laboratory Manual of Plant Cytological Techniques. Royal Botanic Garden, Edinburgh p. 87.
Juchimiuk, J., Maluszynska, J., 2003. Detection of DNA fragmentation caused by chemical mutagens in TUNEL test. In: Maluszynska, J., Plewa, M. (Eds.), Bioassays in Plant Cells for Improvement of Ecosystem and Human Health. Wydawnictwo Uniwersytetu Slaskiego, Katowice, ISBN: 8322613164, pp. 133–138.
Juchimiuk, J., Maluszynska, J., 2005. Transformed roots of *Crepis capillaris*—a sensitive system for the evaluation of abiotic factors. Mutat. Res. 565, 129–138.
Katiyar, R.B., 1977. Radiocytogenetical studies in *Capsicum*: induced desynapsis. Caryologia 30, 347–350.
Katiyar, R.B., 1978. Radiocytogenetical studies on *Capsicum* I. Meiotic anomalies. Cytologia 43, 415–421.
Kawai, T., 1969. Relative effectiveness of physical and chemical mutagens. In: Induced mutations in plants. Proceedings of a Symposium on the Nature, Induction and Utilizaton of Mutations in Plants, Pullman, Washington, 14–18 July 1969. IAEA, Vienna. pp. 137–152. OCLC 73792478.
Konzak, C.F., 1957. Genetic effects of radiation on hihger plants. In: Proceedings of the Symposium on the Effects of Ionizing Radiation on Plants. Quart. Rev. Biol. vol. 32, pp. 27–45.
Konzak, C.F., 1984. Role of induced mutations. In: Vose, P.B., Blixt, S.G. (Eds.), Crop Breeding, A Contemporary Basis. Pergamon Press, Oxford, pp. 216–292.
Kumar, O.A., Raja Rao, K.G., 2003. Cytomorphological studies in gamma-ray induced autotriploids of *Capsicum annuum* L. Cytologia 68, 45–50.
Kumar, G., Gupta, P., 2009. Induced karyomorphological variations in three phenodeviants of *Capsicum annuum* L. Turk. J. Biol. 33, 123–128.
Kyumagai, J., Katoh, H., Kumada, T., Tanaka, A., Tano, S., Miyazaki, T., 2000. Strong resistance of *Arabidopsis thaliana* and *Raphanus sativus* seeds for ionizing radiation as studied by ESR, ENDOR, ESE spectroscopy and germination measurement: effect of long-lived and super-long-lived radicals. Radiat. Phys. Chem. 57, 75–83.
Prina, A.R., Hagberg, A., Favret, E.A., 1986. Inheritable sterility induced by x-rays and sodium azide in barley. Genet. Agrar. 40, 309–320.
Prina, A.R., 1989. Consideraciones sobre la aplicación eficiente de la mutagénesis inducida en fitomejoramiento. Mendeliana 9, 5–49.
Russel, W., 1956. Lack of linearity between mutation rate and dose for x-ray induced mutation in mice. Genetics 41, 655–658.
Saccardo, F., Sree Ramulu, K., 1977. Mutagenesis and cross breeding in *Capsicum* for disease resistance against *Verticillium dahliae*. In: Pochard, E. (Ed.), Capsicum 77, Comptes rendus du 3 degré Congres EUCARPIA sur la genetique et la selection du piment. Institut national de la recherche agronomiqueAvignon-Montfavet, ISBN: 2853400891, pp. 161–169.
Santos, T., Crispim, V., Noualhetas, Y., Macacini, J., Gomes, H., 2005. The study of the effects of low-level exposure to ionizing radiation using a bio-indicator system. Appl. Radiat. Isot. 62, 313–316.
Scaldaferro, M.A., Acosta, M.C., Prina, A.R., Moscone, E.A., 2004. Daños cromosómicos y fisiológicos producidos por radiaciones ionizantes en *Capsicum baccatum* var. *pendulum* (Solanaceae). Actas XXXIII Congreso Argentino de Genética. J. Basic Appl. Genet. 16, 59, Suppl.
Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice in Biological Research, 3rd ed Freeman, New York p. 887.
Stadler, L.J., 1928. The rate of induced mutation in relation to dormancy, temperature and dosage. Anatom. Rec 41, 97.