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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

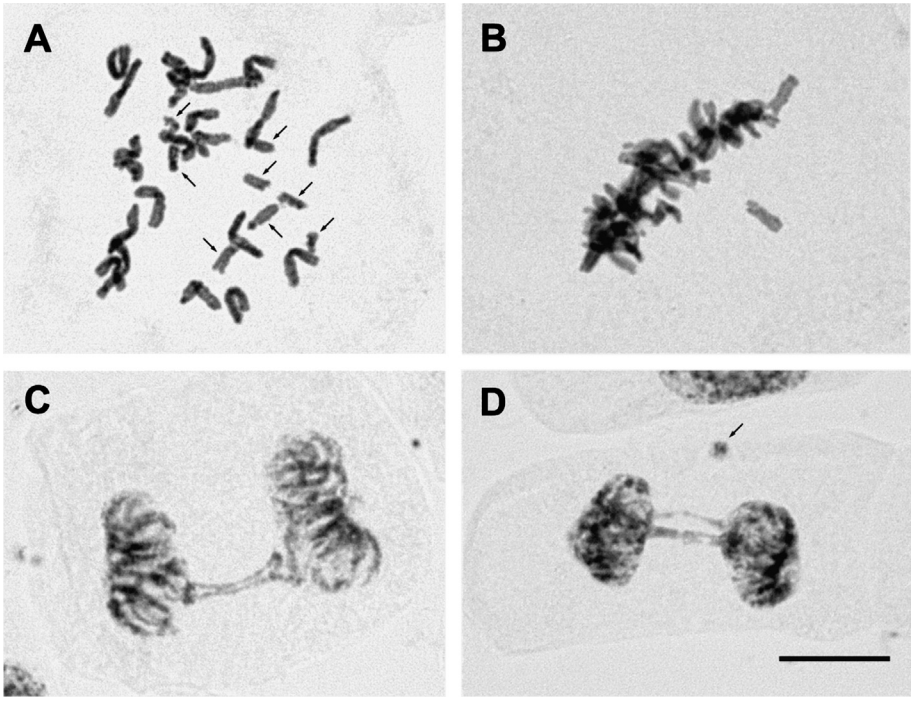

Fig. 1. Mitotic chromosome aberrations in *Capsicum baccatum* var. *pendulum* cv. "Cayenne" ($2n=24$) root meristematic cells after x-ray irradiation of seeds. (A): Metaphase with eight chromosome fragments. (B): Metaphase with one chromosome outside plate. (C–D): Late anaphases with bridges; (C): double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