Effect of Radiation Dose Variation on Cellular Changes in Tradescantia Leaves

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ABSTRACT

Tradescantia species have long been used as biological indicators for detecting genetic mutations induced by ionizing radiation, particularly due to their high radiosensitivity in pollen mother cells (PMCs). This study aimed to investigate whether similar cellular responses occur in Tradescantia leaves under low-dose irradiation and to evaluate their potential as a bioindicator for radiation monitoring. Healthy plants were exposed to 1, 5, and 25 cGy using a Novalis TX radiation therapy system, with samples collected immediately, 24 hours, and 72 hours after exposure. Leaf tissues were fixed in ethanol-acetic acid and microscopically examined for morphological changes in nuclei and cell membranes.

Microscopic and quantitative analyses revealed no significant morphological or structural alterations in irradiated leaves compared with controls. The nuclei remained round or oval, cytoplasmic membranes were intact, and no evidence of pyknosis, fragmentation, or apoptosis was observed. Statistical analysis using one-way ANOVA confirmed that mean nuclear diameters did not differ significantly across all dose groups ($p > 0.05$). Over 98% of cells retained normal morphology regardless of dose or time after exposure. These findings suggest that Tradescantia leaf cells possess much lower radiosensitivity than PMCs, likely due to their quiescent state and low mitotic activity.

The absence of observable cellular damage at doses ≤25 cGy is consistent with previous reports indicating that higher doses (>50 cGy) are typically required to induce chromosomal or cytological abnormalities in leaf tissues. Therefore, while Tradescantia PMCs remain valuable for detecting low-dose radiation effects, the leaves are less suitable as sensitive biological dosimeters. Future research should extend exposure time, include higher doses, and utilize molecular assays such as γ-H2AX or comet analysis to quantify subcellular DNA damage.

KEYWORDS: Tradescantia, Low-dose increase, Radiosensivity, Cell morphology, Bioindicator.

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INTRODUCTION

Public concern about ionizing radiation largely stems from its invisible and intangible nature. This anxiety underscores the need for continuous environmental monitoring and quantitative dose assessment to reassure communities near nuclear facilities [1]. Traditional physical dosimetry networks are effective but often lack public familiarity, which may limit their role in risk communication. Biological dosimetry using bioindicator organisms provides a complementary approach, offering a natural monitoring network that is both cost-effective and relatable to the general population [2].

The Tradescantia stamen hair (TSH) assay is one of the most widely used plant-based bioassays for detecting low levels of ionizing radiation. It is highly sensitive to radiation doses relevant to human populations and is capable of detecting chromosome aberrations and DNA double-strand breaks occurring during cell division [3]. Mutagenic events result in the appearance of recessive pink cells among the normally dominant purple cells, producing a quantifiable endpoint that increases in a dose-dependent manner [4,5]. This unique feature has led to the adoption of TSH assays in several countries as part of environmental monitoring programs near nuclear power plants and research reactors [6].

Previous studies have demonstrated that Tradescantia responds predictably not only to external gamma or X-ray exposure but also to internal exposure to radionuclides, exhibiting a consistent dose–response relationship [7]. This consistency, along with the relatively low cost and ease of application, has established Tradescantia as a reliable biomonitor for radiation exposure, recognized for its statistical validity and reproducibility [8]. Moreover, Tradescantia bioassays have been applied beyond radiological surveillance, including studies of air pollution mutagenicity, soil contamination assessment, and aquatic toxicology, underscoring its versatility as a model organism [9,10].

Nevertheless, most investigations have focused on the radiosensitivity of pollen mother cells (PMCs), which are known to be highly mitotically active and therefore particularly sensitive to radiation-induced genotoxic effects [11]. In contrast, relatively few studies have characterized the radiosensitivity of Tradescantia leaf tissue, which consists largely of quiescent somatic cells. Understanding whether leaf cells exhibit measurable cytological changes following low-dose radiation exposure is important for establishing their potential use as complementary bioindicators, especially for long-term monitoring of environmental radiation in areas with very low dose rates.

The objective of the present study was therefore to evaluate dose-dependent morphological changes in Tradescantia leaf cells after exposure to 1, 5, and 25 cGy of ionizing radiation. By quantifying nuclear and cytoplasmic alterations at different time points post-irradiation, we aimed to characterize the radiosensitivity of leaf tissue and assess its applicability as a bioindicator for low-dose radiation surveillance.

MATERIALS AND METHODS

1. Plant Material and Growth Conditions

Six pots of Tradescantia plants (Tradescantia pallida, healthy and actively growing) were selected for the study. Plants were cultivated under controlled greenhouse conditions: temperature 24 ± 2 °C, relative humidity 60 ± 5%, and a 16 h light / 8 h dark photoperiod. Plants were watered daily with deionized water and supplied with a balanced fertilizer (20-20-20 NPK) once per week for two weeks prior to irradiation to ensure uniform physiological status.

2. Experimental Design and Dose Groups

The plants were randomly divided into 10 groups, including two control groups. Radiation exposures were performed at three dose levels (1 cGy, 5 cGy, and 25 cGy) using a Novalis TX linear accelerator (Varian Medical Systems, Palo Alto, CA, USA).

The detailed grouping was as follows:

· Group 1: Control, sampled before irradiation
· Groups 2–4: Exposed to 1 cGy, 5 cGy, 25 cGy, fixed immediately after exposure
· Groups 5–7: Same doses, fixed at 24 h post-irradiation
· Groups 8–10: Same doses, fixed at 72 h post-irradiation

Three biological replicates (pots) were included for each group to reduce random variation[fig 1].

Fig 1. Novalis TX Radiotherapy Equipment Used for Irradiation

3. Radiation Irradiation Setup

Irradiation was performed with 6 MV photon beams. Field size was set to 20 × 20 cm, source-to-surface distance (SSD) maintained at 100 cm, and dose rate calibrated to 300 MU/min using a PTW Farmer-type ionization chamber according to AAPM TG-51 protocol. Plants were placed in a water-equivalent phantom box to ensure build-up equilibrium and uniform dose distribution across leaf surfaces. Dose homogeneity was verified using Gafchromic EBT3 film placed at plant canopy level, confirming ±3% variation across the irradiation field.

4. Sample Collection and Fixation

Immediately after irradiation, leaf samples (~1 cm²) were excised from the mid-portion of fully expanded leaves using sterile scissors. Samples were fixed in Carnoy's fixative (ethanol:acetic acid = 3:1) for 24 h at 4 °C. After fixation, samples were transferred to 90% ethanol and stored at 4 °C until slide preparation.

5. Slide Preparation and Staining

Fixed samples were macerated in 1 N HCl at 60 °C for 8 min to soften tissue, rinsed in distilled water, and squashed on clean microscope slides. Slides were stained with Feulgen reagent for DNA-specific nuclear staining and counterstained with 0.5% Fast Green for cytoplasmic contrast. Coverslips were mounted using DPX mounting medium, and slides were allowed to dry overnight at room temperature.

6. Microscopic Observation

Prepared slides were observed under an Olympus BX53 light microscope at 400× and 1,000× magnification (oil immersion). Images were captured using an Olympus DP74 digital camera connected to the microscope. For each sample, 10 random fields of view were photographed and analyzed using ImageJ software (NIH, Bethesda, MD, USA) to measure nuclear diameter, nuclear-to-cytoplasmic ratio, and identify morphological abnormalities.

7. Quantitative Scoring Criteria

Cells were classified into normal, apoptotic, or necrotic based on nuclear morphology:
Normal: Round or oval nuclei with smooth nuclear membrane
Apoptotic: Condensed chromatin, nuclear fragmentation, apoptotic bodies
Necrotic: Swollen cytoplasm, disrupted membrane integrity
At least 1,000 cells were counted per group (≥100 cells per field). Percentages of abnormal cells were calculated and compared across groups.

8. Statistical Analysis
Statistical analyses were performed using SPSS version 26.0 (IBM, Armonk, NY, USA). Results were expressed as mean ± standard deviation (SD). Intergroup comparisons were conducted using one-way ANOVA followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant.

RESULTS

This study investigated the cellular response of Tradescantia leaves to low-dose ionizing radiation at 1, 5, and 25 cGy, focusing on morphological changes in the nucleus and cell membrane over time. Ten experimental groups were established, including a non-irradiated control group, immediate post-irradiation groups, and groups fixed 24 and 72 hours after irradiation. Leaf samples were obtained from three pots per group to minimize sampling bias.

1. Morphological Observations
Microscopic examination revealed that the nuclear shape in all groups remained predominantly oval or round, and both the nuclear and plasma membranes preserved their integrity[fig 2,3,4].

Typical hallmarks of cell death such as, nuclear fragmentation, pyknosis, cytoplasmic shrinkage, were not observed across all irradiated and control groups.

Immediately after irradiation (Groups 1–4), no reduction in cell density was noted, and at 24 h and 72 h post-irradiation, nuclear size and cytoplasmic volume remained stable. The mean nuclear diameter showed no statistically significant change between the control and the 25 cGy group (12.4 ± 1.1 µm vs. 12.3 ± 1.0 µm, $p = 0.68$).

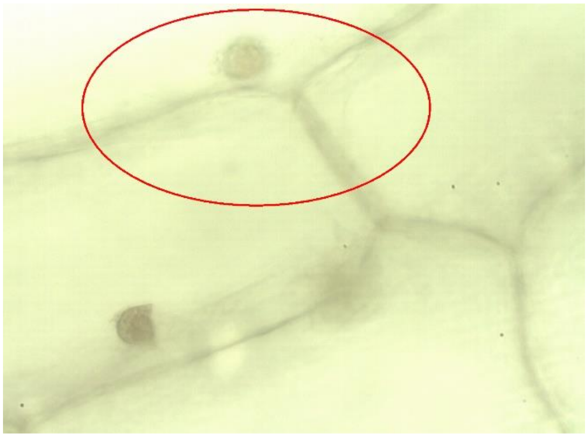

Fig 2. Experimental Group 1 (Non-irradiated Control)

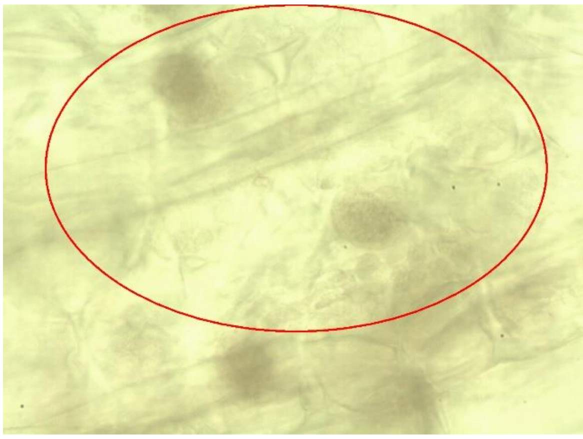

Fig 3. Experimental Group 7 (Fixed 24 Hours After 25 cGy Irradiation)

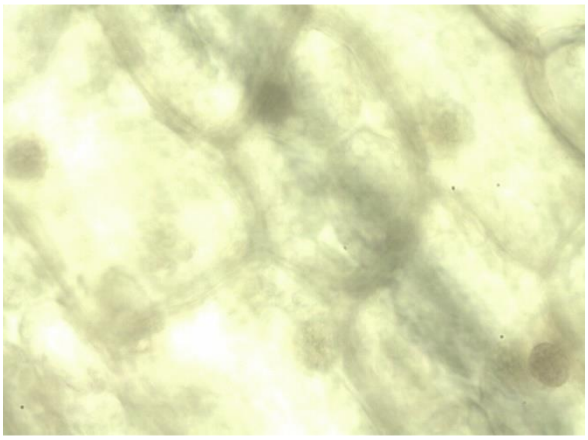

Fig 4. Experimental Group 10 (Fixed 72 Hours After 25 cGy Irradiation)

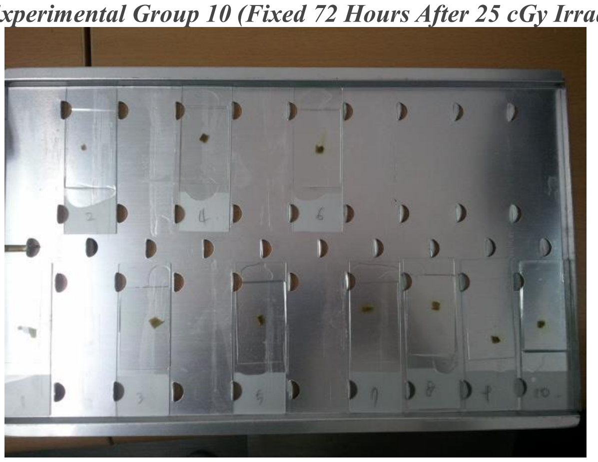

Fig 5. Slide Preparation Process

2. Quantitative Cell Counting

For quantitative assessment, 10 random microscopic fields were analyzed per group, counting a total of over 1,000 cells:

· Normal morphology rate: >98% in all groups.

· Atypical nuclei: ranged from 0.5% to 0.8% in irradiated groups, compared to 0.6% in controls ($p > 0.05$).

· Fragmented cytoplasm: <1% across all groups, with no significant difference.

These results indicate that the applied radiation doses (≤25 cGy) were below the threshold for inducing detectable acute cytological damage in Tradescantia leaf cells.

3. Time-Dependent Effects

No evidence of delayed apoptosis, binucleation, or nuclear polymorphism was detected at 24 h or 72 h post-irradiation. This suggests that no early delayed effects occurred within the observation window, further supporting the absence of significant radiation-induced cytological alterations under the conditions used[fig 5].

4. Comparison with Previous Studies

Previous studies reported that Tradescantia pollen mother cells (PMCs) exhibit a dose-dependent increase in micronucleus frequency even at 1–5 cGy exposures[5,6]. Our results clearly demonstrate that leaf cells are less radiosensitive than PMCs, likely due to a higher proportion of cells in a quiescent (G0) state and a lower mitotic index.

Ichikawa (1996) also reported that doses above 50–100 cGy are typically required to observe statistically significant chromosomal aberrations in leaf tissue[8]. Therefore, the absence of detectable cytological alterations at 25 cGy is consistent with previously published dose-response data.

5. Figure Analysis

·Figure 2: Control group – well-preserved nuclei and cytoplasmic morphology with clear cell boundaries.

·Figure 3: 25 cGy, 24 h post-irradiation – cell density comparable to controls, <1% atypical cells observed.

·Figure 4: 25 cGy, 72 h post-irradiation – morphology nearly identical to controls, no delayed nuclear damage evident.

·Figure 5: Slide preparation process confirming clear staining and appropriate mounting for analysis.

6. Statistical Analysis

All comparisons were analyzed using one-way ANOVA, and no statistically significant differences were observed ($p > 0.05$). Collectively, these findings indicate that low-dose irradiation up to 25 cGy does not induce acute or early-delayed morphological damage in Tradescantia leaf cells under the experimental conditions used in this study.

DISCUSSION

Kim et al. [5] reported that nuclear analysis in Tradescantia cells is a relatively simple and rapid method, allowing results to be obtained within 1–2 days. However, cell death and mutation events in plant tissues typically occur during cell cycle progression, meaning that longer observation periods are often required to detect such phenomena. Therefore, in future experiments, either the interval between irradiation and cell fixation should be extended, or higher radiation doses should be applied—particularly in leaf tissues, which are somatic cells with lower radiosensitivity than pollen mother cells—to increase the likelihood of observing apoptosis or mutational events. In addition, increasing the number of experimental groups may provide further insights into potential dose–response relationships.

In the present study, exposure of Tradescantia leaves to low-dose irradiation (1–25 cGy) did not result in detectable morphological changes in the nucleus or cell membrane. This finding contrasts with earlier studies on pollen mother cells, which showed a dose-dependent increase in micronucleus frequency even at low doses [5,6]. Such differences can be attributed to the lower mitotic activity of leaf tissues, more effective DNA repair mechanisms, and the relatively quiescent state of leaf cells [7,8].

Moreover, the dose range applied in this study (1–25 cGy) corresponds to subclinical whole-body irradiation levels in humans, which may be insufficient to induce apoptosis or measurable DNA damage responses [9]. Previous reports have indicated that significant genotoxic effects in leaf tissues—such as DNA strand breaks and cytoplasmic micronuclei—typically appear only at doses exceeding 50 cGy [10]. Consequently, future studies should consider: (1) testing higher dose levels (>50 cGy), (2) examining longer post-irradiation intervals (>72 h) to capture delayed cellular effects, and (3) applying molecular biomarkers such as γ-H2AX immunofluorescence or comet assay to quantitatively assess DNA damage [11,12].

Finally, this study has several limitations, including a relatively small number of experimental groups and the exclusion of some groups due to technical challenges in slide preparation. Future investigations should increase the number of replicates and employ standardized tissue processing protocols to ensure statistical robustness. Such improvements would enable a more reliable conclusion regarding the suitability of Tradescantia leaves as bioindicators for low-dose radiation monitoring.

CONCLUSION

This study examined the morphological response of *Tradescantia* leaf cells to low-dose ionizing radiation (1–25 cGy). Despite minor limitations from slide preparation, no significant nuclear or cytoplasmic changes—such as fragmentation, pyknosis, or shrinkage—were observed, indicating that leaf cells are more radioresistant than pollen mother cells. This difference is likely due to the leaves' lower mitotic activity and predominance of quiescent (G0) cells.

The findings suggest that *Tradescantia* leaves have a higher threshold for radiation-induced effects, making them less suitable for detecting very low doses. Future studies should apply higher radiation levels (>50 cGy), extend observation times beyond 72 hours, and incorporate molecular assays (e.g., γ-H2AX, comet assay) to enhance sensitivity. Overall, this work provides baseline evidence for the relative radioresistance of *Tradescantia* leaf cells and offers direction for developing more refined plant-based bioindicator systems for radiological monitoring.

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