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Biodosimetry of ionizing radiations at different LET levels through cytogenetic endpoints in *Allium cepa* meristems

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ABSTRACT

- This paper aims to enhance our understanding of the effects of ionizing radiation using radiobiology and biodosimetry techniques applied to living plant organisms. Plants are particularly suitable for this purpose as they are highly sensitive to detecting potential genotoxic agents in the environment and their use allows us to avoid using animals in research in compliance with the 3R principle. Currently, the onion (*Allium cepa*) is recognized as a valid model for the analysis of environmental pollutants but has been relatively unexplored as an indicator of radiation exposure. In this study, analyses of the genotoxicity of X and alpha radiation were conducted using the micronucleus test and mitotic index analysis. Our results indicate that *Allium cepa* can be considered a valid alternative model to animal use for assessing the effects of ionizing radiation. In particular, it was found that alpha radiation caused significant damage, as evidenced by an increased number of micronuclei, which was 20 times higher compared to X-ray radiation. This was further confirmed through the observation of the effective dose parameter, as determined by the analysis of various weight factors associated with different types of radiation.

1. Introduction

Nuclear safety and protection from ionizing radiation are essential to ensure the safe use of radiation and nuclear energy and to preserve human health. Radiation protection aims to safeguard the health and well-being of workers and the general population by reducing risks associated with radiation exposure in activities involving such sources and balancing the benefits to society and its members (ICRP, 2007). In this context, an effective, reliable, and transparent radiation protection system is crucial in industrialized countries to enable the safe conduct of potentially risky activities (ICRP, 2007).

Increased risks of sudden exposure to radiation involving a large number of people make it essential to develop systems for assessing the effects of such radiation on cells, particularly focusing on genetic material (Kimball, 1952; Gerhardt, 2002). This need has driven the search for simple, fast, and easily applicable techniques on a large number of samples. These techniques are based on identifying cytogenetic defects, known as endpoints, which can occur within cells following interaction with radiation (Gerhardt, 2002). Biodosimetry is the discipline that studies the relationship between endpoints and the absorbed dose (World Health Organization, 2011). One of the most widely used tests in biodosimetry is the micronucleus test (Hayashi, 2016). Its widespread adoption is attributed to its analytical simplicity, the low need for specialized operator skills, and the fact that the micronucleus is the most easily detectable defect in this context (Vaijapurkar et al., 2001).

The use of biodosimetry on human models is well-documented in the literature (Da Cruz et al., 1994; Köksal et al., 1996; Ponnaiya et al., 2004; Senthamizhchelvan et al., 2009; Zölzer et al., 2011; World Health Organization, 2011; Chen et al., 2014; Ludovici et al., 2021; Xavier et al., 2021), but most data are related to analyses conducted on exposed

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workers (Köksal et al., 1996; Zölzer et al., 2011) or victims of nuclear disasters (Da Cruz et al., 1994; Chen et al., 2014), presumably individuals who have absorbed high doses, while there is a lack of data on the effects caused by low doses (Xavier et al., 2021; d'Errico et al., 2023; Xavier et al., 2023).

To gain a deeper understanding of the effects of these low-effective doses, it seems appropriate to conduct studies on realistic models, preferably on whole organisms rather than just on cell cultures (Bonciu et al., 2018). It is known that the response of cell cultures can differ from that of a complete organism. Additionally, to obtain reliable data, it is important to consider that the sample size to be studied increases inversely proportional to the square of the effective dose (Brenner et al., 2003). Therefore, the lower the effective dose, the larger the sample must be to ensure the statistical accuracy of the results.

In the 1960s and 1970s, when ethical concerns were less pronounced, extensive studies were conducted on animal models, such as the "Megamouse" project (Russell and Kelly, 1982), to address sample size challenges and gain a better understanding of the effects at low doses. Similar studies are not acceptable today, and the search for alternative models to animals for studying the effects of low effective doses of radiation is a crucial and evolving theme.

The idea of the present work is based on the development and use of a so-called "non-human biota", a living organism of a plant nature, as an alternative model to animal ones (Ulanovsky, 2016; Higley, 2018). In particular, using *Allium cepa*, it seems possible to acquire new information about the effects of low doses of radiation on living organisms by conducting studies on a large number of virtually identical samples.

Interest in this organism arises from the fact that it is a diploid species ($2n = 16$) with a low number of large chromosomes, making it easier to detect DNA damage (Xavier et al., 2021). In addition, *Allium cepa* is widely recognized as a universal indicator of the genotoxic effects of environmental agents on both human health and the environment. Studies have shown a response highly consistent with the responses observed in mammalian cells (Grant, 1978; Leme and Marin-Morales, 2009; Palmieri et al., 2016; Reis et al., 2017). This makes *Allium cepa* a valuable tool for assessing the genotoxic potential of various substances and environmental factors.

Thus, the use of *Allium cepa* as a model organism may provide indications of potential genotoxic effects on both plant and animal cells. This allows for conducting experimental work that applies the principle of the 3 R s (Replacement, Reduction, Refinement), thus enabling the replacement of animal experimentation except in cases where it is strictly necessary (Russell and Burch, 1959).

In the scientific literature, many studies have designated the plant model based on *Allium cepa* as the "gold standard" for assessing the effects of various chemical, physical, and specific categories of radiation (Fiskesjö, 1985; Bagatini et al., 2009; Leme and Marin-Morales, 2009; Bonciu et al., 2018; Bolsunovsky et al., 2019; Xavier et al., 2021, 2023). However, the capacity of this plant model to provide more detailed information, responding differently to stimuli generated by radiation with different Linear Energy Transfer (LET), is not yet clearly defined.

The LET of radiation indicates its ability to transfer energy to matter. Radiation with high LET is characterized by high ionization density, meaning that, interacting with the genetic material of cells, it can generate very close interactions, potentially causing significant damage. On the contrary, low-LET radiation has lower ionization capacity, resulting in more diluted interactions and easily repairable damage within the cell (Goodhead, 2006). Understanding whether the response of *Allium cepa* to radiation of different LETs is different could be very useful in defining its use as a dosimeter for different types of radiation.

Studies on the genotoxicity of X-rays (low LET) and alpha particles (high LET) were conducted through the application of the micronucleus test and the analysis of the mitotic index. Furthermore, the goal was set to "engineer" one phase of the procedure, already known (Xavier et al., 2021), used for preparing samples for microscope analysis to optimize it and make it highly reproducible.

2. Materials and methods

2.1. Germination of Allium cepa seeds

Following a well-established procedure (Xavier et al., 2021) onion seeds, free of pesticides (of the round, red Tropea variety), were placed inside a Petri dish previously prepared with two sheets of filter paper as a culture substrate. Around 50 seeds were scattered at about 1 cm from each other. Subsequently, the filter paper was moistened with 4 ml of distilled water to create a sufficiently humid environment and promote seedling growth. The plates were then sealed and held in an incubator at a constant temperature of (25 ± 1) °C for 3 days until the seedlings reached a length of about 4–5 mm.

2.2. Irradiation

In the Nuclear Measurements Laboratory of the University of Pisa, alpha particle irradiations were performed using a point source of ^{241}Am with a nominal activity of 74 kBq (2 μCi) as of August 11, 2003, and mean energy alpha particle emission of 5.48 MeV (Firestone, 1996). The dose rate was calculated by MCNP Monte Carlo simulations as reported by Xavier et al. (2021). Cylinders 5 mm long and 400 μm thick were used to simulate the roots of *Allium cepa*. The dose rate of α-particles was 7.92 mGy/min at 1 cm from the source. The statistical uncertainty of the Monte Carlo simulations was below 2% (2 SD). The delivered absorbed doses were 20, 40, 60, and 80 mGy.

The experiment with X-rays was conducted at the San Luca Hospital Center in Lucca using a linear accelerator to produce 6 MV X-ray photons. The dose rate was 1 Gy/min, measured with a calibrated PTW 30013 Farmer-type® ionization chamber. The absorbed doses delivered were 150, 300, 450, and 600 mGy.

After exposure, the seedlings were placed back in the incubator for 24 h and then immersed for another 20–24 h in a Carnoy's fixative solution (3:1 absolute ethanol and acetic acid). Finally, they were transferred and stored in 70% ethanol in a refrigerator for up to 20 days (Guerra and de Souza, 2002).

2.3. Preparation and compression of samples

Details of our sample preparation protocol are given by Barco et al. (2024) and are summarized as follows. The protocol for sample preparation begins with placing seeds in a vial containing 1 M HCl, which is then placed in a water bath at (60 ± 2) °C for 5 min to hydrolyze the cell wall, facilitating dye absorption. The seeds are then immersed in distilled water for 10 min to hydrate and make the tissue more visible. Subsequently, the seeds are treated with 45% acetic acid for 3 min to help rupture the cell wall. Staining is done using 2% acetic orcein, which binds to cellular genetic material, highlighting nuclei and micronuclei. The sample is then covered with aluminum foil for 15 min to protect it from light. After this period, the sprout's apical meristem is placed on a microscope slide, separated, and covered with a coverslip. Gentle taps are applied to disintegrate the outer membrane and separate the cells into a monolayer for microscopic analysis. This protocol helps in identifying mitotic cells by highlighting the distinct stages of mitosis.

In earlier studies, compression was performed by applying the necessary pressure manually (Nefic et al., 2013; Xavier et al., 2021). However, this lacks precise control over the applied force, which can vary with each execution of the procedure and depends on the operator involved, making the process quite variable. To address this issue, a mechanical system for compression was designed and developed. This system standardizes one of the most critical phases of the entire process, thus allowing for the production of samples as uniformly as possible.

The device (Fig. 1) consists of a carriage on which the microscope slide with the seedling is placed. Using two rails, the carriage is moved under a bridge, to which the force application system is connected. The latter consists of a roller, constrained to an adjustable spring by a screw.

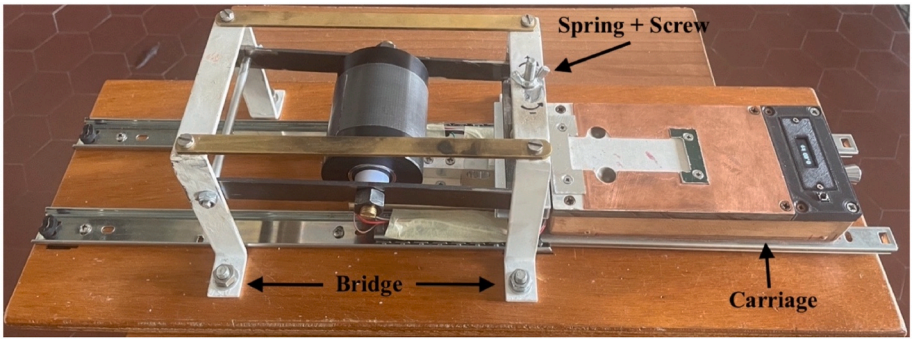

Fig. 1. Cell compression device.

The rotation of the screw allows compressing or loosening the spring, thus adjusting the force applied by the roller on the slide during its passage. To measure the force applied by the roller during the passage, a scale was implemented and integrated into the system.

The system comprises a load cell containing the electronic circuitry and positioned near the carriage. The load cell is managed by a dedicated chip (HX711), connected to an Arduino Nano-compatible board, serving as the system's microcontroller. The board is also connected to a display for viewing the results and a button for calibration. The system is powered by two rechargeable 3.7 V lithium batteries.

The optimization of the force, and hence the pressure, that the roller must apply to produce a monolayer of intact cells free of deformations and suitable for microscopic analysis was conducted as follows. Initially, the force exerted manually (with a thumb) to achieve the optimal compression of the meristems was measured using a kitchen scale. The best results were obtained with a pressure ranging between 2.5 and 4.5 kgf. Subsequently, to narrow down the estimate of the ideal force, the previously described automated compression system was employed. Starting from a minimum value of (2.5 ± 0.1) kgf and incrementing the force in (0.5 ± 0.1) kgf steps up to (4.5 ± 0.1) kgf, multiple tests were performed and results were evaluated in terms of monolayer quality. It was observed that forces close to 2.5 kgf are not sufficient to obtain a monolayer of cells suitable for the microscope, with overlapping cells that make it difficult to determine the presence and position of micronuclei. Similarly, values close to 4.5 kgf produce unusable results, as the high pressure causes the cells to deform or even damage their walls. The best results were obtained with (3.5 ± 0.1) kgf, which allows for the optimal formation of a monolayer of cells with correct dimensions and shapes, and with the intact cytoplasmic membrane, facilitating the scoring of 1000 cells which is the standard for the micronucleus test.

2.4. Sample analysis

The scoring was carried out visually under optical microscopy using a Nikon Eclipse TS100 system with a total magnification of 400x. A thousand cells with intact cytoplasmic membrane were counted for each sample. The analysis was performed on five samples for each dose point, including the negative control. Subsequently, the genotoxicity of the radiation exposure was assessed from the frequency of the number of cells with one or more micronuclei (Fig. 2), and the cytotoxicity from the evaluation of the mitotic index.

Double-blind scoring was independently conducted by two investigators working in parallel. Each micronucleus was recorded as such only when the two investigators agreed. If only one of the two operators marked an anomaly as a micronucleus, it was discarded.

The mitotic index was calculated by counting the number of dividing cells in the sample and dividing this value by the total number of cells counted (in this case, 1000).

The statistical analysis was conducted by applying the Shapiro-Wilk test and the evaluation of the Pearson linear correlation coefficient. The

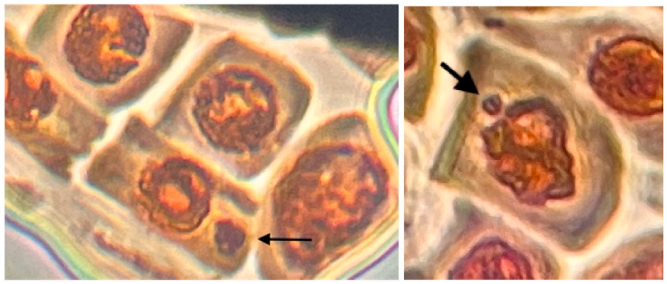

Fig. 2. Examples of micronuclei, obtained with alpha irradiation at an absorbed dose from 20 to 80 mGy (400X magnification).

Shapiro-Wilk test was used to assess the normality of data distributions, with a statistical significance threshold of $p < 0.05$. All statistical tests were performed using the PAST 4 software (Hammer et al., 2001).

3. results

The Shapiro-Wilk test confirmed that the data follow a normal distribution. For this reason, data in our plots are represented as the mean ± 2 standard deviations (SD), to encompass approximately 95% of the expected data (Hazra and Gogtay, 2016). This approach is applied both to the count of micronuclei and to the mitotic index.

The parameters considered for assessing damage as a result of irradiation included the number of cells with at least one micronucleus, the number of cells with more than one micronucleus, and the mitotic index.

3.1. Alpha particles

Consistently with previous studies (e.g., Xavier et al., 2021), exposure to alpha particles was found to cause a variation in the number of micronuclei formed inside the cells. The graph illustrating this relationship (Fig. 3) shows an increase in the number of micronuclei with

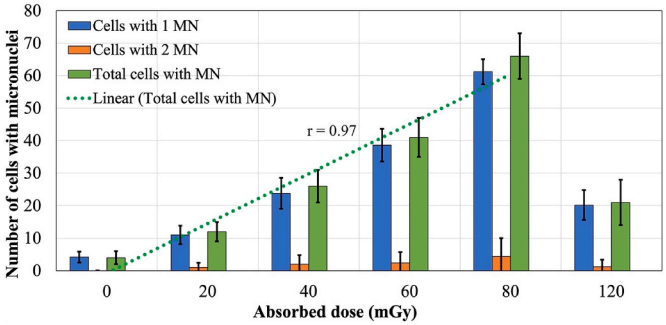

Fig. 3. Number of cells with micronuclei as a function of dose.

increasing dose up to 80 mGy. However, at an absorbed dose of 120 mGy, a decrease in this number was observed. The behavior resembles the results from previous studies on human lymphoblasts, where alpha particle doses higher than 100 mGy caused a decrease in the number of micronuclei (Ren et al., 2013).

Cells with the formation of two micronuclei were observed at doses higher than 20 mGy, although in small numbers. Since the objective of the study was to investigate the correlation between dose and damage, expressed by the appearance of one or more micronuclei, the data were reported and analyzed as the total number of cells exhibiting at least one micronucleus (green bar in the histogram).

The Pearson linear correlation coefficient was assessed for doses ranging from the negative control to 80 mGy, where an increasing trend in the number of micronuclei as a function of dose was observed. A correlation coefficient r = 0.97 was found, indicating an almost perfect positive linear relationship. The correlation of the data within the 0–80 mGy range is therefore consistent with a linear trend, indicating the potential use of *Allium cepa* as a biodosimeter within this dose range.

The analysis of the mitotic index trend (Fig. 4) indicates that, in addition to micronuclei formation, alpha particles have a significant impact on cellular vitality. It is evident that starting from a dose of 20 mGy, the number of dividing cells decreases drastically compared to the negative control. However, no correlation was observed between the increase in dose and the reduction in cellular vitality, despite the observed decline. This result is consistent with the characteristics of high Linear Energy Transfer (LET) radiation, whose high ionization power increases the probability of causing more severe damage, in addition to micronuclei formation, which can lead to cell inactivation.

These data seem to suggest a drop in the mitotic index of *Allium cepa* root cells subjected to doses from 20 to 80 mGy followed by an increase at 120 mGy, which could be linked to damage-induced disordered proliferation (Leme and Marin-Morales, 2009). However, all data fall within the experimental uncertainties and thus warrant further investigation.

3.2. X-rays

Even for the case of exposure to X-rays, the decision was made to include in the graph the number of cells that exhibited one or two micronuclei, along with the total number of cells showing at least one form of anomaly. This choice was made although the number of poly-micronucleated cells turned out to be only two out of a total of 20,000 cells counted (Fig. 5).

In this case, as well, the analysis of the Pearson linear correlation coefficient (r = 0.97), calculated among all considered dose points, revealed an almost perfectly linear trend. The consistency of these findings with a linear trend supports the hypothesis of a causal relationship between X-ray absorption and the occurrence of cellular damage, as indicated by the formation of micronuclei. These results support the use of *Allium cepa* as a biodosimeter for X-ray doses ranging from 0 to 600 mGy.

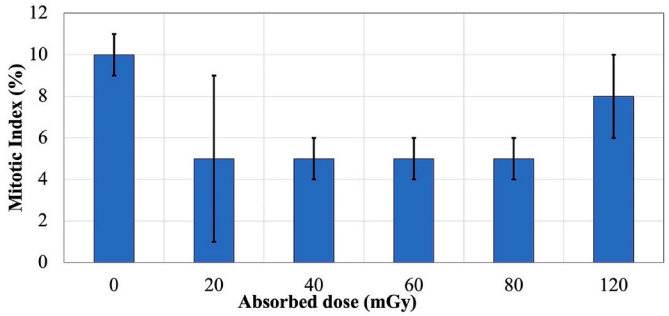

Fig. 4. Mitotic index as a function of dose.

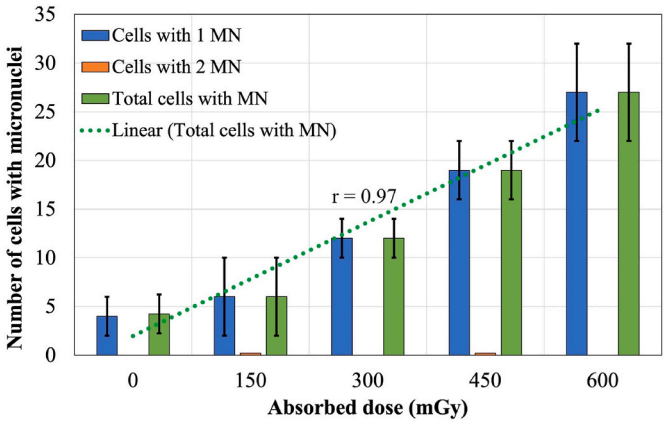

Fig. 5. Number of cells with micronuclei as a function of dose.

Fig. 6 instead shows the trends of the mitotic index values as a function of the dose. In this case, the cellular vitality parameter does not undergo significant variations with varying doses. This trend was also observed in the analysis of the mitotic index when *Allium cepa* sprouts were exposed to other types of low LET radiation, such as gamma rays (Xavier et al., 2023). The trend is consistent with the properties of this type of radiation, where the sparse ionization density makes it difficult to induce double-strand breaks in the DNA filament.

3.3. Comparison

Fig. 7 shows the number of cells with micronuclei varies with the dose, both for alpha particles and for X-rays. Analyzing the results obtained on the apical meristems of *Allium cepa*, it is observed that both in the case of alpha particles and X-rays, the number of cells with micronuclei increases with increasing absorbed dose. This suggests a dependence of the number of cells with micronuclei on the absorbed radiation dose. In the case of alpha radiation, there is a rapid increase in the number of cells with micronuclei, concentrated in a much narrower range of absorbed doses. In the case of X-rays, the increase is slower and occurs over a wider range of doses.

This result agrees with the well-known fact that high Linear Energy Transfer (LET) radiation (alpha particles) is capable of causing greater DNA damage than low-LET radiation (X-rays).

By calculating the ratio between the angular coefficients, a value of about 20 is obtained. This value corresponds to the ratio of radiation weighting factors, which are tabulated based on the radiation hazard. In the case of alpha radiation, the weighting factor is 20, while for X-rays, it is 1. Therefore, the ratio between the factors for high and low LET radiation is confirmed to be 20, in line with the observed ratio between the slopes of the previously described lines. This strengthens the validity of

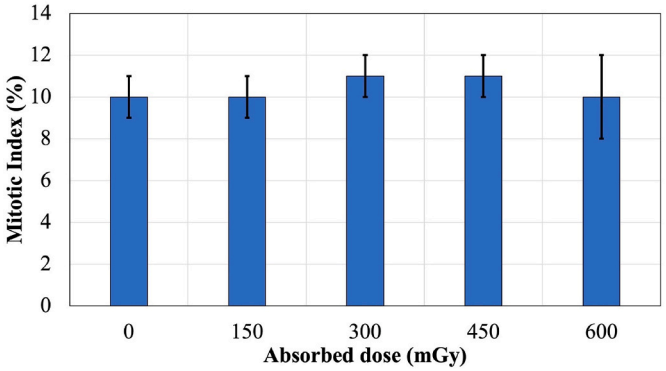

Fig. 6. Mitotic index as a function of dose.

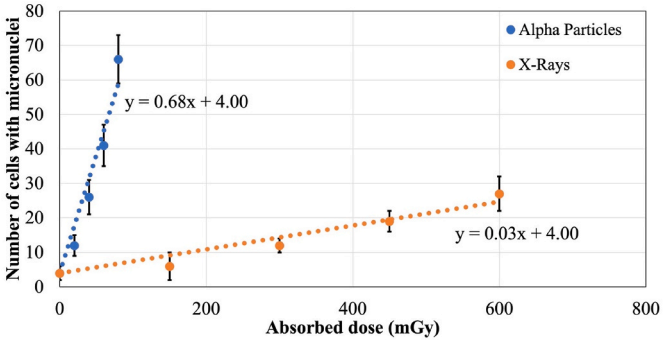

Fig. 7. Number of cells with micronuclei as a function of dose.

the results obtained.

The data related to the mitotic index also show that high-LET radiation, with greater ionization power, has a higher probability of causing damage leading to cellular inactivation and thus a decrease in the mitotic index. This effect was not observed in the case of low-LET radiation, where no significant differences were found between the number of dividing cells in the irradiated samples and the negative control (non-irradiated samples). As a final variable, the number of cells containing two or more micronuclei was examined. The presence of a greater number of DNA defects within a cell may indicate more significant damage to the genetic material. In this analysis, it is confirmed that alpha radiation can generate a greater number of "polymicronucleate" cells compared to X-rays. In detail, considering all the analyzed samples, 40 cells with double micronucleus (in one case even with 3 micronuclei) were found out of a total of 20,000 cells counted in the case of alpha particles. In the case of X-rays, however, only two cells with double micronucleus were counted out of a total of 20,000 cells counted.

4. Conclusions

The objective of this work was to assess the use of *Allium cepa* as an experimental model to investigate the effects of ionizing radiation, with a particular focus on evaluating the response to radiation of different Linear Energy Transfer (LET). *Allium cepa* is a well-established, robust experimental model that is widely used based on several considerations reflecting both scientific and practical considerations.

Chemical similarity between *Allium cepa* and water leads to a similarity in the physical interactions of ionizing radiations in our model and animal tissues. Indeed, *Allium cepa* has shown a response highly consistent with the responses observed in mammalian cells (Grant, 1978; Leme and Marin-Morales, 2009; Palmieri et al., 2016; Reis et al., 2017). This makes *Allium cepa* a valuable tool as its response to radiation exposure can provide indications of potential genotoxic effects on both plant and animal cells.

Moreover, the fact that onion plants are structured organisms provides a unique opportunity to examine the effects of radiation in a biologically relevant and complex context. From an ethical and practical standpoint, tests on *Allium cepa* require fewer approvals compared to animal studies, making them more viable and accessible for scientific research.

Finally, the consistency with a linear trend, observed both for alpha particles in the dose range from 0 mGy to 80 mGy, and for X-rays in the dose range from 0 mGy to 600 mGy, supports the use of Allium cepa as a biodosimeter for these types of radiation within their respective dose ranges. The ability to detect a linear relationship between dose and biological response in these dose ranges underscores the viability of the Allium cepa model in the assessment of radiation effects.

The results of our study confirm the viability of *Allium cepa* as an experimental model, showing significant variations in the response to radiation at different Linear Energy Transfer (LET) values, which are relevant for assessing the risks associated with radiation exposure. Comparing our results with previous studies (Xavier et al., 2021, 2023), it emerges that *Allium cepa* could constitute a valid alternative model to assess the genotoxic effects of low doses of alpha radiation. Additionally, this plant model may reveal variations in the effects induced by different types of radiation, including X-ray radiation.

In conclusion, our investigation strongly suggests that *Allium cepa* represents a valid alternative or complement to animal models for studying the biological effects of ionizing radiations. However, extensive additional experiments and assessment of other cytogenetic endpoints are necessary, as well as work on standardizing and possibly automating the experimental procedures. The latter would increase our data processing speed and reproducibility, thus allowing for large experimentation, e.g., in the analysis of low-dose effects.

CRediT authorship contribution statement

T. Butini: Writing – original draft, Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **F. Barco:** Writing – original draft, Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **M.G. Cascone:** Writing – review & editing, Validation, Supervision. **R. Ciolini:** Investigation. **M. Quattrocchi:** Investigation. **E. Rosellini:** Writing – review & editing, Validation, Supervision. **J.A. Torres Novaes:** Methodology. **M.N. Xavier:** Methodology. **S. de Souza Lalic:** Writing – review & editing, Validation, Supervision. **F. d'Errico:** Writing – review & editing, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Validation, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

References

Bagatini, M.D., Vasconcelos, T.G., Laughinghouse, H.D., Martins, A.F., Tedesco, S.B., 2009. Bio-monitoring hospital effluents by the Allium cepa L. test. Bull. Environ. Contam. Toxicol. 82 (5), 590–592. https://doi.org/10.1007/s00128-009-9666-z.

Barco, F., Butini, T., Cascone, M.G., Chierici, A., Ciolini, R., Rosellini, E., Torres Novaes, J.A., Xavier, M.N., de Souza Lalic, S., d'Errico, F., 2024. Biodosimetry of UV radiation through the detection of cytogenetic endpoints in *Allium cepa* meristems. Submitted for publication in Radiation Measurements 176, 107213. https://doi.org/10.1016/j.radmeas.2024.107213.

Bolsunovsky, A., Dementyev, D., Trofimova, E., Iniatkina, E., Kladko, Y., Petrichenkov, M., 2019. Chromosomal aberrations and micronuclei induced in onion (Allium cepa) by gamma-radiation. J. Environ. Radioact. 207, 1–6. https://doi.org/10.1016/j.jenvrad.2019.05.014.

Bonciu, E., Firbas, P., Fontanetti, C.S., Wusheng, J., Karaismailoğlu, M.C., Liu, D., Menicucci, F., Pesnya, D.S., Popescu, A., Romanovsky, A.V., et al., 2018. An evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay. Caryologia 71 (3), 191–209. https://doi.org/10.1080/00087114.2018.1503496.

Brenner, D.J., Doll, R., Goodhead, D.T., Hall, E.J., Land, C.E., Little, J.B., Lubin, J.H., Preston, D.L., Preston, R.J., Puskin, J.S., et al., 2003. Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. Proc. Natl. Acad. Sci. USA 100 (24), 13761–13766.

Chen, Y., Zhou, P., Zhang, X., Wang, Z., Wang, Y., Darroudi, F., 2014. Cytogenetic studies for a group of people living in Japan 1 year after the Fukushima nuclear accident. Radiat. Protect. Dosim. 159 (1–4), 20–25.

Da Cruz, A.D., McArthur, A.G., Silva, C.C., Curado, M.P., Glickman, B.W., 1994. Human micronucleus counts are correlated with age, smoking, and cesium-137 dose in the Goiania (Brazil) radiological accident. Mutat. Res. Environ. Mutagen Relat. Subj. 313 (1), 57–68.

d'Errico, F., Barco, F., Butini, T., Cascone, M.G., Ciolini, R., Quattrocchi, M., Rosellini, E., Hulber, T., Torres Novaes, J.A., Xavier, M.N., de Souza Lalic, S., 2023. Radiation monitoring with plant-based biotas and an automated micronucleus scoring

approach. EPJ Web Conf. 288, 09007 https://doi.org/10.1051/epjconf/202328809007.
Firestone, R.B., 1996. Table of Isotopes, eighth ed. Wiley, New York.
Fiskesjö, G., 1985. The Allium test as a standard in environmental monitoring. Hereditas 102 (1), 99–112. https://doi.org/10.1111/j.1601-5223.1985.tb00471.x.
Gerhardt, A., 2002. Bioindicator species and their Use in Biomonitoring. In: Environmental Monitoring—Vol. I—Bioindicator Species and Their Use in Biomonitoring, Encyclopedia of Life Support Systems (EOLSS).
Goodhead, D.T., 2006. Energy deposition stochastics and track structure: what about the target? Radiat. Protect. Dosim. 122 (1–4), 3–15.
Grant, W.F., 1978. Chromosome aberrations in plants as a monitoring system. Environmental Health Perspectives 27, 37–43.
Guerra, M., de Souza, M.J., 2002. Como observar cromossomos: um guia de técnicas em citogenética vegetal, animal e humana. Ribeirão Preto, FUNPEC.
Hayashi, M., 2016. The micronucleus test—most widely used in vivo genotoxicity test. Gene Environ. 38, 18. https://doi.org/10.1186/s41021-016-0044-x.
Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. Past: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4 (1), 1–9.
Hazra, A., Gogtay, N., 2016. Biostatistics series module 1: basics of biostatistics. Indian J. Dermatol. 61 (1), 10–20. https://doi.org/10.4103/0019-5154.173988. PMID: 26955089; PMCID: PMC4763618.
Higley, K.A., 2018. Integration of radiological protection of the environment into the system of radiological protection. Ann. ICRP 47 (3–4), 270–284.
ICRP, 2007. The 2007 recommendations of the international commission on radiological protection. ICRP publication 103. Ann. ICRP 37.
Kimball, R.F., 1952. Genetic effects of radiation. Annu. Rev. Nucl. Part Sci. 1, 479–494. https://doi.org/10.1146/annurev.ns.01.120152.002403. ISSN: 1545-4134.
Köksal, G., Dalcí, D.Ö., Pala, F.S., 1996. Micronuclei in human lymphocytes: the Co-60 gamma-ray dose-response. Mutat. Res. Environ. Mutagen Relat. Subj. 359 (2), 151–157. https://doi.org/10.1016/S0165-1161(96)90261-7.
Leme, D.M., Marin-Morales, M.A., 2009. Allium cepa test in environmental monitoring: a review on its application. Mutation research/reviews in mutation research 682 (1), 71–81. https://doi.org/10.1016/j.mrrev.2009.06.002.
Ludovici, G.M., Cascone, M.G., Huber, T., Chierici, A., Gaudio, P., de Souza, S.O., d'Errico, F., Malizia, A., 2021. Cytogenetic biodosimetry techniques in the detection of dicentric chromosomes induced by ionizing radiation: a review. The European Physical Journal Plus 136 (5), 482. https://doi.org/10.1140/epjp/s13360-021-01447-3.
Nefic, H., Musanovic, J., Metovic, A., Kurteshi, K., 2013. Chromosomal and nuclear alterations in root tip cells of allium cepa L. induced by alprazolam. Med. Arch. 67 (6), 388–392. https://doi.org/10.5455/medarh.2013.67.388-392.
Palmieri, M.J., Andrade-Vieira, L.F., Trento, M.V.C., Eleutério, M.W.F., Luber, J., Davide, L.C., Marcussi, S., 2016. Cytogenotoxic effects of spent pot liner (SPL) and its main components on human leukocytes and meristematic cells of Allium cepa. Water, Air, Soil Pollut. 227, 1–10.
Ponnaiya, B., Jenkins-Baker, G., Bigelow, A., Marino, S., Geard, C.R., 2004. Detection of chromosomal instability in α-irradiated and bystander human fibroblasts. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 568 (1), 41–48. https://doi.org/10.1016/j.mrfmmm.2004.06.045.
Reis, G.B., Andrade-Vieira, L.F., Moraes, I.C., César, P.H.S., Marcussi, S., Davide, L.C., 2017. Reliability of plant root comet assay in comparison with human leukocyte comet assay for assessment environmental genotoxic agents. Ecotoxicol. Environ. Saf. 142, 110–116.
Ren, R., He, M., Dong, C., Xie, Y., Ye, S., Yuan, D., Shao, C., 2013. Dose response of micronuclei induced by combination radiation of α-particles and γ-rays in human lymphoblast cells. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 741, 51–56. https://doi.org/10.1016/j.mrfmmm.2012.12.007.
Russell, W.M.S., Burch, R.L., 1959. The Principles of Humane Experimental Technique. Methuen.
Russell, W.L., Kelly, E.M., 1982. Specific-locus mutation frequencies in mouse stem-cell spermatogonia at very low radiation dose rates. Proc. Natl. Acad. Sci. USA 79 (2), 539–541.
Senthamizhchelvan, S., Pant, G.S., Rath, G.K., Julka, P.K., Nair, O., 2009. Biodosimetry using micronucleus assay in acute partial body therapeutic irradiation. Phys. Med. 25 (2), 82–87. https://doi.org/10.1016/j.ejmp.2008.05.004.
Ulanovsky, A., 2016. Dosimetry for animals and plants: contending with biota diversity. Ann. ICRP (Int. Comm. Radiol. Prot.) 45 (1 Suppl. l), 225–238.
Vaijapurkar, S.G., Agarwal, D., Chaudhuri, S.K., Senwar, K.R., Bhatnagar, P.K., 2001. Gamma-irradiated onions as a biological indicator of radiation dose. Radiat. Meas. 33 (5), 833–836. https://doi.org/10.1016/S1350-4487(01)00246-3.
World Health Organization, 2011. Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies. Rapp. Tecn. International Atomic Energy Agency.
Xavier, M.N., Pantaleão, S.M., Scher, R., Ciolini, R., d'Errico, F., Souza, S.O., 2021. Allium cepa used as a dosimetry system in nuclear and radiological emergencies. The European Physical Journal Plus 136 (6). https://doi.org/10.1140/epjp/s13360-021-01674-8.
Xavier, M., Novaes, J., Silva, A., Alves, A., Santos, M., Pantaleão, S., Scher, R., d'Errico, F., Souza, S., 2023. Cytogenetic effects of β-particles in Allium cepa cells used as a biological indicator for radiation damages. J. Environ. Radioact. 259-260, 107109. https://doi.org/10.1016/j.jenvrad.2023.107109.
Zölzer, F., Skalická, Z.F., Havránková, R., Hon, Z., Navrátil, L., Rosina, J., Škopek, J., 2011. Enhanced frequency of micronuclei in lymphocytes from current as opposed to former uranium miners. J. Appl. Biomed. 9 (3), 151–156. https://doi.org/10.2478/v10136-011-0002-7.