Space radiation effects on plant and mammalian cells

C. Arena a,* , V. De Micco b, E. Macaeva c,d, R. Quintens c

a Department of Biology, University of Naples Federico II, Via Cintia 4, 80126 Naples, Italy
b Department of Agricultural and Food Sciences, University of Naples Federico II, via Università 100, 80055 Portici, Naples, Italy
c Radiobiology Unit, Institute of Environment, Health and Safety, Belgian Nuclear Research Centre, SCK•CEN, Boeretang 200, B-2400 Mol, Belgium
d Department of Molecular Biotechnology, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

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A B S T R A C T
The study of the effects of ionizing radiation on organisms is related to different research aims. The current review emphasizes the studies on the effects of different doses of sparsely and densely ionizing radiation on living organisms, with the final purpose of highlighting specific and common effects of space radiation in mammals and plants. This topic is extremely relevant in the context of radiation protection from space environment. The response of different organisms to ionizing radiation depends on the radiation quality/dose and/or the intrinsic characteristics of the living system. Macromolecules, in particular DNA, are the critical targets of radiation, even if there is a strong difference between damages encountered by plant and mammalian cells. The differences in structure and metabolism between the two cell types are responsible for the higher resistance of the plant cell compared with its animal counterpart.

In this review, we report some recent findings from studies performed in Space or on Earth, simulating space-like levels of radiation with ground-based facilities, to understand the effect of ionizing radiation on mammalian and plant cells. In particular, our attention is focused on genetic alterations and repair mechanisms in mammalian cells and on structures and mechanisms conferring radioresistance to plant cells.

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1. Introduction
Understanding the effect of ionizing radiation on organisms is a relevant and complex topic of current research which can be aimed to reach different endpoints within various contexts. The Chernobyl disaster and the more recent one at Fukushima-Daiichi have aroused interest in radioecology which aims to both comprehend the consequences of radiation exposure on living organisms in ecosystems and unravel the mechanisms by which plants and animals are able to counteract the effects of ionizing radiation [1,2,3]. Other fields of ionizing radiation application are in breeding programs to obtain selected plant cultivars [4,5] and in microbial decontamination methods alternative to conventional procedures [6]. In addition, the increasing use of ionizing radiation in medicine, whether for diagnostics or therapy, also supports the need for a better understanding of the long-term health effects of radiation exposure in humans.

Another important field of interest related to the studies on the effects of radiation on living organisms is space research. Humans in space are subjected to galactic cosmic rays (GCR) and solar particle events (SPE) that
cause significant but poorly understood risks of carcinogenesis and degenerative diseases [7]. The GCR spectrum is composed primarily of high-energy protons and atomic nuclei, namely about 87% high energy protons, 12% alpha-particles and 1% heavier ions up to iron (HZE) [8]; SPE consist of low to medium energy protons and alpha-particles. Numerous flight and ground-based experiments have been performed to expand the knowledge on the biological effects of cosmic radiation on humans in the perspective of manned space missions [9–11], although many questions still remain unanswered. At present, radiation risk represents the major constraint for manned exploration and colonization of the Solar system [12,13]. Indeed, the radiation risk in Space is still accompanied by a high degree of vagueness linked to the uncertainty not only of the levels of radiation likely encountered by organisms, but also of the degree of danger associated with different radiation types and organisms [14]. Ionizing radiation may have different outcomes on organisms depending on the radiation quality, the dose (rate) and/or cell characteristics [15,16]. The biological effects of cosmic radiation on other organisms (animals, plants and bacteria) are even less well characterized, but their understanding may be of equal importance in the view of future interplanetary missions aiming to establish permanent inhabited bases because bioregenerative life support systems, which will be needed to sustain such missions, heavily rely on the relationships between humans (animals), plants and bacteria [17–19].

Following the exposure to radiation, a latent period is expected in which the biological effects may not be immediately observed. The extent of the latent period may vary from a few minutes to decades, mainly depending on the irradiation dose and the intrinsic radiosensitivity of the organism. Irradiation at very high doses may cause immediate death or irreversible damages to cells, while both acute and prolonged irradiation with low doses may lead to the development of tumors in animals even decades after the exposure [16]. In addition, the biological effect of ionizing radiation may not only be limited to the organism exposed to irradiation but may also involve next generations. However, transgenerational effects of radiation appear to be very much dependent on the organism. For instance, it has been demonstrated that radiation exposure in mice can lead to increased frequencies in germ cell mutations, followed by malformations and dominant lethal mutations in the offspring [20]. In contrast, in humans no direct evidence of hereditary effects caused by radiation has been reported yet [21].

Generally, the term “high dose” is much different for plant cells than for mammalian cells [22]. The differences in structure and metabolism between the two cell types account for the higher resistance of plant cells compared to mammalian cells. It has been recently demonstrated that doses of X-rays up to 10 Gy do not induce any detrimental effect in leaf anatomical traits of bean plants when irradiation is directed to mature adult tissues [23]. In contrast, a whole body irradiation with such a dose in humans would cause death within days or weeks, mostly due to infections resulting from a depletion of white blood cells. With regard to tissue effects in humans, it is assumed that the threshold dose, below which no deterministic effects occur, is 0.1 Gy for both low- and high-LET (Linear Energy Transfer) radiation [24]. Therefore, for radiation protection in humans doses above 0.1 Gy are considered as high doses. However, despite the evident differences between the two systems, one of the most important common effects is the disruption of cell division. In animal cells, the exposure to radiation even at relatively low doses (below 0.1 Gy) induces a temporary interruption of the cell cycle. Higher doses may cause DNA mutations and chromosomal reorganization which may result in complete cessation of division or destruction of the cells [25]. Because of particular ionization patterns, high-LET ionizing radiations (e.g. protons and heavy ions) are more dangerous than low-LET ones (e.g. X- and gamma-rays), thus generating more cell death and mutations in both plant and mammalian cells [7,15,26]. High-LET radiation research is of particular importance in understanding the biological effects of cosmic radiation since they represent a very large fraction of cosmic radiation.

In this review, we summarize some recent findings related to the effects of low-LET and high-LET ionizing radiation on plant and mammalian cells. In particular, we focus on the main alterations triggered by radiation in mammalian and plant cells, on DNA repair processes in mammalian cells and on structures and mechanisms conferring radioresistance in plant cells.

2. Effects of space radiation on mammalian cells

As mentioned in the introduction, space radiation consists of fairly well described [9] GCR and SPE, of which especially heavy ions are expected to have an important negative impact on the astronaut’s health [27]. Effective shielding might seem to be the easiest and most obvious solution. However, with the current technology, passive shielding is only effective for SPE, whereas it is limited for GCR due to severe mass constraints in spaceflight. Therefore, genetic and biomedical approaches could represent one of the solutions to GCR radiation protection issues [28]. In addition, it is noteworthy that the reduction in primary radiation due to shielding might be counteracted by secondary species (e.g. neutrons) which are formed when primary radiation interacts with the space-craft material. Such a secondary radiation might be even more biologically hazardous [7].

Up to now, the assessment of radiation risk for astronauts is almost completely based on extrapolation from terrestrial experimental and epidemiological data. This situation is unlikely to change in the near future as the number of individuals involved in real space flights is too low and thus comprehensive models are needed [29]. Two excellent reviews have provided an overview of ground-based studies relevant to space flight health risk assessment with respect to cancer [7] and non-cancer effects [30].

It is a well-established fact in radiobiology that physical differences between high-LET and low-LET ionizing radiation have a significant impact on the damage caused in mammalian cells. Because of their physical nature, high-LET particles deposit their energy within a comparatively small volume, thus causing more complex DNA damage and overall biological effects when compared to low-LET radiation of the same energy [31,32]. This, together with
the fact that shielding from these particles is practically almost impossible, makes them the most effective contributors to space radiation health risks [27]. Therefore, ground-based studies of the effect of space radiation, mostly use HZE particles such as carbon and iron. In general, for many relevant cellular effects, as LET increases, the relative biological effectiveness (RBE) of radiation tends to increase as well. In reality, in many studies, significant differences in RBE of particles have been reported with results varying for different physical parameters of the beam and biological endpoint and tissue of choice [7,33]. These observations imply that high-LET radiation may not only cause more serious damage to the cell when compared to low-LET, but also activate different response mechanisms. Nevertheless, because the crucial radiation target in cells is DNA [34], we will mainly focus on DNA damage and repair with respect to low- and high-LET radiation, as well as the induction of chromosome aberrations and effects of microgravity on the above mentioned processes.

Fig. 1. Radiation-induced DNA damage, repair mechanisms and possible consequences in mammalian cells. The major effect of ionizing radiation in cells is DNA damage. Since DNA consists of a pair of complementary strands, breaks of either a single strand or both strands can occur. Depending on the character of DNA damage, an appropriate repair mechanism will be activated. Single-strand breaks (SSBs) and base modifications are eliminated by the base-excision repair (BER) machinery which generally lead to successful repair. Double-strand breaks (DSBs) are repaired by homologous recombination (HR) or, more often, by non-homologous end joining (NHEJ). In case DSB repair processes are erroneous or unsuccessful, the cell can decide to undergo apoptosis or necrosis, become senescent or differentiate prematurely (in the case of stem cells). All of these options would result in removal of damaged cells from the proliferative pool. The inability to activate these pathways in cells with damaged DNA, can lead to malignant transformation of the cell and ultimately cancer development.
2.1. Radiation-induced DNA damage and repair in mammalian cells

A general view of DNA damages, repair mechanisms and consequences in mammalian cells is depicted in Fig. 1. The interaction of radiation with cells is extremely complex, as it can occur through direct interaction of radiation with cell components or through indirect damage caused by elevated radiation-induced ROS production which therefore initiates a complex cellular response. Depending on the type of DNA damage, cells can activate different repair mechanisms. The main types of DNA damage caused by ionizing radiation are (a) abnormal bases and single-strand breaks (SSBs), which are eliminated by the base-excision repair (BER) mechanism, and (b) double-strand breaks (DSBs) repaired by recombination repair mechanisms (mostly non-homologous end joining, NHEJ, or homologous recombination, HR). Whereas SSB and base damages can be usually correctly repaired, this is not always the case for DSBs. Therefore, DSBs are considered to be the most severe because they are more likely to result in chromosome aberrations and genomic instability [35,36] which can ultimately lead to lethal cell damage [37]. Although the number of SSBs and DSBs per absorbed dose are very comparable between different radiation types, the type of DNA damage that is induced by radiation exposure is very much dependent on the radiation quality. As previously mentioned, high-LET radiation induces more complex damage, i.e. clusters of the various possible types of damage within closely localized regions of the DNA, as compared to low-LET radiation [31,32]. This has been linked to the increased RBE of densely ionizing radiation, although it has to be noted that the RBE depends on the measured end-point. For instance, whereas maximal RBE values range from 5 to 20 for chromosome aberrations and cell inactivation, there is no strong dependence on LET for DSBs (maximal RBE between 1 and 2) [38]. The basis of the choice of DSB repair mechanism is still not completely understood but cells of higher eukaryotes appear to preferentially utilize the NHEJ pathway [39]. To a large extent this can be explained by the fact that HR requires the presence of an intact sister chromatid and, as sister chromatids are only available in G2 and late S-phase, this repair mechanism is clearly cell-cycle stage specific. In several studies on mammalian cells irradiated during different cell cycle phases, NHEJ was found to be important in all cell cycle phases and predominant in G1 and early S-phase, while HR is particularly important in late S/G2 phase [39,40]. Currently, there is no evidence that different DNA repair mechanisms are activated after exposure to high- or low-LET radiation although both the quality of the repair as well as the kinetics differ [7]. In human endothe- lial EA.hy926 cells we observed that γH2AX foci disappeared more slowly after irradiation with Nickel ions as compared to X-rays [41,42]. This may be a consequence of the previously mentioned increase in complex damages induced by densely ionizing radiation. Another possible explanation, however, could be that differences exist in the activation of genes involved in DNA repair pathways after exposure to X-rays compared to heavy ions. For instance, we found that exposure of human peripheral blood mononuclear cells to carbon ions and X-rays results in a very similar induction of genes involved in DNA damage response pathways after 8 h post-irradiation. However, whereas most genes returned to background levels after 24 h in X-irradiated cells, their expression remained significantly upregulated in cells irradiated with carbon ions (Macaeva et al., unpublished results). A similar result was found in a human prostate cancer cell line, PC3, in which we observed a transient down-regulation of motility-related genes in X-irradiated cells which lasted longer in carbon-irradiated cells (Suetens et al., unpublished data). Moreover, in these cells, both the number of differentially expressed genes, as well as their magnitude of change was much higher after carbon irradiation [43].

2.2. Radiation-induced chromosome aberrations in mammalian cells

In many ways, the link between unsuccessful or erroneous DSB repair and chromosomal rearrangements remains unclear [44]. The nature of chromosome damage is different for sparsely and densely ionizing radiation with the latter inducing considerably more complex chromosome exchanges in human lymphocytes when assessed at the first cell cycle after exposure [45–48]. These complex chromosome exchanges lead to cell death with a high probability. On the other hand, the fraction of cells carrying stable, transmissible chromosomal aberrations is similar in the progeny of cells irradiated with the same doses of high- and low-LET ionizing radiation [49]. Radiation-induced formation of such aberrations as truncated chromosomes without telomeres, resulting from DSBs at fragile telomeric sites, is of particular interest as it is specifically associated with genomic instability induction [50].

Several cytogenetic tests can be used to assess doses of radiation exposure. Some examples are the micronucleus assay, the premature chromosome condensation assay and the comet assay, each with their different advantages and drawbacks [51,52]. The golden standard for biodosimetry is the measurement of dicentrics in first mitosis lymphocytes [53], which has been mostly used for biodosimetry in cosmonauts [54]. However, because there are large inter-individual differences in the decay rate of dicentrics, this method is considered to be less reliable for biodosimetry of astronauts after long-term missions. Therefore, the NASA biodosimetry program uses the fluorescence in situ hybridisation (FISH) chromosome painting technique to measure chromosomal translocations which are more stable over time [55]. As a consequence, chromosome aberration biodosimetry studies performed on lymphocytes of astronauts and cosmonauts are affected by high experimental uncertainty levels at low-dose exposures and differences in experimental approaches. In a study by Durante and co-authors, mostly based on the dicentric assay, a statistically significant increase in chromosome aberrations was measured in lymphocytes from cosmo- nauts returning from their first long-term space flight. However, for cosmonauts involved in multiple space missions, they found that the frequency of chromosome aberrations was lower than expected and did not seem to be additive. Furthermore, for crew members involved in
two or more space flights, the yield of aberrations at the end of the last mission was close to the background measured before the flights [54]. On the contrary, other studies using FISH painting, do support an additive risk model as all astronauts showed an increase in their levels of chromosome aberrations after both the first and second flight [56,57].

2.3. Interaction between microgravity and radiation-induced DNA damage and repair

Another very important factor during space flight, which is associated with several health consequences for astronauts, is microgravity. Some of its well known health effects are space adaptation syndrome, bone demineralization and muscular atrophy, redistribution of body fluids and weakening of the immune system. At the cellular level, numerous experiments carried out in modeled microgravity on Earth or in-flight showed that altered gravity can induce apoptosis [58,59] and cytoskeletal alterations [60,61], affect cell growth and differentiation [62], and induce the formation of chromosome aberrations [63]. A significant increase in HPRT mutant frequency was observed in human peripheral blood lymphocytes incubated in simulated microgravity conditions after irradiation compared to those maintained in 1g conditions [64]. Another study from the same group provided evidence that simulated microgravity might increase the genotoxic effects of ionizing radiation as measured by delayed kinetics of radiation-induced DSB rejoining in human peripheral blood lymphocytes [65]. However, an earlier study on human fibroblasts showed that in the real microgravity environment, cells are able to repair radiation-induced DNA damage almost normally [66]. Currently, little is known about the possible interaction of the deleterious effects of space radiation and microgravity (especially as in most of the ground-based studies low-LET radiation is used) on the tissue and organ levels, where more influences might be expected. Unpublished results from our group demonstrated that DSB repair is slower in primary mouse neuronal cells subjected to modeled microgravity after acute exposure to X-rays (0.1 and 1 Sv), whereas chronic exposure to low doses (55 mSv) of high-LET neutrons induced more DSBs in cells in microgravity compared to ground conditions (Pani et al., unpublished data).

2.4. Radiation hormesis and the adaptive response to radiation in mammals

It is noteworthy that low doses of ionizing radiation (within the range or slightly above natural background levels) have been proposed to be beneficial by stimulating the activation of protective and repair mechanisms, a phenomenon which is known as radiation hormesis [67]. Another model supporting the possible beneficial effect of ionizing radiation on animals is the adaptive response model, which was first described in an experiment in which human lymphocytes cultured in low concentrations of radioactive thymidine showed fewer chromosomal aberrations in response to a high radiation dose than cultures grown without radioactive thymidine [68]. Several in vitro and animal studies have shown reduced levels of DNA damage and cancers when a higher challenging dose is preceded by a lower priming acute or chronic dose of 1–100 mGy of X or gamma-rays [69–72]. It has even been proposed to exploit inter-individual differences in adaptive response to select crews for interplanetary missions [73]. In the context of manned long-term space missions, it was suggested that adaptive response to space radiation might explain the apparent increase in radiation resistance of cosmonauts [54], however, as described above, the available data on chromosome aberrations in lymphocytes from cosmonauts/astronauts requires further validation. Populations living in high-background radiation areas represent an unique cohort to study the health effects of an environment resembling, to some extent, the chronic exposure of future space colonists to ionizing radiation [74]. Natural background radiation as low dose-rate conditioning exposure has been reported to induce adaptive responses in human lymphocytes, although conflicting results exist, partly because of the use of different techniques for assessing chromosome aberrations [74]. Anyway, it is important to note that no published data have unambiguously shown an increased cancer risk in populations exposed to high natural background radiation. On the contrary, they seem healthier and live longer than people living in areas with normal radiation background, an observation which has been termed “the radiation paradox” [75]. Still, the concepts of radiation hormesis and adaptive response are very controversial and may be related more to low dose rates, rather than low doses as such.

3. Plant cells and ionizing radiation

Plants are more tolerant to ionizing radiation than animals. However, the effect of cosmic radiation (especially energetic heavy ions) on plants’ functions still remains not completely unraveled. Different experiments on seeds and seedlings have been performed in space or on Earth simulating space-like high levels of radiation with ground-based facilities, in order to analyse plant response to ionizing radiation. The effect of radiation varies significantly depending on the type of radiation, delivered dose, species, developmental stage and genetic traits [15,76]. These effects include modifications of both plant morpho-anatomical traits and metabolism [77,78]. The negative outcomes increase at high doses of both low- and high-LET radiation [19,79]. Ionizing radiation on plants can produce: (a) indirect effects, namely phenotypical alterations primed by radiation-induced genetic changes in seeds, meristems or reproductive cells, and (b) the direct damage of adult tissues.

3.1. Effects on genomic material

As in mammalian cells, in plant cells the nucleus is considered the principal site of injury by ionizing radiation [80], which is responsible for random DNA damages and generates different kinds of mutations, such as deletions, base substitutions and chromosomal alterations [15,81,82]. The mutation frequency is significantly higher in cells irradiated with heavy ion beams than with X-rays; however at very low doses of heavy ions, a wide variety of non-lethal mutations may be induced [83,84]. With increasing dose of
3.2. Morphological and anatomical alterations

Heavy ions, significant DNA injuries are expected; just as mentioned for mammalian cells, high-LET radiations also mainly induce DSBs in plant cells [83, 86]. Shikazono et al. reported that about half of the mutations induced by carbon ions with LET of 101–124 keV μm−1 in A. thaliana were small alterations (base substitutions, small insertions/deletions), whereas the other induced mutations consisted of rearrangements such as translocations, inversions, and large insertions/deletions [87]. The detrimental effects of deleterious mutations include death in the embryonic state, the inability to reproduce, occurrence of malformations, increased susceptibility to diseases and decrease in lifespan [15]. Hase et al. reported that in Arabidopsis dry seeds, high-LET ion beams induce DSBs that are not always repaired by the NHEJ pathway [88], and in case this occurs, the viability of the cell becomes compromised, as is the case for mammalian cells (Fig. 1).

There is a direct relationship between the radiosensitivity of a plant and the average volume occupied by a chromosome in the cell nucleus [89]. More specifically, the larger the chromosome volume, the more sensitive is the plant and, therefore, the smaller is the radiation dose necessary to either kill the plant or to cause a strong damage. Moreover, the amount of damage that radiation could produce in the same cell varies at different stages of the plant life cycle because the chromosome volume varies with the cell division stage [89].

The number of chromosomes in a species also determines the susceptibility of a plant to radiation. Generally polyploid species exhibit a decreased sensitivity to radiation compared to those with low chromosome numbers within the same genus.

Polyploidy is widespread in plants and confers an evolutionary advantage driving adaptation and speciation [90, 91, 92]; the success of polyploids in nature can rely not only on positive structural and functional modifications [93], but also on higher phenotypic stability because gene redundancy protects polyploids from the deleterious effect of mutations [94]. In other words, the higher stability of polyploids is due to the presence and expression of multiple copies of the same genes, which, although increasing nuclear volume, determine the presence of additional "non-defective" wild type copies of the same gene which can be correctly expressed, thus hiding possible mutations inducing negative traits.

The alterations to genomic material induced by both high- and low-LET radiations may determine a wide range of phenotypic changes. Such modifications may include visible changes in plant morphology and anatomy, such as abnormal plant development, increased pubescence, occurrence of dwarf growth, altered anatomical structure in various organs, and depleted reproductive capability [23, 95, 96], as well as physiological and cytological alterations such as accumulation of anthocyanins, phenols and antioxidant compounds, changes in the photosynthetic machinery, modulation of the antioxidative system, and dilation of thylakoid membranes [19, 77, 97, 98].

3.2. Morphological and anatomical alterations

Differently from the effects on genomic material, the influence of ionizing radiation on morpho-anatomical traits in plants has been less extensively investigated. Most experiments refer to plants irradiated at the stage of dry seed and support the high radioresistance of plants. In such studies, growth perturbations are considered as phenotypical responses to genetic alterations induced in the irradiated seeds. The phenotypical response at the structural and ultrastructural level is less studied than plant survival, morphology and canopy architecture [19]. Although it is confirmed that high doses of irradiation more likely determine structural modifications, clear-cut dose-dependent tendencies of variations are not always found, as demonstrated in leaf anatomical traits of tomatoes developed from seeds irradiated with low-LET radiation [99].

Apart from growth modifications deriving from genetic alterations, the direct radiation-induced alterations in plants are generally ascribed to the interaction of the radiation itself with atoms and molecules which causes the production of ROS [95]. The accumulation and localization of hydrogen peroxide (a common ROS) and endogenous enzymes counteracting its effect are different in various cell and tissue types [95] which may explain the differences in radioresistance of various plant tissues.

For example, meristematic cells are more sensitive to ionizing radiation than somatic cells. The shoot apex is very sensitive and its cells show different degrees of sensitivity depending on their position [100]. Similarly, irradiation of root tips with C-ion microbeams leads to different inhibition of the gravitropic curvature depending on the position of the target cell [101].

It is generally recognized that the complex intracellular organization of eukaryotes coupled with the multicellular status of higher life forms have played a key role during evolution because they confer increased ability to adapt to harmful and mutagenic effects of the environmental factors, including ionizing radiation [102], Fig. 2. Multicellular organization provides for radioprotection by means of two main mechanisms: one related to genetics, the other basically structural. Complex tissue organization is associated with high resistance to mutagenic effects and the capability to adopt repair mechanisms [102, 103]. On the other hand, the presence of multilayer protective tissues at the organ surface controls the amount of harmful agents penetrating towards inner tissues, especially when accompanied by specialized cuticles and increased pubescence which is a typical response of plants subjected to high levels of radiation [104, 105]. The presence of phenolics among compounds occurring in the cuticle, or filling the trichomes and cells of sub-epidermal layers, confers to external tissues their ability to act as screens. Apart from this photo-protective function, specific phenolic compounds can also play a role as anti-oxidant agents as demonstrated in mesophyll cells [106]. The presence of the cell wall represents another reason for the high radioresistance of the plant cell when a specialized thick secondary wall, encrusted with complex structural phe-}

nolic compounds (e.g. suberin and lignins), is deposited. However, the role of cell wall in radioprotection has not yet been completely clarified since it is a composite structure made of molecules characterized by different degrees of sensitivity to ionizing radiation. Cellulose can be degraded by X-rays and high energy ions, because it
encounters oxidization processes and breaking of bonds [107].

Among matrix components, pectins are the most radiosensitive molecules whose degradation leads to the dissolution of middle lamellae [97]. The degradation of cell wall materials is responsible for macroscopic responses of plants to ionizing radiations, such as tissue softening, due to the dissolution of middle lamellae, and
the increase of seed germination, due to the augmented porosity of the nutritional layers and teguments of seeds which would favor water absorption [108,109]. The radiation-driven alteration of cell wall materials can be considered also responsible for the increase in cell size encountered by irradiated leaves since such degradation phenomena would reduce the mechanical constraints during turgor-driven cell enlargement [23].

Ionizing radiation also influences the content of phenolic compounds in plant tissues. Indeed, phenolic compounds are widespread in plant cell tissues and have a natural role of radiation shielding, which have accompanied the evolution of the first land plants [110]. Their synthesis is commonly stimulated by stress factors, including ionizing radiation; in fact, an increase in the phenolic content has been reported in seedlings and leaves irradiated with various sources of ionizing radiation or subjected to the space environment [19,23,111,112].

Flavonoids and other phenolic compounds, having antioxidant properties, are also extracted from plant tissues and applied for medical purposes following the hypothesis that they can have a role in inducing physiological protection: hence they would reduce the radiation-generated intracellular ROS and protect cells against radiation-induced membrane lipid peroxidation and cellular DNA damage [113–115].

Even when mechanical protection or metabolic responses are not able to impede cell/tissue/organ disruption, plants are more likely to survive than animals since plants are modular systems which can continue to grow after shedding damaged organs or parts of them in a mechanism of response common to other environmental stresses [116].

3.3. Effects on the photosynthetic apparatus

Among the different physiological processes, photosynthesis can be seriously affected by heavy ions, X- and gamma-rays. Specific studies performed on photosynthetic microorganisms and higher plants demonstrated that several components of the photosynthetic machinery may be altered: light-harvesting complexes, electron transport carriers, and enzymes of the carbon reduction cycle [15,117].

Generally, in the chloroplast of both higher plants and cyanobacteria, the photosystem II (PSII) renders the PSII complex more susceptible to radiation-induced damage [118,119].

The main target of the injuries is the PSII reaction centre D1 protein, which mediates the electron transfer between the primary electron donor and the secondary plastoquinone acceptor. The extent of the damage depends on the delivered dose; the loss of PSII functionality and the generation of ROS throughout the cell is expected mostly at high doses [15,120]. It has been demonstrated that high doses of gamma-irradiation (37.5 or 112.5 Gy) are responsible for a decrease of photosynthesis, chlorophyll (Chl) content and photosynthetic electron transport rate in bean and soybean plants [121,122]. A significant decrease in total Chl and carotenoid content, as well as an impairment of Rubisco activity was also found in bean plants after irradiation at 50 and 100 Gy X-rays doses [19,123].

Other studies demonstrated the effectiveness of heavy ion beams in inducing damages such as dilation of thylakoid membranes and loss of granal stacking by inhibiting the synthesis of Chl and LHCCI [97,124]. As a consequence of Chl depletion, the light absorbance spectrum pattern is impaired and a loss of functionality of the whole antenna complexes occurs [125].

It is noteworthy that the developmental stage has an influence on the photosynthetic responses of plants to radiation.

It has been demonstrated that the photosynthetic machinery of Arabidopsis plants at the reproductive stage can be relatively tolerant to gamma-rays even up to 200 Gy [126]. In addition, mature leaves of beans receiving high doses of X-rays (namely 50 and 100 Gy) at adult stage, are less sensitive to radiation injuries than younger ones [123]. The seed phase is more resistant than other life stages but the effect of ionizing radiation may vary significantly depending on whether the seed is wet or dry at the time of irradiation. More specifically, dry seeds are more resistant because the water deprivation limits the radiolysis and, in turn, the overproduction of free radicals [127,128].

3.4. Radioresistance and hormesis in plants

The exposure of plants to ionizing radiation may induce radioresistance. Plants are very radioresistant compared to animals, but the mechanisms underlying such an improved resistance need to be further explored. This phenomenon can be often ascribed to integrated mechanisms of adaptation at genetic, morpho-structural and eco-physiological levels [129].

The occurrence of radioresistance in plants has been mainly observed in radioactively contaminated areas of Chernobyl and adjacent regions where, after the nuclear disaster, plants have experienced both acute and chronic doses of ionizing radiation [120,130].

In these areas, the potential for organisms to adapt to radiation exposure has been attributed to molecular mechanisms such as the regulation of gene expression. In fact, chronic exposure to low doses of ionizing radiation leads to significant differences in the expression of radical scavenging enzyme and DNA-repair genes as well as an increase of the activity of several antioxidant enzymes in plant tissues [120,129].

Recent experiments have demonstrated that the exposure to high doses of X- and gamma-rays (50 and 100 Gy) results in the increasing of some antioxidants and scavenger enzymes activity as well as in the increasing of phenolic compounds in cells, which are effective in the removal of free radicals [19,23,117].

It has also been reported that high doses of X-rays promote the activity of poly(ADP-ribose) polymerases (PARPs), enzymes regulating stress response pathways, which, that are able to recognize damaged DNA and allow its repair [123]. The activation of the scavenging enzymatic machinery and PARPs, and the overproduction of phenolic compounds represent essential strategies to offset cell oxidative damage, thus improving plant radioresistance.
Low doses of ionizing radiation may induce positive responses in plants [131]. Many plant species may take advantage from very low levels of radiation that can be considered harmful for animals [22,128,132].

It has been demonstrated that the exposure of Arabidopsis seedlings to low-dose gamma-rays (1 or 2 Gy) stimulates plant growth [97] and accelerates photosynthesis, respiration and electron transport rate [133,134]. Moreover, the irradiation of lettuce seeds with gamma-rays up to 30 Gy enhanced the content of chlorophyll a, chlorophyll b and carotenoids [135].

At present, no conclusive explanation is available regarding the phenomenon of hormesis, however, it has been hypothesized that low doses of radiation could affect growth by changing the hormonal signaling system or by increasing the antioxidative capacity of the cells to counteract the effects of environmental constraints [133].

4. General conclusions

Ionizing radiation may have different effects on plants and mammals depending on the radiation quality/quantity and/or cell characteristics as well as the developmental stage of the organism, which is generally more sensitive during the initial phase of the life cycle. Moreover, the biological effects of ionizing radiation are not only limited to the organism exposed to irradiation but may also involve next generations, although, in humans no direct evidence of hereditary effects caused by radiation has been reported so far.

There are strong differences in the structure and metabolism between mammalian and plant cells which might be responsible for the higher resistance of plant cells. Thus, non-lethal doses for plants may be very dangerous or fatal for humans, causing death within days or weeks, mostly due to infections resulting from a depletion of white blood cells.

In Fig. 3 we have summarized the main differences and similarities between plants and mammals with regard to ionizing radiation.

The crucial radiation target in both mammalian and plant cells is the DNA. In both systems, high-LET particles, such as HZE and high energy neutrons and protons, cause more complex DNA damage and overall biological effects when compared to low-LET radiation of the same energy. The interaction of radiation with cells can occur through direct interaction of radiation with cell components or through indirect damage caused by elevated radiation-induced ROS production. Ionizing radiation may not only cause serious injuries to the cell but also activate different response mechanisms. Depending on the type of DNA damage, an appropriate repair mechanism is chosen.

Fig. 3. General highlights of radiation response in plants and mammals: similarities and differences.
Generally in mammalian cells, the abnormal bases and SSBs are eliminated by the BER mechanism, whereas DSBs are repaired by recombination repair mechanisms (NHEJ or HR) in both mammalian and plant cells. In plants, the radiosensitivity is provided by several features at cytological (e.g. presence of a thick specialized cell wall, the accumulation of phenolic compounds), genetic (e.g. occurrence of polyploidy) and physiological level (e.g. the activation of specific enzymatic pathways i.e. ROS scavengers and/or of poly(ADP-ribose) polymerases). The loss or instability of genetic material may be lethal or at least lead to malignant transformation in animal cells, but it could not affect at all the fate of cells belonging to polyploid plant lines [136]. Moreover plants, in contrast to animals, are modular organisms, and may suffer important injuries (i.e. leaf abscission or loss of other parts), or even induce organ shedding in case of damage, while still surviving.

Finally, while low dose radiation-induced hormesis has been proposed to confer positive outcomes in plants, such as enhancing growth and photosynthesis, it still remains a controversial issue for human radiation protection. Although it has been shown at the cellular level that low priming doses may reduce different types of DNA damage and apoptosis, the possible beneficial effects at the level of the organism are currently still heavily disputed.

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