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Characterizing dose response relationships: Chronic gamma radiation in *Lemna minor* induces oxidative stress and altered polyploidy level

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ABSTRACT

The biological effects and interactions of different radiation types in plants are still far from understood. Among different radiation types, external gamma radiation treatments have been mostly studied to assess the biological impact of radiation toxicity in organisms. Upon exposure of plants to gamma radiation, ionisation events can cause, either directly or indirectly, severe biological damage to DNA and other biomolecules. However, the biological responses and oxidative stress related mechanisms under chronic radiation conditions are poorly understood in plant systems. In the following study, it was questioned if the *Lemna minor* growth inhibition test is a suitable approach to also assess the radiotoxicity of this freshwater plant. Therefore, *L. minor* plants were continuously exposed for seven days to 12 different dose rate levels covering almost six orders of magnitude starting from 80 μGy h^{-1} up to 1.5 Gy h^{-1}. Subsequently, growth, antioxidative defence system and genomic responses of *L. minor* plants were evaluated. Although *L. minor* plants could survive the exposure treatment at environmental relevant exposure conditions, higher dose rate levels induced dose dependent growth inhibitions starting from approximately 27 mGy h^{-1}. A ten-percentage growth inhibition of frond area Effective Dose Rate (EDR_{10}) was estimated at 95 ± 7 mGy h^{-1}, followed by 153 ± 13 mGy h^{-1} and 169 ± 12 mGy h^{-1} on fresh weight and frond number, respectively. Up to a dose rate of approximately 5 mGy h^{-1}, antioxidative enzymes and metabolites remained unaffected in plants. A significant change in catalase enzyme activity was found at 27 mGy h^{-1} which was accompanied with significant increases of other antioxidative enzyme activities and shifts in ascorbate and glutathione content at higher dose rate levels, indicating an increase in oxidative stress in plants. Recent plant research hypothesized that environmental genotoxic stress conditions can induce endoreduplication events. Here an increase in ploidy level was observed at the highest tested dose rate. In conclusion, the results revealed that in plants several mechanisms and pathways interplay to cope with radiation induced stress.

1. Introduction

Although the environment is continuously exposed to natural ionising radiation of cosmic and terrestrial origin, high levels of ionising radiation could potentially lead to disturbances in population ecosystems. Enhanced radiation levels can be the result of controlled anthropogenic activities from naturally occurring radioactive materials (NORM) industry, nuclear accidents or nuclear power production. During the last decade, international organisations like International Atomic Energy Agency (IAEA) and International Commission on Radiological Protection (ICRP) supported the development of guidance for environmental risk assessment and ecological protection criteria (IAEA, 1992; ICRP, 2003). In the framework of the European funded project ERICA,[1]

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[1] Environmental Risk from Ionising Contaminants: Assessment and Management.

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an effect database was established which holds data on radiation effect observations for non-human biota (Copplestone et al., 2008). From this database, it was clear that most of the studies performed so far deal with responses to acute, high doses rather than more environmentally relevant low dose and chronic exposure conditions (Garnier-Laplace et al., 2004; Esnault et al., 2010). Hence, more biological effect data in chronic, low-dose exposure conditions and on key wildlife groups are required as stated in the strategic research agenda of the ALLIANCE[2] (Hinton et al., 2013).

Gamma emitters and other radionuclides are routinely released into aquatic environments from nuclear power plants and nuclear fuel reprocessing plants. However, the possible biological responses on aquatic organisms and plants in particular, are still far from understood. Aquatic plants are defined as plants whose photosynthetic active parts are permanently or semi-permanently submerged in the water or float on the surface, and thereby play a vital role in healthy ecosystems. In terrestrial plants, both lab and field studies have shown that high external gamma radiation can affect morphology, physiology and reproductive capacity (Daly and Thompson, 1975; Sheppard et al., 1992; Kovalchuk et al., 2000; Kim et al., 2005; Wi et al., 2007). However, irradiation experiments have up to now used rather different plant species and exposure conditions complicating comparative conclusions. Despite these different approaches, literature have pointed out that plant species, plant organs and plant developmental stage show significant differences in radiosensitivity (Sparrow and Miksche, 1961; Kawai and Inoshita, 1965; Killion and Constantin, 1972; Daly and Thompson, 1975; Kovalchuk et al., 2000; Wi et al., 2007). Additionally, depending on the duration and level of the radiation exposure applied, plants showed dissimilarities in gene expression profiles depending on whether plants were acutely or chronically irradiated (Kovalchuk et al., 2000, 2007). In *Arabidopsis*, oxidative stress related genes seemed to be the most represented group in responding to both radiation conditions (Kovalchuk et al., 2007; Gicquel et al., 2012).

Healthy growing plants continuously generate low concentrations of Reactive Oxygen Species (ROS) as by-products of aerobic and anaerobic metabolic pathways in the chloroplasts, mitochondria and peroxisomes. In response to abiotic stress however, ROS can accumulate in plant cells, resulting in oxidative stress possibly leading to severe biological damage (Apel and Hirt, 2004; Gill and Tuteja, 2010). In radiation-exposed plant cells, ROS accumulation is additionally increased among others through the radiolysis of H_2O possibly leading to oxidative stress (Von Sonntag, 1987), although it has been confirmed that the production of ROS by radiolysis is negligible under environmental relevant exposure conditions (Smith et al., 2012). To counteract this ROS induced oxidative stress, plants can modulate antioxidative defence ROS-scavenging enzymes and metabolites (Gill and Tuteja, 2010). Different antioxidative enzymes like catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), ascorbate peroxidase (APOD), syringaldazine peroxidase (SPOD) and guaiacol peroxidase (GPOD) and metabolites like glutathione, flavonoids, phenolic compounds and carotenoids are typically induced in order to reduce increased concentrations of singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) or hydroxyl radical (HO•) in plant cells (Apel and Hirt, 2004). These antioxidative defence systems have their specific reactants, exhibiting different reaction kinetics and locations in subcellular components. For instance, CAT can independently convert H_2O_2 to H_2O and O_2 whereas APOD requires the oxidation of its co-substrate ascorbate to reduce H_2O_2 (Mittler et al., 2011). The metabolites ascorbate (ASC) and glutathione (GSH) are key players in the ASC–GSH cycle, forming an important mechanism of the antioxidative defence system (Noctor and Foyer, 1998).

Plants exposed to gamma radiation face deleterious effects that are either directly or indirectly induced through oxidative stress. The highly reactive ROS species, in particular HO•, can readily target DNA by the oxidation of nucleic acids (Balasubramanian et al., 1998). Additionally, ionising radiation can directly cause DNA lesions by deposition of energy leading to excitations and ionisation events, which may induce a spectrum of rearrangements and modifications to chromosomes. DNA damage affected on only one strand, like single strand breaks or single base conversion, can be repaired relatively easily by the presence of the complementary strand as a template. Hence when double strand breaks are being induced this is presumed to be more severe leading to more detrimental damage (Tuteja et al., 2001). As plants require light for photosynthesis their chloroplasts continuously generate ROS, and hence plants are believed to be constantly exposed to DNA damaging agents (Landry et al., 1995). Therefore plants require a DNA repair machinery of high efficiency and fidelity to protect DNA integrity. As such, when a DNA error is detected by DNA checkpoints, cell cycle progression can be delayed or arrested to gain time for repairing the damage (Britt, 1996). Cyclin-dependent kinases (CDKs) are mitotic regulators that guide two major phases during the cell division cycle. The genome is replicated during the synthesis phase (S-phase) and is afterwards halved during the final step of the mitosis cell cycle (M-phase) (De Schutter et al., 2007). Transition to the latter can only happen if DNA repair has occurred, although, under specific circumstances it is already observed that S-phase can still proceed without subsequent chromosome separation and cytokinesis ending in polyploid cells (De Veylder et al., 2011). This process, called endoreduplication, is well known in a variety of structural plants tissues to achieve growth by cell expansion. However, emerging data in plant research suggest another conserved role for endoreduplication in response to genomic instability caused by genotoxic environmental stressors (De Veylder et al., 2011).

Current literature indicated that for freshwater ecosystems interpretation of radioecological consequences is limited due to a lack in aquatic plant representatives (Garnier-Laplace et al., 2006, 2013). *Lemna minor* is a free-floating freshwater macrophyte for which OECD guidelines have been published to test the environmental toxicity of chemical contaminants in higher aquatic plants (OECD, 2006; Park et al., 2013). In this study, it was questioned if this *L. minor* growth inhibition test is a suitable approach to also assess the radiotoxicity of this freshwater plant. As such the sensitivity of *L. minor* to gamma radiation was first analysed by evaluating the growth inhibition (as described in the OECD guidelines) in the plants exposed to dose rates ranging from background level to 1500 mGy h^{-1}. As radiation induced ROS might be key players in the induction of adverse growth effects, the antioxidative response was further studied in the exposed *L. minor* plants. Additionally, DNA ploidy of *L. minor* plant cells was analysed since it is hypothesized that genotoxic stressors induce endoreduplication events in plants (De Veylder et al., 2011). Linking growth effects of irradiated plants with their respective physiological and genomic transformations, will allow to better understand underlying mechanisms of how chronic induced radiation stress influences plant systems.

2. Material and methods

2.1. Culture stock

L. minor cv. Blarney plants (Serial number 1007, ID number 5500) were obtained from Dr M. Jansen (University College Cork, Ireland) and aseptically cultured in a growth chamber in 250 ml

[2] The European Radioecology Alliance (www.er-alliance.org).