Title

High radiosensitivity in Norway spruce (*Picea abies*) is rendered by less comprehensive mobilisation of protection, repair and stress responses compared to the radiotolerant *A. thaliana*

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Abstract

Following nuclear events, risk assessment and protection of plant communities in contaminated ecosystems require in-depth understanding of radiosensitivity of plants. However, the physiological and molecular factors defining differential sensitivity to chronic ionising radiation exposure are poorly understood. In this study, we compared early molecular events associated with protection, repair, and stress responses as well as phenotypic and cellular effects in gamma-irradiated seedlings of the radiosensitive conifer Norway spruce (*Picea abies*) and the radiotolerant herbaceous *Arabidopsis thaliana*. After 48-h of irradiation, Norway spruce showed reduced growth at 290 mGy h^{-1} and severe organelle damage at ≥ 1 mGy h^{-1} whereas *A. thaliana* showed unaffected development, minor organelle damage at ≥ 100 mGy h^{-1} only and significantly less DNA damage at all dose rates. Comparative transcriptomics revealed that Norway spruce mobilized transcription of DNA damage repair and antioxidant genes at ≥ 40 mGy h^{-1} only while *A. thaliana* showed massive activation of genes related to DNA damage repair, antioxidants, and other stress responses as well as growth-promoting hormones and cell wall components at ≥ 1 mGy h^{-1}. Adverse effects on chloroplasts and mitochondria from low dose rates on and comprehensive downregulation of photosynthetic genes and activation of respiration genes at ≥ 40 mGy h^{-1} in Norway spruce but not in *A. thaliana* may reflect the higher energy demand in Norway spruce to simultaneously maintain its far larger genome and engage protection and repair systems. Hence, the absence of transcriptional response at lower gamma doses and activation of repair and protection at high dose rates only, when accumulated damage is high, is consistent with the high radiosensitivity of Norway spruce. Conversely, the more massive

transcriptional activation of crucial repair and protection pathways even at low dose rates complies with the high radiotolerance of *A. thaliana*.

Key words

Radiosensitivity in plants

Organelle damage

Antioxidant protection

DNA damage repair

Comparative transcriptomics

1. Introduction

Plants are frequently exposed to environmental stress and have therefore evolved stress-management strategies. Stress tolerance capacity in general is highly variable and a defining factor for species niche and habitat preference. Ionising radiation is a stressor that can induce harmful effects to organisms due to damage to biological macromolecules such as DNA, proteins, and lipids (Caplin and Wiley 2018). Field experiments and surveys of radionuclide-contaminated plant communities and ecosystems have shown that species that are sensitive to chronic ionising radiation may succumb and be replaced by more tolerant species (Amiro and Sheppard 1994; Caplin and Willey 2018). Laboratory studies have confirmed that radiosensitivity of different plant species ranges over orders of magnitude (Caplin and Wiley 2018; Blagojevic et al., 2019a). However, the underlying mechanisms conferring tolerance to ionising radiation are not well understood. Such knowledge is needed to improve our ability

to predict environmental consequences of nuclear accidents and release of radionuclides leading to exposure to high levels of ionizing radiation.

The average natural background level of ionising radiation is estimated to 0.3 µGy h^{-1}, while 15 µGy h^{-1} has been measured in areas with naturally elevated radionuclide levels in bedrock, sand and/or sediments (Caplin and Willey 2018). The nuclear power plant accident in Chernobyl in April 1986 as well as other accidents and experiments have shown that acute high doses of 10-1000 Gy can be lethal for plants and that radiosensitivity differs between species (UNSCEAR 1996). In response to sub-lethal chronic radiation, adverse effects such as growth inhibition, reduced apical dominance (increased branching) and leaf chlorosis have been observed, particularly in conifers (Caplin and Wiley 2018; (Kashparova et al., 2020). A dose rate of 10 mGy d^{-1} (417 µGy h^{-1}) has been suggested as a threshold value in the sense that lower dose rates have “no detrimental effects for populations of terrestrial plants in the field” (International Atomic Energy Agency [IAEA]; Caplin and Willey 2018; UNSCEAR). However, this does not appear to hold true for boreal conifer forests. In a long-term field irradiation experiment in Canada persistent effects on forests including conifer trees were observed at chronic dose rates ≥100 µGy h^{-1} (Amiro and Sheppard 1994). In addition to the differential radiosensitivity between species, effects of ionising radiation depend on dose rate, duration (acute or chronic) and thereby total dose, life stages and the co-occurrence of other contaminants as well as environmental conditions (Garnier-Laplace et al. 2013). A range of plant species, including *Arabidopsis thaliana* and rice, have shown high tolerance to ionising radiation compared to conifers (Blagojevic et al., 2019; Vanhoudt et al., 2014; Choi et al., 2021; Hase et al., 2020; *International Atomic Energy Agency, the Annual Report for 1992*).

The sensitivity of plant species to acute irradiation has been suggested to be attributed to the efficiency of DNA repair systems and genome organisation and size (De Micco et al., 2011). *A. thaliana* has a small genome of 135 Mb, whereas conifers like Norway spruce and Scots

pine have giant genomes of 20 and 23 Gb (Nystedt et al., 2013; Zimin et al., 2017). On the other hand, the sensitivity to chronic irradiation appears to be regulated by delicate mechanisms on different levels of biological organisation (Kim et al., 2011; Caplin and Wiley 2018; Kim et al., 2019). However, factors that define sensitivity to chronic exposure are poorly understood and apparently have functional rather than structural origin, depending on the molecular architecture of stress-responsive pathways of different plant species.

Ionising radiation can result in damage to proteins, lipids and nucleic acids both directly and indirectly via the formation of free radicals and subsequent reactive oxygen species (ROS) (Caplin and Willey 2018). Thus, a significant role in plant reactions to chronic radiation is attributed to high levels of ROS conferring oxidative stress. Plants possess potent antioxidant systems involving enzymes such as catalases (CAT), superoxide dismutase (SOD) and peroxidases (PER) as well as metabolites such as ascorbate (AsA), phenolic compounds and glutathione (GSH) that can neutralise ROS and thereby prevent oxidative damage (Dumanović et al. 2020). Studies of specific plant species including *A. thaliana*, have revealed induction of antioxidant-related genes, including *SOD* and *ASCORBATE PEROXIDASE* (*APX*) following ionising radiation exposure but there is limited information about antioxidant homeostasis in conifers (Vandenhove et al., 2009; Vanhoudt et al., 2014; van de Walle et al., 2016; Kim et al., 2011).

DNA strand breaks induced by ROS or directly by the ionising radiation trigger the activation of DNA damage repair (DDR) pathways (Kim et al., 2019). For instance, a range of homologous-recombination (HR) repair-mediated DDR genes known to be activated by the plant-specific transcription factor *SUPRESSOR OF GAMMA RESPONSE 1 (SOG1)* were significantly upregulated by gamma irradiation at 100 Gy in *A. thaliana* (Bourbousse, Vegesna, and Law 2018; Yoshiyama, Sakaguchi, and Kimura 2013). The initiation of the DDR is closely interlinked with the stalling of the cell cycle through the activation of cell

cycle checkpoints by regulating the cyclin (CYC)-cyclin-dependent kinase (CDK)-complexes (Qi and Zhang 2019). This can further lead to DNA damage-induced endoreduplication (Nisa et al., 2019). In addition, transcriptional recruitment of DDR proteins in response to gamma radiation requires chromatin remodelers involved in DNA methylation and histone 2A (H2A) modification (Choi et al., 2019; Kim 2019; Kim et al., 2021). In that context, increased global methylation in Scots pine plants from the Chernobyl exclusion zone has been reported (Kovalchuk et al., 2003). However, there is a lack of knowledge related to molecular events associated with DDR and other relevant pathways such as epigenetic modifications in conifers exposed to ionising radiation.

Although transcriptional regulation in response to high doses of ionising radiation in *A. thaliana* and a few other species has been explored (Choi et al., 2021; Vanhoudt et al., 2014), information on the molecular mechanisms leading to the differential radiosensitivity among plant species is yet to be clarified. Knowledge about mechanisms and factors that determine the radiosensitivity of plant species is of vital importance both with respect to risk assessment and protection of ecosystems from ionising radiation hazard. Hence, by employing gamma irradiation under standardised laboratory conditions, RNA-sequencing (RNA seq), concomitant analyses of growth, organelle, and DNA damage, as well as the dynamics of DDR gene expression, this study aimed to provide an overview of mechanisms involved in plant radiosensitivity. In this respect, early physiological, cellular, and molecular effects were compared in the radiosensitive conifer Norway spruce and the radio-tolerant *A. thaliana* to provide new insight regarding observed effects in plant communities growing in areas contaminated with radionuclides.

2. Materials and methods

2.1. Plant materials and pre-growing conditions

Seeds of the Landsberg erecta (L*er*) accession of *A. thaliana thaliana* (L) Heyhn, and Norway spruce (*Picea abies* L. H. Karst) from the provenance CØ1 from Halden, Norway (59°N latitude, seed lot 98063, Skogfrøverket, Hamar, Norway (skogfroverket.no) were surface-sterilised and sowed according to the protocol in (Blagojevic, et al. 2019 a, b). The seeds were germinated for 6 days at 20 °C under a 16 h photoperiod with a photosynthetic photon flux density (PPFD) of 30 μmol m^{-2} s^{-1} at 400-700 nm (TL-D 58W/840 lamps, Philips, Eindhoven, The Netherlands), as measured at the top of the petri dishes with a Li-Cor Quantum/Radiometer/Photometer (model LI-250, LI-COR, Lincoln, NE, USA).

2.2. Gamma irradiation of seedlings and growing conditions during the exposure

Six days old seedlings of *A. thaliana* and Norway spruce were exposed for 48 h using the FIGARO low dose gamma irradiation facility (^{60}Co; 1173.2 and 1332.5 keV γ-rays) at the Norwegian University of Life Sciences (Lind et al., 2019). Petri dishes (3.5 cm diameter) with seedlings were placed at different distances from the gamma source to obtain the average dose rates to water 1, 10, 20, 40, 100 and 290 mGy h^{-1} (Table 1). The exposure experiments were repeated three times to obtain enough material for all the analyses. For one out three repeated experiments, the highest dose rate was 250 mGy h^{-1} as 290 mGy h^{-1} could not be obtained due to the decay of ^{60}Co in the source (Table S1). The dose rates to water and dose rate intervals (in front and back of the petri dishes) were calculated from the air kerma rates according to (Hansen et al., 2019) as previously described (Blagojevic et al. 2019 a, b). The total doses were calculated from the estimated absorbed dose rates to water, multiplied by the exposure time (48 h). During the exposure the room temperature was 20 ˚C ± 1 °C and the photoperiod 12 h with an irradiance of 55 μmol m^{-2} s^{-1} from high pressure metal halide lamps (HPI-T Plus 250W lamps, Philips, Eindhoven, The Netherlands). The red:far red

(R:FR) ratio was measured to 3.5 using a 660/730 nm Skye sensor (Skye Instruments, Powys, Wales, UK).

2.3. Post-irradiation growing conditions

Since phenotypic effects of gamma irradiation may take some time to be manifested, the post-irradiation effects of the 48 h of gamma exposure were recorded after transfer of seedlings to pots filled with S-soil (Hasselfors Garden AS, Örebro, Sweden), one plant per pot. All plants were kept in growth chambers (manufactured by Norwegian University of Life Sciences) with temperature, relative air humidity (RH), R:FR ratio, and photoperiod maintained as previously described (Blagojevic, et al. 2019).

2.4. Growth parameter recordings

At the end of the gamma irradiation, seedlings from both species were placed between two transparent plastic sheets with a millimetre paper on top and scanned. The shoot and root lengths of Norway spruce and the lengths of the hypocotyl and cotyledons in *A. thaliana* were measured on the scanned seedlings using Image J (US National Institutes of Health, Bethesda, MD, USA; imagej.nih.gov). Per gamma dose rate, 29-40 and 28-35 seedlings of *A. thaliana* and Norway spruce respectively, were measured.

Post-irradiation, the number of rosette leaves as well as the number of plants with floral buds, inflorescence and open flower buds were recorded in 23-24 *A. thaliana* plants per gamma dose rate in time courses up to 75 days post-irradiation (dpi), and percentages of plants with floral buds, inflorescence and open flowers were calculated. Leaf width and leaf length were also measured at 34 and 41 dpi and height of the inflorescence at day 64. In Norway spruce, shoot elongation, the number of needles and plant diameter were recorded in a time course of 59 dpi in 11-22 plants per dose rate. The plant diameter was calculated by averaging two perpendicular measurements from needle tip to needle tip across the plant at the shoot apex.

Also, plant height was measured from the pot edge to the shoot apical meristem and the cumulative shoot elongation was calculated.

2.5. Histological and cytological studies of shoot tips

Histological studies of shoot apical meristems at the end of the gamma irradiation were performed according to (Lee et al. 2017). Shoot tips of Norway spruce and *A. thaliana* were then fixed in 4 % paraformaldehyde and 0.025 % glutaraldehyde in phosphate buffered saline (PBS, pH 7.0), vacuum infiltrated for 1 h at room temperature and stored at 4 °C overnight. The fixed tissues were washed in PBS solution for 30 min and dehydrated in a graded ethanol solution. After dehydration, approximately 3 mm of the shoot tips of each of 5 plants per treatment were infiltrated and embedded in LR White (London Resin Company, London, UK). After polymerisation, 1 µm sections were made using an Ultracut Leica EM UC6 microtome (Leica, Mannheim, Germany), and stained with toluidine blue O to visualise the cells. The stained sections were inspected using a light microscope with bright field optics (DM6B, Leica).

For cytological studies, LR white-embedded samples were sectioned into 70 nm ultrathin sections, using an Ultracut microtome (Leica EMUC6) and stained with uranyl acetate and lead citrate before examining by transmission electron microscopy (TEM) (FEI Morgagni 268, United States).

2.6. Comet assay for analysis of DNA damage

Single and double-strand DNA breaks at the end of the gamma irradiation and 77 dpi were quantified using the COMET assay (Gichner 2003) with some modifications (Blagojevic, et al. 2019). Briefly, this included isolation of cell nuclei and electrophoresis under alkaline pH, resulting in a COMET-like appearance of damaged DNA due to movement out of the nuclei. This was followed by quantification of the DNA in the COMET tail using fluorescence

microscopy. Three biological replicates per dose rate, each consisting of 3-4 mm of shoot tips from 3 plants of Norway spruce and 5 plants of *A. thaliana*, were used to assess DNA damage. For each of the three biological replicates per dose rate, totally about 180-200 cell nuclei were scored respectively, with 60-70 nuclei scored in each of 3 technical replicates (gels) and the overall median values were calculated.

2.7. RNA isolation for RNA sequencing

At the termination of the 48-h gamma irradiation, four biological replicates per gamma dose rate (0, 1, 10, 40, 100 (both species) and 290 mGy h^{-1} (*A. thaliana* only due to lack of growth inhibition and lack of/less organelle and DNA damage at lower dose rates), each containing shoots from 20-30 and four seedlings for *A. thaliana* and Norway spruce, respectively, were harvested in liquid nitrogen. For *A. thaliana*, total RNA was extracted using the RNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol. For Norway spruce, total RNA was extracted using the Masterpure Complete DNA and RNA Purification Kit (Epicenter, Madison, WI, USA) following the manufacturer's protocol, except that 3 µl beta-mercaptoethanol per sample replaced 1,4-dithiothreitol (DTT) and 0.5% polyvinylpyrrolidone (PVP, mw 360 000, Sigma Aldrich, Steinheim, Germany) was added to the extraction buffer. The purified RNA was quantified using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and the quality assessed with an Agilent 2100 Bioanalyzer with an RNA 144000 Nano Kit (Agilent technologies, Palo Alto, CA, USA).

2.8. RNA sequencing

The transcriptomes of the shoots were sequenced on an Illumina HiSeq4000 platform at the Norwegian Sequencing Centre (Oslo, Norway) using the Strand specific 20xTruseq RNA library preparation (paired end, read length 150 bp) as recommended by the manufacturer

(Illumina, San Diego, CA, USA). All samples were sequenced in two repeated lanes on the HiSeq4000. The raw sequence data were submitted to the ArrayExpress under the accession number E-MTAB-8081 for Norway spruce and E-MTAB-11120 for *A. thaliana.*

2.9. Differential gene expression analyses and functional term enrichment

The transcript abundances were estimated using the Salmon program (Patro et al. 2017) comparing each rate with either the Norway spruce genome (*Picea abies* 1.0 assembly, plantgenie.org) (Nystedt, et al., 2013) or *A. thaliana* genome (TAIR10, arabidopsis.org) (Berardini et al. 2015). Gene expression was compared between the samples at different gamma radiation dose rates and the unexposed control samples using the DESeq2 (Love et al., 2014). Genes were classified as differentially expressed genes (DEGs) if the False Discovery Rate (Benjamini & Hochberg correction) adjusted p-values were < 0.05.

Gene Ontology (GO) annotations of Norway spruce genes, as well as spruce to *A. thaliana* orthologs by best sequence matches, were downloaded from plantgenie.org. The *A. thaliana* orthologs were used to associate the Norway spruce genes with KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathways. Enrichment of GO or KEGG terms in sets of DEGs was found using topGO (version 2.34.0) or kegga from the limma package (version 3.38.2), respectively.

2.10. RT-qPCR analysis

Based on the significant regulation in one or both species in response to 48 h gamma irradiation, 11 DNA repair and cell cycle control genes were selected for further RT-qPCR validation and analysis of their regulation after 12, 24 and 48 h of gamma irradiation at 1, 10, 20, 40 and 100 mGy h^{-1} (Table S2 and S3). Four biological replicates per dose rate, each consisting of shoots from eight and 30 seedlings for Norway spruce and *A. thaliana*, respectively, were harvested and RNA was isolated as described above. cDNA was

synthesised from 1250 ng RNA for *A. thaliana* and 2500 ng RNA from Norway spruce in 20 µl reactions using SuperScript™ IV VILO™ cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, US) following the manufacturer's protocol, and no enzyme controls (-RT) were made for each sample.

Gene sequences were retrieved from NCBI (ncbi.nlm.nih.gov/gene) and TAIR (arabidopsis.org) for *A. thaliana* and from plantgenie.org for Norway spruce and exon-exon-spanning primers were designed to avoid non-specific amplification from genomic DNA contamination (if any) in the RNA samples, using the online tools Oligoanalyzer (eu.idtdna.com/calc/analyzer) and Primer Blast (ncbi.nlm.nih.gov/tools/primer-blast) with default parameters. Amplifications were performed in a 7500 Fast Real-time PCR system (Applied Biosystems, Foster city, USA) using 20 µl of Platinum Quantitative PCR Supermix-UDG and SYBRGreen (Thermo Fisher, Carlsbad, CA, US), using standard protocol.

To compare the relative transcript levels of the selected genes in the samples exposed to different gamma radiation dose rates at different time points with the unexposed controls (0 mGy h^{-1}), the comparative ΔΔCT method was used (Livak and Schmittgen 2001). The expression of the target genes was normalised by the mean expression of the three reference genes, *ACTIN* (*ACT*), *ELONGATION FACTOR 1α* (*EF-1α*) and *UBIQUITIN*.

2.11. Statistical analysis for growth recordings, DNA damage and qPCR results

DNA damage, hypocotyl, cotyledon and shoot length, as well as post-irradiation growth parameters at the final sampling time (34 or 41 dpi for *A. thaliana* and 59 dpi for Norway spruce) were tested for normality and homoscedasticity by the Ryan-Joiner and Levene tests, respectively. One-way ANOVA tests (glm model in Minitab 21.1, Minitab Inc, PA, USA) were then used to test for differences in these response variables between plants exposed to 48 h of gamma radiation versus control plants. Due to the lack of normality and

homoscedasticity of the non-transformed RT-qPCR gene expression data, log-transformed data was used in two-way ANOVA for individual species (dose rate and gamma-irradiation duration as factors) and three-way ANOVA for comparison of species (plant species, dose rate and gamma-irradiation duration as factors). Tukey`s post hoc test was used to test for differences between means ($p \leq 0.05$).

3. Results

3.1. Gamma irradiation for 48 h affects growth negatively in Norway spruce but not A. thaliana

In *A. thaliana*, no significant effect of the gamma irradiation on hypocotyl and cotyledon lengths was identified (Fig. 1A and B). At 41 dpi, no significant dose response-relationship was observed for the length, width, or the number of leaves (Fig. S1A, B and C). Although there was slight delay in flowering post-irradiation at 250-290 mGy h^{-1}, eventually all plants flowered (63 dpi) and showed very similar overall morphology, flowering, and silique development (77 dpi) (Fig. S1D, E and F and Fig. 1C). In Norway spruce, the shoot length was significantly reduced (27 %) after 48 h of gamma irradiation at 290 mGy h^{-1} only, but the root length was not affected (Fig. 1D and E). At 59 dpi, the shoot diameter and number of needles were significantly reduced by 5 % and 12 %, in 100 mGy h^{-1} and 9 % and 35 % at 290 mGy h^{-1}, but no such effects were detected at 77 dpi (Fig. S2A and Fig. 1F, G and H). Hence, there was temporary growth impairment in Norway spruce after exposure, while development in *A. thaliana* remained unaffected.

3.2. More severe gamma radiation-induced organelle damage in Norway spruce than in A. thaliana

Normal dome-shaped shoot apical meristems with compact cells were observed in both species after 48 h of gamma irradiation (Fig. 2). In *A. thaliana*, slightly damaged

mitochondria were observed at 100-290 mGy h^{-1}, but the chloroplasts and cell nuclei appeared normal at all dose rates compared to the unexposed control except that a few cells showed less dense nuclei morphology at ≥ 1 mGy h^{-1} (Fig. 3A, B and C). In comparison, in Norway spruce, disrupted nuclear and mitochondrial membranes were observed at ≥ 1 mGy h^{-1}, as well as swollen chloroplasts and disrupted thylakoid membranes at ≥ 40 mGy h^{-1} (Fig. 3D, E and F).

3.3. Higher levels of DNA damage in gamma-irradiated Norway spruce than in A. thaliana

In *A. thaliana*, the Comet assay showed low but significant damage with ≤ 5 % tail DNA at 1-100 mGy h^{-1} and 12 % tail DNA at 290 mGy h^{-1} (Fig. 4A). In comparison, DNA damage was consistently 2- to 8-fold higher in Norway spruce with 10 %, 12 %, 15 %, 17.5% and 40 % DNA in the Comet tail after the 48-h of irradiation at 1, 10, 40, 100 and 290 mGy h^{-1}, respectively (Fig. 4B). At 77 dpi, the DNA damage was substantially reduced but still observed in *A. thaliana* at ≥ 10 mGy h^{-1} with ≤ 3% tail DNA and in Norway spruce seedlings at ≥ 1 mGy h^{-1}, with ≤ 5% tail DNA at all dose rates (Fig. 4 A and B).

3.4 Profound transcriptome differences between gamma-irradiated Norway spruce and A. thaliana

Comparative RNA-seq analysis of gamma-irradiated shoot samples (1, 10, 40, 100 mGy h^{-1} both species, 290 mGy h^{-1} *A. thaliana* only), revealed extensive differences between the species. *A. thaliana* expressed a total of 26,643 genes, of which 11,592 were differentially expressed genes (DEGs) between any dose rate and the unexposed control (43.5 %) in one or multiple gamma dose rates (Fig. 5A). Norway spruce expressed a total of 66,069 genes, of which 5326 were DEGs (8.1%, Fig. 5C). In *A. thaliana*, a high number of DEGs was observed already from 1 mGy h^{-1} (3442 DEGs), with the higher dose rates having ~4400-5500 DEGs (Fig. 5A and B, Fig. S3A). At 100-290 mGy h^{-1}, there was a higher proportion of

upregulated genes with more than 2-fold change as compared to the lower dose rates (Fig. 5A, Fig. S3B). By contrast, in Norway spruce, very few DEGs were observed at 1 and 10 mGy h^{-1} (38 and 43 DEGs), while 822 and 5047 DEGs were observed at 40 and 100 mGy h^{-1}, respectively (Fig. 5C and D). A principal component analysis (PCA) showed that *A. thaliana* samples exposed to 100 and 290 mGy h^{-1} of gamma radiation clustered separately from the low dose rate groups (1-40 mGy h^{-1}) and samples without radiation (0 mGy h^{-1}), reflecting overall differences in both high and low dose rate groups (data not shown). In Norway spruce, samples exposed to 100, and to some extent 40 mGy h^{-1}, clustered separately from other groups, reflecting the overall differences of doses higher than 40 mGy h^{-1} in Norway spruce (Fig. S3D). The increase in DEGs from 40 to 100 mGy h^{-1} was much more pronounced in Norway spruce than in *A. thaliana* (Fig. 5A vs C). The largest overlap of *A. thaliana* DEGs was between 100 and 290 mGy h^{-1}, and between 1, 10 and 40 mGy h^{-1} (Fig. 5B). Upregulation at higher dose rates had more overlap than downregulation. A good proportion of Norway spruce DEGs at 40 and 100 mGy h^{-1} overlapped, showing some consistency in the response even with a large difference in total numbers of DEGs, including many unique ones (Fig. 5D).

3.5. Distinctive pathway enrichment in Norway spruce and A. thaliana at different dose rates

GO and KEGG pathway analyses on sets of DEGs revealed a gamma dose rate-dependent enrichment of different pathways. In *A. thaliana*, DNA replication, DNA repair, cell cycle, endoreduplication, and endoplasmic reticulum (ER) stress were enriched at 1-40 mGy h^{-1} (Fig. S4 and S5). Hormone biosynthesis and signalling pathways showed considerable enrichment along with stress response at 100-290 mGy h^{-1} (Fig. S4 and S5). Oxidative stress, defence response, growth and development, protein synthesis and catabolism as well as mitogen-activated protein kinase (MAPK)-mediated signalling were significantly regulated at all dose rates (Fig. S4 and S5). In Norway spruce, pathways such as DNA replication, DNA

repair, cell cycle, endoreduplication, unfolded protein response (UPR) and endoplasmic reticulum (ER) stress, response to gamma, energy metabolism and growth and development were all enriched at ≥ 40 mGy h^{-1} (Fig. S6 and S7). Photosynthesis and protein biosynthesis and metabolism were enriched in downregulated DEGs at 100 mGy h^{-1} (Fig. S6 and S7) in Norway spruce only (Fig. S6 and S7).

In *A. thaliana* KEGG pathway analysis revealed that the DNA damage repair (DDR) pathways such as HR, mismatch repair (MMR), non-homologous end-joining (NHEJ) and base excision repair (BER) were regulated at 10-40 mGy h^{-1}, and BER also at 290 mGy h^{-1} (Fig. S5). In Norway spruce, NER, MMR, HR and NHEJ pathways were significantly regulated at 40 and 100 mGy h^{-1} and BER at 40 mGy h^{-1} only (Fig. S7).

3.6. Distinct differences in stress response pathways between Norway spruce and A. thaliana

The specific DEGs in DDR, antioxidant protection and other stress response pathways were further analysed to compare *A. thaliana* and Norway spruce response after the exposure to 48 h of gamma radiation.

3.6.1. DNA damage repair

In *A. thaliana*, most DEGs in the DDR pathways showed a 30-70% change in expression (Table S3). Genes involved in DNA double strand break (DSB) repair via HR, including *RAD51*, *SMC6B*, *PROLIFERATING CELL NUCLEAR ANTIGEN 2* (*PCNA2*) and *BRCA2B*, showed significant upregulation at all dose rates in the 10-290 mGy h^{-1} range. In Norway spruce, most DDR DEGs at 40-100 mGy h^{-1} had at least a 60% change in expression (Table S3), and genes involved in DSB repair via HR; *SRS2*, *RAD52*, *RPA1A*, *MCM8*, *XRCC2* and *XRCC3*, were all upregulated at 100 mGy h^{-1}. A few other genes such as *PARP1* and *PARP2* were significantly upregulated at the highest dose rates in both species. Some DEGs showed opposite changes in expression between species; *MRE11*, *RPA2A/ROR1* and *BRCA1* in the

HR pathway, and *MSH7* in the MMR pathway. Unique DEGs to Norway spruce included upregulation of *KU80* in NHEJ at 40-100 mGy h^{-1} and *SOG1* at 100 mGy h^{-1}. Unique to *A. thaliana* was upregulation of *ATM* at 100 mGy h^{-1}.

3.6.2. Cell cycle and endoreduplication

In *A. thaliana*, most cell cycle-related DEGs including *CYCBs* and *CDKB*s, were observed at 1-40 mGy h^{-1} but not 100-290 mGy h^{-1} (Table S4). *CYCB1* was significantly upregulated at all dose rates ≤ 40 mGy h^{-1}, along with many other *CYCBs*. In Norway spruce, no such effects on B type cyclins were observed and most of the cell cycle genes showed changes in expression at ≥ 40 mGy h^{-1} only. Anaphase-promoting factors *APC6* and *13*, chromatin assembly factor *FAS2*, and the cyclin-dependent kinases regulatory subunit *CKS1* showed upregulation at ≥ 40 mGy h^{-1}. Positive regulators of the cell cycle such as D type cyclins (e.g., *CYCD1*) were significantly upregulated in *A. thaliana* at ≤ 40 mGy h^{-1} but downregulated in Norway spruce at 100 mGy h^{-1}. *RBR1* was upregulated in both species. *E2Fb* was upregulated at 40 mGy h^{-1} in Norway spruce only. An important G2/M checkpoint regulator, nuclear kinase *WEE1*, was upregulated at ≤ 40 mGy h^{-1} in *A. thaliana*, but at ≥ 40 mGy h^{-1} in Norway spruce. Most of the cell cycle-related genes, such as *CYC*s and *CDKs*, are also involved in the endocycle and the endoreduplication-related biological pathway was enriched in *A. thaliana* at ≤ 40 mGy h^{-1} but very few such DEGs were observed in Norway spruce (Table S5).

3.6.3. Chromatin remodelers and genes in epigenetic pathways

Significant activation of genes involved in the pathway associated with de-condensation of the nucleosome structure and activation of DDR pathways, was predominantly observed in Norway spruce at 40-100 mGy h^{-1} (Table S6). *In A. thaliana*, a few such genes were upregulated at ≤ 40 mGy h^{-1} only, and a few were downregulated at higher dose rates. In this species, the DNA helicase *DDM1* was upregulated in *A. thaliana* at ≤ 40 mGy h^{-1} and DNA

methyltransferase *CMT3*, histone 2A variant *HTA8* and a component of the core chromatin remodelling complex *ARP6* were significantly downregulated at 100-290 mGy h^{-1} (Table S6). In Norway spruce, histone deacetylase *HOS15* genes involved in the regulation of DNA methylation, such as *DMT1*, *IDN2* and *SUVH4*, as well as the histone 2 A variant *H2AV*, showed significant upregulation at 40-100 mGy h^{-1}.

3.6.4. Hormone biosynthesis and signalling

A. thaliana genes involved in biosynthesis and signalling of main growth-promoting hormones such as auxin, cytokinin, auxin, cytokinin, gibberellin (GA) and brassinosteroid, as well as important stress-response-related hormones such as abscisic acid (ABA) and ethylene, were significantly regulated in response to the gamma irradiation (Table S7) especially at higher dose rates. The auxin-responsive MYB transcription factors *AtMYB93* and *MYB61*, the auxin-responsive genes *IAA13, IAA14, IAA27, SAUR10* and *GH3.6*, the auxin biosynthesis gene *YUC9* as well as the auxin efflux carriers and transporters involved in polar auxin transport, *PIN2*, *PIN3*, *AUX1* and several ABC transporters, were significantly upregulated in this species (Table S6) but downregulated in Norway spruce. Although the auxin-homeostasis-related gene *IAMT1*, and the auxin responsive genes *SAR1* and *SAUR76* showed increased expression at 40-100 mGy h^{-1} in Norway spruce, several auxin signalling genes including *ARF6, TIR1, AIR9* and *IAA26* were significantly downregulated (Table S7). In *A. thaliana*, cytokinin biosynthesis and metabolism as well as cytokinin signalling genes such as *LOG7, AHP1 and SOT8*, cytokinin-responsive genes *ARR2* and *ARR3*, and cytokinin metabolism genes *CKX5*, were upregulated at 40-290 mGy h^{-1} (Table S7). Contrastingly none of these genes showed significant regulation in Norway spruce. Similarly, the gibberellin (GA) biosynthesis genes *GA2* (*ent-kaurene synthase 1*) and *GA 20-OXIDASE 2* (*GA20OX2),* and the brassinosteroid biosynthesis and homeostasis genes *CYP90C1, CYP708A1,* and *CYP85A1*, were upregulated in *A. thaliana* but not Norway spruce.

In general, ABA biosynthesis and signalling genes did not show any gradual up- or downregulation in *A. thaliana* (Table S7). However, a few were downregulated, including the negative regulator of ABA biosynthesis *PUB44*, and the ABA signalling genes *PYL4, CRK29* and *ATHB-6*. Many stress- and ABA-responsive genes including *RCI2A*, *LTP3*, and *BFRUCT3* showed upregulation in 100-290 mGy h^{-1}. Some of the ABA-responsive genes involved in an ABA-mediated signalling pathway such as *ASPG1*, *PPCK1*, and *RD22* showed consistent increasing expression from 10 mGy h^{-1} to 290 mGy h^{-1}. Among the genes regulated in both species, the ABA signalling gene *CIPK3* showed downregulation in all dose rates in *A. thaliana*, and at 100 mGy h^{-1} in Norway spruce, and *ADH1* were upregulated in both species at higher dose rates. In *A. thaliana*, ethylene biosynthesis and signalling genes were mostly downregulated at lower dose rates with few exceptions (Table S6). Ethylene-responsive genes such as *CTR1*, *RAP2-2*, *RAP2-12*, and *ERFs* as well as the ethylene biosynthesis genes *EOL1* and *ACS5* showed significant regulation in *A. thaliana*, but not in Norway spruce.

3.6.5. Genes in antioxidant metabolism pathways

Significant overall upregulation of antioxidant biosynthesis and signalling genes was observed in *A. thaliana* even from 1 mGy h^{-1}. By contrast, in Norway spruce, most of the antioxidant genes were not significantly affected below 100 mGy h^{-1} (Table S8). The key flavonoid and anthocyanin biosynthesis genes *CHS* and *DIHYDROFLAVONOL REDUCTASE* (*DFRA*) were upregulated in *A. thaliana* from 1 mGy h^{-1}. but were significantly downregulated in Norway spruce at 100 mGy h^{-1}. Flavanol synthase (*FLS1*) was upregulated in *A. thaliana* at $\leq$ 10 mGy h^{-1} and Norway spruce at 100 mGy h^{-1}. Many *PEROXIDASE* (*PER*) genes including *PER3* and *PER 11* were regulated in both species but from lower dose rates in *A. thaliana* than Norway spruce. *GLUTATHIONE-S-TRANFERASE* (*GSTU/GSTF*) genes were upregulated in both species.

3.6.6. Cell wall component genes

In *A. thaliana* genes involved in the biosynthesis of cell wall components, cell wall maintenance and fortification, as well as membrane receptors were significantly upregulated at 100-290 mGy h^{-1} with large fold-changes. In Norway spruce, only very few genes related to cell wall biogenesis and maintenance showed regulation, i.e., at 40 and 100 mGy h^{-1} (Table S9). In *A. thaliana*, *PECTIN METHYL TRANSFERASE* (*PMEPCRF*) and *REQUIRES HIGH BORON* (*BOR2*) involved in cellulose and pectin metabolism, *HHT1* involved in pectin and suberin biosynthesis, the cell wall organisation and biosynthesis genes *FATTY ALCOHOL CAFFEOYL-COA CAFFEOYL TRANSFERASE* (*FACT*) and *CYP86B1*, were upregulated at 100-290 mGy h^{-1}. The endoglucanases *GH9C2* and *GH9B13* involved in cell wall organisation were upregulated in all dose rates. Although none of those genes showed any significant changes at any dose rate in Norway spruce, a few other genes such as the *EXPANSINs 4 and 8*, *CELLULOSE SYNTHASE 5 (CESA5*), and *XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASES XTH 5* and *9* were significantly regulated in both species.

3.6.7. Photosynthesis and energy metabolism

Photosynthesis was massively downregulated in Norway spruce while almost no significant changes were observed in *A. thaliana*. Photosystem (PS) I and II-related genes such as *PS I REACTION CENTRE SUBUNIT*s *PSAG*, *PSAK* and *PSAN*, *PHOTOSYSTEM 1 LIGHT HARVESTING COMPLEX 1* (*LHCA1*), PS II assembly factor *HIGH CHLOROPHYLL FLUORESCENCE 36* (*HCF136*), essential enzymes such as *NADPH-PROTOCHLOROPHYLLIDE OXIDOREDUCTASE* (*PORA*), and *FRUCTOSE-1,6-BISPHOSPHATASE* (*CYFBP*), chloroplast binding proteins and membrane efflux carriers, were downregulated in Norway spruce at 100 mGy h^{-1} (Table S10). In *A. thaliana*, only a few genes were regulated, such as upregulation of the PS I and II reaction centre components

PSAB and *PSBB*, and *NAD(P)H-QUININE OXIDOREDUCTASE SUBUNIT 3* (*NDHC*) (Table S10).

Similarly, energy metabolism was far more affected in Norway spruce than *A. thaliana* (Table S11). Norway spruce particularly showed upregulation at ≥ 40 mGy h^{-1} of a wide range of genes involved in glycolysis, TCA cycle and gluconeogenesis, such as *PYRUVATE KINASE*, *GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE* (*GAPC1*), *6-PHOSPHOFRUCTOKINASE 4* (*PFK4*), *TRIOSEPHOSPHATE ISOMERASE* (*CTIMC*), *MALATE DEHYDROGENASE* (*MDH1*), *SUCCINATE DEHYDROGENASE (SDH2-1*), and several homologs of *PHOSPHOENOLPYRUVAT CABOXKINASE* (*PCKA*) and *PFK2*. Additionally, the *CYTOCHROME C OXIDASE 6B1* (*COX6B-1*) and *CYTOCHROME C1* (*CYTC-1*) genes involved in mitochondrial electron transport were significantly upregulated at 100 mGy h^{-1}. *ALCOHOL DEHYDROGENASE 1* (*ADH1*) was significantly upregulated in both species from 100 mGy h^{-1}.

3.7. Differential dynamic regulation of DNA repair genes in A. thaliana and Norway spruce during gamma irradiation

To understand the dynamics of DDR and cell-cycle-related gene expression during radiation stress, both species were exposed to 12, 24 and 48 h of gamma radiation and the transcript levels of selected genes analysed by RT-qPCR. Consistent with the RNA-seq data, significantly increased transcript levels of *WEE1, PARP2, RAD51* and *CYCB1.2* were observed after 48 h in *A. thaliana*, by 5-10-fold as compared to the unexposed controls (Fig. 6). However, after 12 and 24 h exposure no significant effect of the gamma irradiation on their expression was observed. By contrast, in Norway spruce the strongest response was generally observed after 24 h of gamma irradiation (Fig. 7). The transcript levels of *WEE1* increased significantly at 20-100 mGy h^{-1} after 24 h only and those of *SOG1* increased at 40-

100 mGy h^{-1} after 24 h, while *PARP2* expression increased significantly by 10-30-fold at 40-100 mGy h^{-1} after 24 and 48 h. There were no significant effects on expression at 12 h.

4. Discussion

To improve the knowledge base for risk assessment and protection of plant communities in radionuclide-contaminated ecosystems against ionising radiation, the current comparative study has focussed on underlying mechanisms with respect to differential radiosensitivity in the highly radio-sensitive conifer Norway spruce and the radio-tolerant *A. thaliana*. Through exposure of seedlings to gamma radiation under standardised conditions and comparative assessment of phenotypic, cellular, and genotoxic effects, combined with RNA-seq and qPCR analysis, we present a novel in-depth understanding of the differences between the two species in mechanisms involved in defence and repair as well as adverse effects.

Previously, we have shown dose rate-dependent impaired development in Norway spruce seedlings exposed to gamma radiation for 144 h, with significant growth inhibition at ≥ 40 mGy h^{-1} and progressively developing after-effects eventually causing mortality (Dajana Blagojevic et al. 2019). By contrast, *A. thaliana* showed no significant adverse effects at dose rates ≤ 540 mGy h^{-1} (Blagojevic et al., 2019a). Norway spruce recovers better from inhibited growth 77 dpi after 48 h exposure to 290 mGy h^{-1} (Fig. 1 D and F-H) compared to the previous situation (after 144 h exposure to 40 mGy h^{-1}, (Blagojevic, et al. 2019) despite the total dose after 48 h being higher (13,920 mGy) than after 144 h (5,760 mGy), emphasising the severity of chronic exposure. Consistent with previous findings, *A. thaliana* did not show any significant growth or developmental effects in this study (Fig. 1A, B, and C). The RNA-seq analysis revealed, however, profound differences between the two species in response to gamma irradiation (Fig. 5, S4, S5). The fact that *A. thaliana* mounts a gene regulatory

response at lower dose rates than Norway spruce signified that *A. thaliana* was more poised towards a rapid response to inflicted radiation stress.

The energy deposited at a specific gamma radiation dose rate in the two exposed plant species should be similar, with ionization and excitation of electrons occurring uniformly throughout the cells. Thus, induced damage should be randomly distributed in biomolecules such as DNA and relative to the DNA content (genome size) (Einset and Collins 2018). Our previous work as well as this study showed less DNA damage in *A. thaliana* than in Norway spruce and Scots pine, both at the end of the irradiation and during the post-irradiation period (Blagojevic et al., 2019a, b; Fig. 4). We therefore hypothesised that *A. thaliana* has more effective DNA repair and/or protection systems. Yet, the DNA damage at 77 dpi in both species (Fig. 4), albeit at lower level than at the end of the 48-h irradiation, demonstrates that some DNA damage or genomic instability is not critical to survival or development. Although genomic instability may result into detrimental growth effects (Mothersill and Seymour 1998), the lack of clear negative effects on the vegetative growth post-irradiation in *A. thaliana* in contrast to Norway spruce, and only a slight delay in reproductive development after 144 h of gamma irradiation at high dose rates in *A. thaliana*, (Blagojevic et al., 2019a) confirms better tolerance compared to conifers. This is also supported by the mortality observed in Norway spruce and Scots pine at dose rates > 40 mGy h^{-1}, but not in *A. thaliana* even after exposure to 400-540 mGy h^{-1} (Blagojevic et al., 2019a). No mortality after 48 h of gamma irradiation in both species in this study again points to a certain tolerance to damage.

The comparative RNA-seq analysis revealed that both *A. thaliana* and Norway spruce initiated a plethora of DDR pathways to repair the damage to avoid the accumulation of harmful mutations and maintain genome integrity. However, the response of the two species differed in two main aspects (Fig. S9). Firstly, the upregulation of DDR-related genes at lower doses dose rates in *A. thaliana* than in Norway spruce suggested that the former is far

more responsive towards lower levels of DNA damage. Secondly, the prominent DNA repair pathways in *A. thaliana* were mostly HR, and to some extent MMR, whereas in Norway spruce, in addition to HR, several other pathways such as NHEJ, MMR and DNA transleison synthesis were activated. Hence, it is tempting to speculate that Norway spruce needed to engage a wider range of DDR because of the significantly higher levels of DNA damage compared to *A. thaliana*, possibly as a consequence of less antioxidant protection and a larger genome.

One of the key contributor genes in gamma radiation-induced DDR in plants, *SOG1*, which activates several DDR pathways and cell cycle checkpoints (Kim et al., 2019), was not affected at any time points or dose rates tested in the gene expression analysis (Table S3 and Fig. 6). This might indicate that induction of *SOG1* gene expression is only modulated in higher total doses or in acute radiation that generate high level of DNA damage (Bourbousse, Vegesna, and Law 2018). However, no change in *SOG1* expression after 48 h of gamma irradiation does not necessarily indicate the lack of SOG1 activity as that could rather depend on the post-translational modification by ATM- or ATR-kinases (Bourbousse et al., 2018; Yoshiyama et al., 2013). The involvement of *SOG1* in *A. thaliana* is indeed supported by the activation of many primary target genes of SOG1 (i.e., *RAD51*, *BRCA1*, *PARP1* and *2*, *GMI1*) in response to the 48 h of gamma irradiation. By contrast, significant upregulation of *SOG1* in Norway spruce after 24 h at ≥ 40 mGy h^{-1} and 48 h at 100 mGy h^{-1} (Fig. 7) but no significant regulation of ATM (Table S3)3), might imply that elevated *SOG1* expression was triggered in Norway spruce possibly due to higher levels of accumulated DNA damage. Moreover, upregulation of *WEE1*, a direct target of SOG1 and key regulator of CDK-inhibition and stress-induced cell cycle arrest (Kevei et al., 2011; De Schutter et al., 2007), coincided with *SOG1* expression in Norway spruce, strongly suggesting SOG1 involvement

in gamma-induced DNA damage repair and cell cycle arrest (Pedroza-Garcia et al., 2022; Bourbousse et al., 2018).

Transcriptional activation of numerous B type *CYC*- and *CDKB*-genes in *A. thaliana* after 48 h at 1-40 mGy h^{-1} (Table S4) might indicate gamma radiation stress-induced cell cycle arrest and subsequent DNA damage repair through HR by maintaining higher cyclin threshold (Endo et al. 2006). This further supports the previous notion that enhanced CDKB1 activity is involved in ionising radiation-induced cell cycle arrest leading to HR repair in damaged mitotically active cells (Weimer et al., 2016). On the contrary, there were few regulations of B-type *CYC* or *CDK* genes in Norway spruce (Table S4), indicating no significant involvement of such genes in cell cycle arrest. Furthermore, upregulation in *A. thaliana* of the D-type cyclins responsible for cell division progress by repressing the *RBR1-E2F* pathway along with *RBR1* and *E2FB* (Desvoyes and Gutierrez 2020), indicates further repression of cell cycle progression in higher dose rates in contrast to Norway spruce. Hence, the regulation of the key contributors in cell cycle progression in both species, albeit to a larger degree in *A. thaliana*, suggests that the cell cycle is one of the most affected biological pathways in the applied gamma radiation stress. Most of the CYCs and CDKs regulated in *A. thaliana* in our study are also involved in the regulation of the endocycle (Table S5). Although very few genes related to endocycle were regulated in Norway spruce, the observed upregulation of *CKS1* and *APC6* might play a role in endoreduplication (Heyman and De Veylder 2012; De Veylder et al., 2001). However, stress-indued endoreduplication is less common in gymnosperms than angiosperms (De Veylder et al., 2001). A preliminary flow cytometry study did not reveal any effect of 48 h gamma radiation at 100 mGy h^{-1} on the endoreduplication status in *A. thaliana* and Norway spruce (results not shown), suggesting that the applied dose was not sufficient to induce the endocycle. Increased endoreduplication

was previously shown in *A. thaliana* at 1 Gy min^{-1} (150 Gy totally) and *Lemna minor* at 1500 mGy h^{-1} for 7 days, but not at lower dose rates (Adachi et al., 2009; Van Hoeck et al., 2015).

DDR is closely related to epigenetic regulation and chromatin structure must be decondensed for the DDR proteins to reach the damaged sites to carry out efficient repair (Choi et al., 2019; Kim et al., 2019). Although upregulation of epigenetically-related genes were shown previously in *A. thaliana* exposed to ionising radiation (Sidler et al., 2015), very few such genes were regulated in *A. thaliana* in our study. By contrast, the upregulation of *H2A* variants, DNA methylation and deacetylation-related genes particularly in Norway spruce (Table S6, Fig. S6), clearly indicate chromatin modification at ≥ 40 mGy h^{-1} in this species. The strong enrichment of DNA methylation regulation is consistent with the previously reported DNA hypermethylation in *P. sylvestris* trees exposed to high doses of ionising radiation in the Chernobyl area, a mechanism suggested to stabilise the genome (Kovalchuk et al., 2003). Despite the genome of Norway spruce being 150 times bigger than that of *A. thaliana*, it only contains 2.5 times more genes (Nystedt et al., 2013), and has large heterochromatin regions which requires the activation of chromatin remodelers for decondensation of chromatin regions for efficient DDR (Fig. S9). Moreover, gamma radiation-induced damage to heterochromatin regions does not initiate rapid activation of DDR pathways (Falk et al., 2008). It is thus plausible that Norway spruce requires a higher level of DNA damage for the activation of DDR and chromosome remodelers only when the damage was significantly high.

Our previous study with gamma irradiation for 144 or 360 h showed that the apical meristems of the two species were affected differently with adverse effects in Norway spruce only (Blagojevic et al., 2019). In this study the 48-h exposure did not affect the structural integrity of the apical meristems (Fig. 2). We therefore proceeded to investigate the effect of the radiation exposure on organelles. TEM image analysis revealed that integrity of thylakoid

and nuclear membranes was compromised in Norway spruce, but not in *A. thaliana* (Fig. 3). Notably, in both species the mitochondria appeared to be the most vulnerable organelle. Furthermore, the fact that disrupted mitochondria were evident from 1 mGy h^{-1} in Norway spruce, while *A. thaliana* showed no damage below 100 mGy h^{-1}, indicating a major difference in their capacity to maintain cell homeostasis.

Previous studies of gamma-irradiated plant species such as *A. thaliana* and *L. minor* have demonstrated increased activities of ROS-scavenging enzymes such as SOD and APX (Vanhoudt, et al. 2014; Van Hoeck et al. 2017; Xie et al. 2019), but such information was lacking in conifers. Consistent with the notion that species-specific antioxidant response determines oxidative stress-induced damage, less activation of such genes occurred in Norway spruce < 40 mGy h^{-1}, while *A. thaliana* showed comprehensive modulation of enzymatic and non-enzymatic antioxidant-related genes (*PERs*, *CHSs*, *GSTs*, anthocyanin and flavanol synthesis genes) (Table S8). This could at least partly explain the massive damage to mitochondria, chloroplasts, and nuclear membranes in Norway spruce in contrast to only slight mitochondrial damage in *A. thaliana* (Fig. 2 and 3). These observations imply that *A. thaliana* could have a higher capacity to ameliorate cellular ROS generated by gamma irradiation, thus preventing damage to organelles and indirect DNA damage. Moreover, antioxidants such as *PERs* can modulate cell wall composition during stress (Díaz-Tielas et al. 2012). Cellulose and pectin were previously shown to be damaged by very high doses of gamma radiation (1-2.5 kGy) (Kovács and Keresztes 2002). Hence, in contrast to Norway spruce which was negatively affected, the radical upregulation of cell wall component genes even at lower gamma dose rates in *A. thaliana* (Table S9) might suggest efficient replacement of damaged cell wall components and cell wall fortification in *A. thaliana* (Le Gall et al. 2015).

Antioxidants such as *GST*s regulate auxin-responsive genes which modulate plant growth in presence of ABA to induce tolerance towards abiotic stress (Sylvestre-Gonon et al. 2019; Jiang et al. 2010). Thus, activation of the *GST*s in Norway spruce might have affected plant growth in addition to counteracting oxidants. This is consistent with the observed growth retardation after 48 h irradiation (290 mGy h^{-1}) and during the post-irradiation period (100-290 mGy h^{-1}) in Norway spruce only (Fig. 1 and Fig. S1), and the downregulation or no regulation of growth- promoting hormones in Norway spruce (Table S7). By contrast, the gamma-irradiation-induced upregulation of auxin and cytokinin-related genes (biosynthesis, metabolism and signalling as well as auxin transport) in *A. thaliana* complies with the maintenance of meristem function to sustain normal vegetative growth. These results are in line with the previously demonstrated activation of such genes in response to abiotic stress, indicating the importance of growth-hormone homeostasis under stress (Verma, Ravindran, and Kumar 2016). Similar to previous reports on the induction of ABA biosynthesis and signalling genes triggered by ROS in gamma-irradiated *A. thaliana* (Vishwakarma et al. 2017), upregulation of many such ABA-responsive genes were observed in *A. thaliana* in our study even though the total doses applied were much lower. Also, upregulation of the ethylene-responsive oxidative stress-pathway (multiple *ERFs*, *ACSs* and *MPK6*) and SA-responsive genes (*WRKY* and *MYB* transcription factors) in *A. thaliana*, but not Norway spruce (Table S7), indicate less induction of hormone response to gamma stress in the latter and is consistent with the greater tolerance of *A. thaliana* to gamma radiation. Hence, in *A. thaliana* better homeostasis of antioxidants, cell wall biosynthesis/fortification and hormone signalling might contribute to the tolerance to the gamma irradiation. Conversely, less transcriptional activation of the same protective pathways in Norway spruce are likely to have resulted in adverse effects on overall growth and higher susceptibility to radiation-induced oxidative damage.

The RNA-seq analysis also provided evidence that energy metabolism and photosynthesis in Norway spruce were strongly affected by gamma irradiation in Norway spruce, but only marginally in *A. thaliana*. In Norway spruce, the reduced expression of photosynthesis-related genes coincided with the reduced growth and chloroplast impairment at higher dose rates (≥ 40 mGy h^{-1}). Concurringly, the strong upregulation of energy metabolism-related genes (glycolysis, Krebs cycle, mitochondrial electron transport-related genes) (Table S11) in this species may support the hypothesis that there is a high ATP requirement for damage repair including maintenance of the large genome, and overall growth. Furthermore, the fact that energy metabolism genes were upregulated, may indicate a compensatory effect to counteract the damage to mitochondria. Hence, it appears that radiation induces energy deficiency, which in turn requires increased energy expenditure on damage repair at the expense of growth and is reflected by the downregulation of growth-promoting hormones in Norway spruce at higher dose rates. However, the upregulation of energy-metabolism-genes might additionally indicate that the damage to mitochondria at high dose rates could have led to inefficient energy production and thus a need for the activation of respiratory genes to meet the energy demands.

5. Conclusions

This study demonstrates that compared to the radiosensitive Norway spruce, radiotolerant *A. thaliana* mobilises more comprehensive gamma radiation-induced defence, repair, and homeostasis-maintaining stress responses, even at very low dose rates. Despite significant adverse effects in Norway spruce related to DNA and organelle damage and growth at low dose rates, transcriptional activation of DNA damage repair was evident at dose rates higher dose than 40-100 mGy h^{-1}. Furthermore, in Norway spruce, mobilization of antioxidants, as well as modulation of growth-regulatory and stress-related hormones at lower dose was less efficient compared to *A. thaliana*. Thus, the short-lived *A. thaliana* with quick generation-

time, and small genome with less energy requirement for maintenance, showed better plasticity in response to radiation stress compared to the long-lived Norway spruce with lengthy generation time and gigantic genome. These observations expand our understanding of differential radiosensitivity in plants, and thus improves the knowledge base for risk assessment and protection of ecosystems from ionising radiation exposure. In the context of environmental impact, the findings strongly support that under severe ecosystem exposure situations, radiosensitive conifers require special attention with respect to remediating measures.

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CRediT authorship contribution statement

Payel Bhattacharjee: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing; Dajana Blagojevic: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review and editing; YeonKyeong Lee: Methodology, Visualization, Formal analysis, Writing – review and editing; Gareth B Gillard: Data curation, Formal analysis, Visualization, Writing - review & editing; Lars Grønvold, Torgeir R Hvidsten, Simen R Sandve: Data curation, Formal analysis, Writing – review and editing;

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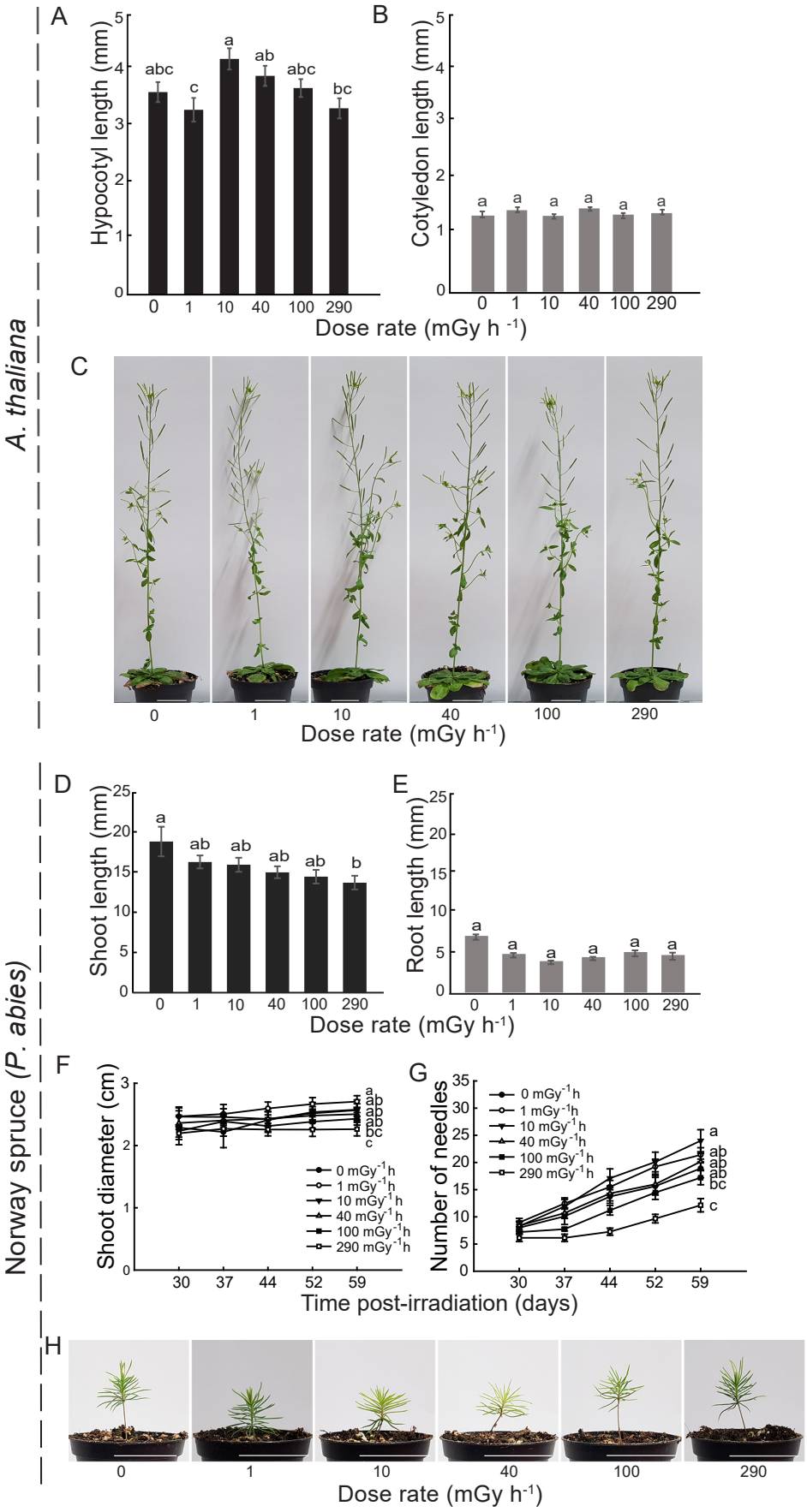

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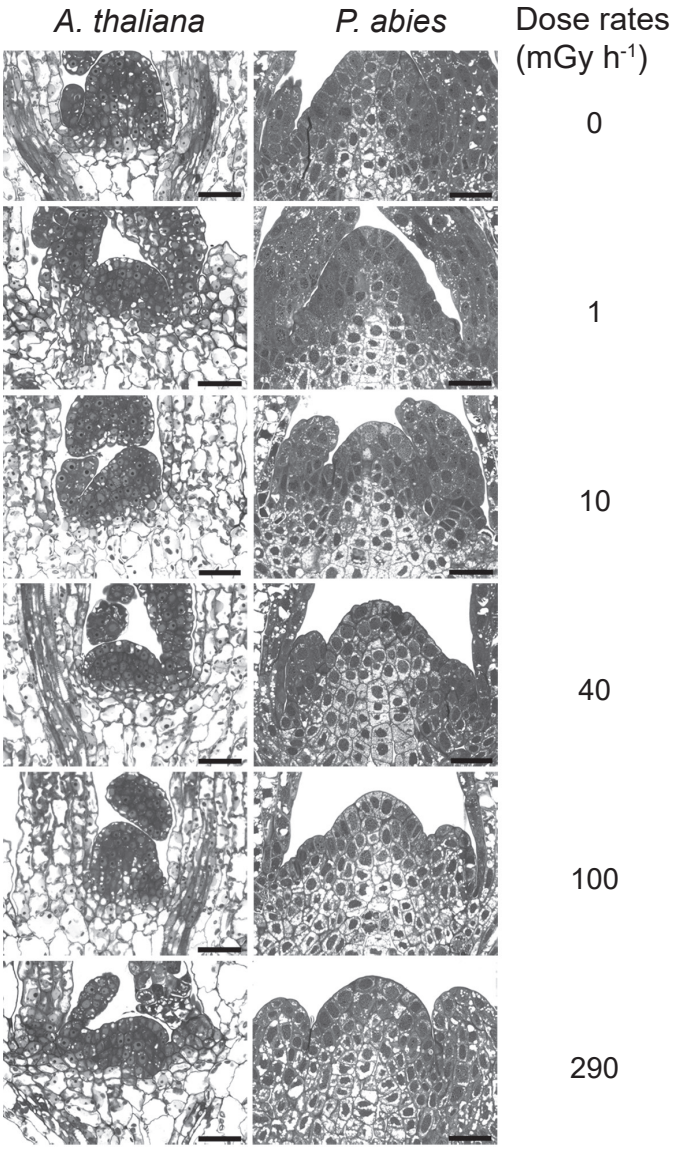

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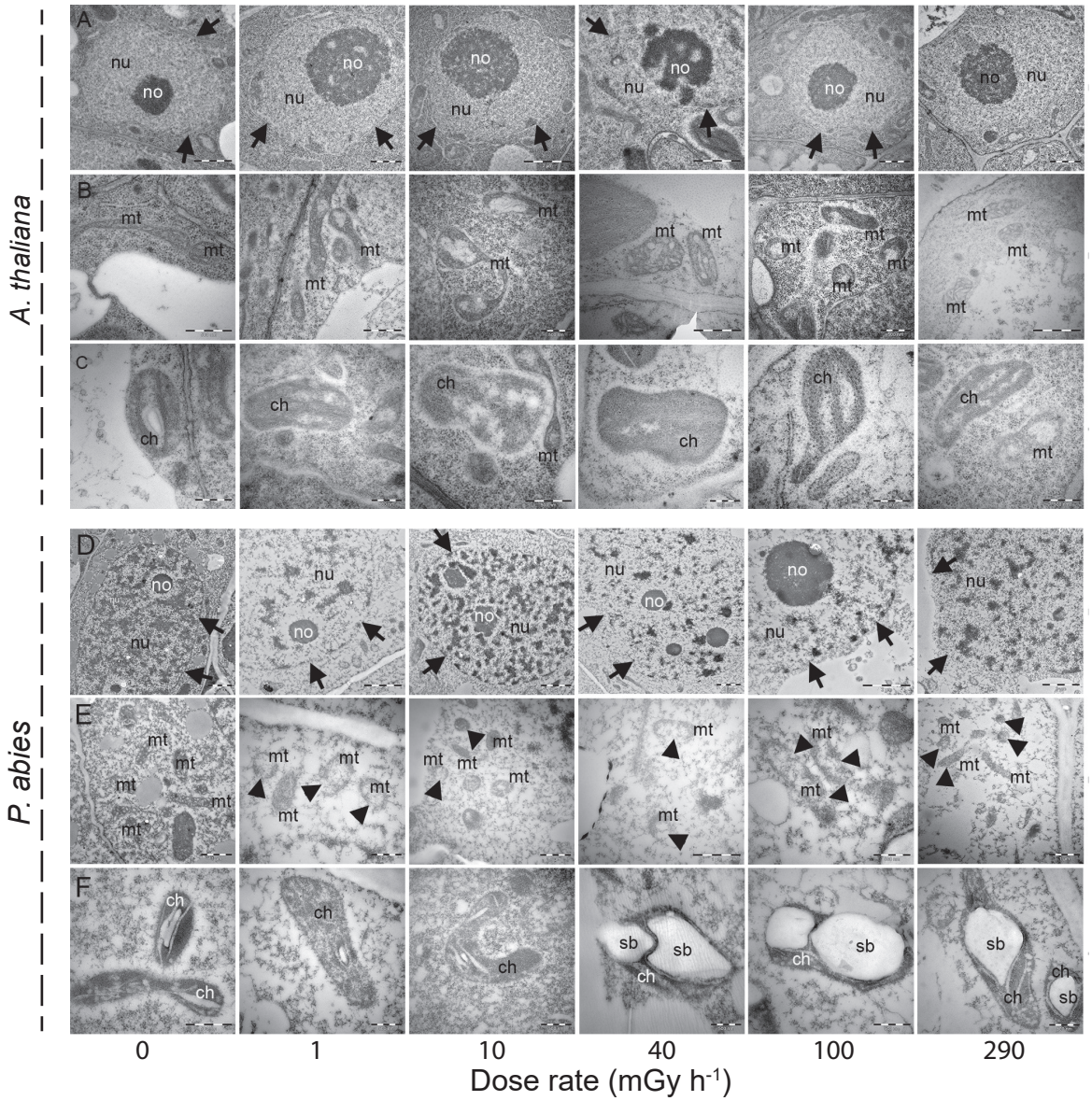

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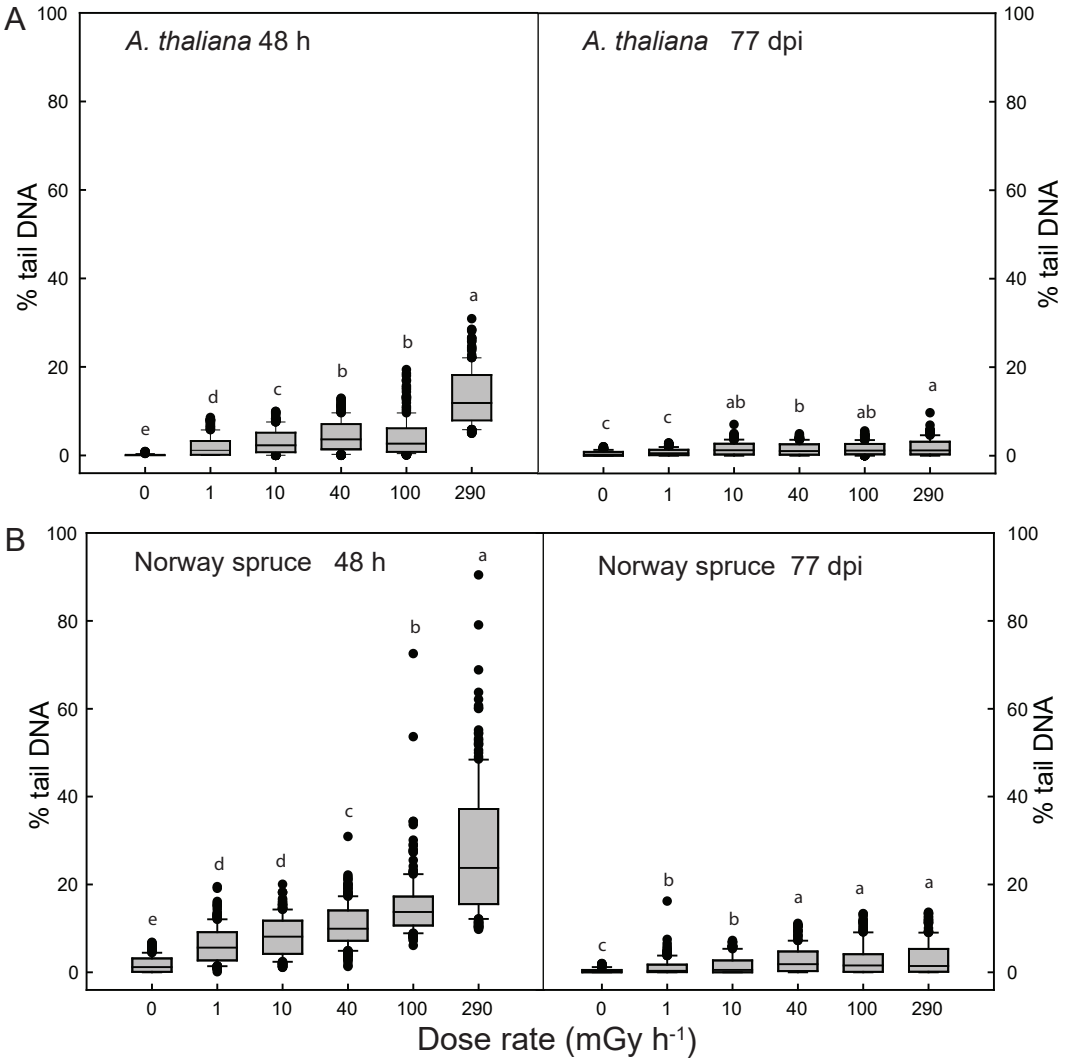

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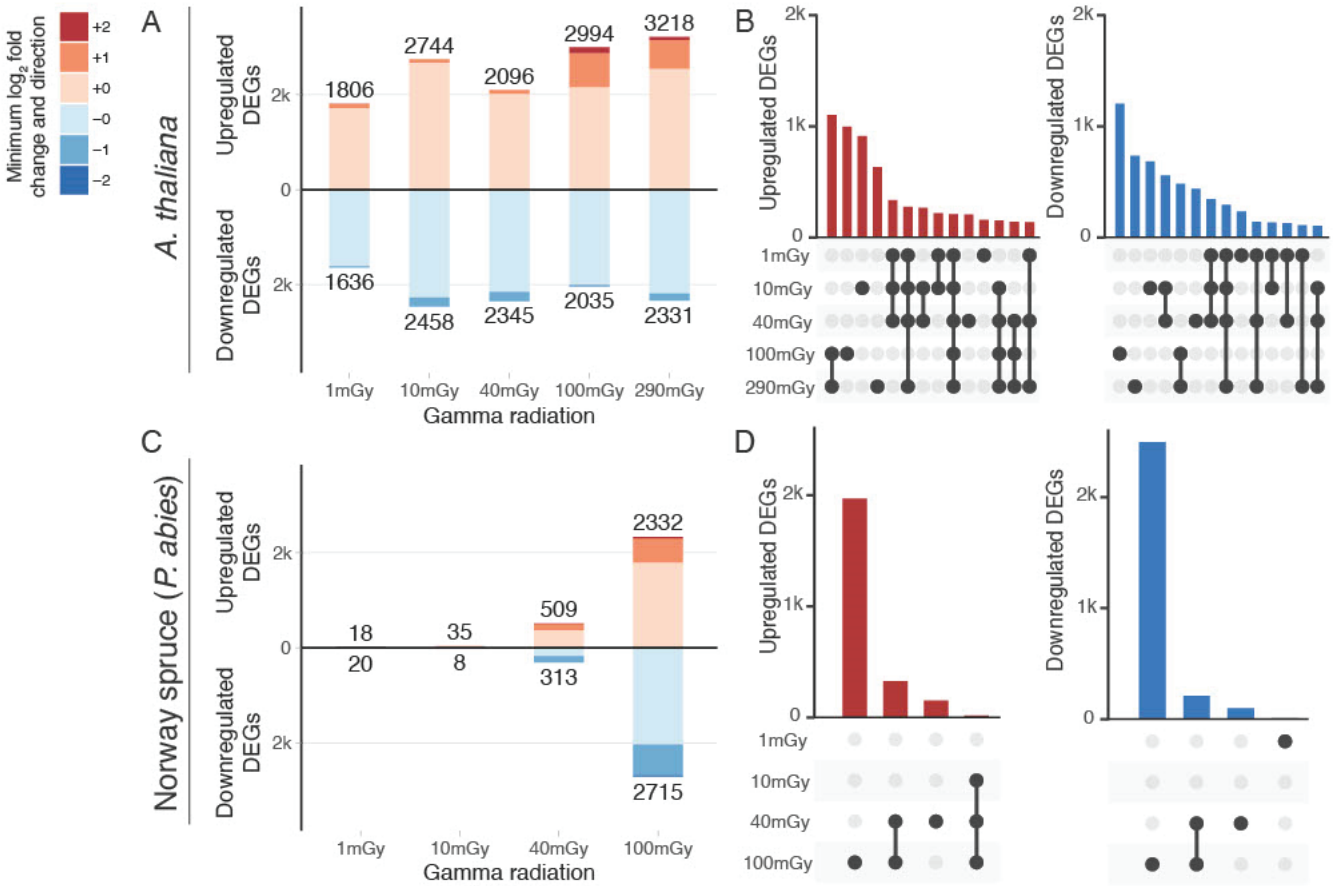

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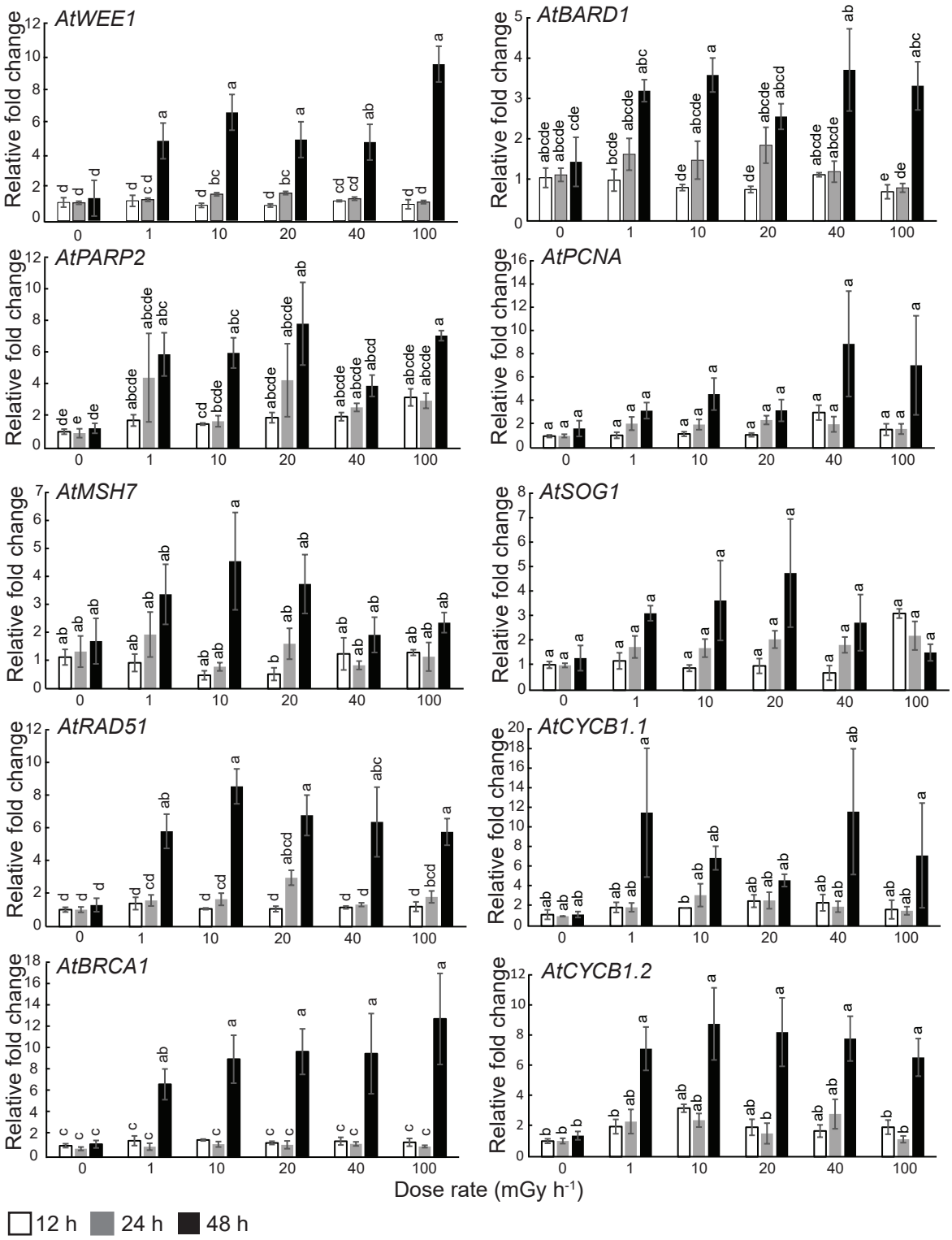

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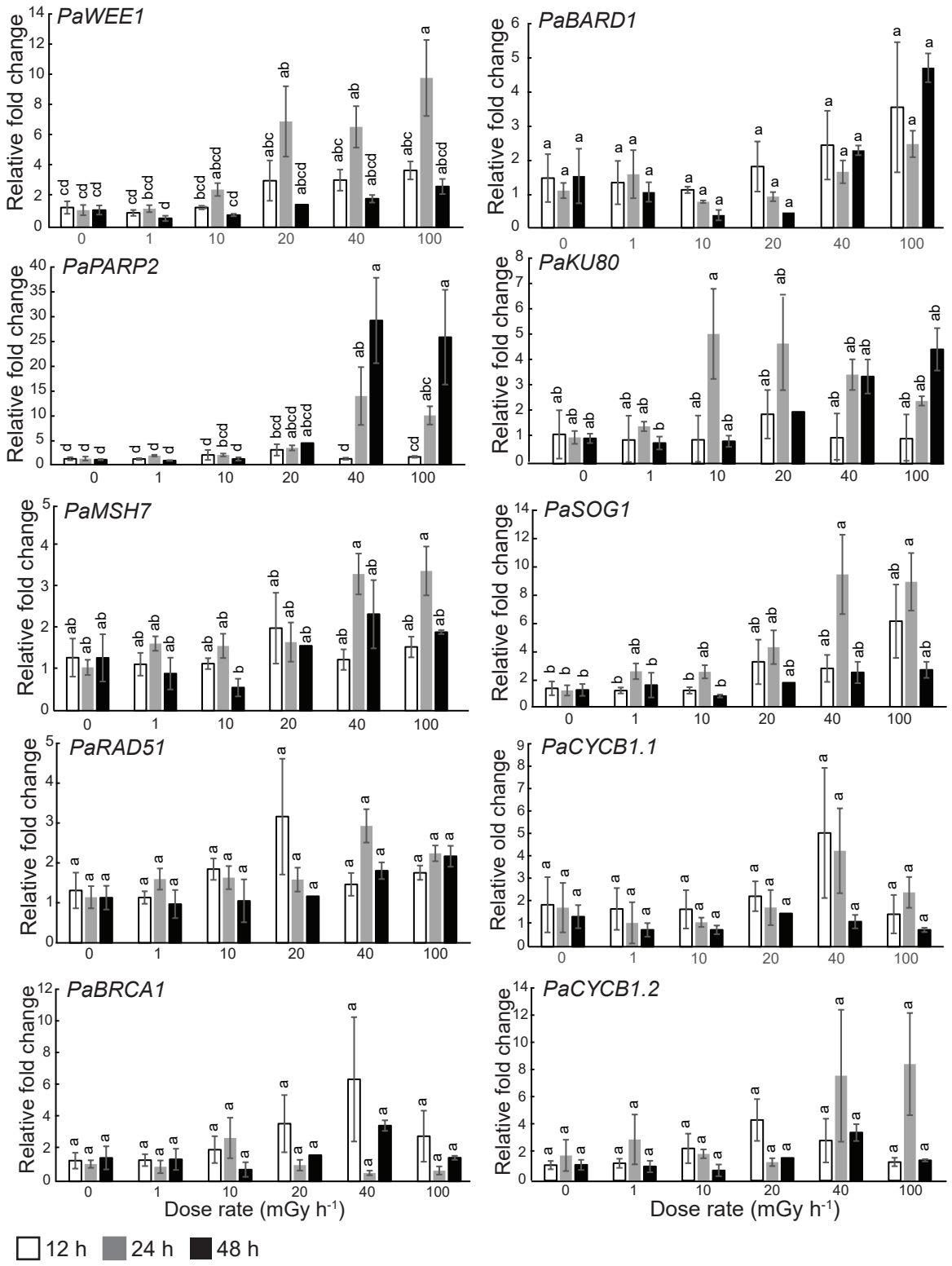

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Figure legends

Fig. 1. Effect of 48 h of gamma irradiation from day 7 after sowing on growth in *A. thaliana* and Norway spruce (*P. abies*). A. Hypocotyl and B. cotyledon length in *A. thaliana (*n = 29-40) after the 48-h irradiation and C. morphology 77 days post-irradiation (dpi). D. Shoot and E. root length of Norway spruce (n = 28-35) after the 48-h irradiation and F. shoot diameter and G. number of needles in time courses (n = 11-22) and F. morphology 77 dpi. Scale bars: 3 cm. Means ± standard errors are shown. Different letters within each figure denotes significant difference ($p \leq 0.05$) based on analysis of variance followed by Tukey`s test.

Fig. 2. Light micrographs of shoot apical meristems after 48 h of gamma irradiation from day 7 after sowing in *A. thaliana* and Norway spruce (*P. abies*). Five plants were investigated per treatment. Scale bars: 25 µm.

Fig. 3. Transmission electron micrographs showing cellular organelles after 48 h of gamma irradiation from day 7 after sowing in A – C *A. thaliana* and D - E Norway spruce (*P. abies*). nu: nucleus, no: nucleoli, mt: mitochondrion, ch: chloroplast, sb: starch body. Arrows indicate nuclear membrane. Arrowheads indicate disrupted mitochondria. Five plants were investigated per treatment. Scale bars: Scale nu: 1 µm, ch: 500 nm, mt: 500 nm.

Fig. 4. Effect of 48 h of gamma irradiation from day 7 after sowing on DNA damage detected by the COMET assay in A. *A. thaliana* and B. Norway spruce after the 48-h irradiation and day 77 post-irradiation. The line in each box represents the median values of 3 samples with 200 nuclei scored in each. Lower and upper box boundaries = 25 and 75 percentiles, error bars = 10 and 90 percentiles with data points outside these shown as dots. Different letters within each figure indicate significant difference ($p \leq 0.05$) based on analysis of variance followed by Tukey's test.

Fig. 5. Number of differentially expressed genes (DEGs) and their $\log_2$ fold change after 48 h of gamma irradiation from day 7 after sowing relative to unexposed controls of A-B. *A. thaliana* and C-D Norway spruce, with false discovery rate (FDR) < 0.05 (n = 4). Overlap of upregulated (red numbers) and downregulated (blue numbers) genes at different dose rates in (b) *A. thaliana* and (d) Norway spruce.

Fig. 6. Relative transcript levels of DNA repair and cell cycle genes after 12, 24 and 48 h of gamma irradiation from day 7 after sowing in shoots of *A. thaliana*. Transcript levels were normalized against *ACTIN*, *ELONGATION FACTOR 1 α* and *UBIQUITIN 10* and shown relative to the unexposed controls at 12 h. The results are mean ± SE (n = 3-4 with 3-4 technical replicates). Different letters within each gene indicate significant difference ($p \leq 0.05$) based on analysis of variance followed by Tukey's post-hoc test.

Fig. 7. Relative transcript level of DNA repair and cell cycle genes after 12, 24 and 48 h of gamma irradiation from day 7 after sowing in shoots of Norway spruce. Transcript levels were normalized against *ACTIN*, *ELONGATION FACTOR 1 α* and *UBIQUITIN 10* and shown relative to the unexposed controls at 12 h. The results are mean ±SE (n = 3-4 with 3-4 technical replicates). Different letters within each gene indicate significant differences ($p \leq 0.05$) based on analysis of variance followed by Tukey's post-hoc test.

Table 1. The gamma radiation dose rates and total doses applied in experiments with 48 h exposure of seedlings of *Arabidopsis thaliana* and Norway spruce from day 7 after sowing, using a ^{60}Co source.

Dose rate (mGy h^{-1})	Dose rate interval (mGy h^{-1})		Total dose (Gy) after 48 h exposure	Total dose interval (Gy) after 48 h exposure)	
	Minimum	Maximum		Minimum	Maximum
290	255	325	13.9	12.2	15.6
100	88	112	4.8	4.3	5.4
40	35	45	1.9	1.69	2.15
10	8,8	11,2	0.48	0.41	0.55
1	0,88	1,12	0.048	0.041	0.055
0	-	-	0	-	-