Are **Arabidopsis thaliana** plants able to recover from exposure to gamma radiation? A molecular perspective

Highlights

- Young *Arabidopsis* plants recover from 14d (20-100 mGy/h) gamma radiation
- Plant growth, hormones and gene expression responded in dose-dependent manner
- Chronic 14d irradiation induces early flowering and senescence during recovery
- Long-term differences in gene transcription were found for the highest dose rates

Abstract

Most plant research focuses on the responses immediately after exposure to ionizing irradiation (IR). However, it is as important to investigate how plants recover after exposure since this has a profound effect on future plant growth and development and hence on the long-term consequences of exposure to stress. This study aimed to investigate the IR-induced responses after exposure and during recovery by exposing 1-week old A. thaliana seedlings to gamma dose rates ranging from 27 to 103.7 mGy/h for 2 weeks and allowing them to recover for 4 days. A high-throughput RNAsequencing analysis was carried out. An enrichment of GO terms related to the metabolism of hormones was observed both after irradiation and during recovery at all dose rates. While plants exposed to the lowest dose rate activate defence responses after irradiation, they recover from the IR by resuming normal growth during the recovery period. Plants exposed to the intermediate dose rate invest in signalling and defence after irradiation. During recovery, in the plants exposed to the highest dose rate, fundamental metabolic processes such as photosynthesis and RNA modification were still affected. This might lead to detrimental effects in the long-term or in the next generations of those irradiated plants.

Title Page (with Author Details)

Are *Arabidopsis thaliana* plants able to recover from exposure to gamma radiation? A molecular perspective

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1 Introduction

All living organisms are constantly exposed to a natural background of ionizing radiation (IR) as well as to radiation produced from man-made activities (Eisler, 1994). Gamma radiation is an important type of IR because of its highly penetrative capabilities. Numerous plant studies have shown that gamma radiation can elicit a wide array of changes at the plant, organ, tissue and cellular/subcellular levels (Biermans et al., 2015; Esnault et al., 2010; Kim et al., 2005; Van Hoeck et al., 2015; Vanhoudt et al., 2014; Wi et al., 2007). At the plant level, high levels of gamma radiation have been observed to inhibit growth, reduce plant biomass and induce various morphological abnormalities (Kim et al., 2005; Watanabe et al., 2015; Wi et al., 2007). In addition, gamma radiation can cause significant changes to the expression of genes involved in the regulation of various physiological and biochemical processes, such as DNA damage repair, cell cycle control, antioxidative stress and various other defence responses (Biermans et al., 2015; Culligan et al., 2006; Kariuki et al., 2019; Sahr et al., 2005; van de Walle et al., 2016).

In the recent past, microarray and next generation sequencing platforms have made it possible to carry out in-depth genome-wide transcriptome analysis of the response to gamma radiation in the popular molecular model plant *Arabidopsis thaliana.* Hence, to date, there is substantial information from different experimental set-ups on the influence of gamma radiation on the *A. thaliana* transcriptome. For example, in *A. thaliana* leaves that were exposed to gamma radiation at the reproductive stage genes involved in antioxidative defence, photosynthesis and chlorophyll synthesis (Kim et al., 2007) as well as genes involved in secondary metabolism and nucleotide metabolism (Hwang et al., 2016) were differentially expressed. In *A. thaliana* leaves exposed to gamma radiation at the vegetative stage, metabolic and reproductive-related genes as well as genes related to response to stimuli were differentially expressed (Kim et al. (2014). Gicquel et al. (2012) compared the transcriptomes of *A. thaliana* plantlets exposed to moderate and low doses of ionising radiation for different durations (2 and

26 hours) and observed that genes involved in protein maintenance, DNA damage repair and cell cycle checkpoints were regulated in a dose-dependent manner. Nagata et al. (2005) investigated the effects elicited following plant exposure to acute high doses of radiation and observed an alteration of genes involved in redox reactions, signal transduction and stress response. However, to date only in a selective number of studies the long term consequences of a chronic exposure were studies using an holistic approach such as RNA sequencing. To this end, Kovalchuk et al. (2007) compared the transcriptomic profile of plants acutely and chronically exposed to low levels of radiation and observed that chronic radiation exposure caused more drastic changes to the transcriptome compared to acute exposure. Furthermore, they observed a differential expression of genes related to the hormone response, including auxin-responsive genes. Also other studies reported changes in the expression of genes related to auxin metabolism or auxin response after exposure to ionizing radiation in different plant species (Bitarishvili et al., 2018; Fortunati et al., 2010; Hwang et al., 2014; Hwang et al., 2016; Kim et al., 2007; Kovalchuk et al., 2007; Latif et al., 2011). Ricaud et al. (2007) suggested an important role for auxin in organ survival after ionizing radiation. Ionizing radiation has also been shown to affect the expression of genes related to other hormones, including abscisic acid (Bitarishvili et al., 2018; Qi et al., 2015), gibberelins (Hwang et al., 2014; Latif et al., 2011), cytokinins (Bitarishvili et al., 2018), ethylene (Gicquel et al., 2012; Kovalchuk et al., 2007) and jasmonic acid (Hwang et al., 2016). Since phytohormones coordinate almost all physiological processes in higher plants, changes in the hormonal status will have an effect on the development of the plant and its adaptation to the environment.

Besides studying the direct effects of irradiation, it is as important to investigate the recovery phase following cessation of stress treatments (Kosová et al., 2018). More often than not, this phase is ignored in research. However, in the long run, it has a profound effect on future plant growth and development and hence on the long-term consequences of exposure to stress. As such, it has been recommended that plant stress response studies should take the mechanisms and processes occurring during the recovery phase into account to predict long-term consequences (Crisp et al., 2016). There are several publications on the recovery of individual plants from abiotic stresses such as drought (Yin and Bauerle, 2017; Zhang et al., 2018), excess light (Crisp et al., 2017) and nutrient stress (Secco et al., 2015). In addition, Kariuki et al. (2019) investigated the recovery in *Oryza sativa* after exposure to different doses of ionizing radiation. They reported that the plants were able to recover from ionizing radiation, but that

a new equilibrium or homeostasis is established. In newly developed plant tissues, that were not directly irradiated, a radiation-induced stress signature was present leading to the establishment of a systemic acquired acclimation. Recovery was also observed in *A. thaliana* plants 12 and 24 hours after neutron irradiation. However, on the long term (i.e. 20 days after irradiation) some negative effects of the irradiation persisted, such as accelerated senescence (Fortunati et al., 2010).

Despite the substantial amount of knowledge regarding the immediate effects and responses triggered by gamma radiation in plants, there is currently very limited information on the long-term consequences of radiation exposure in plants e.g. chronically or historically exposed to radiation. Comparisons between irradiation and recovery responses may provide a better understanding on various aspects such as: (1) the mechanisms behind plant radiosensitivity to ionising radiation and (2) the processes involved in linking radiation-induced effects from molecular to individual levels of complexity. In this framework, the aim of the present study was to compare the elicited effects after chronic exposure to and during recovery from gamma radiation in *A. thaliana* plants, including the transcriptomic changes. Understanding the underlying molecular responses of recovery will contribute towards strengthening current legislation that has been formulated for radiation protection of the environment.

2 Materials and Methods

2.1 *Arabidopsis thaliana* culture in hydroponics

A. thaliana (Columbia ecotype) seeds were incubated in the dark on moist filter paper for 3 days at 4 °C to synchronize germination. Plants were grown as described before by Vanhoudt et al. (2014). Briefly, the seeds were grown on plugs made from 1.5 mL polyethylene centrifuge tubes filled with modified Hoagland solution (1 mM KNO_3, 0.3 mM $Ca(NO_3)_2$, 0.2 mM $MgSO_4$, 0.1 mM $NH_4H_2PO_4$, 1.62 µM $FeSO_4 \bullet 7H_2O$, 0.78 µM Na_2EDTA, 4.6 µM H_3BO_3, 0.9 µM $MnCl_2$, 32 nM $CuSO_4$, 55.6 nM H_2MoO_4, and 76.5 nM $ZnSO_4 \bullet 7H_2O$) solidified with 0.6% agar. The plugs were positioned on racks which could fit 36 plants per rack and placed on rectangular containers (10 x 20 x 10 cm) containing distilled water. Once the roots emerged through the agar 1 week later, the distilled water was replaced with modified Hoagland solution. Plants were grown in a climate chamber (Microclima 1000E, Snijders Scientific B.V) at 22/18 ºC day-night temperatures, 14h photoperiod, 65% relative humidity and a photosynthetic photon flux

density of 165 µmol m^{-2} s^{-1} at the leaf level. Roots were aerated during the entire course of the experiment.

2.2 Gamma irradiation and recovery

One-week old *A. thaliana* seedlings were transferred to the radiation facility at SCK CEN where they were exposed to gamma radiation for 14 days emitted from a ^{137}Cs panoramic source. Non-irradiated control plants were grown in a separate chamber under similar conditions. Different dose rates were obtained by placing the plant containers 2 m, 1.5 m, 1 m and 0.5 m from the radiation source. The dose rates obtained were: 27.2 mGy/h, 48.8 mGy/h and 103.5 mGy/h, which 2 weeks later resulted in a cumulative dose of 9 Gy, 16 Gy and 34 Gy, respectively. Dose rates were chosen based on previous experiments as inducing molecular and biochemical changes in *A. thaliana* (Biermans et al., 2015; Laanen et al., 2021). After 2 weeks irradiation, the plants were divided into two groups; one group was harvested immediately while the second group was returned to the climate chamber and allowed to recover for 4 days. The rosette fresh weight (FW) was measured and the collected samples were snap-frozen in liquid nitrogen and stored at -80 °C until further analysis. In summary, one-week old seedlings (day 7 after seeding) were irradiated for 14 days to one of the gamma dose rates or kept in control conditions. After the 14 days (day 21 after seeding) samples were taken for growth, biochemical and molecular analysis and all leftover plants were transferred to the control chamber, At day 25, plants were again sampled for growth, biochemical and molecular analysis. The leftover plants were further grown and scored for flowering at day 35 (when plants were 6 weeks old).

2.3 Hormone measurements

Endogenous levels of plant hormones (auxins and jasmonic acid) were determined in 20 mg FW according to the method described by (Šimura et al., 2018). Briefly, the phytohormones were extracted using an aqueous solution of acetonitrile (50% ACN/H_2O, v/v). A cocktail of stable isotope-labelled standards was added (all from Olchemim Ltd, Czech Republic) per sample to validate the LC-MS method. The extracts were purified using Oasis HLB columns (30 mg/1 ml, Waters) and analytes were eluted using 30% ACN/H_2O (v/v). Eluent containing plant hormones and their metabolites was gently evaporated to dryness under a stream of nitrogen. Separation was performed on an Acquity I-Class System (Waters, Milford, MA, USA) equipped with an Acquity UPLC® CSH C18 RP column (150×2.1 mm, 1.7 µm; Waters),

and the effluent was introduced into the electrospray ion source of a triple quadrupole mass spectrometer Xevo™ TQ-S (Waters), operating in multiple reaction monitoring (MRM) mode. Using the standard isotope dilution method (Rittenberg and Foster, 1940), concentrations of all the analytes were calculated as ratios of non-labelled compounds to labelled internal standards or closely eluting stable isotope-labeled tracers (Šimura et al., 2018).

2.4 RNA isolation and sequencing

For both irradiated and recovery plants, about 80 mg of frozen leaf tissue in 2 ml tubes were ground using two stainless steel beads (3 mm) in a Mixer Mill (MM 400, Retsch) for 3.5 minutes at 30 Hz. RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The quantity and purity were assessed at 230, 260 and 280 nm using the NanoDrop® ND-1000 spectrophotometer (Isogen Life Science) whereas RNA integrity was verified using RNA Nano Chips on an Agilent 2100 Bioanalyzer. Sequencing and subsequent data analysis was carried out as previously described by Krivoshiev et al. (2018). Briefly, a total of 24 sequencing libraries were prepared from both irradiated and recovery plants (3 independent biological replicates for the 4 conditions *i.e.* controls, 27.2, 48.8 and 10.3.5 mGy/h) using the TruSeq® Stranded mRNA Sample Preparation kit (Illumina, USA) following the manufacturer's protocol. Prepared libraries were 2 × 50 bp paired-end sequenced using the Illumina HiSeq® 1500 platform (Illumina, USA).

2.5 Mapping, annotation and identification of differentially expressed genes (DEGs)

Reads were mapped to the *A. thaliana* reference genome (Tair10) and only reads that were uniquely mapped and mapped concordantly in pairs were retained. Alignment, mapping, and annotation steps were performed with CLC Genomics Workbench (version 9.0.1, CLC Bio, DEN) using default parameters and the Araport11 annotation. Samples were normalized by quantile normalization. The abundances of gene expression were reported as reads per kilobase of transcript million mapped reads (RPKM). Differential expression p-values were generated using Baggerly's test statistic (Baggerly et al., 2003). These p-values were subsequently corrected with the Benjamini-Hochberg procedure to limit the false discovery rate (FDR) to 5% of the significant genes (Benjamini and Hochberg, 1995). To visualize the distance of the relationship between each biological replicate for irradiated and recovery plants, a multi-dimensional scaling (MDS) plot for the normalized count data was used. The cut-off

values: | fold-change| ≥ 2 and FDR ≤ 0.05 were used to determine differentially expressed genes (DEGs) at different dose rates for both irradiated and recovery plants compared to their respective non-exposed control plants.

2.6 GO and KEGG enrichment analysis

Enrichment analyses of Gene Ontology (GO) terms for DEGs characterizing biological processes and KEGG pathways were performed using the web-based tool Metascape (Zhou et al., 2019) (http://metascape.org). The tool uses all the genes in the *A. thaliana* genome as the enrichment background. Terms with a p-value < 0.05, a minimum count of 3, and an enrichment factor > 1.5 *i.e.* the ratio between the observed counts and the counts expected by chance, are collected and grouped into clusters based on their membership similarities. P-values are calculated based on the accumulative hypergeometric distribution and corrected using the Benjamini-Hochberg procedure to account for multiple testing. GO and KEGG enrichment analyses were performed separately for up- and down-regulated DEGs of each dose rate for the 2 weeks irradiated and 4 days recovery plants relative to their respective control plants.

2.7 RT-qPCR for gene expression analysis and validation of RNA-seq data

RNA for gene expression analysis as validation of RNA-seq data was isolated as previously described **(section 2.4)**. Contaminating genomic DNA was removed by treating the samples with the TURBO DNA-free™ Kit (Ambion, Thermo Fisher Scientific). An equal RNA input of 1 µg was used for each sample. cDNA synthesis was carried out using the PrimeScript™ RT Reagent Kit (Perfect Real Time, Takara Bio Inc., Westburg, The Netherlands) according to the manufacturer's protocol then it was diluted eight times in nuclease free water and stored at −20 °C. Quantitative real-time PCR (qPCR) was performed using the Rotor gene Q real-time PCR cycler (Qiagen) and the Fast SYBR® Green Master Mix (Applied Biosystems, Thermo Fisher Scientific). Reactions contained 2.5 µL diluted cDNA sample, 5 µL 2x Fast SYBR® Green Master Mix, 1.9 µL RNase-free H_2O and 300 nM each of the forward and reverse primers in a total reaction volume of 10 µL. Amplification occurred at universal cycling conditions (20 s at 95 °C, 40 cycles of 3 s at 95 °C and 30 s at 60 °C) followed by the generation of a dissociation curve to verify specificity of amplification. Relative gene expression levels were determined via the $2^{-\Delta Cq}$ method and normalized against the expression of multiple *A. thaliana* reference genes. These

reference genes were selected based on the GrayNorm algorithm (Remans et al., 2014). Primers used for RNA-seq validation and gene expression analysis are shown in Suppl. Table S1. The amplification efficiency (E) of these primers was calculated by making a 4-fold serial dilution series of a mixed sample over at least five dilution points and only those that were greater than 80% ($E = 10^{-1/\text{slope}}$) were accepted. Suppl. Table S2 shows the qPCR parameters according to the Minimum Information for publication of qPCR Experiments (MIQE) guidelines (Bustin et al., 2009).

2.8 Statistical analysis

Statistical analysis was performed using the open source software package Rstudio (RStudio Team, 2015). Normal distribution and homoscedasticity were tested with a Shapiro-Wilk and Bartlett's test, respectively. Where required, a logarithmic, inverse, square root or exponential transformation was applied. To identify statistical differences at different gamma dose rates, a one-way ANOVA was performed and when significant differences (p-value < 0.05) were found, a Tukey post-hoc test was applied to discriminate significantly different groups. If the transformed data did not meet the normality assumption, a non-parametric Kruskal-Wallis test was used, followed by a post-hoc Wilcoxon rank-sum test.

3 Results

3.1 Growth and development

The first goal of this experiment was to investigate the impact of different dose rates of gamma irradiation (control, 27.2 mGy/h, 48.8 mGy/h, 103.5 mGy/h) and a subsequent recovery period on the growth and development of *A. thaliana.* Therefore, the fresh weight (FW) of plants was determined after 2 weeks of irradiation and following a recovery period of 4 days. In addition, some plants were allowed to recover during 2 weeks after irradiation. Within this 2-week time frame plants transformed from the vegetative to the reproductive phase, enabling us to determine some flowering-related endpoints during recovery.

After 2 weeks of irradiation, the rosette FW of plants exposed to the two lowest dose rates was significantly reduced compared to that of control plants. Plants exposed to the highest dose rate did not significantly differ from control plants (Table 1). After recovering for 4 days, the differences in rosette FW were similar to that observed in irradiated plants with a significant decline at the two lowest dose rates and no significant change at the highest dose rate when compared to control plants (Table 1). However, when expressed as percentage growth relative to the 2 weeks irradiated plants, an increase with 143% and 155% was observed after exposure to 27.2mGy/h and 48.8 mGy/h, respectively, while this increase was 132% and 129% for control plants and plants exposed to 103.5 mGy/h, respectively.

When plants were allowed to recover for 2 weeks, early flowering was observed in IR-exposed plants compared to control plants, irrespective of the used dose rate. This was based on the assessment of flowering-related endpoints such as the fresh weight and height of the inflorescence stem, the number of siliques and the number of flowers (Figure 1D), which were all significantly increased after exposure to ionizing radiation.

3.2 Transcriptional changes after gamma exposure and following recovery

To analyse the molecular mechanisms that lie at the basis of the ionizing radiation-induced changes in growth and development, the transcriptome of *A. thaliana* plants was sequenced after irradiation and after a 4-day recovery period. To obtain a general overview of the variation between the different biological replicates for each experimental condition, a multi-dimensional scaling (MDS) plot of the raw

normalised reads was constructed. This plot clusters samples from both irradiated and recovering plants depending on the treatment (controls and the 3 dose rates; Figure 2).

After irradiation, the clustering of samples exposed to the lowest (27.2 mGy/h) and intermediate dose rates (48.8 mGy/h) shifted away from the control samples cluster in a dose rate-dependent manner along both the first and second axis. On the other hand, at the highest dose rate (103.5 mGy/h), the cluster shift of samples was evident after irradiation along the second axis relative to the control samples cluster, although this shift was not present along the first axis.

In recovering plants, a cluster shift of the lowest and intermediate dose rate experimental groups was only visible along the secondary axis. The cluster of the highest dose rate shifted from the control plants along the first and second axis (Figure 2). In addition, the spread in recovering plants is smaller than in plants directly after irradiation as there is a near overlap in the clustering of samples exposed to the intermediate and highest dose rates along both the first and second axes in recovering plants.

A total of 4074 (2119 up- & 1995 down-regulated) and 2086 (814 up- & 1148 down-regulated) DEGs were identified in irradiated and recovering plants relative to their corresponding control samples, respectively, among the 3 tested dose rates (Suppl. file S1). The overall increase in both up- and down-regulated DEGs was dose rate-dependent (Figure 3A). Analysis of the Venn diagrams (Figure 3B) revealed that more than 40% (387 up-regulated) and more than 30% (277 down-regulated) DEGs were shared between the intermediate and highest dose rates, after irradiation.

Next, the functions of the DEGs in irradiated and recovering plants were studied. Figure 4 and Figure 5 show an overview of the significantly altered GO classes while the affected KEGG pathways are presented in Suppl. file S2.

Immediately after irradiation, up-regulated DEGs across the 3 tested dose rates were enriched for GO terms for various metabolic responses (e.g. ‘polyamine metabolic process’, ‘secondary metabolic process’ and ‘hormone metabolic process’), defence responses (e.g. ‘response to wounding’, ‘response to fungus’ and ‘response to oxidative stress’) and hormone related processes (e.g. ‘response to jasmonic acid’, ‘regulation of hormone levels’, ‘auxin metabolic process’, ‘response to gibberellin’). The down-regulated DEGs immediately after irradiation were enriched for GO terms related to growth/development at the

lowest and intermediate dose rate (e.g. 'flower development', 'positive regulation of growth', 'tissue development' and 'regulation of leaf senescence'). At the highest dose rate, however, the majority of the down-regulated DEGs were enriched for GO terms related to pathways involved in basic fundamental processes (e.g. 'nuclear DNA replication' and 'reductive pentose phosphate cycle').

In recovering plants exposed to the lowest dose rate, up-regulated DEGs were enriched in growth/developmental-related processes such as: 'response to auxin', 'auxin polar transport', 'response to blue light' and 'vegetative to reproductive phase transition of meristem'. The enriched GO terms associated with the downregulated genes in those plants are linked nutrient-associated processes as indicated by 'sulfur compound metabolic process', 'response to nitrogen compound' and 'ion transport'. After exposure to the intermediate and highest dose rate, similar processes seemed to be affected, including enrichment for the GO terms 'RNA modification', 'mitochondrial RNA modification' and 'negative regulation of RNA metabolic process' among the up-regulated DEGs and 'photosynthesis', 'regulation of photosynthesis', 'chloroplast organisation', 'photosynthetic electron transport in photosystem I', 'photosystem II assembly', 'chlorophyll metabolic process' and 'photosynthesis, light harvesting' among the downregulated genes.

In addition to the transcriptomic profiling, the expression profiles of some senescence and flowering-related genes was determined by RT-qPCR. Twelve genes, 6 related to senescence and 6 to flowering, were selected based on the DEGs and GO analyses and their expression profile was determined via RT-qPCR. In addition, these data served as a validation of our RNA-seq data. The expression profiles were consistent for senescence- (Figure 6 A to F) and flowering-related genes (Figure 6 G to L), thus confirming both up- and down-regulation trends of all the genes analysed.

The transcription of senescence-related genes increased in a dose-rate dependent manner after exposure to ionizing radiation, except for *ACS4,* where a significant increase was only present after exposure to 27.2 mGy/h and 48.8 mGy/h. During recovery, the dose-rate dependent increase in expression was still present for *COR15*A, *COR15B* and *TAG1*, while no significant increases were present in the expression profile of *ACS4* and *SEN1*. The transcript levels of *RNS1* only increased significantly during recovery in plants that were exposed to 48.8 mGy/h.

For the flowering-related genes, an increased expression after exposure to gamma radiation, irrespective of the used dose rate, was present in the expression profile for *FLP2* and *GA20OX1*. A significant increase in the transcript levels for *FKF1* and *GRP7* was only observed after exposure to 103.5 mGy/h, while no changes were observed in the expression level of *FLP1*. For *LHY1*, a dose-rate dependent decrease was observed after exposure to gamma radiation. During recovery, the transcript levels of *LHY1* still showed the same trend, while no significant changes were present in the expression levels for *GA20OX1* and *FLP2*. For *FLP1*, *FKF1* and *GRP7*, a significant increase was present at all dose rates during recovery.

3.3 Hormone analyses

To validate the importance of different hormones in the response of *A. thaliana* after exposure to ionizing radiation and in the subsequent recovery period as suggested by the enrichment analysis, the concentration of different phytohormones was determined (Table 2). The levels of the JA precursors *cis*- and dinor-12-oxo-phytodienoic acid (cis-OPDA and dnOPDA, respectively) significantly increased at the highest dose rate after irradiation whereas following recovery, a significant decline in these precursors was apparent for all tested dose rates. On the other hand, JA levels themselves significantly declined at the highest dose rate following irradiation while after recovery the decline was significant both at the intermediate and highest dose rates. The levels of the JA derivative 9,10-dihydrojasmonic acid (DHJA) did not change significantly after irradiation. During the recovery period, however, a significant increase in the levels of this hormone was present at the highest dose rate.

In addition, different auxin precursors and conjugates were quantified. After irradiation, the levels of the auxin precursor indole-3-acetonitrile (IAN) significantly increased at all dose rates, whereas that of the auxin catabolite, 2-oxoindole-3-acetic acid (oxIAA), significantly increased only at the two highest dose rates. In recovering plants, IAN levels fluctuated at the different dose rates, while oxIAA levels significantly increased at the two lowest dose rates compared to control levels. In both irradiated and recovering plants, there was no significant change in the levels of indol-3-acetic acid (IAA).

4 Discussion

The aim of this study was assessing the irradiation and recovery responses in *A. thaliana* as well as to unravel the potential strategies that are employed by plants at the molecular level to recover from increasing doses of gamma radiation. Therefore, a transcriptome analysis of irradiated and recovering

298 plants was carried out using an mRNA sequencing approach. The dose rates used in this study (27 to
299 103.7 mGy/h) are above what is designated as a low dose ≤ 6 mGy/h (see references in Blagojevic et
300 al., 2019) and above the ICRP (2008) proposed 'Derived Consideration Reference Levels' DCRL for plants
301 (0.04-0.4 mGy/h) which are positioned as a dose rate band within which there is likely to be some
302 chances or deleterious effects of IR to occur to individuals as well as environmental levels found at the
303 present day in accidental affected areas such as the Chornobyl exclusion zone (0.0002 mGy/h to 0.4
304 mGy/h (Horemans et al., 2019; Raines et al., 2020). However, they were chosen based on previous
305 studies in which they were shown to induce molecular and biochemical changes in the relatively
306 radioresistent *A. thaliana* plants (Biermans et al., 2015; Laanen et al., 2021).

307 Immediately after irradiation the plants showed a decreased FW at the lowest (27.2 mGy/h) and
308 intermediate (48.8 mGy/h) dose rate (Table 1) that can be linked to the down-regulated DEGs
309 immediately after irradiation that were enriched for GO terms related to growth/development such as
310 'positive regulation of growth', 'tissue development', 'phloem or xylem biogenesis', 'regulation of leaf
311 senescence' and 'regulation of cell death'. In addition, it seems that at those dose rates, metabolic
312 processes ('polyamine metabolic process', 'secondary metabolic process' and 'hormone metabolic
313 process') and defences responses (e.g. 'response to wounding', 'cellular response to abiotic stimulus',
314 'response to fungus') are switched on. A similar enrichment of GO terms related to metabolism, signalling
315 and defence was observed in rosette leaves of *A. thaliana* plants exposed to 100, 200 and 800 Gy gamma
316 radiation during the reproductive stage (Hwang et al., 2016; Kim et al., 2007) and in plants exposed to
317 100 and 800 Gy during the vegetative stage (Kim et al., 2014). It has previously been proposed that
318 up-regulation of metabolic processes modulates signal transduction cascades that switch on plant
319 defence responses following exposure to biotic stress (Rojas et al., 2014). This possibly indicates that
320 plants when exposed to the lowest and intermediate dose rate are shifting their energy from growth-
321 related processes to defence responses, as has also been suggested before by Van Hoeck et al. (2017)
322 for gamma exposed *Lemna minor* plants. However, at the highest dose rate, we suggest that other
323 mechanisms come into play after irradiation. As such, it is clear in Figure 2 that the cluster of the highest
324 dose rate is shifted from the lowest and intermediate dose rate. In addition, fundamental day-to-day
325 processes are affected at the highest dose rate, which is not the case at the lowest and intermediate
326 dose rate.

Gamma radiation has been reported before to affect plant growth in various ways ranging from hormesis (van de Walle et al., 2016) to no effects (Kariuki et al., 2019; Vanhoudt et al., 2014) to growth inhibition (Van Hoeck et al., 2015). The inconsistencies reported here in the plant biomass responses between the present and other studies may be attributed to differences in growth conditions that the plants were subjected to at the time of irradiation, the developmental stage of the plants at the time of exposure and the plant species used as was also suggested by Biermans et al. (2015). In addition, also the experimental conditions, such as dose rate, duration of exposure and total dose delivered might impact these responses. Therefore, we suggest that plant biomass is an integrative endpoint that should be considered with care when assessing IR-induced effects in plants.

A 4-day recovery period had no effect on the rosette FW of *A. thaliana* seedlings (Table 1). However, by comparing the percentage growth relative to the 2 weeks irradiated plants, it seems that *A. thaliana* plants have recovered from the stress induced by gamma radiation at the lowest and intermediate dose rate, which was reflected in a higher growth rate. Similar results have been reported before by Kariuki et al. (2019), where rice plants exposed to gamma radiation during 2 weeks seem to recover during a 2-week recovery period. Kariuki and co-workers (2019) suggested the establishment of a new homeostasis during the recovery period. A reprogramming of the physiological status in order to tolerate ionizing radiation-induced stress has also been reported by Van Hoeck et al. (2017) in *L. minor* plants exposed to dose rates up to 232 mGy/h.

Within the current exposure set up, when the *A. thaliana* plants were allowed to recover for 2 weeks, they showed early flowering (Figure 1C&D). Previous studies have reported that diverse abiotic (drought, salt, heat, cold, nutrients) and biotic stresses altered the time and development of inflorescence emergence in plants (for a detailed review see Kazan and Lyons, 2016). This switch from vegetative to reproductive growth under stress conditions is considered to be critical, since proper timing of flowering ensures the success of the next generation and the continuity of the species (Takeno, 2016). Kovalchuk et al. (2007) observed early flowering in chronically irradiated *A. thaliana* plants as compared to both control and acutely irradiated plants. In addition, an induction of a selection of flowering-related genes was detected in these chronically irradiated plants. Hwang et al. (2016) observed that the alterations in transcript levels of flowering-related genes led to changes in the onset of flowering time in plants exposed to 100 and 200 Gy. In addition, a larger number of siliques was observed as compared to control plants

(Hwang et al., 2016). Consistent with the observed induction in early flowering in the present study, also DEGs were identified enriched for growth/developmental-related GO terms in recovering plants at the lowest dose rate ('vegetative to reproductive phase transition of meristem), intermediate dose rate (e.g. 'seed development') and highest dose rate (e.g. 'regulation of seed germination'). The data coming from the RNAseq analysis were further confirmed by measuring the expression level of a selection of flowering-related genes (Figure 6). The increased expression of *FLP2* and *FLP1* observed in this study, after irradiation and during recovery, respectively, can be linked to early flowering, as suggested before by Borner et al (2000). The Flavin-binding, Kelch repeat, F-box 1 (*FKF1*) has an important role in stabilizing CONSTANS and FLOWERING LOCUS T. Both proteins play a central role in the flowering time controlling network (Blumel et al., 2015; Song et al., 2012), with an increased expression leading to promotion of flowering. Also *GRP7* promotes floral transition, partly by downregulating Flowering Locus C (Streitner et al., 2008). Finally, the Late Elongated Hypocotyl (*LHY*) is involved in regulating the timing of flowering where a downregulation is associated with early flowering (Park et al., 2016). Although the effects on the transcript levels of flowering-related genes seemed to be dose-rate dependent, this was not the case for the transition to the reproductive stage which was switched on after plants were stressed, independent of the used dose rate. Similar effects were reported before by Keunen et al. (2011) where the effects of 5 and 10 µM Cd were investigated on the life cycle of *A. thaliana* plants. While they reported a concentration-dependent effect on the vegetative growth of the plant, the influence on the inflorescence emergence was switched on after Cd-treatment, independent of the used concentration.

The above observations on flowering led us to hypothesize that radiation stress induced early aging. In support of this, Kim et al. (2018) reported a strong correlation between the timing of flowering and leaf senescence in *A. thaliana* and the reason behind this was that both developmental processes are coupled to the circadian clock. As such, our hypothesis was investigated further by measuring the transcript levels of a number of genes involved in senescence (Figure 6). An increased expression of *SEN1* is already known to be involved in leaf senescence (Oh et al., 1996). In addition, Schenk et al. (2005) suggested *SEN1* as a marker gene to link plant defence and senescence responses since it is regulated by signals of these two important pathways. Buchanan-Wollaston et al. (2005) and Yang et al. (2011) reported before an increased expression of *COR15A* and *COR15B* in senescing leaves. *TAG1*, also known as diacylglycerol acyltransferase (*DGAT1*), is involved in recruitment of membrane carbon from

senescing leaves to growing parts of the plant by increasing the levels of triacylglycerol (TAG) containing fatty acids. The formation of TAG may be an intermediate step in the conversion of thylakoid fatty acids to phloem-mobile sucrose (Kaup et al., 2002). After exposure to radiation, the transcript levels of *RNS1* significantly increased, with a 470-fold induction after exposure to 103.5 mGy/h. This increased expression has been reported to be important in senescing leaves and can be linked to an increased degradation of RNA leading to the remobilization of Pi to non-senescing organs (Bariola et al., 1994; Shane et al., 2014) As such, the strong upregulation of senescence related genes such as *RNS1* in the plants exposed to the highest dose rates potentially indicates that under ionising irradiation plants might go into an early senescence. In this respect, our lab-based data seem to be coherent with the observations made in the initial phase after the Mayak as well as the Chornobyl accident where the trees of the highest exposed areas went into early senescence (Arkhipov et al., 1994; UNSCEAR, 1996).

Taken together, it is shown here that the observed early flowering in *A. thaliana* plants recovering from 14-days of radiation is marked by a changed expression of genes related to both senescence and flowering, which was initiated during the exposure. Laanen et al. (2023) recently reviewed the available literature and reported that plants exposed to ionising irradiation definitely can alter their flowering pattern, however, the fine tuning and regulation of this interaction remains to be elucidated. Gicquel et al. (2012) stated that, in the event that damages from radiation exposure are excessive such that repairing processes are not fully successful, senescence is one of the measures plants take at the molecular, tissue and whole-organism level in order to acclimatize and ensure survival, in addition to nutrient recycling and regulation of programmed cell death. Actually, for various abiotic stresses, accelerated senescence resulting in the initiation of early seed production has been suggested as a strategy to facilitate survival of the next generation and one of the tactics used by plants to cope with adverse conditions (Sade et al., 2017).

Phytohormones are essential in plant growth and development and in the adaption to adverse environmental conditions as they mediate elaborate signalling networks (for detailed review see: Verma et al., 2016). A general observation that stood out across all 3 dose rates for both irradiated and recovering plants, was the enrichment of up- and down-regulated DEGs for GO terms related to the metabolism of phytohormones. DEGs were enriched for the term 'response to jasmonic acid' after 2 weeks irradiation across all dose rates and 'auxin metabolic process' was enriched in plants exposed to

*the intermediate and highest dose rate (Figure 4) whereas after 4 days recovery, 'response to auxin' was enriched at the 2 lowest dose rates and 'auxin homeostasis' was enriched at the highest dose rate (*Figure 5*). To this end, the levels of jasmonates and auxins were quantified in the rosette leaves of the plants immediately after exposure and after 4 days recovery as shown in* ***Table 2*****: Phytohormone levels in *A. thaliana* rosettes in response to different levels of irradiation.**

Jasmonate and Auxin metabolites after 2 weeks irradiation and 4 days recovery of 1-week old plants following exposure to different gamma dose rates. Data represents the average ± SE of 5 biological replicates. Green and red colours indicate significant increase and decrease, respectively, in compound levels. cis-OPDA: cis-12-oxo-phytodienoic acid; dn-OPDA: dinor-12-oxo-phytodienoic acid; JA: jasmonic acid; 9,10-DHJA: 9,10-dihydrojasmonic acid; IAN: indole-3-acetonitrile; oxIAA: 2-oxoindole-3-acetic acid; IAA: indole-3-acetic acid.Table 2.

Literature evidence suggests that *cis*-OPDA in particular plays a distinct role in plant signalling in response to environmental stress stimuli as well as developmental cues (Dave and Graham, 2012). This may explain why, unlike JA, *cis*-OPDA levels increased (significantly at the lowest and highest dose rates) in the here studied *A. thaliana* plants following exposure to radiation stress. The other JA precursor, dnOPDA, was also shown in a previous study to play a role in signalling, more specifically, wound signalling in *A. thaliana* and potato leaves (Weber et al., 1997). The levels of DHJA, a derivative of JA, did not significantly change after irradiation but significantly increased at the highest dose rate after recovery. There is limited information on the specific role of DHJA, but it has been previously suggested that this derivative is converted to various JA metabolites through glucosylation, hydroxylation and conjugation with amino acids (Yoshihara et al., 1996). These JA metabolites may be inactive (following glucosylation), partially active (following hydroxylation) or fully active (following conjugation with an amino acid such as isoleucine). It has been suggested that formation of the various JA metabolites having different modes of action, allows plants to respond specifically and flexibly to alterations in the environment (Wasternack and Strnad, 2016). A possible role for jasmonates in the root-to-shoot signalling in *A. thaliana* after alpha particle radiation of the roots has been suggested before by Wang et al. (2016). Furthermore, Volkova et al. (2020) reported a differential expression of genes related to JA metabolism and biosynthesis after low-dose irradiation of barley seeds. Taken together, it can be concluded that jasmonates seem to play a critical role in stress signalling in plants after exposure to

ionizing radiation whereas following recovery the response is fine-tuned through the action of different JA metabolites in accordance with the needs of the plants.

From the RNA-seq data, the GO terms 'response to auxin' and 'auxin homeostasis' were mainly enriched after the recovery period and not following irradiation, but quantification of the various auxin compounds revealed that their levels were altered in both irradiated and recovering plants. It has been previously reported that oxIAA has little biological activity and is formed rapidly and irreversibly in response to increases in auxin levels (Pěnčík et al., 2013). This serves to regulate IAA homeostasis, consequently modulating developmentally important auxin gradients and auxin maxima/minima within plants (Pěnčík et al., 2013). Thus, in the present study, increased levels of the IAA precursor (IAN) and catabolite (oxIAA) most likely serve to ensure that IAA levels are maintained at optimal levels and may be the reason why overall levels of this hormone remain unchanged in both irradiated and recovering plants. Optimum auxin levels can be presumed to play an essential role in enabling plants to withstand radiation stress following exposure as well as aid in recovery thereafter. However, in the present study the auxin concentration was determined at rosette level. Since it is known that not the overall concentration, but often the tissue gradient of auxins plays an important role in the coordination of growth and development (Tognetti et al., 2017), future studies should take this gradient into account in e.g. different leaves or in the shoot apical meristem of plants after exposure to ionizing radiation and during the recovery period.

As discussed above and summarised in Figure 7 the GO enrichment analyses of DEGs after irradiation revealed an enrichment of GO terms related to signalling processes, various metabolic processes and defence responses among all tested dose rates. Among the downregulated genes, GO terms related to growth/development were affected at the lowest and intermediate dose rate. Further assessment of the GO enrichment analyses revealed that at the highest dose rate the majority of down-regulated DEGs were enriched for GO terms related to pathways involved in fundamental day-to-day processes. For example, the most enriched GO term among the downregulated genes was for the process 'nuclear DNA replication', which is essential for cell division and, if affected, may compromise genome integrity (Jossen and Bermejo, 2013). Down-regulation of genes involved in DNA synthesis was also observed in *A. thaliana* plants acutely exposed to high doses (3.0 kGy/h) of gamma radiation (Nagata et al., 2005). In addition, the GO term 'reductive pentose phosphate cycle' was enriched among the downregulated genes. This cycle, which is also known as the Calvin cycle, is the main biochemical pathway for the

conversion of atmospheric CO_2 to organic compounds. Gamma radiation has previously been observed to cause alterations in photosynthesis or in non-photochemical quenching in plants (Kim et al., 2004; Kim et al., 2005; Vanhoudt et al., 2014). Previous transcriptome studies in *A. thaliana* also reported differential changes in photosynthesis-related genes after irradiation (Hwang et al., 2016; Kim et al., 2007; Kovalchuk et al., 2007). Photosynthesis is a fundamental physiological process necessary in plants for provision of energy required for metabolism, but also for stress adaptation and stress survival. Therefore, the observed effects on photosynthesis-related processes at the molecular level, in our study, indicate that radiation exposure has a negative impact that occurred to a greater extent in plants exposed to the highest dose rate compared to plants exposed to the intermediate and lowest dose rate.

By analysing the molecular responses during recovery in the plants exposed to the lowest dose rate, it seemed there is a disturbance in nutrient-related pathways during the recovery period indicated by the 'sulfur-compound metabolic process' and 'ion transport' enriched GO terms. A disturbed nutrient profile in *A. thaliana* leaves has been reported before by Vanhoudt et al. (2011) after 72 h exposure to 50 mGy/h or 400 mGy/h. Previous studies have indicated that plants integrate their energy/nutrient status to regulate growth and stress responses via signaling pathways (Bechtold and Field, 2018). This may explain the observed disturbance in the nutrient metabolic pathways following exposure to gamma radiation and recovery thereafter. However, when plants are exposed to the lowest dose rate, they seem to resume normal growth and are able to establish a new homeostasis (Figure 7), indicating that those plants can probably fully recover from the irradiation.

The processes affected in recovering plants exposed to the intermediate and highest dose rate were very similar, but distinct from those of the lowest dose rate. This is consistent with the near overlap of the intermediate and highest dose recovering plant samples along the second axis of the MDS plot (Figure 2). For example, after 4-days recovery at both dose rates up-regulated DEGs were enriched for the GO term 'photosynthesis'. Also, several other photosynthesis-related GO terms were commonly enriched for by down-regulated DEGs at these two dose rates (e.g. 'chloroplast organization', 'photosystem II assembly', 'regulation of photosynthesis'). In addition, down-regulated DEGs were enriched for the GO term 'RNA modification', a function that influences many fundamental processes such as gene and protein expression as well as RNA metabolism. RNA modifications, also referred to as epitranscriptome,

are an additional layer of information deposited and recognised by proteins resulting in effects on various downstream functions (Shen et al., 2019; Vandivier and Gregory, 2018). In plants, studies have linked RNA modifications to important biological outcomes such as leaf morphogenesis, shoot apical meristem maintenance, floral transition and root development (for a detailed review, see Vandivier and Gregory, 2018). Information on how RNA modifications affect plant responses to various environmental stresses, and in particular gamma radiation, is scarce. However, the present study indicates that *A thaliana* plants exposed to the highest dose rates despite some underlying stress responses could resume growth and flower. RNA modifications which include methylation of adenine [m^6 A] (Liang et al., 2018), is potentially an important regulator during recovery from IR-induced stress responses in both the intermediate and highest dose rates.

5 Conclusion

In this study it was shown that *A. thaliana* plants exposed for 14 days to different dose rates of gamma radiation (ranging from 27.2-103.5 mGy/h), were able to recover and transition to flowering despite the induction of radiation stress responses. However, the timing of senescence and flowering is affected in the plants as well as resisting radiation effects were present during the recovery phase. Different coping strategies were employed depending both on the dose-rate and the phase (exposure or recovery). In addition to the hormone metabolism, epigenetic changes including mRNA modifications were put forward to regulate growth, senescence and flowering in a multifactorial way. Affecting fundamental processes such as senescence and flowering can possibly have consequences for the next generations of those irradiated plants. Therefore, it would be interesting to extend in future research the experimental period to investigate whether these coping strategies become apparent within subsequent generations. Finally, it is of interest to study if plants exposed in the field e.g. in the Chornobyl accident affected areas show similar underlying molecular changes as this will increase the knowledge base for environmental radiation protection as well as to identify common factors allowing extrapolation to other organisms or trophical levels.

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735 Tables

736 **Table 1: Effect of different gamma dose rates on rosette fresh weight.**

737 Fresh weight (mg) after 2 weeks irradiation and 4 days recovery of 1-week old *A. thaliana* plants. Small letters
738 indicate significant differences ($p < 0.05$; one-way ANOVA) within irradiated plants between exposed and control
739 plants; capital letters indicate significant differences ($p < 0.05$; one-way ANOVA) within recovering plants between
740 exposed and control plants. Data represent the average ± SE of at least 50 biological replicates. The percentage
741 growth after recovery relative to the irradiated plants is presented between brackets.

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Dose rate	2 weeks irradiated	4 days recovery	
Control	85.39 ± 1.08^{a}	198.33 ± 3.62^{A}	(+132%)
27.2 mGy/h	74.86 ± 1.01^{b}	181.97 ± 3.71^{B}	(+143%)
48.8 mGy/h	68.02 ± 1.20^{b}	173.52 ± 4.01^{B}	(+155%)
103.5 mGy/h	86.72 ± 1.27^{a}	198.68 ± 5.03^{A}	(+129%)

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747 ***Table 2*: Phytohormone levels in *A. thaliana* rosettes in response to different levels of irradiation.**

748 Jasmonate and Auxin metabolites after 2 weeks irradiation and 4 days recovery of 1-week old plants following
749 exposure to different gamma dose rates. Data represents the average ± SE of 5 biological replicates. Green and red
750 colours indicate significant increase and decrease, respectively, in compound levels. cis-OPDA: cis-12-oxo-
751 phytodienoic acid; dn-OPDA: dinor-12-oxo-phytodienoic acid; JA: jasmonic acid; 9,10-DHJA: 9,10-dihydrojasmonic
752 acid; IAN: indole-3-acetonitrile; oxIAA: 2-oxoindole-3-acetic acid; IAA: indole-3-acetic acid.

			pmol/g FW			
	Hormone	Compound	Control	27.2 mGy/h	48.8 mGy/h	103.5mGy/h
2 weeks irradiated	Jasmonates	*cis-OPDA*	676 ± 68	1109 ± 156	736 ± 58	2142 ± 265
		dnOPDA	1116 ± 282	1630 ± 387	781 ± 154	2593 ± 807
		JA	19 ± 4	17 ± 6	23 ± 6	6 ± 2
		9,10-DHJA	1.2 ± 0.5	1.3 ± 0.4	2.5 ± 0.3	2.2 ± 0.4
	Auxins	IAN	4706 ± 347	6264 ± 569	7509 ± 728	7207 ± 268
		oxIAA	276 ± 5	314 ± 22	362 ± 27	324 ± 19
		IAA	164 ± 15	181 ± 4	136 ± 12	164 ± 6
4 days recovery	Jasmonates	*cis-OPDA*	723 ± 58	394 ± 11	543 ± 66	543 ± 37
		dnOPDA	734 ± 77	416 ± 64	430 ± 115	381 ± 40
		JA	16 ± 3	14 ± 3	7 ± 2	10 ± 1
		9,10-DHJA	2.8 ± 0.3	3.5 ± 0.6	3.0 ± 0.5	6.4 ± 0.3
	Auxins	IAN	5685 ± 722	11198 ± 2440	5412 ± 469	8700 ± 594
		oxIAA	231 ± 13	277 ± 19	273 ± 18	254 ± 29
		IAA	106 ± 4	106 ± 3	119 ± 6	95 ± 4

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Figures

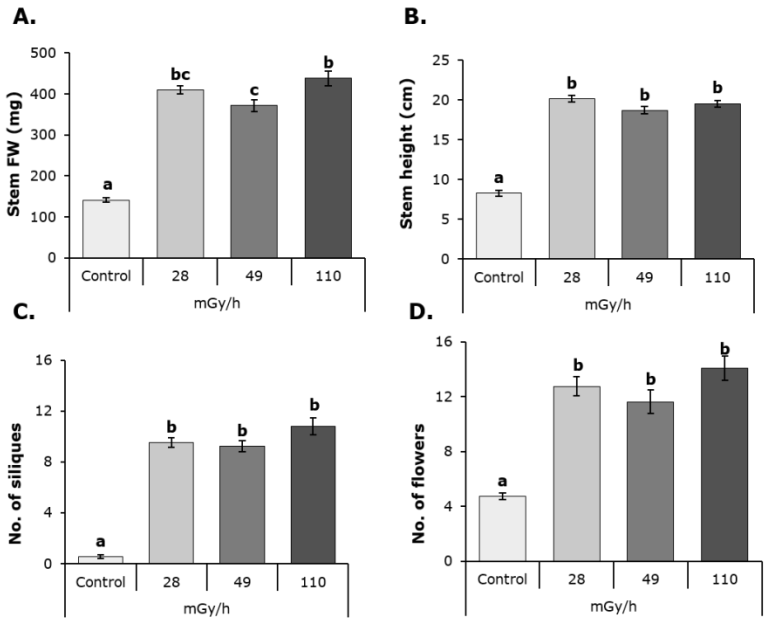
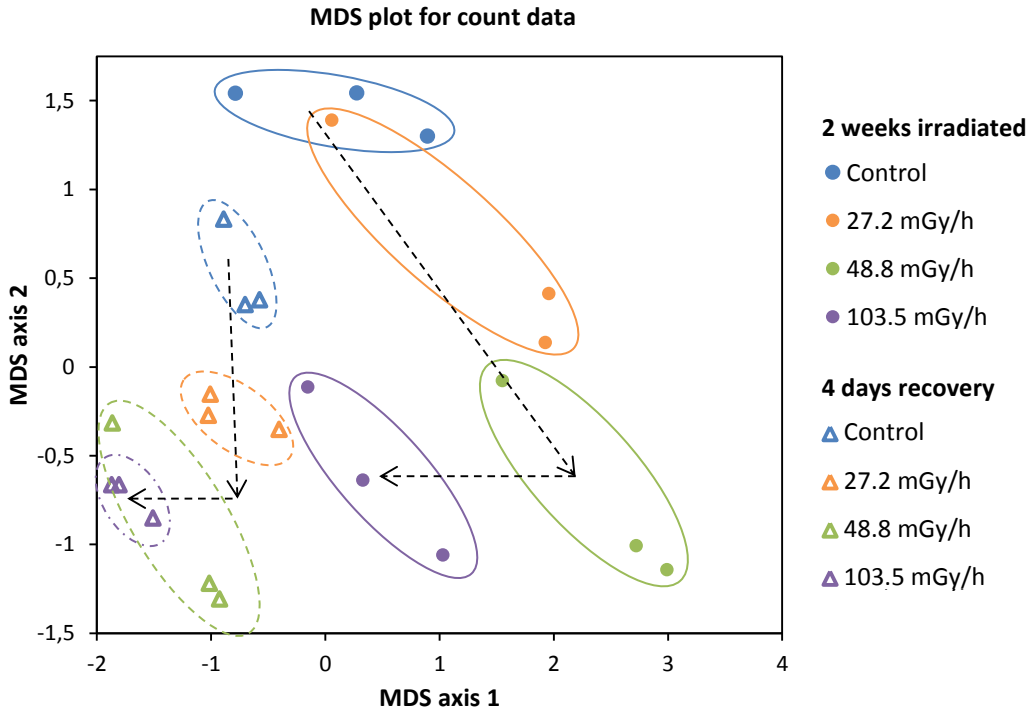


Figure 1: Effect of different gamma dose rates on flowering-related endpoints. (**A**) inflorescence stem fresh weight; (**B**) inflorescence stem height; (**C**) number of siliques and (**D**) number of flowers after 2 weeks irradiation and 2 weeks recovery of 1-week old *A. thaliana* plants. Small letters indicate significant differences ($p < 0.05$; one-way ANOVA). Error bars represent the average ± SE of 40 biological replicates.



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763 **Figure 2: Multi-dimensional scaling (MDS) plot of the RNA-seq gene expression profile.** 1-week old *A.*
764 *thaliana* plants were exposed to different doses of gamma radiation for 2 weeks and after allowing them to recover
765 for 4 days. Samples of irradiated plant are represented by circles; samples of recovering plants are represented by
766 circles; different colours correspond to the different treatments. Four distinct clusters were observed for both
767 irradiated and recovering plants inclusive of their respective controls. The distances correspond to differences in the
768 biological variation between samples.

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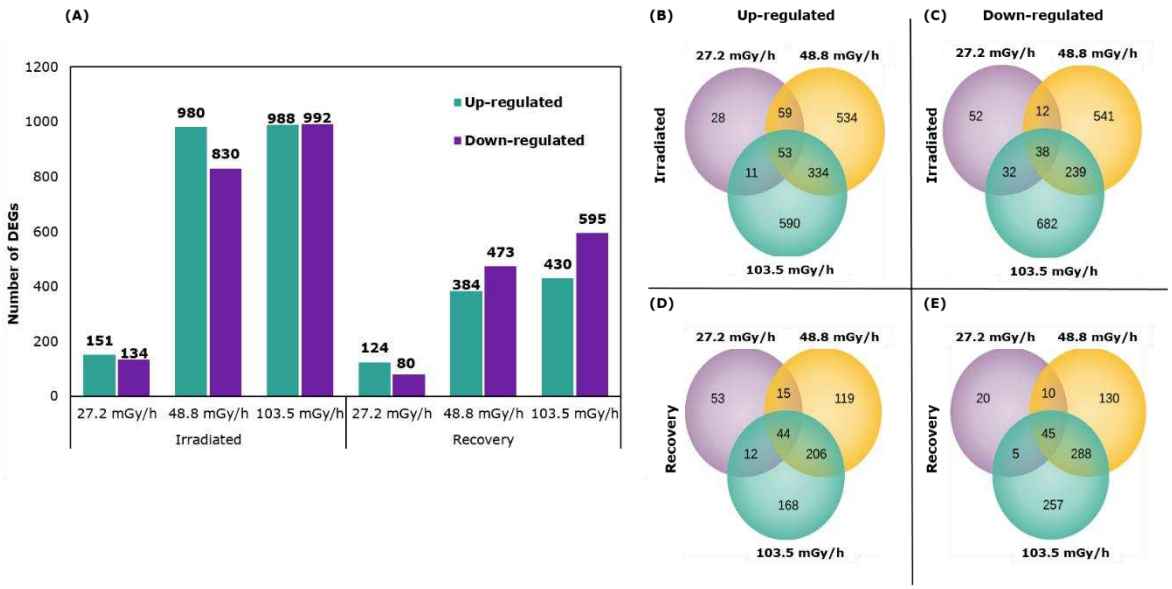

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772 Figure 3**: Comparison of differentially expressed genes upon different doses of irradiation and**
773 **subsequent recovery.** Expression profiles of DEGs(| log2(fold-change)| ≥ 2 and FDR < 0.05) after exposing 1-
774 week old *A. thaliana* plants to different doses of gamma radiation for 2 weeks and after allowing them to recover for
775 4 days: (**A**) Number of up- and down-regulated DEGs in irradiated and recovering plants; (**B, C, D & E**) Venn
776 diagrams showing the overlap of DEGs that were up-and down-regulated for each dose rate relative to their
777 respective controls in irradiated and recovering plants.

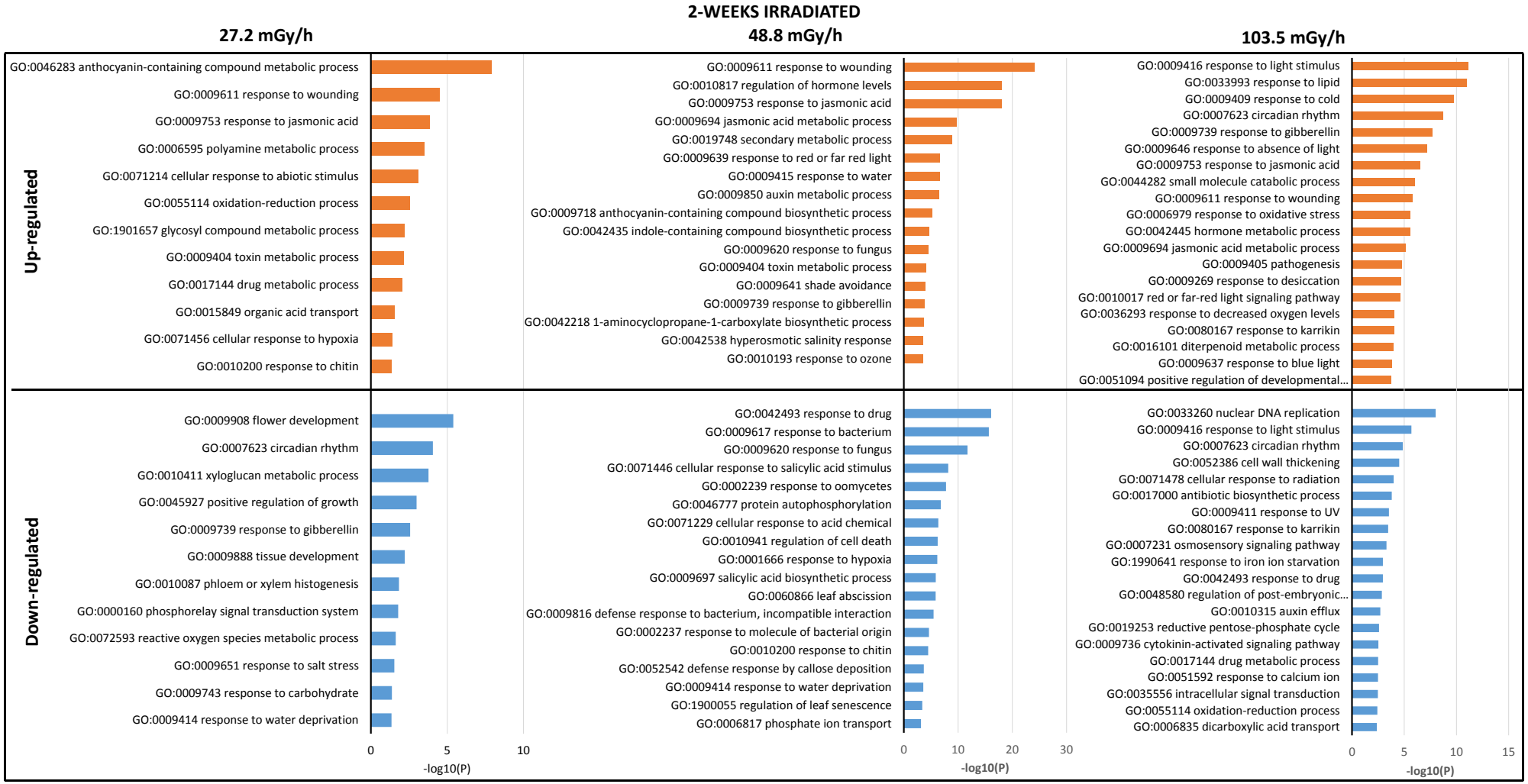


Figure 4: Gene ontology of significantly enriched GO terms.

(hypergeometric test and p-value < 0.05) for biological processes among up-regulated (orange bars) and down-regulated (blue bars) DEGs after exposure of 1-week old A. thaliana *plants to different gamma radiation dose rates for 2 weeks.*

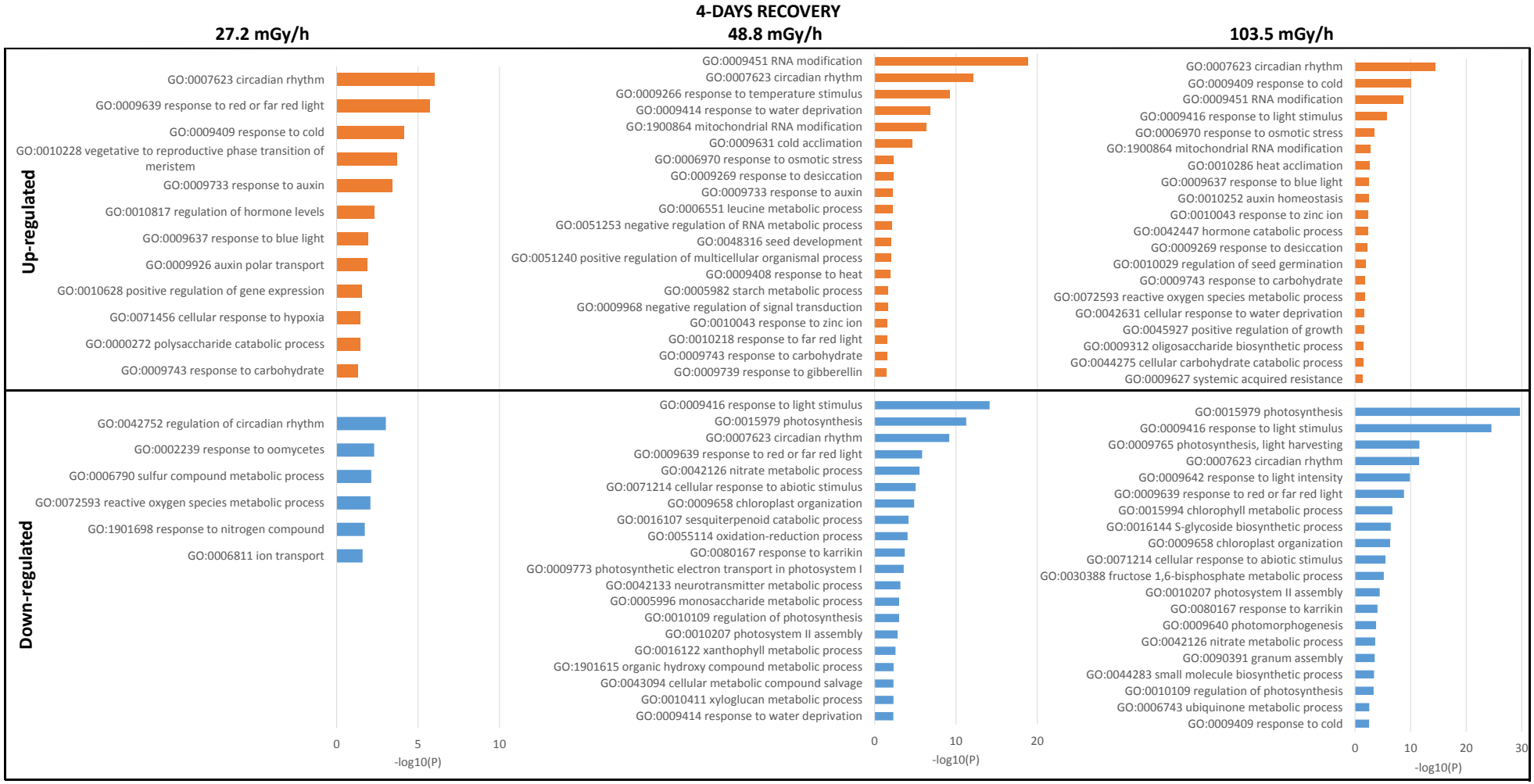
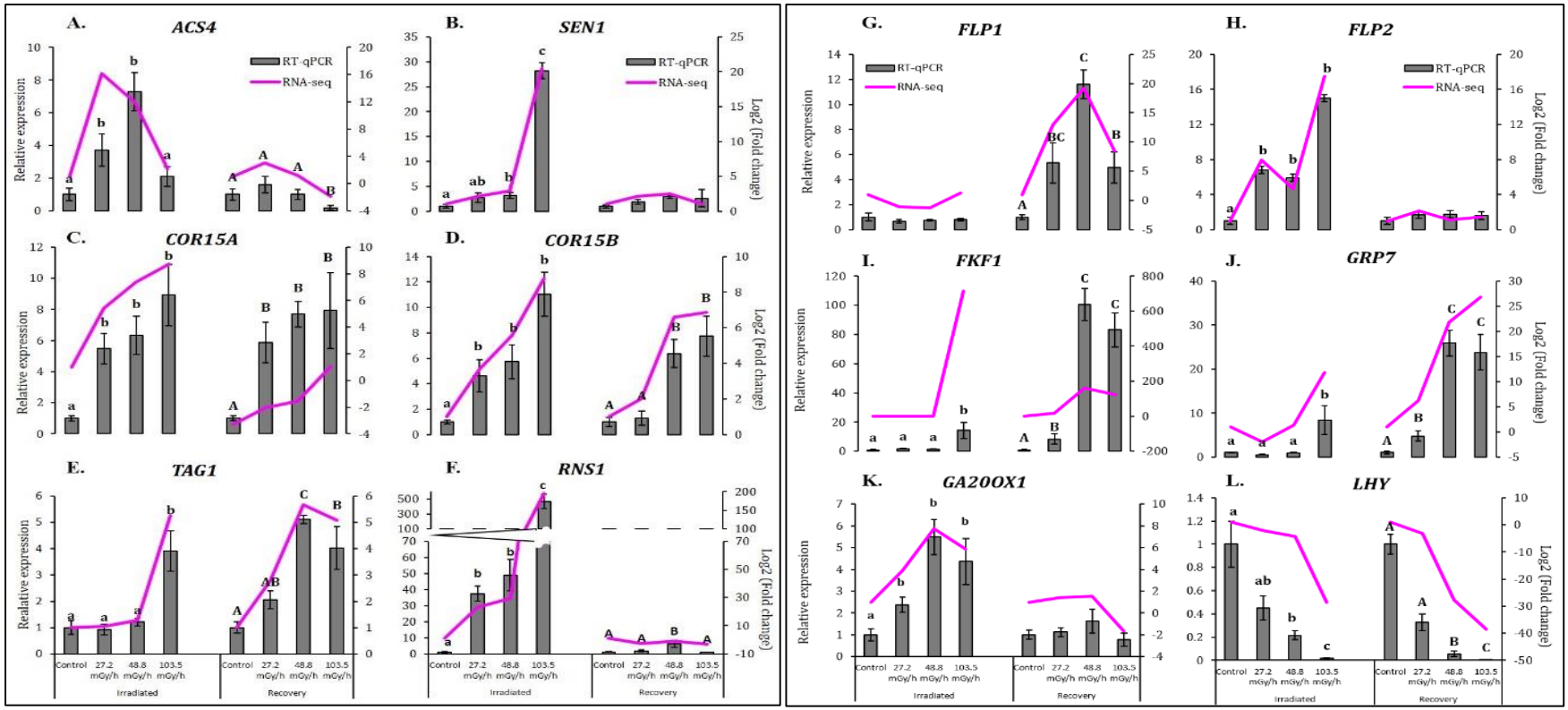


Figure 5: Gene ontology of significantly enriched GO terms.

(hypergeometric test and p-value < 0.05) for biological processes among up-regulated (orange bars) and down-regulated (blue bars) DEGs after allowing a 4-day recovery period of *A. thaliana* plants that had been exposed for 2 weeks to different doses of gamma radiation.



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788 **Figure 6: RT-qPCR validation of RNA-seq data.** Relative expression profiles of senescence and flowering-related DEGs in 1-week old *A. thaliana* plants after 2 weeks
789 gamma irradiation and 4 days recovery. Relative expression levels based on RNA-seq data and RT-qPCR are indicated by solid pink lines and shaded columns, respectively.
790 Small letters indicate significant differences ($p < 0.05$; one-way ANOVA) within irradiated plants between exposed and control plants; capital letters indicate significant
791 differences ($p < 0.05$; one-way ANOVA) within recovery plants between exposed and control plants, no letters indicates no significant difference was found in any of the
792 comparisons. Error bars represent the average ± SE of 4 biological replicates. **Abbreviations:** *ACS4* = 1-aminocyclopropane-1-carboxylate synthase 4, *SEN1* = Senescence
793 1; *COR15A/B* = Cold-regulated 15A/B; *TAG1* = Triacylglycerol 1; *RNS1*= Ribonuclease 1; *FLP1/2* = Flowering-promoting factor 1-like protein 1/2; *FKF1* = Flavin-binding, kelch
794 repeat, F box 1; *GRP7* = Gibberellin 20 oxidase 1; *LHY* = Late elongated hypocotyl.

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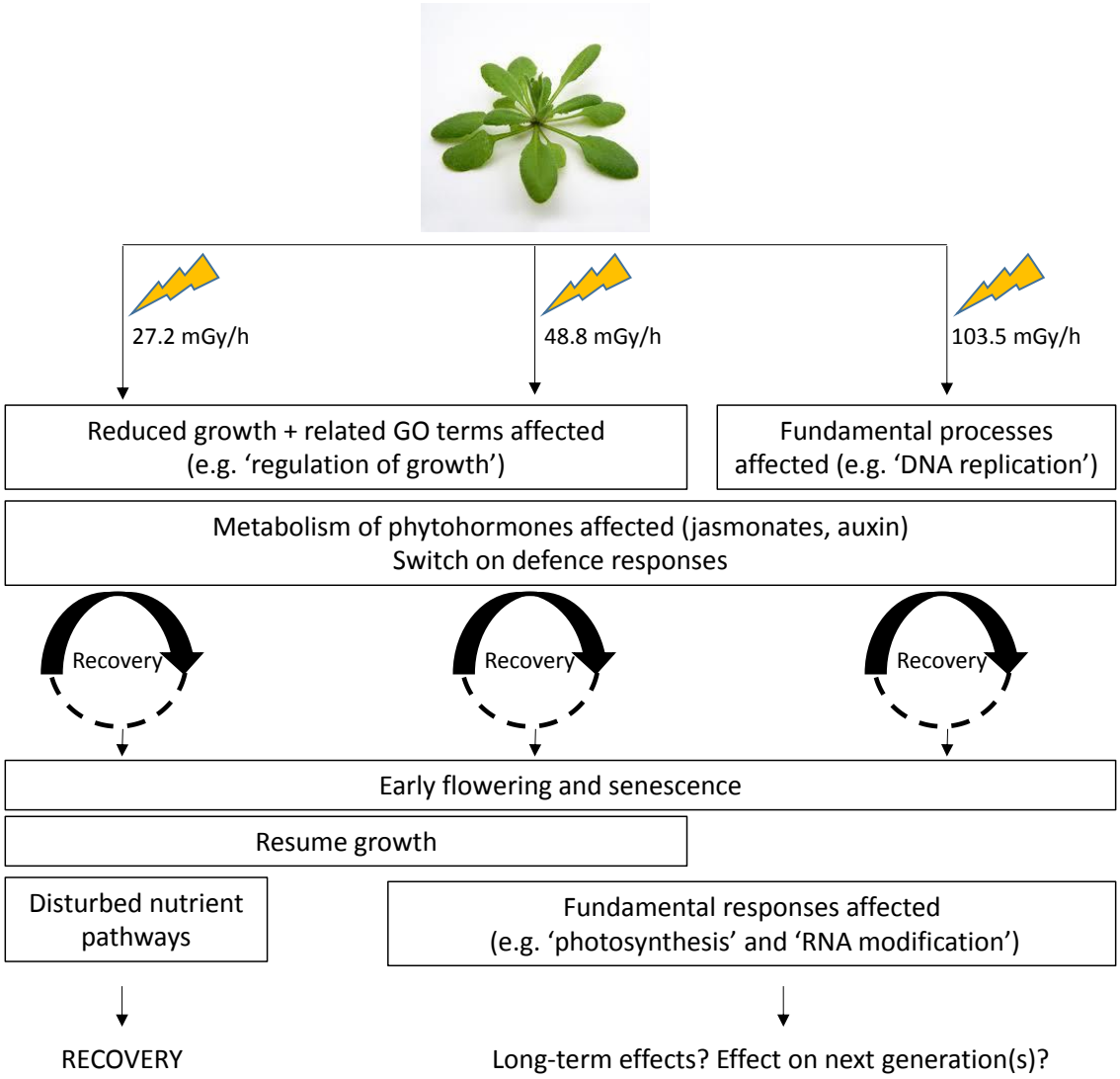

Figure 7: General overview of the effects *in A. thaliana* plants following exposure to different dose rates of gamma radiation and a subsequent recovery period.