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Plant acclimation to ionising radiation requires activation of a detoxification pathway against carbonyl-containing lipid oxidation products

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Abstract

Ionising γ radiation produces reactive oxygen species by water radiolysis, providing an interesting model approach for studying oxidative stress in plants. Three-week old plants of *Arabidopsis thaliana* were exposed to a low dose rate (25 mGy h⁻¹) of γ radiation for up to 21 days. This treatment had no effect on plant growth and morphology, but it induced chronic oxidation of lipids which was associated with an accumulation of reactive carbonyl species (RCS). However, contrary to lipid peroxidation, lipid RCS accumulation was transient only, being maximal after 1 day of irradiation and decreasing back to the initial level during the subsequent days of continuous irradiation. This indicates the induction of a carbonyl-metabolising process during chronic ionising radiation. Accordingly, the γ -radiation treatment induced the expression of xenobiotic detoxification-related genes (*AER*, *SDR1*, *SDR3*, *ALDH4*, and *ANAC102*). The transcriptomic response of some of those genes (*AER*, *SDR1*, and *ANAC102*) was deregulated in the *tga256* mutant affected in three TGAI transcription factors, leading to enhanced and/or prolonged accumulation of RCS and to a marked inhibition of plant growth during irradiation compared to the wild type. These results show that *Arabidopsis* is able to acclimate to chronic oxidative stress and that this phenomenon requires activation of a carbonyl detoxification mechanism controlled by TGAI. This acclimation did not occur when plants were exposed to an acute γ radiation stress (100 Gy) which led to persistent accumulation of RCS and marked inhibition of plant growth. This study shows the role of secondary products of lipid peroxidation in the detrimental effects of reactive oxygen species.

KEYWORDS

detoxification, lipid peroxidation, reactive carbonyl species, spontaneous chemiluminescence, TGAI transcription factor, ultraweak photon emission, γ radiation

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1 | INTRODUCTION

Living organisms like plants are exposed to a variety of environmental constraints including ionising radiation from natural sources such as cosmic radiation and naturally radioactive materials contained in bedrocks, sediments and soils. Radiation in the environment also originates from controlled or accidental releases of radionuclides from anthropogenic activities. There are multiple forms of ionising radiation including radioactive particles (α , β and neutrons) and electromagnetic waves (γ , X-ray radiation) (IAEA, 2023). Of the different types of electromagnetic radiation, γ radiation has the highest energy and penetration power.

Exposure of plants to γ rays can result in a number of biological responses such as oxidative stress, DNA damage, growth reduction, reproduction impairment and morphological alterations (Caplin & Willey, 2018; De Micco et al., 2011; Dowlath et al., 2021). These effects can arise either through direct ionisation of biomolecules or indirectly via radiolysis of water molecules producing free radicals followed by recombination to reactive oxygen species (ROS). The indirect effect is considered to be predominant, particularly for DNA damage (Esnault et al., 2010; Srinivas et al., 2019). However, the occurrence and extend of these responses are very dependent on the dose and dose rate of ionising radiation. Based on their effect on living organisms, low doses of radiation are usually defined as about 10 to about 100 mGy and moderate doses as about 100 mGy to 1 Gy (UNSCEAR, 2020). However, many experimental studies, especially on animals, have considered doses equal to or below 200–500 mGy to be low (e.g., Barjaktarovic et al., 2013; Filchenkov et al., 2014; Shin et al., 2010). Plant radioresistance is also dependent on development stage, with the sensitivity to chronic exposure to γ radiation decreasing with increasing age (Biermans et al., 2015; Vanhoudt et al., 2010; Vandenhove et al., 2010). This differential radioresistance between developmental stages was interpreted as resulting from differences in regulatory processes and defense mechanisms such as ROS scavenging and lipid signalling (Biermans et al., 2015).

The impact of acute γ radiation stress, that is, high dose rates applied in a short time span, has been extensively studied and characterised in plants. In contrast, the biological effects of low doses of ionising radiation are not yet fully understood, in part because the induced responses are weak and hence difficult to quantify. Previous studies on various organisms have discussed the effects of low-dose radiation, with most of them suggesting beneficial or no effects, while others reported negative effects (e.g., Jaipo et al., 2019; Podlutskii et al., 2022; Singh & Datta, 2010; Tang & Loganovsky, 2018). Beneficial effects observed in plants after low doses of γ radiation are induction of adaptive responses, increased growth rate, enhancement of antioxidant defense, and increased resistance to high dose of ionising radiation or to other abiotic stresses (Volkova et al., 2022).

Arabidopsis thaliana is a rather radioresistant species, with the lethal dose exceeding 500 Gy (Chang et al., 2020). In this work, we

have exposed *Arabidopsis* plants to γ rays at a rather low dose rate of 25 mGy h⁻¹ for a few hours to several days (corresponding to total radiation doses from 50 mGy to 2 Gy) which did not cause any phenotypic change. One of the objectives of this study is to provide information on the molecular mechanisms underlying the resistance to radiation stress. Moreover, in such conditions of exposure to γ rays, it is possible to induce a continuous production of ROS and a chronic oxidative stress in plants, which can be difficult to achieve experimentally with other environmental factors. Thus, the results of this study may be of interest in a broader context of oxidative stress in plants. From an evolutionary point of view, ionising radiation is considered to be a primeval stressor as all life on Earth has evolved in the presence of various naturally occurring radiations whose intensity has changed over geological time (Caplin & Willey, 2018; Duarte et al., 2023; Zagórski & Kornacka, 2012). Then, adaptive mechanisms to ionising radiation are likely to function also in response to other pro-oxidative environmental conditions that occurs in natural environments such as climatic stressors. The similarity found between transcriptional profiles of plants exposed to chronic ionising radiation and those exposed to climatic stressors (Duarte et al., 2023; Podlutskii et al., 2022) indicates that the strategies developed to cope with ionising radiation could potentially be utilised to enhance the resilience of crop species to various environmental constraints. In line with this idea, a few studies have previously shown enhanced resistance of crop plants to salt stress or drought stress after a pretreatment with γ rays (Moussa, 2011; Qi et al., 2014). γ -Radiation could also be used as a proxy for studying adaptation to other ROS-producing and/or genotoxic agents, such as heavy metals, UV-C in space environment or various chemicals. Moreover, identifying the determinants of radioresistance would be useful for future space exploration and colonisation efforts. In the absence of protection by the Earth's atmosphere and magnetic field, ionising radiation in space constitutes a potential threat to living organisms (Restier-Verlet et al., 2021). It appears essential to understand the long-term effects of ionising radiation on plant growth, development, and metabolism, which could directly impact the viability of a plant cultivation in space. Finally, knowledge of the underlying mechanisms conferring tolerance to ionising radiation is needed to improve our ability to predict environmental consequence of accidental release of radionuclides. The present study reports the identification of an acclimatory mechanism in *Arabidopsis* involving accelerated turnover of toxic carbonyls, which is essential for maintaining plant growth rate under chronic oxidative stress induced by γ radiation.

2 | MATERIALS AND METHODS

2.1 | Plant material and growth conditions

The experiments were performed on 3-week old *Arabidopsis* plants (*A. thaliana* L. Heynh). The following genotypes were used: the wild type (WT, ecotype Col-0), the *tga256* triple knockout mutant (D'Alessandro et al., 2018) and the *pAER:LUC* line (Rac et al., 2021).

Plants were grown on potting soil under controlled environmental conditions in phytotrons of the Phytotec platform at CEA/Cadarache: photon flux density was $130 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, photoperiod was 8 h and day/night temperatures were 20/18°C.

2.2 | γ -Irradiation

Arabidopsis plants were chronically exposed for up to 21 days to γ radiation provided by a ^{137}Cs source in the MICADOLab experimental irradiation facility (IRSN, Cadarache). The dose rate was 25 mGy h^{-1} . To confirm the dose rates of exposure, we used operational dosimetry with radio-photoluminescent dosimeters (RPL, GD-301 type; Chiyoda Technol Corporation Japan), and the effective dose rates were estimated by MCNPX v2.70. Plants were exposed to white light of $130 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by HQI or LED lamps with a photoperiod of 8 h. Control plants were grown under the same conditions in the MICADOLab installation in a zone that was not exposed to γ radiation.

High-dose γ irradiations were performed in a GSR D1 irradiator from GSM Company in the irradiation platform of iRCM at CEA/Fontenay-aux-Roses (France). It is a self-shielded irradiator with four sources of ^{137}Cs with a total activity of around 180.28 TBq in March 2014. Arabidopsis plants were exposed to a single dose of 100 Gy with an output of 2.59 Gy min^{-1} . Before irradiation, dosimetry was performed. A cylindrical ionising chamber 31010 by PTW was used following the recommendation of the American Association of Physicists in Medicine (protocole TG-61). This ionising chamber has a cavity of 0.125 cm^3 calibrated in ^{137}Cs air kerma at the PTB reference facility number 210382701. The polarity and the ion recombination were measured for this ^{137}Cs source. Each measurement was corrected by the KTP factor to take the variations of temperature and atmospheric pressure into account.

2.3 | Biochemical analyses of lipid peroxidation

Lipid peroxidation was estimated by quantifying the hydroxy linolenic acid levels (HOTEs, hydroxyoctadecatrienoic acids) as detailed in Ksas and Havaux (2022). Lipids were extracted from leaf samples (ca. 200 mg fresh weight) in methanol/chloroform, and HOTEs were quantified by HPLC-UV. ROS- and LOX-mediated HOTE levels were quantified separately as described in Montillet et al. (2004) and Ksas and Havaux (2022).

2.4 | Luminescence imaging of lipid peroxidation

Spontaneous photon emission by Arabidopsis plants linked to lipid peroxidation was measured with a high-sensitivity CCD camera cooled with liquid nitrogen (Roper) operating at -110°C as described in Birtic et al. (2011). Acquisition time was 20 min and pixel binning

on the CCD was 2×2 . The images thus obtained were analysed with the ImageJ software.

2.5 | Luciferase/luciferine luminescence

The Arabidopsis *pAER:LUC* line expressing the firefly luciferase gene under the control of the promoter of the AER gene (*ALKENAL REDUCTASE*) was used to monitor the expression levels of AER (Rac et al., 2021). Plants were sprayed with a 5 mM aqueous solution of luciferin containing 0.01% Triton X-100. Luciferin/luciferase luminescence was measured with the imaging system described above for lipid peroxide imaging. Acquisition time was 1 min, and the on-CCD pixel binning was 2×2 .

2.6 | Biochemical analyses of carbonyls

Carbonyls were measured in leaf samples (200 mg fresh weight) by HPLC-UV after derivatization with DNPH (dinitrophenylhydrazine). We used the protocol detailed in Mano and Biswas (2018) with a few minor modifications. Addition of DNPH was done after centrifugation (7000g for 5 min), rather than decantation, and 50 μL (rather than 20 μL) were injected in the HPLC system. DNPH was obtained from Molekula, and carbonyl standards were obtained from Sigma-Aldrich and AccuStandard. Most of the standards were purchased in the derivatized form. The other standards were derivatized with DNPH as detailed in Mano and Biswas (2018).

2.7 | H_2O_2 measurements

H_2O_2 levels were visualised in seedlings by DAB staining (3,3'-diaminobenzidine) using the method described by Daudi and O'Brien (2012). In brief, Arabidopsis seedlings were vacuum-infiltrated for 15 min with 10 mM Na_2HPO_4 DAB staining solution (1 mg DAB mL^{-1}). The samples were then incubated in the DAB solution under stirring in the dark for 5 h. The samples were bleached for 15 min in ethanol/acetic acid/glycerol (3/1/1, v/v/v) at 90–95°C. The intensity of the brown colour of oxidised DAB was quantified using ImageJ software.

2.8 | qRT-PCR analyses of gene expressions

Total RNA was extracted from 50 mg of frozen shoot powder using the Direct-zol RNA Miniprep Plus Kit (Zymoresearch) and treated with DNase I (Zymoresearch) according to the manufacturer's instructions. Reverse transcription (RT) was performed on 400 ng total RNA using the qScript cDNA SuperMix (Quantabio). Real-time quantitative reverse transcription PCR was performed

using a LightCycler® 480 real-time thermocycler (Roche) and the LightCycler® 480 SYBR Green I Master (Roche) with 10 μ M primers and 0.1 μ L of RT reaction product in a total volume of 5 μ L per reaction. The specific primers used for PCR amplification are listed in Supporting Information S2: Table S1. *UPL7* (AT3G53090) was used as a reference gene for normalisation.

3 | RESULTS

3.1 | Plant growth and oxidation status

Arabidopsis plants aged 3 weeks were exposed for up to 21 days to γ radiation at a dose rate of 25 mGy h^{-1} . This treatment had no significant effect on plant growth. The plant rosettes did not exhibit any morphological changes (Figure 1a), and their biomass (shoot dry

weight and fresh weight) was not significantly affected after 21-day irradiation (Figure 1b).

Figure 2a shows the spontaneous luminescence emitted by *Arabidopsis* plants exposed for 7 days to γ rays. Irradiated plants appeared to emit more photons than nonirradiated control plants. Quantification of the signal indicated that spontaneous photon emission was increased by a factor of 2 to 3, and this phenomenon persisted during the γ -radiation treatment (Figure 2b). Spontaneous luminescence emission by plants is attributed to oxidation phenomena (Cifra & Pospíšil, 2014), especially lipid peroxidation (Birtic et al., 2011; Havaux & Ksas, 2022), that lead to the generation of light-emitting by-products. In particular, unstable lipid peroxides spontaneously break down, producing luminescent species such as triplet-excited carbonyls and singlet oxygen (Cifra & Pospíšil, 2014; Prasad et al., 2018). Thus, the spontaneous luminescence images

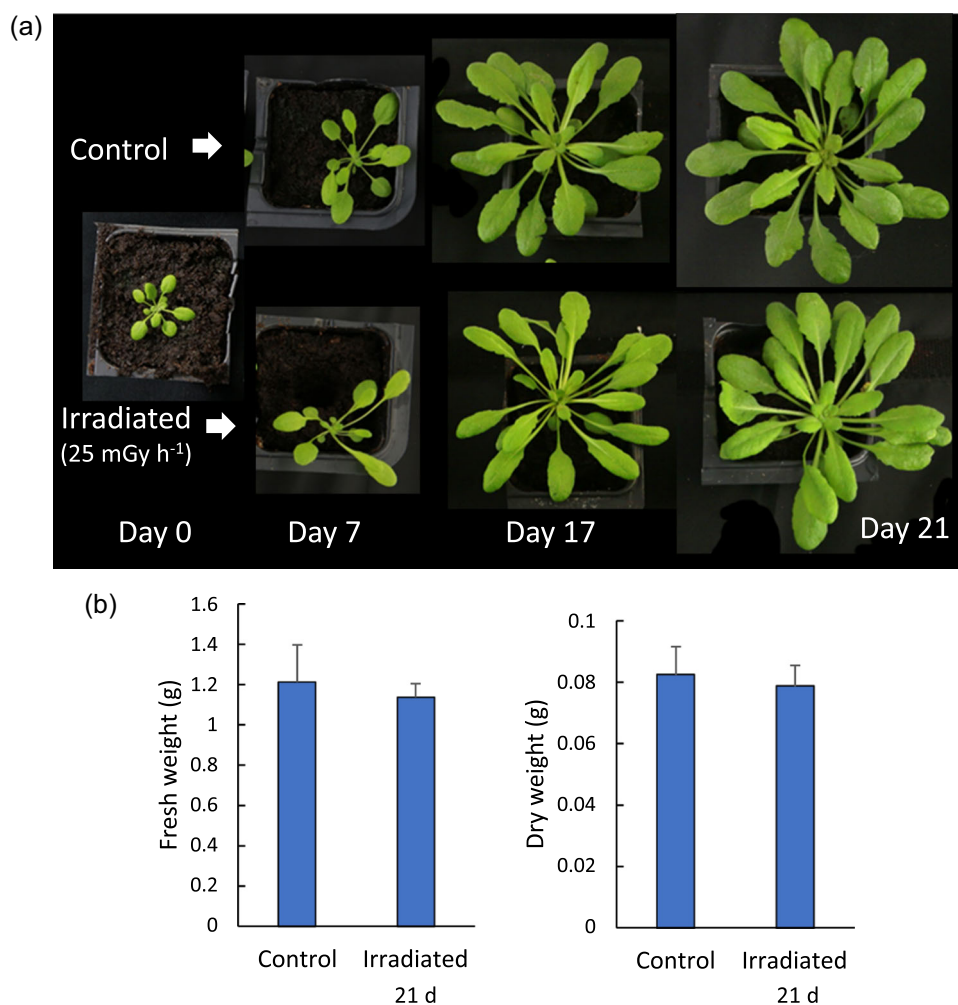


FIGURE 1 Effect of γ radiation on the growth of *Arabidopsis* plants. (a) Pictures of wild-type (WT) *Arabidopsis* plants exposed for 7, 17 or 21 days to constant γ radiation at a dose rate of 25 mGy h^{-1} . Plants were transferred to γ radiation at the age of 3 weeks. Control plants were grown under the same environmental conditions as the irradiated plants except that they were not exposed to the γ radiation treatment. (b) Biomass (fresh weight and dry weight) of the plants after 21 days of γ irradiation. Data are mean values of five biological replicates \pm standard deviation.

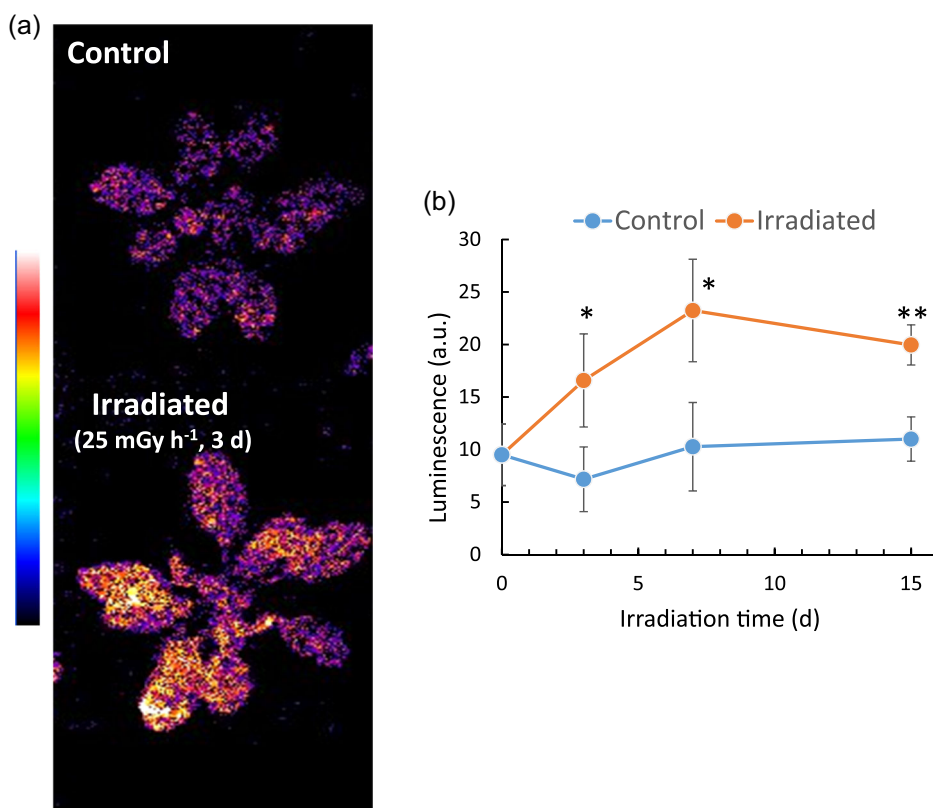


FIGURE 2 Spontaneous luminescence imaging of control and γ -irradiated Arabidopsis plants. (a) Spontaneous luminescence images of control and irradiated plants (3 days under a dose rate of 25 mGy h^{-1}). The colour palette indicates the signal intensity from low (dark blue) to high values (white). (b) Quantification of the signal intensity from plant exposed for 0, 3, 7 or 15 days to γ radiation at a dose rate of 25 mGy h^{-1} . Data are mean values of three biological replicates \pm standard deviation. * and **, different from control at $p < 0.05$ and 0.01 , respectively (Student's *t*-test). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pcel.14994)]

of Figure 2a indicate a change in the oxidation status of the irradiated plants and suggest that γ radiation brought about chronic oxidation of lipids. To confirm this phenomenon, we performed a biochemical analysis of lipid peroxidation by measuring hydroxy linolenic acid levels (HOTEs) in leaves by HPLC combined with UV detection. Depending on which double bond is oxidised in linolenic acid, six different HOTE stereoisomers can be formed: 9-, 10-, 12-, 13-, 15- and 16-HOTE. 10- and 15-HOTE are specific to oxidation by singlet oxygen and are not quantified here because they do not absorb UV light; mass spectrometry is required for their detection (Triantaphylides et al., 2008). The stereoisomers 12-HOTE and 16-HOTE are specific markers of ROS-induced lipid peroxidation (singlet oxygen and free radicals). 9- and 13-HOTEs can be formed enzymatically under the catalytic action of lipoxygenases. The protocol that we used in this study allows to separate the 9-HOTE and 13-HOTE produced by lipoxygenases (LOX) from the 9- and 13-HOTE produced nonenzymatically (Montillet et al., 2004; Ksas & Havaux, 2022). ROS-induced lipid peroxidation is obtained by adding the latter HOTEs to 12-HOTE and 16-HOTE. Figure 3a confirms that ROS-induced lipid peroxidation

was substantially enhanced in γ -irradiated plants relative to control plants. In contrast, the intensity of LOX-mediated lipid peroxidation did not appear to be modified by γ radiation (Figure 3b).

3.2 | Reactive carbonyls and their detoxification

The decomposition of lipid peroxides can generate a variety of toxic compounds, such as reactive carbonyl species (RCS), that can propagate oxidative stress in the cells (Biswas & Mano, 2021; Sousa et al., 2017). We analysed some of those RCS by HPLC-UV in control Arabidopsis plants and in plants exposed to chronic γ radiation (Figure 3c). The chromatogram of Figure 3c shows that several carbonyl compounds substantially increased in leaves exposed for 3 days to γ rays. In Figure 4, we were able to quantify the concentration of eight carbonyls, including RCSs, using authentic standards. In WT plants, we observed a burst of carbonyls after 1-day exposure to γ radiation (Figure 4, blue symbols/lines). The levels of hydroxy hexenal (HHE), *trans*-pentenal, butanal, nonanal and hydroxy nonenal (HNE)

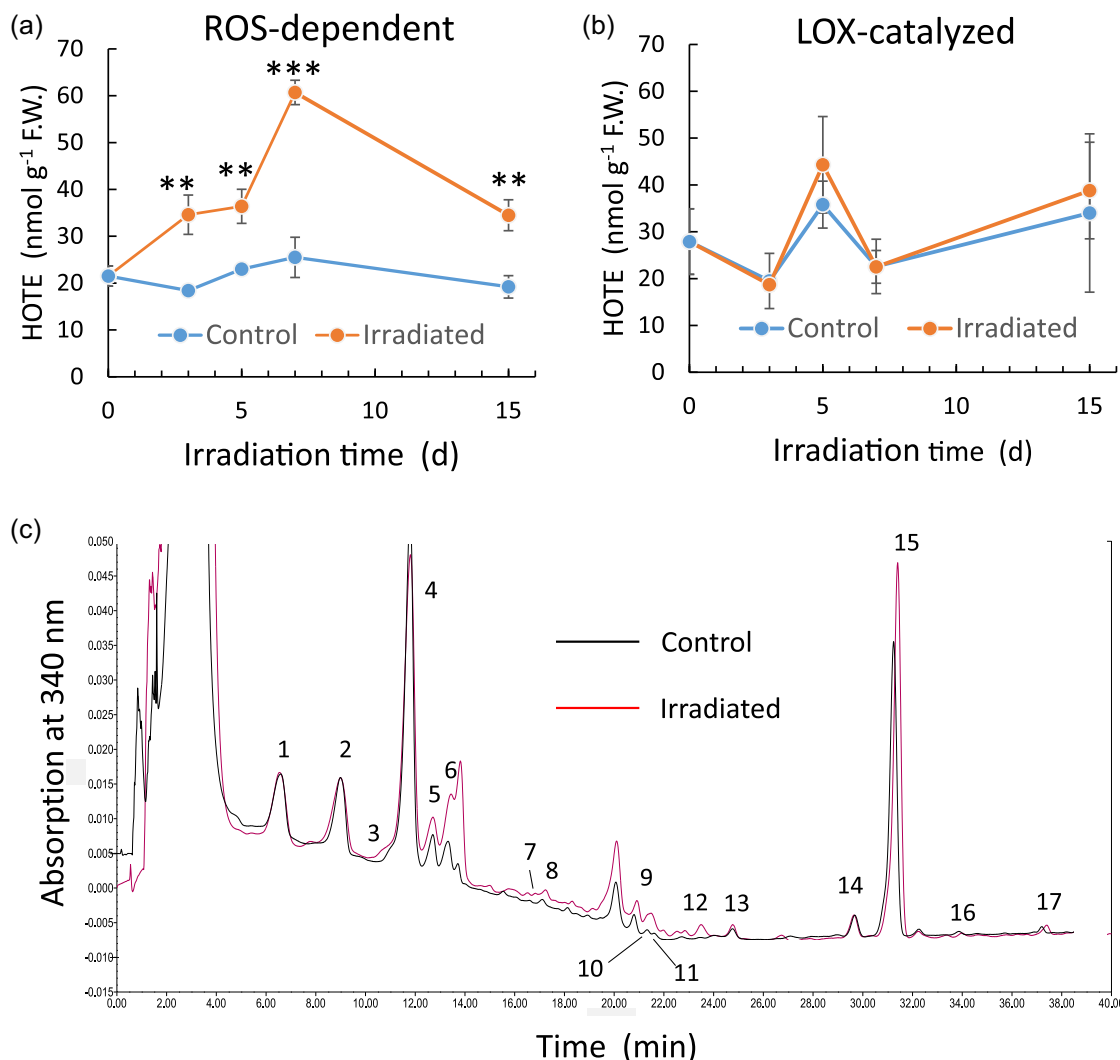


FIGURE 3 Lipid peroxidation (hydroxy linolenic acid levels [HOTE] levels) and reactive carbonyl species (RCS) in leaves of *Arabidopsis* plants exposed for 3, 5, 7 and 15 days to γ rays at a dose rate of 25 mGy h^{-1} . (a) ROS-mediated oxidation. (b) Lipoxygenases (LOX)-dependent oxidation. Data are mean values of three biological replicates \pm standard deviation. ** and ***, different from control at $p < 0.01$ and 0.001 , respectively (Student's *t*-test). (c) Chromatogram of carbonyls extracted from control leaves and leaves irradiated for 3 days at 25 mGy h^{-1} . Carbonyl identification: 1, Formaldehyde; 2, Acetaldehyde; 3, HHE; 4, Acetone; 5, Acrolein; 6, Propionaldehyde; 7, *trans*-2-Butenal; 8, Butanal; 9, *trans*-2-Pentenal; 10, Pentanal; 11, HNE; 12, *trans*-2-Hexenal; 13, Hexanal; 14, Heptanal; 15, internal standard (2-Ethylhexanal); 16, Octanal; 17, Nonanal. [Color figure can be viewed at wileyonlinelibrary.com]

significantly increased in irradiated plants and then decreased back to the control levels measured before irradiation after 3 or 7 days of irradiation. This tendency was also observed for *trans*-hexenal, acrolein and pentanal, but the amplitude of the changes was much lower and below statistical significance. Since lipid peroxidation occurred throughout the γ irradiation treatment (Figures 2b and 3a), accumulation of carbonyls followed by a decline suggests the occurrence of a carbonyl-scavenging mechanism which rapidly eliminates these potentially toxic compounds.

RCS detoxification in response to several abiotic stresses is known to involve various enzymes with dehydrogenase or oxidoreductase activities

which can transform RCS into less reactive compounds such as alcohols or carboxylic acids (Biswas & Mano, 2021). One such enzyme is alkenal reductase (AER) which specifically reduces the unsaturated double bond activated by an adjacent carbonyl group (Mano et al., 2005). The promoter activity of the AER gene (AT5G16970) was monitored in the *pAER:LUC* line by luciferase/luciferin luminescence. The luminescence images of Figure 5a show induction of AER expression after 5 h and 1 day of γ irradiation. The signal subsequently faded away in plants irradiated for 7 days. Figure 5b details the time course of the changes in luciferase luminescence during γ irradiation. AER expression was rapidly and significantly increased after 2-h irradiation only (γ radiation dose of $\sim 50 \text{ mGy}$), reached a maximum after 1 day ($\sim 600 \text{ mGy}$) and then

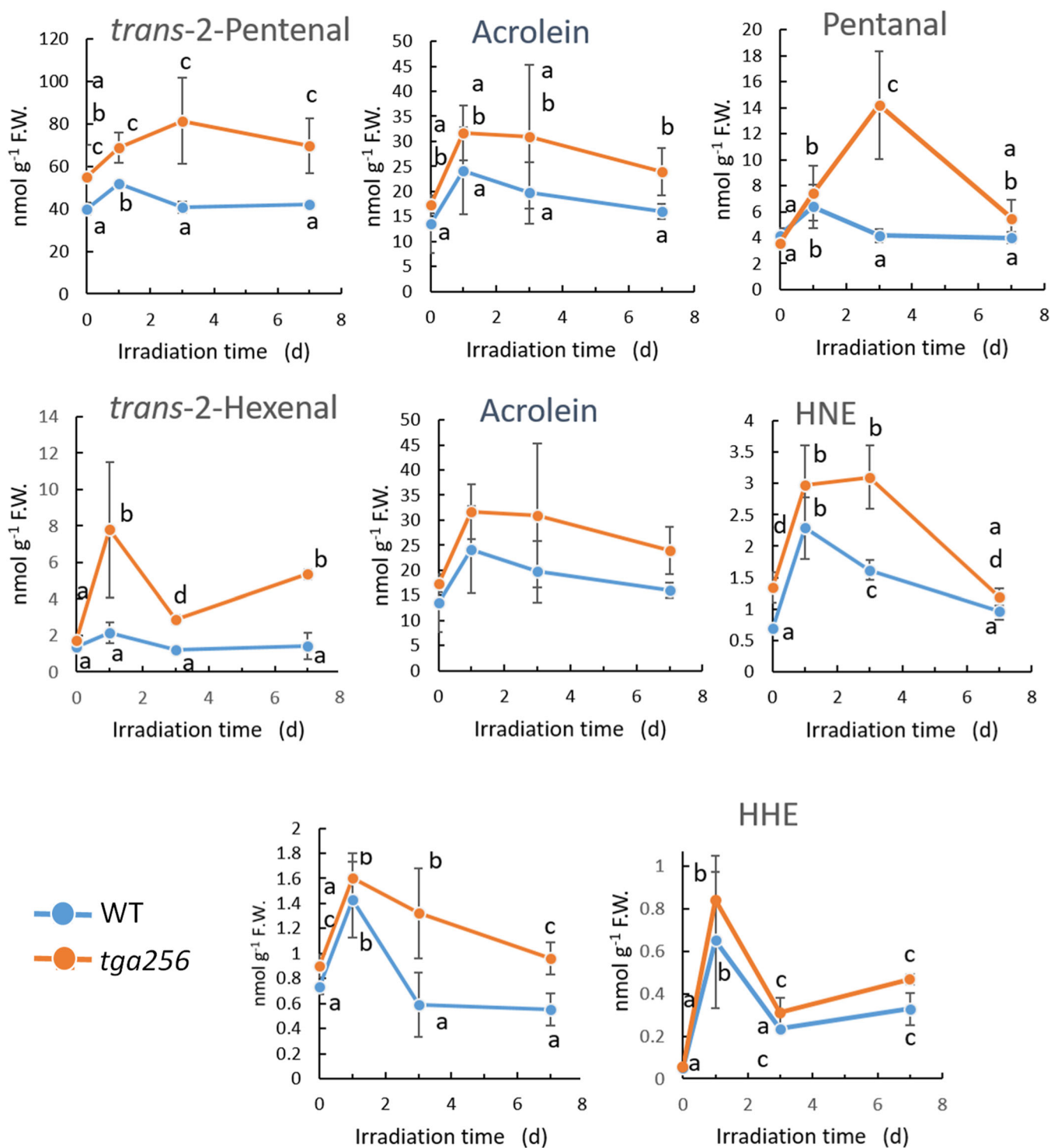


FIGURE 4 Reactive carbonyl species in leaves of Arabidopsis plants (wild-type [WT] and *tga256* triple mutant) exposed for different times (0, 1, 3 and 7 days) to γ radiation at a dose of 25 mGy h^{-1} . Data are mean values of three biological replicates \pm standard deviation. Statistical analysis was performed using analysis of variance/Fisher's LSD test. Different letters indicate statistical differences at $p < 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

progressively decreased reaching the initial level after 11 d of irradiation. Thus, AER induction is an early event in the response of plants to low γ -radiation doses. This response of AER to γ radiation was confirmed by qRT-PCR (Figure 6). A transient expression of other genes coding for detoxifying enzymes (*SHORT CHAIN DEHYDROGENASE/REDUCTASE 1*

and 3, *SDR1* (AT3G61220), *SDR3* (AT2G47130)) or the transcription factor (TF) *ANAC102* (AT5G63790) (Figure 6). AER has a rather broad spectrum of substrates, acting on a variety of lipid RCS, with a special affinity for HNE (Mano et al., 2002; Mano et al., 2005; Youn et al., 2006). *SDR1* is an aldehyde reductase that catalyses the reduction of the

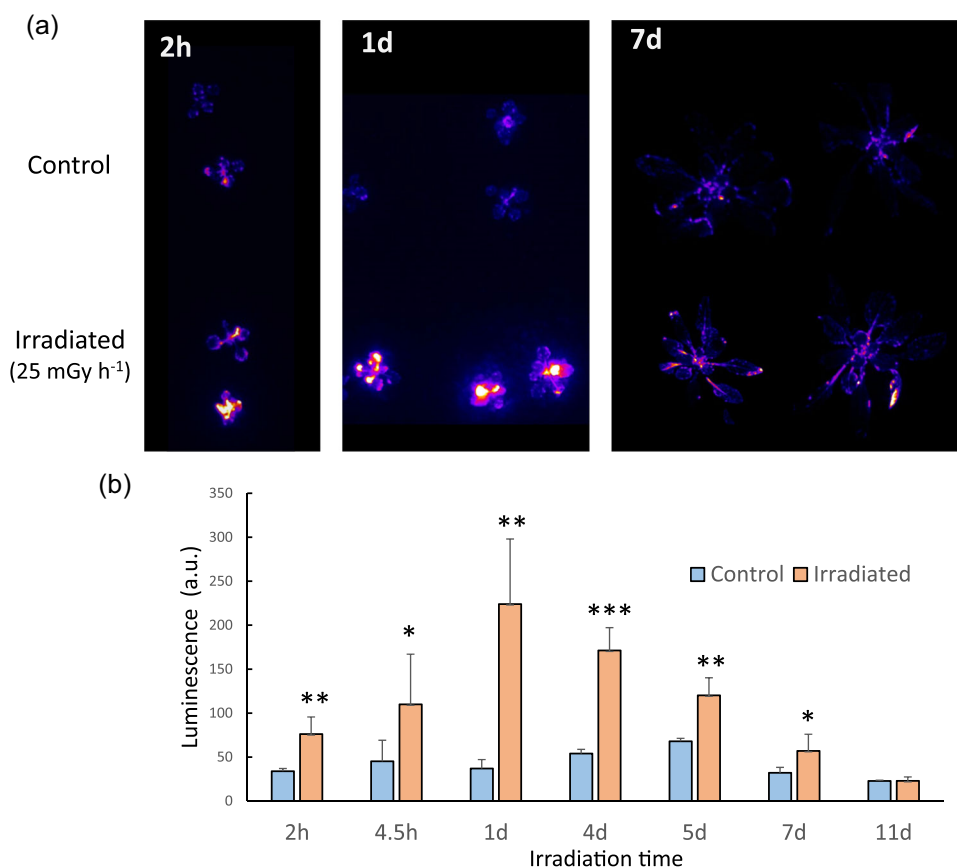


FIGURE 5 Luciferase/luciferin luminescence of *Arabidopsis pAER:LUC* plants exposed to γ radiation. (a) Luminescence images. (b) Quantification of the signal intensity. Data are mean values of four or five biological replicates \pm standard deviation. *, **, ***, different from control at $p < 0.05$, 0.01 and 0.001 , respectively (Student's t -test). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pce.14994)]

aldehyde carbonyl group on α,β -unsaturated aldehydes with more than 5 carbons in vitro (Yamauchi et al., 2021). SDR3 is a stress-inducible SDR whose substrate specificity is not well characterised; SDR3 has a β -hydroxysteroid dehydrogenase activity (Munkert et al., 2014) and plays a role in plant defense response to bacteria (Hwang et al., 2012). ANAC102 is located downstream of TGAI in the detoxification pathway, controlling a large range of aldehyde-detoxifying enzymes such as AER, SDR1, chloroplastic aldehyde reductase and chloroplastic aldo-keto reductase (D'Alessandro et al., 2018). The response of *ALDH4* (*ALDEHYDE DEHYDROGENASE 4*, AT1G44170) was slower, showing a significant increase in expression after 3 days of irradiation only (Figure 6). *ALDH4* oxidises variable substrates, including saturated aliphatic aldehydes and α,β -unsaturated aldehydes (Kirch et al., 2001, 2004).

3.3 | RCS detoxification and radioresistance

AER, ANAC102 and SDR1 belong to the so-called xenobiotic detoxification pathway that is controlled by TGAI TFs (Clade II TGACG-binding transcription factors) (D'Alessandro et al., 2018; Fode et al., 2008). Accordingly, the expression levels of AER, SDR1 and ANAC102 was very low in a *tga256* triple mutant

deficient in three TGAI TFs (TGAI-2, TGAI-5 and TGAI-6) and was insensitive to γ irradiation (Figure 6). In contrast, *SDR3* and *ALDH4* are independent of those TGAI TFs, with their expression increasing in both WT and *tga256* in response to exposure to γ radiation. However, the profile of expression for these genes as a function of the irradiation time was not the same in WT and *tga256*.

Inhibition of the xenobiotic pathway in the triple *tga256* mutant was found to cause prolonged maintenance of high RCS levels compared to WT during irradiation (Figure 4, orange symbols/lines). This effect was marked for all the measured RCS except HHE. Maintenance of increased levels of RCS in irradiated plants is likely to have negative effects on the plant physiology and development since those compounds are toxic. This was confirmed when the growth rate of the plants was measured (Figure 7). Growth of the triple mutant was noticeably slowed down by γ rays. Please note that, under control conditions, WT and *tga256* grow similarly (Figure 7b and Supporting Information S1: Figure S1).

TGAI is known to regulate a variety of genes related to cellular detoxification. For instance, UV-B stress was reported to induce genes encoding peroxide-scavenging enzymes under the

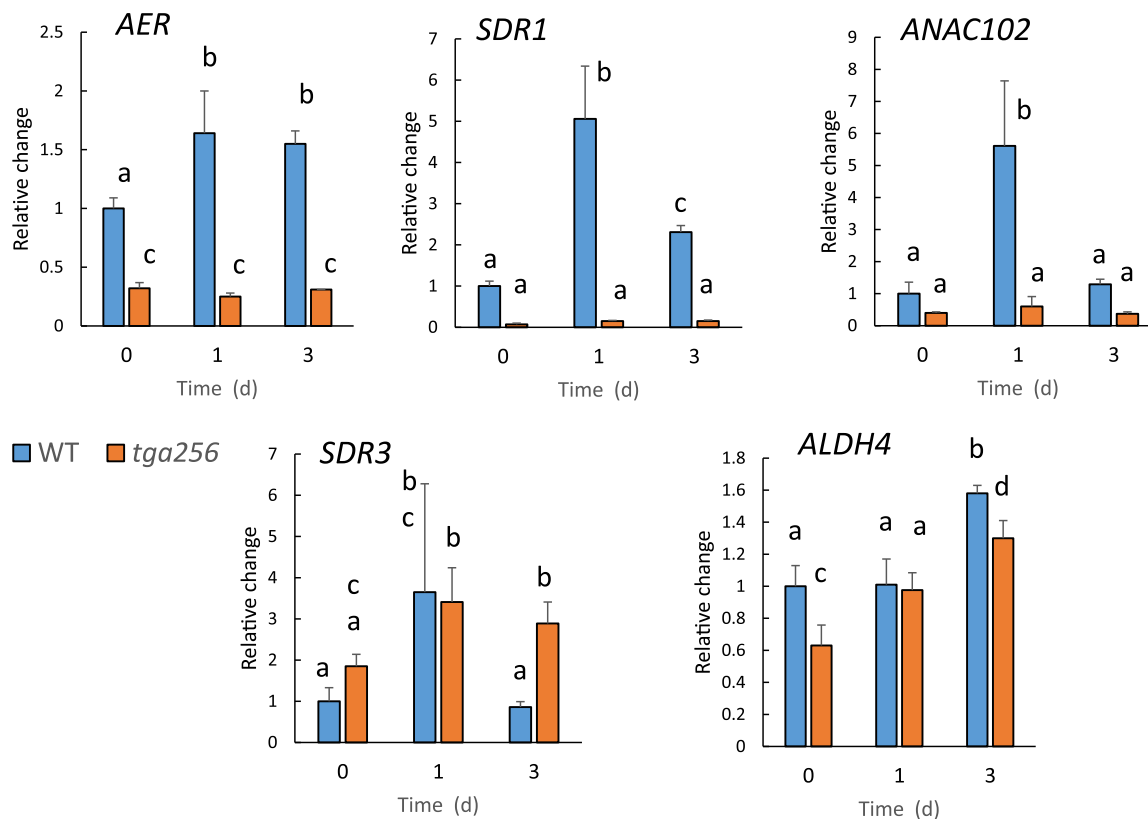


FIGURE 6 qRT-PCR analysis of the expression of detoxification-related genes. *AER*, *SDR1*, *ANAC102*, *SDR3* and *ALDH4* genes were analysed in leaves of Arabidopsis plants (WT and *tga256* triple mutant) exposed to γ radiation at a dose of 25 mGy h^{-1} . Data are mean values of three repetitions \pm standard deviation. Statistical analysis was performed using analysis of variance/Fisher's LSD test. Different letters indicate statistical differences at $p < 0.05$. [Color figure can be viewed at wileyonlinelibrary.com]

control of TGAI, and the *tga256* mutant was found to accumulate more H_2O_2 under UV-B stress than WT (Herrera-Vásquez et al., 2021). Therefore, we cannot exclude that γ radiation led to different H_2O_2 levels in WT and *tga256*. To test this possibility, H_2O_2 levels were quantified in control and irradiated plants by in situ staining using DAB (Figure 8a). Exposure of plants to γ radiation slightly enhanced the brown coloration of oxidised DAB both in WT and *tga256* seedlings. The effect was maximal after 1 day of irradiation, subsequently decreasing and reaching the control level after 7 days of irradiation. No significant difference in DAB coloration was observed between WT and *tga256* plants. We also analysed the expression of two H_2O_2 -responsive genes (*CATALASE 2* (*CAT2*), *ASCORBATE PEROXIDASE 1* (*APX1*)) by qRT-PCR. Both genes were moderately and transiently induced after 1 day of irradiation, and this effect was not enhanced in *tga256* leaves compared to WT leaves (Figure 8c). After 3 days of irradiation, the expression levels were similar in irradiated and nonirradiated plants. Globally, H_2O_2 production in γ -irradiated Arabidopsis plants appeared to be rather low. In fact, H_2O_2 is only a secondary product of water radiolysis (LaVerne, 2000; Sartorio et al., 2022). Radicals, such as $\bullet\text{OH}$ and $\text{H}\bullet$, are the predominant species generated by this mechanism. We also compared lipid peroxidation in WT and *tga256* (Figure 8b). The ROS-induced

HOTE levels increased by a factor between 3 and 5 relative to the nonirradiated controls in both WT and *tga256* (Figure 8b). Importantly, those changes in HOTE levels were similar in both genotypes. We can conclude from the data of Figure 8 that γ radiation-induced increase in RCS levels in *tga256* compared to WT is not attributable to higher levels of lipid peroxidation in the former genotype, supporting differential metabolism of RCS between the two genotypes.

3.4 | High dose rates of γ irradiation

The results presented above show that acclimation of Arabidopsis to relatively low doses of γ rays requires activation of a detoxification mechanism that enhances the turnover of toxic carbonyls derived from lipid peroxidation. We wanted to know if this mechanism also takes place in plants exposed to acute γ -ray stress. To this end, Arabidopsis plants were exposed to a high dose rate of 2.59 Gy min^{-1} delivered in 38 min (total dose of 100 Gy). This treatment had a noticeable impact on the subsequent growth of the plants (Figure 9a). Not only was growth slowed down, but plant morphology was also affected, with the appearance of leaf deformations. The 100-Gy radiation treatment

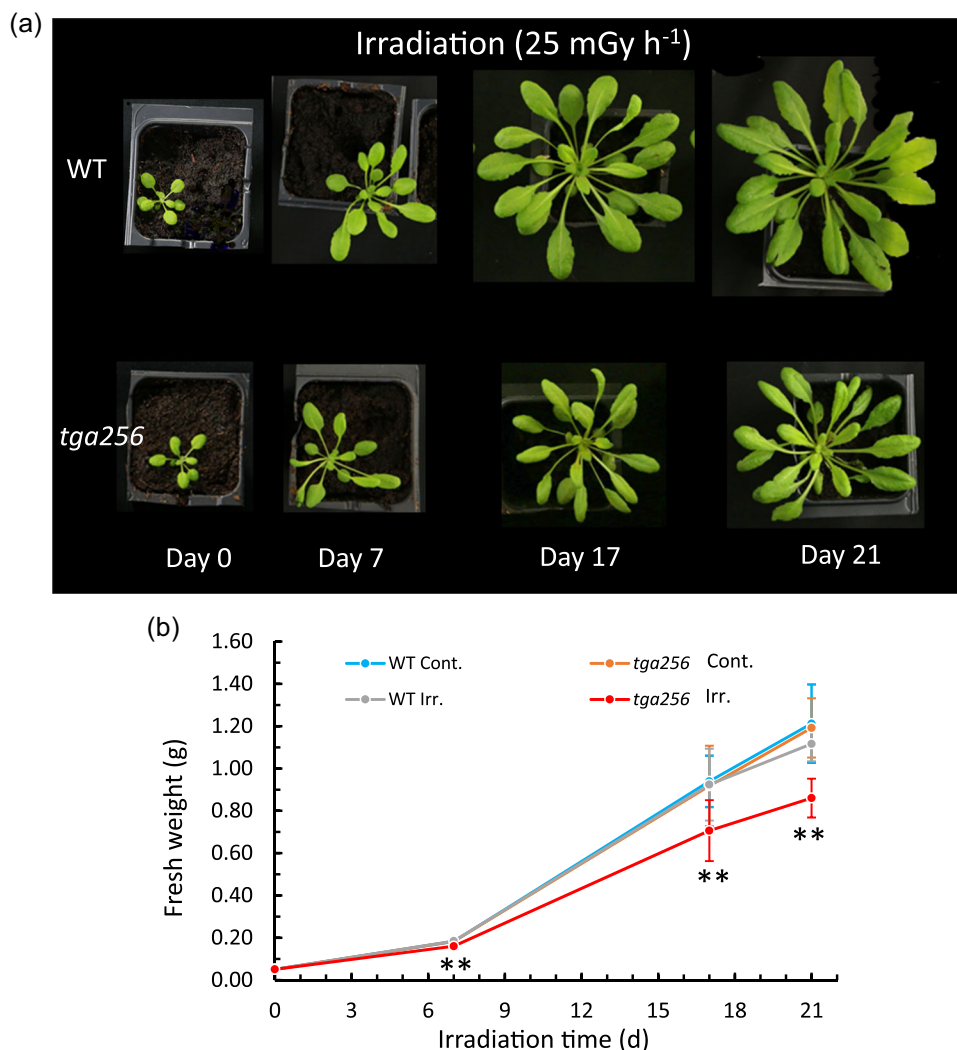


FIGURE 7 Relative growth of wild-type [WT] *Arabidopsis* and the *tga256* triple mutant during exposure to γ radiation. (a) Picture of the plants during the irradiation treatment for 0, 7, 17 and 21 days. (b) Growth curves of WT and *tga256* under control (Cont) and irradiation (Irr) conditions. Data are mean values of 6–8 repetitions \pm standard deviation. ** indicates significant difference between irradiated *tga256* and the other conditions at $p < 0.01$ (analysis of variance/Fisher's LSD test). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pc.14994)]

was associated with a substantial increase in LOX-mediated lipid peroxidation while ROS-induced lipid peroxidation was similar to that in control plants (Figure 9b), which is the opposite of what we observed in response to low dose rates of γ radiations (Figure 3). The bioluminescence of the *pAER:LUC* line exposed to the 100-Gy treatment showed no induction of the AER gene expression in comparison of control, nonirradiated plants (Figure 10a,b). This phenomenon was confirmed by qRT-PCR analyses of the expression of AER, SDR1 and ANAC102 (Figure 10c). No upregulation of gene expression was observed at 1, 3 and 7 days after the 100-Gy treatment. Rather, all the tested genes were downregulated, in particular at Day 3 after irradiation for SDR1 and ANAC102. We also measured RCS levels in leaves 3 and 7 days after the γ -ray pulse. Several carbonyls (HNE, HHE, acrolein, *trans*-2-butenal, butanal, pentanal, hexanal, octanal, nonanal, proprionaldehyde) accumulated in the irradiated

leaves (Figure 11). This accumulation persisted for at least 7 days. For a number of carbonyls and RCS (HNE, octanal, acrolein, butanal, hexanal, pentanal and nonanal), the concentrations were even higher after 7 days compared to 3 days. Very few carbonyls did not change significantly (*trans*-hexenal, hexanal, heptanal) in response to the acute ionising treatment (Supporting Information S1: Figure S2). Then, it appears that acute γ -radiation stress did not trigger the xenobiotic detoxification pathway, leading to prolonged accumulation of RCS and impairing plant development.

4 | DISCUSSION

A. thaliana is considered to be a rather radioresistant plant species, with the median lethal dose LD50 exceeding 600 Gy (Blagojevic et al., 2019; Chang et al., 2020). Although *Arabidopsis*

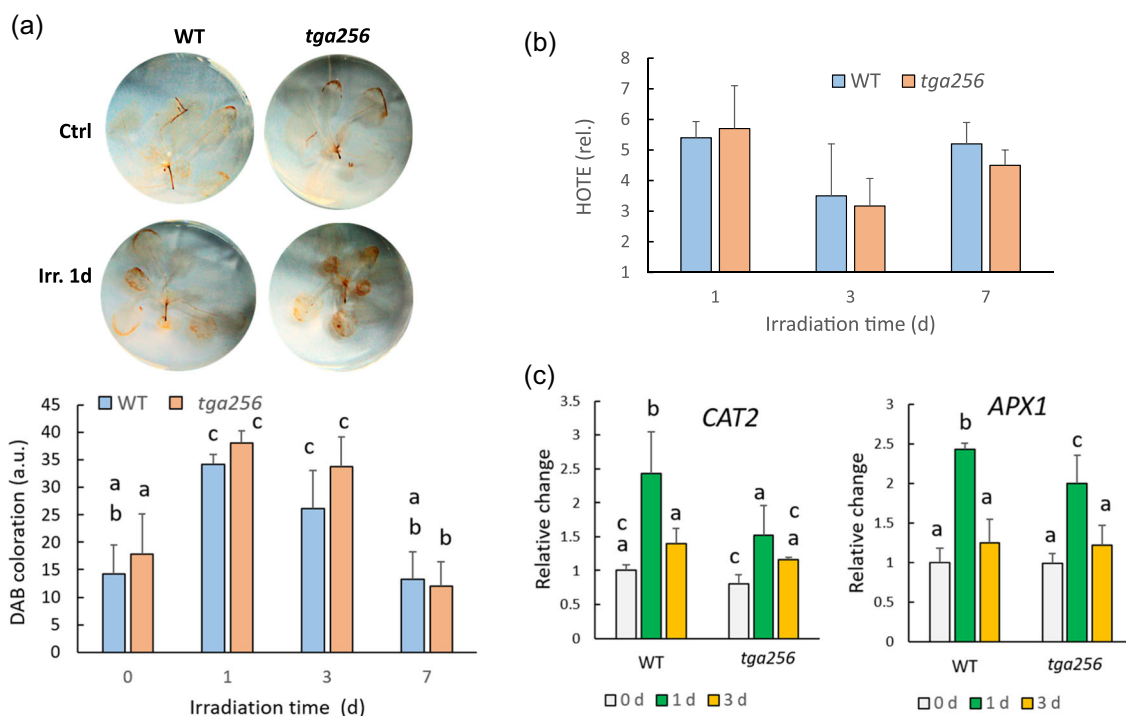


FIGURE 8 H_2O_2 production and oxidative stress in wild-type [WT] and *tga256* Arabidopsis plants exposed to γ radiation. Plants were exposed for 0, 3 and 7 days to a dose rate of 25 mGy h^{-1} . (a) H_2O_2 levels using 3,3'-diaminobenzidine (DAB) staining. Top: examples of DAB coloration of WT and *tga256* plants before (Ctrl) and after 1-day γ irradiation. Bottom: Average intensity of DAB coloration after 0, 1, 3 and 7 days of γ irradiation. (b) Increase in reactive oxygen species (ROS)-induced HOTE levels in WT and *tga256* leaves after 1, 3 and 7 days of γ irradiation. Data are normalised to the respective nonirradiated control. Mean values of 3–5 measurements \pm standard deviation. (c) Expression levels of two H_2O_2 -responsive genes in WT and *tga256* plants after 0, 1 and 3 days of γ irradiation. Statistical analyses were performed using analysis of variance/Fisher's LSD test. Different letters indicate statistical differences at $p < 0.05$. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pcel.14994)]

leaf biomass was not affected by low or moderate doses of γ radiation, we observed chronic oxidation of lipids throughout the irradiation treatment. This is consistent with previous studies showing that lipids are targets of ionising radiations (Choi et al., 2021; Kang et al., 2020; Kurt-Celebi et al., 2023; Vanhoudt et al., 2010; Xie et al., 2019). Strikingly, enzymatic lipid peroxidation was predominant in Arabidopsis leaves after the high radiation treatment while lipid peroxidation was mainly nonenzymatic under low radiation doses. This difference could be related to the expression of LOX-encoding genes. Previous studies have shown that LOX genes are induced by high γ -radiation levels (35 Gy), not by low levels (3.5 Gy) (Vanhoudt et al., 2010, 2011). Also, low doses were observed to induce a more pronounced lipid peroxidation than high doses (Vanhoudt et al., 2011). The fact that high radiation levels did not induce significant changes in ROS-catalysed lipid peroxidation (Figure 9) is surprising, but we can speculate that high ROS production under acute γ -radiation stress caused multiple oxidations of the fatty acids followed by rapid decomposition of the highly oxidised compounds.

Lipid peroxidation is problematic because lipid peroxides are unstable and fragment in a myriad of secondary oxidation products such as carbonyls which can be very reactive and

potentially damaging (Biswas & Mano, 2021). We observed that several RCS accumulated in Arabidopsis leaves exposed for 1 day to 25 mGy h^{-1} . To the best of our knowledge, there is no other study providing extensive data on RCS in irradiated plants. In the present study, accumulation of distinct aldehydes was quantified in irradiated plants whereas the estimation of lipid peroxidation in previous studies was restricted to the TBARS assay as a proxy of malondialdehyde (MDA) (e.g., Choi et al., 2021; Kang et al., 2020; Kurt-Celebi et al., 2023; Vanhoudt et al., 2011; Volkova et al., 2017; Xie et al., 2019). Although those previous works did not directly analyze MDA, they are consistent with our results since MDA is an RCS. Consistently, lipid oxidation by γ -irradiation of liposomes was accompanied by the accumulation of fragmentation products (Shadyro et al., 2002). The carbonyls that changed the most in WT Arabidopsis leaves in response to γ radiation were HHE, HNE, butanal and nonanal. The *tga256* mutation had a marked impact on the levels of those compounds, except HHE, and also on *trans*-2-hexenal, pentanal, and *trans*-2-pentenal. HNE, *trans*-2-hexenal and *trans*-2-pentenal are toxic RCS that can potentially affect growth of the *tga2* mutant compared to WT.

RCS can propagate oxidative stress within cells and also they can make adducts to various macromolecules (Biswas &

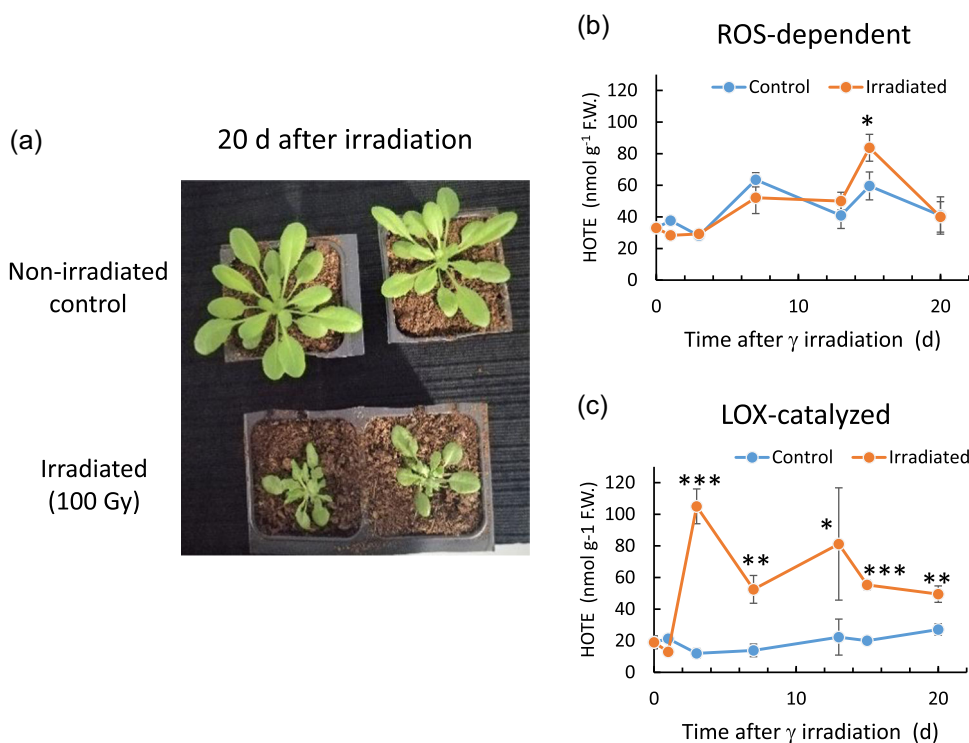


FIGURE 9 Effects of high doses of γ radiation (2.59 Gy min^{-1} for 38 min = 100 Gy) on growth and lipid peroxidation. (a) Arabidopsis growth (20 days after irradiation), and (b) reactive oxygen species (ROS)-dependent and (c) lipoxygenases (LOX)-catalysed lipid peroxidation (1, 3, 7, 13, 15 and 20 days after irradiation). ***, **, different from control at $p < 0.05$, 0.01 and 0.001 (Student's t -test). [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pce.14994)]

Mano, 2021; Moldogazieva et al., 2023; Sousa et al., 2017). Because RCS are harmful, protection against ROS and oxidative stress requires their detoxification. In fact, RCS detoxification processes are known to play a role in plant acclimatisation to abiotic stresses. For example, overexpression of AER makes plants resistant to multifarious oxidative stress conditions (Mano et al., 2005) and to nitrogen deficiency (Wang et al., 2021). AKR (Aldo-keto reductase) protects plants against heat stress or salt stress (Kanayama et al., 2014; Turóczy et al., 2011). Similarly, overexpression of various aldehyde dehydrogenases increased plant tolerance to oxidative and osmotic stresses, as well as to heavy metals (Kotchoni et al., 2006; Sunkar et al., 2003). Stimulation of the xenobiotic detoxification pathway by application of a chemical inducer led to high resistance of Arabidopsis to water stress (D'Alessandro et al., 2019). A transcriptomic analysis of Arabidopsis seedlings exposed to 28 mGy h^{-1} for 14 days showed the induction of genes related to oxido-reduction processes (including detoxification), to toxin and drug metabolic processes (Horemans et al., 2023), in agreement with the idea that the xenobiotic detoxification pathway is sensitive to low dose rates of γ radiation. Interestingly, in mammalian cells too, studies have shown that detoxification enzymes (aldehyde dehydrogenase (ALDH) or carbonyl reductase) modulate responses to ionising radiation such as radiation-induced

carcinogenesis or the effects of radiotherapy (Wright et al., 2015; Yun et al., 2018). For example, activation of ALDH2 by an allosteric agonist in mice decreases HNE levels and delays the onset of dermatitis symptoms after irradiation (Ning et al., 2012).

As shown in the present study, the TGAI-regulated xenobiotic detoxification pathway is also recruited by Arabidopsis cells in response to chronic ionising γ -radiations. Partial block of this pathway in the *tga256* triple mutant led to a radiosensitive phenotype, indicating the importance of TGAI-controlled RCS metabolization in plant acclimation to low or moderate doses of γ radiation. The TGA transcription factors, which belong to the basic region leucine zipper (bZIP) family, can affect a wide range of cellular processes (Gatz, 2013; Herrera-Vásquez et al., 2021; Tomaž et al., 2022). More specifically, TGAI factors have been previously related to protection against chemical stress and ROS. In Arabidopsis plants treated with phytoprostanes (linolenic acid oxidation products), TGAI factors are required for the expression of 60% of the induced genes, most of them being related to detoxification, stress responses, and secondary metabolism (Mueller et al., 2008). Accordingly, beside TGAI-dependent genes, we found genes coding for detoxification enzymes that are still inducible (but with modified kinetics) by γ radiations in the absence of TGAI-2, -5 and -6, such as *ALDH4* and *SDR3*, indicating a network of detoxification pathways.

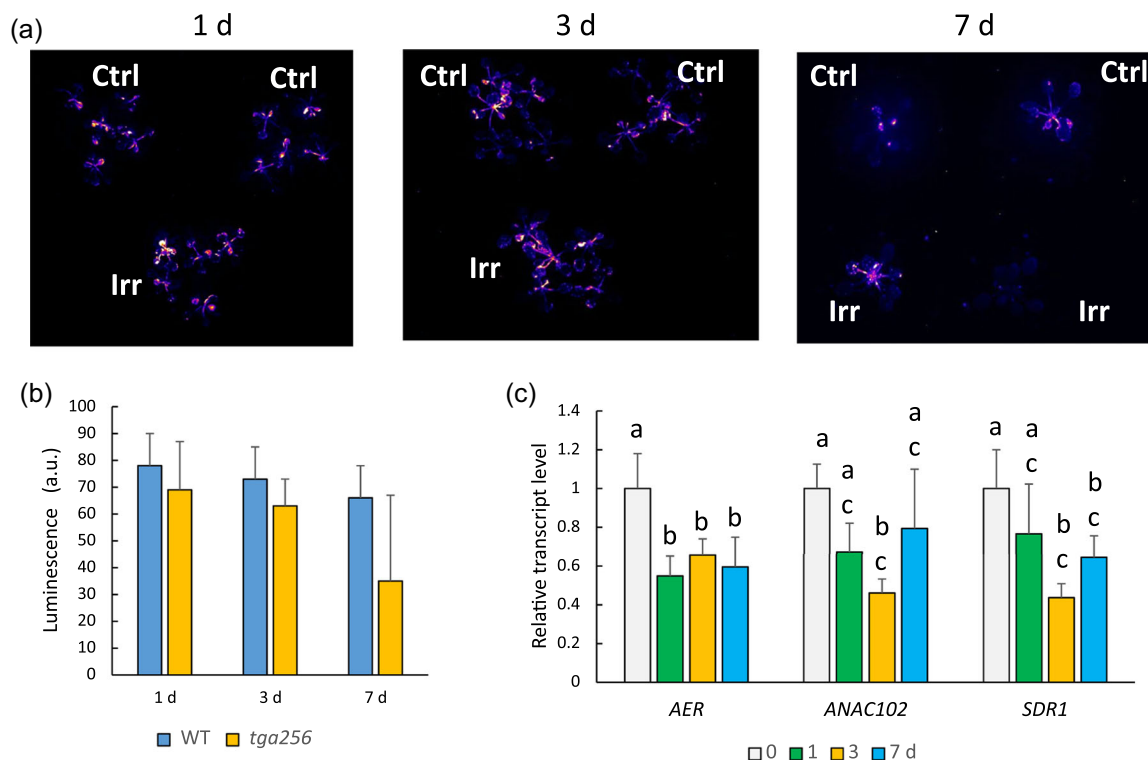


FIGURE 10 Effects of high doses of γ radiation (2.59 Gy min^{-1} for 38 min = 100 Gy) on detoxification-related genes (*AER*, *SDR1*, *ANAC102*). (a) *AER* gene expression as measured by luciferase/luciferin luminescence of *pAER:LUC* plants. Luminescence was measured 1, 3 and 7 days after the irradiation treatment. Ctrl, control; Irr, irradiated. (b) Quantification of the luciferase/luciferin luminescence intensity (1, 3 and 7 days after irradiation). Data are mean values of 4–8 plants \pm standard deviation. No statistically significant difference between WT and *tga256*. (c) qRT-PCR analysis of *AER*, *SDR1* and *ANAC102* transcripts (1, 3 and 7 days after irradiation). Statistical analysis was performed using analysis of variance/Fisher's LSD test. Different letters indicate statistical differences at $p < 0.05$. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pcel.14994)]

When *Arabidopsis* was exposed to a high dose of 100 Gy, none of the detoxification genes tested was induced, and a persistent accumulation of many RCS was observed. This is at variance with the responses to low doses of γ radiation. The 100-Gy treatment also caused a marked inhibition of plant growth. It is tempting to link this effect of acute γ -radiation stress on plant development with RCS accumulation. A recent study of salt-stressed *Arabidopsis* plants has correlated stress-induced growth inhibition with the levels of several RCS, and their scavenging by exogenously applied histidine-containing dipeptides was found to alleviate the symptoms of salt stress and to restore growth (Sultana et al., 2022). RCS are known to form adducts to proteins and DNA, which could alter the normal cell functioning and indirectly impact plant development (Biswas & Mano, 2021; Moldogazieva et al., 2023). RCS can also act as molecular intermediates in several cell signalling pathways in plants and animals, including stress responses, cell death events, kinase/phosphatase activity regulation and nuclear transcription function (Alm eras et al., 2003; Islam et al., 2019; Mano et al., 2019; Mueller et al., 2008; Semchyshyn, 2014; Weber et al., 2004). Based on some data on yeast, potential interplay between certain

RCS and TOR signalling cascade cannot be excluded (Semchyshyn, 2014). Consequently, in the absence of rapid removal of RCS (e.g. after exposure to 100 Gy), those signalling compounds can reach concentration levels capable of triggering various processes that ultimately affect plant development.

In conclusion, this study has shown that *Arabidopsis* acclimates to moderate dose rates of γ -radiation through a TGAI-mediated defense mechanism which avoids chronic accumulation of potentially harmful lipid-derived compounds. Under the doses of γ radiation used in this study, this protective mechanism is necessary to maintain the plant growth rate. As detailed above, detoxification enzymes are involved in the defense response of plants against a wide range of abiotic stresses and pollutants. The common factor between all those stress conditions and ionising radiations are the short-lived ROS and their more stable derived products, the RCS. Ionising radiations are naturally present, deriving from radioactive minerals in soil and from cosmic rays which interact with the gaseous and particulate elements of the atmosphere, producing radioisotopes (Duarte et al., 2023). It must be remembered that ionising radiation incidence in earlier Earth environments was higher than nowadays (Duarte et al., 2023).

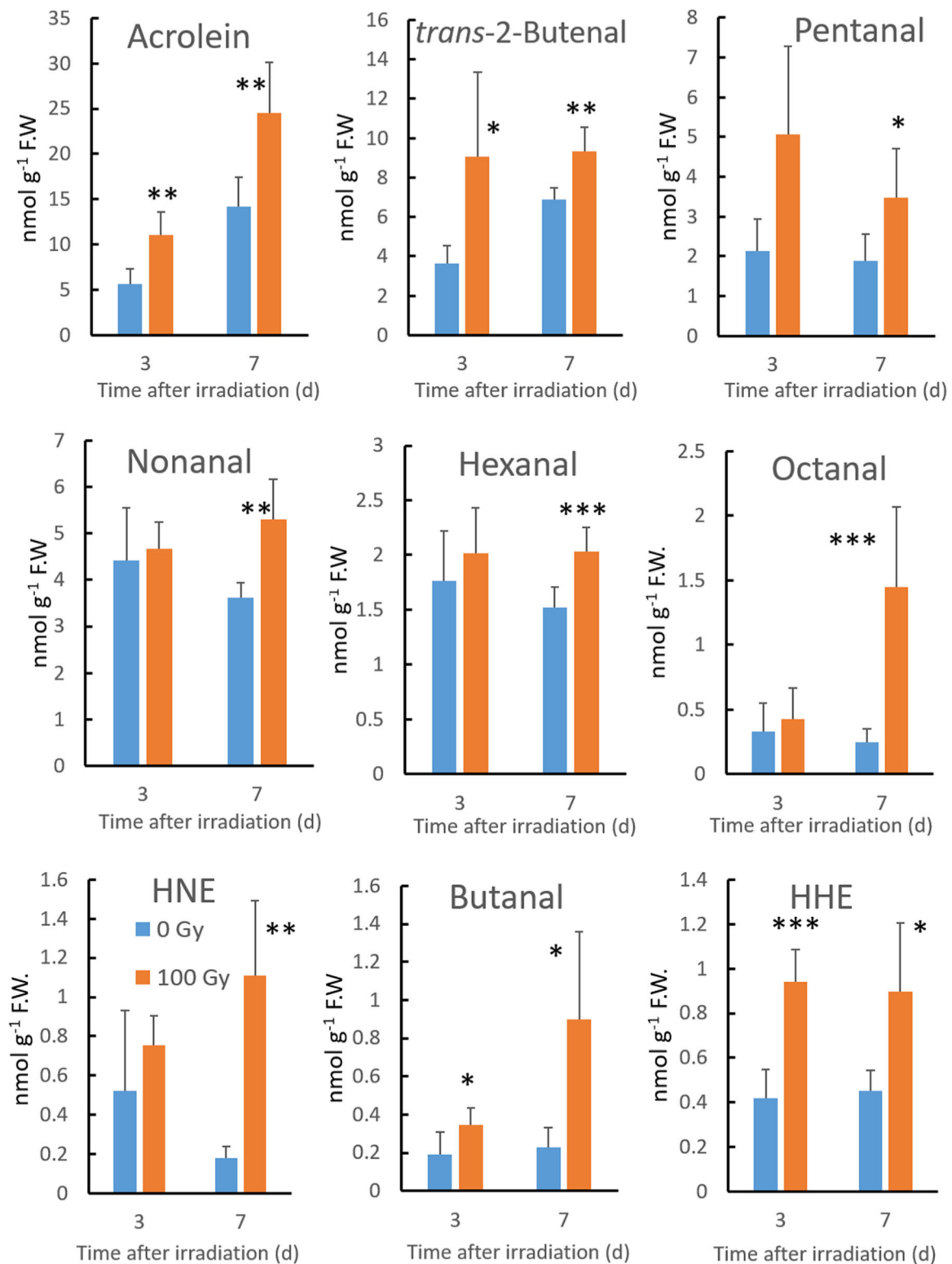


FIGURE 11 Carbonyl compounds in *Arabidopsis* leaves exposed to a high dose of γ radiation (2.6 Gy min^{-1} for 38 min = 100 Gy). Samples were harvested 3 and 7 days after irradiation. In blue, nonirradiated controls; in orange, irradiated plants. *, **, ***, different from 0 Gy at $p < 0.05$, 0.01 and 0.001, respectively (Student's *t*-test). [Color figure can be viewed at wileyonlinelibrary.com]

Therefore, through ROS/RCS production, chronic ionising radiations possibly participated in the evolutionary driving force leading to the establishment of a general, versatile detoxification mechanism in living organisms.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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