A pivotal role of the jasmonic acid signal pathway in mediating radiation-induced bystander effects in *Arabidopsis thaliana*

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ABSTRACT

Although radiation-induced bystander effects (RIBE) in *Arabidopsis thaliana* have been well demonstrated *in vivo*, little is known about their underlying mechanisms, particularly with regard to the participating signaling molecules and signaling pathways. In higher plants, jasmonic acid (JA) and its bioactive derivatives are well accepted as systemic signal transducers that are produced in response to various environmental stresses. It is therefore speculated that the JA signal pathway might play a potential role in mediating radiation-induced bystander signaling of root-to-shoot. In the present study, pretreatment of seedlings with Salicylhydroxamic acid, an inhibitor of lipoxigenase (LOX) in JA biosynthesis, significantly suppressed RIBE-mediated expression of the *AtRAD54* gene. After root irradiation, the aerial parts of *A. thaliana* mutants deficient in JA biosynthesis (*aos*) and signaling cascades (*jar1-1*) showed suppressed induction of the *AtRAD54* and *AtRAD51* genes and *TSI* and *180-bp* repeats, which have been extensively used as endpoints of bystander genetic and epigenetic effects in plants. These results suggest an involvement of the JA signal pathway in the RIBE of plants. Using the root micro-grafting technique, the JA signal pathway was shown to participate in both the generation of bystander signals in irradiated root cells and radiation responses in the bystander aerial parts of plants. The over-accumulation of endogenous JA in mutant *fatty acid oxygenation up-regulated 2* (*fou2*), in which mutation of the Two Pore Channel 1 (*TPC1*) gene up-regulates expression of the *LOX* and *allene oxide synthase* (*AOS*) genes, inhibited RIBE-mediated expression of the *AtRAD54* gene, but up-regulated expression of the *AtKU70* and *AtLIG4* genes in the non-homologous end joining (NHEJ) pathway. Considering that NHEJ is employed by plants with increased DNA damage, the switch from HR to NHEJ suggests that over-accumulation of endogenous JA might enhance the radiosensitivity of plants in terms of RIBE.

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1. Introduction

Radiation-induced bystander effects (RIBE) represent a paradigm shift in our understanding of the radiobiological effects of ionizing radiation, in which radiation responses in non-hit cells can be induced by the signals from nearby hit cells [1]. Bystander effects have been well demonstrated using a wide range of biological endpoints in single-cell culture models [2–5], multi-cellular tissue models [6–11], and whole animals [12–19]. Recently, our team has started to investigate RIBE using the model plant *Arabidopsis thaliana*. Following microbeam-localized irradiation of naked seed embryos and low-energy ion irradiation of intact seeds, some post-embryonic developmental phenotypes were significantly changed, differentiating from the non-irradiated shoot apical meristem cells and root apical meristem cells, respectively [20,21]. The enhanced level of DNA strand breaks, up-regulated expressions of the DNA damage repair gene *AtRAD54*, and increased induction of DNA homologous recombination (HR) have been observed in the non-irradiated aerial parts of plants after local irradiation of roots with alpha particles and dormant seeds with low-energy argon ions, indicating the occurrence of bystander mutagenic effects

in plants [22,23]. Bystander effects in plants were also found to mediate changes at the epigenetic level, such as the level and pattern of DNA methylation, and the alleviation of transcriptional gene silencing (TGS) [24]. Interestingly, in contrast to normal experimental conditions, the bystander effects in plants have some distinct manifestations under modeled microgravity conditions, due to modulation of the generation and/or transportation of bystander signaling molecules by microgravity in irradiated root cells [25]. In order to investigate the time course of root-to-shoot bystander signaling, young seedlings of *A. thaliana* were treated with a combination of root micro-grafting and root irradiation, whereby the root-to-shoot bystander signaling could easily be stopped or started at specific time points [26]. On the basis of this technique, we further demonstrated temporal and spatial features of bystander signaling and the synergistic effects of multiple bystander signals in *A. thaliana* [26,27].

However, the molecular mechanisms underlying RIBE in plants remain to be determined, particularly with regard to the signaling molecules and signaling cascades. It has been reported that in cell culture models, some signal factors are released from hit cells and induce radiation responses in bystander cells through diffusion in medium and/or cellular gap-junctions [1,2]. Several signaling cascades, including MAPK, NF-kB/cox-2, NO−, and inflammation-related pathways, are thought to mediate RIBE transduction [1,28–30]. Although RIBE have also been demonstrated in whole animal models, there is little information as to the nature of long-distance bystander communication [15,17,31]. In marked contrast to DNA damage in irradiated tissues, which is mainly caused by direct energy deposition on DNA strands and/or oxidative damage by free radicals produced by lysis of water [32], the DNA damage and resultant DNA repairs in bystander tissues is generally attributable to enhanced oxidative stress triggered by the incoming bystander signals [33]. Plant hormones such as jasmonic acid (JA) and salicylic acid have been reported to increase the induction of HR and activation of TGS loci [34,35]. Interestingly, the distribution and level of the plant hormone auxin in bystander tissues can be changed by microbeam-localized irradiation of naked seeds of *A. thaliana* [20]. Accordingly, it is proposed that plant hormones might be the potential signaling molecules that mediate root-to-shoot bystander signaling.

JA and its bioactive derivatives are collectively referred as to jasmonates, and play important roles in plant growth and development [36]. Synthesis of the JA begins with α-linolenic acid liberated from membrane phospholipids. The linolenic acid is first oxygenated by lipoxigenase (LOX) to form 13(S)-hydroxylinolenic acid (13-HPOT), which is then converted to 12-oxo-phytodeinoic acid (12-OPDA) by allene oxide synthase (AOS) and allene oxide cyclase (AOC). The 12-OPDA subsequently undergoes a reduction and three cycles of β-oxidation in the peroxisomes to produce JA [37,38], which is further modified in the cytosol to produce various JA derivatives, such as methyl jasmonate (MeJA) [38–41]. In the subsequent signaling cascades, JA is first activated by its conjugation to L-isoleucine by a JA-amino synthetase encoded by the *JASMONATE RESISTANT 1* (*JAR1*) gene. The resultant JA-Ile binds to the COI1-JAZ complex to promote degradation of the JAZ proteins, which initiates expression of down-stream JA response genes by freeing their transcription factors [42]. Mutation of the *JAR1* gene impairs JA perception and signaling cascades in plants, which exhibit decreased sensitivity to exogenous JA [43]. Although JA has been reported to act as a long-distance systemic signal transducer in response to biotic and abiotic stress [44–46], there is yet no evidence showing that JA signals can mediate RIBE in plants.

In the present study, we adopted *A. thaliana* mutants deficient in JA biosynthesis (*aos*) and signaling cascades (*jar1-1*), and investigated whether the JA signal pathway could mediate RIBE in plants. The results showed that deficiencies in both processes blocked the
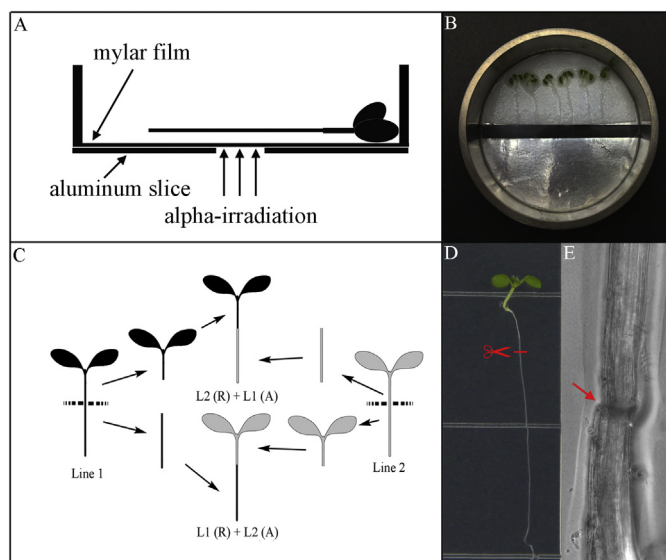

Fig 1. Root-localized irradiation and root micro-grafting of *Arabidopsis thaliana* seedlings. (A) Schematic of the root-localized irradiation of *A. thaliana* seedling with alpha particles; (B) positioning of *A. thaliana* seedlings on the radiation dish, with 100-μm-thick aluminum shielding the aerial parts of plants from alpha irradiation; (C) Schematic procedure of root micro-grafting of *A. thaliana* seedling; (D) The seedlings prepared for root micro-grafting, the red line indicates the cutting position for root grafting; (F) The graft junction at 24 h after root grafting, as indicated by red arrow.

induction of bystander genetic and epigenetic effects, suggesting an important role of the JA signal pathway in mediating RIBE in plants. Using the root micro-grafting technique, the JA signal pathway was shown to participate in both the generation of bystander signals in irradiated root cells and radiation responses in bystander aerial tissues. The over-accumulation of endogenous JA suppressed RIBE-mediated HR repair, but up-regulated the non-homologous end joining (NHEJ) mechanism.

2. Materials and methods

2.1. Transgenic *A. thaliana* lines and plant growth

A. thaliana line 15-6# carrying the *AtRAD54 promoter-GFP+ GUS* construct was presented by Dr. Seiichi Toki (Plant Genetic Engineering Research Unit, National Institute of Agrobiological Sciences, Japan) [47]. The line *fatty acid oxygenation up-regulated 2 (fou2)*, in which a missense mutation in the *Two Pore Channel 1* (*TPC1*) gene up-regulates expression of the *LOX* and *AOS* genes [48], was kindly donated by Prof. Edward Farmer (University of Lausanne, Switzerland). *A. thaliana* lines *aos* and *jar1-1* are mutants with loss of function of the *AOS* (CYP74A) and *JAR1* genes, respectively [49,50]. *A. thaliana* line A1 is transgenic for two copies of the *pAOS:uidA* reporter [51]. The *A. thaliana* wild-type (Col-0), A1, *aos*, and *jar1-1* lines were obtained from the NASC (Nottingham *Arabidopsis* Stock Centre, UK).

Surface-sterilized seeds of *A. thaliana* were sown on growth medium [1 × Murashige and Skoog (MS) mineral salts, agar at 0.8% (w/v), and sucrose at 1% (w/v)] in a square Petri dish. After 48 h of jarovization at 4 °C, the Petri dish was placed in a growth chamber at 22 °C with continuous illumination of approximately 100 μMm^2 s^{-1} in a vertical orientation so that the roots would grow along the agar surface.
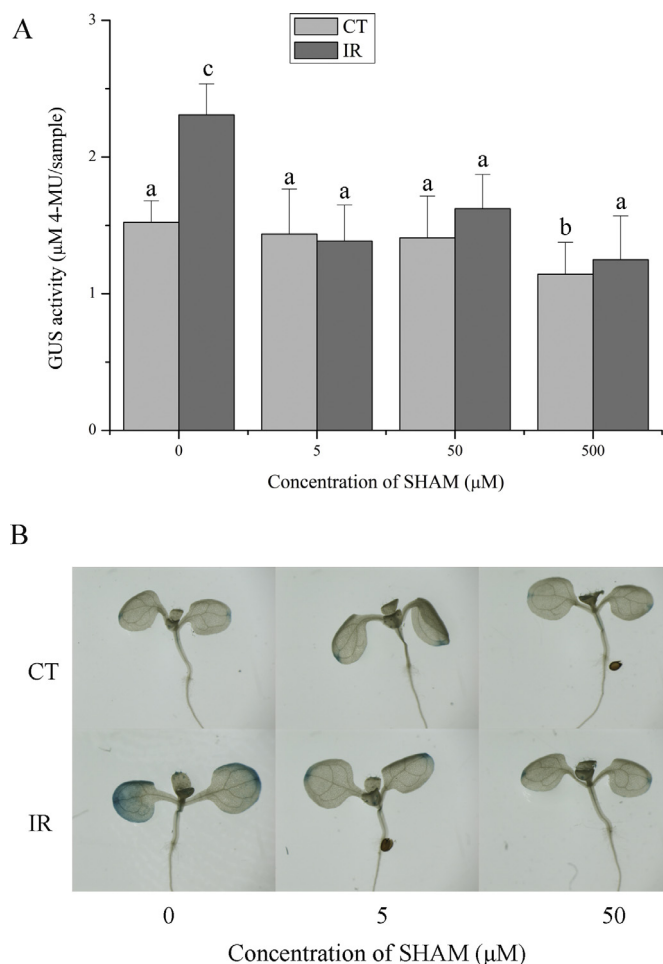
A

B

Fig. 2. The repressive effect of SHAM on RIBE in *A. thaliana* plants. (A) The effect of the indicated concentrations of SHAM on RIBE-mediated expression of the *AtRAD54* gene; (B) The histochemical staining of GUS activity in seedlings of line 15-6# co-treated with 0, 5, and 50 μM SHAM and/or root irradiation. The results are means ± SD [n > 10 for GUS activity, the same lowercase letter denotes a non-significant difference between means (*p* < 0.05)].

2.2. Root irradiation of A. thaliana *seedlings with alpha-particles*

The irradiation of roots of 7-day-old seedlings with alpha parti-cles was performed using a Rotate-Adjustable alpha particle Source Facility in our laboratory (Fig. 1A and B), as previously described [22,24,26]. Before each experiment, the energy spectrum of alpha particles was measured using a silicon surface barrier detector (ORTEC, USA). In this study, the average energy of alpha particles measured in the seedlings was 3.3 MeV, with a maximum value of 4.02 MeV. The alpha particles were delivered at a dose rate of 1.05 cGys^{-1}.

2.3. Protocols for root micro-grafting

In order to determine the mediating mechanism of the JA sig-nal pathway in RIBE in plants, root micro-grafting of *A. thaliana* seedlings was carried out according to the general protocols described previously [26]. In this study, the seedlings of wild-type, 15-6#, *aos*, and *jar1-1* were used in the grafting combinations of L1(R)-L2(A) and L1(A)-L2(R), where L1 and L2 represent the names of *A. thaliana* lines, R represents for rootstock, and A represents aerial scions, as shown in Fig. 1C, D and E.

2.4. Treatment with saliccylhydroxamic acid(SHAM)and exogenous MeJA

For the treatment of seedlings with SHAM, 5-day-old seedlings of line 15-6# were transferred onto MS medium containing var-ious concentrations of SHAM (0, 5, 50, and 500 μM) for 2 days. After root irradiation, the seedlings were again transferred onto the same SHAM medium for 24 h, and then the aerial parts of seedlings without hypocotyls were sampled for quantitative assay of β-glucuronidase (GUS) activity.

For the treatment of seedlings with exogenous MeJA, seedlings of line *aos* were transferred onto MS medium containing various concentrations of MeJA (0, 50, and 100 μM) for 6 h. After root irradi-ation, the seedlings were again transferred onto the same medium for 24 h, and then their aerial parts were collected for qRT-PCR analysis.

2.5. Determination of RNA level using qRT- PCR

RNA levels of the *AtRAD54, AtRAD51, AOS, AOC2, LOX2, PDF1.2, AtKU70,* and *AtLIG4* genes and *transcriptionally silent information* (*TSI*) and *180-bp* repeats were measured using qRT-PCR, their cor-responding PCR primers are listed in Supplementary material Table S1 in the online version at DOI: 10.1016/j.mrfmmm.2016.07.002). Fifteen to twenty *A. thaliana* plants were collected from each inde-pendent experiment. The extraction and reverse transcription of total RNA were carried out according to our previous work [24,25]. qRT-PCR was performed under the following conditions: one cycle of 95 °C for 10 s followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The *ACTIN* and *AtEF1αA4* genes were used as internal controls. The final data are the average of 3 independent experiments, with 3 technical replicates for each experiment.

2.6. Quantitative analysis of GUS activity in the aerial parts of plants

The quantitative analysis of GUS activity in plants of line 15-6# was carried out according to our previous description [25]. A popu-lation of 7-day-old seedlings with uniform size (rosette diameter) was prepared for the same batch of experiments. In an experi-ment, the seedlings were randomly divided into specified groups, with 6 seedlings in each group. After treatments, the aerial parts of seedlings were sampled. Quantitative assay of GUS activity was performed according to protocols previously described [52]. The samples in each group were separately collected and then incu-bated in 4-methylumbelliferyl glucuronide assay solution for 16 h. The resultant 4-methyl umbelliferone (4-MU) was measured using a luminescence spectrophotometer equipped with an ELISA plate reader (Spectra Max M2, Molecular Devices). Final data for each group are the average of more than 10 single samples from 3 inde-pendent experiments.

For detection of the distribution of GUS activity in plants of lines 15-6# and A1, samples were collected at 24 h after root irradiation, and then assayed by histochemical staining, as described previously [53].

2.7. Statistical analysis

All data were evaluated in terms of means and standard devia-tions. Determinations of the statistical significance between treated and control groups or between treated groups were made using Student's *t*-test. A *P* value of 0.05 or less between groups was con-sidered to be significant.
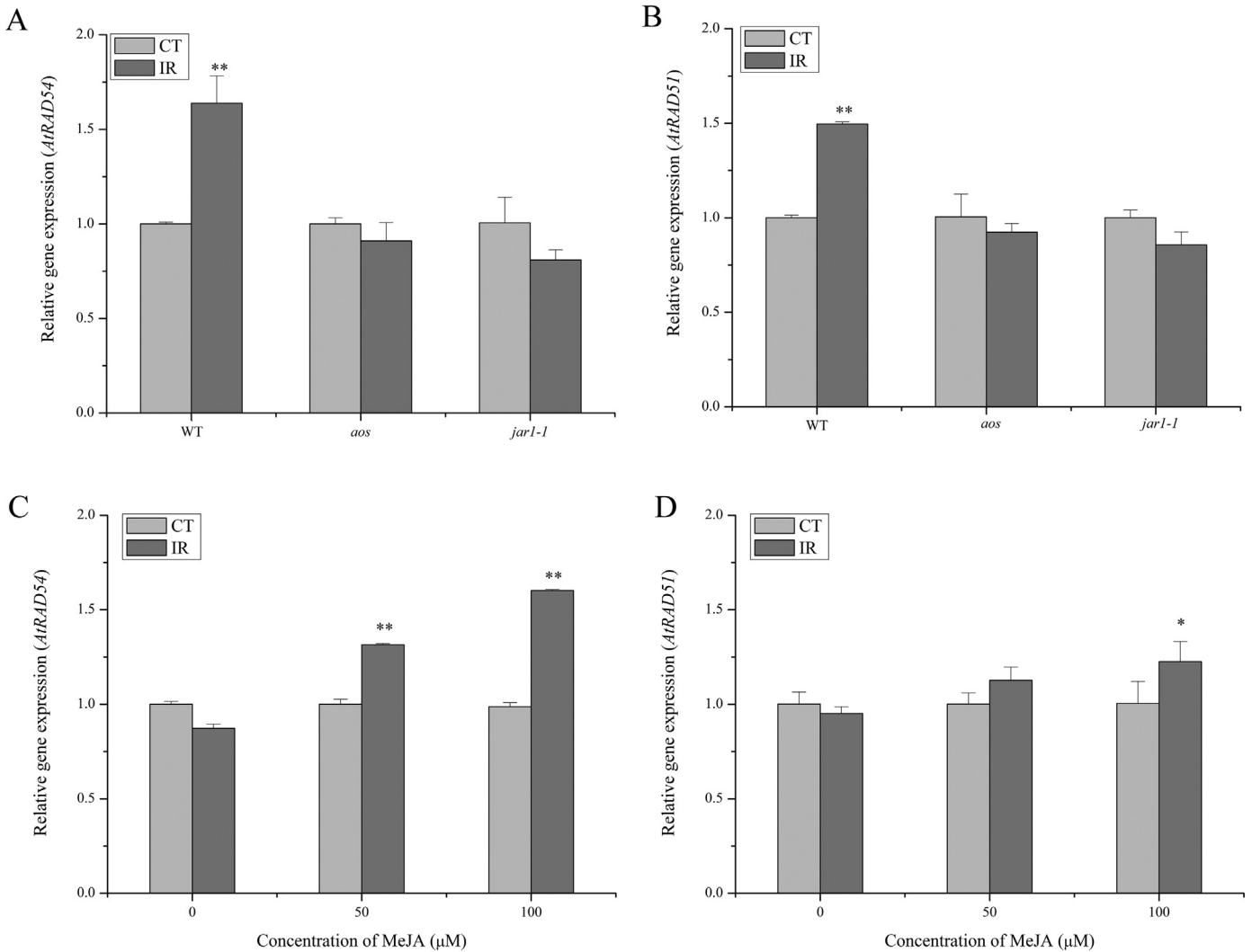
Fig. 3. RIBE in the mutants deficient in JA biosynthesis and signaling cascades. (A) mRNA level of the *AtRAD54* gene in the aerial tissues of lines *aos* and *jar1-1* after root irradiation; (B) mRNA level of the *AtRAD51* gene in the aerial tissues of lines *aos* and *jar1-1* after root irradiation; (C) mRNA level of the *AtRAD54* gene in the aerial parts of *aos* plants co-treated with exogenous MeJA and root irradiation; D) mRNA level of the *AtRAD51* gene in the aerial parts of *aos* plants co-treated with exogenous MeJA and root irradiation. The results are means ± SD (n = 3 for RNA level, *t* test *$P < 0.05$ and **$P < 0.01$).

3. Results

3.1. Repressive effect of SHAM on RIBE in plants

SHAM is well accepted as an inhibitor of JA biosynthesis in plants through inhibiting the enzymatic activity of LOX [54]. Accordingly, we first investigated the effect of SHAM on RIBE in plants, using expression of the *AtRAD54* gene (GUS activity) as a biological endpoint. In our previous study, the bystander effects in plants can be induced by root-localized irradiation with 10 Gy of alpha particles [22,24]. In this study, the same dose of alpha particles was used. Five-day-old seedlings of line 15-6# were transferred onto medium containing various concentrations of SHAM for 2 days. After root irradiation, the seedlings continued to grow on the same SHAM medium until GUS activity was examined at 24 h after root irradiation. As shown in Fig. 2A, the application of 5 and 50 μM SHAM alone did not significantly change the GUS activity in seedlings of line 15-6# (in both cases, $P > 0.05$). However, there was a slight decrease in GUS activity following application of 500 μM SHAM ($P < 0.05$) compared to seedlings without SHAM treatment, possibly due to a slight suppression of *A. thaliana* seedling growth at

the higher concentration of SHAM. In agreement with our previous study [22], root irradiation alone significantly increased expression of the *AtRAD54* gene compared to control plants ($P < 0.01$). However, in the presence of SHAM, root irradiation made no difference to expression of the *AtRAD54* gene (in all cases, $P > 0.05$), as shown in Fig. 2A. The histochemical staining of GUS activity in plants of line 15-6# subjected to 5 and 50 μM SHAM was also performed at 24 h after root irradiation. Control plants showed a low background of GUS activity in small regions in cotyledons and true leaves. Root irradiation alone increased the distribution of GUS activity in the upper part of cotyledons, whereas the distribution of GUS activity was not changed by co-treatment with SHAM and root irradiation, as shown in Fig. 2B. These results initially suggested that the JA signal pathway might play a pivotal role in mediating RIBE in plants.

3.2. The effects of deficiencies in JA biosynthesis and signaling cascades on RIBE

In order to verify the above supposition, we examined *A. thaliana* mutants deficient in JA biosynthesis (*aos*) and signaling cascades (*jar1-1*). Transcript levels of the *AtRAD54* gene in the aerial parts

of plants were measured using qRT-PCR at 24 h after root irradiation. As shown in Fig. 3A, root irradiation-induced expression of the *AtRAD54* gene was significantly repressed in plants of lines *aos* and *jar1-1* (in both cases, $P > 0.05$). In addition to the time point of 24 h, repressed expression of the *AtRAD54* gene was also observed at 8 and 36 h after root irradiation, as shown in Supplementary material Fig. S1B and C in the online version at DOI: 10.1016/j.mrfmmm.2016.07.002. Transcript levels of the *AtRAD51* gene, encoding another important component in HR repair pathway, were also measured at 24 h after root irradiation. Similarly, the induction of *AtRAD51* expression was significantly repressed in plants of lines *aos* and *jar1-1* (in both cases, $P > 0.05$), as shown in Fig. 3B. In order to further verify the role of JA in mediating RIBE, *A. thaliana* seedlings of line *aos* were pretreated with exogenous MeJA before root irradiation. As can be seen in Fig. 3C and D, the application of exogenous MeJA significantly restored RIBE-mediated expression of the *AtRAD54* gene in seedlings of line *aos* (in both cases, $P < 0.01$), as shown in Fig. 3C, but only a weakly restored expression of the *AtRAD51* gene ($P < 0.05$ for 100 μM MeJA) (Fig. 3D). These results suggest that the JA signal pathway might be involved in RIBE in plants.

3.3. RIBE-mediated TGS alleviation in aos and jar1-1 mutants

In our previous work, we also reported radiation-induced bystander epigenetic effects in *A. thaliana* plants, in which TGS-silenced DNA repetitive elements were epigenetically activated via RIBE [24]. In the present study, we further wanted to determine whether the JA signal pathway likewise mediates the bystander epigenetic effects. For this purpose, transcript levels of the *TSI* and *180-bp* repeats in seedlings of lines *aos* and *jar1-1* were examined using qRT-PCR at 24 h after root irradiation. As shown in Fig. 4A and B, RIBE-mediated activation of the *TSI* and *180-bp* repeats was significantly repressed in plants of lines *aos* and *jar1-1* compared to the wild-type plants with root irradiation (in all cases, $P < 0.01$), although with enhanced activation compared with their non-irradiated control plants (in all cases, $P < 0.01$). These results suggest that the JA signal pathway might also be involved in the radiation-induced bystander epigenetic effects.

3.4. Roles of the JA signal pathway in generation of bystander signals and bystander radiation responses

After confirming the involvement of the JA signal pathway in plant RIBE, we speculated on which step(s) the pathway mediates in RIBEs, generation of bystander signals in hit roots or radiation responses in bystander aerial tissues, or both. To address this, the well-established technique of root micro-grafting was adopted [26], as shown in Fig. 1C. When we conducted grafting of *aos* (R)-15-6# (A) and *jar1-1*(R)-15-6# (A), in which roots of lines *aos* and *jar1-1* were respectively grafted to aerial plants of line 15-6#, RIBE-mediated expression of the *AtRAD54* gene (GUS activity) was completely inhibited compared to the control grafted plants without root irradiation (in both cases, $P > 0.05$), as shown in Fig. 5A. These results suggest that the JA signal pathway might participate in the generation of bystander signals in irradiated roots.

Following grafting of WT(R)-*aos*(A) and WT(R)-*jar1-1*(A), RIBE-mediated expression of the *AtRAD54* gene was similarly inhibited compared to the control grafting without root irradiation (in both cases, $P > 0.05$), as shown in Fig. 5B and C, suggesting that JA biosynthesis and signaling cascades might both be involved in the induction of radiation responses in bystander aerial parts of plants. In order to further confirm this, we examined the mRNA levels of several important genes involved in JA biosynthesis in bystander aerial parts of plants, and found that root irradiation changed the expression of the *LOX2*, *AOC2*, and *AOS* genes at dif-
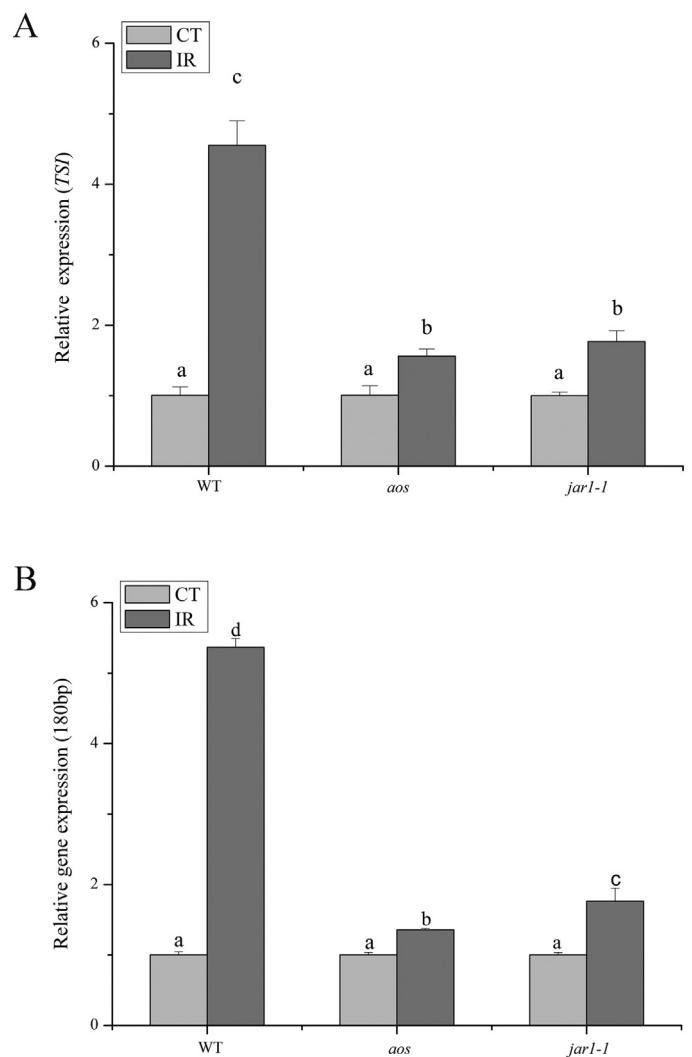

Fig. 4. The RIBE-mediated alleviation of TGS in mutant plants deficient in JA biosynthesis and signaling cascades. (A) RNA level of *TSI* in aerial plants of lines *aos* and *jar1-1* after root irradiation; (B) RNA level of *180-bp* repeats in aerial plants of lines *aos* and *jar1-1* after root irradiation. The results are means $\pm$ SD [n = 3 for RNA level, the same lowercase letter denotes a non-significant difference between means ($P < 0.05$)].

ferent time points after root irradiation (Fig. 6A), suggesting a *de novo* biosynthesis of JA in bystander aerial tissues after root irradiation. Histochemical staining of GUS activity in seedlings of line A1 showed an enhanced expression of the *AOS* gene only in some specific regions of leaves (Fig. 6B). At the same time, expression of the *PDF1.2* gene, a downstream effector of JA signaling cascades [37], was significantly up-regulated after root irradiation (Fig. 6A).

3.5. Effects of over-accumulation of endogenous JA on RIBE

In the above experiment, we found that deficiency of JA biosynthesis in plants can inhibit RIBE. We further wanted to determine the effect of over-accumulation of endogenous JA on RIBE. For this purpose, we used a mutant of line *fou2*, in which a missense mutation in the putative voltage sensor of *TPC1* gene leads to an enhanced level of endogenous JA through up-regulating expression of the *LOX* and *AOS* genes [51]. Surprisingly, the over-accumulation of endogenous JA inhibited RIBE-mediated expression of the *AtRAD54* gene compared to *fou2* plants without root irradiation ($P > 0.05$), as shown in Fig. 7A. We further investigated the effect of over-accumulation of JA only in roots on RIBE using
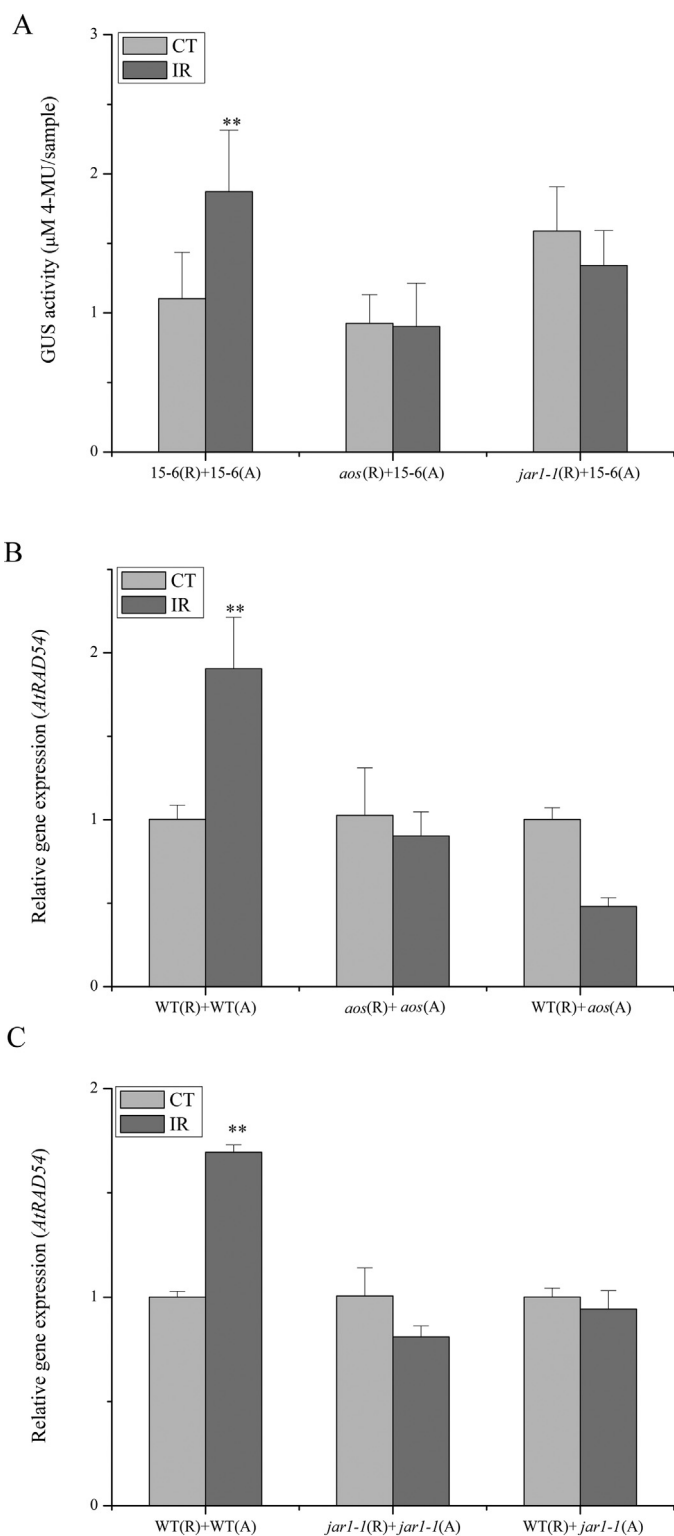
Fig. 5. Roles of the JA signal pathway in generation of bystander signals and bystander responses. (A) GUS activity (*AtRAD54-GUS*) in the aerial scions of line 15-6#, which were grafted to rootstocks of line *aos* or *jar1-1*; (B) mRNA level of the *AtRAD54* gene in the aerial scion of line *aos*, which were grafted to wild-type rootstocks; (C) mRNA level of the *AtRAD54* gene in the aerial scions of line *jar1-1*, which were grafted to wild-type rootstocks. Results are means ± SD (n ≥ 12 for GUS activity, n = 3 for RNA level, *t* test *$P < 0.05$ and **$P < 0.01$).
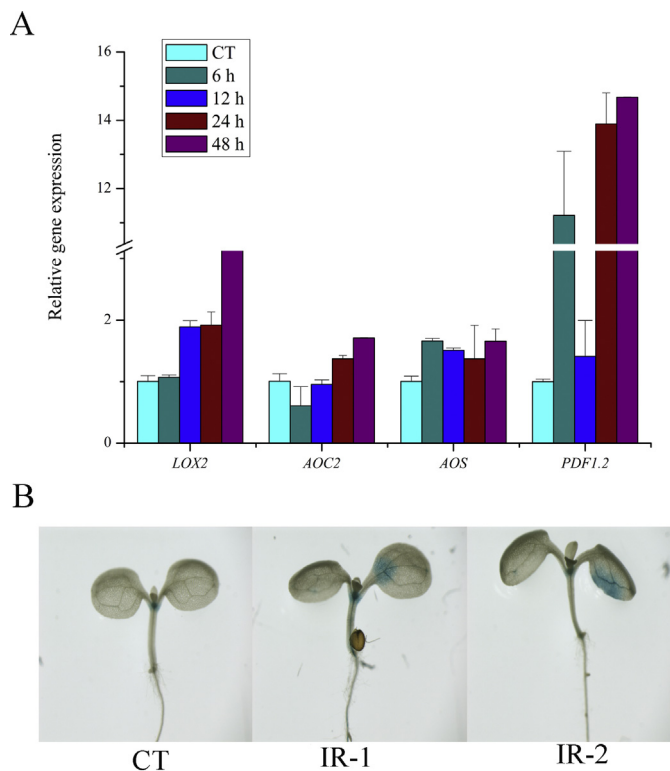
Fig. 6. The effects of root irradiation on JA biosynthesis and signaling pathways in the aerial parts of plants. (A) mRNA level of the *LOX2*, *AOC2*, *AOS*, and *PDF1.2* genes in the aerial tissues at indicated time-points after 10 Gy of root-irradiation; (B) The histochemical staining of line A1 plants after 10 Gy of root irradiation. Results are means ± SD (n = 3 for RNA level, *t* test *$P < 0.05$ and **$P < 0.01$).

fou2(R)-15-6#(A) grafting, and found that the over-accumulation of JA in roots did not affect the induction of *AtRAD54* gene expression in bystander aerial tissues, as shown in Fig. 7B. The results suggest that higher concentrations of endogenous JA did not interfere with the generation of bystander signals in roots.

3.6. Effects of over-accumulation of endogenous JA on RIBE-mediated NHEJ

In addition to HR, NHEJ is an alternative repair mechanism for DNA double-strand breaks. In higher plants, HR is usually switched to NHEJ as DNA damage increases [55]. Therefore, it is likely that in a background of endogenous JA over-accumulation, the repressed expression of the *AtRAD54* gene is due to the switch from HR to NHEJ. To examine this, we measured expression of the *AtKU70* and *AtLIG4* genes, encoding two important components in NHEJ [56,57]. It was found that wild-type plants exhibited down-regulated expression of the *AtKU70* gene at 8 and 24 h after root irradiation (in both cases, $P < 0.05$) and the *AtLIG4* gene at 24 h ($P < 0.05$), as shown in Fig. 8A. The results suggest that in a wild-type background, plants might preferentially employ HR in response to root irradiation. Interestingly, the mutant of line *fou2* exhibited significantly up-regulated expression of the *AtKU70* and *AtLIG4* genes after root irradiation, as shown in Fig. 8B, indicating RIBE-mediated up-regulation of NHEJ in the background of endogenous JA over-accumulation.

4. Discussion

Although radiation-induced bystander effects have been well demonstrated in plants, the signaling molecules and signaling cascades involved in the long-distance bystander communication are
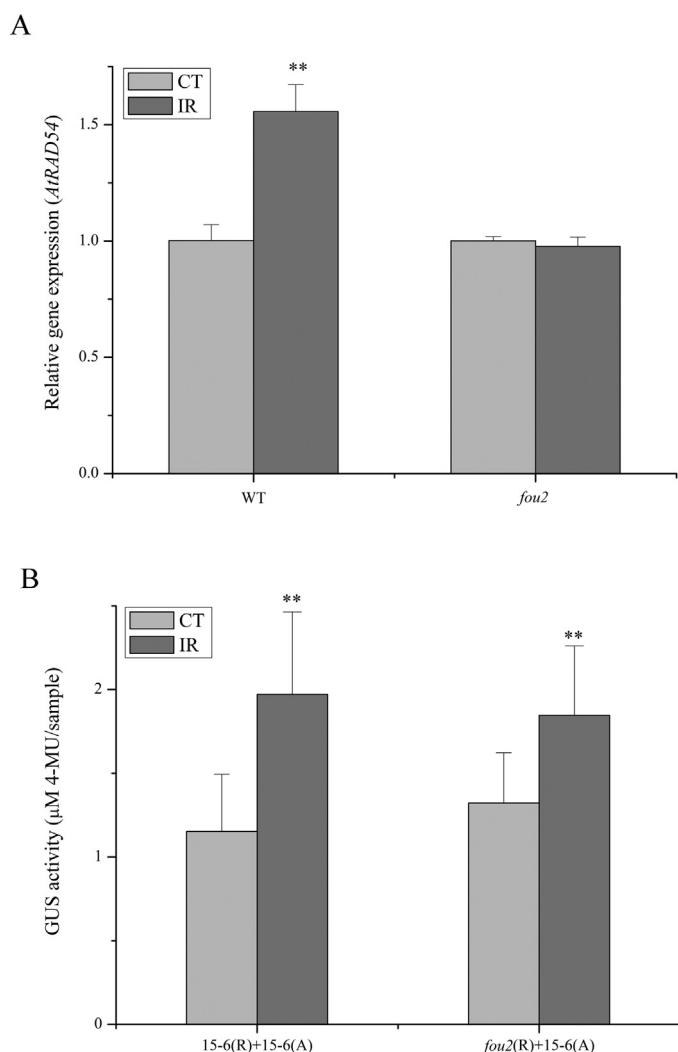
A

B

Fig. 7. The effects of over-accumulation of endogenous JA on RIBE in plants. (A) mRNA level of the *AtRAD54* gene in aerial tissues of line *fou2* after root irradiation; (B) GUS activity (*AtRAD54-GUS*) in the aerial scions of line 15-6#, which were grafted to rootstocks of line *fou2*. Results are means ± SD (n ≥ 12 for GUS activity, n = 3 for RNA level, *t* test *$P < 0.05$ and **$P < 0.01$).

A

B

Fig. 8. Employment of NHEJ in plants of line *fou2* after root irradiation. (A) mRNA level of the *AtKU70* and *AtLIG4* genes in the aerial parts of wild-type plants after root irradiation; (B) mRNA level of the *AtKU70* and *AtLIG4* genes in aerial tissues of line *fou2* after root irradiation. Results are means ± SD (n = 3 for RNA level, *t* test *$P < 0.05$ and **$P < 0.01$).

largely unknown. In this study, we present robust evidence showing that the JA signal pathway is involved in the RIBE in plants. Interestingly, the application of exogenous MeJA readily restored RIBEs in the background of JA biosynthesis deficiency. However, application of exogenous MeJA alone did not up-regulate expression of the *AtRAD54* and *AtRAD51* genes in plants of lines *aos* (Fig. 3C and D), and had no effect on expression of the *AtRAD54* gene (GUS activity) in plants of line 15-6# (wild-type background) (see Supplementary material Fig. S2 in the online version at DOI: 10.1016/j.mrfmmm.2016.07.002). Therefore, it is likely that RIBE in plants might be mediated jointly by the JA signal pathway and other potential signal pathway(s), such as those of salicylic acid, ethylene, auxin, abscisic acid and gibberellin acid, which have been reported to cross-talk with the JA signaling pathway [58–60]. In the cell culture systems, it is well accepted that RIBE rely on multiple signal transduction pathways with a unified model, and that the cooperation of these signals contributed to the final consequences [61,62].

In this study, it was shown that JA biosynthesis is involved in both the generation of bystander signals in irradiated root cells and radiation responses in bystander aerial tissues. We also found that root irradiation led to up-regulated expression of the *LOX2*,
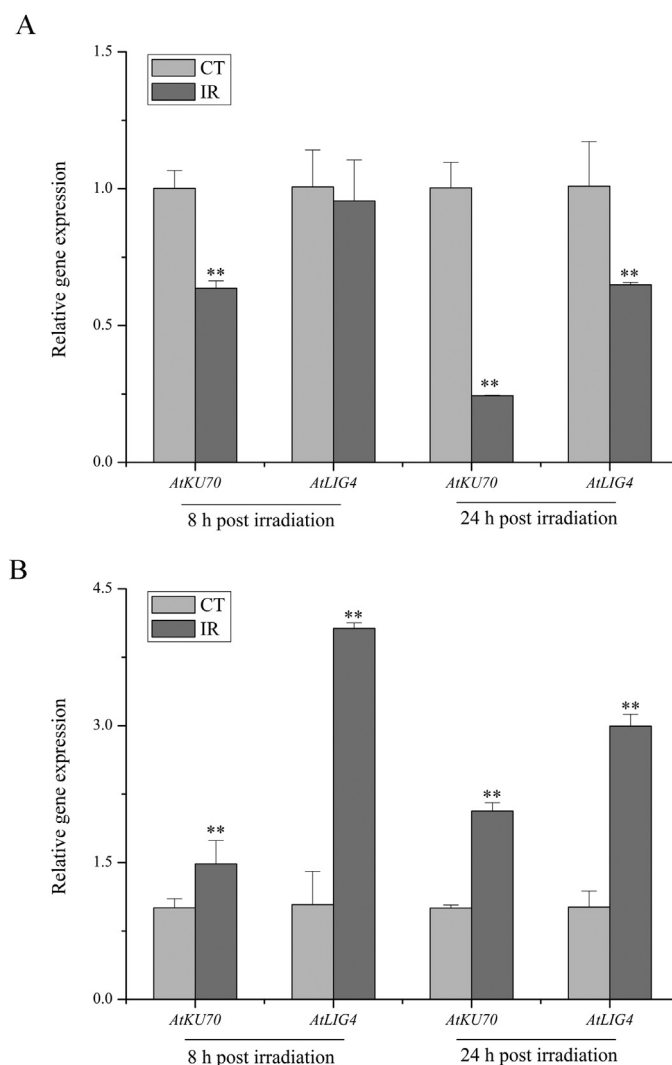
AOC2, and *AOS* genes in aerial parts of plants (see Fig. 6A and B), suggesting a *de novo* biosynthesis of JA in bystander aerial tissues. However, despite their confirmed role as long-distance mobile signals in systemic wound response [44–46], it remains undetermined whether JA or its derivatives act directly as long-distance mobile signals in the root-to-shoot bystander communication. It has been reported that some physical signals, such as hydraulic signals, are transferred from stressed roots to shoots via vascular systems, and then converted into chemical signals such as JA in the aerial parts of plants [63,64]. Therefore, we cannot excluded the possibility that in the root-to-shoot bystander signaling, non-JA factors act as long-distance mobile bystander signals. If this is the case, the JA signal pathway in irradiated roots might only mediate the generation of bystander signals in a cell-autonomous manner. Thus, it is likely that in bystander aerial tissues, the mobile signals conveyed from roots trigger JA biosynthesis and subsequent JA signaling cascades. This then raises the question of how the JA signal pathway in bystander aerial tissues is coupled to the DNA damage repair mechanisms. It is well known that oxidative stress in cells can cause damage to cell structures, including DNA [65], in response to which cells promote their DNA
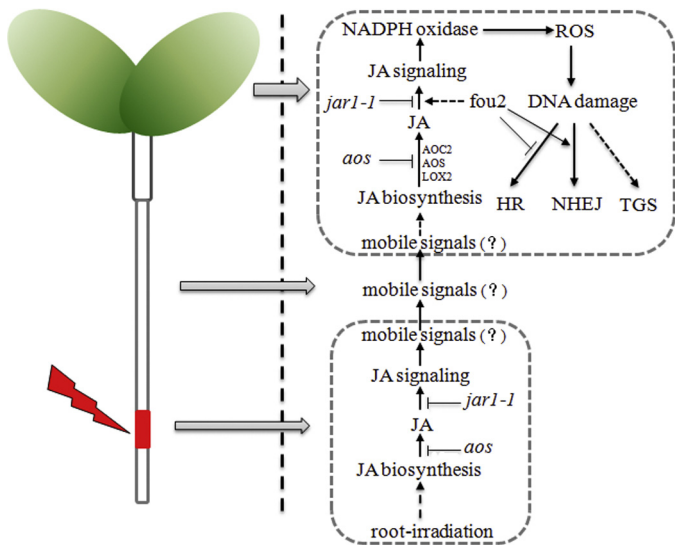
Fig. 9. A schematic model for the role of the JA signal pathway in mediating RIBE in plants. (The solid arrow indicates a certain process, the dashed arrow represents a possible process, and "?" indicates the undermined nature of mobile signals).

repair mechanisms [32], possibly accompanied by the alleviation of TGS [66]. Our previous study showed the increased levels of reactive oxygen species (ROS) and DNA damage in bystander tissues, and we demonstrated that pretreatment with dimethylsulfoxide, a scavenger of ROS, can significantly inhibit the induction of HR and alleviation of TGS [22,24]. Of more importance is the fact that the JA signal pathway can evoke the production of ROS in plants subjected to environmental stress [67]. On the basis of these findings, a schematic model for the role of the JA signal pathway in RIBE in plants is proposed in Fig. 9.

In contrast to HR, NHEJ is an error-prone repair mechanism for DNA double-strand breaks. Under normal conditions, HR is the predominant mechanism by which plants repair DNA damage in an error-free manner [68]. However, with an increase in DNA damage, plants can switch from HR to NHEJ [55]. In the genetic background of *fou2*, NHEJ was up-regulated after root irradiation, whereas HR was suppressed (see Figs. 7A and 8B). Therefore, it is suggested that over-accumulation of endogenous JA might enhance radiosensitivity in terms of RIBE. However, treatment of line *aos* with exogenous MeJA, even at a high concentration of $100\,\mu M$, did not lead to the switch from HR to NHEJ, indicating normal induction of the *AtKU70* and *AtLIG4* genes in plants of line *aos*, (see Fig. 3C and D and Supplementary material Fig. S3 in the online version at DOI: 10.1016/j.mrfmmm.2016.07.002). In this study, it was found that increased activity of AOS only occurred in some specific regions of leaves (see Fig. 6B), and that the expressions of the JA synthesis-related genes were up-regulated at different time-points after root irradiation (see Fig. 6A). These results indicate that biosynthesis of endogenous JA in plants has a unique temporal and spatial pattern, upon which the biological function of JA might largely depend [69,70]. It is clear that treatment with exogenous MeJA does not establish the same temporal and spatial distribution of JA in plant tissues. Accordingly, it is likely that application of exogenous MeJA might only weakly restore the JA signal pathway in plants of line *aos*.

Generally, in this study, the JA signal pathway was shown to mediate the RIBE in plants, which involves in the generation of bystander signals and bystander radiation responses. It is also found that radiosensitivity might be modulated by the level of endogenous JA. However, it still remains undetermined how alpha-irradiation promotes the JA signal pathway in roots and whether JA or its derivatives directly act as root-to-shoot mobile signals.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

References

[1] T.K. Hei, Cyclooxygenase-2 as a signaling molecule in radiation-induced bystander effect, Mol. Carcinog. 45 (2006) 455–460.

[2] W.F. Morgan, Non-targeted and delayed effects of exposure to ionizing radiation: I. Radiation induced genomic instability and bystander effects *in vitro*, Radiat. Res. 159 (2003) 567–580.

[3] J.B. Little, Genomic instability and bystander effects: a historical perspective, Oncogene 22 (2003) 6978–6987.

[4] C. Mothersill, C.B. Seymour, Radiation-induced bystander effects—implications for cancer, Nat. Rev. Cancer 4 (2004) 158–164.

[5] N. Hamada, H. Matsumoto, T. Hara, Y. Kobayashi, Intercellular and intracellular signaling pathways mediating ionizing radiation-induced bystander effects, Radiat. Res. 48 (2007) 87–95.

[6] O.V. Belyakov, M. Folkard, C. Mothersill, K.M. Prise, B.D. Michael, Bystander-induced apoptosis and premature differentiation in primary urothelial explants after charged particle microbeam irradiation, Radiat. Prot. Dosimetry 99 (2002) 249–251.

[7] O.V. Belyakov, M. Folkard, C. Mothersill, K.M. Prise, B.D. Michael, A proliferation-dependent bystander effect in primary porcine and human urothelial explants in response to targeted irradiation, Br. J. Cancer 88 (2003) 767–774.

[8] O.V. Belyakov, S.A. Mitchell, D. Parikh, G. Randers-Pehrson, S.A. Marino, S.A. Amundson, C.R. Geard, D.J. Brenner, Biological effects in unirradiated human tissue induced by radiation damage up to 1 mm away, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 14203–14208.

[9] R. Persaud, H. Zhou, S.E. Baker, T.K. Hei, E.J. Hall, Assessment of low linear energy transfer radiation-induced bystander mutagenesis in a three-dimensional culture model, Cancer Res. 65 (2005) 9876–9882.

[10] O.V. Belyakov, M. Folkard, C. Mothersill, K.M. Prise, B.D. Michael, Bystander induced differentiation: a major response to targeted irradiation of a urothelial explant model, Mutat. Res. 597 (2006) 43–49.

[11] O.A. Sedelnikova, A. Nakamural, O. Kovalchuk, I. Koturbash, S.A. Mitchell, S.A. Marino, D.J. Brenner, W.M. Bonner, DNA double-strand breaks form in bystander cells after microbeam irradiation of three-dimensional human tissue models, Cancer Res. 67 (2007) 4295–4302.

[12] G.E. Watson, S.A. Lorimore, D.A. Macdonald, E.G. Wright, Chromosomal instability in unirradiated cells induced *in vivo* by a bystander effect of ionizing radiation, Cancer Res. 60 (2000) 5608–5611.

[13] L.Y. Xue, N.J. Butler, G.M. Makrigiorgos, S.J. Adelstein, A.I. Kassis, Bystander effect produced by radiolabeled tumor cells *in vivo*, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 13765–13770.

[14] I. Koturbash, R.E. Rugo, C.A. Hendricks, J. Loree, B. Thibault, K. Kutanzi, I. Pogribny, J.C. Yanch, B.P. Engelward, O. Kovalchuk, Irradiation induces DNA damage and modulates epigenetic effectors in distant bystander tissue *in vivo*, Oncogene 25 (2006) 4267–4275.

[15] I. Koturbash, A. Boyko, R. Rodriquez-Juarez, R.J. McDonald, V.P. Tryndyak, I. Kovalchuk, I.P. Pogribny, O. Kovalchuk, Role of epigenetic effectors in maintenance of the long-term persistent bystander effect in spleen *in vivo*, Carcinogenesis 28 (2007) 1831–1838.

[16] I. Koturbash, K. Kutanzi, K. Hendrickson, R. Rodriquez-Juarez, D. Kogosov, O. Kovalchuk, Radiation-induced bystander effects *in vivo* are sex specific, Mutat. Res. 642 (2008) 28–36.

[17] M. Mancuso, E. Pasquali, S. Leonardi, M. Tanori, S. Rebessi, V. Di Majo, S. Pazzaglia, M.P. Toni, M. Pimpinella, V. Covelli, A. Saran, Oncogenic bystander radiation effects in patched heterozygous mouse cerebellum, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 12445–12450.

[18] A. Bertucci, R.D. Pocock, G. Randers-Peherson, D.J. Brenner, Microbeam irradiation of the *C. elegans* nematode, Radiat. Res. 50 (2009) A49–A54.

[19] X.Y. Guo, J. Sun, P. Bian, L.Y. Chen, F.R. Zhan, J. Wang, A. Xu, Y.G. Wang, T.K. Hei, L.J. Wu, Radiation-induced bystander signaling from somatic cells to germ cells in *Caenorhabditis elegans*, Radiat. Res. 180 (2013) 268–275.

[20] G. Yang, L. Wu, L. Chen, B. Pei, Y. Wang, F. Zhan, Y. Wu, Z. Yu, Targeted irradiation of shoot apical meristem of *Arabidopsis* embryos induces long-distance bystander/abscopal effects, Radiat. Res. 167 (2007) 298–305.

[21] G. Yang, T. Mei, H. Yuan, W.M. Zhang, L.Y. Chen, J.M. Xue, L.J. Wu, Y.G. Wang, Bystander/abscopal effects induced by low-energy ion irradiation on intact *Arabidopsis* seeds, Radiat. Res. 170 (2008) 372–380.

[22] F.H. Li, P. Liu, T. Wang, P. Bian, Y.J. Wu, L.J. Wu, Z.L. Yu, The induction of bystander mutagenic effects *in vivo* by α-irradiation in whole *Arabidopsis thaliana* plants, Radiat. Res. 174 (2010) 228–237.

[23] F.H. Li, T. Wang, S.Y. Xu, H. Yuan, P. Bian, Y.J. Wu, L.J. Wu, Z.L. Yu, Abscopal mutagenic effect of low-energy-ions in *Arabidopsis thaliana* seeds, Int. J. Radiat. Biol. 87 (2011) 984–992.

[24] W. Xu, T. Wang, S.Y. Xu, S.X. Xu, L.J. Wu, Y.J. Wu, P. Bian, Radiation-induced bystander epigenetic effects demonstrated in *Arabidopsis thaliana*, Radiat. Res. 183 (2015) 511–524.

[25] T. Wang, Q. Sun, W. Xu, H.S. Li, J.Y. Lu, L.J. Wu, Y.J. Wu, M. Liu, P. Bian, Repressive effect of modeled microgravity on early bystander responses in *Arabidopsis thaliana*, Mutat. Res. 773 (2015) 27–36.

[26] T. Wang, F.H. Li, S.Y. Xu, P. Bian, Y.J. Wu, L.J. Wu, Z.L. Yu, The time course of long-distance signaling in radiation-induced bystander effect *in vivo* in *Arabidopsis thaliana* demonstrated using root micro-grafting, Radiat. Res. 176 (2011) 234–243.

[27] T. Wang, F.H. Li, W. Xu, P. Bian, Y.J. Wu, L.J. Wu, Novel features of radiation-induced bystander signaling in *Arabidopsis thaliana* demonstrated using root micro-grafting, Plant Signal. Behav. 7 (2012) 1566–1572.

[28] K.M. Prise, J.M. O'sullivan, Radiation-induced bystander signalling in cancer therapy, Nat. Rev. Cancer 9 (2009) 351–360.

[29] R. Baskar, Emerging role of radiation induced bystander effects: cell communications and carcinogenesis, Genome Integr. 1 (2010) 13.

[30] W.F. Morgan, M.B. Sowa, Non-targeted bystander effects induced by ionizing radiation, Mutat. Res. 616 (2007) 159–164.

[31] V.W.Y. Choi, C.Y.P. Ng, A. Kobayashi, T. Konishi, N. Suya, T. Ishikawa, S.H. Cheng, K.N. Yu, Bystander effect between zebra fish embryos *in vivo* induced by high-dose X-rays, Environ. Sci. Technol. 47 (2013) 6368–6376.

[32] T.B. Kryston, A.B. Georgiev, P. Pissis, A.G. Georgakilas, Role of oxidative stress and DNA damage in human carcinogenesis, Mutat. Res. 711 (2011) 193–201.

[33] E.I. Azzam, S.M. de Toledo, J.B. Little, Oxidative metabolism, gap junctions and the ionizing radiation-induced bystander effect, Oncogene 22 (2003) 7050–7057.

[34] Y. Yao, C.H. Danna, F.J. Zemp, V. Titov, O.N. Ciftci, R. Przybylski, F.M. Ausubel, I. Kovalchuk, UV-C-Irradiated *Arabidopsis* and tobacco emit volatiles that trigger genomic instability in neighboring plants, Plant Cell 23 (2011) 3842–3852.

[35] W. Xu, T. Wang, S. Wu, F. Li, C. Deng, L. Wu, Y. Wu, P. Bian, UV-C-induced alleviation of transcriptional gene silencing through plant–plant communication: key roles of jasmonic acid and salicylic acid pathways, Mutat. Res. (2016) 56–67, http://dx.doi.org/10.1016/j.mrfmmm.2016.04.003.

[36] R.A. Creelman, J.E. Mullet, Biosynthesis and action of jasmonates in plants, Ann. Rev. Plant Physiol. Plant Mol. Biol. 48 (1997) 355–381.

[37] J.G. Turner, E. Christine, A. Devoto, The jasmonate signal pathway, Plant Cell 14 (2002) S153–S164.

[38] C. Wasternack, B. Hause, Jasmonates and octadecanoids: signals in plant stress responses and development, Prog. Nucleic Acids Res. Mol. Biol. 72 (2002) 165–221.

[39] M.H. Beale, J.L. Ward, Jasmonates: key players in the plant defence, Nat. Prod. Rep. 15 (1998) 533–548.

[40] B. Hause, I. Stenzel, O. Miersch, H. Maucher, R. Kramell, J. Ziegler, C. Wasternack, Tissue-specific oxylipin signature of tomato flowers: allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles, Plant J. 24 (2000) 113–126.

[41] F. Schaller, A. Schaller, A. Stintzi, Biosynthesis and metabolism of jasmonates, J. Plant Growth Regul. 123 (2005) 179–199.

[42] S. Fonseca, J.M. Chico, R. Solano, The jasmonate pathway: the ligand, the receptor and the core signalling module, Curr. Opin. Plant Biol. 12 (2009) 539–547.

[43] P.E. Staswick, W. Su, S.H. Howell, Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 6837–6840.

[44] G.A. Howe, Jasmonates as signals in the wound response, J. Plant Growth Regul. 23 (2004) 223–237.

[45] E.E. Farmer, E. Almeras, V. Krishnamurthy, Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory, Curr. Opin. Plant Biol. 6 (2003) 372–378.

[46] L. Li, C. Li, G.I. Lee, G.A. Howe, Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 6416–6421.

[47] K. Osakabe, K. Abe, T. Yoshioka, Y. Osakabe, S. Todoriki, H. Ichikawa, B. Hohn, S. Toki, Isolation and characterization of the RAD54 gene from *Arabidopsis thaliana*, Plant J. 48 (2006) 827–842.

[48] G. Bonaventure, A. Gfeller, W.M. Proebsting, S. Hortensteiner, A. Chetelat, E. Martinoia, E.E. Farmer, A gain-of-function allele of TPC1 activates oxylipin biogenesis after leaf wounding in *Arabidopsis*, Plant J. 49 (2007) 889–898.

[49] J.H. Park, R. Halitschke, H.B. Kim, I.T. Baldwin, K.A. Feldman, R. Feyereisen, A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis, Plant J. 31 (2002) 1–12.

[50] P.E. Staswick, I. Tiryaki, M.L. Rowe, Jasmonate response locus JAR1 and several related *Arabidopsis* genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation, Plant Cell 14 (2002) 1405–1415.

[51] I. Kubigsteltig, D. Laudert, E.W. Weiler, Structure and regulation of the *Arabidopsis thaliana* allene oxide synthase gene, Planta 208 (1999) 463–471.

[52] M. Blazquez, Quantitative GUS activity assay, in: D. Weigel, J. Glazebrook (Eds.), *Arabidopsis*: a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2002, pp. 249–252.

[53] L.L. Li, S. Santerre-Ayotte, E.B. Boivin, M. Jean, F. Belzile, A novel reporter for intrachromosomal homoeologous recombination in *Arabidopsis*, Plant J. 40 (2004) 1007–1015.

[54] W. Maksymiec, Z. Krupa, Effects of methyl jasmonate and excess copper on root and leaf growth, Biol. Plant. 51 (2007) 322–326.

[55] A. Boyko, F. Zemp, J. Filkowski, I. Kovalchuk, Double-strand break repair in plants is developmentally regulated, Plant Physiol. 141 (2006) 488–497.

[56] P.O. Mari, B.I. Florea, S.P. Persengiev, N.S. Verkaik, H.T. Bruggenwirth, M. Modesti, G. Giglia-Mari, K. Bezstarosti, J.A. Demmers, T.M. Luider, A.B. Houtsmuller, D.C. van Gent, Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 18597–18602.

[57] S. Costantini, L. Woodbine, L. Andreoli, P.A. Jeggo, A. Vindigni, Interaction of the Ku heterodimer with the DNA ligase IV/Xrcc4 complex and its regulation by DNA-PK, DNA Repair (Amst.) 6 (2007) 712–722.

[58] K. Kazan, J.M. Manners, Jasmonate signaling: toward an integrated view, Plant Physiol. 146 (2008) 1459–1468.

[59] J.J. Cheong, Y.D. Choi, Signaling pathways for the biosynthesis and action of jasmonate, J. Plant Biol. 50 (2007) 122–131.

[60] M.B. Traw, J. Bergelson, Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*, Plant Physiol. 133 (2003) 1367–1375.

[61] T.K. Hei, H. Zhou, V.N. Ivanov, M. Hong, H.B. Lieberman, D.J. Brenner, S.A. Amundson, C.R. Geard, Mechanism of radiation-induced bystander effects: a unifying model, J. Pharm. Pharmacol. 60 (2008) 943–950.

[62] H. Zhou, V.N. Ivanov, Y.C. Lien, M. Davidson, T.K. Hei, Mitochondrial function and NF-kB mediated signaling in radiation-induced bystander effects, Cancer Res. 68 (2008) 2233–2240.

[63] Y. Sogabe, H. Nakamura, T. Nakagawa, S. Hasegawa, T. Asano, H. Ohta, K. Yamaguchi, M.J. Mueller, H. Kodama, T. Nishiuchi, Visualization of wounding-induced root-to-shoot communication in *Arabidopsis*, Plant Signal. Behav. 6 (2011) 1037–1039.

[64] V. Hlavackova, J. Naus, Chemical signal as a rapid long distance information messenger after local wounding of a plant? Plant Signal. Behav. 2 (2007) 103–105.

[65] I.M. Moller, P.E. Jensen, A. Hansson, Oxidative modifications to cellular components in plants, Annu. Rev. Plant Biol. 58 (2007) 459–481.

[66] Chun-Hsin Liu, Andreas Finke, Mariana Diaz, Wilfried Rozhon, Brigitte Poppenberger, Tuncay Baubec, Ales Pecinka, Repair of DNA damage induced by the cytidine analog Zebularine requires ATR and ATM in *Arabidopsis*, Plant Cell 27 (2015) 1788–1800.

[67] Shintaro Munemasa, Izumi C. Mori, Yoshiyuki Murata, Methyl jasmonate signaling and signal crosstalk between methyl jasmonate and abscisic acid in guard cells, Plant Signal. Behav. 6 (2011) 939–941.

[68] D. Schuermann, J. Molinier, O. Fritsch, B. Hohn, The dual nature of homologous recombination in plants, Trends Genet. 21 (2005) 172–181.

[69] G. Glauser, E. Grata, L. Dubugnon, S. Rudaz, E.E. Farmer, J.L. Wolfender, Spatial and temporal dynamics of jasmonate synthesis and accumulation in *Arabidopsis* in response to wounding, J. Biol. Chem. 283 (2008) 16400–16407.

[70] T.O. Tytgat, K.J. Verhoeven, J.J. Jansen, C.E. Raaijmakers, T. Bakx-Schotman, L.M. Mclntyre, W.H. van der Putten, A. Biere, N.M. van Dam, Plants know where it hurts: root and shoot jasmonic acid induction elicit differential responses in Brassica oleracea, PLoS One 8 (2013) e65502.