Cell death resulted from loss of fumarylacetoacetate hydrolase in *Arabidopsis* is related to phytohormone jasmonate but not salicylic acid

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Fumarylacetoacetate hydrolase (FAH) catalyzes the final step in Tyr degradation pathway essential to animals but not well understood in plants. Previously, we found that mutation of *SSCD1* encoding *Arabidopsis* FAH causes cell death under short day, which uncovered an important role of Tyr degradation pathway in plants. Since phytohormones salicylic acid (SA) and jasmonate (JA) are involved in programmed cell death, in this study, we investigated whether *sscd1* cell death is related to SA and JA, and found that (1) it is accompanied by up-regulation of JA- and SA-inducible genes as well as accumulation of JA but not SA; (2) it is repressed by breakdown of JA signaling but not SA signaling; (3) the up-regulation of reactive oxygen species marker genes in *sscd1* is repressed by breakdown of JA signaling; (4) treatment of wild-type *Arabidopsis* with succinylacetone, an abnormal metabolite caused by loss of FAH, induces expression of JA-inducible genes whereas treatment with JA induces expression of some Tyr degradation genes with dependence of JA signaling. These results demonstrated that cell death resulted from loss of FAH in *Arabidopsis* is related to JA but not SA, and suggested that JA signaling positively regulates *sscd1* cell death by up-regulating Tyr degradation.

Programmed cell death (PCD) is a sequence of genetically regulated events resulting in the elimination of specific cells, tissues, or whole organs¹, which is required both for normal development and to face stress conditions²-⁴. In plants, one well-characterized example of PCD is hypersensitive response taking place on incompatible plant–pathogen interactions³, which leads to cell death and then forms visible lesions at the site infected by an avirulent pathogen, as a result, limits the pathogen spread⁴. Phytohormones including salicylic acid (SA) and jasmonate (JA) appear to be key players for hypersensitive response regulation⁵.

To date, a large number of mutants displaying spontaneous cell death lesions have been identified in plants including *Arabidopsis*, rice, barley, maize, and so on⁶-⁹. These mutants have been named as lesion-mimic mutants (LMM) because of the form of lesions in the absence of pathogen infection¹⁰. In some of LMM, the SA or JA signaling has been activated¹¹. By isolating LMM's genes, many of regulators that play important roles in PCD and SA or JA signal defense responses have been identified, including ACCELERATED CELL DEATH¹¹, LESION SIMULATING DISEASE¹, and NICOTIANA BENTHAMIANA HOMEOBO¹²-¹⁴.

SA is involved in plant defense and cell death¹⁵,¹⁶. The level of SA correlates with the expression of *PATHOGENESIS-RELATED1* (*PRI1*) gene and resistance to pathogen attack¹⁷,¹⁸. The NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (*NPR1*) gene is required for SA-induced expression of *PRI1* gene and resistance in *Arabidopsis*¹⁹,²⁰.

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Jasmonates (JAs) including jasmonic acid, methyl jasmonate (MeJA), and other derivatives, are a basic class of plant hormones involved in different processes, including plant growth, development, and responses to biotic and abiotic stresses23–25. JA signaling pathway is closely involved in plant PCD24,25. The F-box protein CORONATINE INSENSITIVE1 (COI1) has been found to be an indispensable component of the JA signaling pathway26–28. JA induces expression of many genes including those for vegetative storage proteins (VSPs), a thionin (THI2.1), and a plant defensin (PDF1.2), which is abolished in the coi1 mutant29,30.

In plants, PCD also correlates to reactive oxygen species (ROS), which produces in plants as byproducts of aerobic metabolism and controls a variety of physiological functions including responses to abiotic and biotic stress and plant growth and development30,31. The generation of ROS is one of the most normal responses to PCD32–34 and the genes associated with oxidative stress are up-regulated during PCD35–38. For example, the expression of ascorbate peroxidase 2 (APX2) is rapidly induced by oxidative stress37. Oxidative signal inducible 1 (OXI1) is regulated by ROS and the OXI1 expression is specifically induced by stress conditions that cause cell death38,39. The expressions of bonzai1-associated protein1 (BAP1) and a putative c2h2 zinc finger transcription factor (ZP) are induced specifically by singlet oxygen, one form of ROS40.

In addition, plant PCD is resulted from blockage of some metabolic pathways such as Tyr degradation41, an essential pathway to animals42. The Tyr degradation pathway includes five-step enzymatic reactions42. First, Tyr aminotransferase (TAT) catalyzes the conversion of Tyr into 4-hydroxyphenylpyruvate, which is then converted into homogentisate by 4-hydroxyphenylpyruvate dioxygenase. Next, homogentisate dioxygenase (HGO) catalyzes homogentisate to yield maleylacetoacetate that is isomerized by maleylacetoacetate isomerase (MAAI) to fumarylacetoacetate, and finally fumarylacetoacetate hydrolase (FAH) hydrolyzes fumarylacetoacetate to fumarate and acetoacetate42. Loss of FAH results in the accumulation of fumarylacetoacetate and maleylacetoacetate, both of which undergo spontaneous reduction to succinylacetoacetate that is converted to succinylacetone (SUAC) by spontaneous non-enzymatic decarboxylation42. SUAC is toxic to cells and tissues resulting in severe metabolic disorders in mammals43–44. In Arabidopsis, we have identified one LMM named as short-day sensitive cell death 1 (sscd1) displaying spontaneous cell death lesions under short day (SD) conditions, and isolated the SSSD1 gene encoding the Arabidopsis putative FAH, which uncovered the role of Tyr degradation pathway in plant41.

To investigate whether the appearance of spontaneous cell death lesions in the sscd1 mutant is related to SA and JA, in this study, we first analyzed expression of some SA- and JA-inducible genes and then generated double mutants of sscd1 with npr1 and coi1, respectively, and found that cell death in sscd1 is accompanied by JA accumulation and repressed by mutation of COI1, however, it is unrelated to SA although it is accompanied by up-regulation of SA-inducible PR1. Furthermore, we found that the up-regulation of ROS marker genes such as APX2, OXI1, BAP1, and ZP in the sscd1 mutant is also repressed by mutation of COI1. In addition, we found that treatment of Arabidopsis seedlings with SUAC induces expression of JA-inducible genes. However, treatment with JA induces expression of some Tyr degradation pathway genes including TAT3 encoding an Arabidopsis putative TAT45, HGO, and MAAI, which is dependence of COI1. Our work uncovered a crosstalk between JA signaling and Tyr degradation pathway in the regulation of sscd1 cell death, i.e. JA signaling positively regulates sscd1 cell death by up-regulating Tyr degradation.

Results

Cell death in sscd1 is uncorrelated to SA signaling although it is accompanied by up-regulation of SA-inducible PR1. The sscd1 mutant grows normally under long day (LD), but displays obvious cell death symptoms after transferred to SD for 3 days41. To investigate whether cell death in sscd1 is related to SA, we first analyzed expression of PR1, one of SA-inducible genes, in wild-type and sscd1 seedlings transferred from LD to SD for 1, 2 and 3 days by quantitative real-time polymerase chain reaction (RT-qPCR). As shown in Fig. 1a, no significant difference in the expression level of PR1 between wild type and sscd1 was observed before seedlings were transferred to SD or after they were transferred to SD for 1 day, however, the expression level of PR1 was significantly increased in sscd1 compared to wild type when seedlings were transferred to SD for 2 days and that this increase was much more obvious after seedlings were transferred to SD for 3 days.

Since SA-inducible gene PR1 was significantly up-regulated in the sscd1 mutant compared to wild type when seedlings were transferred from LD to SD for 2–3 days (Fig. 1a), we next measured the content of SA to investigate whether up-regulation of PR1 is resulted from accumulation of SA in the sscd1 mutant. Unexpected, the content of SA was not significantly increased in sscd1 compared to wild type before seedlings were transferred to SD or after they were transferred to SD for 2 or 3 days (Fig. 1b). Therefore, the up-regulation of PR1 in sscd1 was not related to SA.

NPR1 is a SA receptor in SA signaling46 and expression of SA-inducible PR1 is abolished in the npr1 mutant19. To investigate whether loss of NPR1 influences the up-regulation of PR1 as well as the cell death in sscd1, a double mutant of sscd1 and npr1-1 was generated and then expression of PR1 was analyzed as well as the seedlings phenotype was observed. As shown in Fig. 1c, expression of PR1 was almost undetected in the npr1-1 mutant whereas it was significantly induced in the sscd1npr1 double mutant although its level was much lower than that in the sscd1 single mutant after seedlings were transferred to SD for 3 days. Furthermore, the rate of seedlings death in sscd1npr1 was similar to that in sscd1 (Fig. 1d). These results demonstrated that both up-regulation of PR1 and cell death in sscd1 are independent of NPR1 and that cell death in sscd1 is uncorrelated to SA signaling although it is accompanied by the up-regulation of SA-inducible PR1.

Cell death in sscd1 is accompanied by up-regulation of JA-inducible genes and accumulation of jasmonic acid. Since cell death in sscd1 is uncorrelated to SA signaling (Fig. 1), we next investigated whether it is related to JA signaling. We first analyzed the expression of JA-inducible genes including VSP2, PDF1.2, and THI2.1 in wild-type and sscd1 seedlings which were transferred from LD to SD for 1, 2 and 3 days. The results
showed that the expression level of these genes was similar in wild type and sscd1 before seedlings were trans-ferred to SD or after they were transferred to SD for 1 day; however, it was significantly increased in sscd1 com-pared to wild type after seedlings were transferred to SD for 2 days and that this increase was much more obvious after seedlings were transferred to SD for 3 days (Fig. 2a–c). Then, we measured the content of jasmonic acid in wild type and sscd1 before seedlings were transferred to SD or after they were transferred to SD for 2 and 3 days to investigate whether the up-regulation of these genes is resulted from the accumulation of jasmonic acid. The result showed that there was no significant difference in the content of jasmonic acid between wild type and sscd1 before seedlings were transferred to SD or after they were transferred to SD for 2 and 3 days (Fig. 2d). These results indicated that the cell death in sscd1 is accompanied by both up-regulation of JA-inducible genes and accumulation of jasmonic acid and suggested that the up-regulation of JA-inducible genes is caused by the accumulation of jasmonic acid.

**Figure 1.** Cell death in sscd1 is uncorrelated to SA signaling although it is accompanied by the up-regulation of SA-inducible PR1. (a) Relative expression level of SA-inducible genes PR1 in wild-type (WT) and sscd1 seedlings that were grown under LD for 3 weeks, and then transferred to SD for 0, 1, 2 and 3 days. (b) The content of SA in wild-type (WT) and sscd1 seedlings that were grown under LD for 3 weeks, and then transferred to SD for 0, 2 and 3 days. (c) Relative expression level of SA-inducible genes PR1 in wild-type (WT), npr1-1, sscd1 and sscd1npr1 seedlings that were grown under LD for 3 weeks, and then transferred to SD for 3 days. (d) The rate of seedlings death in sscd1 and sscd1npr1 seedlings grown on MS under SD for 6–9 days. LD, long day; SD, short day. The expression of gene was analyzed by RT-qPCR, relative expression level was normalized to those of ACTIN2 and the control (in wild type) was set to 1. Mean ± SE from three biological replicates. Asterisk ** represents the significance of differences (two-tailed Student’s t-test) at the level of P < 0.01.

**Cell death in sscd1 is repressed by mutation of COI1.** COI1 is a JA receptor in JA signaling. To investigate whether JA signaling mediates the sscd1 cell death, we generated the sscd1/coi1 double mutant through a cross of sscd1 with coi1-2 to break the JA signaling, and then observed the phenotype of seedlings. It was interesting that the phenotype of seedlings death was obviously rescued in sscd1/coi1 compared to sscd1 (Fig. 3a). For example, 65% of 7-old sscd1 seedlings grown under SD were dead whereas the rate of sscd1/coi1 seedlings death was only 43% (Fig. 3b). This result suggested that the cell death in sscd1 is repressed by breakdown of JA signaling through mutation of COI1 and that JA signaling positively regulates the sscd1 cell death.
Figure 2. Cell death in sscd1 is accompanied by the up-regulation of JA-inducible genes and the accumulation of jasmonic acid. (a–c) Relative expression level of JA-inducible genes VSP2 (a), PDF1.2 (b), THI2.1 (c) in wild-type (WT) and sscd1 seedlings that were grown under LD for 3 weeks, and then transferred to SD for 0, 1, 2 and 3 days. (d) The content of jasmonic acid in wild-type (WT) and sscd1 seedlings that were grown under LD for 3 weeks, and then transferred to SD for 0, 2 and 3 days. LD, long day; SD, short day. The expression of genes was analyzed by RT-qPCR, relative expression level was normalized to those of ACTIN2 and the control (in wild type) was set to 1. Mean ± SE from three biological replicates. Asterisk * and ** represent the significance of differences (two-tailed Student’s t-test) at the levels of P < 0.05 and P < 0.01, respectively.

Figure 3. Cell death in sscd1 is reduced by mutation of COI1. (a) The phenotype of wild-type (WT), sscd1, coi1-2 and sscd1coi1 seedlings grown on MS under SD for 7 days. (b) The rate of seedlings death in coi1-2 and sscd1coi1 seedlings grown on MS under SD for 7 days. SD, short day. Mean ± SE from three biological replicates. Asterisk ** represents the significance of differences (two-tailed student’s t-test) at the level of P < 0.01.
Mutation of COI1 suppresses the up-regulation of ROS marker genes in sscd1. Previously, we found that ROS marker genes such as APX2, OXI1, BAP1 and ZP were up-regulated before an occurrence of cell death in the sscd1 mutant48, so, we next investigated whether the repression of cell death in sscd1 by mutation of COI1 is correlated with the expression of these genes. Since the cell death phenotype of sscd1 seedlings that were grown under SD appeared on the 6th day41,49, therefore, we tested the expression of APX2, OXI1, BAP1 and ZP in seedlings grown under SD for 5 days. As shown in Fig. 4, the expression pattern of APX2, OXI1, BAP1 and ZP was similar in both WT and coi1-2, however, the up-regulation of these genes in sscd1 was significantly suppressed in sscd1coi1 (Fig. 4), which indicated that the up-regulation of ROS marker genes in sscd1 could be suppressed by the mutation of COI1.

SUAC treatment activates the expression of JA-inducible genes. Previously, we speculated that the cell death in sscd1 is resulted from the accumulation of SUAC and also found that treatment of Arabidopsis wild-type seedlings with SUAC mimicked the cell death phenotype of sscd141. We next investigated whether SUAC treatment activates the expression of JA-inducible genes. To this end, we analyzed the expression of VSP2 and THI2.1 in wild-type seedlings treated with SUAC, in which some leaves started wilting. The result showed the expression of both VSP2 and THI2.1 was significantly increased upon SUAC treatment (Fig. 5), indicating that SUAC treatment could activate the expression of JA-inducible genes.

Treatment with MeJA causes the COI1-dependent up-regulation of some Tyr degradation pathway genes. Since the cell death in sscd1 is accompanied by the accumulation of jasmonic acid (Fig. 2d) and could be repressed by breakdown of JA signaling through mutation of COI1 (Fig. 3), we next investigated whether treatment of Arabidopsis wild-type and coi1-2 seedlings with MeJA influences the Tyr degradation pathway by analyzing the expression of Tyr degradation pathway genes including TAT3, HGO, MAAI, and SCD1. The results showed that the expression level of TAT3, HGO, and MAAI except SCD1 was significantly increased in wild type upon MeJA treatment (Fig. 6), especially, an increase of TAT3 expression level in wild type treated with MeJA was much more significant compared with HGO and MAAI (Fig. 6a–c). However, it was interesting that the expression level of these genes was not significantly increased in the coi1-2 mutant upon MeJA treatment (Fig. 6). These results suggested that MeJA up-regulates the expression of some Tyr degradation pathway genes, which would promote Tyr degradation, however, the breakdown of JA signaling through mutation of COI1 could eliminate an effect of JA on Tyr degradation pathway.
Discussion

Tyr degradation pathway is essential to animals42 but it is not well understood in plants. Previously, we found that mutation of \textit{SSCD1} encoding \textit{Arabidopsis} FAH, an enzyme catalyzing the final step of Tyr degradation pathway, results in spontaneous cell death under SD, which uncovered an important role of Tyr degradation pathway in plants41. Afterwards, we found that sugar suppresses cell death caused by disruption of FAH in \textit{Arabidopsis}, indicating that Tyr degradation is regulated by sugar in plants49. Recently, we found that cell death resulted from loss of FAH in \textit{sscd1} is related to chlorophyll (Chl) biosynthesis, suggesting a crosstalk between Tyr degradation and Chl biosynthetic pathways in mediating the \textit{sscd1} cell death48. Phytohormones such as SA and JA are involved in PCD13–16,24,25,50. In this study, the investigation whether cell death resulted from loss of FAH in \textit{Arabidopsis} is related to SA and JA would expand our understandings on the regulation of Tyr degradation pathway in plants.

Through testing expression of SA-inducible \textit{PR1} and content of SA, we found that cell death in \textit{sscd1} was accompanied by the up-regulation of SA-inducible \textit{PR1} and content of SA. Similarly, an increase of \textit{PR1} expression in the \textit{loh1} mutant displaying spontaneous cell death phenotype is also independent of SA43. Breakdown of SA signaling by mutation of \textit{NPR1} that encodes a receptor of SA46 represses expression of \textit{PR1}19. In our study, the expression of \textit{PR1} was also repressed in \textit{sscd1npr1} compared to \textit{sscd1} (Fig. 1c), however, the rate of seedlings death was similar in \textit{sscd1npr1} and \textit{sscd1} (Fig. 1d), suggesting that the cell death in \textit{sscd1} is uncorrelated to both SA signaling and the up-regulation of \textit{PR1}. In addition, we also generated the \textit{sscd1nahG} double mutant by crossing \textit{sscd1} with \textit{nahG} harboring a bacterial gene encoding salicylate hydroxylase that catalyzes the decarboxylation of SA52,53 and found that the degree of cell death was similar between \textit{sscd1nahG} and \textit{sscd1} (data not shown), indicating that the degradation of SA would not affect the cell death in \textit{sscd1}, which further confirmed the \textit{sscd1} cell death is not related to SA.
However, cell death in sscd1 was accompanied by the up-regulation of JA-inducible genes as well as the accumulation of jasmonic acid (Fig. 2). The up-regulation of JA-inducible genes in sscd1 should be resulted from the accumulation of jasmonic acid, but why the cell death of sscd1 is accompanied by the accumulation of jasmonic acid? In animals, loss of FAH results in the accumulation of Tyr degradation pathway's abnormal metabolite SUAC that is toxic to cells and tissues resulting in severe metabolic disorder diseases42. In plants, we have found that treatment of Arabidopsis wild-type seedlings with SUAC mimicked the sscd1 cell death phenotype41 and demonstrated that the cell death of sscd1 seedlings correlates with the accumulation of SUAC54. Recently, we found that SUAC affects Chl biosynthesis, resulting in the generation of ROS and then inducing cell death48. Some researcher's work has shown that JA could be synthesized in response to singlet oxygen that is one form of ROS25,55. Singlet oxygen is very unstable and difficult to detect within a cell55, however, some genes were specifically induced by singlet oxygen40. Recently, we found that the genes induced specifically by singlet oxygen40 were up-regulated in sscd148, suggesting that an effect of SUAC on Chl biosynthesis results in the generation of singlet oxygen in the sscd1 mutant. Furthermore, we found that treatment of Arabidopsis wild-type seedlings with SUAC activated the expression of JA-inducible genes (Fig. 5). Taken together, we concluded that cell death in sscd1 was accompanied by the accumulation of JA (Fig. 2d) is due to the synthesis of JA in response to singlet oxygen.

TAT catalyzes the first step in Tyr degradation pathway56. For the first time, Titarenko et al.57 reported that TAT could be induced by wounding as well as by JA. The gene for the F-box protein COI1 was identified for its irreplaceable role in JA signal transduction26–28. Mutations in the COI1 gene result in plants compromised in all known JA responses: defense against biotic and abiotic stresses, growth inhibition, and fertility26–28. Titarenko et al.57 reported that wounding induced TAT in wild type but not in the coi1-2 mutant, suggesting that wound-induced TAT is dependent on JA signaling. Brosché and Kangasjärvi58 reported that expression of TAT3 encoding Arabidopsis putative TAT45 was induced by JA. In this study, we not only confirmed that expression of TAT3 was induced by JA (Fig. 6a) but also found that expression of some of Tyr degradation pathway's genes including HGO and MAAIL was also induced by JA (Fig. 6b,c), however, the expression of these genes in the coi1-2 mutant was not significantly induced by JA (Fig. 6a–c), which suggested that JA signaling up-regulates Tyr degradation in plants.

JA plays an important role in cell death regulation. Singlet oxygen- and JA-mediated cell death in irradiated fhu plants is likely to be a form of PCD59. Inactivation of the EXECUTER1 protein abrogates not only singlet

Figure 6. Exogenous MeJA up-regulates the expression of Tyr degradation pathway genes TAT3, HGO, and MAAIL and this up-regulation is dependent on COI1. (a–d) Relative expression level of Tyr degradation pathway genes TAT3 (a), HGO (b), MAAIL (c) and SSSC1 (d) in wild-type (WT) and coi1-2 seedlings that were grown under LD for 7 days, and then transferred to SD and treated with ddH2O or 100 μM MeJA for 3 days. MeJA, methyl jasmonate; LD, long day; SD, short day. The expression of genes was analyzed by RT-qPCR, relative expression level was normalized to those of ACTIN2 and the control (in wild type without MeJA treatment) was set to 1. Mean ± SE from three biological replicates. Asterisk * and ** represent the significance of differences (two-tailed Student’s t-test) at the levels of P < 0.05 and P < 0.01, respectively.
oxygen-mediated cell death of *flu* plants but also accumulation of JA, however, inactivation of JA biosynthesis in the *aos/flu* double mutant does not affect singlet oxygen-mediated cell death\(^5\), hence, JA does not act as second messengers during singlet oxygen-mediated cell death but forms an integral part of a stress-related signaling cascade activated by singlet oxygen that encompasses several signaling pathways known to be activated by abiotic and biotic stressors\(^5\). In our study, the cell death of *sscd1* seedlings was repressed by mutation of COI1 (Fig. 3). Accordingly, the up-regulation of ROS-inducible genes *APX2* and *OXI1*, as well as singlet oxygen specifically induced genes *BAPI* and *ZP* was also repressed by mutation of COI1 (Fig. 4), suggesting that the breakdown of JA signaling reduces the generation of ROS in the *sscd1* mutant. We have just discussed above that JA signaling up-regulates Tyr degradation. Therefore, the accumulation of JA in *sscd1* would promote cell death by up-regulating Tyr degradation producing more SUAC. However, blockage of JA signaling by mutation of COI1 breaks the action of JA in Tyr degradation in *sscd1*, resulting in repression of cell death.

Taken all together, we concluded that cell death resulted from loss of FAH in *Arabidopsis* is related to JA but not SA, and proposed a model for the relationship between JA and Tyr degradation pathway in mediating the *sscd1* cell death. In the *sscd1* mutant, the accumulation of SUAC results in the generation of singlet oxygen, which induces cell death as well as JA synthesis. The accumulation of JA in *sscd1* accelerates Tyr degradation by up-regulating Tyr degradation pathway, producing more SUAC, which promotes cell death. Once JA signaling is broken by mutation of COI1, the up-regulation of Tyr degradation by JA in *sscd1* is eliminated, reducing production of SUAC, as a result, the *sscd1* cell death is repressed.

**Methods**

**Plant material and growth conditions.** The *sscd1* mutant was isolated previously in our laboratory\(^4\). The *coi1-2* mutant\(^2\) was kindly provided by Professor Xie (Tsinghua University). The *npr1-1* mutant\(^19\) was obtained from the Arabidopsis Biological Resource Center (ABRC; Ohio State University, Columbus, OH, USA).

Seeds were surfaced sterilized and plated on Murashige & Skoog (MS) medium in which 1% sucrose was added. Plates were chilled at 4°C in darkness for 3 days and then transferred to a growth chamber with LD (16 h of light/8 h of dark) or SD (8 h of light/16 h of dark) under 150 μmol photons m\(^{-2}\) s\(^{-1}\), controlled temperature (22 ± 2°C).

For RT-qPCR analysis and determination of SA and jasmonic acid in Figs. 1 and 2, the seeds were germinated on MS medium and grown under LD for 1 week and then the seedlings were transplanted to a new MS medium for additional 2 weeks’ growth under LD, and then transferred to SD.

**Construction of double mutants.** The *sscd1coi1* double mutant was created by first selecting F\(_2\) individuals from a cross between *sscd1* and *coi1-2* on plates containing 25 mM MeJA by screening for decreased sensitivity to JA\(^2\), and then F\(_3\) lines were selected by sequencing the *SSCD1* gene\(^4\). The primers for sequencing the *SSCD1* gene are as follows: forward primer is 5’-CCTCGTCTCTGCCGTCGCTAT-3’ and reverse primer is 5’-CTTGTTGAATGGCCCTGACCT-3’.

The *sscd1npr1* double mutant was created by selecting F\(_2\) individuals from a cross between *sscd1* and *npr1-1* (a recessive mutation with a single base mutation in *NPR1*\(^19\)) by sequencing *SSCD1* and *NPR1*, respectively. The primers for sequencing the *NPR1* gene are as follows: forward primer is 5’-GTTGCTCTTTATTGCGTGCGTATGTTTCTTGATGTA-3’ and reverse primer is 5’-ACCCGGTATGTTTCTTTGAATGTA-3’.

**RT-qPCR analysis.** RT-qPCR analysis were performed as described\(^4\). Total RNA was isolated using TRI-ZOL reagent (LIFE TECHNOLOGIES, https://www.thermofisher.com/us/en/home/brands/life-technologies.html). After incubation with DNase I (RNase Free, THERMO FISHER SCIENTIFIC, https://www.thermofisher.com/) at 37 °C for 30 min and then at 65 °C for 10 min to remove genomic DNA, RNA concentrations and purities were measured spectrophotometrically using OD260/OD280 and OD260/OD230 ratios (ND-1000, NanoDrop, THERMO FISHER SCIENTIFIC). Complementary DNA was synthesized from the mixture of oligo-dT primers and random primers using a ReverTraAce qPCR RT kit (perfect real time) according to the manufacturer’s instructions (TOYOBO, https://www.toyobo-global.com/).

RT-qPCR was performed in 96-well blocks using a SYBR qPCR mix (ROCHE, https://lifescience.roche.com/) with a BIO-RAD CFX CONNECT Real-Time PCR detection system (https://www.biorad.com/) following the manufacturer’s instructions. The RT-qPCR amplifications were performed under the following conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The primers of genes tested by RT-qPCR were listed in Table 1, and *ACTIN2* was used as an internal control. The gene expression for each sample was calculated on three analytical replicates, and the relative expression was quantified using the 2\(^-\Delta\Delta\text{Ct}\) method. The experiment was performed in three independent biological repeats. The significance of differences between datasets was evaluated using the two-tailed Student’s t-test.

**Determination of the dead seedlings.** Seedlings of *sscd1* and *sscd1npr1* were grown under SD and the number of dead seedling (all leaves were completely bleached) was counted from day 6 to 9. Seedlings of *sscd1* and *sscd1coi1* were grown under SD for 7 days and the number of dead seedlings was counted. The rate of seedling death was calculated as the percentage of dead seedlings from 250 to 300 seedlings. At least three independent biological repeats were performed.

**Detection of jasmonic acid and SA.** 0.5 g of leaves from WT and *sscd1* seedlings that were grown under LD for 3 weeks and then transferred to SD for 0, 2 and 3 days was harvested for jasmonic acid and SA extraction. The harvested tissues were immediately ground to a fine powder in liquid N\(_2\), and then exposed to extraction buffer (1.0 mL of 80% methanol) at 4 °C overnight. The samples were centrifuged at 10,000g for 5 min, and the
residues were re-extracted with 0.6 mL of 80% methanol (HPLC grade methanol, Merck, Germany). The supernatants were vacuum freeze dried to dryness at −60 °C, then dissolved in 200 μL of 0.1 M sodium phosphate buffer (pH 7.8), and extracted with 200 μL of petroleum ether. The aqueous phase was purified using a Waters Sep-Pak C18 cartridge (Waters, USA). The cartridge was washed with 200 μL of ddH2O and then eluted with 1.5 mL of 80% methanol. The eluate with 80% methanol was vacuum freeze dried. The dried extract was dissolved in 40 μL of 50% methanol and used for LC/MS assay in a WATERS ACQUITY SQD (LC/MS) system according to Liu et al.60.

**MeJA treatments.** For MeJA treatment, the seedlings of WT and coi1-2 were first grown under LD for 7 days, and then transferred to SD for 3 days. Once transferred to SD, plants were sprayed with 100 μM MeJA or ddH2O (as a control) under light once per day for 3 days. After treatment for 3 days, the plants were harvested and used for RT-qPCR analysis. The experiment was performed in three independent biological repeats.

**Treatment with SUAC.** The seeds were germinated on MS medium and grown under LD for 1 week and then the seedlings were transplanted to a new MS medium for additional 2 weeks' growth under LD, and then transferred to SD and sprayed with 1.280 μg mL−1 SUAC (SIGMA) or ddH2O (as a control) twice per day for 3 days. After treatment for 3 days, the plants were harvested and used for RT-qPCR analysis. The concentration of SUAC treatment was determined following our previous work49. The experiment was performed in three independent biological repeats.

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**Table 1. Primers of genes tested by RT-qPCR.**

| Gene      | Forward primer                  | Reverse primer                  |
|-----------|---------------------------------|---------------------------------|
| PRR1 (AT2G14610) | 5′-AATGACTAAGCTAGCTGCCGCGAA-3′      | 5′-CGAGTGACATGACCTGTCTTCT-3′     |
| VSR2 (AT5G24770) | 5′-GGATGACCCCATATACACATCAG-3′      | 5′-CAGGAGATCTCTTCGCCACATT-3′     |
| PDD1.1 (AT5G44420) | 5′-GCTCTCTCTCTACTCTCAATCC-3′      | 5′-TTGGCTTCTTGGCAGACATT-3′      |
| THII.1 (AT1G72260) | 5′-GTTGGGAAAGGGCATTCT-3′          | 5′-CATTGTCAGGCTTCCATT-3′        |
| APX2 (AT3G99640) | 5′-CAAAAGTTGAGCAACCTCTT-3′        | 5′-AAGGTTTGTCGCAACAGAA-3′       |
| OXI1 (AT3G25250) | 5′-GTGGAAGGAAATCAGGGTCTAG-3′      | 5′-TGACGAGATTTCCACCATCT-3′      |
| BAPI (AT3G61190) | 5′-ATCGGATCCACCAAGACATACG-3′      | 5′-AATCTCGGGCTCACAAGACAG-3′     |
| ZP (AT5G84540) | 5′-TACGAGAAAGAGAAGGAGGACG-3′      | 5′-GGTATGCGGGAATGTTGAAAG-3′     |
| TAT3 (AT2G24850) | 5′-CTCCGGACTTCCAACTCTCA-3′        | 5′-ATTACGACCCGCCCTTCTA-3′       |
| HGO (AT5G54080) | 5′-GGAGATGTTGTTGGATGGTTGGT-3′     | 5′-GCGGATGGTTCTTATCTGTGGTGA-3′ |
| MAAI (AT2G23980) | 5′-GCTGGACTCTGCTTACCTCAAGA-3′     | 5′-AGGCGGATACGGACGATG-3′        |
| SSDD1 (AT1G12050) | 5′-GACCTGGACCTCCCTATACAG-3′        | 5′-GACCATCGAAACCGCCAGT-3′       |
| ACTIN2 (AT3G18780) | 5′-AGCCTCTGCGACCAAGACGATG-3′       | 5′-ACAGATTCCTGACCTTGCTCCTAC-3′ |

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References
1. Lockshin, R. A. & Zakeri, Z. Apoptosis, autophagy, and more. *Int. J. Biochem. Cell Biol.* **36**, 2405–2419 (2004).
2. Jones, A. M. Programmed cell death in development and defense. *Plant Physiol.* **125**, 94–97 (2001).
3. Lam, E. Controlled cell death, plant survival and development. *Nat. Rev. Mol. Cell. Biol.* **5**, 305–315 (2004).
4. Morel, J. B. & Dangl, J. L. The hypersensitive response and the induction of cell death in plants. *Cell Death Differ.* **4**, 671–683 (1997).
5. Overmyer, K., Brosché, M. & Kangasjärvi, J. Reactive oxygen species and hormonal control of cell death. *Trends Plant Sci.* **8**, 335–342 (2003).
6. Marchetti, M., Bollich, C. & Uecker, F. Spontaneous occurrence of the sekiguchi lesion in two American rice lines: Its induction, inheritance, and utilization. *Phytopathology* **73**, 603–606 (1983).
7. Wolter, M. et al. The nlo resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defence mimic phenotype. *Mol. Gen. Genet.* **239**, 122–128 (1993).
8. Gray, J. et al. A novel suppressor of cell death in plants encoded by the Ls1 gene of maize. *Cell Death Differ.* **4**, 671–683 (1997).
9. Lorrain, S., Vailleau, F., Balagué, C. & Roby, D. Lesion mimic mutants: Keys for deciphering cell death and defense pathways in plants? *Trends Plant Sci.* **8**, 263–271 (2003).
10. Bruggeman, Q., Raynaud, C., Banchamed, M. & Delarue, M. To die or not to die? Lessons from lesion mimic mutants. *Front. Plant Sci.* **6**, 24 (2015).
11. Ianda, M. & Ruelland, E. Magical mystery tour: Salicylic acid signalling. *Environ. Exp. Bot.* **114**, 117–128 (2015).
12. Kliebenstein, D. J., Dietrich, R. A., Martin, A. C., Last, R. L. & Dangl, J. L. LSD1 regulates salicylic acid induction of copper zinc superoxide dismutase in *Arabidopsis thaliana*. *Mol. Plant. Microbe. Interact.* **12**, 1022–1026 (1999).
13. Brodersen, P., Malinovsky, F. G., Hématy, K., Newman, M. A. & Mondy, J. The role of salicylic acid in the induction of cell death in *Arabidopsis* acd11. *Plant Physiol.* **138**, 1037–1045 (2005).
14. Yoon, J., Chung, W. I. & Choi, D. NbHB1, *Nicotiana benthamiana* homeobox 1, is a jasmonic acid-dependent positive regulator of pathogen-induced plant cell death. *New Phytop.* **184**, 71–84 (2009).
15. Draper, J. Salicylate, superoxide synthesis and cell suicide in plant defence. *Trends Plant Sci.* **2**, 162–165 (1997).
16. Alvarez, M. E. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. *Plant. Mol. Biol.* **44**, 429–442 (2000).
17. Durner, J., Shah, J. & Klessig, D. F. Salicylic acid and disease resistance in plants. *Trends Plant Sci.* **2**, 266–274 (1997).
18. Shah, J. & Klessig, D. F. Salicylic acid: Signal perception and transduction. *New Compr. Biochem.* **33**, 513–541 (1999).
19. Cao, H., Bowling, S. A., Gordon, A. S. & Dong, X. Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* **6**, 1583–1592 (1994).
20. Pieterse, C. M. & Van Loon, L. C. NPR1: The spider in the web of induced resistance signaling pathways. *Curr. Opin. Plant Biol.* **7**, 456–464 (2004).
21. Browse, J. Jasmonate passes muster: A receptor and targets for the defense hormone. *Annu. Rev. Plant Biol.* **60**, 183–205 (2009).
22. Wasternack, C. Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann. Bot.* **100**, 681–697 (2007).
23. Avanci, N. C., Luche, D. D., Goldman, G. H. & Goldman, M. H. Jasmonates are phytohormones with multiple functions, including plant defense and reproduction. *Genet. Mol. Res.* **9**, 484–505 (2010).
24. Rao, M. V., Lee, H., Creelman, R. A., Mullet, J. E. & Davis, K. R. Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. *Plant Cell** **12**, 1653–1660 (2000).
25. Reinbothe, C., Springer, A., Samol, I. & Reinbothe, S. Plant oxylipins: Role of jasmonic acid during programmed cell death, defence and leaf senescence. *FEBS J.* **276**, 4666–4681 (2009).
26. Xie, D. X., Fays, B. F., James, S., Nieto-Rostro, M. & Turner, J. G. COI1: An Arabidopsis gene required for jasmonate-regulated defense and fertility. *Science** **280**, 1091–1094 (1998).
27. Devoto, A. et al. COII links jasmonate signalling and fertility to the SCF ubiquitin-ligase complex in Arabidopsis. *Plant J.* **32**, 457–466 (2002).
28. Xu, L. et al. The SCF(COI1) ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. *Plant Cell** **14**, 1919–1935 (2002).
29. Benedetti, C. E., Xie, D. & Turner, J. G. COI1-dependent expression of an Arabidopsis vegetative storage protein in flowers and siliques and in response to coronatine or methyl jasmonate. *Plant Physiol.* **109**, 567–572 (1995).
30. Apel, K. & Hirt, H. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **55**, 373–399 (2004).
31. Mittler, R., Vanderauwera, S., Gollery, M. & Breusegem, F. V. Reactive oxygen gene network of plants. *Trends Plant Sci.* **9**, 490–498 (2004).
32. Dat, J. F., Pellinen, R., Beeckman, T., Cotte, B. V. D. & Breusegem, F. V. Changes in hydrogen peroxide homeostasis trigger an active cell death program in tobacco. *Plant J.* **33**, 621–632 (2003).
33. Van Beusegem, F. & Dat, J. F. Reactive oxygen species in plant cell death. *Plant Physiol.* **141**, 384–390 (2006).
34. Petrov, V., Hille, J., Mueller-Roeber, B. & Gechev, T. S. ROS-mediated abiotic stress-induced programmed cell death in plants. *Front. Plant Sci.* **6**, 69 (2015).
35. Conklin, P. L. & Last, R. I. Differential accumulation of antioxidant mRNAs in Arabidopsis thaliana exposed to ozone. *Plant Physiol.* **109**, 203–212 (1995).
36. Inze, D. & Montagu, M. V. Oxidative stress in plants. *Curr. Opin. Biotechnol.* **6**, 153–158 (1995).
37. Karpinski, S. et al. Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. *Science** **284**, 654–657 (1999).
38. Rentel, M. C. Oxylipins as signals for plant defense and reproduction. *Genet. Mol. Res.* **9**, 484–505 (2010).
39. Shumbe, L. et al. COI1-dependent stress response program in the flu mutant of Arabidopsis thaliana. *Ann. Rev. Plant Biol.* **63**, 16–25 (2016).
40. Liu, X. et al. Determination of both jasmonic acid and methyl jasmonate in plant samples by liquid chromatography tandem mass spectrometry. *Chin. Sci. Bull.* **55**, 2231–2235 (2010).

**Acknowledgements**

This work was supported by grants from the Program for Key Basic Research of the Ministry of Science and Technology of China (2014CB160308), and the National Science Foundation of China (31571802).
Author contributions
C.R. conceived and designed research. Z.Z., C.H. and Z.P. constructed of double mutants, Z.Z. and T.Z. performed MeJA/SUAC treatment and RT-qPCR experiments, R.W. and J.T. performed detection of SA/jasmonic acid, T.Z., Z.Z. and C.R. analyzed data. Z.Z., T.Z., Q.Z. and C.R. wrote the manuscript. All authors read and approved the manuscript.

Competing interests
The authors declare no competing interests.

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