Phenylalanine suppresses cell death caused by loss of fumarylacetoacetate hydrolase in Arabidopsis

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Fumarylacetoacetate hydrolase (FAH) catalyzes the final step of Tyrosine (Tyr) degradation pathway essential to animals and the deficiency of FAH causes an inborn lethal disease. In plants, a role of this pathway was unknown until we found that mutation of Short-day Sensitive Cell Death1 (SSCD1), encoding Arabidopsis FAH, results in cell death under short day. Phenylalanine (Phe) could be converted to Tyr and then degraded in both animals and plants. Phe ingestion in animals worsens the disease caused by FAH defect. However, in this study we found that Phe represses cell death caused by FAH defect in plants. Phe treatment promoted chlorophyll biosynthesis and suppressed the up-regulation of reactive oxygen species marker genes in the sscd1 mutant. Furthermore, the repression of sscd1 cell death by Phe could be reduced by α-aminooxy-β-phenylpropionic acid but increased by methyl jasmonate, which inhibits or activates Phe ammonia-lyase catalyzing the first step of phenylpropanoid pathway, respectively. In addition, we found that jasmonate signaling up-regulates Phe ammonia-lyase 1 and mediates the methyl jasmonate enhanced repression of sscd1 cell death by Phe. These results uncovered the relation between chlorophyll biosynthesis, phenylpropanoid pathway and jasmonate signaling in regulating the cell death resulting from loss of FAH in plants.

Tyrosine (Tyr) degradation pathway includes the five-step enzymatic reactions, in which Tyr is first converted to 4-hydroxyphenylpyruvate by Tyr aminotransferase, then transformed into homogentisate by 4-hydroxyphenylpyruvate dioxygenase. Next, homogentisate dioxygenase catalyzes homogentisate to form maleylacetocetate (MAA) that is isomerized by MAA isomerase to fumarylacetoacetate (FAA). Last, FAA is hydrolyzed by fumarylacetoacetate hydrolase (FAH) to fumarate and acetoacetate1,2. Tyr degradation is essential to animals, blockage of this pathway results in metabolic disorder diseases, in which the most severe disorder in humans is hereditary tyrosinemia type I (HT1), an inborn lethal disease caused by deficiency of FAH3,4. Loss of FAH in HT1 patients results in the accumulation of Tyr degradation intermediates including FAA and MAA, and then both would undergo spontaneous reduction to succinylacetoacetate followed by spontaneous nonenzymatic decarboxylation to succinylacetone (SUAC) toxic to cells and tissues1. Although the homologous genes putatively encoding homogentisate dioxygenase, MAA isomerase, and FAH were demonstrated to exist in plants2,5, the role of the Tyr degradation pathway in plants had been unclear until we cloned the short-day sensitive cell death 1 (SSCD1) gene encoding an Arabidopsis putative FAH6. Loss of FAH in the sscd1 mutant leads to spontaneous cell death under short-day conditions (SD)6 and the accumulation of SUAC7. SUAC inhibits the activity of δ-aminolevulinic acid dehydratase involved in Chlorophyll (Chl) biosynthesis, resulting in high production of the Chl biosynthesis intermediate protochlorophyllide (Pchlide) in the dark under SD8. The excessive accumulation of Pchlide induces the production of reactive oxygen species (ROS) upon light irradiation and thereby causes cell death8.

Phenylalanine (Phe) is catalyzed by Phe hydroxylase to Tyr and then degraded in animals, and the dietary restriction of Tyr and Phe can improve the condition of HT1 patients6,9. In plants, Phe could also be converted into Tyr and then via homogentisate, to plastquinones and tocopherols, or to degradation of the aromatic ring10. However, Phe can carry through phenylpropanoid metabolism to produce secondary metabolites10. Phenylpropanoids are precursors to flavonoids, isoflavonoids, cumarins, and stilbenes, which have important functions...
in plant defense against pathogens and other predators, as UV light protectants, and as regulatory molecules in signal transduction and communication with other organisms\(^1\). In phenylpropanoid pathway, the first step is that Phe ammonia-lyase (PAL) catalyzes the deamination of Phe to give cinnamic acid\(^2,12,13\). The α-amino-oxy-β-phenylpropionic acid (AOPP) is an inhibitor of PAL, treatment with AOPP could reduce the activity of PAL\(^14,16\). Jasmonates (JAs) include jasmonic acid, methyl jasmonate (MeJA), and other derivatives, are a basic class of plant hormones involved in plant growth, development, and responses to biotic and abiotic stresses\(^17-20\). Treatment with MeJA could increase the activity of PAL in plants such as Chinese bayberry\(^21\), wheat\(^22\), and tuberose\(^18\). The PAL gene expression is responsive to a variety of environmental stimuli including pathogen infection, wounding, nutrient depletion, UV irradiation, extreme temperatures, and other stress conditions\(^23-26\).

To investigate whether the uptake of Phe in plants increases the cell death caused by loss of FAH as it does in animals, in this study the sscd1 mutant was treated with Phe and it was found that the death of sscd1 seedlings is not increased but suppressed by Phe treatment. With further investigation, we found that Phe treatment promotes Chl biosynthesis and represses the up-regulation of ROS marker genes in the sscd1 mutant. Furthermore, the repression of sscd1 cell death by Phe is reduced by AOPP whereas enhanced by MeJA. In addition, MeJA up-regulates the PAL1 gene and enhances the repression of sscd1 cell death by Phe through JA signaling. Our study shows that Phe has different effects on the cell death caused by FAH loss in plants and animals and uncovers the relation between Chl biosynthesis, phenylpropanoid pathway, and JA signaling in regulating the cell death resulting from loss of FAH, which will help to further investigate the regulation of Tyr degradation, phenylpropanoid biosynthesis and the cell death in plants.

**Results**

**Phe treatment suppresses the death of sscd1 seedlings.** Since Phe could be converted into Tyr and then degraded in plants\(^2,9\), we wondered whether Phe treatment promotes the death of sscd1 seedlings. To this end, the seeds of wild type and the sscd1 mutant were plated on MS medium without or with 0.1, 0.5, 1 and 2 mM Phe and grown under SD. Unexpectedly, the death of sscd1 seedlings treated with Phe was not increased, on the contrary, it was reduced (Fig. 1). When the medium was supplemented with 0.1 mM Phe, the rate of death seedlings was slightly reduced compared to that without Phe, however, it was significantly reduced as concentrations of Phe were increased (Fig. 1a). For example, on the 7th day, more than 80% of sscd1 seedlings in the medium without Phe were dead, however, when the medium was supplemented with 0.5, 1, and 2 mM Phe, the rates of death seedlings were only about 70%, 30%, and 5%, respectively (Fig. 1a). The phenotype of seedlings shown in Fig. 1b clearly displayed that Phe treatment reduced the death of sscd1 seedlings. The sscd1 seedlings are normal under long-day conditions (LD), however, once transferred to SD and grown for several days, some leaves of sscd1 seedlings are dead and show bleaching, but the seedlings would not die\(^9\). To test whether the death of sscd1 seedling leaves is also weakened by Phe, the seedlings growing under LD were treated with Phe and then transferred to SD. As shown in Fig. 1c, when sscd1 seedlings growing in the medium with Phe under LD were transferred to SD, the extent of seedling leaf death was obviously attenuated. All these results indicated that Phe treatment suppresses the death of sscd1 seedlings.

**The up-regulation of ROS marker genes in sscd1 could be repressed by Phe treatment.** Previously, we have speculated that the ROS resulting from excessive accumulation of Pchlide causes the sscd1 cell death\(^9\). Because ROS marker genes such as ascorbate peroxidase 2 (APX2)\(^20,22,23\), oxidative signal inducible 1 (OXI1)\(^20,22,23\), bonzai-associated protein 1 (BAP1), and transcription factor (ZP)\(^29\) were up-regulated in the sscd1 mutant\(^27,31\), we next investigated whether repression of sscd1 cell death by Phe is correlated with the expression of these genes. As shown in Fig. 2, the expression levels of APX2, OXI1, BAP1, and ZP in the sscd1 mutant were significantly increased compared to that in wild type, however, they were clearly reduced after Phe treatment. Therefore, the up-regulation of ROS marker genes in sscd1 could be repressed by Phe treatment. Since the expression level of ROS marker genes is positively correlated to the content of ROS\(^27,29,30\), the repression of the up-regulation of ROS marker genes in sscd1 by Phe treatment (Fig. 2) indicated the reduction of ROS after Phe treatment.

**Chl biosynthesis could be promoted by Phe treatment.** Since the sscd1 cell death is mediated by Chl biosynthetic pathway\(^9\), we next investigated whether the Phe treatment influences Chl biosynthesis. We first determined the content of Chl and found that it was increased after Phe treatment (Fig. 3a). In the Chl biosynthetic pathway, there are two pivotal control points, one is the formation of the initial precursor, δ-aminolevulinic acid, by glutamyl-tRNA reductase, and another is the metal-ion insertion step by Mg-chelatase\(^32-34\). In Arabidopsis, the HEMA1 gene encodes the glutamyl-tRNA reductase and the CHLH gene encodes the H subunit of Mg-chelatase\(^32,34\). The transcriptional regulation of both HEMA1 and CHLH could affect Chl biosynthesis\(^35\). Thus, we next tested whether the transcription of these genes changes after Phe treatment by RT-qPCR. As we expected, the expression levels of HEMA1 and CHLH in both wild type and sscd1 were also increased after Phe treatment (Fig. 3b). Therefore, Phe treatment promotes Chl biosynthesis.

**Repression of the sscd1 cell death by Phe could be reduced by AOPP and enhanced by MeJA.** Phe is a precursor of phenylpropanoid biosynthesis and PAL catalyzes the first step of this pathway\(^36\). If the repression of sscd1 cell death by Phe is related to phenylpropanoid pathway, it would be changed by inhibition or activation of PAL. To confirm that, firstly, the sscd1 seedlings were treated with AOPP, a potent inhibitor of PAL\(^35\), on the basis of Phe treatment. As we expected, treated with Phe and AOPP, the death sscd1 seedlings were clearly increased compared to that treated with Phe alone (Fig. 4a). For example, the death rate of 7-d-old sscd1 seedlings treated with 1 mM Phe was approximately 30%, however, when seedlings were treated with
1 mM Phe and 100 μM AOPP, it was increased to approximately 72% (Fig. 4b). These results indicated that the inhibition of PAL activity by AOPP could reduce repression of the \textit{sscd1} cell death by Phe.

Since the activity of PAL could be activated by MeJA\textsuperscript{16}, we next investigated whether treatment with MeJA enhances the repression of \textit{sscd1} cell death by Phe. As shown in Fig. 5a, in the absence of Phe, treatment with 5 μM MeJA did not distinctly affect the phenotype of both wild type and \textit{sscd1} seedlings; however, after treatment with 5 μM MeJA and 0.5 mM Phe, the death seedlings of \textit{sscd1} obviously reduced compared to those only treated with 0.5 mM Phe. The death rate of 7-d-old \textit{sscd1} seedlings treated with 0.5 mM Phe was approximately 64% whereas it was less than 40% once treated with 5 μM MeJA and 0.5 mM Phe (Fig. 5b). Therefore, the activation of PAL activity by MeJA could enhance repression of the \textit{sscd1} cell death by Phe.

**Treatment with MeJA causes the COI1-dependent up-regulation of PAL1.** In Arabidopsis, PAL is encoded by a small gene family including \textit{PAL1, PAL2, PAL3}, and \textit{PAL4}\textsuperscript{26,37}. We next investigated whether treat-
Figure 2. Expression of ROS marker genes in sscd1 was down-regulated by treatment with Phe. Relative expression levels of APX2, OXI1, BAP1 and ZP in WT and sscd1 seedlings which were first grown in MS medium under LD for 7 days and transplanted to MS medium added without (−) or with (+) 2 mM Phe under LD for an additional 7-day growth, and then transferred to SD for a 3-d growth. ACTIN2 expression was used as the internal control. Each value is the mean of three independent biological replicates ± standard deviation. An asterisk represents the significance of differences (two-tailed Student’s t-test) at the level of $P < 0.05$. WT wild type, Col-0; Phe phenylalanine; LD long day; SD short day.

Figure 3. Chlorophyll synthesis was increased after Phe treatment. (a) The content of chlorophyll in WT and sscd1 seedlings which were first grown in MS medium under LD for 7 days and transplanted to MS medium added without (−) or with (+) 2 mM Phe under LD for an additional 7-day growth, and then transferred to SD for a 3-day growth. (b) The relative expression levels of CHLH and HEMA1 in WT and sscd1 seedlings which were first grown in MS medium under LD for 7 days and transplanted to MS medium added without (−) or with (+) 2 mM Phe under LD for additional 7-d growth, and then transferred to SD for a 3-d growth. ACTIN2 expression was used as the internal control. Each value is the mean of three independent biological replicates ± standard deviation. An asterisk represents the significance of differences (two-tailed Student’s t-test) at the levels of $P < 0.05$. WT wild type, Col-0; LD long day; Phe phenylalanine; SD short day.
ment with MeJA affects some or all of these genes' expression, and if so, is it dependent on COI1? To this end, the seedlings of wild type and the coi1-2 mutant, a coi1 leaky mutant, were treated with MeJA and the expression levels of PAL1, PAL2, PAL3, and PAL4 were assessed by an analysis of RT-qPCR. As shown in Fig. 6, after treatment with MeJA, the expression level of PAL1 was significantly increased in wild type but not in coi1-2. However, the expression levels of PAL2, PAL3, and PAL4 were not significantly altered in both wild type and coi1-2 after being treated with MeJA (Fig. 6). These results suggested that treatment with MeJA up-regulates PAL1, and this up-regulation is dependent on COI1.

MeJA's enhancement of Phe's inhibition of sscd1 cell death depends on COI1. Since treatment with MeJA enhanced the repression of sscd1 cell death by Phe (Fig. 5) as well as up-regulated PAL1 in dependence of COI1 (Fig. 6), we next investigated whether the enhancement of MeJA on Phe in inhibiting the sscd1 cell death is also dependent on COI1. The seeds of the sscd1 single mutant and the sscd1coi1 double mutant were plated on medium added without or with 0.5 mM Phe or/and 5 μM MeJA and grown under SD, and then the death seedlings were counted. As shown in Fig. 7a, the death rate of sscd1coi1 seedlings was lower than that of sscd1, which is due to JA signaling positively regulating the sscd1 cell death. Phe treatment also significantly reduced the death rate of sscd1coi1 seedlings (Fig. 7a, right), and that the reduction of seedling mortality was greater in sscd1coi1 (Fig. 7a, right) than in sscd1 (Fig. 7a, left), which is mainly resulted from that the blockage of JA signaling in sscd1coi1 suppresses the sscd1 cell death and Phe could also be degraded through the Tyr degradation pathway. However, the death rate of sscd1coi1 seedlings treated with MeJA and Phe was not significantly reduced compared to that treated with Phe alone (Fig. 7a, right), which is unlike in sscd1 (Fig. 7a, left). From the Fig. 7b, we could see that MeJA treatment clearly enhances the repression of sscd1 seedlings death by Phe, however, this enhancement was not obvious in sscd1coi1 (Fig. 7b, right). Therefore, the MeJAs enhancement of Phe's inhibition of sscd1 cell death depends on COI1.

Discussion
FAH catalyzes the final step of the Tyr degradation pathway and the deficiency of FAH in animals causes an inborn lethal disease, which was named HT1 in humans. Phe could be converted to Tyr and then degraded in animals, and the dietary restriction of Tyr as well as Phe can improve the condition of HT1 patients. In plants, the SSCD1 gene encodes the Arabidopsis FAH and the mutation of SSCD1 results in spontaneous cell death under SD. Like as in animals, Phe could also be converted into Tyr in plants and then degraded. However, in our study, the death of sscd1 seedlings was not increased but repressed by Phe treatment (Fig. 1). So, why would Phe treatment repress the cell death resulting from loss of FAH in plants?
Figure 5. Treatment with MeJA enhanced the inhibitory effect of Phe on the sscd1 seedlings death. (a) The phenotype of WT and sscd1 seedlings which were grown in medium added without (−) or with 0.5 mM Phe or/and 5 μM MeJA under SD for 7 days. (b) The death rate of sscd1 seedlings which were grown in medium added without (control) or with 5 μM MeJA or/and 0.5 mM Phe under SD for 7 days. Error bars represent standard deviations (n > 30). The experiment was performed in three independent biological repeats. An asterisk represents the significance of differences (two-tailed Student’s t-test) at the level of P < 0.05. WT wild type, Col-0; Phe phenylalanine; MeJA methyl jasmonate; SD short day.
Previously, we demonstrated that the ssd1 cell death is mediated by Chl biosynthesis. The inhibition of the δ-aminolevulinic acid dehydratase activity by SUAC in the ssd1 mutant influences Chl biosynthesis resulting in impairment of feedback inhibition of Chl biosynthesis from the light–dark transition under SD, which activates Chl biosynthesis and accumulation of Pchlide in the dark, and then upon re-illumination the excessive accumulation of Pchlide induces the mass production of ROS and thereby causes cell death. The main form of ROS induced by Pchlide is singlet oxygen, which is unstable and difficult to be quantitatively detected, however, it could be assessed through the analysis of the expression levels of some genes including ZP and BAP1 activated specifically by singlet oxygen. In this study, treatment of ssd1 seedlings with Phe distinctly repressed the up-regulation of ZP and BAP1 as well as other ROS marker genes such as APX2 and OXI1.

In plants, Phe could be metabolized through the phenylpropanoid pathway to produce secondary metabolites, which plays an important role in plant stress including UV-light, drought, and pathogen attack, due to their antioxidant function. PAL catalyzes the first step of the phenylpropanoid pathway, which is a key step in phenylpropanoid biosynthesis. The activity of PAL could be inhibited by AOPP and promoted by MeJA. Treatment with AOPP prevents the increase in resistance to B. cinerea due to the application of external Phe. In our study, the repression of ssd1 seedlings death by Phe was reduced by AOPP (Fig. 4), however, it was enhanced by MeJA (Fig. 5), which suggested that the suppression of ssd1 cell death by Phe is related to the phenylpropanoid pathway. Catechins, a class of flavonoids produced from Phe through phenylpropanoid pathway, have antioxidant activity. In our study, treatment with catechins also suppressed the death of ssd1 seedlings (Fig. S2). Since the secondary metabolites produced by Phe metabolism through the phenylpropanoid pathway have antioxidant function, ROS could also be reduced by the metabolism of Phe through the phenylpropanoid pathway, which should be another important cause for the repression of ssd1 cell death by Phe.

Previously, we found that JA signaling is involved in the ssd1 cell death. In the ssd1 mutant, the accumulation of SUAC results in the generation of ROS, which induces cell death as well as JA synthesis. JA up-regulates the Tyr degradation pathway, producing more SUAC, which promotes cell death. Once JA signaling is broken specifically by singlet oxygen, the up-regulation of Tyr degradation pathway by JA is eliminated, reducing the production of SUAC, as a result, the ssd1 cell death is repressed. MeJA is an activator of PAL catalyzing the first step of phenylpropanoid pathway. In this study, MeJA treatment markedly increased the expression level of PAL1 in wild type but not in the coi1-2 mutant (Fig. 6), indicating that JA signaling can up-regulate the phenylpropanoid pathway through activating PAL. The repression of ssd1 cell death by Phe could be enhanced by MeJA treatment in the ssd1 mutant (Figs. 5 and 7) but not in the ssd1coi1 mutant (Fig. 7), which suggested that MeJA treatment enhances Phe inhibition of the ssd1 cell death through JA signaling. Therefore, JA...

**Figure 6.** An analysis of expression of the PAL genes upon MeJA treatment by RT-qPCR. The relative expression levels of PAL1, PAL2, PAL3 and PAL4 in WT and coi1-2 seedlings which were first grown in medium under LD for 12 days and removed to SD for a 3-d growth, and then treated with ddH2O (−) or 100 μM MeJA (+) for 1 day. ACTIN2 expression was used as the internal control. Each value is the mean of three independent biological replicates ± standard deviation. An asterisk represents the significance of differences (two-tailed Student’s t-test) at the levels of P<0.05. WT wild type, Col-0; MeJA methyl jasmonate; LD long day; SD short day.
has a dual regulatory effect on the sscd1 cell death. On the one hand, JA up-regulates the Tyr degradation pathway, promoting the sscd1 cell death, on the other hand, JA up-regulates the phenylpropanoid pathway, inhibiting the sscd1 cell death. For this reason, the death of sscd1 seedlings was not increased by MeJA treatment, it seemed to
decrease slightly (Figs. 5 and 7), which suggested that the effect of MeJA treatment on the sscd1 cell death through the phenylpropanoid pathway might be greater than that through Tyr degradation pathway.

In addition, we observed that with Phe treatment alone the decrease of death seedlings in sscd1coi1 is greater than that in sscd1 at 8 days (Fig. 7). One main reason for that should be that the blockage of JA signaling in sscd1coi1 decreases the seedlings death because JA signaling positively regulates the sscd1 cell death31. Another main reason for that should be that Phe could also be degraded through the Tyr degradation pathway10, inducing production of more ROS and subsequent cell death, however, blockage of JA signaling in sscd1coi1 could suppress the sscd1 cell death31. As a result, with Phe treatment alone the suppression of the seedlings death in the double mutant sscd1coi1 should be more obvious than that in the single mutant sscd1.

In conclusion, although Phe can be degraded through the Tyr degradation pathway, unlike in animals, Phe treatment does not increase the cell death resulting from loss of FAH in plants, instead, it represses the cell death. A possible mechanism for the repression of sscd1 cell death by Phe treatment can be described as follows (Fig. 8). Loss of FAH in the sscd1 mutant results in a decline of Chl biosynthesis, which impairs the feedback inhibition of Chl biosynthesis from light–dark transition under SD, leading to the accumulation of ROS and then cell death. Phe treatment, on the one hand, promotes Chl biosynthesis, increasing the feedback inhibition of Chl biosynthesis from light–dark transition under SD, and on the other hand, activates the phenylpropanoid pathway, both of which reduce ROS and subsequent cell death. In addition, in the sscd1 mutant ROS induces cell death as well as JA synthesis. JA signaling up-regulates the Tyr degradation pathway, promoting the sscd1 cell death, however, it also up-regulates PAL1 which activates the phenylpropanoid pathway, repressing the sscd1 cell death. Since the effect of MeJA treatment on the sscd1 cell death through the phenylpropanoid pathway might be greater than that through the Tyr degradation pathway, the repression of sscd1 cell death by Phe could be enhanced by MeJA treatment.

Methods

Plant materials and growth conditions. Arabidopsis thaliana L. ecotype Columbia-0 (Col-0) was obtained from the Arabidopsis Biological Resource Center (ABRC; Ohio State University, Columbus, OH, USA) and the mutants used in this study are in Col-0 background. The sscd1 mutant6 was isolated by Han et al. in our laboratory. The coil-2 mutant39 was kindly provided by Professor Xie (Tsinghua University, Beijing, China) and the sscd1coil double mutant31 was generated through a cross of sscd1 with coil-2 by Zhou et al. in our laboratory. Experimental research on plants including the collection of plant material was performed in accordance with relevant institutional, national, and international guidelines and legislation.

Seeds were surface sterilized with 20% (v/v) chlorine bleach containing 0.1% (v/v) Triton X-100 for 10 min and washed three to five times with sterile water, then plated on Murashige & Skoog medium supplemented with 1% (m/v) sucrose and 0.7% (w/v) agar (pH 5.8) (MS). The different concentrations of Phe (SIGMA) were added to the MS medium. Plates were chilled at 4 °C in darkness for 3 days and then transferred to a growth chamber with LD (16-h light/8-h dark) or SD (8-h light/16-h dark) under 150 μmol photons m−2 s−1, controlled temperature (22 ± 2 °C).

For RT-qPCR analysis (Figs. 2, 3b) and determination of Chl content (Fig. 3a), 1-week-old seedlings growing under LD were transplanted onto MS added without or with Phe and grown under LD for an additional 1 week's growth, and then transferred to SD for three days growth. Since the cell death of sscd1 seedlings occurs on the 4th day after transferred from LD to SD, the seedlings were harvested at the end of the third day's light for the determination of Chl content or harvested at 2 h light after three days for RT-qPCR analysis.

Determination of the dead seedlings. A dead seedling is one for which all leaves were completely bleached. The rate of seedling death was calculated as the percentage of dead seedlings. The number of seedlings counted was approximately 100, and the experiment was performed in three independent biological repeats.

RT-qPCR analysis. Total RNA was isolated using TRIZOL reagent (Life Technologies, https://www.thermoscientific.com/us/en/home/brands/life-technologies.html). After incubation with DNase I (RNase Free, Thermo Fisher Scientific, https://www.thermoscientific.com/) at 37 °C for 30 min and then at 65 °C for 10 min to remove genomic DNA, the RNA concentration and purity 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, http://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 (http://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 are 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−ΔΔCt 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 Chl content. The content of Chl was determined referring to the method described by Lichtenthaler46. Weighed segments of frozen crushed material (about 0.04 g) were homogenized in 1 mL 80% acetone and stood for 5–6 h, then centrifuged for 10 min at 5000 rpm at 4 °C and assayed spectrophotometrically at 663 nm and 645 nm. Result calculation: \(C_{\text{mg/g}} = (17.32 \, A_{663} + 7.18 \, A_{645})/m_{\text{g}}\). The experiment was performed in three independent biological repeats.
AOPP treatment. Seeds were germinated on MS added with 1 mM Phe (SIGMA) and grown under SD. On the third day seedlings were sprayed with 100 μM AOPP (Wako) or ddH2O (as a control) once a day for
5 days, then the rate of death seedlings was counted, and the seedlings were photographed. The experiment was performed in three independent biological repeats.

**MeJA treatment.** For determination of dead seedlings (Figs. 5 and 7), seeds were germinated on MS added without or with 0.5 mM Phe and/or 5 μM MeJA (SIGMA) and grown under SD for 6–8 days. For RT-qPCR analysis (Fig. 6), about 2-week-old seedlings growing under LD were transferred to SD and on the fourth day the seedlings were sprayed with 100 μM MeJA or ddH2O (as a control). After MeJA treatment for one day, the seedlings were harvested and used for RT-qPCR analysis. The experiment was performed in three independent biological repeats.

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| Gene     | Forward primer | Reverse primer |
|----------|----------------|----------------|
| APX2     | 5′-CTGTAATCCACTGTTCTTCCC-3′ | 5′-TTTATGAGATTCCACCAACCCAAC-3′ |
| OX2      | 5′-CTGACAGTAATGTCCACACATCC-3′ | 5′-GCTGCGGATCTTCCACACATCC-3′ |
| ZP       | 5′-TTTATAATCCACTGTTCTTCCC-3′ | 5′-GCTGCGGATCTTCCACACATCC-3′ |
| CHLH     | 5′-GACCGGAGATCCTTGTCTGC-3′ | 5′-ACCTGCTTCTTGCACCATC-3′ |
| BAPI     | 5′-ATCCTCCAACGCCACTGTTCTTCCC-3′ | 5′-GATGTCGCCACCAAGAAGAC-3′ |
| HEMA1    | 5′-GCTGCTGCAACCAAGAAGAC-3′ | 5′-ATATCCCATGCTTCAAC-3′ |
| PAL1     | 5′-GCTGCTGCAACCAAGAAGAC-3′ | 5′-ATATCCCATGCTTCAAC-3′ |
| PAL2     | 5′-GCTGCTGCAACCAAGAAGAC-3′ | 5′-ATATCCCATGCTTCAAC-3′ |
| PAL3     | 5′-GCTGCTGCAACCAAGAAGAC-3′ | 5′-ATATCCCATGCTTCAAC-3′ |
| PAL4     | 5′-GCTGCTGCAACCAAGAAGAC-3′ | 5′-ATATCCCATGCTTCAAC-3′ |
| ACTIN2   | 5′-GCTGCTGCAACCAAGAAGAC-3′ | 5′-ATATCCCATGCTTCAAC-3′ |

Table 1. Primers of genes tested by real-time quantitative PCRs.
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**Author contributions**

C.R. conceived, designed and supervised the experiments; Y.J. and T.Z. performed the experiments; Q.Z. and H.Y. provided technical assistance to Y.J.; Y.J., Q.Z. and C.R. analyzed the data; Y.J. wrote the article with contributions from all authors; and C.R. supervised, modified and complemented the writing.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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