Introduction

Abscission is the natural shedding of plant tissues such as leaves, flowers, seed pods, and fruits, which plays an essential role in plant survival as a means of seed dispersal or removal of vulnerable or diseased tissues (Addicott, 1982). The specialized cell layers responsible for abscission are called abscission zones (AZs) and are usually formed together in early stages of organogenesis. When abscission is activated, cell wall-hydrolysing enzymes are secreted to disrupt the cell wall at the AZ in a highly coordinated process that integrates various developmental and environmental cues (Patharkar & Walker, 2019).

The molecular mechanism of abscission has been largely elucidated for the floral organs of Arabidopsis (Arabidopsis thaliana) (Cho et al., 2008; McKim et al., 2008; Liu et al., 2013; Lee et al., 2018). As constituents of reproductive organs, petals play an important role in attracting pollinators but are also easily exposed to predators and are vulnerable to environmental stress, making their timely removal after fertilization critical for plant survival. Various phytohormones, including ethylene, auxin, abscisic acid (ABA), and jasmonic acid (JA), are involved in activating abscission after fertilization has occurred (Patterson & Bleecker, 2004; Kim et al., 2013; Meir et al., 2019). The receptor-like kinases HAESA (HAE) and its cognate peptide ligand INFLORESCENCE DEFICIENT IN ABSCISSION (IDA), the key signalling components of abscission, was accelerated in msd2 mutants, suggesting that MSD2 acts upstream of IDA-HAE. Further transcriptome and pharmacological analyses revealed that abscisic acid and nitric oxide facilitate abscission by regulating the expression of IDA and HAE during MSD2-mediated signalling.

These results suggest that MSD2-dependent ROS metabolism is an important regulatory point integrating environmental stimuli into the developmental programme leading to abscission.

Summary

- The timely removal of end-of-purpose flowering organs is as essential for reproduction and plant survival as timely flowering. Despite much progress in understanding the molecular mechanisms of floral organ abscission, little is known about how various environmental factors are integrated into developmental programmes that determine the timing of abscission.
- Here, we investigated whether reactive oxygen species (ROS), mediators of various stress-related signalling pathways, are involved in determining the timing of abscission and, if so, how they are integrated with the developmental pathway in Arabidopsis thaliana.
- MSD2, encoding a secretory manganese superoxide dismutase, was preferentially expressed in the abscission zone of flowers, and floral organ abscission was accelerated by the accumulation of ROS in msd2 mutants. The expression of the genes encoding the receptor-like kinase HAESA (HAE) and its cognate peptide ligand INFLORESCENCE DEFICIENT IN ABSCISSION (IDA), the key signalling components of abscission, was accelerated in msd2 mutants, suggesting that MSD2 acts upstream of IDA-HAE. Further transcriptome and pharmacological analyses revealed that abscisic acid and nitric oxide facilitate abscission by regulating the expression of IDA and HAE during MSD2-mediated signalling.
- These results suggest that MSD2-dependent ROS metabolism is an important regulatory point integrating environmental stimuli into the developmental programme leading to abscission.

Key words: abscisic acid, Arabidopsis thaliana, HAE, MSD2, nitric oxide, organ abscission, reactive oxygen species, superoxide dismutases.

Jinsu Lee1,2, Huize Chen1,3, Gisuk Lee4, Aurélia Emonet5, Sang-Gyu Kim4,5, Donghwan Shim6 and Yuree Lee2,7,8

1Research Institute of Basic Sciences, Seoul National University, Seoul 08826, Korea; 2Research Centre for Plant Plasticity, Seoul National University, Seoul 08826, Korea; 3Higher Education Key Laboratory of Plant Molecular and Environmental Stress Response in Shanxi Province, Shanxi Normal University, Taiyuan 030000, Shanxi, China; 4Department of Biological Sciences, Korea Advanced Institute for Science and Technology, Daejeon 34141, Korea; 5Department of Plant Molecular Biology, University of Lausanne 1015, Lausanne, Switzerland; 6Department of Biological Sciences, Chungnam National University, Daejeon 34134, Korea; 7School of Biological Sciences, Seoul National University, Seoul 08826, Korea; 8Plant Genomics and Breeding Institute, Seoul National University, Seoul 08826, Korea

Author for correspondence:
Yuree Lee
Email: yuree.lee@snu.ac.kr

Received: 8 December 2021
Accepted: 27 May 2022

New Phytologist (2022) 235: 2466–2480
doi: 10.1111/nph.18303

© 2022 The Authors
New Phytologist © 2022 New Phytologist Foundation.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
Shi et al., 2018). Additionally, the expression levels of IDA and IDA-LIKE (IDL) increase in response to abiotic and biotic stress conditions (Vie et al., 2015), suggesting that the IDA-HAE module plays a role in linking stress responses to development. However, how various environmental stimuli modulate IDA-HAE activity is not well understood.

Redox homeostasis plays critical roles in plant development and stress responses (Mhamdi & Van Breusegem, 2018; Huang et al., 2019). Reactive oxygen species (ROS) are by-products of various cellular processes, including photosynthesis and mitochondrial respiration, in different intracellular compartments. Plants have not only developed various systems for detoxifying ROS but also evolved mechanisms that can integrate ROS as signalling molecules, thus linking metabolism and responses to highly variable environments (Waszczyk et al., 2018). Reactive oxygen species accumulation in the AZ has been reported in various species, including Arabidopsis, with roles in abscission signalling and cell wall remodelling (Sakamoto et al., 2008a,b; Bar-Dror et al., 2011; Yang et al., 2015; Liao et al., 2016; Lee et al., 2018). Various enzymes, such as NADPH oxidases, peroxidases, and polyamine oxidases, might be involved in ROS production in the AZ, but how their roles are interconnected and regulated is not clear.

NADPH oxidases located at the cell membrane generate superoxide that might be used as a signal in various developmental and stress conditions (Mittler et al., 2011; Huang et al., 2019). The Arabidopsis genome harbours 10 genes, RbohA–RbohJ (RESPIRATORY BURST OXIDASE HOMOLOG), encoding NADPH oxidases with functions in various developmental stages, including root and hypocotyl elongation, root hair development, fruit ripening, and cell wall remodelling during seed germination (Dunand et al., 2007; Muller et al., 2009; Yan et al., 2016). RbohD and RbohF are highly expressed in the AZ and provide the ROS substrates needed for peroxidase-dependent lignin formation, which forms a physical apoplastic barrier to precisely control the localization of cell wall enzymes (Lee et al., 2018). However, it remains unknown whether RBOHs are also involved in signalling to regulate the timing of abscission or cell wall loosening. How the generated ROS are metabolized is also unknown.

Extracellular superoxides (O$_2^-$) produced by NADPH oxidases can be dismutated to hydrogen peroxide (H$_2$O$_2$) either spontaneously or enzymatically via apoplastic superoxide dismutases (SODs) and transported to the cytoplasm via aquaporins (Qi et al., 2017; Mhamdi & Van Breusegem, 2018). Superoxide dismutases can be divided into three classes as a function of the metal ions in their active centres: manganese (Mn), iron (Fe), and copper and zinc (Cu/Zn). Arabidopsis has eight known SODs: three Cu/Zn SODs (CSD1–3), three Fe SODs (FSD1–3), and two Mn SODs (MSD1–2) (Kliebenstein et al., 1998; Chen et al., 2022). Their subcellular localizations vary, with CSD2 and FSD1–3 in chloroplasts (Kliebenstein et al., 1998; Myouga et al., 2008; Dvorak et al., 2021), MSD1 in mitochondria (Morgan et al., 2008), CSD3 in peroxisomes (Kliebenstein et al., 1998; Huang et al., 2012), CSD1 and FSD1 in the cytoplasm (Kliebenstein et al., 1998; Dvorak et al., 2021), and FSD1 in the nucleus (Dvorak et al., 2021). MSD2 is an apoplastic SOD with Mn SOD activity that is secreted into vacuoles or the apoplast (Chen et al., 2022). SODs, which convert O$_2^-$ into H$_2$O$_2$, not only detoxify O$_2^-$ accumulated from various stress conditions and metabolic processes, but also affect the redox balance. Given the recent reports that different types of ROS perform distinct functions (Tsukagoshi et al., 2010; Lee et al., 2018), the role of SOD in influencing the balance between O$_2^-$ and H$_2$O$_2$ may serve as an important signalling rheostat along with ROS-generating enzymes. Although several factors regulating the expression of SODs and the function of the encoded enzymes have recently been identified (Yamasaki et al., 2007; Xing et al., 2013; Dvorak et al., 2020; Hu et al., 2021), our understanding of their regulatory mechanisms and their relationship with other signalling pathways is still fragmentary.

In this study, we demonstrated that MSD2, a recently identified secretory SOD (Chen et al., 2022), is involved in the regulation of abscission signalling. MSD2 was preferentially expressed in the AZ of flowers, and the encoded MSD2 enzyme was secreted into the vacuole and extracellular spaces. In msd2 mutants, superoxide accumulated earlier than in the wild-type and was accompanied by an acceleration of floral organ shedding. Transcriptome analysis revealed that the expression of nitric oxide (NO)- and ABA-related genes is upregulated in msd2 mutants. NO and ABA abundance increased upon activation of abscission, and an exogenous supply of NO and ABA accelerated abscission, while treating plants with an NO scavenger blocked the accelerated abscission observed in msd2 mutants. We also established that the expression of IDA4 and HAE is affected by NO and ABA. These results suggest that the regulation of ROS metabolism by MSD2 affects the onset of abscission through the NO and ABA signalling pathways upstream of IDA-HAE.

**Materials and Methods**

**Plant materials and growth conditions**

For all experiments, 7-wk-old Arabidopsis thaliana (accession Col-0) plants were used. After stratification for 2 d (at 4°C in the dark), seeds were sown on a soil : sand mixture (4 : 1, w/w) and cultivated in a climate chamber with 60% relative humidity under long-day conditions (16 h : 8 h, dark : light cycles, 22°C : 18°C, day : night regime, 70 μmol m$^{-2}$ s$^{-1}$ photon flux density). The ida and hae-1 hsl2-1 mutants were described before (Lee et al., 2018). Msd2-1 (GABI_100H05), msd2-2 (SM_3_35975), UBQ10pro:MSD2-mCherry, and MSD2pro:nlsGFP-GUS were described by Chen et al. (2022).

**NO, cPTIO, and ABA treatments**

To test the effect of chemicals on floral abscission, drugs were mixed with lanolin wax (Sigma) melted at 50°C and applied using sterile wooden toothpicks to the primary inflorescence of plants 35 d after germination. All chemicals were diluted from 100 mM stock solutions in dimethyl sulfoxide (Sigma) to the working concentrations of 50 μM for ABA (Duchefa), 500 μM for peroxynitrite (ONOO$^-$; Calbiochem), or 500 μM for the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl3-oxide (cPTIO; Sigma).
Microscopy and histology

For toluidine blue (TB, cat. no. 92-31-9; Sigma) staining, flowers were dipped in 0.025% (w/v) TB solution in water for 2–3 min and washed with distilled water for 2 min. For β-glucoronidase (GUS) staining, flowers were incubated in GUS solution (3 mM potassium ferrocyanide, 3 mM potassium ferricyanide, 1 mM X-Gluc, 1 M NaH₂PO₄, 1 M Na₂HPO₄, 0.5 M EDTA, pH 8.0) for 6 h at 37°C and then rinsed in 70% (v/v) ethanol for at least 5 min. After removing chlorophyll in clearing solution (6 : 1 ethanol : acetic acid, v/v) several times, samples were rinsed using 70% (v/v) ethanol. To visualize superoxide accumulation in AZs, inflorescences were stained with nitroblue tetrazolium (NBT; Sigma) and washed with distilled water for 2 min. For toluidine blue (TB, cat. no. 92-31-9; Sigma) staining, flowers suitable for the stage were collected only from the primary inflorescence. Total RNA was extracted from the AZ samples using an RNeasy Plant Mini kit (Qiagen) according to the manufacturer’s instructions. Total RNA concentration and quality were measured using an ND-2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The RNA optical density (OD) 260 : 280 ratios were 1.9–2.1, and OD 260 : 230 ratios were 2.0–2.5. RNA quality was monitored by running all samples on a TapeStation RNA screentape (Agilent, Santa Clara, CA, USA). Samples with RNA integrity number > 8.0 were used for RNA-sequencing (RNA-Seq) library construction.

A first-strand synthesis kit (GenDepot) with oligo(dT) primers was used for complementary DNA (cDNA) synthesis from 2 μg of total RNA according to the manufacturer’s instructions. The resulting cDNAs were used for quantitative polymerase chain reaction (qPCR) with a Quant Studio 1 (Applied Biosystems) instrument using SYBR Green Real-time PCR Master Mix (Applied Biosystems). Primer sequences are listed in Supporting Information Table S1. Threshold cycle (Ct) values were used to calculate 2−ΔΔCt for expression analysis, where ΔΔCt for treated plants was determined as follows: (Ct target gene – Ct ACTIN gene) – control plant (Ct target gene – Ct ACTIN gene) (Livak & Schmittgen, 2001).

Transcriptome deep sequencing (RNA-Seq) library preparation and sequencing

RNA-Seq libraries were independently prepared from 1 μg of total RNA for each sample using the TruSeq RNA Sample Prep Kit v2 (Illumina, San Diego, CA, USA) according to the manufacturer’s manual. Library quality and titre were verified using KAPA Library Quantification Kits for Illumina Sequencing platforms according to the qPCR Quantification Protocol Guide (Kapa Biosystems). Sequencing was performed on an Illumina NovaSeq instrument (Illumina), generating 100-bp paired-end reads. Library preparation and sequencing were performed by Macrogen (Seoul, South Korea).

RNA-Seq analysis and functional annotation

Reads for each sample were mapped to the reference genome (TAIR10) and counted using RSEM 1.3.0 software and trimmed mean of M value-normalized transcripts per million (TPM) values were determined for each transcript (Li & Dewey, 2011). Differentially expressed genes (DEGs) were identified using edgeR v.3.16.5 to calculate the negative binomial dispersion across conditions (Robinson et al., 2010). Genes were determined to be differentially expressed if they showed a minimum two-fold change in expression, with a false discovery rate (FDR)-adjusted P value of < 0.05. Functional annotation of DEGs was performed using Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analyses using DAVID (v.6.8) (Huang et al., 2009), and Web Gene Ontology Annotation Plot (WEGO) analysis using WEGO v.2.0 (Ye et al., 2018).

Statistical analysis

All statistical analyses were done with the Stata statistical software package (v.26; IBM, Armonk, NY, USA). One-way analysis of
variance (ANOVA) with post hoc Tukey or Kruskal–Wallis with Dunn’s multiple comparisons test was performed. Each experiment was repeated three times.

Results

Floral organ abscission is accelerated in msd2 mutants

The timing of floral organ abscission is determined through the complex integration of various environmental changes and developmental signals (Sawicki et al., 2015). Reactive oxygen species are important mediators of various environmental stress-related signalling pathways (Huang et al., 2019) and might contribute to organ abscission, as they also accumulate in AZs (Sakamoto et al., 2008a; Bar-Dror et al., 2011; Yang et al., 2015; Liao et al., 2016; Lee et al., 2018). To investigate the link between ROS and the timing of abscission, we focused on the secretory Mn SOD MSD2 (Chen et al., 2022) and tested its role in regulating abscission. MSD2 was preferentially expressed in the floral AZ starting in S13 flowers, when the buds open (Smyth et al., 1990); MSD2 expression levels increased as flower development progressed (Figs 1a, S1a). At S16, when floral organs wither and begin to fall, MSD2 expression decreased in the AZ and showed a strong signal in the nectary, as determined with MSD2pro:GUS transgenic lines (Fig. 1a). MSD2 harbours a secretory signal peptide in its N terminus and localizes in the secretory pathways, including the vacuole or apoplast in seedlings (Chen et al., 2022). We determined the accumulation pattern of MSD2 in the AZ of transgenic plants that ubiquitously express MSD2-mCherry under the control of the UBIQUITIN 10 (UBQ10) promoter. We mainly detected MSD2-mCherry in the vacuole of residual cells (RECs), which are AZ cells of the receptacle, and in the apoplast of secession cells (SECs), which are AZ cells of the separating organs (Lee et al., 2018; Fig. 1b).

To investigate the effects of MSD2 on abscission, we analysed the abscission phenotype of two independent T-DNA insertion mutants (Fig. S1b,c; Chen et al., 2022). In the msd2-1 and msd2-2 mutants, floral organ abscission occurred when fruit length was markedly shorter than that of the wild-type (Fig. 1c), suggesting that abscission starts earlier in msd2 mutants. We obtained similar results when we analysed the embryonic developmental stage at the beginning of abscission. In the wild-type, abscission took place mainly at the 2/4-cell stage of the embryo, whereas abscission occurred at a younger stage of embryonic development in msd2 mutants, with the proportion of 1-cell stage embryos being higher in the S16 silique of the msd2-1 mutant compared to the wild-type (Fig. S1d). Neither the number of siliques per inflorescence (Fig. S1e,f) nor the germination rates of seeds (Fig. S1g,h) were significantly different between the wild-type and msd2 mutants. Since abscission is closely related to flower development, abscission can be accelerated by promoting seed development or by promoting the onset of abscission. The earlier results suggest that MSD2 has a direct effect on the onset of abscission without significantly affecting embryo development.

To quantify the phenotypes of msd2 mutants, we measured siliquae length and the position of the first flowers at S16, when the floral organs begin to wither and fall (Fig. 1d,e). Using both quantification methods, we confirmed the accelerated abscission in msd2-1 and msd2-2 mutant plants; this phenotype was rescued to that of the wild-type upon overexpression of MSD2 under the control of the UBQ10 promoter in the msd2-1 mutant background (Fig. 1c–e). The phenotype of msd2-2 was slightly weaker than that of msd2-1 (Fig. 1e), which could be attributed to the low level of RNA remaining msd2-2 (Fig. S1c). During abscission, the AZ becomes permeable as the cell wall is hydrolysed, which can be visualized by staining with a hydrophilic dye such as TB (Tanaka et al., 2004). In agreement with the results earlier, we confirmed the acceleration of abscission in msd2 mutants by TB staining (Fig. 1f).

To explore the possible correlation between the onset of abscission and ROS accumulation, we investigated the pattern of ROS accumulation by staining with NBT, which is highly sensitive to superoxide (Straus et al., 2010). We observed earlier NBT staining of the AZ in msd2-1 and msd2-2 mutant plants compared to the wild-type; again, this phenotype was restored to that seen in the wild-type in the msd2-1 UBQ10pro:MSD2 transgenic lines (Fig. 1g). These results suggest that MSD2, which is involved in ROS metabolism, negatively regulates the timing of abscission.

MSD2 regulates the expression of IDA and HAE in the AZ

The IDA-HAE signalling pathway plays an important role in regulating the expression of cell wall-modifying enzymes responsible for cell wall hydrolysis during abscission (Cho et al., 2008; Stenvik et al., 2008; Aalen et al., 2013; Patharkar & Walker, 2015). To investigate the possible relationship between MSD2 and the IDA-HAE pathway for the regulation of abscission onset, we determined the expression of IDA and HAE in the msd2-1 mutant with the GUS reporter gene driven by the IDA or HAE promoters. The expression of IDA and HAE increased during S15–S16, when abscission is activated and floral organs begin to shed (Butenko et al., 2003; Cai & Lashbrook, 2008; Leslie et al., 2010; Patharkar & Walker, 2015). We observed a similar expression pattern with our IDApro:GUS and HAEpro:GUS reporter lines, which was altered in msd2-1 mutants (Fig. 2a). In the msd2-1 mutant, the spatial expression patterns of IDA and HAE were similar to those in the wild-type, but the temporal patterns were altered. We detected GUS staining in an earlier position compared to the wild-type for both IDA and HAE reporters, which was consistent with the early onset of abscission in the msd2-1 mutant (Fig. 1c–e). We confirmed the acceleration of the temporal expression pattern of IDA and HAE through reverse-transcription (RT)-qPCR analysis by position, and observed a similar earlier shift in the expression of HSL2 (Fig. 2b). Unlike the change in the timing of their expression, the expression levels of these genes did not differ significantly between the wild-type and the msd2-1 mutant (Fig. S2), suggesting that the temporal difference rather than the quantitative difference in IDA and HAE transcript levels is the major change associated with the msd2-1 mutant. The accelerated abscission caused by the loss of MSD2 function was abolished when the msd2-1 mutation was introduced into the hae hsl2 double mutant background. Indeed,
the hae hsl2 msd2-1 triple mutant exhibited abscission phenotypes similar to the hae hsl2 double mutant (Fig. 2c). These results suggest that MSD2 acts upstream of the IDA-HAE pathway, regulating the expression of the encoding genes and thus influencing the timing of abscission.

**Genome-wide transcriptome analysis**

To explore the pathways regulating the expression of IDA and HAE in the msd2-1 mutant, we conducted a comparative transcriptome analysis between the wild-type and the msd2-1 mutant by RNA-Seq. To this end, we collected AZ samples by hand-cutting AZ regions from approximately 50 flowers at S13 and S15 from the wild-type and the msd2-1 mutant. The progression of flower development from S13 to S15 was accompanied by changes in the expression of many genes in both genotypes, with 3966 DEGs in the wild-type and 3511 DEGs in the msd2-1 mutant (Fig. 3a,b). When comparing the wild-type and mutant, we identified 875 DEGs at S13 (406 genes upregulated and 469 genes downregulated in the msd2-1 mutant) and 295 DEGs at S15 (185 genes upregulated and 110 genes downregulated in the msd2-1 mutant) (Fig. 3b,c; Table S2). To understand the biological functions of these DEGs in the msd2-1 mutant, we performed a GO enrichment analysis, using the DEGs at each stage (Tables S3–S5). This analysis revealed terms related to phytohormone responses including ABA, auxin, and JA, and responses to...
a wide range of stress stimuli, including nitrate, oxidative stress, cold, and pathogens (Fig. 3d; Tables S3–S5). KEGG pathway analysis also indicated that the expression of genes involved in NO compound biosynthetic processes is affected in the msd2-1 mutant (Fig. S3; Table S6).

The timing of abscission is determined by integrating environmental changes along with a programmed developmental process. Therefore, we hypothesized that ABA and NO, which are important signalling molecules responding to environmental stress (Mur et al., 2013; Ma et al., 2018), may also play a role in MSD2-related abscission. The total number of DEGs between the wild-type and the msd2-1 mutant was lower at S15 compared to S13, but we identified more DEGs associated with NO and ABA responses at S15 (Fig. 3c–f). Among the NO- and ABA-responsive genes with altered expression levels at S15 flowers of the msd2-1 mutant compared to the wild-type, many showed stage-dependent expression changes in the wild-type (Fig. 3c,f). Therefore, the expression of these genes in the wild-type were closely linked with abscission development, and their expression patterns were further enhanced in the msd2-1 mutant. This result suggested that NO and ABA responses are part of the intrinsic abscission process and that these responses are enhanced in the msd2-1 mutant. In contrast to the ABA-responsive genes that showed higher expression levels at S15 in the msd2-1 mutant compared to the wild-type, the expression pattern of genes involved in ABA biosynthesis or recognition of ABA signalling were not significantly different between the wild-type and the mutant. In addition, genes encoding negative regulators of ABA signalling were strongly upregulated in the msd2-1 mutant compared to the wild-type at S15 (Figs 3g, S4). We validated these observations by RT-qPCR (Fig. S5). These results suggest that MSD2 affects abscission by modulating ABA signalling rather than via ABA biosynthetic pathways, and that the process is accompanied by a feedback loop that upregulates the expression of negative regulators such as protein phosphatase 2Cs (PP2Cs).

Fig. 2 The timing of IDA and HAE expression in the abscission zone (AZ) accelerates in the msd2-1 Arabidopsis mutant. (a) Promoter analysis of IDA and HAE in the wild-type (WT) (Col-0) and the msd2-1 mutant harbouring the reporter constructs IDApro:GUS and HAEpro:GUS. Yellow arrowheads indicate the first flower showing β-glucuronidase (GUS) pattern. (b) Relative expression levels of the indicated genes in the AZ of WT and msd2-1 plants by floral position, as determined by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). ACTIN2 served as a reference gene. Values represent mean ± standard error of the mean (SEM) of three independent experiments. Different letters indicate significant differences (n = 3; P < 0.05, one-way ANOVA with post hoc Tukey test). (c) Abscission phenotype in the hae hsl2 double mutant and the hae hsl2 msd2-1 triple mutant. P, flower position counted after anthesis (a, c). Bar, 1 mm.
Fig. 3 Changes in gene expression in msd2-1 assessed by RNA-sequencing (RNA-Seq). (a) Heatmap representation of log$_2$-normalized counts per million across all samples for differentially expressed genes (DEGs) in the mutant relative to the wild-type (WT). A cut-off probability $P$-value $< 0.05$, false discovery rate (FDR) $< 0.05$, and |Fold-change| $\geq 2$ were applied to genes expressed in each sample. (b) DEG counts among sets C13 vs m13 (control S13 vs msd2-1 S13, bold), C15 vs m15 (control S15 vs msd2-1 S15, bold), C13 vs C15, and m13 vs m15. (c) Venn diagram showing the overlap between DEG sets between C13 vs m13 (upper panel) and C15 vs m15 (lower panel). (d) Gene Ontology (GO) term enrichment analysis. Web Gene Ontology Annotation Plot (WEGO) output for GO enrichment analysis for biological functions within the DEGs between C13 vs m13 and C15 vs m15 with adjusted $P$-value $< 0.05$. (e) Venn diagram (upper panel) and heatmap representation of expression levels of abscisic acid (ABA)-responsive DEGs between C13 vs C15 and C15 vs m15. (f) Venn diagram (upper panel) and heatmap representation of expression levels of nitric oxide (NO)-responsive DEGs between C13 vs C15 and C15 vs m15. (g) Heatmap representation of transcript levels for DEGs involved in ABA signalling pathways. In heatmaps, relative expression is scaled from red (high expression) to blue (low expression). Each column represents each replicate labelled 1 through 3, and each row represents a gene (a, d, e, f).
Accelerated abscission in the msd2-1 mutant is mediated by NO

RNA-Seq analysis suggested that NO may contribute to regulating abscission and that MSD2 may negatively regulate NO-mediated signalling. To determine whether NO plays a role in abscission, we visualized NO accumulation in the AZ by 4,5-diaminofluorescein diacetate (DAF-2DA) staining (He et al., 2004; Planchet & Kaiser, 2006; Vishwakarma et al., 2019; Duan et al., 2020). Fluorescence measurement is possible only after the floral organs are removed. To avoid damage and induction of wounding signalling, we performed this experiment on S16 flowers, when floral organs naturally detach. We detected DAF-2DA fluorescence in S16 AZs that decreased upon treatment with the NO scavenger cPTIO (Planchet & Kaiser, 2006; Vishwakarma et al., 2019; Duan et al., 2020), demonstrating the specificity of the signal (Fig. 4a). The msd2-1 mutant exhibited higher fluorescence intensity than the wild-type (Fig. 4a,b), suggesting higher NO accumulation in the msd2-1 mutant.

To investigate whether NO induces abscission, we applied lanolin wax containing 500 μM peroxynitrite (ONOO−) to inflorescence stems and recorded the state of floral organ abscission 5 d later. Peroxynitrite treatment dramatically accelerated abscission, whereas cPTIO treatment delayed abscission (Fig. 4c–e). Simultaneous treatment of inflorescences with peroxynitrite and cPTIO blocked the abscission-promoting effects of peroxynitrite (Fig. 4c–e). Similarly, treating the msd2-1 mutant with cPTIO also suppressed the abscission-promoting effects associated with the loss of MSD2 (Fig. 4f–h). These results suggest that accelerated abscission in msd2 mutants is mediated by NO.

Interaction of ABA with NO is important for regulating abscission

The RNA-Seq analysis earlier also suggested ABA signalling as another downstream target for the MSD2-mediated regulation of abscission. To test the role of ABA in floral organ abscission in Arabidopsis, we measured ABA contents in the AZ: ABA levels increased with developmental stage in both wild-type and msd2-1 flowers (Fig. 5a). To directly observe the effects of ABA on floral abscission, we applied lanolin wax containing ABA to inflorescence stems and scored floral abscission 5 d after treatment. We observed a dramatic promotion of abscission by ABA (Fig. 5b–d). Abscission was delayed in the abi5-7 mutant (ABA insensitive 5), providing an important control for the specificity of the response (Fig. 5e–g).

To elucidate the interaction between ABA and NO during abscission, we tested the effect of simultaneous treatment with ABA and the NO scavenger. Co-treatment of inflorescences with cPTIO and ABA suppressed the promotion of abscission mediated by ABA (Fig. 5b–d). In addition, the expression levels of ABI5 and the ABA-responsive genes ABI1 and ABRE BINDING FACTOR4 (ABF4) rose in response to exogenously supplied peroxynitrite (Fig. 5h). Just as the expression of ABA-responsive genes was regulated by NO, we determined that the expression of NO-responsive genes is also regulated by ABA. Expression of the NO-responsive genes GLUTATHIONE S-TRANSFERASE6 (GST6) and LIPOXGENASE 4 (LOX4) (Ahlfors et al., 2009; Gaudinier et al., 2018) was upregulated by ABA treatment as well as peroxynitrite treatment (Fig. 5b). These results suggest that ABA and NO interact to determine the timing of abscission.

NO and ABA affect abscission by regulating the expression of IDA and HAE

To determine the relationship between NO, ABA, the IDA-HAE module, we tested the effects of NO and ABA on abscission in *ida* and *hae hsl2* mutants. We observed that abscission is slightly accelerated by NO and ABA treatment in *ida* mutants (Fig. 6a–c), while similar treatments had no effect in the *hae hsl2* double mutant (Fig. 6d). However, the expression of ABA- and NO-responsive genes increased in S15 flowers of both the *ida* and *hae hsl2* mutants after NO and ABA treatment (Fig. 6e), suggesting that NO and ABA act upstream of the IDA-HAE module. To assess whether the expression of *IDA* and *HAE* is modulated by NO and ABA, we dissected the spatiotemporal expression patterns of *IDA* and *HAE* using our GUS reporter lines. Treatment with peroxynitrite or ABA did not affect the spatial expression pattern of these genes but did affect their temporal expression pattern; we detected GUS signals earlier under these treatments than under the control condition (Fig. 6f,g). We confirmed these results by RT-qPCR for *IDA* and *HAE* transcript levels under control and treated conditions in S15 flowers (Fig. 6h). Taken together, our data suggest that alterations in ROS metabolism in the msd2-1 mutant promote the accumulation of NO and activate ABA signalling, which in turn induces abscission by regulating the expression timing of *IDA* and *HAE*.

Discussion

Floral organ abscission is directly affected by flower development and fertilization but also reacts to changes in the outside environment (Addicott, 1982; Taylor & Whitelaw, 2001). Although a multi-layered regulatory mechanism to initiate abscission is likely to be required to comprehensively integrate these various external factors, our understanding of the entire signalling cascade is still fragmentary. In this study, we propose that ROS metabolism regulated by MSD2 contributes to one of the regulatory layers of abscission. While the regulation of ROS-generating enzymes has been intensively studied, the metabolism of the ROS they generate and the pathways activated by ROS remain largely unknown in the context of abscission, largely because the extracellular SOD had not been identified in Arabidopsis. None of the seven classic SODs reported in Arabidopsis have a secretory signal peptide (Kliebenstein et al., 1998). In this study, we show that MSD2, recently shown to encode a secretory Mn SOD (Chen et al., 2022), is preferentially expressed in the AZ and is involved in the regulation of ROS metabolism. Reactive oxygen species levels regulated by MSD2 appeared to play an essential role in the correct timing of abscission, with both NO and ABA signals contributing to this effect, to regulate the expression of *IDA* and *HAE* as the downstream targets of ROS and MSD2. Our findings...
suggest another layer that regulates ROS signalling, which helps to understand the complexity of the integration of external signals into endogenous developmental programmes.

The name ABA was coined in light of the discovery that the phytohormone induced abscission in young cotton (*Gossypium hirsutum*) fruit (Ohkuma et al., 1963; Cornforth et al., 1965; Addicott et al., 1968). However, it was later suggested that the observed abscission was an indirect effect of elevated ethylene levels (Cracker & Abeles, 1969), and the roles of ABA in abscission have not received much attention since. Indirect roles of ABA on floral abscission were recently demonstrated in Arabidopsis as well (Ogawa et al., 2009). The ABA-deficient mutant *aba2-2* exhibits normal floral organ abscission, while the *ein2-1 aba2-2* double mutant shows an abscission phenotype similar to that of the *ein2-1* (*ethylene insensitive 2-1*) single mutant. However, a triple mutant between *ein2-1*, *aba2-2*, and *aos* (defective in ALLENE OXIDE SYNTHASE, involved in JA biosynthesis) displayed a very severe abscission delay relative to the wild-type and the *ein2-1* mutant, suggesting partially redundant roles for the three phytohormones ethylene, ABA, and JA in abscission. Stage-specific transcriptome analysis also confirmed the interplay between the three phytohormones (Niederhuth et al., 2013). The expression of ethylene and ABA signalling and biosynthesis-related genes increases at S15, while the expression of JA signalling-related genes decreases, in both the wild-type and the *hae hsl2* double mutant, suggesting that phytohormone regulation is independent of HAE.

In contrast to previous studies in which abscission was normal in the ABA-deficient mutant *aba2-2* (Niederhuth et al., 2013), we observed a delay in floral organ abscission in the *abi5-7* mutant (Fig. 5). These results suggest the possibility that ABI5 may regulate abscission separately from its role in ABA signalling. ABI5 is a basic leucine zipper transcription factor whose activity is regulated via protein–protein interactions and posttranslational modifications. Although ABI5 is a well-known master regulator of ABA signalling, ABI5 expression and ABI5 activity and the *ein2-1* mutant, suggesting partially redundant roles for the three phytohormones ethylene, ABA, and JA in abscission. Stage-specific transcriptome analysis also confirmed the interplay between the three phytohormones (Niederhuth et al., 2013). The expression of ethylene and ABA signalling and biosynthesis-related genes increases at S15, while the expression of JA signalling-related genes decreases, in both the wild-type and the *hae hsl2* double mutant, suggesting that phytohormone regulation is independent of HAE.

In contrast to previous studies in which abscission was normal in the ABA-deficient mutant *aba2-2* (Niederhuth et al., 2013), we observed a delay in floral organ abscission in the *abi5-7* mutant (Fig. 5). These results suggest the possibility that ABI5 may regulate abscission separately from its role in ABA signalling. ABI5 is a basic leucine zipper transcription factor whose activity is regulated via protein–protein interactions and posttranslational modifications. Although ABI5 is a well-known master regulator of ABA signalling, ABI5 expression and ABI5 activity and the *ein2-1* mutant, suggesting partially redundant roles for the three phytohormones ethylene, ABA, and JA in abscission. Stage-specific transcriptome analysis also confirmed the interplay between the three phytohormones (Niederhuth et al., 2013). The expression of ethylene and ABA signalling and biosynthesis-related genes increases at S15, while the expression of JA signalling-related genes decreases, in both the wild-type and the *hae hsl2* double mutant, suggesting that phytohormone regulation is independent of HAE.

In contrast to previous studies in which abscission was normal in the ABA-deficient mutant *aba2-2* (Niederhuth et al., 2013), we observed a delay in floral organ abscission in the *abi5-7* mutant (Fig. 5). These results suggest the possibility that ABI5 may regulate abscission separately from its role in ABA signalling. ABI5 is a basic leucine zipper transcription factor whose activity is regulated via protein–protein interactions and posttranslational modifications. Although ABI5 is a well-known master regulator of ABA signalling, ABI5 expression and ABI5 activity and
Mitogen-activated protein kinases activated by the ABA core signalling pathway (de Zellicourt et al., 2016) are also downstream targets of ROS (Lee et al., 2016). Among the various possibilities of activating ABA signalling by ROS, elucidating which pathways are actually regulated in msd2 mutants remains a major challenge.

There is growing evidence that NO acts as a plant physiological mediator in various developmental and stress responses (Domingos et al., 2015; Farnese et al., 2016). NO affects senescence and abscission of the rudimentary leaves in lychee (Litchi chinensis) (Yang et al., 2018). Reactive oxygen species might participate in the signalling cascade leading to NO biosynthesis (Gaupels et al., 2011; Farnese et al., 2016), but the understanding of this process is very limited, mainly because NO biosynthesis is not yet fully understood. Nitrate reductase (NR), a cytosolic enzyme essential for nitrogen assimilation, has been proposed to be involved in NO production in a variety of physiological processes (Hao et al., 2010; Mur et al., 2013; Chamizo-Ampudia et al., 2017). However, important issues remain unresolved. Under

stability are regulated by various phytohormones, and ABI5 serves as an integration hub (Skubacz et al., 2016; Collin et al., 2021). Evaluating how the activity of ABI5 is regulated in the AZ will be important for understanding the effects of ABA on abscission and crosstalk with other phytohormones. Furthermore, we observed no significant changes for the expression of ABA-responsive genes such as ABF3, NINE-CIS-EPoxyCAROTENOID DIOXYGENASE5 (NCED5), or NCED6 (Fig. 3) or for ABA contents (Fig. 5) in the msd2-1 mutant. Since these changes were not quantified over time, we cannot exclude that the timing of ABA biosynthesis in the msd2-1 mutant may have accelerated as well. However, it is also possible that the increased expression levels of ABA-responsive genes in the msd2-1 mutant are the result of direct control of ABA signalling rather than ABA biosynthesis. The PP2Cs ABI1 and ABI2 are negative regulators of ABA signalling and are redox-sensitive, as their activity can be inactivated by ROS (Meinhard & Grill, 2001; Meinhard et al., 2002; Sierla et al., 2016).
Fig. 6 Nitric oxide (NO) and abscisic acid (ABA) regulate abscission by modulating the expression of IDA and HAE SA. (a–c) Analysis of abscission in ida (a–c) and hae hsl2 (d) mutants 5 d after treatment with 50 μM ABA or 500 μM peroxynitrite (ONOO−) in S13 flowers. Silique lengths extending above S16 flowers (b), the position of S16 flowers (c) in the ida mutant (n = 15). Yellow arrowheads indicate the first flower showing permeable staining with toluidine blue (TB) (a). (e) Relative expression levels of the indicated genes in S15 flowers of ida (upper panel) and hae hsl2 (lower panel) mutants, as determined by reverse-transcription quantitative polymerase chain reaction (RT-qPCR). Control values were set to 1 (n = 3). (f, g) Promoter activity analysis of IDA and HAE using the reporter lines IDApro::GUS (f) and HAEpro::GUS (g). Yellow arrowheads indicate the first flower showing β-glucuronidase (GUS) pattern. (h) Relative expression levels of IDA and HAE in S15 flowers after 5 d treatment with peroxynitrite or ABA, as determined by RT-qPCR. ACT2 served as a reference gene. Control values were set to 1. Values represent mean ± standard error of the mean (SEM) of three independent experiments (n = 3). Different letters indicate significant differences (b, c, e, h; P < 0.05, one-way ANOVA with post hoc Tukey test). P, flower position counted after anthesis (a, d, f, g). Bars: 1 mm (a, d, f, g).
normal growth conditions, NR preferentially reduces nitrate (NO$_3^-$) to nitrite (NO$_2^-$) because NR prefers nitrate over nitrite. Currently, NR appears to require specific conditions, such as anaerobic conditions or high nitrate concentrations, to produce any significant amounts of NO (Mur et al., 2013; Farnese et al., 2016). Nitric oxide synthase (NOS), the main enzymatic source for NO in animals, has also been widely considered as another candidate for NO biosynthesis. Several reports acknowledge the possible existence of NOS activity in plants (Corpas et al., 2009; Astier et al., 2018), but the associated protein has yet to be identified (Jandroz et al., 2016; Santolini et al., 2017). Future exploration into the interactions between ROS, NO, and ABA that take place during abscission may uncover as-yet-unknown upstream regulatory pathways of NO biosynthesis or novel NO-related messengers.

The onset of floral organ abscission is determined by integrating fertilization, the age of floral organs, and environmental conditions, at the centre of which the interaction between the plant phytohormones ethylene, ABA, and JA plays a fundamental role (Ogawa et al., 2009; Estornell et al., 2013; Sawicki et al., 2015). In particular, ethylene plays central roles in determining the onset of floral organ abscission in Arabidopsis (Meir et al., 2019). Floral organ abscission does occur in the ethylene-insensitive mutants ctr1-1 (ethylene triple response 1-1) and ein2-1, though with a dramatic delay relative to the wild-type (Patterson & Bleecker, 2004). While the interplay between phytohormones is important to determine the onset of abscission, IDA-HAE may play a key role in the execution of organ shedding (Roberts & Gonzalez-Carranza, 2007; Cho et al., 2008). The signalling cascades and transcription factor networks downstream of IDA-HAE are well described (Cho et al., 2008; Patharkar & Walker, 2015), but the regulatory mechanisms upstream of IDA-HAE are relatively poorly understood. The expression of IDA and IDL is dependent on ethylene signalling (Butenko et al., 2006) and responds to abiotic and biotic stress conditions (Vie et al., 2015), suggesting that the IDA-HAE module plays a role in linking stress responses to development. The elucidation of additional regulatory circuits regulating the expression of IDA and HAE is necessary to reflect the underlying complexity associated with the integration of multiple signals.

In this study, we propose that ROS metabolism, which is regulated by MSD2 and affects NO and ABA signalling components, is a novel upstream module that regulates the expression of IDA and HAE. Further elucidation of how the activity of MSD2 or the expression of MSD2 is regulated under various environmental conditions is necessary for a comprehensive understanding of the biological significance of ROS metabolism regulated by MSD2. Reactive oxygen species and IDA-HAE are involved in cell separation and responses to stress in various cell types, the mechanisms of which are conserved in various plants (Tucker & Yang, 2012; Ventimilla et al., 2020, 2021), suggesting that our findings should be applicable to other cell types and other plant species, including crops. Protein concentration is often controlled not only through synthesis but also through the balance of synthesis and degradation (Vierstra, 1993), which has the advantage of being able to respond quickly to needs. Our findings suggest that ROS concentrations are similarly regulated through a balance between production and degradation, which is thought to be broadly applicable to various signal transduction processes beyond abscission.

**Acknowledgements**

The authors thank Niko Geldner (UNIL) for critical reading of the manuscript and H. Ryu (Chungbuk National University) for sharing abh5-7 mutant seeds. YL was funded by the Suh Kyungbuk Foundation (SUHF-19010003) and the National Research Foundation of Korea (NRF-2020R1A2C2013176 and NRF-2021R1A5A1032428). JL was funded by the National Research Foundation of Korea (NRF-2020R1I1A1A01068615). HC was supported by the China Scholarship Council (CSC202008140062) and the Natural Science Foundation of China (NSFC31900251).

**Competing interests**

None declared.

**Author contributions**

YL and JL conceived the study and designed the experiments. JL, HC, GL, AE and S-GK performed the experiments. DS and JL analysed the RNA-Seq data. YL and JL wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

**ORCID**

Sang-Gyu Kim https://orcid.org/0000-0003-2574-3233
Jinsu Lee https://orcid.org/0000-0003-3825-288X
Yuree Lee https://orcid.org/0000-0002-4663-6974

**Data availability**

The raw data files for the RNA-Seq analysis reported in this article can be found at GenBank under the accession number PRJNA786431.

**References**

Aalen RB, Wildhagen M, Sto IM, Butenko MA. 2013. IDA: a peptide ligand regulating cell separation processes in Arabidopsis. *Journal of Experimental Botany* 64: 5253–5261.

Addicott FT. 1982. *Abscission*. Berkeley, CA, USA: University of California Press.

Addicott FT, Lyon JL, Ohkuma K, Thiessen WE, Carns HR, Smith OE, Cornforth JW, Milborrow BV, Ryback G, Wareing PF. 1968. Absciscic acid: a new name for abscisin II (dormin). *Science* 159: 1493.

Ahlfors R, Brosché M, Kollist H, Kangasjärvi J. 2009. Nitric oxide modulates ozone-induced cell death, hormone biosynthesis and gene expression in *Arabidopsis thaliana*. *The Plant Journal* 58: 1–12.

Astier J, Gross I, Durner J. 2018. Nitric oxide production in plants: an update. *Journal of Experimental Botany* 69: 3401–3411.

Bar-Dror T, Dermastia M, Kladnik A, Znidaric MT, Novak MP, Meir S, Burd S, Philosoph-Hadas S, Ori N, Songeon L et al. 2011. Programmed cell death occurs asymmetrically during abscission in tomato. *Plant Cell* 23: 4146–4163.
Huang CH, Kuo WY, Weiss C, Jinn TL. 2012. Copper chaperone-dependent and -independent activation of three copper-zinc superoxide dismutase homologs localized in different cellular compartments in Arabidopsis. *Plant Physiology* 158: 737–746.

Huang DW, Sherman BT, Lempecki RA. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols* 4: 44–57.

Huang H, Ullah F, Zhou D-X, Yi M, Zhao Y. 2019. Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in Plant Science* 10: 800.

Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Mellkonian M, Tian Z, Zhu Y, Carpenter EJ, Wong GK, Wendehenne D. 2016. Occurrence, structure, and evolution of nitric oxide synthase-like proteins in the plant kingdom. *Science Signaling* 9: re2.

Joo Y, Kim H, Kang M, Lee G, Choug S, Kaur H, Oh S, Choi JW, Ralph J, Baldwin IT. 2021. Pith-specific lignification in *Nicotiana attenuata* as a defense against a stem-boring herbivore. *New Phytologist* 232: 332–344.

Kim J, Dotson B, Rey C, Lindsey J, Bleecker AB, Binder BM, Patterson SE. 2013. New clothes for the jasmonic acid receptor COI1: delayed abscission, meristem arrest and apical dominance. *PLoS ONE* 8: e60505.

Kliebenstein DJ, Monde RA, Last RL. 1998. Superoxide dismutase in Arabidopsis: an ecletic enzyme family with disparate regulation and protein localization. *Plant Physiology* 118: 637–650.

Kumpf RP, Shi C-L, Larriue A, Sun JM, Butenko MA, Pérez B, Rüser ES, Bennett MJ, Aalen RB. 2013. Floral organ abscission peptide IDA and its HAE/HSL2 receptors control cell separation during lateral root emergence. *Proceedings of the National Academy of Sciences, USA* 110: 5235–5240.

Lee Y, Kim YJ, Kim MH, Kwak JM. 2016. MAPK cascades in guard cell signal transduction. *Frontiers in Plant Science* 7: 80.

Lee Y, Yoon TH, Lee J, Jeon SY, Lee JH, Lee MK, Chen H, Yun J, Oh SY, Wen X et al. 2018. A lignin molecular brace controls precision processing of cell walls critical for surface integrity in Arabidopsis. *Cell* 173: 1468–1480 e1469.

Leslie ME, Lewis MW, Youn J-Y, Daniels MJ, Liljegren SJ. 2010. The EVERSHELD receptor-like kinase modulates floral organ shedding in Arabidopsis. *Development* 137: 467–476.

Li B, Dewey CN. 2011. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 12: 1–16.

Liao W, Wang G, Li Y, Wang B, Zhang P, Peng M. 2016. Reactive oxygen species regulate leaf pulvinus abscission zone cell separation in response to water-deficit stress in cassava. *Scientific Reports* 6: 21542.

Liu B, Butenko MA, Shi CL, Belivar JL, Wang P, Stenvik GE, Vie AK, Leslie ME, Brembu T, Kristiansen W et al. 2013. NEVERSHED and INFLORESCENCE DEFICIENT IN ABSCESSION are differentially required for cell expansion and cell separation during floral organ abscission in Arabidopsis thaliana. *Journal of Experimental Botany* 64: 5345–5357.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression using real-time quantitative PCR and the 2− ΔΔCT method. *Methods* 25: 402–408.

Ma Y, Cao J, He J, Chen Q, Li X, Yang Y. 2018. Molecular mechanism for the regulation of ABA homeostasis during plant development and stress responses. *International Journal of Molecular Sciences* 19: 3643.

McKim SM, Stenvik GE, Butenko MA, Kristiansen W, Cho SK, Hepworth SR, Aalen RB, Haughn GW. 2008. The BLADE-ON-PETIOLE genes are essential for abscission zone formation in Arabidopsis. *Development* 135: 1537–1546.

Meinhard M, Grill E. 2001. Hydrogen peroxide is a regulator of ABA1, a protein phosphatase 2C from *Arabidopsis*. *FEBS Letters* 508: 443–446.

Meinhard M, Rodriguez PL, Grill E. 2002. The sensitivity of ABA2 to hydrogen peroxide links the abscisic acid-response regulator to redox signalling. *Planta* 214: 775–782.

Meir S, Philosop-Hadas S, Rov J, Tucker ML, Patterson SE, Roberts JA. 2019. Re-evaluation of the ethylene-dependent and -independent pathways in the regulation of floral and organ abscission. *Journal of Experimental Botany* 70: 1461–1467.

Meng X, Zhou J, Tang J, Li B, de Oliveira MV, Chai J, He P, Shan L. 2016. Ligand-induced receptor-like kinase complex regulates floral organ abscission in *Arabidopsis*. *Cell Reports* 14: 1330–1338.

Mhamdi A, Van Breusegem F. 2018. Reactive oxygen species in plant development. *Development* 145: dev164376.
Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Characterization of *msd2* mutant plants.

**Fig. S2** Relative expression level of the indicated genes in the abscission zone of wild-type and *msd2-1* plants by abscission stage, as determined by reverse-transcription quantitative polymerase chain reaction.

**Fig. S3** Functional heatmap of select enzymes in the nitric oxide biosynthesis pathway.

**Fig. S4** Functional heatmaps of selected enzymes in abscisic acid (ABA) metabolism (a) and ABA signaling pathway (b).

**Fig. S5** Validation of RNA-sequence data by reverse-transcription quantitative polymerase chain reaction for differential gene expression.

**Table S1** Primers and constructs in this study.

**Table S2** Differentially expressed genes transcripts per million values.

**Table S3** List of Gene Ontology terms in C13 vs m13.

**Table S4** List of Gene Ontology terms in C15 vs m15.

**Table S5** List of Gene Ontology terms in Web Gene Ontology Annotation Plot (WEGO) analysis.

**Table S6** List of Kyoto Encyclopaedia of Genes and Genomes pathways.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.