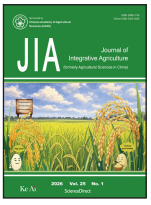
Journal of Integrative Agriculture 2026, 25(1): 118–126

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

SlIDL6–SlHSL1/2/3 ligand-receptor pairs regulate tomato pedicel abscission

Yanyun Tu[1], Lina Cheng[1], Xianfeng Liu[1], Marta Hammerstad[2], Chunlin Shi[3], Sida Meng[1], Mingfang Qi[1], Tianlai Li[1], Tao Xu[1#]

[1] *College of Horticulture/Key Laboratory of Protected Horticulture of Ministry of Education/Modern Protected Horticulture Engineering & Technology Center, Shenyang Agricultural University, Shenyang 110866, China*
[2] *Department of Biosciences, University of Oslo, Oslo 0371, Norway*
[3] *ANGENOVO AS, Oslo 0753, Norway*

Highlights

- We isolated three HAESA-like homologs, SlHSL1/2/3, which are involved in tomato flower abscission.
- SlHSL1/2/3 are the receptors of SlIDL6 in pedicel abscission and flower abscission under low light.
- Ethylene action inhibitor 1-methylcyclopropene (1-MCP) can significantly depress the expression of *SlHSL1/2/3*, and the abscission regulated by SlHSL1/2/3 is partially dependent on ethylene.

Abstract

Flower and fruit abscission reduce crop yield, so decreasing abscission is a significant agricultural issue. HAESA (HAE) and HAESA-like2 (HSL2) kinases and their ligand, INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) peptide, have been confirmed to be the core elements regulating floral organ abscission in *Arabidopsis thaliana*. Our earlier research revealed that SlIDL6, a homolog of IDA in tomato, functions similarly to AtIDA, regulating the abscission of tomato flower organs. Here, we further isolated three HAESA-like homologs, SlHSL1/2/3, which are involved in tomato flower abscission. *SlHSL1/2/3* are highly expressed in the abscission zone (AZ). The knockout mutant lines of *Slhsl1*, *Slhsl2*, and *Slhsl3* showed lower flower pedicel abscission than wild type (WT). The double mutant of *Slhsl1Slhsl2*, *Slhsl1Slhsl3*, and *Slhsl2Slhsl3* further depressed abscission than each of the single mutant lines, while triple mutants *Slhsl1Slhsl2Slhsl3* exhibited the lowest abscission, indicating that SlHSL1/2/3 mediated abscission is non-redundancy, at least partially. Treating tomato pedicel explants with SlIDL6 peptide significantly accelerated pedicel abscission in WT. However, it had little effect on the abscission rate of *SlHSL1*/2/3 knockout lines, indicating that SlHSL1/2/3 are the receptors of SlIDL6 in pedicel abscission. Ethylene action inhibitor 1-methylcyclopropene (1-MCP) can significantly depress the expression of *SlHSL1/2/3*. Ethylene can significantly accelerate the abscission of WT, while less abscission was found in *SlHSL1/2/3* knockout lines. Our findings indicate that SlHSL1/2/3 can act as receptors for SlIDL6 to positively regulate tomato pedicel abscission, and the abscission regulated by SlHSL1/2/3 was partially dependent on ethylene.

Keywords: tomato, abscission, SlHSL, SlIDL6, ethylene

1. Introduction

Plant reproductive organs abscise at a specific region known as the abscission zone (AZ), induced by environmental and developmental factors, leading to diminished crop yields (Taylor and Whitelaw 2001). Although ethylene is crucial in mediating this process (Estornell *et al.* 2013), the involvement of other factors in abscission needs further study.

Receptor-like kinases (RLKs) are an important class of receptors that primarily function to perceive and convey extracellular signals, consequently regulating the development and growth of plants. The subgroup of leucine-rich repeat RLKs (LRR-RLKs) represents the most abundant RLKs

Received 4 November 2024; Received in revised form 7 January 2025; Accepted 14 Feburary 2025; Available online 22 March 2025
#Correspondence Tao Xu, E-mail: syauxutao@syau.edu.cn

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.03.018

class in *Arabidopsis thaliana*, consisting of approximately 200 members (Shiu and Bleecker 2001; Hohmann *et al.* 2017). Proteins with LRR domains can be found in all living organisms (Kobe and Kajava 2001). A typical LRR-RLK comprises an external LRR domain of ligand binding, an intracellular kinase domain, and a transmembrane domain. Only a limited number of LRR-RLKs have been featured to date (Gou and Li 2020; Ou *et al.* 2021; Cui *et al.* 2022; Li *et al.* 2022). LRR-RLKs mainly perform four tasks. Firstly, they perceive extracellular signals with high specificity, whether these are foreign or natural small peptides, molecules, or protein ligands. Secondly, the activity of intracellular kinase domains changes after ligand recognition. Thirdly, the activated LRR-RLKs regulate the activity of downstream signal cascades, resulting in signal output. Fourthly, LRR-RLKs offer protein interaction sites that expose them to various regulatory mechanisms and integrate them into extensive signaling networks (Hohmann *et al.* 2017).

Arabidopsis HAESA (AtHAE) belongs to the LRR XI RLK subfamily and has two paralogs, HAESA-LIKE1 (AtHSL1) and HAESA-LIKE2 (AtHSL2), which exhibit analogous intracellular Ser/Thr protein kinase domain and extracellular LRR domain (Shiu and Bleecker 2001). In *Arabidopsis*, AtHAE and AtHSL2 redundantly and actively regulate the process of floral organ abscission (Cho *et al.* 2008). Mutations in *HAE* and *HSL2* result in the complete absence of abscission (Cho *et al.* 2008; Stenvik *et al.* 2008). INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) is essential for regulating flower abscission since the *ida* mutants were, like *hae hsl2* double mutants, completely lacking the ability to abscise, while *IDA* overexpression results in considerable early abscission occurrences (Butenko *et al.* 2003, 2006; Stenvik *et al.* 2008). Communication between plant cells can be regulated by the unique structure of receptor-ligands (Butenko *et al.* 2014). Further studies have shown that the function of IDA in floral organ abscission depends on its receptor HAE/HSL2 (Cho *et al.* 2008; Stenvik *et al.* 2008; Santiago *et al.* 2016). The homologous sequence of IDA-HAE/HSL2 has been discovered and expressed in AZs of fruits, leaves, and flowers of all flowering plants, indicating that the IDA-HAE/HSL2 module can initiate the abscission process of species other than *Arabidopsis* (Stø *et al.* 2015). So far, some cases have confirmed the conserved role of IDA-HAE/HSL2 across several organs and species through *Arabidopsis ida* mutants. Recent research has also indicated that AtIDA-induced abscission is conserved in other species, such as citrus (*Citrus unshiu*), mango (*Mangifera indica* L.), tobacco (*Nicotiana benthamiana*), bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) (Tucker and Yang 2012; Estornell *et al.* 2015; Rai *et al.* 2021; Ventimilla *et al.* 2021). Furthermore, LcHSL2 is a homolog of AtHAE/AtHSL2 in litchi (*Litchi chinensis* Sonn.) and is presumably implicated in the fruitlet abscission, as ectopic expression of *LcHSL2* can effectively rescue abscission defects when expressed in the presence of *hae hsl2* mutation background (Wang *et al.* 2019). In *N. benthamiana*, after silencing *NbenIDA* and *NbenHAE*, corolla abscission was significantly slower compared to the control group (Ventimilla *et al.* 2021).

After IDA activates HAE/HSL2, the mitogen-activated protein kinase (MAPK) cascade is initiated, including MAP kinase kinase kinase (MAPKKK), YODA (YDA, also referred to as MAPKKK4), MAPKKK3/5, MAPK kinase 4 (MKK4)/MKK5, and MAPK 3 (MPK3)/MPK6 (Cho *et al.* 2008; Stenvik *et al.* 2008; Wang *et al.* 2022), leading to suppression of the KNOTTED1-LIKEHOMEOBOX (KNOX) transcription factor and ultimately induction of genes encoding enzymes involved in cell wall remodeling (Cho *et al.* 2008; Stenvik *et al.* 2008; Shi *et al.* 2011).

Cutting off flowers depletes the auxin of AZs and rapidly enhances the sensitivity of ethylene, accelerating the tomato pedicel abscission (Meir *et al.* 2010). Low light stress can promote the abscission of fruits and flowers, associating the alterations in ethylene content (Taylor and Whitelaw 2001; Zhu *et al.* 2011; Domingos *et al.* 2015). The homolog of AtIDA in tomato SlIDL6 has been shown to positively regulate flower abscission under low light stress, with SlIDL6-induced abscission partially independent on the ethylene pathway (Li *et al.* 2021). However, there remains an insufficiency of evidence about the conservative role of IDA-HAE/HSL2 in regulating tomato abscission.

Tomato is a critically significant vegetable crop, with flower drop resulting in diminished yields and substantial economic losses, so decreasing abscission is extremely important. In this study, we identified SlHSL1/2/3, homologs of AtHAE/AtHSL2, acting as receptors for SlIDL6 to positively regulate tomato pedicel abscission.

2. Materials and methods

2.1. Plant materials and growth conditions

Transgenic tomato plants were produced utilizing the wild type (WT) *Solanum lycopersicum* cv. Ailsa Craig (AC). Transgenic tomato plants from transformant (T2) isolate were used for genotyping and phenotype analysis. The WT and transgenic plants were cultivated in the greenhouse with 16 hours (h) of light at 25°C and 8 h of darkness at 15°C. The shading treatment was conducted as earlier stated (Li *et al.* 2021). The levels of photosynthetically active radiation under low light and normal conditions were 60 and 600 μmol, respectively. Tomato flower pedicel explants were cultured in a glass container containing 0.9% agar, which was infused with ethylene to reach an ultimate concentration of 20 μL L^{-1}. In the 1-methylcyclopropene (1-MCP, ethylene action inhibitor) treatment, pedicel explants were incubated with 1-MCP at a final concentration of 0.4 μL L^{-1} at 23°C for 16 h. All samples were promptly frozen using liquid nitrogen and preserved at –80°C until analysis.

Nicotiana benthamiana was nurtured in the growth chamber under the following conditions: 25°C during the day and 18°C at night, a time period of 16 h of light and 8 h of darkness, relative humidity set at 70%, and a photosynthetic photon flux density (PPFD) of 300 μmol m^{-2} s^{-1}.

2.2. Plasmid construction and plant transformation

To make deletions in the *SlHSL1*, *SlHSL2*, and *SlHSL3* coding sequence and produce knockout lines, two individual guide RNAs (sgRNAs) have been established to target *SlHSL1*, *SlHSL2*, and *SlHSL3* to generate genome-edited mutants in the corresponding genes. The U6-26-

SlHSL1/2/3-gRNA cassettes were inserted into the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated nuclease 9 (Cas9) binary vector, pCBC-DT1T2-tomatoU6, to obtain pCBC-DT1T2-tomatoU6-SlHSL1/2/3. Each primary transformant (T0) underwent genotyping through sequencing of PCR products produced from the relevant target areas. The identification of the genome editing system was completed by Sanger sequencing of PCR products generated from specific regions of interest. Perform phenotype analysis by identifying Cas9 transgenic homozygous lines through PCR. The primers utilized in this experimental analysis are all recorded in Appendix A.

2.3. Subcellular localization analysis

The sequences of *SlHSL1*, *SlHSL2*, and *SlHSL3* were fused into a pCAMBIA1300-35S-GFP vector, which generated the constructs *35S*::SlHSL1-GFP, *35S*::SlHSL2-GFP, and *35S*::SlHSL3-GFP, each tagged with a green fluorescent protein (GFP). Then, *35S*::SlHSL1-GFP, *35S*::SlHSL2-GFP, and *35S*::SlHSL3-GFP constructs were injected into *A. tumefaciens* strain GV3101 and subsequently converted into the leaves of tobacco (*N. benthamiana*). The GFP was detected using the TCS SP8 confocal microscope (Leica, Wetzlar, Germany) 72 h after infiltration. The excitation/emission wavelengths for GFP were 488 nm/500 to 530 nm. FM4-64 is a red fluorescent dye acting as the cell membrane marker. Bars measure 50 μm. The primers utilized in this study are in Appendix A.

2.4. Virus-induced gene silencing (VIGS)

The silencing fragments for *SlHSL1*, *SlHSL2*, *SlHSL3*, and *SlHSL5* were designed utilizing the VIGS tool available on the Sol Genomics Network website (https://solgenomics.net/). These fragments were subsequently amplified *via* PCR and fused into the pTRV2 vector to obtain the pTRV2-*SlHSL1*, pTRV2-*SlHSL2*, pTRV2-*SlHSL3*, and pTRV2-*SlHSL5* constructs. The pTRV2 vector, pTRV2-*SlHSL*, and pTRV1 vectors were separately introduced into *A. tumefaciens* strain GV3101. The culture mixture, including pTRV2-*SlHSL* and pTRV1, was utilized for infection, as earlier delineated (Liu *et al.* 2002). The pedicel abscission rate was investigated using the pedicels of blooming flowers at least 14 days after infiltration, and the silencing efficiency was detected with the corresponding flowers. The primers utilized in this experimental analysis are all recorded in Appendix A.

2.5. RNA extraction and RT-qPCR analysis

Total RNA was collected from the tomato flower pedicel AZs utilizing an RNA pure plant kit (CWBIO, Cambridge, MA, USA). One microgram of total RNA was utilized for first-strand cDNA generation employing Prime Script RT Master Mix (TaKaRa, Kusatsu, Japan). RT-qPCR was conducted with primers that are gene-specific and the TB Green® Premix *Ex Taq* II (TaKaRa) reaction system as previously outlined (Cheng *et al.* 2022). The internal control for RT-qPCR was the tomato *ACTIN* gene (NCBI: NM_001330119.1). The primers utilized in this experimental analysis are all recorded in Appendix A.

2.6. Peptide treatment

Explants of tomato flower pedicel were collected at the anthesis phase, rapidly washed in 70% ethanol and filtered water, and subsequently cultivated on 0.9% agar plates with 5 μmol L^{-1} doses of the SlIDL6 peptide and 12 aa (PFLGVYYHKNNK) control peptide supplants (Li *et al.* 2021). These plates were positioned beneath a glass cover in the growth chamber at 25°C for 8 h in darkness and 16 h in light. These data were gathered and examined from at least three distinct treatments. These peptides were manufactured by the Shenggong Company (China), with a purity of over 90%.

2.7. Phylogenetic analysis

The conserved protein sequences from AtHAE and AtHSL1/2 were examined against the tomato genome database (https://www.solgenomics.net/) using a BLASTp search. Phylogenetic analysis was conducted on the protein sequences of AtHAE, AtHSL1/2, and SlHSL1–11 using MEGA7 software with a neighbor-joining method. The CDS and amino acid sequences of *SlHSL1–11* are provided in Appendix B.

2.8. Computational modeling

Three-dimensional structures were generated for the ectodomain of SlHSL1, SlHSL2, and SlHSL3 utilizing the AtHAE-AtIDA crystal structure (PDBid: 5ixq) and homology modeling (SWISS-model). The interactions between SlIDL6 peptide and SlHSL1/2/3 receptors were investigated according to (Shi *et al.* 2019). All structure figures were prepared using PyMOL (Schrödinger, LLC, CA, USA).

2.9. Oxidative burst assays

Ox-burst (Oxidative burst) experiments were carried out in accordance with the method described by Butenko *et al.* (2014), employing a luminol-dependent assay. The constructs *35S*::SlHSL1-GFP, *35S*::SlHSL2-GFP, and *35S*::SlHSL3-GFP were transformed into tobacco leaves (*N. benthamiana*). After 24 h, the GFP signal was detected using the TCS SP8 confocal microscope (Leica, Wetzlar, Germany) at 488 nm. Subsequently, the tobacco leaves were cut and then left floating on water overnight. The following day, the leaves were placed in 96-well plates, with each plate containing 100 μL of water, 10 μg mL^{-1} of horseradish peroxidase, and 20 μmol L^{-1} luminol. Additionally, 5 μmol L^{-1} doses of the SlIDL6 peptide were added to the wells. The emission of light was measured using an LB 96 microplate luminometer (Groedig, Austria).

2.10. Statistical analysis

All trials were conducted a minimum of three times. The Student's *t*-test is employed to evaluate the statistical significance of the results, as delineated in the relevant sections of the methodology and illustrations.

2.11. Accession numbers

Sequence data from this paper is available in the tomato genome database (http://www.solgenomics.net/) or the National Center for Biotechnology Information (https://

www.ncbi.nlm.nih.gov/) under accession numbers *SlHSL1* (Solyc02g077630); *SlHSL2* (Solyc03g006300); *SlHSL3* (Solyc07g053600); *SlHSL4* (Solyc06g065260); *SlHSL5* (Solyc11g020280); *SlHSL6* (Solyc04g077010); *SlHSL7* (Solyc02g091860); *SlHSL8* (Solyc08g066270); *SlHSL9* (Solyc08g066320); *SlHSL10* (Solyc04g072570); *SlHSL11* (Solyc04g005390).

3. Results

3.1. *SlHSL1/2/3/5* are highly expressed in AZs

Eleven HAESA-like genes from *SlHAESA-like-1* to *SlHAESA-like-11* (*SlHSL1–11*) were identified, including the previously identified *SlHSL1–7* (Lu *et al.* 2023), in tomato through a TBLASTN search (https://blast.ncbi.nlm.nih.gov) using AtHAE and AtHSL1/2 protein sequences (Appendix B). The SlHSL1–11 belongs to the LRR-RLKs family, each comprising conserved extracellular tandem repeats of LRR, an intracellular Ser/Thr kinase domain, and a transmembrane domain (Appendix C). A phylogenetic tree was created utilizing the neighbor-joining approach and bootstrap analysis (1,000 replicates) through MEGA7 software. The analysis revealed that AtHAE and AtHSL1/2 are highly similar to SlHSL1, SlHSL2, SlHSL3, and SlHSL7 (Fig. 1-A).

We investigated the expression levels of *SlHSL1–11* in AZs by RT-qPCR. We demonstrated that *SlHSL1*, *SlHSL2*, *SlHSL3*, and *SlHSL5* are highly expressed in AZs, while *SlHSL4*, *SlHSL6*, *SlHSL7*, *SlHSL8*, *SlHSL9*, *SlHSL10*, and *SlHSL11* are lowly expressed in AZs (Fig. 1-B).

3.2. *SlHSL1/2/3* positively regulate tomato pedicel abscission

To investigate their potential role in abscission, we generated a set of tomato lines individually silencing *SlHSL1*, *SlHSL2*, *SlHSL3*, and *SlHSL5* utilizing VIGS (Appendix D). There was no significant difference in pedicel abscission between plants infiltrated with the pTRV2-*SlHSL5* and pTRV2-empty vector control (Appendices D and E). However, a reduction in *SlHSL1*, *SlHSL2*, and *SlHSL3* transcript levels significantly delayed abscission (Appendices D and E). Furthermore, we demonstrated that *SlHSL1*, *SlHSL2*, and *SlHSL3* were highly expressed in AZs with a lower expression in the proximal and distal regions by RT-qPCR (Appendix F).

To further elucidate the function of *SlHSL1*, *SlHSL2*, and *SlHSL3* in abscission, we created knockout mutants for *SlHSL1*, *SlHSL2*, and *SlHSL3* utilizing CRISPR/Cas9 genome editing (Appendices G–J). Two target areas were established for *SlHSL1*, *SlHSL2*, and *SlHSL3*, respectively. We acquired at least 3 transformants (T2s) with lesions in *SlHSL1*, *SlHSL2*, and *SlHSL3*, and three independent transgenic lines were selected for further studies. All knockout lines exhibited delayed abscission. The WT plants showed 96% abscission at 40 h after flower removal, while the *Slhsl1*, *Slhsl2*, and *Slhsl3* knockout lines exhibited significantly delayed abscission, with pedicels displaying 64, 69, and 66% abscission, respectively (Fig. 2-A–C).

To verify whether there is functional redundancy among *SlHSL1*, *SlHSL2*, and *SlHSL3*, we obtained double and triple mutants using hybridization technology. Then, we analyzed the abscission rate of double and triple mutants after removing flowers. The abscission of *Slhsl1Slhsl2*, *Slhsl1Slhsl3*, and *Slhsl2Slhsl3* double mutants had significantly slower than WT, *Slhsl1*, *Slhsl2*, and *Slhsl3* with pedicels that demonstrated 51, 54, and 55% abscission at 40 h after flower removal. Further, the abscission of *Slhsl1Slhsl2Slhsl3* triple mutants exhibited more markedly delayed abscission, with flower pedicels demonstrating 38% abscission at 40 h after flower removal (Fig. 2-D). These results show that *SlHSL1/2/3* are at least partially functionally nonredundant in tomato pedicel abscission.

3.3. SlHSL1/2/3 are located in the plasma membrane

To determine the subcellular localization of SlHSL1/2/3, fusion proteins, SlHSL1-GFP, SlHSL2-GFP, and SlHSL3-GFP, were constructed and temporarily expressed under the regulation of the *35S* promoter in *N. benthamiana*. We transiently expressed SlHSL1-GFP, SlHSL2-GFP, and SlHSL3-GFP fusion protein in *N. benthamiana* leaves. The SlHSL1-GFP, SlHSL2-GFP, and SlHSL3-GFP proteins were detected exclusively in the plasma membrane (Fig. 3), indicating that SlHSL1-GFP, SlHSL2-GFP, and SlHSL3-GFP are membrane proteins.

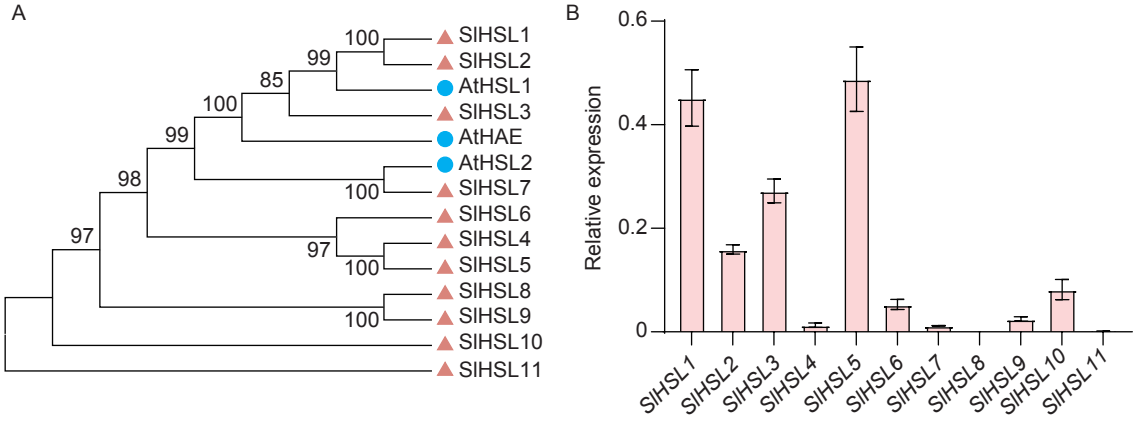


Fig. 1 Phenologenetic and expression analysis of *SlHSL1–11* in abscission zones (AZs). A, phylogenetic relationships between *Arabidopsis* AtHAE/AtHSL and tomato SlHSL1–11 proteins utilizing the MEGA7 software with the neighbor-joining method. B, expression levels of *SlHSL1–11* in AZs by RT-qPCR. These data are mean±SD (*n*=3), with a minimum of 30 samples per replication.

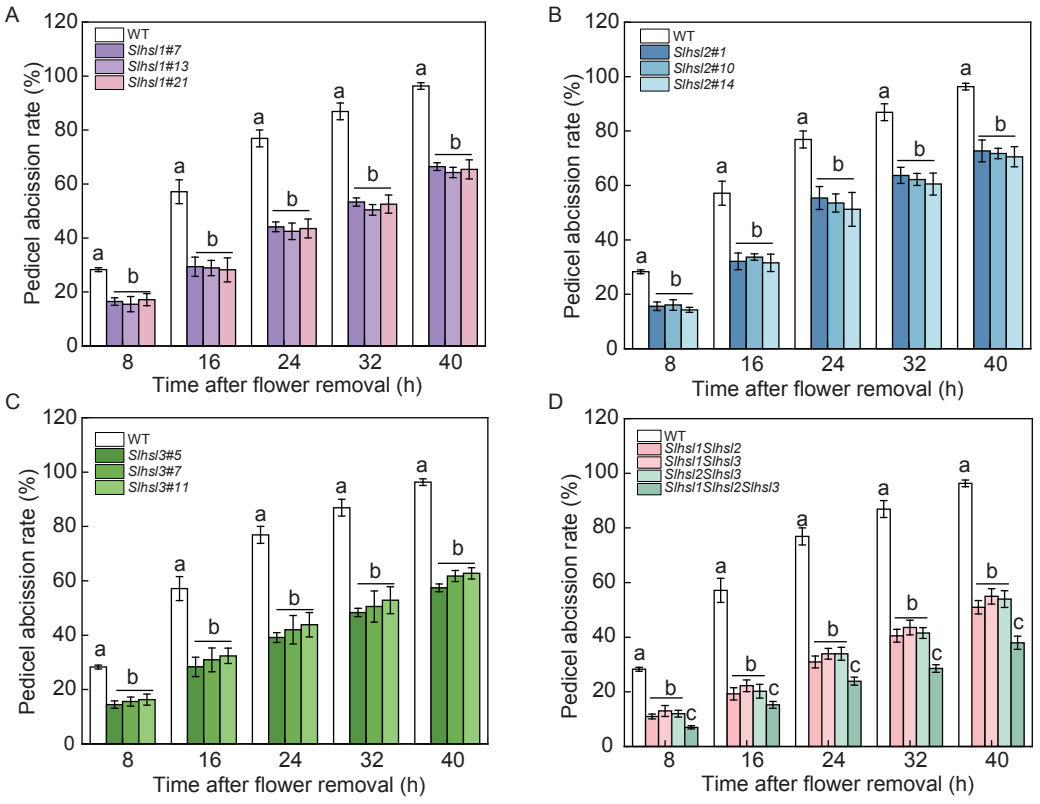


Fig. 2 Effects of *SlHSL1*, *SlHSL2*, and *SlHSL3* knockout on flower pedicel abscission in wild-type (WT) plants and the mutants. A, *Slhsl1*. B, *Slhsl2*. C, *Slhsl3*. D, *Slhsl1Slhsl2*, *Slhsl1Slhsl3*, *Slhsl2Slhsl3*, and *Slhsl1Slhsl2Slhsl3*. These data are mean±SD (n=3), with a minimum of 30 samples per replication. Lowercase letters denote statistically significant differences (Student's t-test, P<0.05).

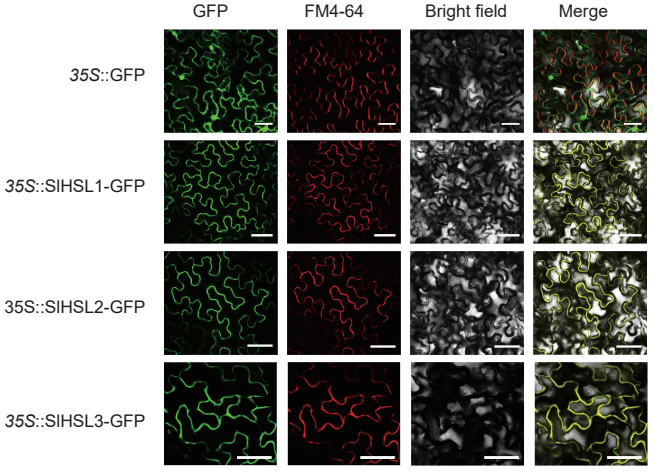


Fig. 3 Subcellular localization analysis of SlHSL1-GFP, SlHSL2-GFP, and SlHSL3-GFP in epidermis cells of tobacco leaves. FM4-64 is a red fluorescent dye acting as the cell membrane marker. Bars measure 50 μm.

3.4. SlIDL6 is a ligand of SlHSL1/2/3 in regulating tomato abscission

Our earlier research indicated that SlIDL6 is highly expressed in AZs and plays a positive role in tomato pedicel abscission (Li *et al.* 2021). The C-terminal proline-rich domain of the SlIDL6 peptide, the so-called PIP domain, conserved the amino acid residues Ser62^{AtIDA}, Pro64^{AtIDA}, Ser65^{AtIDA}, and Asn69^{AtIDA} that were crucial in the interaction of AtIDA with the AtHAE/AtHSL2 receptors (Appendix K). The amino acid residues within the peptide binding pocket of AtHAE, essential for interaction with AtIDA, are also preserved in the SlHSL1/2/3 receptors, which retain the crucial amino acid residues necessary for ligand-receptor binding (Glu266^{AtHAE}, Phe289^{AtHAE}, Ser311^{AtHAE}, Arg407^{AtHAE}, and Arg409^{AtHAE}) (Santiago *et al.* 2016) (Appendix K). Based on the *Arabidopsis* IDA-HAE/HSL2 binding model, we conducted a similar analysis on tomato and found that SlIDL6 is highly likely to bind to SlHSL1/2/3 (Santiago *et al.* 2016; Shi *et al.* 2019) (Fig. 4-A–C).

Our previous research has shown that 5 μmol L^{-1} SlIDL6 peptide significantly enhances tomato pedicel abscission (Li *et al.* 2021). To test whether SlHSL1/2/3 function in SlIDL6 signaling in AZs, 5 μmol L^{-1} SlIDL6 peptide used to treat the flower pedicel explants, and it was found that the abscission of WT, *Slhsl1*, *Slhsl2*, *Slhsl3* single mutants, *Slhsl1Slhsl2*, *Slhsl1Slhsl3*, and *Slhsl2Slhsl3* double mutants were accelerated, while the abscission of the *Slhsl1Slhsl2Slhsl3* triple mutants remained basically unchanged. After 24 h of SlIDL6 peptide application, 94% for WT, 56% for *Slhsl1*, 58% for *Slhsl2*, 57% for *Slhsl3*, 47% for *Slhsl1Slhsl2*, 45% for *Slhsl1Slhsl3*, 44% for *Slhsl2Slhsl3*, and 24% for *Slhsl1Slhsl2Slhsl3* plants pedicels had abscised, while the corresponding values from the contrast to 12 aa control peptides, 82% for WT, 42% for *Slhsl1*, 47% for *Slhsl2*, 44% for *Slhsl3*, 30% for *Slhsl1Slhsl2*, 34% for *Slhsl1Slhsl3*,

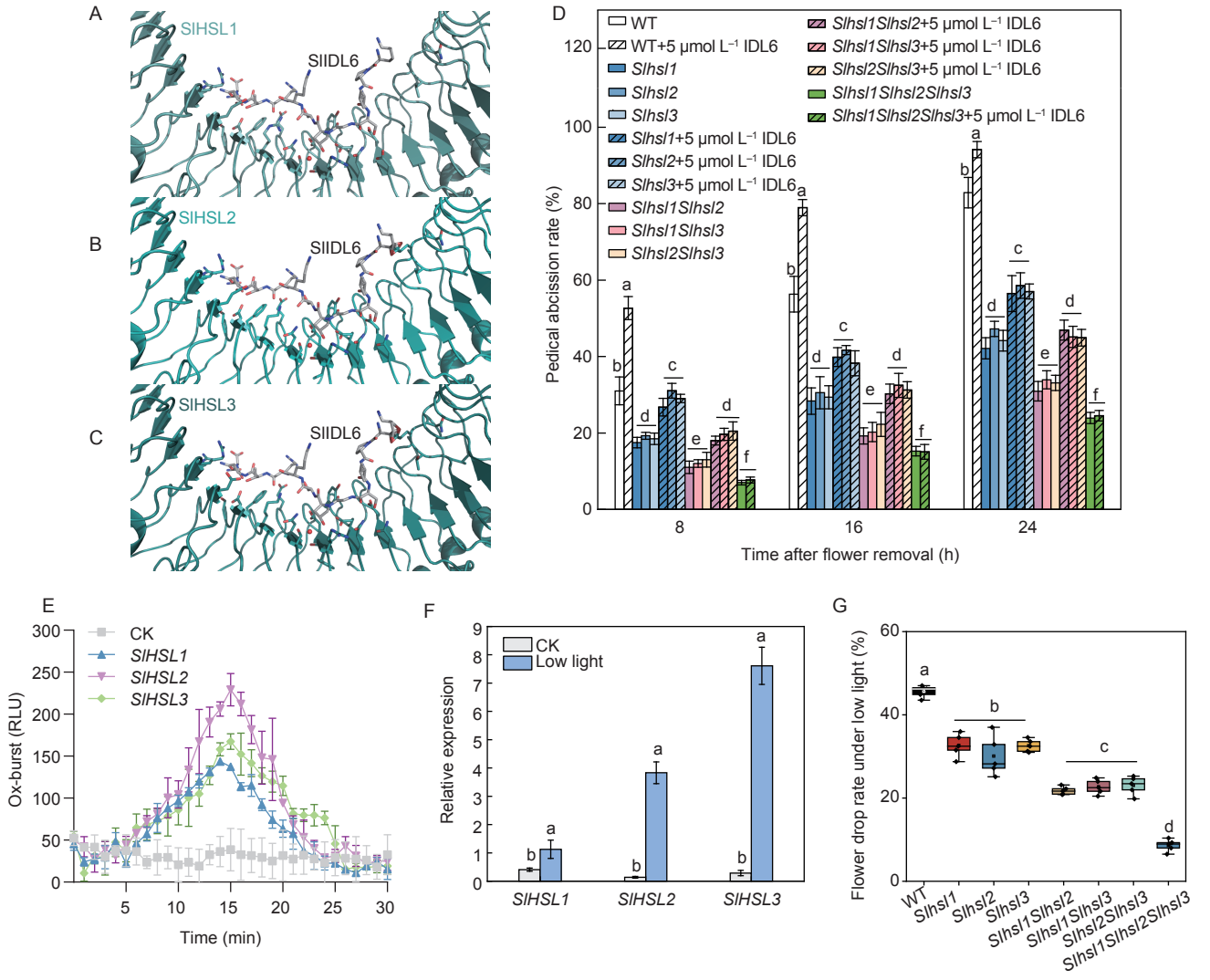


Fig. 4 SlIDL6 serves as the ligand for SlHSL1/2/3, participating in regulating abscission under low light stress. A–C, structures of SlHSL1 (A), SlHSL2 (B), and SlHSL3 (C) bind to SlIDL6 peptides. All structure figures were prepared using PyMOL (Schrödinger, LLC). D, the impact of 5 μmol L^{-1} SlIDL6 peptide and 12 aa control peptide (PFLGVYYHKNNK) concentrations on the abscission of mutants and WT flower pedicel. These data are mean±SD (n=3), with a minimum of 30 samples per replication. Lowercase letters suggest statistically significant differences (Student's t-test, P<0.05). E, SlIDL6 peptide triggers the production of reactive oxygen species (ROS) through SlHSL1, SlHSL2, and SlHSL3 in *N. benthamiana*. Oxidative burst (Ox-burst) was measured in leaves of untransformed (CK) or leaves transiently expressing the *35S*::SlHSL1-GFP, *35S*::SlHSL2-GFP, and *35S*::SlHSL3-GFP constructs. Leaf pieces were then exposed to the SlIDL6 peptide. Ox-burst was monitored over time as relative light units (RLU) using a luminol-based assay. These data are mean±SD (n=3). F, comparative analysis of the expression levels of *SlHSL1*, *SlHSL2*, and *SlHSL3* in the pedicel AZs under low light treatment and the control (CK). These data are the averages of three replicates±SD, with a minimum of 30 samples per replication. Lowercase letters suggest statistically significant differences (Student's t-test, P<0.05). G, low light stress induces the abscission of flowers in mutants and WT after fruit setting. These data are the averages of three replicates±SD, with a minimum of 25 samples per replication. Lowercase letters suggest statistically significant differences (Student's t-test, P<0.05).

34% for *Slhsl2Slhsl3*, and 24% for *Slhsl1Slhsl2Slhsl3* plants pedicels had abscised, respectively (Fig. 4-D). To further investigate whether SlIDL6 and SlHSL1/2/3 act as ligand-receptor pairs, we carried out oxidative burst assays (Ox-burst), which is a highly sensitive approach for detecting ligand-receptor interactions (Butenko *et al.* 2014). Remarkably, after treating the tobacco leaves expressing SlHSL1/2/3 with the SlIDL6 peptide, a significant Ox-burst was detected by means of luminol-dependent assays (Fig. 4-E). These results demonstrated that SlIDL6-mediated abscission requires SlHSL1/2/3 to accelerate abscission, and SlIDL6 may serve as a ligand for SlHSL1/2/3 to regulate tomato pedicel abscission.

Prior research has demonstrated that AtHAE/AtHSL2 plays a significant role in regulating plant organ abscission under stress conditions (Vie *et al.* 2015; Patharkar and Walker 2016), while SlIDL6 regulates the abscission of tomato flower pedicels under low light (Li *et al.* 2021; Fu *et al.* 2024). Consequently, we evaluated the expression levels of *SlHSL1/2/3* under low light stress. Low light stress can markedly induce the expression of *SlHSL1/2/3* (Fig. 4-F). Furthermore, 46% of WT flowers were abscised under low

light stress, compared to 32% for *Slhsl1*, 37% for *Slhsl2*, 34% for *Slhsl3*, 21% for *Slhsl1Slhsl2*, 21% for *Slhsl1Slhsl3*, 25% for *Slhsl2Slhsl3*, and 9% for *Slhsl1Slhsl2Slhsl3* (Fig. 4-G). Those results further support SlIDL6-SlHSL1/2/3 ligand-receptor pairs mediating low light-induced flower drop.

3.5. Ethylene employs SlHSL1/2/3 to accelerate abscission

To investigate whether SlHSL1/2/3 induced abscission can be mediated *via* ethylene, we measured the gene expression of *SlHSL1/2/3* by RT-qPCR with or without 1-MCP treatment. The expression levels of *SlHSL1/2/3* were significantly depressed after 16 h of 1-MCP treatment compared to control (Fig. 5-A), indicating that ethylene can promote *SlHSL1/2/3* expression. We measured WT, *Slhsl1*, *Slhsl2*, *Slhsl3*, *Slhsl1Slhsl2*, *Slhsl1Slhsl3*, *Slhsl2Slhsl3*, and *Slhsl1Slhsl2Slhsl3* abscission rate after treating them with 20 μL L^{-1} of ethylene. After 16 h of ethylene treatment, 91% of WT pedicel explants were abscised, in contrast to 70% for *Slhsl1*, 72% for *Slhsl2*, 71% for *Slhsl3*, 59% for *Slhsl1Slhsl2*, 58% for *Slhsl1Slhsl3*, 61% for *Slhsl2Slhsl3*, and 46% for *Slhsl1Slhsl2Slhsl3* plants (Fig. 5-B). Compared to WT, the abscission of mutants was significantly delayed, indicating that the ethylene-induced abscission required SlHSL1/2/3.

4. Discussion

Multiple receptors regulated abscission, among which AtHAE was the earliest identified (Jinn *et al.* 2000; Cho *et al.* 2008; Stenvik *et al.* 2008; Patharkar and Walker 2016; Reichardt *et al.* 2020; Li *et al.* 2021; Cheng *et al.* 2022). Besides *Arabidopsis*, *HAESA-like* genes have been found in other species. For example, *HAESA-like* genes are highly expressed in AZs of citrus, lychee, and yellow lupine and are involved in regulating abscission (Tucker and Yang 2012; Estornell *et al.* 2013; Estornell *et al.* 2015; Wang *et al.* 2019; Ventimilla *et al.* 2021). Our study identified that SlHSL1/2/3, homologs of AtHAE and AtHSL1/2, are highly expressed in AZs (Fig. 1-B; Appendix F). Knocking out *SlHSL1/2/3* separately, all exhibited delayed abscission, while double or triple mutants exhibited more delayed abscission (Fig. 2), indicating that SlHSL1/2/3 have nonredundant functions in regulating tomato pedicel abscission, at least partially.

We conducted a similar analysis on tomato based on the *Arabidopsis* IDA-HAE/HSL2 binding model and found that SlIDL6 is highly likely to bind to SlHSL1/2/3 (Fig. 4-A–C). Then, we used SlIDL6 peptide to treat the flower explants of WT and mutants. Compared to WT significantly accelerated abscission, the abscission of *Slhsl1*, *Slhsl2*, *Slhsl3*, *Slhsl1Slhsl2*, *Slhsl1Slhsl3*, and *Slhsl2Slhsl3* was accelerated but not restored to the level of WT (Fig. 4-D). However, the abscission of *Slhsl1Slhsll2Slhsl3* had little alteration (Fig. 4-D), indicating that the abscission induced by SlIDL6 depends on SlHSL1/2/3. Meanwhile, *SlIDL6* and *SlHSL1/2/3* are all highly expressed in the AZ (Li *et al.* 2021) (Fig. 1-B; Appendix F). Based on the above results, we speculate that SlHSL1/2/3 are the receptors of SlIDA6. IDA-HAE/HSL2 is a finely studied ligand-receptor module to date, capable of regulating *Arabidopsis* floral organ abscission and lateral root emergence (Cho *et al.* 2008; Stenvik *et al.* 2008; Shi *et al.* 2019; Zhu *et al.* 2019). Our previous research has shown that SlIDL6 positively regulates tomato pedicel abscission under low light stress (Li *et al.* 2021). This study shows that low light promotes the expression of *SlHSL1/2/3* and the mutants abscise slower than WT under low light (Fig. 4-F–G), indicating that SlHSL1/2/3 are also involved in tomato pedicel abscission under low light stress.

Ethylene is a proven promoter of flower abscission (Meir *et al.* 2010; Wilson *et al.* 2011; Estornell *et al.* 2013; Botton and Ruperti 2019). In *Arabidopsis*, the *ida* mutant exhibited typical ethylene triple reaction and aging after ethylene treatment but did not abscise (Butenko *et al.* 2006). The latest findings have shown that ethylene can mediate *IDA*

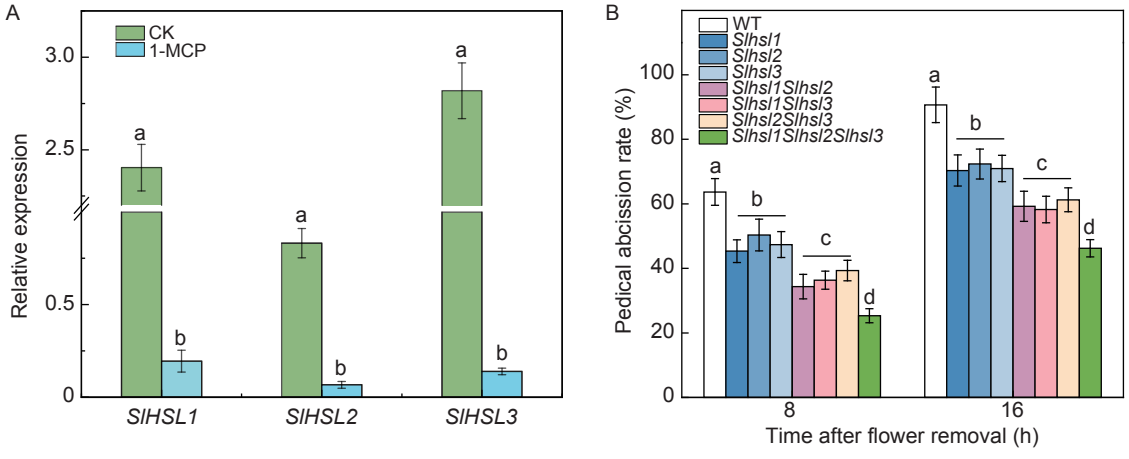


Fig. 5 Knockout of *SlHSL1/2/3* impaired ethylene-induced abscission. A, the impact of 0.4 μL L^{-1} 1-methylcyclopropene (1-MCP) on *SlHSL1*, *SlHSL2*, and *SlHSL3* expression in the pedicel abscission zones (AZs). These data are mean±SD (n=3), with a minimum of 30 samples per replication. Lowercase letters suggest statistically significant differences (Student's t-test, P<0.05). B, pedicel explants abscission assay for *SlHSL1/2/3* knockout and WT plants with 20 μL L^{-1} ethylene treatment. These data are the averages of three replicates±SD, with a minimum of 25 samples per replication. Lowercase letters suggest statistically significant differences (Student's t-test, P<0.05).

expression (Meir *et al.* 2019). Additionally, research indicated that ethylene enhanced the expression of *HSL* and *IDA* genes in AZs of oil palm fruits and in AZs of lupine flowers (Stø *et al.* 2015; Wilmowicz *et al.* 2018). Our prior research has demonstrated that pedicles treated with ethylene increased the expression of *SlIDL6* (Li *et al.* 2021). After ethylene treatment, there was a significant delay in the abscission of *Slidl6* plants compared to WT. 1-MCP treatment can inhibit WT pedicel abscission, while SlIDL6 peptide can significantly accelerate this abscission, indicating that SlIDL6 can partially induce abscission through the ethylene-independent pathway (Li *et al.* 2021). Our findings indicate that the expression level of *SlHSL1/2/3* decreased after 1-MCP treatment (Fig. 5-A). After ethylene treatment, compared to WT, the abscission of mutants was significantly delayed (Fig. 5-B), indicating that SlHSL1/2/3 are involved in ethylene-induced abscission.

5. Conclusion

In this study, we identified that *SlHSL1/2/3* are highly expressed in AZs and positively regulate tomato flower pedicel abscission. SlIDL6 acts as the ligand for SlHSL1/2/3 to positively regulate tomato pedicel abscission and SlIDL6-SlHSL1/2/3 ligand-receptor pairs actively respond to low light stress. Ethylene partially accelerates the abscission process by enhancing the transcription of *SlIDL6-SlHSL1/2/3* (Fig. 6).

Acknowledgements

This work was supported by the Liaoning Science and Technology Innovation Team Project, China (JYTTD2024007).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.03.018

References

Botton A, Ruperti B. 2019. The yes and no of the ethylene involvement in abscission. *Plants*, **8**, 187.

Butenko M A, Patterson S E, Grini P E, Stenvik G, Amundsen S S, Mandal A, Aalen R B. 2003. Inflorescence deficient in abscission controls floral organ abscission in *Arabidopsis* and identifies a novel family of putative ligands in plants. *The Plant Cell*, **15**, 2296–2307.

Butenko M A, Stenvik G, Alm V, Sæther B, Patterson S E, Aalen R B. 2006. Ethylene-dependent and -independent pathways controlling floral abscission are revealed to converge using promoter::reporter gene constructs in the *ida* abscission mutant. *Journal of Experimental Botany*, **57**, 3627–3637.

Butenko M A, Wildhagen M, Albert M, Jehle A, Kalbacher H, Aalen R B, Felix G. 2014. Tools and strategies to match peptide-ligand receptor pairs. *The Plant Cell*, **26**, 1838–1847.

Cheng L, Li R, Wang X, Ge S, Wang S, Liu X, He J, Jiang C, Qi M, Xu T. 2022. A SlCLV3-SlWUS module regulates auxin and ethylene homeostasis in low light-induced tomato flower abscission. *The Plant Cell*, **34**, 4388–4408.

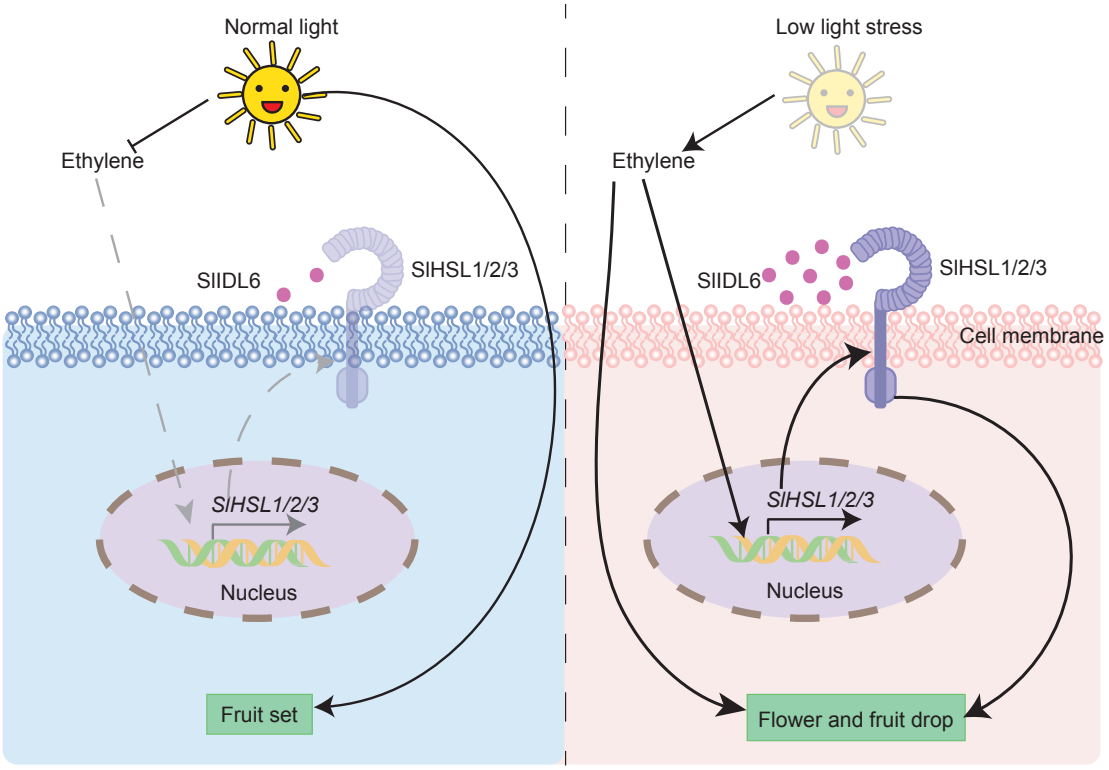


Fig. 6 The role of SlIDL6-SlHSL1/2/3 ligand-receptor pairs in tomato pedicel abscission. Normal light promotes fruit setting by inhibiting ethylene. Low light stress enhances ethylene signaling, which induces the expression of *SlHSL1/2/3* in abscission zones (AZs). SlHSL1/2/3 receptor accelerates tomato flower abscission after sensing the SlIDL6 peptide.

Cho S K, Larue C T, Chevalier D, Wang H, Jinn T, Zhang S, Walker JC. 2008. Regulation of floral organ abscission in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 15629–15634.

Cui Y, Lu X, Gou X. 2022. Receptor-like protein kinases in plant reproduction: Current understanding and future perspectives. *Plant Communications*, **3**, 100273.

Domingos S, Scafidi P, Cardoso V, Leitao AE, Di Lorenzo R, Oliveira C M, Goulao L F. 2015. Flower abscission in *Vitis vinifera* L. triggered by gibberellic acid and shade discloses differences in the underlying metabolic pathways. *Frontiers in Plant Science*, **6**, 457.

Estornell L H, Agustí J, Merelo P, Talón M, Tadeo F R. 2013. Elucidating mechanisms underlying organ abscission. *Plant Science*, **199–200,** 48–60.

Estornell L H, Wildhagen M, Pérez-Amador M A, Talón M, Tadeo F R, Butenko M A. 2015. The IDA peptide controls abscission in *Arabidopsis* and *Citrus*. *Frontiers in Plant Science*, **6**, 1003.

Fu X, Li R, Liu X, Cheng L, Ge S, Wang S, Cai Y, Zhang T, Shi C, Meng S. 2024. Kinase CPK10 regulates low light-induced tomato flower drop downstream of IDL6 in a calcium-dependent manner. *Plant Physiology*, **196**, 2014–2029.

Gou X, Li J. 2020. Paired receptor and coreceptor kinases perceive extracellular signals to control plant development. *Plant Physiology*, **182**, 1667–1681.

Hohmann U, Lau K, Hothorn M. 2017. The structural basis of ligand perception and signal activation by receptor kinases. *Annual Review of Plant Biology*, **68**, 109–137.

Jinn T, Stone J M, Walker J C. 2000. HAESA, an *Arabidopsis* leucine-rich repeat receptor kinase, controls floral organ abscission. *Genes & Development*, **14**, 108–117.

Kobe B, Kajava A V. 2001. The leucine-rich repeat as a protein recognition motif. *Current Opinion in Structural Biology*, **11**, 725–732.

Li R, Shi C, Wang X, Meng Y, Cheng L, Jiang C, Qi M, Xu T, Li T. 2021. Inflorescence abscission protein SlIDL6 promotes low light intensity-induced tomato flower abscission. *Plant Physiology*, **186**, 1288–1301.

Li X, Zhang J, Shi H, Li B, Li J. 2022. Rapid responses: Receptor-like kinases directly regulate the functions of membrane transport proteins in plants. *Journal of Integrative Plant Biology*, **64**, 1303–1309.

Liu Y, Schiff M, Dinesh-Kumar S P. 2002. Virus-induced gene silencing in tomato. *The Plant Journal*, **31**, 777–786.

Lu L, Arif S, Yu J M, Lee J W, Park Y H, Tucker M L, Kim J. 2023. Involvement of IDA-HAE module in natural development of tomato flower abscission. *Plants*, **12**,185.

Meir S, Philosoph-Hadas S, Riov J, Tucker M L, Patterson S E, Roberts J A. 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.

Meir S, Philosoph-Hadas S, Sundaresan S, Selvaraj K V, Burd S, Ophir R, Kochanek B, Reid M S, Jiang C, Lers A. 2010. Microarray analysis of the abscission-related transcriptome in the tomato flower abscission zone in response to auxin depletion. *Plant Physiology*, **154**, 1929–1956.

Ou Y, Kui H, Li J. 2021. Receptor-like kinases in root development: Current progress and future directions. *Molecular Plant*, **14**, 166–185.

Patharkar O R, Walker J C. 2016. Core mechanisms regulating developmentally timed and environmentally triggered abscission. *Plant Physiology*, **172**, 510–520.

Rai A C, Halon E, Zemach H, Zviran T, Sisai I, Philosoph-Hadas S, Meir S, Cohen Y, Irihimovitch V. 2021. Characterization of two ethephon-induced IDA-like genes from mango, and elucidation of their involvement in regulating organ abscission. *Genes*, **12**, 439.

Reichardt S, Piepho H, Stintzi A, Schaller A. 2020. Peptide signaling for drought-induced tomato flower drop. *Science*, **367**, 1482–1485.

Santiago J, Brandt B, Wildhagen M, Hohmann U, Hothorn L A, Butenko M A, Hothorn M. 2016. Mechanistic insight into a peptide hormone signaling complex mediating floral organ abscission. *eLife*, **5**, e15075.

Shi C, Alling R M, Hammerstad M, Aalen R B. 2019. Control of organ abscission and other cell separation processes by evolutionary conserved peptide signaling. *Plants*, **8**, 225.

Shi C, Stenvik G, Vie A K, Bones A M, Pautot V, Proveniers M, Aalen R B, Butenko M A. 2011. *Arabidopsis* class I KNOTTED-like homeobox proteins act downstream in the IDA-HAE/HSL2 floral abscission signaling pathway. *The Plant Cell*, **23**, 2553–2567.

Shiu S, Bleecker A B. 2001. Plant receptor-like kinase gene family: Diversity, function, and signaling. *Science's STKE*, **2001**, re22.

Stenvik G, Tandstad N M, Guo Y, Shi C, Kristiansen W, Holmgren A, Clark S E, Aalen R B, Butenko M A. 2008. The EPIP peptide of INFLORESCENCE DEFICIENT IN ABSCISSION is sufficient to induce abscission in *Arabidopsis* through the receptor-like kinases HAESA and HAESA-LIKE2. *The Plant Cell*, **20**, 1805–1817.

Stø I M, Orr R J, Fooyontphanich K, Jin X, Knutsen J M, Fischer U, Tranbarger T J, Nordal I, Aalen R B. 2015. Conservation of the abscission signaling peptide IDA during Angiosperm evolution: Withstanding genome duplications and gain and loss of the receptors HAE/HSL2. *Frontiers in Plant Science*, **6**, 931.

Taylor J E, Whitelaw C A. 2001. Signals in abscission. *New Phytologist*, **151**, 323–340.

Tucker M L, Yang R. 2012. IDA-like gene expression in soybean and tomato leaf abscission and requirement for a diffusible stelar abscission signal. *AoB Plants*, **2012**, pls35.

Ventimilla D, Velázquez K, Ruiz-Ruiz S, Terol J, Pérez-Amador M A, Vives M C, Guerri J, Talon M, Tadeo F R. 2021. IDA (INFLORESCENCE DEFICIENT IN ABSCISSION)-like peptides and HAE (HAESA)-like receptors regulate corolla abscission in *Nicotiana benthamiana* flowers. *BMC Plant Biology*, **21**, 226.

Vie A K, Najafi J, Liu B, Winge P, Butenko M A, Hornslien K S, Kumpf R, Aalen R B, Bones A M, Brembu T. 2015. The IDA/IDA-LIKE and PIP/PIP-LIKE gene families in *Arabidopsis*: Phylogenetic relationship, expression patterns, and transcriptional effect of the PIPL3 peptide. *Journal of Experimental Botany*, **66**, 5351–5365.

Wang F, Zheng Z, Yuan Y, Li J, Zhao M. 2019. Identification and characterization of HAESA-like genes involved in the fruitlet abscission in litchi. *International Journal of Molecular Sciences*, **20**, 5945.

Wang Y, Wu Y, Zhang H, Wang P, Xia Y. 2022. *Arabidopsis* MAPKK kinases YODA, MAPKKK3, and MAPKKK5 are functionally redundant in development and immunity. *Plant Physiology*, **190**, 206–210.

Wilmowicz E, Kućko A, Ostrowski M, Panek K. 2018. INFLORESCENCE DEFICIENT IN ABSCISSION-like is an abscission-associated and phytohormone-regulated gene in flower separation of *Lupinus luteus*. *Plant Growth Regulation*, **85**, 91–100.

Wilson Z A, Song J, Taylor B, Yang C. 2011. The final split: The regulation of anther dehiscence. *Journal of Experimental Botany*, **62**, 1633–1649.

Zhu H, Dardick C D, Beers E P, Callanhan A M, Xia R, Yuan R. 2011. Transcriptomics of shading-induced and NAA-induced abscission in apple (*Malus domestica*) reveals a shared pathway involving reduced photosynthesis, alterations in carbohydrate transport and signaling and hormone crosstalk. *BMC Plant Biology*, **11**, 1–20.

Zhu Q, Shao Y, Ge S, Zhang M, Zhang T, Hu X, Liu Y, Walker J, Zhang S, Xu J. 2019. A MAPK cascade downstream of IDA-HAE/HSL2 ligand-receptor pair in lateral root emergence. *Nature Plants*, **5**, 414–423.

Executive Editor-in-Chief Sanwen Huang
Managing Editor Lingyun Weng