

# The miR167–ARF8 module coordinates stamen–pistil development and fruit set in tomato

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## Summary

- Auxin is an important regulator of reproductive development. The families of the AUXIN RESPONSE FACTOR (ARF) genes, important components of the auxin signal, have expanded in flowering plants. Tomato (*Solanum lycopersicum*) has two copies of ARF8, which are negatively regulated by miR167. Here, we investigated the involvement of this expanded SlmiR167–SIARF8 module in flower development.
- Mutants were generated in 5 SlmiR167 and 2 SIARF8 genes, and flower development and fruit set were characterized in mutant combinations.
- We show that the SlmiR167–SIARF8 module regulates flower-organ development and fruit set. The two SIARF8 paralogs are found to have both overlapping and distinct roles. They additively affect stigma position by oppositely affecting stamen and pistil growth, while SIARF8B has evolved a unique role in the inhibition of anther dehiscence. Loss of SIARF8B in the *Slmir167a* background uncovers a unique reproductive syndrome of fast, early, fertilization-independent fruit set and increased yield under cold stress, mediated by the deregulation of SIARF8A.
- These results demonstrate how gene duplication within the miR167–ARF8 module enables fine-tuning of auxin signaling to achieve coordinated, robust reproductive development, with implications for the control of fruit set in tomato.

## Introduction

Efficient reproduction in flowering plants (angiosperms) relies on synchronized development of the flower reproductive organs. The pistil consists of a stigma, a style, and an ovary, which hosts the female gamete-containing ovules (Smyth *et al.*, 1990; Smyth, 2022; Ramos-Pulido & de Folter, 2023). The stamen consists of the filament and the anther, which contains the pollen grains, housing the male gametes (Wilson *et al.*, 2011). At anthesis, the day the flower opens, the anthers dehisce and release pollen grains onto the stigma (Gorguet *et al.*, 2009; Wilson *et al.*, 2011). Tomato anthers each consist of a pair of thecae, each composed of two pollen-containing locules. The locules are separated by the connective (also referred to as the connective tissue) and a septum (Sanders *et al.*, 2005; Senatore *et al.*, 2009; Liu *et al.*, 2025). During anther dehiscence, the septum and the connective undergo lysis, leading to the merging of each pair of adjacent locules. In parallel, the endothecium, a sub-epidermal layer of the anther wall, undergoes secondary-wall thickening in the upper third of the anther, characterized by a U-shape lignification

termed reticulate ribs (Bonner & Dickinson, 1989; García, 2002; García & Barboza, 2006). The stomium, a specialized strip of weak cells structurally continuous with the septum, then ruptures, and following desiccation, the anther opens and releases pollen (Wilson *et al.*, 2011; Liu *et al.*, 2025).

Stigma position at anthesis can be categorized as exerted, when the stigma protrudes beyond the stamens, as flush, when the stigma and the stamens are of equal length, or as inserted, when the stigma is positioned below the stamens (Chen & Tanksley, 2004; Shang *et al.*, 2021). Tomato (*Solanum lycopersicum* L.) domestication involved a reduction in style length relative to the anthers, which led to an inserted-stigma position, and consequently increased self-pollination and reduced outcrossing (Rick, 1988; Chen *et al.*, 2007; Shang *et al.*, 2021).

Successful fertilization leads to the initiation of fruit development, termed fruit set. In the absence of fertilization, ovaries typically abort (Gillaspy *et al.*, 1993; Shinozaki *et al.*, 2015). However, in some cases, unpollinated ovaries can still develop into a seedless fruit, a process known as parthenocarpy (Joldersma & Liu, 2018). In tomato, several mutations, including those

affecting auxin signaling, have been shown to induce either obligate or facultative parthenocarpy. Abiotic stress can also trigger facultative parthenocarpy in specific tomato genetic backgrounds. Therefore, the coupling between fertilization and fruit set is sensitive to the genetic and environmental circumstances (Hawthorn, 1937; Gorguet *et al.*, 2005; Molesini *et al.*, 2009; He *et al.*, 2021; Ezura *et al.*, 2023; Guan *et al.*, 2024; Lu *et al.*, 2025).

Auxin plays a central role in regulating flower-organ development and fruit set (Gustafson, 1936, 1942; Sjut & Bangerth, 1982; Rotino *et al.*, 1997; Wang *et al.*, 2005; Ren *et al.*, 2011; Pattison *et al.*, 2014; Mazzucato *et al.*, 2015; Cucinotta *et al.*, 2021; Fenn & Giovannoni, 2021). AUXIN RESPONSE FACTORS (ARF) function as the primary transcription factors that mediate auxin signaling. They fall into three classes: A, B, and C, where class A is considered to include mainly transcriptional activators and classes B and C mainly repressors (Guilfoyle & Hagen, 2007; Finet *et al.*, 2013; Chandler, 2016; Weijers & Wagner, 2016). In angiosperms, ARF proteins belong to protein families with multiple members. The A-ARF gene ARF8 is involved in flower and fruit development (Goetz *et al.*, 2006; Tabata *et al.*, 2010; Varaud *et al.*, 2011; Reeves *et al.*, 2012; Liu *et al.*, 2014; Du *et al.*, 2016; Ghelli *et al.*, 2018; Hu *et al.*, 2023; Israeli *et al.*, 2023; Hua *et al.*, 2024). ARF8 is negatively regulated by the conserved microRNA miR167 (Liu *et al.*, 2021). In tomato, there are two copies of ARF8, *SLARF8A* and *SLARF8B* (Zouine *et al.*, 2014), and five or six *SIMIR167*-related sequences (Liu *et al.*, 2014; Jodder *et al.*, 2018; Arazi & Khedia, 2022). The biological significance of this gene duplication is intriguing in the context of the expansion of auxin-response gene families (Israeli *et al.*, 2020). *Slarf8a Slarf8b* double mutants have shortened styles and reduced stigma, and produce parthenocarpic, seedless fruit (Hu *et al.*, 2023; Israeli *et al.*, 2023; Hua *et al.*, 2024). However, the biological roles of *SIMIR167* genes and their relationship with the two *SLARF8* genes are largely unexplored.

In Arabidopsis, four *AtMIR167* genes have been identified (*MIR167a-d*), among which *AtMIR167a* is the most prominent. *AtMIR167a* is involved in multiple aspects of flower development via *AtARF8* and *AtARF6* (Wu *et al.*, 2006; Yao *et al.*, 2019; Zheng *et al.*, 2019). In tomato, overexpression of the Arabidopsis *AtmiR167a* led to shortened styles and impaired stigma receptivity and fruit development, partially overlapping with the *Slarf8a Slarf8b* double-mutant phenotypes (Liu *et al.*, 2014; Hua *et al.*, 2024). At least five *SIMIR167* genes have been described, encoding at least two mature SlmiR167 species, SlmiR167a and SlmiR167b (Arazi & Khedia, 2022). Four genes (*SIMIR167a*, *SIMIR167c*, *SIMIR167d*, and *SIMIR167e*) encode SlmiR167a, which is identical to AtmiR167a, and *SIMIR167b* encodes a second mature miR167 species, SlmiR167b (Liu *et al.*, 2014; Jodder *et al.*, 2018). One additional *SIMIR167* sequence has been mentioned in the literature, but it is not clear whether it is functional (Liu *et al.*, 2014; Chen *et al.*, 2021). SlmiR167a is the major isoform in leaves (Jodder *et al.*, 2018).

Here, we sought to understand how the interplay between multiple SlmiR167 and *SLARF8* genes orchestrates tomato flower and fruit development (Hu *et al.*, 2023; Israeli *et al.*, 2023; Hua

*et al.*, 2024) by generating and characterizing mutant combinations involving the 5 validated *SIMIR167* genes, and the two *Slarf8* mutants. We show that *SIMIR167a* plays a central role in flower and fruit development via SLARF8s. The SlmiR167-SLARF8 module tunes stigma position in a dosage-dependent manner by oppositely influencing style and stamen elongation and is a central regulator of anther dehiscence. SlmiR167 is shown to act through different SLARF8 targets in a developmental-context-specific manner. A specific interaction between *Slmir167a* and *Slarf8b* stabilized fruit set and yield in extreme temperatures. The results show how the plant uses expanded gene families of auxin-related transcription factors and their microRNA regulators to tune flower development and fruit set.

## Materials and Methods

### Plant material and growth conditions

Tomato (*Solanum lycopersicum* L.) cv M82 (LA3475 in the Tomato Genetic Resource Center (TGRC) collection) was used as the background genotype and is referred to as wild type. Seeds were germinated in a controlled growth chamber for 3–4 wk and subsequently transferred to a glasshouse under natural daylight with controlled temperatures (*c.* 26°C : *c.* 20°C, day : night), to a glasshouse with mild heating, or to a net house with no temperature control. The *Slarf8a* and *Slarf8b* mutants were described previously (Israeli *et al.*, 2023). The *Slmir167a-e* mutants and the reporter line *pARF8B:mNeonGreen-N7 pARF8A:mScarletI-N7* were generated during this study.

*Nicotiana benthamiana* plants were grown in soil under glasshouse conditions with day : night temperatures and photoperiod of 16 h : 8 h, 24°C : 18°C, light : dark.

### Clustered regularly interspaced short palindromic / CRISPR-associated protein 9 (CRISPR/Cas9) and reporter construct design

Two CRISPR/Cas9 plasmids were used to target the *SIMIR167* genes. The first contained four sgRNAs targeting *SIMIR167a* and *SIMIR167d* (two guides for each); the second also contained four sgRNAs, two guides targeting *SIMIR167b*, one guide targeting *SIMIR167c*, and one guide targeting *SIMIR167e*. sgRNAs were designed using the CRISPR-P server (<http://cbi.hzau.edu.cn/crispr>) and assembled by the Golden Gate method (Engler *et al.*, 2014). Briefly, each sgRNA was amplified from the level 0 vector using a specific forward primer containing the sgRNA sequence and a BsaI target site as a tail, and a general reverse primer. The sgRNA-containing fragment was inserted into Level 1 vectors, stitched together with the U6 promoter, and subsequently cloned into the binary vector pAGM4723, which also contains the Cas9 sequence. For the reporter construct, 2412-bp (*SLARF8A*) and 2649-bp (*SLARF8B*) long promoter regions upstream of the ATG were cloned into pAGM4723 using Golden Gate-based assembly and inserted upstream of *mScarletI-N7* and *mNeonGreen-N7*, respectively. Primers sequences are listed in Supporting Information Table S1. The promoter

sequence of *SLARF8A*, cloned into the *mScarletI* reporter construct, was found to bear a single-nucleotide polymorphism (SNP) at position 70 bp and a single-nucleotide deletion at position 12. These mutations do not substantially alter predicted *cis*-regulatory elements (Tian *et al.*, 2020). In addition, the expression driven by this promoter is similar to that of *SLARF8B* and to public databases. These mutations therefore likely do not substantially affect the activity of the *SLARF8A* promoter.

### Plant transformation and tissue culture

Binary vectors were introduced into the *Agrobacterium tumefaciens* strain GV3101 by electroporation. M82 cotyledon transformation was performed using a standard protocol (McCormick, 1991). Briefly, sterile seedlings were grown on Nitsch medium for 7–10 d, and cotyledons were excised and preincubated in the dark for 24 h with acetosyringone. Then the cotyledons were infected with *Agrobacterium* ( $OD_{600} = 0.35$ – $0.4$ ) for 48 h in the dark, with acetosyringone, and transferred through J1, J2, and J3 into the rooting tissue culture medium, as previously described in detail (Israeli *et al.*, 2019, 2023). T0 plants were then transferred to the soil in a protected container for genotyping, recovery, and development.

To plants were screened by PCR to confirm the presence of the transgene. Subsequently, the target regions were amplified and sequenced to identify and characterize induced mutations. Following mutation verification, the single mutants were separated from each other by self-pollination or by crossing with M82. In the *Slmir167a Slmir167d* lines, the transgene was also segregated out. In *Slmir167b*, *Slmir167c*, and *Slmir167e*, the transgene remained and occasionally produced additional alleles.

### Cold experiment

Plants were grown for 4 wk in a controlled chamber, genotyped, and transferred to a net house equipped with a glass roof in a completely randomized design. Yield was measured 149 d after sowing (DAS). Plants were maintained under standard horticultural practices by the Agricultural Research Organization (ARO) – Volcani Center, Israel (*c.* 32.0031°N, 34.8141°E), including routine irrigation, fertilization, and disease monitoring. Temperatures were continuously recorded using two independent data loggers and are provided in Tables S2 and S3. The number of inflorescences per plant and the total number of fruits per plant were recorded along the experiment. The following parameters were measured at the end of the experiment: total weight of green and red fruits, number of green and red fruits per plant, and fresh vegetative biomass.

### RNA extraction and qRT-PCR analysis

Total RNA was extracted using the Ribospin™ Plant Kit (GeneAII) with DNase treatment. cDNA was synthesized using the UltraScript cDNA Synthesis Kit (PCR Biosystems, London, UK) or the Superscript II reverse transcriptase (Invitrogen), using 0.4 or 1 µg of RNA, respectively. Quantitative reverse transcription

polymerase chain reaction (qRT-PCR) was performed using SYBR premix on a Corbett Rotor-Gene 6000 real-time PCR machine. Expression levels were normalized to the *EXPRESSED* (*EXP*; Expósito-rodríguez *et al.*, 2008) reference gene using standard curves. Statistical significance was assessed using a *t*-test. Primer sequences are provided in Table S1.

### Developmental stages of flowers and fruits

Flower development stages were defined according to previously characterized morphological criteria (Dobritsch *et al.*, 2015; Israeli *et al.*, 2023), relative to the flower opening day (anthesis): Stages 1, 2, 3, and 4 (s1–4) are buds at 7, 5, 3, and 1–2 d before anthesis, respectively. Stage 5 (s5) is the anthesis day. During fruit development, the days from the anthesis day are referred to as days post anthesis (DPA). For characterization of reproductive development and yield, plant age was defined by DAS. As not all flowers normally set fruit, we defined the timing of fruit set in each plant as the time of the first appearance of a visible emerging fruit (*c.* 5 mm in diameter).

### Anther dehiscence and water-content measurement

For dehiscence analysis, flowers at 2 DPA were excised, their pedicels were placed in water, and the stamens were manually dissected and scored under a stereomicroscope. Anther dehiscence and opening were determined visually. While most anthers were classified as either open or closed, a few anthers had a minor longitudinal slit without visible pollen release. Those anthers were grouped and counted together with the closed anthers. Anthers that were damaged or ruptured during dissection were excluded from the analysis. For water-content measurement, fresh anther cones were separated and weighed, dried at 60°C for 5 d, and reweighed. Water content was calculated as  $1 - (\text{dry weight}/\text{fresh weight})$ .

### Stamen-cone histological sections

Stamen cones were collected from flowers of 8 genotypes (M82, *Slarf8a*, *Slarf8b*, *Slarf8a Slarf8b*, *SlmiR167a*, *Slarf8a SlmiR167a*, *Slarf8b SlmiR167a*, and *Slarf8a Slarf8b SlmiR167a*), 1 DPA ( $n > 3$ ). Tissue was immediately fixed in FAA solution (10% [v/v] formaldehyde, 5% [v/v] acetic acid, and 50% [v/v] ethanol in H<sub>2</sub>O), vacuumed for 30 min, and left overnight at 4°C. Samples were then dehydrated through a graded ethanol series followed by a gradual ethanol: K-Clear series and cleared in a 100% K-Clear (Kalktek srl, Saonara, Italy). Specimens were then infiltrated with paraffin (Paraplast Plus, Leica Biosystems) and embedded into heated molds. Transverse sections (10 µm thickness) obtained from above, through, and below the region of normal anther opening were transferred onto slides, deparaffinized with K-Clear, hydrated, and stained with either 0.5% (w/v) Toluidine blue for 30 s for the morphological characterization or with 1% (w/v) Safranin O for 3 h followed by a 0.3% (w/v) Fast Green for 40 s for lignin characterization. After the wash and dehydration stages, conducted according to each dye protocol in

ethanol/clove oil and K-Clear, sections were enclosed in Entellan (Merck, Darmstadt, Germany).

### Pollen-viability assay

Flowers at anthesis were collected from either M82 or *SlmiR167a* plants grown under normal conditions (*c.* 28°C : 22°C, day : night). Stamen cones of each bud were dissected to release pollen grains, which were then fixed on a microscope slide using 10 µl Alexander staining solution with minor adaptations as described by (Peterson *et al.*, 2010). Viable pollen grains stained magenta-red, and nonviable grains stained light blue-green. At least eight biological repeats (flowers) were measured from each genotype, and six images were taken from each slide. Pollen grains were counted using the multipoint tool of the IMAGEJ software (Schneider *et al.*, 2012).

### Imaging and morphological measurement

Fluorescent reporter expression was imaged using a Nikon SMZ18 stereomicroscope equipped with the proper filter combinations for mNeonGreen and mScarletI and DS-Ri2 camera. Stamen and pistil imaging and length measurements were performed using a Nikon SMZ1270 stereomicroscope with a DS-Ri2 camera and the NIS-ELEMENT software.

Images of stamen-cone cross-sections and pollen grains (for the pollen-viability test) were taken using a light microscope (DM500; Leica, ×10 objective) equipped with a digital camera (ICC50 W; Leica). Measurements of stamen-cone sections were conducted using the straight tool of the IMAGEJ software (Schneider *et al.*, 2012).

### Transactivation assay

Transient transactivation assays were performed in *N. benthamiana* leaves according to (Hellens *et al.*, 2005; Panda *et al.*, 2022). For the reporter construct, the upstream regions (UPRs) of *SLARF8A* (2412 bp) and *SLARF8B* (2649 bp) were amplified from *S. lycopersicum* cv M82 genomic DNA using gene-specific oligonucleotides (Table S1) and cloned into the pCambia1302 vector upstream of the firefly luciferase (LUC) gene using the ClonExpress II One Step Cloning Kit (Takara, Kusatsu, Japan). Within the same construct, a *Renilla* luciferase (REN) gene driven by the CaMV 35S promoter served as an internal control to normalize transient expression levels, and reporter activity was expressed as the LUC/REN ratio. For the effector construct, the full-length coding sequences of *SLARF8A* and *SLARF8B*, amplified from *S. lycopersicum* cv Micro-Tom cDNA, were cloned into the pDGB3α2 vector downstream of the *UBQ10* promoter with the ClonExpress II One Step Cloning Kit. As a negative control, an empty pDGB3α2 vector was used. All constructs were introduced into *A. tumefaciens* strain GV3101 by electroporation.

Agrobacterium strains carrying reporter or effector constructs were grown for 24 h at 28°C in LB medium containing kanamycin and gentamicin, harvested, and resuspended in infiltration

buffer to an OD<sub>600</sub> of 0.2. Reporter and effector strains were mixed at a 1 : 9 ratio, incubated for 2 h at room temperature, and infiltrated into the abaxial side of leaves from 4- to 5-wk-old *N. benthamiana* plants using a 1-ml needleless syringe. Plants were maintained under glasshouse conditions for 2 d after infiltration.

Eight biological replicates, each consisting of 3-mm-diameter leaf disks, were harvested into 1.2-ml collection tubes containing two 3-mm tungsten beads and immediately frozen on dry ice. Frozen tissue was homogenized using a TissueLyser, returned to dry ice, and then lysed by adding extraction buffer and vortexing. Samples were centrifuged at 2465 *g* for 10 min at 4°C, and supernatants were collected for luminescence measurement using a Modulus Microplate Luminometer (Turner Biosystems, Sunnyvale, CA, USA) by mixing 20 µl of sample extract with 80 µl of either LUC substrate or REN substrate.

### Statistical analysis

All Statistical analyses were performed using the JMP software (SAS Institute Inc., Cary, NC, USA), and GRAPHPAD PRISM (v.11.0.0 for Windows; GraphPad Software, Boston, MA USA, [www.graphpad.com](http://www.graphpad.com)).

## Results

### *SIMIR167a* is the predominant *SIMIR167* among *SIMIR167a-e*

To understand the role of tomato miR167 and its interaction with *SLARF8* in tomato flower and fruit development, we generated mutants in five *SIMIR167* genes, *SIMIR167a-e*, using CRISPR/CAS9-mediated gene editing. We used two constructs: one targeting *SIMIR167a* and *SIMIR167d*, and the other targeting *SIMIR167b*, *SIMIR167c*, and *SIMIR167e* (Jodder *et al.*, 2018; Fig. S1). The resulting plants contained various combinations of mutations, which were separated from each other by crosses to the wild-type (WT). The single homozygous *Slmir167a* mutant produced seedless fruits under all tested growth conditions and was similar to the quintuple mutant *Slmir167a-e* (Fig. 1). Conversely, *Slmir167b*, *Slmir167c*, *Slmir167d*, and *Slmir167e* single mutants, as well as *Slmir167a/+* and *Slmir167a/+ b-e* (homozygous for *Slmir167b-e* and heterozygous for *Slmir167a*), were fertile and similar to the WT (Fig. 1).

These findings indicate that the *SIMIR167a* gene is the predominant *SIMIR167* gene affecting reproductive development, although a role for an additional putative *SIMIR167* gene not targeted here cannot be excluded. As reproductive development in the quintuple mutant was apparently similar to *Slmir167a*, we focused further research on the single *Slmir167a* mutant. Expression of *SLARF8A*, *SLARF8B*, and *SLARF6A* was increased in stage s3 *Slmir167a* pistils (3 d before anthesis) compared with the WT and was unchanged in stages s2 and s4 pistils. *SLARF8B* expression also increased in s4 and s5 (anthesis) ovaries (Fig. S2a–e, see also below).



**Fig. 1** *SlMIR167a* is the functionally prominent *SlMIR167* gene in tomato fruit development. Fruits of wild-type (WT), single *Slmir167a* (*mir167a*), *Slmir167a/+ b,c,d,e* (*mir167a/+ b-e*), which is heterozygous for *Slmir167a* and homozygous for the other *Slmir167* mutations, and the quintuple *Slmir167a,b,c,d,e* (*mir167a-e*) mutant. *Slmir167a* and *Slmir167a-e* fruits were smaller than the WT and seedless, while *Slmir167a/+ b,c,d,e* was similar to the WT. Bar, 3 cm.

### Compromised inflorescence development in *Slmir167a* mutants

Characterization of inflorescence development in *Slmir167a* revealed diverse phenotypic effects and severities. A large proportion of the *Slmir167a* inflorescences failed to develop normally (Fig. S3a–d). Two main categories of aberrant phenotypes could be observed. In the first category, inflorescences were small and yellowish and stopped developing prematurely (Fig. S3c,e). In the second category, inflorescences were atrophied and very small (Fig. S3d,e). Both types of abnormal inflorescences contained all organs (Fig. S3f) but senesced without producing fruit. A low proportion of these aberrant inflorescence forms also appeared in WT plants. Quantification of the different categories showed that in the *Slmir167a* mutant only 43% of the inflorescences developed normally, compared with 83% in the WT (Fig. S3g). Further analysis of flower development was conducted on normal inflorescences.

### The *SlmiR167a*-*SlARF8* module coordinates the positions of the stamen and pistil

Previous studies showed that mutations in *SlARF8A/B* or overexpression of *miR167a* reduce style length (Liu *et al.*, 2014; Hu *et al.*, 2023; Israeli *et al.*, 2023), which is expected to affect stigma position (Rick & Dempsey, 1969; Saeed *et al.*, 2007; Pan *et al.*, 2017, 2019). We therefore examined the effect of *Slmir167a* and its interaction with *Slarf8a* and *Slarf8b* on pistil and stamen lengths, and on the resulting position of the stigma. We first examined the expression of the *SlARF8* promoters during pistil and stamen development by generating transgenic tomato plants that simultaneously express the fluorescent markers mNeonGreen and mScarletI under the control of the *SlARF8B* and *SlARF8A* promoters, respectively. Both promoters drove expression in young pistils and stamens (Fig. 2). The expression is in agreement with publicly available expression data (Fei *et al.*, 2010; Zouine *et al.*, 2017; Proost & Mutwil, 2018; Bae *et al.*, 2021).

To dissect the role of *SlARF8* and *SlmiR167a* in stigma position, we measured stamen and pistil lengths and the stamen-to-pistil length ratio (S : P) in single mutants and

mutant combinations involving *Slarf8a*, *Slarf8b*, and *Slmir167a*. The mean S : P ratio of WT was consistently > 1, corresponding to its typical inserted stigma. Stamen length was gradually increased, and pistil length gradually decreased with the reduction in *SlARF8* dosage, with *Slarf8b* showing a stronger effect than *Slarf8a*, and the double mutant exhibiting the most pronounced change (Fig. 3a–c). Accordingly, the S : P ratio was gradually and substantially increased with the decrease in *SlARF8* dose (Fig. 3c). Conversely, stamen length was slightly but statistically significantly decreased in *Slmir167a* mutants. *Slmir167a* exhibited considerable variation in pistil length, and although the mean was slightly longer than that of the WT, the difference was not statistically significant. However, the S : P ratio was substantially and statistically significantly decreased, consistently reaching < 1, corresponding to exerted stigma (Fig. 3).

To understand the relative contributions of *SlARF8A* and *B* to the exerted-stigma phenotype of *Slmir167a*, we generated mutant combinations among *Slmir167a*, *Slarf8a*, and *Slarf8b*. Stamen and pistil lengths of *Slarf8a Slmir167a* and *Slarf8b Slmir167a* were intermediate between the lengths of their respective single mutants (Fig. 3a,b). Notably, *Slarf8a Slarf8b* was completely epistatic over *Slmir167a*, such that stamen and pistil length in the *Slarf8a Slarf8b Slmir167a* triple mutant was nearly identical to that of the *Slarf8a Slarf8b* double mutant (Fig. 3a,b). This suggests that increased activities of *SlARF8A* and *B* are together responsible for the effect of *SlmiR167a* on stamen and pistil lengths. However, the style of the triple mutant was slightly shorter than that of the *Slarf8a Slarf8b* double mutant, resulting in an even higher S : P ratio (Figs 3c, S4). The reduction in pistil length in the *Slarf8* mutants resulted from a shortened style, since *Slarf8a Slarf8b* ovaries were longer than those of the WT (Fig. S4). Conversely, the *Slmir167a* style length was similar to the WT, but the ovary was longer (Fig. S4). Together, these results indicate that the *SlmiR167a*-*SlARF8* module regulates stigma position by oppositely affecting stamen and pistil lengths. In the WT, *SlARF8A* and *B* promote pistil elongation and inhibit stamen elongation in an additive manner, with *SlARF8B* having a greater impact. In the *Slmir167a* mutant, *SlARF8A* and *B* are de-regulated, leading to shortened stamens. In the *Slarf8a Slmir167a* and *Slarf8b Slmir167a* double mutants, there is partial

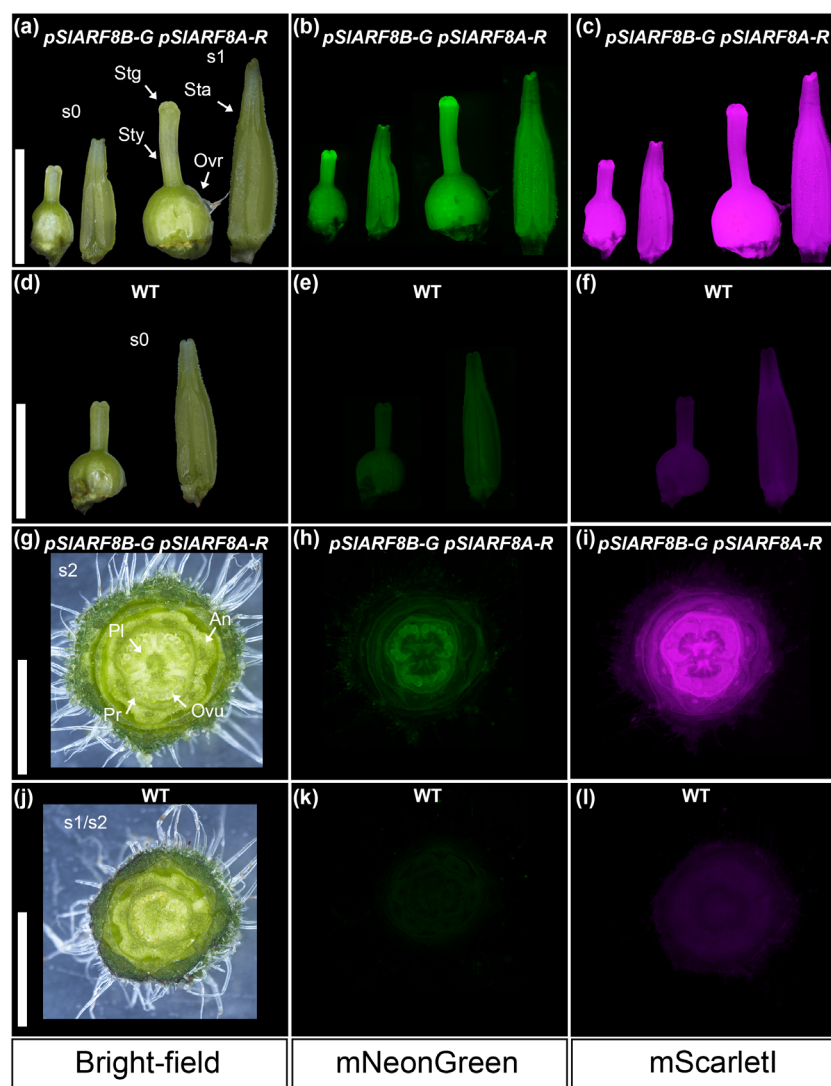


**Fig. 2** Promoters of *SIARF8B* (*pSIARF8B*) and *SIARF8A* (*pSIARF8A*) drive strong expression in tomato pistils and anthers. Stereomicroscope images show the same flower organs from the *pSIARF8A/B* dual-reporter line imaged using the filter modules for mNeonGreen or mScarletl. *pSIARF8B-G* and *pSIARF8A-R*, denote *pSIARF8B:mNeonGreen-N7* (green) and *pSIARF8A:mScarletl-N7* (magenta), respectively. For improved accessibility, the red fluorescence channel is artificially displayed in magenta. s1 and s2 are closed buds c. 7 and 5 d before anthesis, respectively. s0 designates a bud younger than s1. WT indicates nontransformed wild-type plants. (a–f) Pistils and stamens of the indicated genotypes and developmental stages. (g–l) Cross-sections of buds at the c. s1–s2 stage of flower development. Bars, 2 mm. An, anther; Ovr, ovary; Ovu, ovule; Pl, placenta; Pr, pericarp; Sta, stamen; Stg, stigma; Sty, style.

rescue of stamen length, and the pistil length is intermediate between the single mutants.

The *Slmir167a-SIARF8* module controls anther dehiscence, with a central role for *SIARF8B*

In *Arabidopsis*, both the *Atarf8 Atarf6* double mutants and the *Atmir167a* mutants exhibit anther indehiscence phenotypes (Nagpal *et al.*, 2005; Tabata *et al.*, 2010; Yao *et al.*, 2019; Zheng *et al.*, 2019). We therefore assessed anther development and dehiscence in the genotypes with altered activity of the *Slmir167a-SIARF8* module. Tomato anthers typically crack open and release pollen between the morning and the afternoon of the anthesis day, although environmental conditions can influence this timing. At 1 or 2 DPA, opening could be observed in almost all WT anthers. By contrast, although *Slmir167a* anthers appeared morphologically normal, they remained closed or exhibited only slight cracking, with no pollen release. No dehiscence defect was observed in *Slarf8a*, *Slarf8b*, and *Slarf8a Slarf8b* anthers (Figs 4a–d, S5a–d). In the mutant combinations,

75%/73% and 80%/97% of *Slarf8b Slmir167a* and *Slarf8a Slarf8b Slmir167a* anthers, respectively, dehisced successfully in two separate experiments, whereas only one of 28 *Slarf8a Slmir167a* anthers (4%) dehisced in one experiment, and none in a second experiment (Figs 4a,b,e–g, S5a). These data indicate that the *Slmir167a* indehiscent phenotype is mainly due to misregulation of *SIARF8B*, with a possible minor contribution of *SIARF8A* when *SIARF8B* is absent.

Desiccation does not restore anther dehiscence in *Slmir167a*

The final stage of anther dehiscence involves dehydration of the anther wall (Wilson *et al.*, 2011). Comparison of water content in stamens of the different genotypes showed that *Slmir167a* had a slightly but significantly higher water content relative to the WT 1 DPA, but not at other time points (Fig. S5e). To examine whether desiccation could induce anther dehiscence, anthers were excised on the morning of anthesis (c. 9:30 h), when most anthers were still closed, and air-dried at room temperature. After

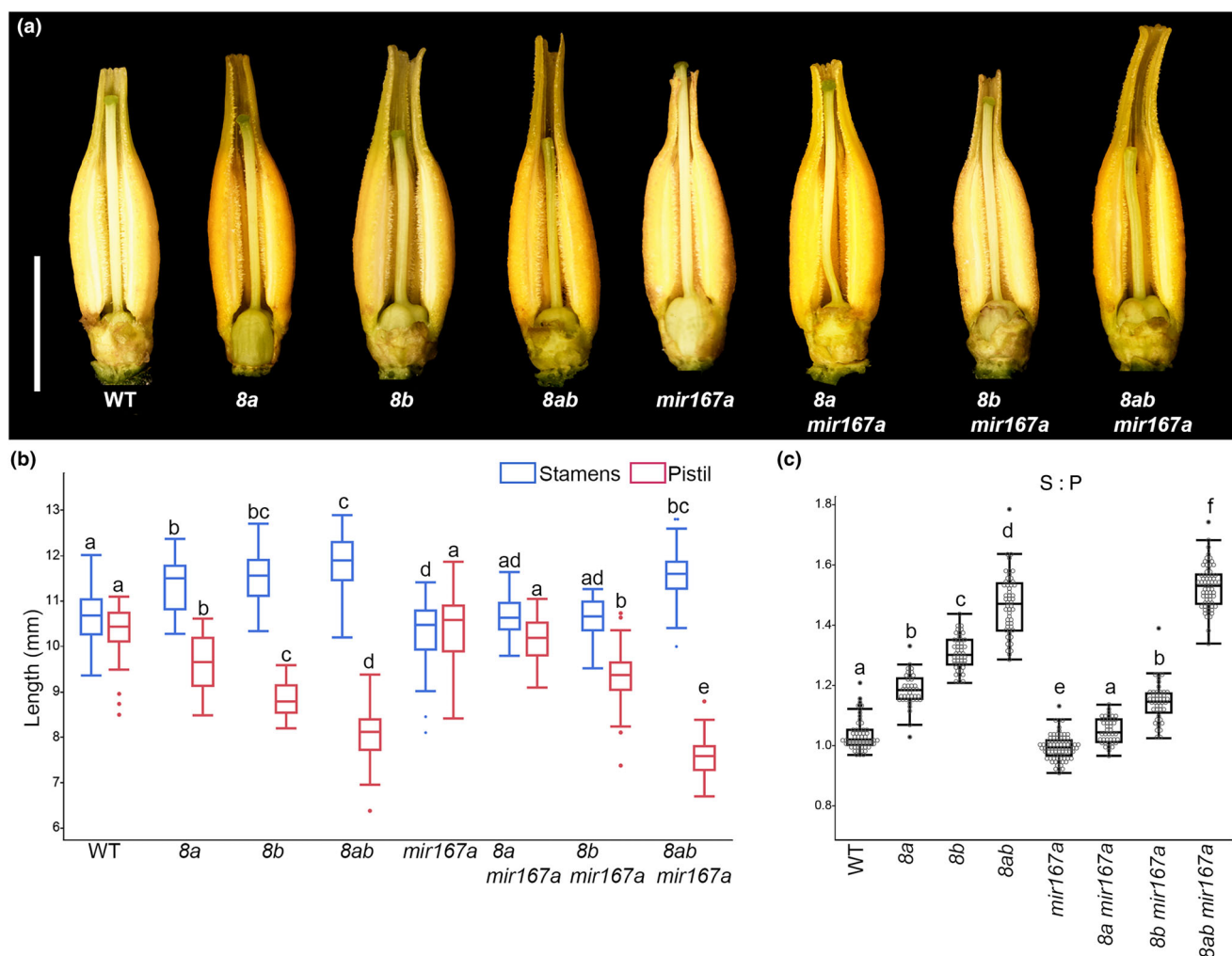


**Fig. 3** Tomato *SlMIR167a-SIARF8* module affects the relative position of the stamens and pistil. (a) Pistil and stamens of the indicated genotypes at anthesis. Part of the stamen cone was removed to expose the pistil. Bar, 5 mm. (b) Average lengths of the stamens and the pistil of the indicated genotypes at anthesis. (c) Stamen-to-pistil length ratio (S : P) of the indicated genotypes at anthesis.  $n = 38-79$ . In the box plot in (b, c), lower, middle, and upper horizontal lines indicate the first quartile, median, and third quartile, respectively. Whiskers extend to 1.5 times the interquartile range. Outliers are plotted individually. Letters above the bars indicate statistical differences between genotypes (provided separately for the stamens and pistil in (b)) using Tukey–Kramer multiple comparisons,  $P < 0.05$ . Genotype abbreviations: 8a – *Slarf8a*; 8b – *Slarf8b*; 8ab – *Slarf8a Slarf8b*; mir167a – *Slmir167a*.

4 h of desiccation, 82% of WT anthers dehiscid, compared with only 7% of *Slmir167a* anthers, and only a few additional anthers dehiscid in the next 5 d (Fig. 5a–c). Further experiments identified a time window in the late morning where WT anthers dehiscid only following desiccation, after which they dehiscid on the plant. By contrast, *Slmir167a* anthers mostly failed to dehiscid regardless of the time and desiccation (Fig. S5f–n). Therefore, the indehiscent phenotype of *Slmir167a* anthers could not be restored by desiccation and could not be attributed to the higher water content.

### The *Slmir167a-SIARF8* module controls endothecium thickening

Anther dehiscence involves septum, connective, and stomium breakdown, and secondary-wall thickening of the endothecium

layer (Wilson *et al.*, 2011), characterized in tomato by U-shaped cell-wall lignification (Bonner & Dickinson, 1989; García & Barboza, 2006). We therefore examined these features in *Slmir167a* anthers. Unlike the WT, *Slmir167a* septum and connective remained partially intact, leading to the maintenance of four separate locules (Fig. 4c,d). The endothecium layer was thicker in *Slmir167a* anthers compared with the WT, due to longer cells, without an effect on the number of cell layers (Fig. 5d–f). The partially intact septum and connective and the endothecium cell length were suppressed by *Slarf8b* and by *Slarf8a Slarf8b*, but not by *Slarf8a*, in correlation with dehiscence (Figs 4e–g, 5d–f). Differential staining with safranin O and fast green at 1 DPA revealed that there was no detectable lignification in *Slmir167a* endothecia, in contrast to the clear U-shape lignified secondary cell-wall thickening observed in WT and *Slarf8* endothecia (Fig. 5g–k). *Slarf8b* and *Slarf8a Slarf8b* suppressed

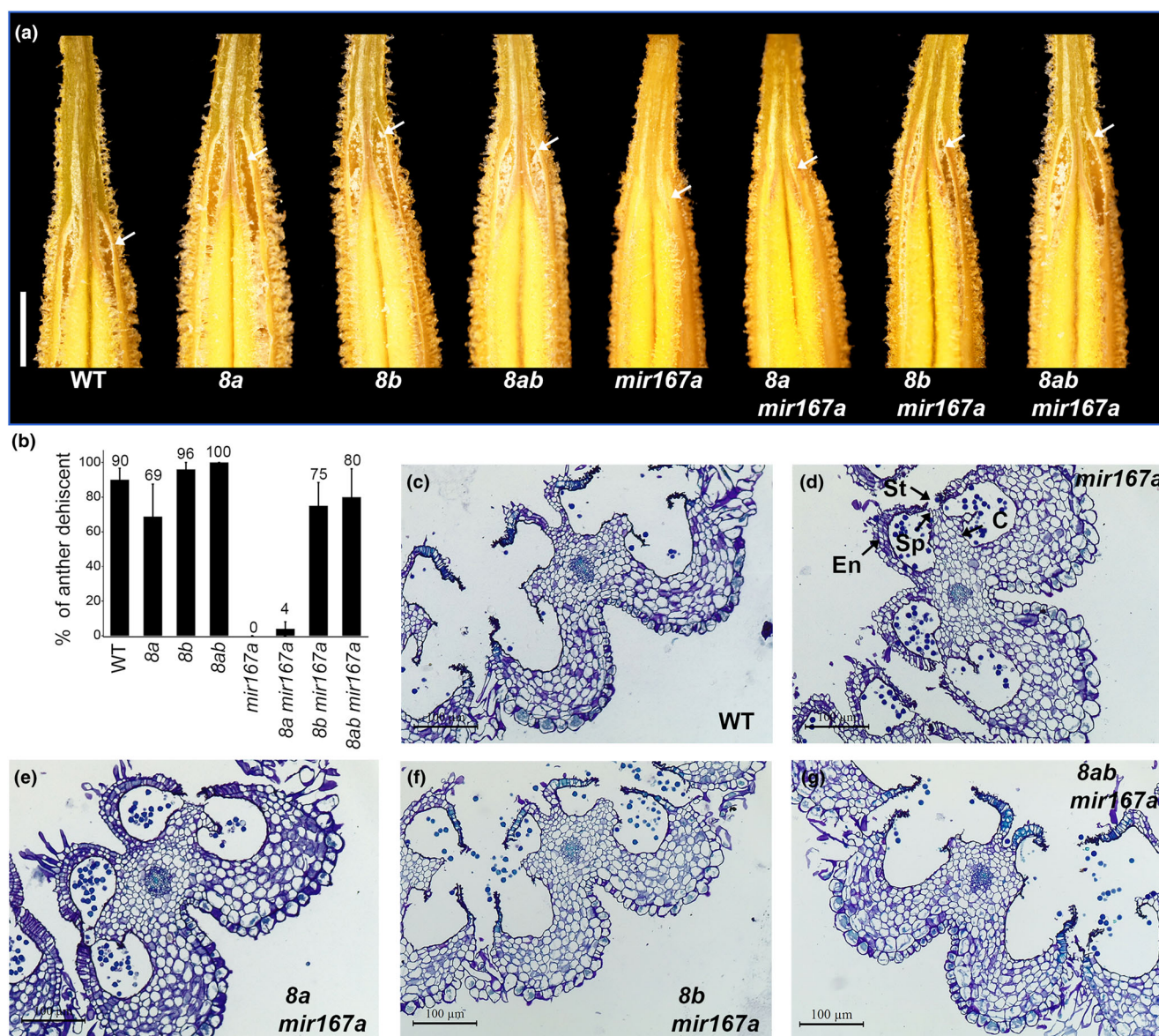


**Fig. 4** Tomato *Slmir167a* anthers fail to dehisce. (a) Anthers of the indicated genotypes at 1 d post anthesis (DPA). White arrows point to the anther-opening site in the stomium area. (b) Quantification of dehiscing anthers in flowers two DPA.  $n$  = between 27 and 46 anthers from four to eight flowers. Error bars represent SE. (c–g) Cross-section of anthers (1 DPA) of the indicated genotypes. Genotype abbreviations: 8a – *Slarf8a*; 8b – *Slarf8b*; 8ab – *Slarf8a Slarf8b*; *mir167a* – *Slmir167a*. Bars: (a) 1 mm; (c–g) 100  $\mu$ m. The relevant tissues are labeled in (d) as follows: C, connective; En, endothecium; Sp, septum; St, stomium.

this phenotype, and only a weak lignin signal could be detected in *Slarf8a Slmir167a* endothecia, likely insufficient to suppress the indehiscent phenotype (Fig. 5L–N). Endothecium lignification in tomato is restricted to the upper part of the anther (Bonner & Dickinson, 1989). In agreement, lignin staining was observed in a restricted region along the anther. In *Slmir167a*, anther lignification was not detected in any part of the anther (Fig. S6). Together, these results indicate that impaired lignification in the endothecium, intact septum, and remnants of the connective prevent anther dehiscence in *Slmir167a*, perhaps alongside some contribution from the slightly higher water content. The indehiscent phenotypes result mainly from misregulation of *SLARF8B*,

with minor contributions from *SLARF8A*, indicating that the *SLARF8* duplication enabled the evolution of unique roles for the two paralogs.

### Extremely delayed fruit set in *Slmir167a* mutants

We and others have previously shown that *SLARF8*s affect fruit set (Goetz *et al.*, 2006, 2007; Hu *et al.*, 2023; Israeli *et al.*, 2023; Hua *et al.*, 2024). We therefore assessed the effect of *Slmir167* on fruit set. WT and *Slmir167a* flowered and reached anthesis at similar times, in several seasons and locations (Table S4). However, fruit set was severely delayed in *Slmir167a* (Fig. 6a,b).

*Slmir167a* plants began to set fruit only at 94–99 DAS, when WT plants had already developed red fruits (Fig. 6a,b). These late *Slmir167a* fruits usually developed from early inflorescences, from flowers that had opened several weeks earlier and remained attached to the plant, unlike WT unfertilized flowers, which typically abscise (Fig. 6b).

To understand the cause of the impaired fruit set in *Slmir167a*, we first quantified pollen viability in WT and *Slmir167a* plants. Pollen viability was slightly lower in *Slmir167a* plants compared with the WT (Fig. S7a–c), but 90% of the pollen was viable (Fig. S7b,c). In addition, *Slmir167a*<sup>+</sup> plants segregated homozygous *Slmir167a* progeny at the expected Mendelian rate of 1/4 (Table S5). We therefore hypothesized that the lack of anther dehiscence, rather than reduced pollen viability, is the main cause of the impaired fruit set in *Slmir167a* plants. To test this hypothesis, flowers from the first six inflorescences of WT and *Slmir167a* plants were either left to self-pollinate naturally ('untouched') or emasculated and manually self-pollinated shortly after flowering. Two weeks later, 40% of the untouched WT flowers and none of the untouched *Slmir167a* flowers had set fruit (Table S6). Manual self-pollination yielded 95% and 65% fruit set in WT and *Slmir167a* flowers, respectively (Table S6), with comparable fruit growth and ripening (Fig. 6c,d). These results indicate that anther indehiscence is a central cause of the delayed fruit set in *Slmir167a* plants, resulting in a substantial reduction in final fruit number (Fig. 6e,f). The mechanism that triggers some of the ovaries to resume development and set fruit weeks later remains unknown, as they mostly originated from older flowers lacking functional reproductive organs (Fig. 6b). Notably, fruits resulting from manual self-pollination had pseudo-seeds, which were absent in naturally developing *Slmir167a* seedless fruits (Figs 1, S8).

### Loss of *SLARF8* genes rescues *Slmir167a* fruit set but not seed formation

To test whether deregulated *SLARF8* activity underlies the *Slmir167a* fruit-set phenotype, we examined the respective mutant combinations. As described above, *Slmir167a* plants produced very few fruits compared with the WT and *Slarf8* mutants, all of which were still green at 127 DAS. Single or double *Slarf8* mutants suppressed this delay, supporting a role for *SLARF8* deregulation in this phenotype (Figs 6e–h, S9).

Despite the restoration of fruit-set timing, neither the *Slarf8a Slmir167a* nor the *Slarf8b Slmir167a* double mutant produced viable, fully developed seeds (Fig. S8). To assess whether reduced *SLARF8* dosage might restore seed formation in the *Slmir167a* background, we manually self-pollinated the different mutant combinations. After 10 d, developing seeds in *Slmir167a*, *Slarf8a Slmir167a*, or *Slarf8b Slmir167a* appeared similar to the WT (Fig. S10a–d). However, 18- or 21-d post-pollination, seed development stopped, the ovules contained no embryos, and the seeds remained green, unlike in WT (Fig. S10e–k). Ovules of *Slarf8a Slarf8b*<sup>+</sup> *Slmir167a* exhibited seed-like morphology (Fig. S10g) after 18 DPA, but they were all empty.

### *Slarf8b Slmir167a* exhibit early and rapid fruit production

Tracking the timing of fruit set across different mutant combinations revealed that *Slarf8a* and *Slarf8b* had distinct effects, alone and in combination with *Slmir167a*. The *Slarf8a* single mutant set more fruit and earlier than the WT, meaning that visible fruits appeared earlier and the number of fruits was greater at each time point during development (Fig. 6f). Fruit set in the *Slarf8a Slmir167a* double mutants was delayed compared with the WT but earlier compared with the *Slmir167a* single mutant (Fig. 6f). Therefore, *Slarf8a* only partially suppressed the late fruit set of the *Slmir167a* mutant. The partial restoration of fruit set in *Slarf8a Slmir167a* despite the indehiscence of anthers suggests that fruit development is partially independent of anther dehiscence in this genotype. Strikingly, while the *Slarf8b* single mutant set fruit at a similar timing and rate as the WT, *Slarf8b Slmir167a* double mutants set fruit much earlier, and at a much faster rate than both corresponding single mutants and even the earliest single mutant, *Slarf8a*. Therefore, this combination displayed a novel phenotype of extreme early fruit set, not seen by any of the single mutants (Fig. 6g). The *Slarf8a Slarf8b* double mutant was nearly epistatic over *Slmir167a* with respect to fruit set, except for an earlier termination of fruit set in the triple mutant compared with the *Slarf8a Slarf8b* double mutant (Fig. 6h). Fruit set in *Slarf8b Slmir167a* was even earlier and reached a plateau more rapidly than in *Slarf8a Slarf8b Slmir167a* (Fig. 6g). Monitoring the percentage of flowers that set fruit in the first five inflorescences revealed that fruit production in the *Slarf8b Slmir167a* was strongly biased toward the earliest inflorescences, with progressively fewer fruits formed in later ones (Fig. S11). A similar but less pronounced trend was also observed in the *Slarf8b* single mutant, whereas the WT displayed a relatively uniform fruit distribution across inflorescences (Fig. S11).

### *Slarf8b Slmir167a* plants exhibit accelerated fruit growth and set fruit independently of pollination

In addition to the high fruit-set rate, *Slarf8b Slmir167a* fruits grew and matured much faster than WT and *Slarf8b* fruits (Figs 6i–l, S12a), ultimately reaching a final size similar to the WT. By contrast, fruits produced by the single mutants *Slarf8b* and *Slmir167a* were significantly smaller than WT fruits (Fig. S12b). Such rapid fruit growth is characteristic of parthenocarpic fruits, which develop independently of fertilization. We therefore monitored the percentage of fruit set in the absence of fertilization by removing anther cones before anthesis. More than 70% of emasculated *Slarf8b Slmir167a* flowers developed parthenocarpic fruits (Fig. 6m,n), in contrast to emasculated flowers of WT, *Slarf8a*, *Slmir167a*, and, notably, *Slarf8a Slmir167a*, all of which did not develop fruits, and to single *Slarf8b* flowers, of which only 1 out of 44 emasculated flowers produced fruits (Fig. 6n). Therefore, the *Slarf8b Slmir167a* double mutant uniquely tunes the ARF8 dose to enable fertilization-independent fruit set.

Hence, the *SLARF8* duplication enabled the evolution of unique roles for the paralogs, also with respect to fruit set,

enabling novel fruit-development-related phenotypes through the interplay between the two *SlARF8s* and *SlmiR167a*.

### Increased yield in *Slarf8b Slmir167a* under cold stress

Tomato is susceptible to environmental conditions, with both high and low temperatures decreasing yield (Charles & Harris, 1972).

The Mediterranean winter is characterized by relatively moderate cold nights (5–10°C), which can impair pollen viability and subsequently limit fruit set and yield (Charles & Harris, 1972). As a result, the growing season for processing tomatoes is typically confined to the spring, as summer is also unsuitable due to high temperatures. Because of the fertilization-independent fruit set and robust, rapid fruit formation and development in *Slarf8b*

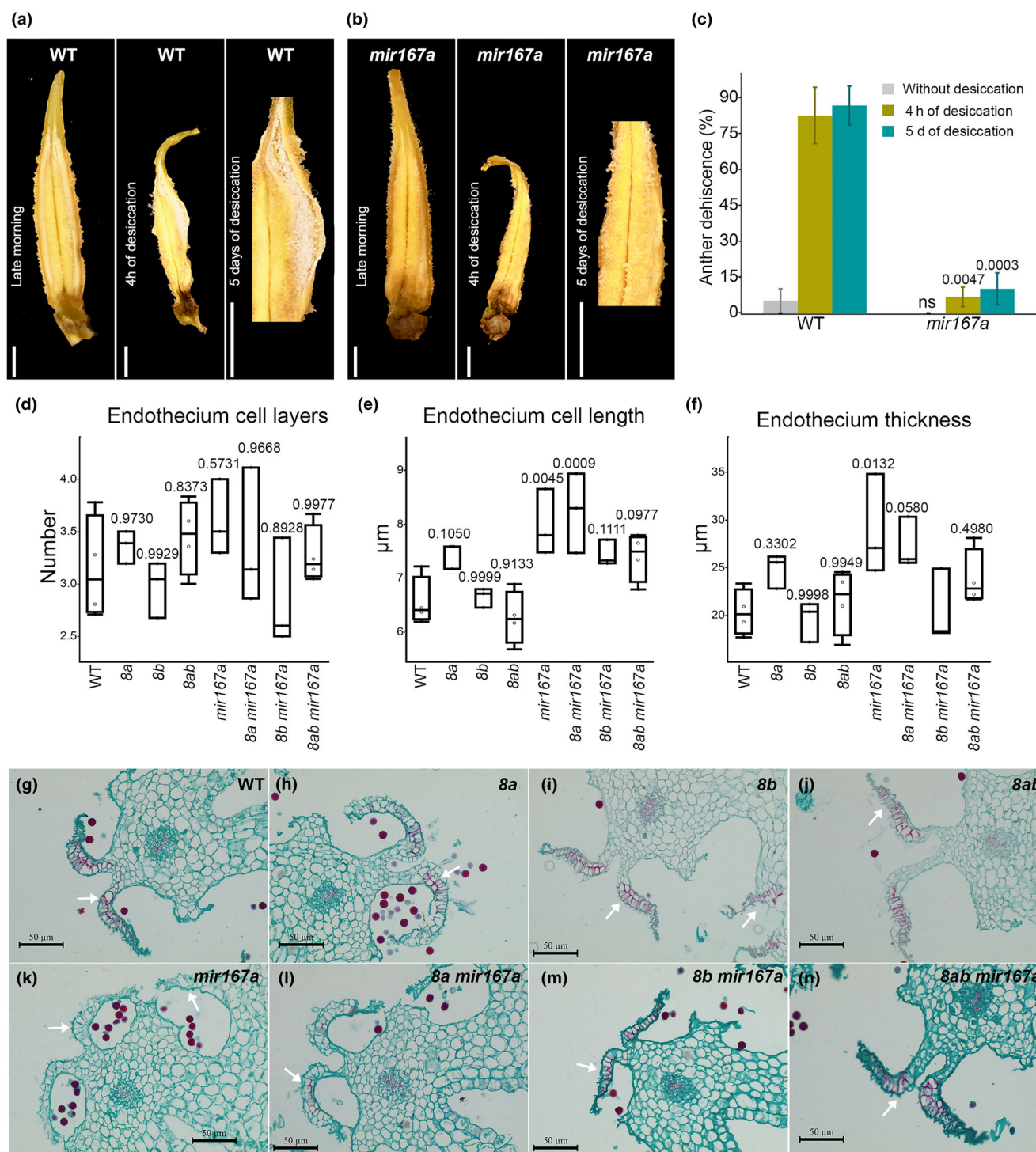


**Fig. 5** *Slmir167a* tomato mutant fails to dehisce after desiccation and lacks lignification in the endothecium. (a, b) Representative images of anthers from the indicated genotypes, dissected on the morning of anthesis (between 9:30 h and 10:00 h), shortly before dehiscence in the wild-type (WT). Left – freshly dissected anthers, middle – the same anthers after 4 h of dehydration at room temperature, and right – a magnified view of the dried anther after 5 d of dehydration. Note that the anthers of both genotypes shrank, but only the WT anthers dehisce. (c) Percentage of dehisced anthers of WT and *Slmir167a* at the indicated times after detachment;  $n = 22$  stamens for WT and 30 for *Slmir167a* from four and five flowers, respectively. Error bars represent SE.  $P$ -values in (c) indicate differences as determined by  $t$ -test between WT and *Slmir167a*. (d–f) Quantification of histological characteristics at 1 d post anthesis (DPA) obtained from the anther sections shown in Fig. 4 and Supporting Information Fig. S5: number of endothecium cell layers (d), endothecium cell length (e), and endothecium tissue thickness (f).  $n = 4$  stamen cones, each consisting of six anthers. In the box plot, lower, middle, and upper horizontal lines indicate the first quartile, median, and third quartile, respectively. Whiskers extend to 1.5 times the interquartile range. Outliers are plotted individually.  $P$ -values indicate differences from WT as determined by Dunnett test. (g–n) Anther transverse sections of the indicated genotypes at 1 DPA, focused on the adaxial dehiscence zone. Endothecium lignification appears in red. White arrows indicate lignified regions or a lack of lignification. Note the absence of lignin staining in *Slmir167a*. Genotype abbreviations: 8a – *Slarf8a*; 8b – *Slarf8b*; 8ab – *Slarf8a Slarf8b*; mir167a – *Slmir167a*. Bars: (a–b) 1 mm; (g–n) 50  $\mu$ m.

*Slmir167a* mutants, we wondered whether they would show decreased sensitivity to cold stress, thus producing stable yield. To test this possibility, we conducted a yield experiment during the Mediterranean winter at the Volcani Center, Israel. The experiment was carried out without temperature control, in a net house with a glass roof. The plants were exposed to night temperatures of  $c. 10^{\circ}\text{C}$  for 6 to 8 h during December, January, and February (Fig. S13a; Tables S2, S3).

One-month-old plants were planted in late November. Flower production and fruit set were monitored until mid-January (76 DAS) and until the end of January (93 DAS), respectively. Yield traits were assessed at the end of March (149 DAS). While all genotypes flowered at the same time (Fig. S13b), WT and *Slmir167a* plants nearly failed to produce fruit by 93 DAS (Fig. S13c). At the same time, *Slarf8b* and *Slarf8b Slmir167a* produced more than 7 and more than 18 times more fruits than the WT, respectively (Fig. S13c). At the last month of the experiment, when the weather started to warm up, WT and *Slmir167a* plants produced many small fruits that almost did not contribute to fruit weight at harvest (Figs S13c, 7a–c). We therefore measured red and green fruit yield separately. *Slarf8b Slmir167a* plants produced 6 times more red fruits and 10 times higher red fruit weight than the WT (Fig. 7a–c). They had almost exclusively red fruits, while in the other genotypes, most of the fruits were still green at the time of harvest (Fig. 7a,c). The average individual fruit weight was also much higher in *Slarf8b Slmir167a* (Fig. 7d). The higher yield combined with a reduced vegetative mass led to a dramatically increased harvest index in *Slarf8b Slmir167a* relative to the WT (Fig. 7e). This indicates a reallocation of resources toward reproductive growth at the expense of vegetative growth in *Slarf8b Slmir167a*, resulting in a compact, high-yielding plant.

### A regulatory network within the *Slmir167a*-*SlARF8* module controls the timing of fruit development

In most developmental processes described above, *SLMIR167a* and *SLARF8* have opposite effects, with *SLARF8A* and *SLARF8B* showing unique, process-dependent effects. Surprisingly, with respect to fruit set and parthenocarpy, *Slarf8b Slmir167a* but not *Slarf8a Slmir167a* plants have a unique fruit-development-related phenotypic syndrome of extremely early and robust fruit set, rapid fruit growth, and fertilization-independent fruit set, not seen in the respective single mutants. Interestingly, removing

*SLARF8A* activity suppressed this phenotypic syndrome, with *Slarf8a Slarf8b Slmir167a* being almost identical to the double *Slarf8a Slarf8b* mutants. Therefore, the unique fruit-related syndrome of *Slarf8b Slmir167a* depends on intact *SLARF8A* activity (Fig. 6h). Previous studies suggested that *SLARF8* can both inhibit and promote fruit set (Fu, 2013; Du *et al.*, 2016; Hu *et al.*, 2023). We therefore hypothesized that the unique phenotypes of *Slarf8b Slmir167a* may result from synergistic deregulation of *SLARF8A* expression. To test this, we examined *SLARF8A* expression in the mutant combinations. Unlike its upregulation in s3 pistils, at s5 *SLARF8A* expression was not significantly different from the WT in *Slmir167a* pistils (Figs S2e, 8a). In *Slarf8b* pistils, *SLARF8A* expression was not increased in most experiments, though a slight upregulation was occasionally observed. Notably, *SLARF8A* expression was significantly elevated in the *Slarf8b Slmir167a* double mutant in most experiments, exceeding its levels in either single mutant (Fig. 8a). *Slarf8a* mutants did not affect *SLARF8A/B* expression, and *SLARF8B* expression was not affected in either *Slarf8a* or *Slarf8b* (Fig. 8a,b).

These results suggest that *SLARF8B* and *Slmir167a* synergistically repress *SLARF8A* expression. We therefore tested the effect of *SLARF8A* and *SLARF8B* on expression from the *SLARF8A* and *SLARF8B* promoters using a Luciferase-based transient transactivation assay. *SLARF8B* repressed transcription from both the *SLARF8A* promoter and its own promoter, compared with the basal activation of the empty vector (Fig. 8c,d). *SLARF8A* also repressed its own promoter and that of *SLARF8B* (Fig. 8c,d).

Together, the data suggest that *SLARF8A*, *SLARF8B*, and *SLMIR167a* form a coordinated inhibitory circuit that modulates *SLARF8A/B* activity during pistil development and fruit set (Fig. 8e). Upregulation of *SLARF8B* in the *Slmir167a* mutant may restrict *SLARF8A* expression, and in the absence of both *SLARF8B* and *Slmir167a*, *SLARF8A* may escape the repression and reach a threshold sufficient to induce rapid, robust fruit set and development (Fig. 8f).

## Discussion

### The *Slmir167*-*SlARF8* module orchestrates flower-organ development

In the current work, we show both evolutionarily conserved features and tomato-specific characteristics of the *Slmir167*-

regulate the S : P ratio in an additive, dose-dependent manner (Figs 3, S4), while *SLARF8B* evolved unique roles in restricting anther dehiscence (Figs 4, 5), both functions antagonized by

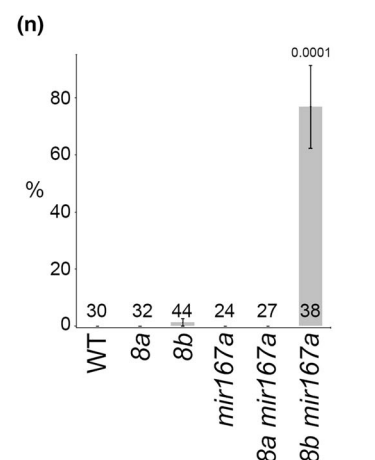


**Fig. 6** *Slmir167a* tomato mutants set fruit late, and *Slarf8b Slmir167a* mutants have an extremely early and robust fruit set. (a, b) First inflorescences of wild-type (WT) (a) and *Slmir167a* (b) at 99 d after sowing (DAS), corresponding to 50 and 51 d post anthesis (DPA), respectively. In (b), initial development of a fruit can be observed (white arrow). (c, d) Inflorescence/fruit branches after manual self-pollination at c. 48 DPA. Fruits resulting from manual pollination are marked with a blue ring. (c) A WT branch containing fruits from manual self-pollination alongside naturally self-pollinated fruits. (d) A *Slmir167a* branch with fruits from manual self-pollination alongside undeveloped flowers. (e) Representative first fruit branch of the indicated genotypes at 127 DAS. Two branches from different plants are shown for *Slmir167a*. (f–h) Quantification of fruit production over time in the indicated genotypes. Error bars represent SE. Plants were grown during the winter season in a closed glasshouse with mild heating. For clarity, the results are presented in three separate graphs, each showing the same data for WT and *Slmir167a*.  $n = 5–6$  plants per genotype.  $P$ -values indicate the differences from WT as determined by Dunnett test; the  $P$ -values of *Slmir167a* are indicated in (f) only. In (h), the  $P$ -values with the asterisk belong to *arf8a arf8b mir167a*, and the ones without to *arf8a arf8b*. (i–l) 1<sup>st</sup> fruit of the indicated genotypes at 8 DPA. (m) Developing *Slarf8b Slmir167a* fruits following emasculum. (n) Percentage of fruits developing after emasculum in the indicated genotypes. The number at the base of the graph represents the number of analyzed flowers/fruits (n). Error bars represent SE. Genotype abbreviations: 8a – *Slarf8a*; 8b – *Slarf8b*; 8ab – *Slarf8a Slarf8b*; *mir167a* – *Slmir167a*. Bars: (a–d) 3 cm; (e) 5 cm; (i–m) 1 cm. P +, manual self-pollination; P–, without pollination.

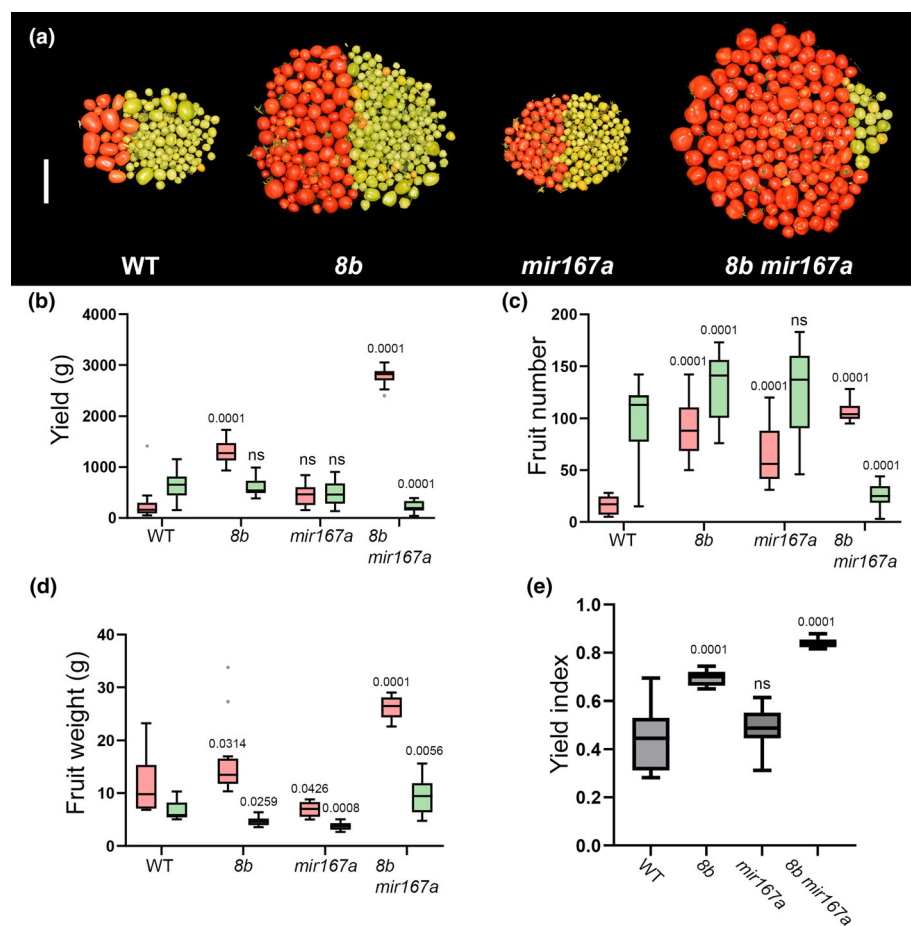


**Fig. 7** *Slarf8b Slmir167a* tomato plants are resilient to cold stress. (a) Images of total fruits from one representative plant of the indicated genotypes, 149 d after sowing (DAS). Bar, 10 cm. (b) Total yield of red and green fruits. (c) Number of red and green fruits. (d) Average weight of an individual red and green fruit. (e) Yield index, calculated as the ratio of total fruit weight to plant vegetative and fruit weight. In (b–d), pink (left) and green (right) values correspond to red and green fruits, respectively, for each genotype. In the box plot, lower, middle, and upper horizontal lines indicate the first quartile, median, and third quartile, respectively. Whiskers extend to 1.5 times the interquartile range. Outliers are plotted individually.  $P$ -values in (b–e) indicate the differences from the respective wild-type (WT) as determined by Dunnett test.  $n$  (number of plants) = WT – 14; *Slarf8b*, *Slmir167a*, and *Slarf8b Slmir167a* – 13. Genotype abbreviations: 8b – *Slarf8b*; *mir167a* – *Slmir167a*. ns, not significant.

*SlmiR167a*. We further expose a unique interaction between *SlmiR167a* and *SLARF8B* in tuning fruit set and development (Fig. 6).

### The role of the *miR167-ARF8* module in stigma position

The S : P ratio is a key morphological trait that determines the position of the stigma relative to the anther cone, which affects pollination strategy (Rick, 1950, 1988; Rick *et al.*, 1978; Chen *et al.*, 2007; Shang *et al.*, 2021). Here, we demonstrate that

*SLARF8A/B* and *SlmiR167a* form an antagonistic regulatory module that differently tunes reproductive organ growth. *SLARF8A* and *SLARF8B* promote style elongation while repressing stamen elongation in a dose-dependent manner, with a stronger contribution from *SLARF8B* (Figs 3, S4). Notably, *SLARF8B* transcripts are upregulated in pistil bulks from cultivated tomato accessions with stable exerted stigma compared with inserted-stigma accessions, suggesting a central role in stigma position control across diverse germplasm (Riccini *et al.*, 2021). The stigma position is also affected by environmental stress. Prolonged exposure to heat

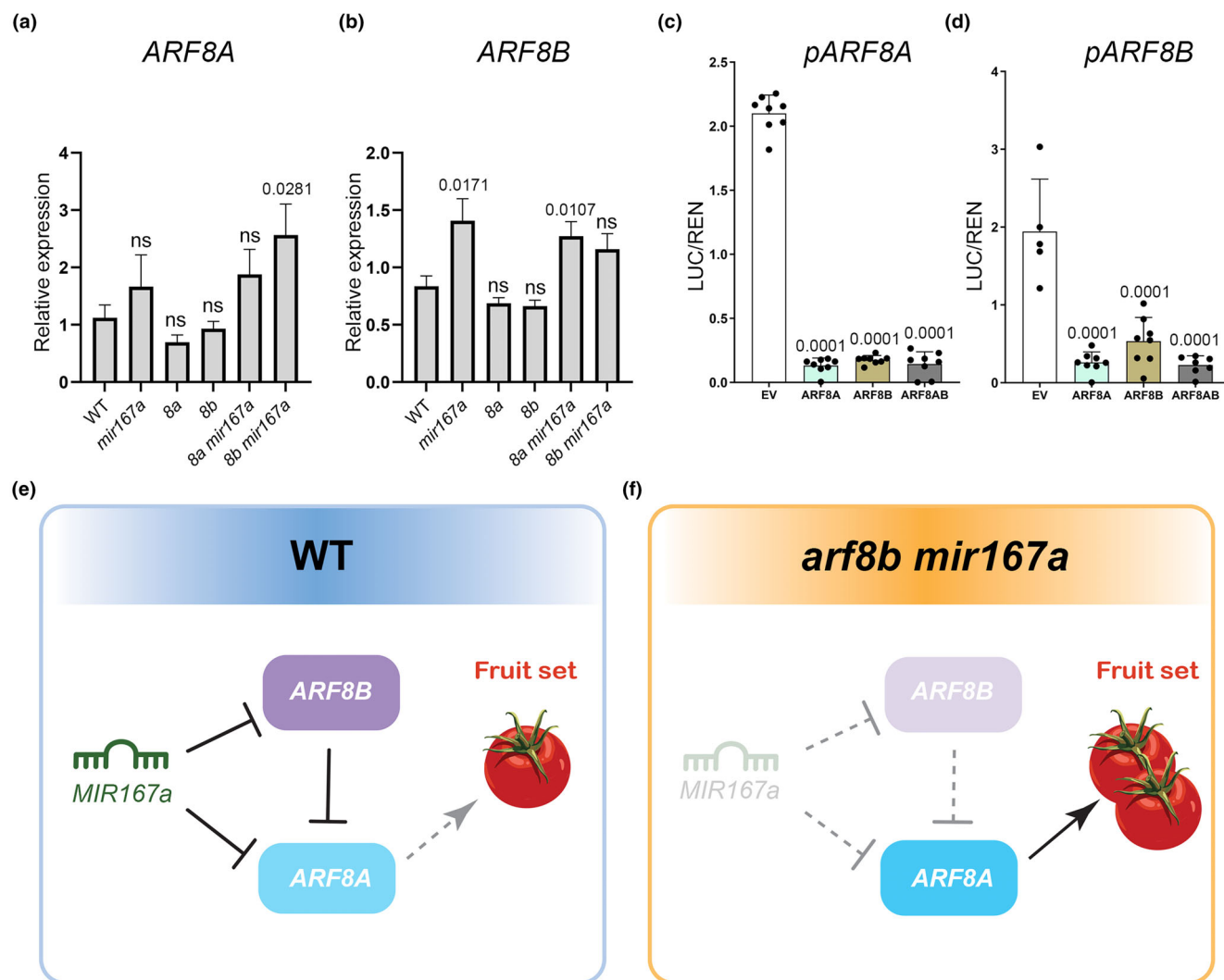


**Fig. 8** *SIARF8B* and *SIMIR167a* repress *SIARF8A* in tomato pistils at anthesis. (a–b) Relative expression of *SIARF8A* (a) and *SIARF8B* (b) in pistils at anthesis of the indicated genotypes. Error bars represent SE. Statistical significance was determined using a *t*-test between wild-type (WT) and the other genotypes. ns, not significant. Note that transcripts of *SIARF8A* and *SIARF8B* remain unaltered in the respective single mutants, since the edited frame shift leads to a premature stop codon resulting in a nonfunctional AUXIN RESPONSE FACTOR (ARF) protein but does not cause diminished transcription. (c, d) Transactivation assays in *Nicotiana benthamiana* leaves show the repression of the reporter-gene expression (luciferase) from the promoters of *SIARF8A* (*pARF8A*) (c) and *SIARF8B* (*pARF8B*) (d), by co-expression with *SIARF8A* and *SIARF8B* or both as effectors. Both effectors similarly repress expression from both promoters, either alone or together. Each column represents the mean fold change in the luciferase (LUC) to *Renilla* luciferase (REN) luminescence ratio, and error bars indicate the SD. *P*-values above each column indicate statistical significance relative to the empty vector (EV) control, as determined by Dunnett test. (e–f) A proposed scheme of the complex inter-regulation in the *Slmir167-SIARF8* module in the context of fruit set. In the WT at anthesis, *Slmir167a* represses *SIARF8A* and *SIARF8B*, with *SIARF8B* further inhibiting *SIARF8A* (e). In the *Slarf8b Slmir167a* double mutant, *SIARF8A* expression is elevated (f). *SIARF8A* promotes fruit set and growth, and its deregulation in *Slarf8b Slmir167a* promotes robust, rapid, fertilization-independent fruit set and growth. Blunt ended arrows represent inhibition. Dashed, gray arrows and blunt-ended arrows indicate reduced activation and repression, respectively. Bolded arrow represents increased activation. *n* = in (a) and (b), 11–12 biological replicates per genotype, with four to seven pistils analyzed in each replicate; In (c) and (d), five to eight biological replicates in two and three independent experiments, respectively.

stress promotes stigma exertion, reducing self-pollination efficacy and decreasing yield (Lohar & Peat, 1998; Saeed *et al.*, 2007; Srivastava *et al.*, 2012; Pan *et al.*, 2019). Therefore, an increased S : P ratio, as seen in *Slarf8* mutants, may help mitigate the negative effects of heat stress on reproductive success (Israeli *et al.*, 2023).

Although the basis for the opposite effect of *SIARF8* on the elongation of different organs remains unresolved, a similar functional duality is seen during fruit development, where *SIARF8*

represses fruit initiation yet promotes placenta formation and fruit growth (Hu *et al.*, 2023; Israeli *et al.*, 2023; Hua *et al.*, 2024). Moreover, different direct *SIARF8* target genes respond in opposite directions to *SIARF8* absence, with some increasing and others decreasing in expression (Hu *et al.*, 2023). Here, we provide additional evidence that *SIARF8A/B* can also act as transcriptional repressors, using the transactivation assay (Fig. 8c,d). This supports a dual, context-dependent role for *SIARF8*s as both

transcriptional activators and repressors. The activity of Arabidopsis ARFs was recently shown to differ according to the developmental and molecular context and depend on interactions among the ARFs and with other regulators, as well as the specific bound *cis*-elements (Martin-Arevalillo *et al.*, 2025).

Arabidopsis *Atarf8* mutants also exhibit a dual effect on flower-organ elongation, but in the opposite direction, with shorter stamens and longer carpels (Varaud *et al.*, 2011; Ghelli *et al.*, 2018). Although the prevailing model proposes that low auxin levels promote formation of a repressive ARF-Aux/IAA complex, whereas high auxin levels relieve this inhibition and activate transcription through the same and/or other transcription factors (Chandler, 2016; Weijers & Wagner, 2016; Kato *et al.*, 2017; Yu *et al.*, 2022), other studies show that the ARF8 protein forms a growth-repressing complex with petal-specific variants of transcription factors outside of the auxin signaling pathway (Varaud *et al.*, 2011), thereby leading to opposite effects in different tissues. Moreover, *AtARF8* also generates tissue-specific isoforms with distinct activities (Ghelli *et al.*, 2018). *SLARF8A* and *SLARF8B* undergo alternative splicing as well (Zouine *et al.*, 2014), which may underlie their dual functionality.

### The role of the *miR167*-ARF8 module in anther dehiscence

Anthers of *Slmir167a* flowers were indehiscent (Figs 4, S5), which was largely rescued by *Slarf8b* (Figs 4g, S5a). Indehiscent anthers were also observed by loss of Arabidopsis *AtMIR167a* or expression of *miR167*-resistant *AtARF6* and *AtARF8* forms (*mAtARF6/8*). In agreement, *Atarf6/8* rescued *Atmir167a* indehiscent anthers (Wu *et al.*, 2006; Yao *et al.*, 2019; Zheng *et al.*, 2019). In addition, in both tomato and Arabidopsis, *miR167* is abundant in the final developmental stages of stamens (Wu *et al.*, 2006; Pan *et al.*, 2017; Yao *et al.*, 2019; Zheng *et al.*, 2019), indicating a conserved role for *miR167*-ARF8 regulation in anther dehiscence. However, in Arabidopsis, the *miR167*-ARF8/6 module affects anther dehiscence through the regulation of anther growth, which affects its desiccation. As a result, desiccation restored anther dehiscence in *Atmir167a* (Zheng *et al.*, 2019). By contrast, desiccation did not restore anther dehiscence in tomato *Slmir167a* (Figs 5, S5). Instead, *Slmir167a* affected structural aspects of anther development, including the persistence of the septum and the connective, and the lack of endothecium lignification (Figs 4, 5). Interestingly, while in Arabidopsis, *AtARF8* plays a positive role in endothecium lignification (Ghelli *et al.*, 2018; Zheng *et al.*, 2019), in tomato *SLARF8B* appears to inhibit endothecium lignification and dehiscence, as reflected by the complete loss of endothecium lignification in *Slmir167a* and the suppression of this phenotype by *Slarf8b* (Figs 5k,m,n, S6). The inhibition of endothecium lignification is in agreement, however, with the negative regulation of endothecium lignification by auxin in Arabidopsis (Cecchetti *et al.*, 2008). Jasmonate (JA) was also shown to affect anther dehiscence in an opposite manner in tomato and Arabidopsis (Li *et al.*, 2004, 2022; Dobritzsch *et al.*, 2015; Schubert *et al.*, 2019). ARF8 affects JA accumulation in both Arabidopsis and tomato

(Nagpal *et al.*, 2005; Tabata *et al.*, 2010; Israeli *et al.*, 2023), suggesting a possible involvement of JA in these differential functions.

### Expansion of the *SLARF8* family, and the interaction with *SlmiR167a* enabled flexible tuning of fruit development

The families of auxin-signaling genes have expanded during the evolution of flowering plants, posing the question of the biological significance of this expansion (Israeli *et al.*, 2020). The unique existence of two *SLARF8* genes in tomato is particularly interesting in the context of reproductive development. We found that this expansion enabled diverse, process-specific modes of relationship among the two *SLARF8* members and their *SlmiR167* regulator. For example, in the regulation of stigma position, *SLARF8A* and *SLARF8B* appear to play additive, partially overlapping roles, while in anther dehiscence, *SLARF8B* has acquired a unique role.

We further expose a unique role for the specific interaction between *SLMIR167a* and *SLARF8B* to repress fruit initiation and growth (Fig. 6m,n). The phenotypic syndrome of parthenocarpic fruits, early fruit set, and fast fruit growth, seen in the *Slarf8b Slmir167a* double mutants, is not seen in the respective single mutants, *Slarf8a Slarf8b*, or in Arabidopsis *Atmir167* mutants (Wu *et al.*, 2006; Liu *et al.*, 2014, 2021; Yao *et al.*, 2019; Zheng *et al.*, 2019). Moreover, the syndrome was absent in the *Slarf8a Slmir167a* double mutant that failed to set fruits following emasculation, and instead exhibited intermediate dynamics of fruit development between those observed in the *Slarf8a* and *Slmir167a* single mutants, and may reflect the persistence of stamens, which can occasionally induce fruit set without fertilization (Okabe *et al.*, 2019; Nomura *et al.*, 2025). This suggests a delicate, highly regulated balance between *SLARF8s* and *SlmiR167*, with unique roles of the two *SLARF8s*, enabled by the gene duplication. The genetic, expression, and transactivation data, and the suppression of the *Slarf8b Slmir167a* syndrome by *Slarf8a*, suggest that *SLARF8B* and *SLMIR167a* cooperatively repress *SLARF8A* to prevent premature fruit initiation (Fig. 8). Reciprocal repression between *SLARF8A* and *SLARF8B* further fine-tunes this balance. Expression data from the tomato expression atlas indicate that *SLARF8B* is abundant in the placenta at anthesis and downregulated after fruit set, whereas *SLARF8A* increases post-anthesis, and its uncontrolled expression leads to parthenocarp (Shinozaki *et al.*, 2018; Hu *et al.*, 2023). Thus, *SLARF8B* and *SLMIR167a* likely constrain *SLARF8A* activity during the early stages of fruit initiation in the ovular and placental tissues (Fig. 8e,f).

*Slarf8b Slmir167a* plants produced stable yield under Mediterranean winter conditions, where low temperatures typically impair pollen viability and prevent fertilization. This enhanced yield stability and accelerated maturation may enable the extension of the growing season to earlier, cooler months, alongside heat-resistant genotypes that can cope with increased heat (Israeli *et al.*, 2023). Commercial use of parthenocarpic tomatoes is currently hampered by quality concerns and commercial considerations of seed companies. However, they still have a large

potential for application in growth conditions in which fertilization is prevented, particularly for processing tomatoes (Huang *et al.*, 2021), in which the lack of jelly can be an advantage.

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## Competing interests

None declared.

## Author contributions

NM, NO, BH and NT conceptualized and designed the research. TA and AI identified the *SLMIR167* gene sequences and designed the CRISPR guide RNAs. NM, NT, AB and MF performed the experiments. NM and NO wrote the first draft of the manuscript. All authors contributed to the writing and editing of the manuscript. BH and NO supervised the research and acquired funding.

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## Data availability

The raw data that support the findings of this study are available in Dataset S1, in the Supporting Information of this article. The gene sequences used in this article are available from the SOL Genomics Network (<https://solgenomics.net/>). Sequence data used in this study can be found in the Sol Genomics Network under the following accession nos.: *SLARF8A* (Soly03g031970); *SLARF8B* (Soly02g037530); and *SLARF6A* (Soly00g196060). The genomic locations of *SLMIR167* genes are shown in Fig. S1.

## References

Arazi T, Khedia J. 2022. Tomato microRNAs and their functions. *International Journal of Molecular Sciences* 23: 1–20.

- Bae SH, Park J, Park SJ, Han J, Oh JH. 2021. Transcriptome data for tissue-specific genes in four reproductive organs at three developmental stages of micro-tom tomato. *Data in Brief* 34: 106715.
- Bonner LJ, Dickinson HG. 1989. Anther dehiscence in *Lycopersicon esculentum* Mill. I. Structural aspects. *New Phytologist* 113: 97–115.
- Cecchetti V, Altamura MM, Falasca G, Costantino P, Cardarelli M. 2008. Auxin regulates Arabidopsis anther dehiscence, pollen maturation, and filament elongation. *Plant Cell* 20: 1760–1774.
- Chandler JW. 2016. Auxin response factors. *Plant, Cell and Environment* 39: 1014–1028.
- Charles WB, Harris RE. 1972. Tomato fruit-set at high and low temperatures. *Canadian Journal of Plant Science* 52: 497–506.
- Chen C, Li J, Feng J, Liu B, Feng L, Yu X, Li G, Zhai J, Meyers BC, Xia R. 2021. sRNAanno—a database repository of uniformly annotated small RNAs in plants. *Horticulture Research* 8: 45.
- Chen KY, Cong B, Wing R, Vrebalov J, Tanksley SD. 2007. Changes in regulation of a transcription factor lead to autogamy in cultivated tomatoes. *Science* 318: 643–645.
- Chen KY, Tanksley SD. 2004. High-resolution mapping and functional analysis of se2.1: a major stigma exertion quantitative trait locus associated with the evolution from allogamy to autogamy in the genus *Lycopersicon*. *Genetics* 168: 1563–1573.
- Cucinotta M, Cavalleri A, Chandler JW, Colombo L. 2021. Auxin and flower development: a blossoming field. *Cold Spring Harbor Perspectives in Biology* 13: a039974.
- Dobritsch S, Weyhe M, Schubert R, Dindas J, Hause G, Kopka J, Hause B. 2015. Dissection of jasmonate functions in tomato stamen development by transcriptome and metabolome analyses. *BMC Biology* 13: 28.
- Du L, Bao C, Hu T, Zhu Q, Hu H, He Q, Mao W. 2016. SmARF8, a transcription factor involved in parthenocarp in eggplant. *Molecular Genetics and Genomics* 291: 93–105.
- Engler C, Youles M, Gruetznert R, Ehnert TM, Werner S, Jones JDG, Patron NJ, Marillonnet S. 2014. A golden gate modular cloning toolbox for plants. *ACS Synthetic Biology* 3: 839–843.
- Expósito-rodríguez M, Borges AA, Borges-pérez A, Pérez JA. 2008. Selection of internal control genes for quantitative real-time RT-PCR studies during tomato development process. *BMC Plant Biology* 8: 131.
- Ezura K, Nomura Y, Ariizumi T. 2023. Molecular, hormonal, and metabolic mechanisms of fruit set, the ovary-to-fruit transition, in horticultural crops. *Journal of Experimental Botany* 74: 6254–6268.
- Fei Z, Joung JG, Tang X, Zheng Y, Huang M, Lee JM, McQuinn R, Tieman DM, Alba R, Klee HJ *et al.* 2010. Tomato functional genomics database: a comprehensive resource and analysis package for tomato functional genomics. *Nucleic Acids Research* 39: D1156–D1163.
- Fenn MA, Giovannoni JJ. 2021. Phytohormones in fruit development and maturation. *The Plant Journal* 105: 446–458.
- Finet C, Berne-Dedieu A, Scutt CP, Marlétaz F. 2013. Evolution of the ARF gene family in land plants: old domains, new tricks. *Molecular Biology and Evolution* 30: 45–56.
- Fu Y. 2013. *Identification des Membres de la Famille de Genes Codant Pour les ARF Chez la Tomate et Décryptage du Rôle Central du Gene SLARF8 Dans le Mécanisme Contrôlant la Formation des Fruits et la Parthénocarpie*. PhD Thesis, Vegetal Biology. Toulouse, France: Institut National Polytechnique de Toulouse.
- García CC. 2002. An approach to the diversity of endothelial thickenings in Solanaceae. *Flora* 197: 214–223.
- García CC, Barboza GE. 2006. Anther wall development and structure in wild tomatoes (*Solanum* sect. *Lycopersicon*): functional inferences. *Australian Journal of Botany* 54: 83–89.
- Ghelli R, Brunetti P, Napoli N, De Paolis A, Cecchetti V, Tsuge T, Serino G, Matsui M, Mele G, Rinaldi G *et al.* 2018. A newly identified flower-specific splice variant of AUXIN RESPONSE FACTOR8 regulates stamen elongation and endothecium lignification in Arabidopsis. *Plant Cell* 30: 620–637.
- Gillaspy G, Ben-David H, Gruissem W. 1993. Fruits: a developmental perspective. *Plant Cell* 5: 1439–1451.
- Goetz M, Hooper LC, Johnson SD, Rodrigues JCM, Vivian-Smith A, Koltunow AM. 2007. Expression of aberrant forms of AUXIN RESPONSE

- FACTOR8 stimulates parthenocarp in Arabidopsis and tomato. *Plant Physiology* 145: 351–366.
- Goetz M, Vivian-Smith A, Johnson SD, Koltunow AM. 2006. AUXIN RESPONSE FACTOR8 is a negative regulator of fruit initiation in Arabidopsis. *Plant Cell* 18: 1873–1886.
- Gorguet B, Schipper D, Van Lammeren A, Visser RGF, Van Heusden AW. 2009. *ps-2*, the gene responsible for functional sterility in tomato, due to non-dehiscent anthers, is the result of a mutation in a novel polygalacturonase gene. *Theoretical and Applied Genetics* 118: 1199–1209.
- Gorguet B, Van Heusden AW, Lindhout P. 2005. Parthenocarpic fruit development in tomato. *Plant Biology* 7: 131–139.
- Guan H, Yang X, Lin Y, Xie B, Zhang X, Ma C, Xia R, Chen R, Hao Y. 2024. The hormone regulatory mechanism underlying parthenocarpic fruit formation in tomato. *Frontiers in Plant Science* 15: 1404980.
- Guilfoyle TJ, Hagen G. 2007. Auxin response factors. *Current Opinion in Plant Biology* 10: 453–460.
- Gustafson FG. 1936. Inducement of fruit development by growth-promoting chemicals. *Proceedings of the National Academy of Sciences, USA* 22: 628–636.
- Gustafson FG. 1942. Parthenocarp: natural and artificial. *The Botanical Review* 8: 599–654.
- Hawthorn LR. 1937. Seedlessness in tomatoes. *Science* 85: 199.
- He M, Song S, Zhu X, Lin Y, Pan Z, Chen L, Chen D, Hu G, Huang B, Chen M *et al.* 2021. SITPL1 silencing induces facultative parthenocarp in tomato. *Frontiers in Plant Science* 12: 672232.
- Hellens RP, Allan AC, Friel EN, Bolitho K, Grafton K, Templeton MD, Karunairatnam S, Gleave AP, Laing WA. 2005. Transient expression vectors for functional genomics, quantification of promoter activity and RNA silencing in plants. *Plant Methods* 1: 13.
- Hu J, Li X, Sun TP. 2023. Four class A AUXIN RESPONSE FACTORs promote tomato fruit growth despite suppressing fruit set. *Nature Plants* 9: 706–719.
- Hua B, Wu J, Han X, Bian X, Xu Z, Sun C, Wang R, Zhang W, Liang F, Zhang H *et al.* 2024. Auxin homeostasis is maintained by sly-miR167-SIARF8A/B-SIGH3.4 feedback module in the development of locular and placental tissues of tomato fruits. *New Phytologist* 241: 1177–1192.
- Huang B, Hu G, Wang K, Frasse P, Maza E, Djari A, Deng W, Pirrello J, Burlat V, Pons C *et al.* 2021. Interaction of two MADS-box genes leads to growth phenotype divergence of all-flesh type of tomatoes. *Nature Communications* 12: 6892.
- Israeli A, Capua Y, Shwartz I, Tal L, Meir Z, Levy M, Bar M, Efroni I, Ori N. 2019. Multiple auxin-response regulators enable stability and variability in leaf development. *Current Biology* 29: 1746–1759.
- Israeli A, Reed JW, Ori N. 2020. Genetic dissection of the auxin response network. *Nature Plants* 6: 1082–1090.
- Israeli A, Schubert R, Man N, Teboul N, Carlos J, Yarcé S, Rosowski EE, Wu M, Levy M, Efroni I *et al.* 2023. Modulating auxin response stabilizes tomato fruit set. *Plant Physiology* 192: 2336–2355.
- Jodder J, Das R, Sarkar D, Bhattacharjee P, Kundu P. 2018. Distinct transcriptional and processing regulations control miR167a level in tomato during stress. *RNA Biology* 15: 130–143.
- Joldersma D, Liu Z. 2018. The making of virgin fruit: the molecular and genetic basis of parthenocarp. *Journal of Experimental Botany* 69: 955–962.
- Kato H, Kouno M, Takeda M, Suzuki H, Ishizaki K, Nishihama R, Kohchi T. 2017. The roles of the sole activator-type auxin response factor in pattern formation of *Marchantia polymorpha*. *Plant and Cell Physiology* 58: 1642–1651.
- Li C, Xu M, Cai X, Han Z, Si J, Chen D. 2022. Jasmonate signaling pathway modulates plant defense, growth, and their trade-offs. *International Journal of Molecular Sciences* 23: 3945.
- Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA. 2004. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell* 16: 126–143.
- Liu J, Wu S, Shi H. 2025. Circular cell clusters and calcium oxalate crystals: critical players in Solanaceae anther dehiscence. *Planta* 261: 120.
- Liu N, Wu S, Van Houten J, Wang Y, Ding B, Fei Z, Clarke TH, Reed JW, Van Der Knaap E. 2014. Down-regulation of AUXIN RESPONSE FACTORS 6 and 8 by microRNA 167 leads to floral development defects and female sterility in tomato. *Journal of Experimental Botany* 65: 2507–2520.
- Liu X, Huang S, Xie H. 2021. Advances in the regulation of plant development and stress response by miR167. *Frontiers in Bioscience-Landmark* 26: 655–665.
- Lohar DP, Peat WE. 1998. Floral characteristics of heat-tolerant and heat-sensitive tomato (*Lycopersicon esculentum* Mill.) cultivars at high temperature. *Scientia Horticulturae* 73: 53–60.
- Lu X, Wu J, Shi QQ, Sun S, Cheng Y, Zhou G, Li R, Wang H, van der Knaap E, Cui X. 2025. A feedback loop at the THERMOSENSITIVE PARTHENOCAIRY 4 locus controls tomato fruit set under heat stress. *Nature Communications* 16: 4184.
- Martin-Arevalillo R, Guillotin B, Dumas R, Scho J, Hugues A, Gerentes M, Tang K. 2025. Synthetic deconvolution of an auxin-dependent transcriptional code. *Cell* 188: 2872–2889.
- Mazzucato A, Cellini F, Bouzayen M, Zouine M, Mila I, Minoia S, Petrozza A, Picarella ME, Ruii F, Carriero F. 2015. A TILLING allele of the tomato Aux/IAA9 gene offers new insights into fruit set mechanisms and perspectives for breeding seedless tomatoes. *Molecular Breeding* 35: 22.
- McCormick S. 1991. Transformation of tomato with *Agrobacterium tumefaciens*. In: *Plant tissue culture manual: supplement 7*. Dordrecht, the Netherlands: Springer Netherlands, 311–319.
- Molesini B, Pandolfini T, Rotino GL, Dani V, Spena A. 2009. Aucsa gene silencing causes parthenocarpic fruit development in tomato. *Plant Physiology* 149: 534–548.
- Nagpal P, Ellis CM, Weber H, Ploense SE, Barkawi LS, Guilfoyle TJ, Hagen G, Alonso JM, Cohen JD, Farmer EE *et al.* 2005. Auxin response factors ARF6 and ARF8 promote jasmonic acid production and flower maturation. *Development* 132: 4107–4118.
- Nomura Y, Lu Y, Harada K, Enomoto H, Yano R, Ezura K, Kojima M, Takebayashi Y, Sakakibara H, Ezura H *et al.* 2025. Spatiotemporal localization of jasmonate in the regulation of fruit set in tomato. *Journal of Experimental Botany* 76: 6527–6547.
- Okabe Y, Yamaoka T, Ariizumi T, Ushijima K, Kojima M, Takebayashi Y, Sakakibara H, Kusano M, Shinozaki Y, Pulungan SI *et al.* 2019. Aberrant stamen development is associated with parthenocarpic fruit set through up-regulation of gibberellin biosynthesis in tomato. *Plant and Cell Physiology* 60: 38–51.
- Pan C, Yang D, Zhao X, Jiao C, Yan Y, Lamin-Samu AT, Wang Q, Xu X, Fei Z, Lu G. 2019. Tomato stigma exertion induced by high temperature is associated with the jasmonate signalling pathway. *Plant, Cell & Environment* 42: 1205–1221.
- Pan C, Ye L, Zheng Y, Wang Y, Yang D, Liu X, Chen L, Zhang Y, Fei Z, Lu G. 2017. Identification and expression profiling of microRNAs involved in the stigma exertion under high-temperature stress in tomato. *BMC Genomics* 18: 843.
- Panda S, Jozwiak A, Sonawane PD, Szymanski J, Kazachkova Y, Vainer A, Vasuki Kilambi H, Almekias-Siegl E, Dikaya V, Bocobza S *et al.* 2022. Steroidal alkaloids defence metabolism and plant growth are modulated by the joint action of gibberellin and jasmonate signalling. *New Phytologist* 233: 1220–1237.
- Pattison RJ, Csukasi F, Catalá C. 2014. Mechanisms regulating auxin action during fruit development. *Physiologia Plantarum* 151: 62–72.
- Peterson R, Slovin JP, Chen C. 2010. A simplified method for differential staining of aborted and non-aborted pollen grains. *International Journal of Plant Biology* 1: 66–69.
- Proost S, Mutwil M. 2018. CoNekT: an open-source framework for comparative genomic and transcriptomic network analyses. *Nucleic Acids Research* 46: W133–W140.
- Ramos-Pulido J, de Folter S. 2023. Organogenic events during gynoecium and fruit development in Arabidopsis. *Current Opinion in Plant Biology* 75: 102440.
- Reeves PH, Ellis CM, Ploense SE, Wu M-F, Yadav V, Tholl D, Chételat A, Haupt I, Kennerly BJ, Hodgens C *et al.* 2012. A regulatory network for coordinated flower maturation. *PLoS Genetics* 8: e1002506.
- Ren Z, Li Z, Miao Q, Yang Y, Deng W, Hao Y. 2011. The auxin receptor homologue in *Solanum lycopersicum* stimulates tomato fruit set and leaf morphogenesis. *Journal of Experimental Botany* 62: 2815–2826.

- Riccini A, Picarella ME, De Angelis F, Mazzucato A. 2021. Bulk RNA-Seq analysis to dissect the regulation of stigma position in tomato. *Plant Molecular Biology* 105: 263–285.
- Rick CM. 1950. Pollination relations of *Lycopersicon esculentum* in native and foreign regions. *Evolution* 4: 110–122.
- Rick CM. 1988. Evolution of mating systems in cultivated plants. In: Gottlieb LD, Jain SK, eds. *Plant evolutionary biology*. Dordrecht, the Netherlands: Springer, 133–147.
- Rick CM, Dempsey WH. 1969. Position of the stigma in relation to fruit setting of the tomato. *Botanical Gazette* 130: 180–186.
- Rick CM, Holle M, Thorp RW. 1978. Rates of cross-pollination in *Lycopersicon pimpinellifolium*: impact of genetic variation in floral characters. *Plant Systematics and Evolution* 129: 31–44.
- Rotino GL, Perri E, Zottini M, Sommer H, Spena A. 1997. Genetic engineering of parthenocarpic plants. *Nature Biotechnology* 15: 1398–1401.
- Saeed A, Hayat K, Ali Khan AA, Iqbal S. 2007. Heat tolerance studies in tomato (*Lycopersicon esculentum* Mill.). *International Journal of Agriculture and Biology* 9: 649–652.
- Sanders PM, Bui AQ, Le BH, Goldberg RB. 2005. Differentiation and degeneration of cells that play a major role in tobacco anther dehiscence. *Sexual Plant Reproduction* 17: 219–241.
- Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to IMAGEJ: 25 years of image analysis. *Nature Methods* 9: 671–675.
- Schubert R, Dobritsch S, Gruber C, Hause G, Athmer B, Schreiber T, Marillonnet S, Okabe Y, Ezura H, Acosta IF *et al.* 2019. Tomato myb21 acts in ovules to mediate jasmonate-regulated fertility. *Plant Cell* 31: 1043–1062.
- Senatore A, Trobacher CP, Greenwood JS. 2009. Ricinosomes predict programmed cell death leading to anther dehiscence in tomato. *Plant Physiology* 149: 775–790.
- Shang L, Song J, Yu H, Wang X, Yu C, Wang Y, Li F, Lu Y, Wang T, Ouyang B *et al.* 2021. A mutation in a C2H2-type zinc finger transcription factor contributed to the transition toward self-pollination in cultivated tomato. *Plant Cell* 33: 3293–3308.
- Shinozaki Y, Hao S, Kojima M, Sakakibara H, Ozeki-Iida Y, Zheng Y, Fei Z, Zhong S, Giovannoni JJ, Rose JKC *et al.* 2015. Ethylene suppresses tomato (*Solanum lycopersicum*) fruit set through modification of gibberellin metabolism. *The Plant Journal* 83: 237–251.
- Shinozaki Y, Nicolas P, Fernandez-Pozo N, Ma Q, Evanich DJ, Shi Y, Xu Y, Zheng Y, Snyder SI, Martin LBB *et al.* 2018. High-resolution spatiotemporal transcriptome mapping of tomato fruit development and ripening. *Nature Communications* 9: 364.
- Sjut V, Bangerth F. 1982. Induced parthenocarpy—a way of changing the levels of endogenous hormones in tomato fruits (*Lycopersicon esculentum* Mill.) 1. Extractable hormones. *Plant Growth Regulation* 1: 243–251.
- Smyth DR. 2022. Carpels. In: *eLS*. Chichester, UK: John Wiley & Sons, Ltd, 1–8.
- Smyth DR, Bowman JL, Meyerowitz EM. 1990. Early flower development in Arabidopsis. *Plant Cell* 2: 755–767.
- Srivastava K, Kumar S, Kumar S, Prakash P, Vaishampayan A. 2012. Screening of tomato genotypes for reproductive characters under high temperature stress conditions. *SABRAO Journal of Breeding and Genetics* 44: 263–276.
- Tabata R, Ikezaki M, Fujibe T, Aida M, Tian CE, Ueno Y, Yamamoto KT, MacHida Y, Nakamura K, Ishiguro S. 2010. Arabidopsis AUXIN RESPONSE FACTOR6 and 8 regulate jasmonic acid biosynthesis and floral organ development via repression of class 1 KNOX genes. *Plant and Cell Physiology* 51: 164–175.
- Tian F, Yang DC, Meng YQ, Jin J, Gao G. 2020. PlantRegMap: charting functional regulatory maps in plants. *Nucleic Acids Research* 48: D1104–D1113.
- Varaud E, Brioudes F, Szécsi J, Leroux J, Brown S, Perrot-Rechenmann C, Bendahmane M. 2011. AUXIN RESPONSE FACTOR8 regulates Arabidopsis petal growth by interacting with the bHLH transcription factor BIGPETALp. *Plant Cell* 23: 973–983.
- Wang H, Jones B, Li Z, Frasse P, Delalande C, Regad F, Chaabouni S, Latché A, Pech JC, Bouzayen M. 2005. The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. *Plant Cell* 17: 2676–2692.
- Weijers D, Wagner D. 2016. Transcriptional responses to the auxin hormone. *Annual Review of Plant Biology* 67: 539–574.
- Wilson ZA, Song J, Taylor B, Yang C. 2011. The final split: the regulation of anther dehiscence. *Journal of Experimental Botany* 62: 1633–1649.
- Wu MF, Tian Q, Reed JW. 2006. Arabidopsis microRNA167 controls patterns of ARF6 and ARF8 expression, and regulates both female and male reproduction. *Development* 133: 4211–4218.
- Yao X, Chen J, Zhou J, Yu H, Ge C, Zhang M, Gao X, Dai X, Yang ZNN, Zhao Y. 2019. An essential role for miRNA167 in maternal control of embryonic and seed development. *Plant Physiology* 180: 453–464.
- Yu Z, Zhang F, Friml J, Ding Z. 2022. Auxin signaling: research advances over the past 30 years. *Journal of Integrative Plant Biology* 64: 371–392.
- Zheng L, Nagpal P, Villarino G, Trinidad B, Bird L, Huang Y, Reed JW. 2019. miR167 limits anther growth to potentiate anther dehiscence. *Development* 146: dev174375.
- Zouine M, Fu Y, Chateigner-Boutin AL, Mila I, Frasse P, Wang H, Audran C, Roustan JP, Bouzayen M. 2014. Characterization of the tomato ARF gene family uncovers a multi-levels post-transcriptional regulation including alternative splicing. *PLoS ONE* 9: e84203.
- Zouine M, Maza E, Djari A, Lauvernier M, Frasse P, Smouni A, Pirrello J, Bouzayen M. 2017. TomExpress, a unified tomato RNA-Seq platform for visualization of expression data, clustering and correlation networks. *The Plant Journal* 92: 727–735.

## Supporting Information

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

**Dataset S1** Raw data for all experiments.

**Fig. S1** *Slmir167* mutants.

**Fig. S2** *SlMIR167* targets are upregulated at specific stages of pistil development in *Slmir167a* mutants.

**Fig. S3** Abnormal inflorescence development in *Slmir167a* mutants.

**Fig. S4** Reduced pistil length of *Slarf8* mutants stems from differences in the style and not the ovary.

**Fig. S5** WT but not *Slmir167a* anthers gradually acquire dehiscence competence during the anthesis day.

**Fig. S6** Serial transverse sections of the distal anther region showing the spatial lignification window and its absence in *Slmir167a*.

**Fig. S7** Slightly reduced pollen-grain viability in *Slmir167a* at anthesis.

**Fig. S8** *Slmir167a* fruits fail to produce seeds, and following manual self-pollination, produce pseudo-seeds.

**Fig. S9** Impaired and delayed fruit set in *Slmir167a* mutants.

**Fig. S10** *Slmir167a* fails to develop normal seeds.

**Fig. S11** Increased fruit set in *Slarf8b* and *Slarf8b Slmir167a*.

**Fig. S12** *Slarf8b Slmir167a* exhibits accelerated fruit growth, with final fruit size similar to WT.

**Fig. S13** Flowering time and fruit accumulation under ambient cold conditions.

**Table S1** Primer list.

**Table S2** Temperature and humidity data recorded on data logger 1 (Cold stress experiment).

**Table S3** Temperature and humidity data recorded on data logger 2 (Cold stress experiment).

**Table S4** Biological and chronological time to first inflorescence and time to first anthesis.

**Table S5** Normal Mendelian segregation of the *Slmir167a* mutation in *Slmir167a*<sup>+/−</sup> F2 progeny.

**Table S6** Manual self-pollination restores the fruit-set timing in *Slmir167a*.

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