

Chr Position; physical position of mutation on the tomato reference genome (SL2.40). Reference (Micro-Tom); Sequence of WT tomato Micro-Tom genome (SL2.40). DP4; Four numbers correspond to number of forward ref alleles, reverse ref, forward non-ref, and reverse non-ref alleles, used in variant calling. Locus, mapped position for *s/sep* mutation. Mutation position; Exon indicates that homozygous indel or SNPs found in *s/sep* mutant are located within the exon region, while intron indicates that the indel or SNPs are located within the intron region. IGR, intergenic region. ITAG name, ITAG gene number defined in SL2.40. Effect of mutation; Missense mutation causes non-synonymous mutation. Frame shift indicates the gap of amino acid transration.

Supplementary Table S2. List of primers

Primer name	Purpose	Forward Primer Sequence	Reverse Primer Sequence	Restriction Enzyme
<i>SISES</i> marker-1	DNA marker (<i>SES</i> mutation)	CGAGTAAAAATGGACAGAAGAAGAA	TGATGAAACAGGAGTTAATTTAGGG	-
<i>SISES</i> marker-2	DNA marker (<i>SES</i> mutation)	ACACCATAGCTAGCCAGACCATGT	ACAATGAGCCACCAGTTGAAGCTC	<i>Bsl</i> I
<i>NPT</i> II marker	DNA marker (<i>NPT</i> II)	ATGATTGAACAAGATGGATTGCAC	TCAGAAGAACTCGTCAAGAAGGCG	-
Whole <i>SISES</i> gene	In-Fusion	GATTACGCCAAGCTTCAATTAGCGATGGACGAAGTICTAGGACGCGTCGACCTCACTTCTAGACTGCCATAAAGC		-

Supplementary Table S3. Homology with SISES

Protein		Identity	Similarity	Gaps
SPL/NZZ	AT4G27330	26.4%	39.2%	33.7%
TIE1	AT4G28840	12.6%	21.3%	60.0%
TIE2	AT2G20080	12.9%	18.4%	67.9%
TIE3	AT1G29010	21.1%	31.8%	36.3%
TIE4	AT2G34010	17.1%	28.3%	38.0%

Each Homology was calculated with The European Bioinformatics Institute.

SPL/NZZ, SPOROCTELESS/NOZZLE

TIE, TCP INTERACTOR CONTAINING EAR MOTIF PROTEIN

Supplementary Table S4. *t*-butyl alcohol series

	<i>t</i> -butyl alcohol	Ethanol	DW
1	10 mL	40 mL	50 mL
2	20 mL	50 mL	30 mL
3	35 mL	50 mL	15 mL
4	55 mL	45 mL	0 mL
5	75 mL	25 mL	0 mL
6	100 mL	0 mL	0 mL

Each solution are composed with *t*-butyl alcohol, ethanol and distilled water (DW).

Supplementary Table S5. List of primers for qRT-PCR

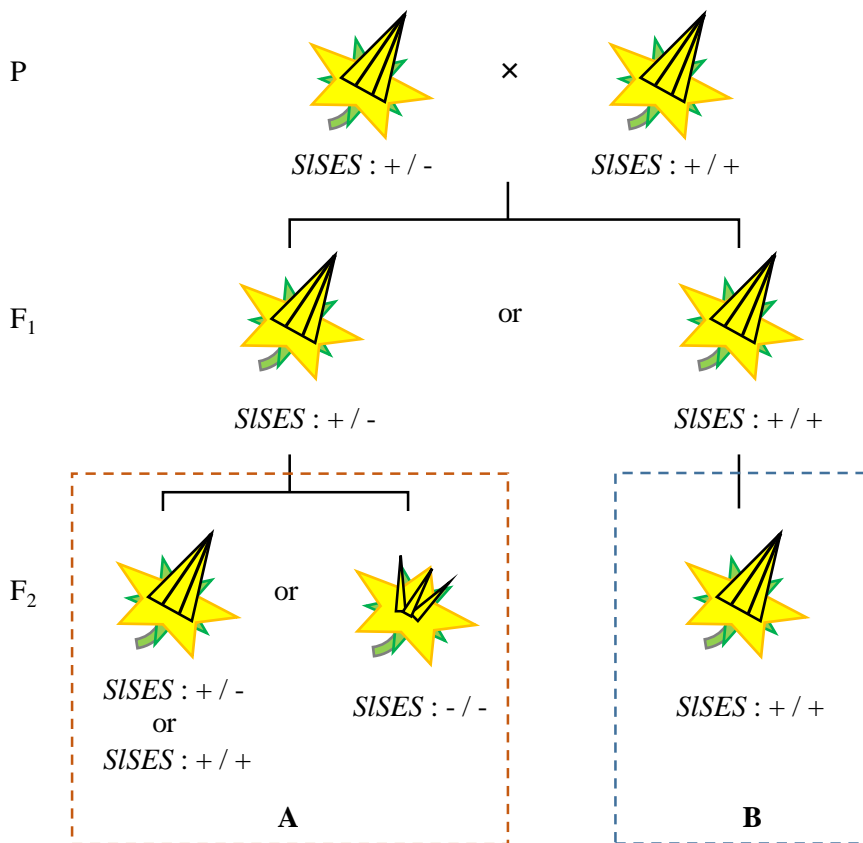
Gene	Solyc ID	Forward Primer Sequence (5'→3')	Reverse Primer Sequence (5'→3')
<i>SISES</i>	<i>Solyc07g063670</i>	AGCCTTTGCTTCACTCATACAGTT	ACTCATCATCGTTGCTTCATTCTC
<i>MS10³⁵</i>	<i>Solyc02g079810</i>	AAATGACACCAGTGCCACTG	AGTAGATTCCGTGGATGATCTCC
<i>BAM1/2-like</i>	<i>Solyc02g091840</i>	AATTCCCAGGGACACAAAAG	ATAAACCCCGAGCCTAGAAAG
<i>EMS1/EXS-like</i>	<i>Solyc09g098420</i>	TTTCGCGAACTGTTCAAGCC	ATTGTTGCCGAGTGAGATCCC
<i>TAG1</i>	<i>Solyc02g071730</i>	GCAGAAGAGGGAAGTTGATTTACAC	GTCTAGGGTAATGGTTGTTGGTTTG
<i>SIWUS</i>	<i>Solyc02g083950</i>	AAGAAGAGGCTCATTGCTGCTG	ACCCCATGTGAAGATGGTGATG
<i>SIINO</i>	<i>Solyc05g005240</i>	TACCCCAACATGACTCACAAGC	TTGCTATCTCTTGGGACCATGG
<i>ANT-like</i>	<i>Solyc04g077490</i>	CTGCTGCCTCATTAGTCTTTGC	AAAGACTGGTAGGTGAGCCATG
<i>PIN1-like</i>	<i>Solyc03g118740</i>	CCCACAACCCATAAACCAAAAAC	GCACAACAGCAGTCATAACATGG
<i>BEL1-like</i>	<i>Solyc08g081400</i>	TGATGGAGACTCATCCTTGGAG	CCAGTTTGGCGAGCTAAAATG
<i>SIREV</i>	<i>Solyc11g069470</i>	ACTCGACATGCTGGAGACAAC	AGATACCACCAGGCAAACACG
<i>CNA-like</i>	<i>Solyc03g120910</i>	GTCTAGTGCCGTTTCAGTTTGG	ACCAGCTCTGTTACTCTGCTTC
<i>PHB/PHV-like</i>	<i>Solyc02g024070</i>	GCTCTCGTTCTCTTGGAGGAG	TCTCCTCTCTGTAAACACAAGGC
<i>GOB</i>	<i>Solyc07g062840</i>	TGCATGAATATCGCCTTGATGG	AGTAGCGGCACCATTAGAACC

SISES, SEXUAL STERILITY; *MS10³⁵*, MALE STERILE 10³⁵; *BAM1/2*, BARELY ANY MERISTEM1 and 2;

EMS1/EXS, EXCESS MICROSPOROCTES1/EXTRA SPOROGENEOUS CELLS; *TAG1*, TOMATO AGAMOUS1; *SIWUS*, WUSCHEL;

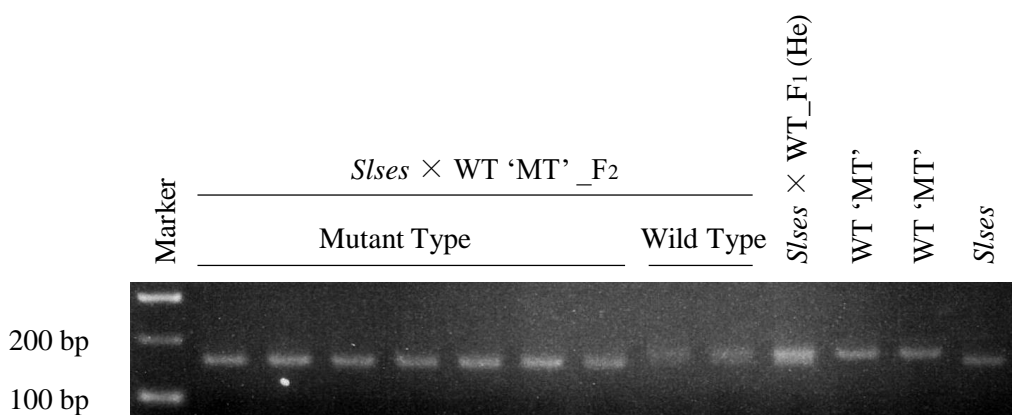
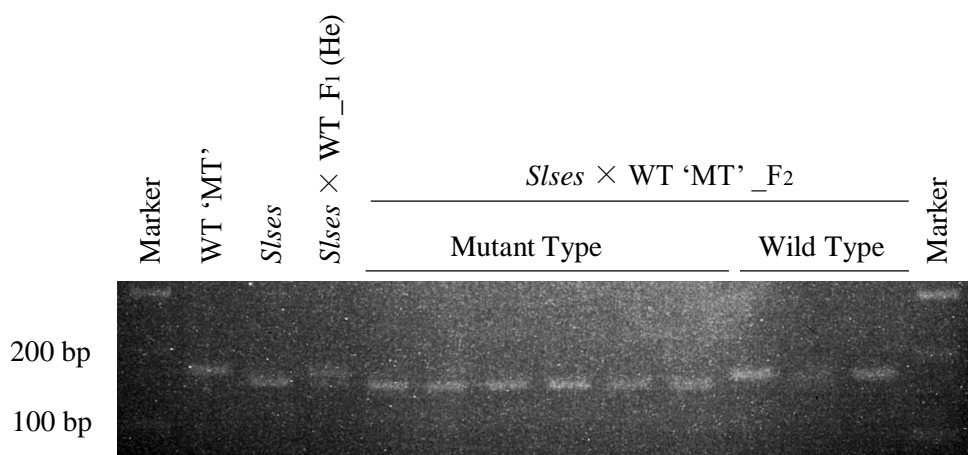
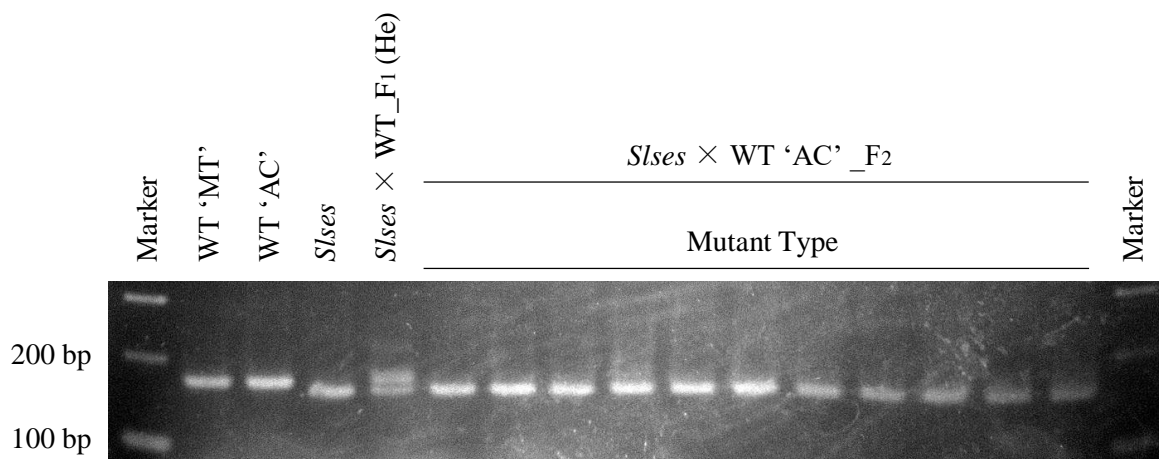
SIINO, INNER NO OUTER; *ANT*, AINTEGUMENTA; *PIN1*, PIN-FORMED1; *BEL1*, BELLI; *SIREV*, REVOLUTA; *CNA*, CORONA;

PHB/PHV, PHABULOSA/PHAVOLUTA; *GOB*, GOBLET



Supplementary Fig. S1. Schematic diagram of crossing analysis.

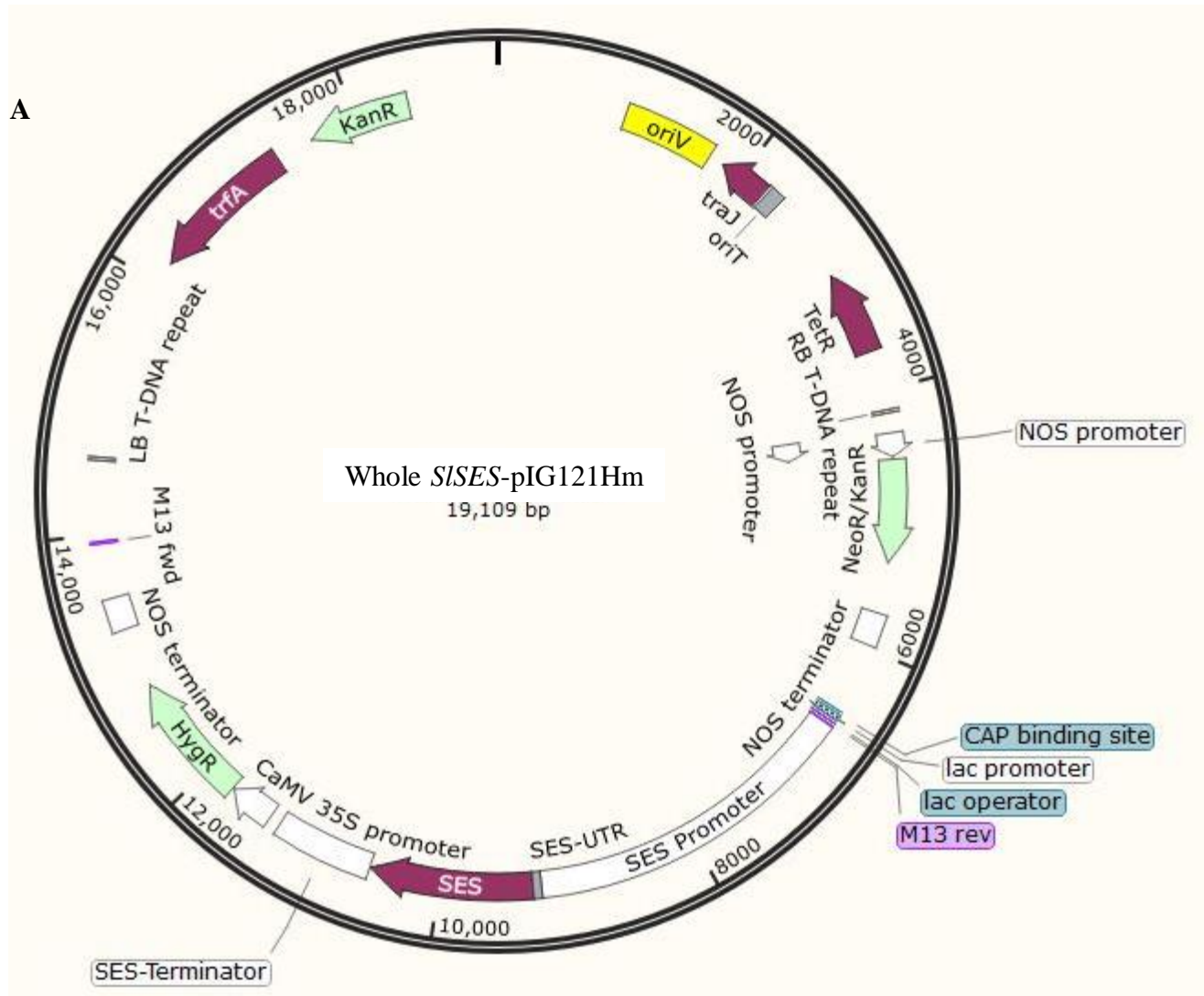
The *SISES* homozygous mutant plants showed sexual sterility, and therefore the *SISES* heterozygous mutant plants were used for crossing. All of F₁ plants showed wild type phenotype, but there were two types of genotypes. One type was the *SISES* heterozygous genotype, and F₂ plants from this genotype were segregated to WT phenotype (left image) and mutant phenotype (right image) (A). Another type was WT genotype, and F₂ plants from this genotype showed WT phenotype (B). + indicates wild type *SISES* and - indicates mutated *SISES*.



Supplementary Fig. S2. Linkage analysis of the 13-bp deletion on *Solyc07g063670* by *SlSES* marker-1.

Micro-Tom WT and Ailsa Craig showed upper band (173-bp), the *slses* mutant showed lower band (160-bp) and the heterozygous showed both bands. All of F₂ plants which showed mutant phenotype had the 13-bp deletion. The F₂ population were constructed from crossed with 'Micro-Tom (MT)' WT or 'Ailsa Craig (AC)'. He, heterozygous.

A

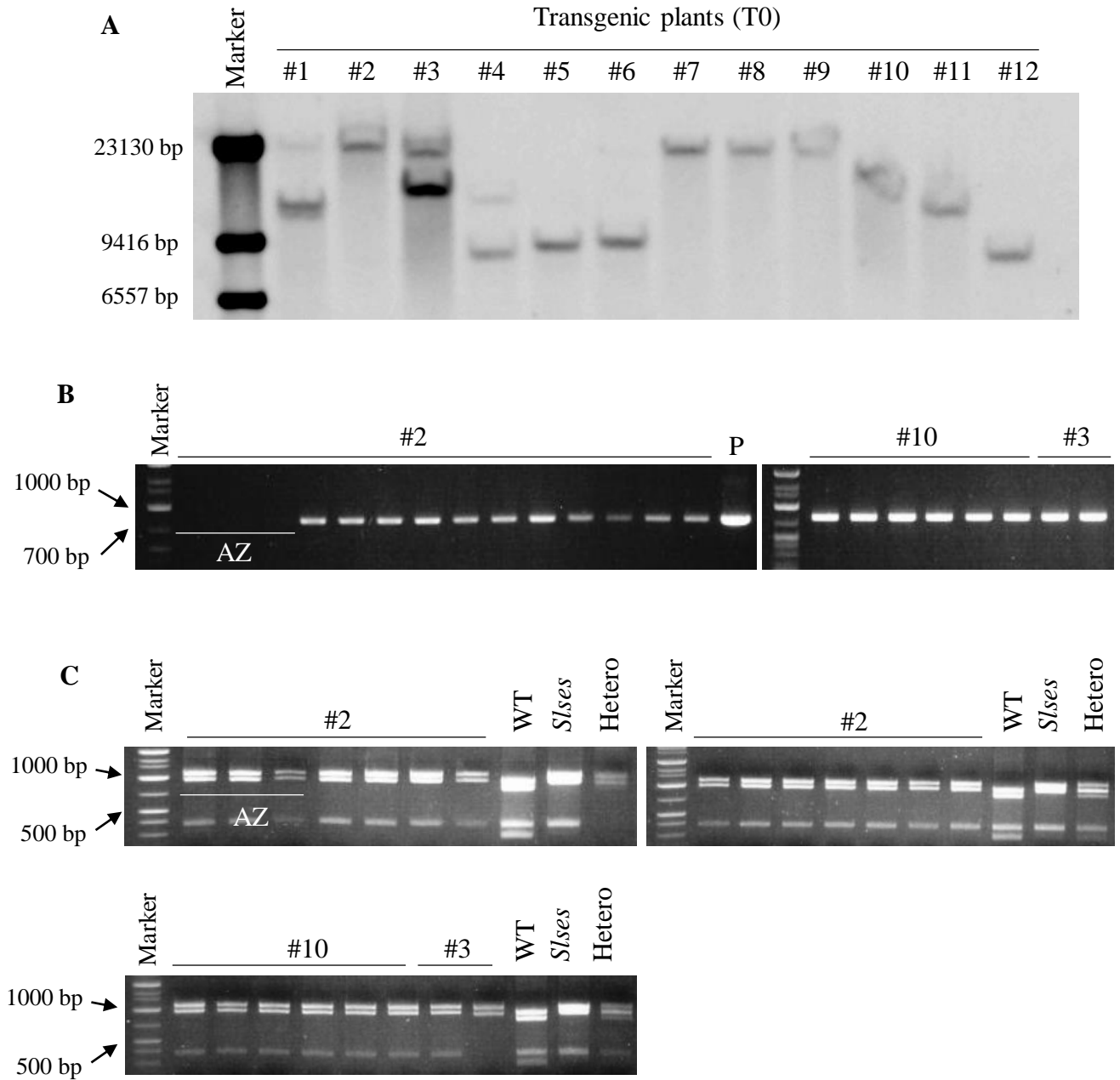


B



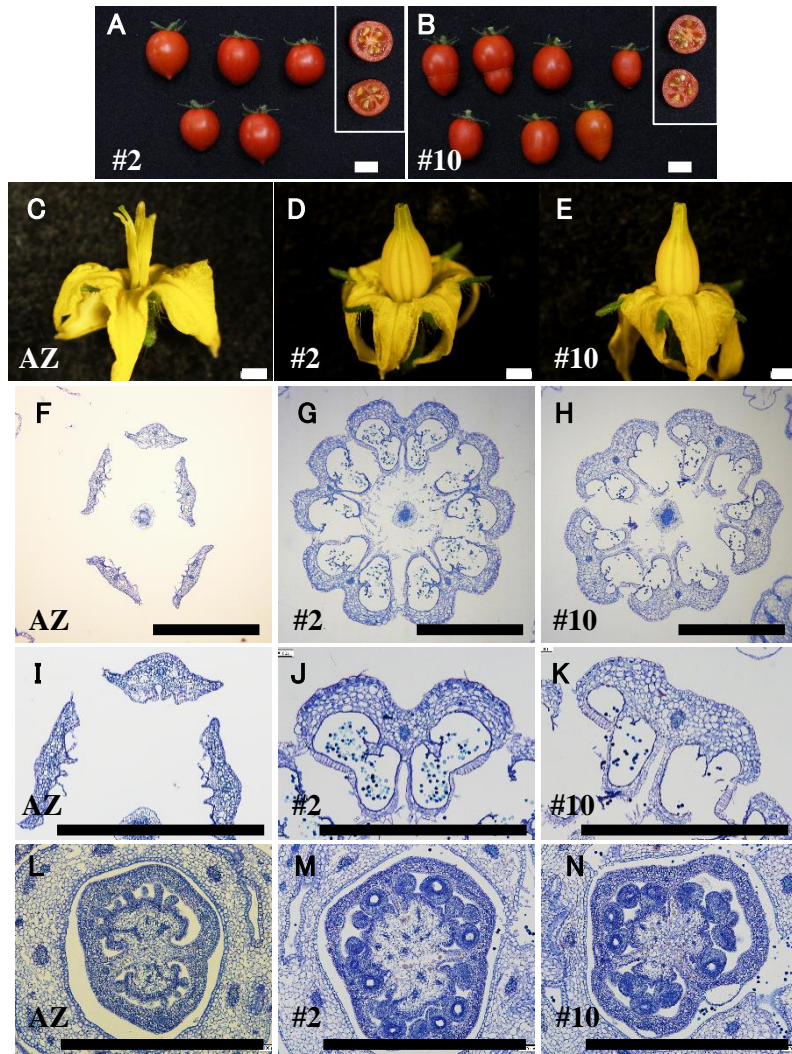
Supplementary Fig. S3. The vector map for the whole *SISES* gene transgenic plant.

(A) Entire vector map constructed from pIG121Hm vector. (B) The transferred region to the plant, from right border (RB) to left border (LB). *NPTII* indicates kanamycin resistance gene. *HPT* indicates hygromycin resistance gene.

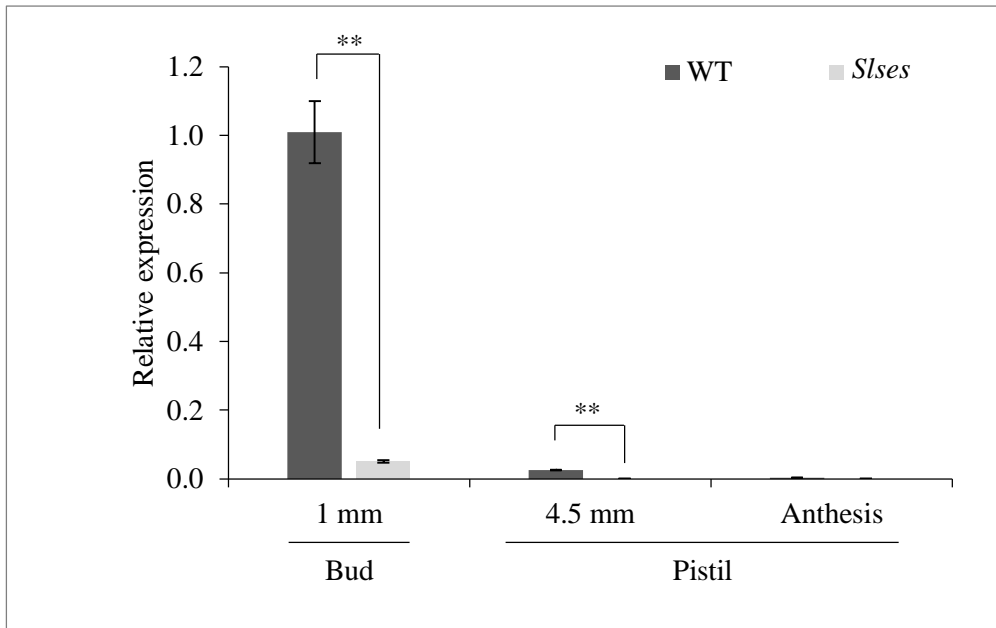


Supplementary Fig. S4. Selection of the plants which not only have transgene but also be the *Sises* homozygous genotype.

(A) Confirmation of copy numbers of transgene in the T0 plants by southern blotting analysis. The probe recognized *NPTII*. (B) *NPTII* existence confirmation in the T1 plants by *NPTII* marker (See supplementary Table S2). (C) The genotype analysis of the T1 plants by *SISES* marker-2 (See supplementary Table S2). #2, #3, #10 indicate line number and P indicates used vector as a positive control. AZ, azygous; Hetero, heterozygous.



Supplementary Fig. S5. Complementation experiment verified *Solyc07g063670* was responsible for the *SlSes* phenotypes. (A, B) The red ripe fruits of the transgenic *SlSes* mutant in which *SISES* whole gene was induced. Bar = 1 cm. (C-E) The anthesis flower. (F-N) The cross section of the flower at anthesis flower, (F-K) the anther and pollen, (L-N) the ovary and ovules. Bar = 1 mm. (A, D, G, J, M) The transgenic line #2, (B, E, H, K, N) the transgenic line #10, (C, F, I, L) azygous (AZ). AZ plant doesn't have transgenes.



Supplementary Fig. S6. The expression analysis of *SISES* in WT and the *Sises* mutant by qRT-PCR.

The *SISES* expression of the *Sises* mutant at 1 mm bud and 2 stages of pistils from 4.5 mm bud and anthesis stages. n = 3, error bar = SE, statistical analysis was realized using the Student's *t*-test, **P<0.01; *P<0.05.