Supporting Information

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flat	row	column	ML14_137K	ML14_138K	ML14_141K	ML14_150K	LAR1	ML14_153K	ML14_158K	ML14_170K	ML14_189K
2	B	7	C	H	H	H	H	H	H	H	H
3	B	12	C	C	C	C	C	H	H	H	H
3	F	1	C	C	C	C	C	C	C	C	H
4	A	6	C	C	C	C	C	C	C	C	H
4	G	11	H	H	H	H	H	H	C	C	C
9	F	1	C	C	C	H	H	H	H	H	H
10	A	2	H	H	H	H	H	H	H	C	C
11	B	9	C	C	C	C	C	C	C	C	H
11	E	12	H	H	H	H	H	H	H	H	C
11	F	4	H	H	H	H	H	H	H	C	C
12	F	1	H	H	H	H	H	H	H	H	C

Fig. S1. Fine-scale recombination mapping that located *LAR1* to the interval between ML14_141K and ML14_153K, which contains only three genes. H: heterozygous for LF10 and CE10; C: homozygous for CE10.

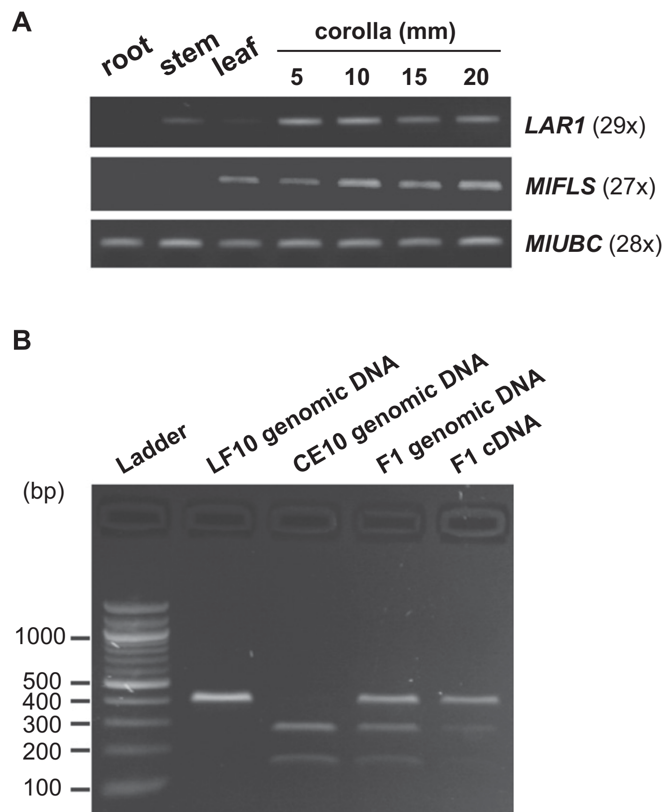

Fig. S2. Gene expression analyses of *LAR1* and *MlFLS*. (*A*) RT-PCR of *LAR1* and *MlFLS* in various tissue types and four stages of corolla development. In this case, whole corollas (including the corolla tube and the petal lobes) were used. *LAR1* expression is relatively high in early developmental stages and starts decreasing after 10-mm. *MlFLS* expression is relatively low in early corolla and becomes strong at 10-mm and later stages. *MlUBC* was used as a reference gene to control for cDNA concentration and quality. *MlFLS* expression is detectable in the leaf but *LAR1* is not, indicating that *MlFLS* may be regulated by other transcription factors in the leaf. (*B*) Assay of relative expression level of the *M. lewisii* (LF10) and *M. cardinalis* (CE10) *LAR1* alleles in the F1 using allele-specific restriction enzyme digestion. A 400-bp fragment in the third exon of *LAR1* was amplified (29 PCR cycles) from the F1 genomic DNA and petal lobe cDNA (10-mm stage) using the primer pair 5′-TCAGGTCCATTACAGGTGACTC-3′ and 5′-TGTTTTCRCATCGAGACCTCAC-3′. The restriction enzyme AluI cuts the CE10 allele into two smaller fragments (270 bp and 130 bp), but does not cut the LF10 allele. The F1 genomic DNA has equal representation of the LF10 allele and the CE10 allele. Note that the smaller sizes of the digested CE10 bands accounts for their lower brightness compared with the undigested LF10 band (second to the last lane). For the F1 cDNA, the CE10 allele is barely detectable (last lane) compared with the LF10 allele, indicating that the latter is preferentially expressed in the F1. The LF10 and CE10 genomic DNA amplicons were used as control for complete restriction digestion.

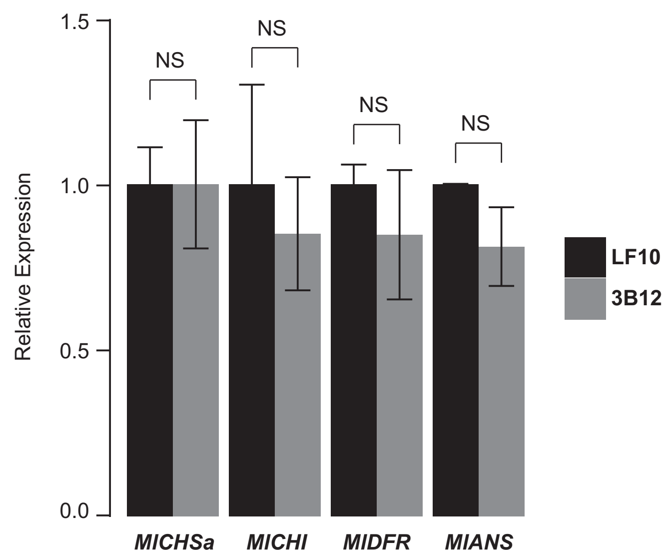

Fig. S3. qRT-PCR of four anthocyanin biosynthetic genes in the petal lobe of 3B12 (the *lar1*/*lar1* NIL) compared with wild-type LF10 at the 10-mm corolla stage. Expression level was standardized to LF10. None of these genes shows significant difference between wild-type and 3B12 NIL (two-tailed *t* test: $P > 0.1$). Error bars represent 1 SD from three biological replicates.

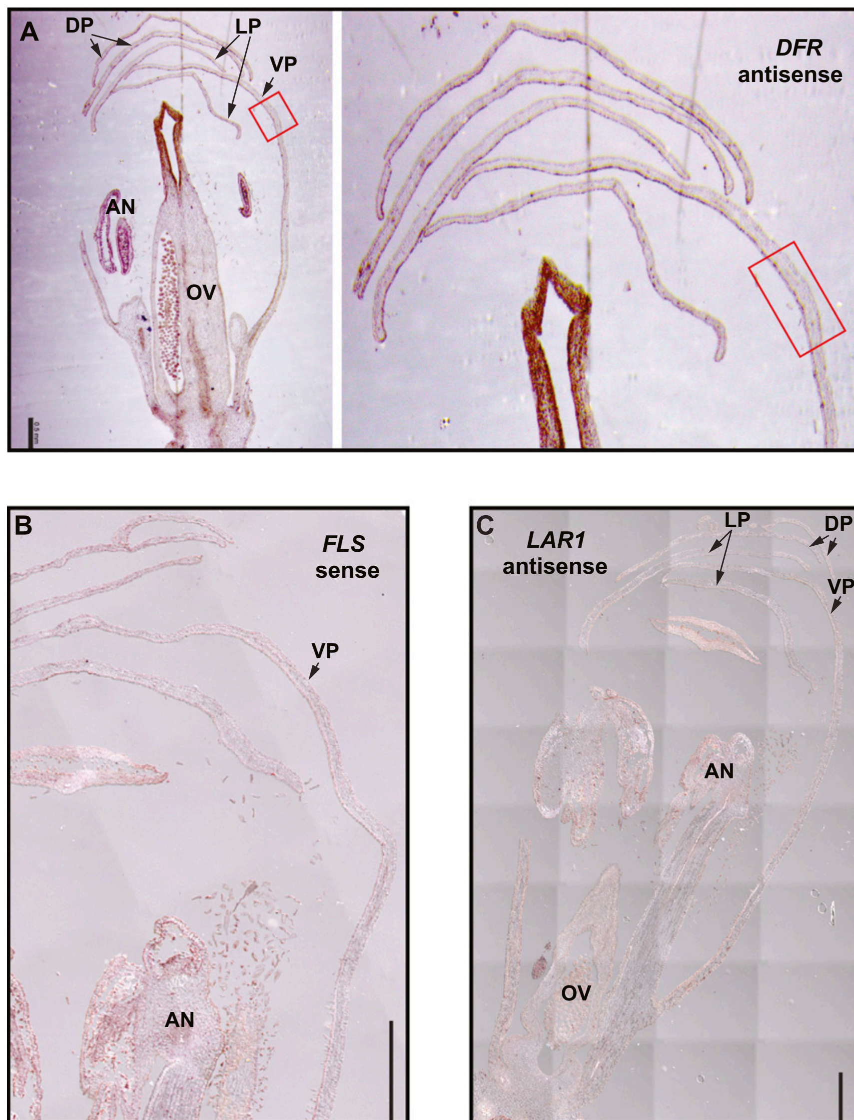

Fig. S4. In situ hybridizations using *DFR* antisense probe (*A*), *FLS* sense probe (*B*), and *LAR1* antisense probe (*C*) at 7-mm corolla stage. Red boxes in *A* mark the light area region (the transition zone between the corolla tube and corolla limb) of the ventral petal. The right panel in *A* is a 3× zoom-in of the light area region. In contrast to the *FLS* expression pattern (Fig. 5*E*), *DFR* expression shows no conspicuous difference between the light areas and the pink areas. AN, anther; DP, dorsal petal; LP, lateral petal; OV, ovary; VP, ventral petal. (Scale bars, 0.5 mm.)

Table S1. Primers used for fine mapping

Primer	Sequence (5′-3′)	LF10 amplicon (bp)	CE10 amplicon (bp)
ML14_137K_F1	GCTTATGCAAGTGGCTGTGTTATC	190	155
ML14_137K_R1	CTTGGCTTCCTCCATCATATTGAC		
ML14_138K_F1	TGGAAGCACCGTGAGTCATGTCATATC	230	270
ML14_138K_R1	GTGTTCTTCAATGTATATGGAGCAGAG		
ML14_141K_F1	CCCAAGATTCCGGCTGATTACGT	190	155
ML14_141K_R1	GTCCCTTTGATTTGCAGGCCGTT		
ML14_150K_F1	CTCATTCGATTGGAACGAGCTC	230	160
ML14_150K_R1	ATGGCTGCTGGAAACCAAGACCAA		
ML14_153K_F1	AGATGTCGGAGCGATATTCAAC	190	220
ML14_153K_R1	ATGGGATACTGGTCGCCGGCGAAGA		
ML14_158K_F1	GGCTTGGACTCTGCAGTCTGT	93	120
ML14_158K_R1	TTCATCGTTGTTTTCCGACGAC		
ML14_170K_F1	AGCGATGAAGTAATAGTAACGAC	340	280
ML14_170K_R1	GCAGTACTGCCACCTGCTACAA		
ML14_189K_F1	ATGAGTGACAGTACTCCGGTCA	440	270
ML14_189K_R1	GACGGAAGATGGGATATTGGTC		

Table S2. Primers used for plasmid constructions

Primer	Sequence (5′-3′)	Fragment size (bp)
*LAR1*_RNAi_F	**GTTCTAGACCATGG**AGGTGACTCCACCACACTCTCTG	193
*LAR1*_RNAi_R	**GTGGATCCGGCGCGCC**ACCCTCGTTTTGCACCACAGTTGT	
*FLS*_RNAi_F	**GTTCTAGACCATGG**GTATCCCAAATGAAGTCATTTCA	343
*FLS*_RNAi_R	**GTGGATCCGGCGCGCC**GCCACCTTCAATTCTTGTTCTT	
*LAR1*_ProF1	CACCTTTGTGAGGACAACAACACTGC	3,157 (promoter:
*LAR1*_cdsR	CGACCAAATGAAGATATCACCTTC	1,483; gene: 1,674)

The sequences in bold contain the restriction sites. The sequence underlined ("CACC") is the 4-bp sequence necessary for pENTR/D-TOPO cloning.

Table S3. RT-PCR primers used in this study

Primer	Forward (5′-3′)	Reverse (5′-3′)
LAR1	TCAACGAGCAAGAATTTAGCAG	CGACCAAATGAAGATATCACCTTC
MlFLS	CTTATCATACACATTGGCGACCA	CAAGACATCCTCGTACTCTCCT
MlCHS	GCACCGTCCTCCGCATGGCCAAG	TGCCCGACGAGACTGTCCAAATG
MlCHI	AAACTTCCCGCCTGGGAGCTCCA	TACACCATGCCTCCCAATGATCGA
MlDFR	TCGAGGATCCCACAGCACAAGGA	TGGCTTCTCTAAACATGTCCTCCA
MlANS	CCCAAGATTCCGGCTGATTACGT	TCGTGTTCTAATCCTAGTCCGGT
MlUBC	GGCTTGGACTCTGCAGTCTGT	TCTTCGGCATGGCAGCAAGTC

Table S4. Primers used for amplifying in situ hybridization probes

Primer	Sequence (5′-3′)	Fragment size (bp)
*MlFLS*_insitu_F	AGGATCGATAAACGAAGCTAG	527
*MlFLS*_insitu_R	CGTTAGGTACGAGGATTGTGA	
*MlDFR*_insitu_F	ATGGATTTCGAATCCGAAGATC	557
*MlDFR*_insitu_R	TCAGGCCATTTGTCTCTGATC	
*LAR1*_insitu_F	AGGTGACTCCACCACACTCTCTG	441
*LAR1*_insitu_R	GTGGTCCCCACAATCCGTATTGTT	