Supplemental data:



Supplemental Figure 1. Phenotypes of tomato fruit on the vine at different stages of ripening and over-ripening. For each stage, 8-12 fruit were checked and representative fruit are shown.





	MG	Breaker	B+1W	B+2W	B+4W	B+6W	B+8W	B+10W
MicroTom	а	ab	ab	b	ab	b	ab	ab
Del/Ros1	а	b	cd	e	de	de	de	c
AtMYB12	а	а	b	d	cd	cd	bc	bc
Indigo	а	а	b	d	d	d	cd	bc

в

Α



Supplemental Figure 2. The high antioxidant capacity of *AtMYB12* tomatoes is not maintained as long as for *Del/Ros1* fruit.

(A) Total antioxidant capacity of hydrophilic compounds in different tomato lines during ripening. Different letters indicate significantly different values at p < 0.05 (one way ANOVA, Tukey post hoc test) within the same genotype.

(**B**) MDA content of different tomato lines during ripening. Error bars show SEM (n=3). Different letters indicate significantly different values at p < 0.05 (one way ANOVA, Tukey post hoc test) within the same genotype.



Supplemental Figure 3. VIGS of anthocyanin biosynthetic genes in *Del/Ros1* tomatoes alters the anthocyanin biosynthetic pathway.

(A). From left to right, phenotype of WT, VIGS-*Del/Ros1*, VIGS-*SlF3H*, VIGS-*SlDFR*, VIGS-*SlANS* and *Del/Ros1* MoneyMaker fruit. Pictures were taken at two weeks after breaker. (B) Expression of anthocyanin biosynthetic genes in VIGS fruit. Data are represented by comparing the expression of silenced sectors to the non-silenced sectors on the same fruit. Error bars show SEM ($n\geq 2$).



Supplemental Figure 4. Silencing of anthocyanin biosynthetic genes in *Del/Ros1* tomatoes causes the accumulation of different flavonoid compounds

(A) Comparative HPLC analysis of methanol extracts of tomato fruit recorded at 525 nm showing the accumulation of anthocyanins in different fruit. (B) Comparative HPLC analysis of methanol extracts of tomato fruit recorded at 280 nm showing the accumulation of phenylpropanoid compounds in different fruit. Compounds identified: 1. Cholorgenic acid, 2. Delphinidin 3-(trans-coumaroyl)-rutinoside-5-glucoside (Del-Cou-Rut-Glc), 3. Petunidin 3-(trans-coumaroyl)-rutinoside-5-glucoside (Pet-Cou-Rut-Glc), 4. Rutin, 5. Malvidin 3-(trans-coumaroyl)-rutinoside-glucoside (Mal-Cou-Rut-Glc), 6. Kaempfero-3-O-rutinoside, 7. Naringin, 8. Myricetin-coumaroyl-rutinoside (Myr-Cou-Rut), 9. Methyl myricetin-coumaroyl-rutinoside (MeMyr-Cou-Rut), 10. Quercetin-coumaroyl-rutinoside (Que-Cou-Rut). 11. Naringenin.

Α	Compound	RT (min)	mass Other masses		Reference
	Del-Glc-Cou-Rut	15.86	919.1	757	Butelli et al. 2008
	Pet-Glc-Cou-Rut	17.26	933.1	771	Butelli et al. 2008
	Rutin	17.83	633	303, 465	Luo et al. 2008
	Kae-3-Rut	19.64	617	287, 449	Luo et al. 2008
	Myr-Cou-Rut	23.53	795	319	This work



Supplemental Figure 5. Compounds identified by LC-MS. (**A**) List of major compounds identified by LC-MS. Abbreviations were used as described in Figure S4. (**B**) MS and UV spectrum for Myr-Cou-Rut.



Supplemental Figure 6. Crossing anthocyanin mutants with *Del/Ros1* tomato generates hybrids containing different flavonoid compounds.

(A) Comparative HPLC analysis of methanol extracts of tomato fruit recorded at 525 nm showing the accumulation of anthocyanins in fruit of different lines.

(**B**) Comparative HPLC analysis of methanol extracts of tomato fruit recorded at 280 nm showing the accumulation of flavonoids and other phenylpropanoid compounds in different fruit. Annotation of compounds is the same as in Supplemental Figure 7.



Supplemental Figure 7. *AtMYB12* MoneyMaker tomatoes are susceptible to *B. cinerea*.

From left to right, WT, *Del/Ros1* and *AtMYB12* MoneyMaker tomatoes infected with *B. cinerea*. White dots represent lesion margins. Fruit were harvested at 7 dpb. Pictures were taken at 3 dpi.



Supplemental Figure 8. Expression of markers for pathogen response in WT and *AtMYB12* tomatoes following *B. cinerea* inoculation.

(A)-(E) RT-qPCR of pathogen response genes before and after *B. cinerea* infection. (A) *Chitinase* (*SlChi*), (B) β -1,3-glucanase (*SlGLU*), (C) *Metacaspase* 7(*SlMCA7*), (D) *Pathogenesis-related protein* 1(*SlPR1*) and (E) *Hypersensitivity-related gene* 203 (*SlHSR203*). (F) The total antioxidant capacity of uninfected and infected fruit. Both wound only and wound + infection fruit were analyzed at 3 dpi. Error bars show the SEM, n=2. * indicates p<0.05 compared to wound only samples.



Supplemental Figure 9. Preparative HPLC to purify anthocyanins from the fruit of delphinidin-producing *Del/Ros1* tomatoes.

(A) Chromatogram of the preparative HPLC of total anthocyanins (535 nm) and other polyphenols (280 nm) run on the Phenomenex[®] 5 μ m Gemini[®] C18 column using a Varian ProStar 210 preparative HPLC.

(**B**) Chromatogram of the preparative HPLC of a single anthocyanin fraction previously separated in (A) and corresponding to delphinidin 3-(coumaroyl)-rutinoside-5-glucoside with a mass of 919 run on the Waters 5 μ m XBridgeTM C18 prep column using a Waters Prep 2000 HPLC system.



Supplemental Figure 10. Effects of flavonoid derivatives added to the culture medium on the growth of *B. cinerea*. 1 mM of different compounds was added to the standard ¹/₄ strength PDB medium containing 5×10^4 spores/mL *B. cinerea* GFP-labeled spores. 64 mg/L Triadiminol was added as a negative control. The fluorescence of each inoculation was measured at t0 and 24 hpi and the ratio (24 hpi v.s. t0) was calculated. Error bars show SEM (n=4). ** (p<0.01) indicates significant difference compared to inoculation only with PDB.



1/4 PDB

+1% MeOH

+1 mg/ml Kanamycin



+1 mM Del-Cou-Rut-Glc +1 mM Pet-Cou-Rut-Glc +1 mM Myr-Cou-Rut



+1 mM Rutin

+1 mM Kae-3-O-Rut

Supplemental Figure 11. Effects of flavonoid derivatives added to the culture medium on the germination of *B. cinerea*. 1 mM of different compounds was added to the standard ¹/₄ strength PDB medium containing spores (5×10^4 spores/mL) of *B. cinerea*. Pictures were taken at 16 hpi.



Α

D

Ε

Myricetin 3-O-Rhamnoside (Myr-Rha)





1 mM Myr-Rha





Myricetin 3-O-(coumaroyl)-rutinoside (Myr-Cou-Rut)





1 mM Myr-Cou-Rut



Supplemental Figure 12. The structure of B-ring is an important factor determining the scavenging ability of different flavonoids.

(A) Structures of purchased myricetin 3-O-rhammanoside (Myr-Rha) and myricetin 3-O-(coumaroyl)-rutinoside (Myr-Cou-Rut) purified from tomato fruit.

(**B**) No significant difference was observed in the scavenging ability between Myr-Rha and Myr-Cou-Rut. Error bars show SEM (n=4). The data for Myr-Cou-Rut are drawn from the data shown in **Figure 8D**.

(C) Both Myr-Rha and Myr-Cou-Rut showed no direct inhibition effect on *B. cinerea* growth *in vitro*. Error bars show SEM (n=4). The data for Myr-Cou-Rut are drawn from the data shown in **Figure 8C**.

(D) Both Myr-Rha and Myr-Cou-Rut showed no direct inhibition on *B. cinerea* germination *in vitro*. The data for Myr-Cou-Rut are drawn from the data shown in Supplemental Figure 9.

(E) Both Myr-Rha and Myr-Cou-Rut supplements to inocula can significantly reduce pathogen susceptibility on WT tomato fruit. ** indicates significant difference between the tested concentration and the PDB control (p<0.01) in Student's T-test. The data for Myr-Cou-Rut are drawn from the data shown in **Figure 8B**.

Compounds (mg/g FW)	WT AtMYB12		Del/Ros1	Indigo
Chlorogenic Acid	0.014 ± 0.002	0.047 ± 0.016	0.007 ± 0.004	0.04 ± 0.005
Kaempferol-3-Rutinoside	0.040 ± 0.001	2.860 ± 0.384	0.052 ± 0.002	0.718 ± 0.026
Rutin	0.047 ± 0.000	1.471 ± 0.124	0.152 ± 0.042	0.312 ± 0.007
Delphinidin-(Coumaroy)- Rutinoside-Glucoside	-	-	1.034 ± 0.299	2.107 ± 0.378
Petunidin-(Coumaroyl)- Rutinoside-Glucoside	-	-	1.188 ± 0.308	2.568 ± 0.458

Table S1. Levels of major phenylpropanoids in WT, AtMYB12, Del/Ros1 and Indigo (AtMYB12x Del/Ros1) MicroTom tomato fruit. Values are means ±SEM of determinations on two samples.

Table S2. Primers used in this research

Gene	ID	Primer Name	Purpose	Seq
P aincrea autings A	Z69264	BcCutA-g-F	aDCD	ATTCCACAATATGGCATGAAATC
Б. cinerea cuinase A		BcCutA-g-R	ЧРСК	ATGTTATCTCCAGCGTGACAAAT
SIACTIN	Solyc11g005330	SlACTIN-g-F	DCD	ACAACTTTCCAACAAGGGAAGAT
		SIACTIN-g-R	фрск	TGTATGTTGCTATTCAGGCTGTG
SIANS	Solyc08g080040	SIANS-attB1	GW Cloning	GGGGACAAGTTTGTACAAAAAGCAGGCTTAGTCCCCAAC CAGAACTAG
		SIANS-attB2		GGGGACCACTTTGTACAAGAAAGCTGGGTATCTTCTCCTTT GGAGGCTC
SIDFR	Solyc02g085020	SIDFR-attB1	GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATTGATTTC ATTAGCATC
		SIDFR-attB2	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTACTGGCCATTT CTGTCGCAC
SIF3H	Solyc02g083860	SIF3H-attB1	GW	GGGGACAAGTTTGTACAAAAAAGCAGGCTTATGGATAGGT GTAACTGAG
		SIF3H-attB2	Cloning	GGGGACCACTTTGTACAAGAAAGCTGGGTAATTATCTTTA GTGGCTTG
Ubiquitin 3	Solyc01g056940	SIUBI-RT-F	RT-qPCR	GCCAAAGAAGATCAAGCACA
*		SIUBI-RT-R	1	TCAGCATTAGGGCACTCCTT
AmDelila	M84913	AmDel-RT-F	RT-qPCR	AGATTACTTGAGAGGGCTTGAGAGG
		AmDel-RT-R		TGGCATCGTGTAGTTTAGTTTTTGT
AmRosea1	DQ275529	AmRos1-RT-F	RT-aPCR	TGGTCGCTGATTGCTGGTAG
		AmRos1-RT-R	1 -	ATCGTTCTCCATCCTCGCCTA
SIPAL	Solyc00g282510	SlPAL-qRT-F	RT-aPCR	AATTGCTTCGAGTCGTGGATAG
		SlPAL-qRT-R	1 -	ACAAGGACTTGTCTCAGCTTCTG
SICHS1	Solyc09g091510	SICHS1-RT-F	RT-aPCR	CCTTTATTTGAACTCGTCTCAGC
5,01,01		SICHS1-RT-R		CAGGAACATCCTTGAGTAAGTGG
SICHI	Solyc05g010320	SICHI-qRT-F	RT-qPCR	TTGTCAACTCGGTCTAATGTGTC
siem		SICHI-qRT-R		TAAAGTGGGACCTTATTGCACAC
SIF3H	Solyc02g083860	SIF3H-RT-F	RT-qPCR	ATGGATGAGCCGATTACATTTG
SIFSE		SIF3H-RT-R		TGGCCTCTTCAGTTTGTATCTTC
SlF3'5'H	Solyc11g066580	SIF3'5'H-RT-F	RT-qPCR	СТСААСGCCACTAAATCTCCCTA
		SIF3'5'H-RT-R		TTGCCCATATGTTGACACTAAGC
SIFLS	Solyc11g013110	SIFLS-RT-F	RT-aPCP	TGTCCCATATCACCCTTCTTGTC
		SIFLS-RT-R		TCACCAATGTGGACAATTATAGCA
SIDFR	Solyc02g085020	SIDFR-qRT-F	RT-qPCR	GACTTGCCGACAGAAGCAAT
		SIDFR-qRT-R		GTGCATTCTCCTTGCCACTT

SIANS	Solyc08g080040	SIANS-qRT-F	RT-qPCR	ACGAACAGGATTTTGCTGCT
		51/11/5-4/(1-1		
Chitinase	Solyc02g082920	SIChitinase-qRT- F	RT-qPCR	GAGGACCTATCCAATTGACACACC
		SIChitinase-qRT- R		CGCAACTAAATCAGGGTTGTTCAC
β -1,3-glucanase	Solyc01g060020	SlGLU-qRT-F	RT-qPCR	GGATCGCGTCAATATAGGAACTT
		SIGLU-qRT-R		TTCCTACAGATCCTCCTCTGTT
Metacaspase 7	Solyc09g098150	SIMCA7-qRT-F	RT-qPCR	AGGAGGTTTATGCCGGTTCAGG
		SIMCA7-qRT-R		TTGGTCTGTTTGGCACCCACTG
Pathogenesis-related protein 1	Solyc09g007010	SlPR1-qRT-F	RT-qPCR	ATACTCAAGTAGTCTGGCGCAAC
		SlPR1-qRT-R	-	CAGTTGCCTACAGGATCGTAGTT
Hypersensitivity- related gene 203	Solyc02g069800	SIHSR203-qRT-F	RT-qPCR	TTCAGTAGACCGGACATGGACTGG
		SIHSR203-qRT-R		AAGTCGTCATGCGGTGGAACAG