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Appendix

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Photoreceptor-induced sinapate synthesis contributes to photoprotection in
Arabidopsis

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Ulm, Roman; Demarsy, Emilie

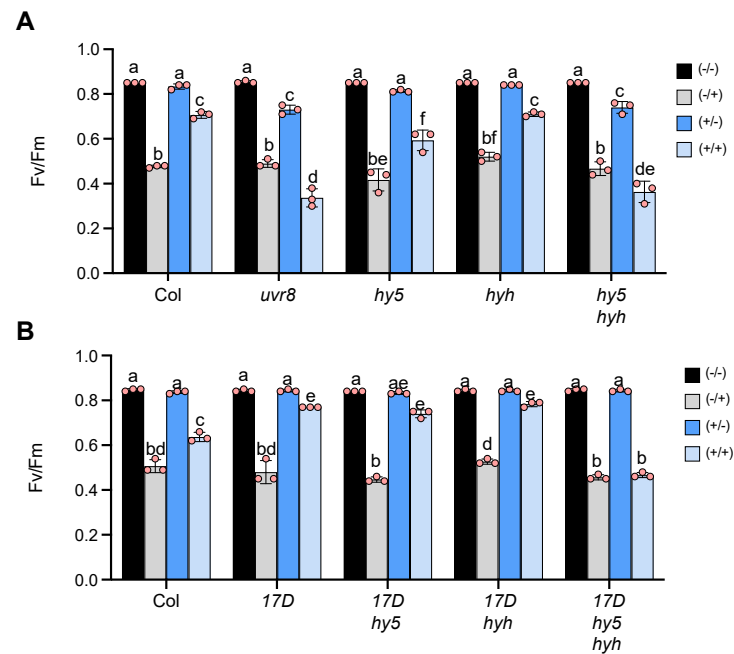
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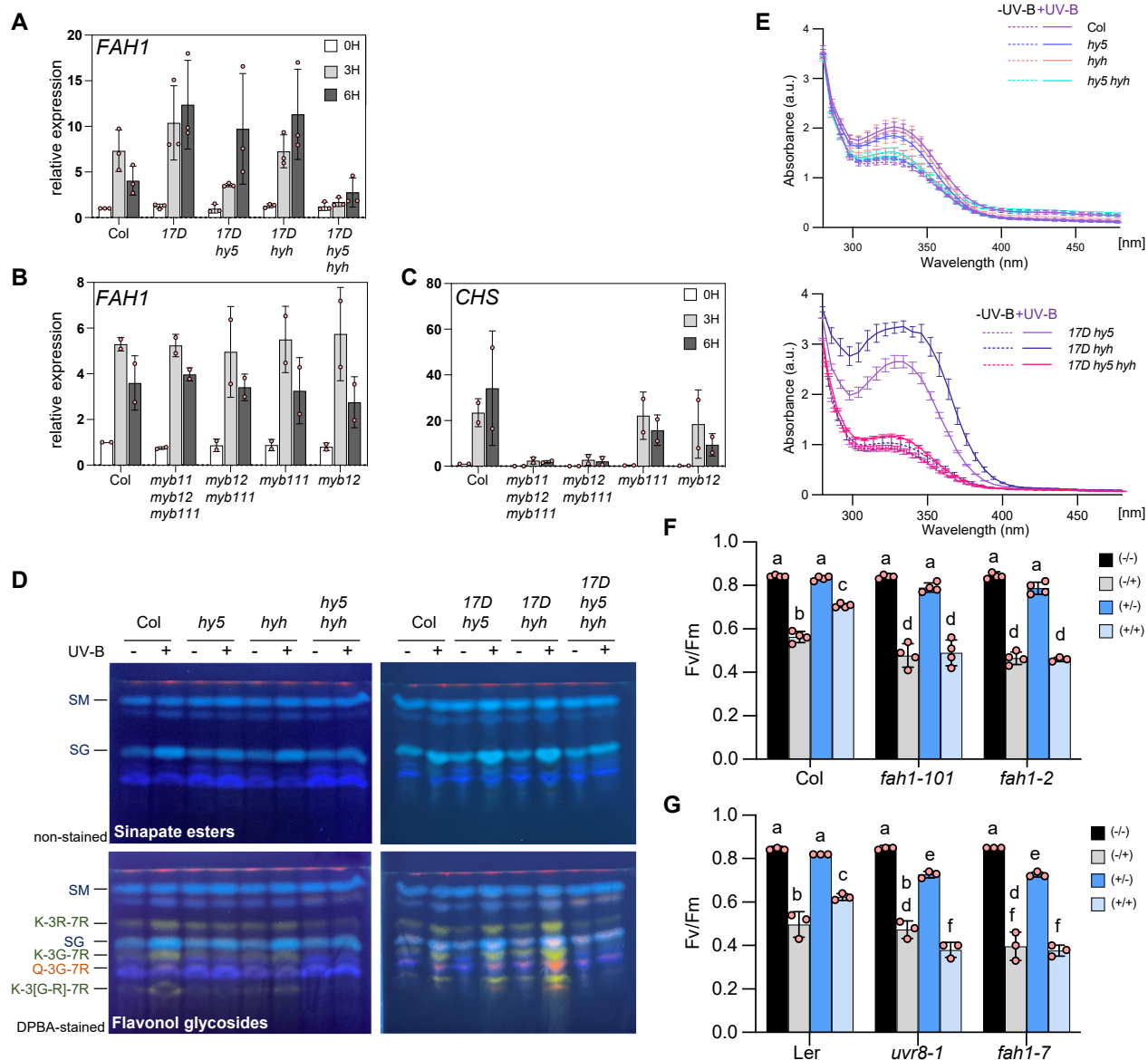
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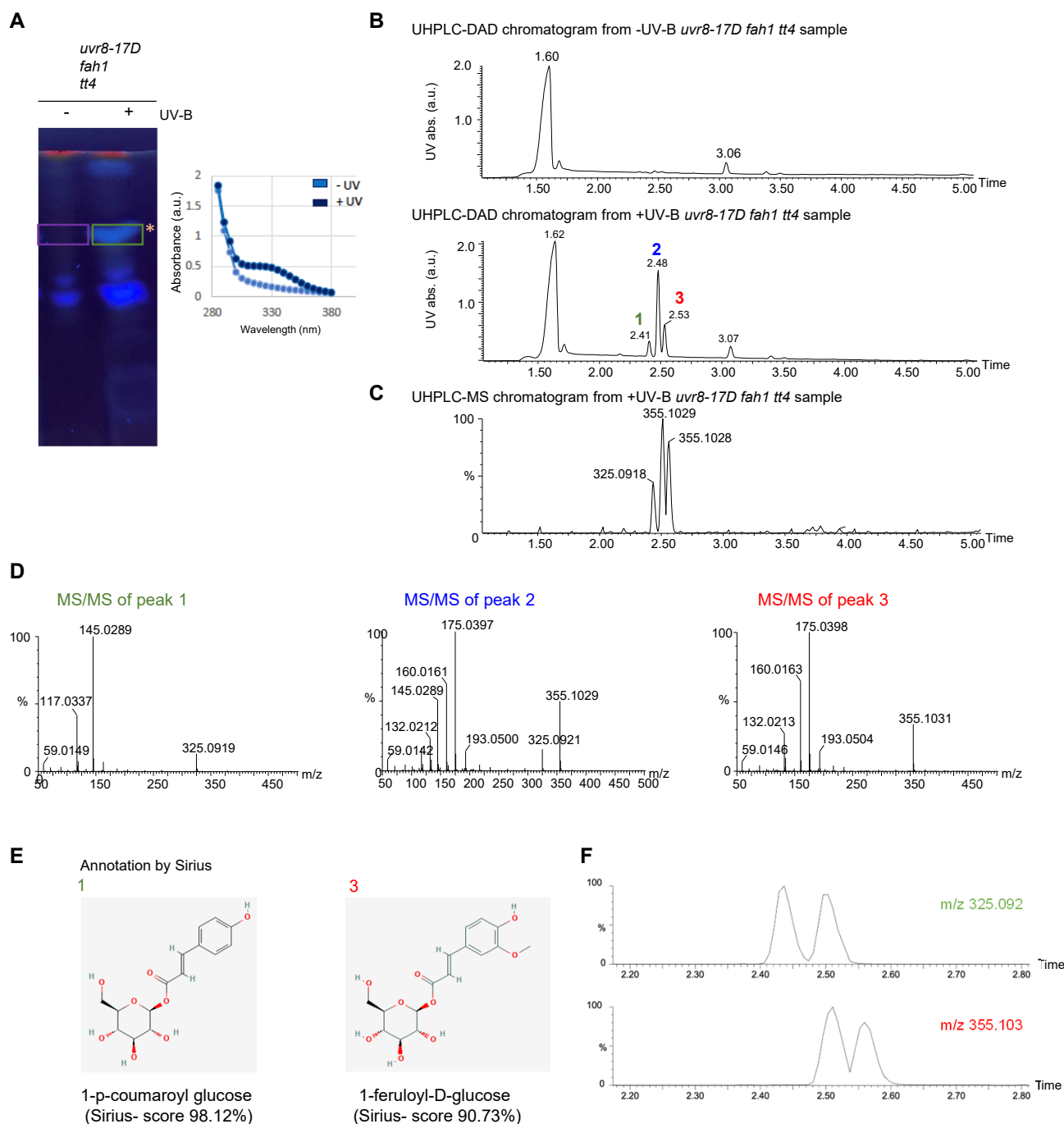
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Supplementary Figure S1. Enhanced photoprotection through UVR8-mediated UV-B acclimation depends on HY5 and HYH.

(A, B) Maximum efficiency of PSII (Fv/Fm) in (A) wild type (Col), *uvr8-6 (uvr8)*, *hy5*, *hyh*, and *hy5 hyh*, and in (B) Col, *uvr8-17D (17D)*, *uvr8-17D hy5 (17D hy5)*, *uvr8-17D hyh (17D hyh)*, and *uvr8-17D hy5 hyh (17D hy5 hyh)* grown as described in Figure 1A. Values of independent experiments (orange points) and means $\pm$ SD are shown ($n = 3$). Shared letters indicate no statistically significant difference between the means ($P > 0.05$), as determined by two-way ANOVAs followed by Tukey's test for multiple comparisons. -/-, not acclimated, not stressed; -/+, not acclimated, stressed; +/-, acclimated, not stressed; +/+, acclimated, stressed.





Supplementary Figure S3. Enhanced UVR8 signaling promotes accumulation of coumaroyl glucose and feruloyl glucose.

(A) (left panel) HPTLC of sinapate esters in 10-d-old *uvr8-17D fah1 tt4* seedlings grown for 7 days in white light ($20 \mu\text{mol m}^{-2} \text{s}^{-1}$) and exposed to supplemental UV-B (0.08 mW cm^{-2}) for 3 days (UV-B: +) or not (UV-B: -). The asterisk (*) indicates the specific metabolite(s) mainly detectable in the *fah1* mutant extracts (see Figure 3C, D); (right panel) Absorption spectra between 280–480 nm of UV-absorbing compounds in methanolic extracts prepared from bands scraped off the silica plate at the indicated positions (-UV corresponds to purple rectangle on HPTLC, +UV corresponds to green rectangle). a.u., arbitrary units.

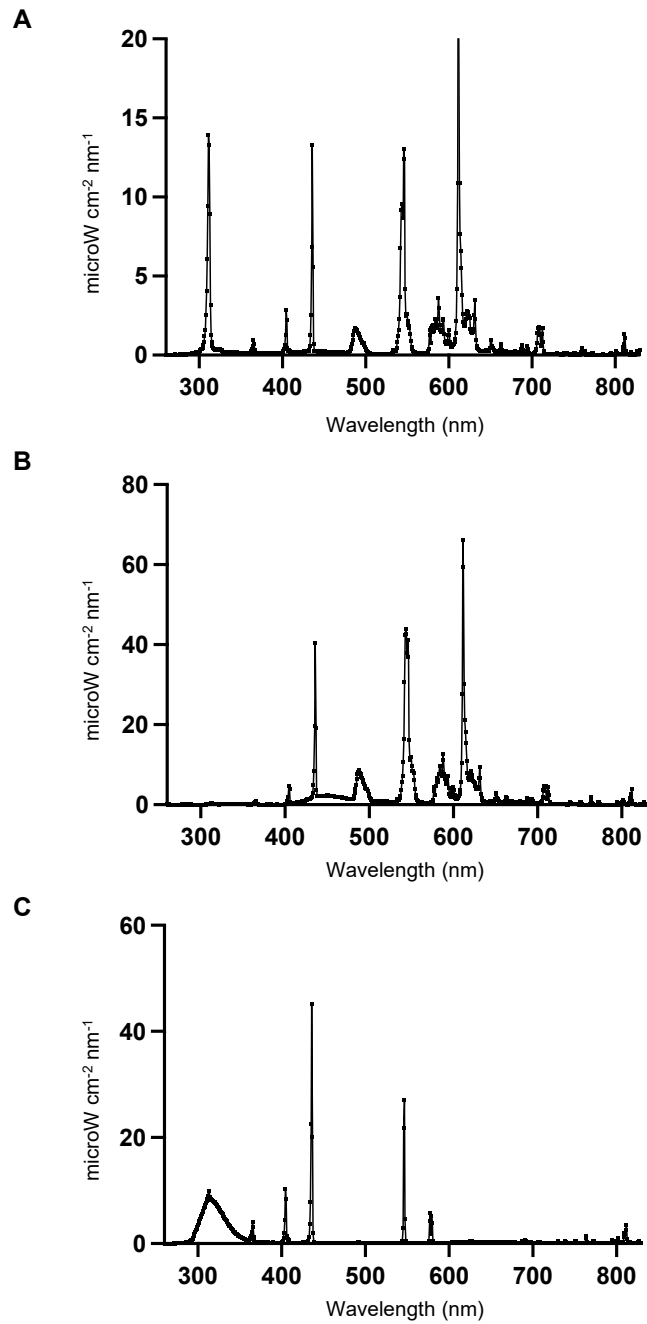
(B) UHPLC-DAD chromatograms of methanolic extracts from silica scraped off from the HPTLC plate region indicated by the purple rectangle in Supplementary Figure S3A (-UV-B) (upper panel) or from the region indicated by the green rectangle (+UV-B) (lower panel). a.u., arbitrary unit. 1 (green), 2 (blue), 3 (red) indicate peaks present in the + UV-B but not -UV-B extracts.

(C) UHPLC-MS chromatogram of methanolic extracts from silica scraped off from the HPTLC plate region indicated by the green rectangle (Supplementary Figure S3A; +UV-B).

(D) UHPLC-MS/MS spectra of peaks 1, 2 and 3 indicated in Supplementary Figure S3B.

(E) Chemical structures of 1-p-coumaroyl glucose and 1-feruloyl-D-glucose, identified with the highest score by the program Sirius as the major compounds in peaks 1 and 3, respectively.

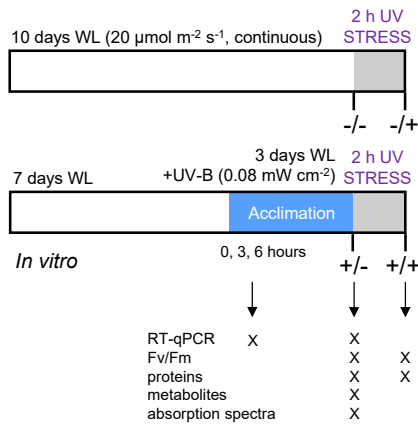
(F) UHPLC-MS extracted ion chromatograms at m/z 325.092 and m/z 355.103 showing the presence of two isomers for both coumaroyl-glucose and feruloyl-glucose. Peak 2 contains a mixture both compounds detected in peaks 1 and 3.



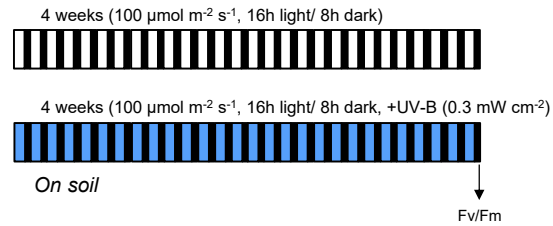
Supplementary Figure S4. Spectra of the different light sources used in the study.

(A) Spectra of the Osram L18W/30 white-light fluorescent tubes ($20 \mu\text{mol m}^{-2} \text{ s}^{-1}$), supplemented with Philips TL20W/01RS narrowband UV-B tubes (0.08 mW cm^{-2}), (B) spectrum of Philips Alto II F17T8/TL841 white-light tubes ($100 \mu\text{mol m}^{-2} \text{ s}^{-1}$), (C) spectrum of the broadband UV-B tubes (Philips TL20W/12RS; 2.2 mW cm^{-2}). (A-C) Spectral irradiance was measured in 0.8 nm intervals using an Ocean Optics QE65000 spectrometer.

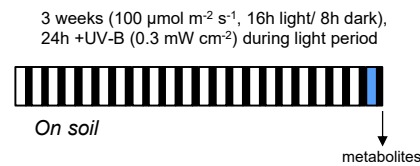
A Figures 1, 2, 3, S1, S2



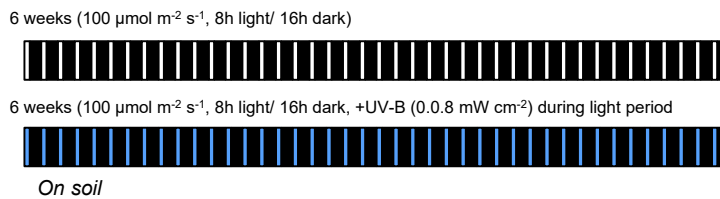
B Figure 4A, B



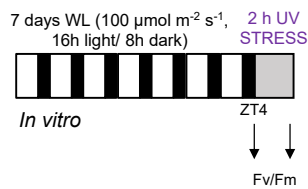
C Figure 4C-H



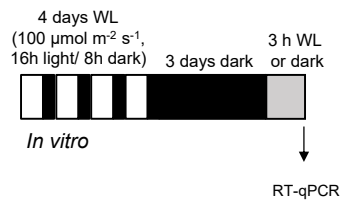
D Figure 5A



E Figure 5B



F Figure 5C



Supplementary Figure S5. Graphical representation of the experimental setups used in this study.

Experimental conditions used to generate the data presented in (A) Figures 1, 2, and 3, and Supplementary Figures S1 and S2, (B, C) in Figure 4, and (D-F) in Figure 5. Black bars and white bars represent the dark and light periods, respectively. Blue Bars represent light period with additional UV-B. Grey bars represent the UV treatment under broadband UV lamps.

Supplementary Table S1. Quantification of hydroxycinnamic acids (HCAs) and flavonol glycosides in 10-day-old seedlings grown in vitro (related to Figure 2).

condition	genotype	Sinapoyl-glucose		Sinapoyl-malate		diSinapoyl-glucose		diSinapoyl-glucose isomer		K-3G-7R		Q-3G-7R		K-3R-7R		K-3[G-R]-7R	
		average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD
- UV	Col	68.0	7.3	2.5	0.6	9.0	1.8	2.5	0.6	2.8	0.5	10.3	1.3	31.5	3.5	16.5	1.3
	<i>uvr8</i>	68.3	5.2	4.3	0.5	8.0	0.8	3.0	0.0	3.3	0.5	12.3	1.0	37.3	2.2	19.5	2.5
	<i>rup1 rup2</i>	64.8	8.2	4.3	0.5	9.3	2.1	3.5	0.6	4.5	0.6	10.0	1.8	32.5	4.2	17.8	2.4
	<i>uvr8-17D</i>	62.3	8.5	5.8	0.5	8.8	0.5	2.5	0.6	5.5	0.6	12.8	1.3	34.8	3.1	17.5	1.7
	<i>hy5</i>	90.3	9.6	2.3	0.5	14.3	1.9	6.0	0.8	0.8	0.5	6.8	1.0	23.0	2.2	17.5	3.1
	<i>hyh</i>	66.5	5.9	4.0	0.8	8.0	1.4	2.0	1.4	4.0	1.2	12.5	3.1	39.0	7.9	15.3	2.8
	<i>hy5 hyh</i>	101.8	7.9	4.5	0.6	10.0	1.2	4.3	1.0	1.3	1.3	8.8	1.3	24.8	4.1	12.3	1.7
	<i>fah1-101</i>	19.0	14.9	0.3	0.5	1.0	1.2	0.5	0.6	2.8	0.5	6.8	1.0	26.3	4.4	10.0	0.8
	<i>tt4</i>	61.0	3.6	4.5	0.6	3.8	0.5	1.5	0.6	0.8	1.0	0.8	1.0	5.3	3.4	2.3	2.2
+ UV	Col	214.3	34.9	6.0	1.4	11.8	2.4	20.0	5.6	17.3	4.9	22.5	7.2	88.5	31.2	29.5	5.1
	<i>uvr8</i>	73.0	9.1	4.8	0.5	7.0	0.8	2.0	0.8	3.5	0.6	13.5	1.7	39.0	3.7	21.0	1.4
	<i>rup1 rup2</i>	556.0	14.6	7.8	0.5	21.0	3.8	56.3	2.1	128.5	13.8	104.8	18.5	367.8	35.8	151.3	17.2
	<i>uvr8-17D</i>	349.5	23.1	9.3	1.3	16.3	1.5	29.3	4.1	58.8	7.8	44.5	11.4	188.0	20.3	69.3	5.5
	<i>hy5</i>	130.3	37.2	4.0	1.4	9.5	2.6	8.0	2.4	1.3	0.5	7.3	1.0	24.8	4.3	17.0	2.2
	<i>hyh</i>	218.5	50.4	7.5	2.4	10.5	2.5	17.0	5.9	18.8	5.1	29.8	6.2	91.3	19.6	32.5	7.2
	<i>hy5 hyh</i>	99.8	6.8	4.3	0.5	8.0	2.2	3.0	0.0	0.3	0.5	6.5	1.3	19.0	2.2	10.3	1.7
	<i>fah1-101</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.8	1.3	19.8	2.1	79.5	7.0	24.5	1.3
	<i>tt4</i>	228.0	35.3	11.5	3.7	6.8	0.5	22.5	7.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Average in µg equivalent of sinapate /g FW for HCA esters, in µg equivalent of rutin /g FW for flavonol glycosides.

SD= Standard Deviation of the mean (n=4)

K-3G-7R, kaempferol-3-O-glucoside-7-O-rhamnoside; Q-3G-7R, quercetin-3-O-glucoside-7-O-rhamnoside; K-3R-7R, kaempferol-3-O-rhamnoside-7-O-rhamnoside; K-3[G-R]-7R, kaempferol 3-O-[rhamnosyl-glucoside]-7-O-rhamnoside

Supplementary Table S2. Quantification of hydroxycinnamic acids (HCAs) and flavonol glycosides in 10-day-old seedlings grown in vitro (related to Figure 3).

condition	genotype	Sinapoyl-glucose		Sinapoyl-malate		diSinapoyl-glucose isomer 1		diSinapoyl-glucose isomer 2		Coumaroyl-glucose isomer 1		Coumaroyl-glucose isomer 2		Feruloyl -glucose isomer 1		Feruloyl -glucose isomer 2		K-3G-7R		Q-3G-7R		K-3R-7R		K-3[G-R]-7R	
		average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD
- UV	Col	49.0	3.5	nd		6.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	1.0	7.0	1.2	29.5	3.4	17.0	2.6
	<i>uvr8</i>	52.5	7.7	nd		7.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5	1.9	14.5	3.4	52.0	8.3	26.0	3.7
	<i>rup1 rup2</i>	60.0	5.9	nd		8.5	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0	1.2	12.5	1.0	39.0	2.6	28.0	4.6
	<i>uvr8-17D</i>	60.0	3.7	nd		10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	1.0	17.0	2.6	60.5	2.5	33.5	1.9
	<i>fah1</i>	0.5	1.0	nd		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	1.6	16.5	2.5	33.5	7.0	17.5	4.4
	<i>tt4</i>	56.0	4.9	nd		4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>uvr8-17D fah1</i>	0.0	0.0	nd		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5	1.0	12.0	1.6	31.5	2.5	23.0	1.2
	<i>uvr8-17D tt4</i>	68.5	3.4	nd		4.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>uvr8-17D fah1 tt4</i>	1.0	1.2	nd		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	1.0	0.0	0.0
+ UV	Col	210.5	9.6	nd		8.0	0.0	10.5	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.5	4.7	35.0	7.7	113.0	21.7	42.0	2.8
	<i>uvr8</i>	64.0	21.4	nd		3.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.5	1.0	16.0	1.6	54.0	8.2	32.5	10.9
	<i>rup1 rup2</i>	605.0	44.0	nd		16.5	3.4	42.5	5.7	5.5	1.0	9.0	1.2	5.0	1.2	2.0	0.0	151.5	16.8	158.5	14.0	484.5	26.1	223.5	20.7
	<i>uvr8-17D</i>	504.5	51.8	nd		15.0	1.2	33.0	4.2	2.0	0.0	3.5	1.9	1.5	1.0	0.0	0.0	105.0	10.6	103.0	14.3	336.5	24.6	136.0	13.9
	<i>fah1</i>	0.0	0.0	nd		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.5	1.0	5.5	1.0	40.0	11.3	45.5	9.7	141.5	30.5	60.0	14.5
	<i>tt4</i>	168.0	27.8	nd		6.5	1.9	9.0	3.8	0.5	1.0	0.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>uvr8-17D fah1</i>	0.0	0.0	nd		0.0	0.0	0.0	0.0	5.0	1.2	8.0	2.8	63.0	12.3	21.5	3.0	100.0	27.9	70.5	18.0	332.5	74.2	144.5	41.5
	<i>uvr8-17D tt4</i>	488.0	48.7	nd		13.5	4.4	46.0	6.3	14.0	3.7	18.5	3.0	9.5	1.9	3.5	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	<i>uvr8-17D fah1 tt4</i>	0.0	0.0	nd		0.0	0.0	0.0	0.0	36.5	4.7	48.0	5.4	240.5	25.3	89.5	5.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Average in µg equivalent of sinapate /g FW for HCA esters, in µg equivalent of rutin /g FW for flavonol glycosides

SD= Standard Deviation of the mean (n=4)

K-3G-7R, kaempferol-3-O-glucoside-7-O-rhamnoside; Q-3G-7R, quercetin-3-O-glucoside-7-O-rhamnoside; K-3R-7R, kaempferol-3-O-rhamnoside-7-O-rhamnoside; K-3[G-R]-7R, kaempferol 3-O-[rhamnosyl-glucoside]-7-O-rhamnoside

Supplementary Table S3. Quantification of hydroxycinnamic acids (HCAs) and flavonol glycosides in soil-grown plants (related to Figure 4).

condition	genotype	Sinapoyl-glucose		Sinapoyl-malate		diSinapoyl-glucose isomer 1		diSinapoyl-glucose isomer 2		Coumaroyl-glucose isomer 1		Coumaroyl-glucose isomer 2		Feruloyl -glucose isomer 1		Feruloyl -glucose isomer 2		K-3G-7R		Q-3G-7R		K-3R-7R		K-3[G-R]-7R	
		average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD
- UV	Col	28.0	15.6	60.8	48.0	38.1	20.2	41.8	17.1	0.1	0.3	0.3	0.3	0.0	0.0	0.1	0.3	30.1	18.1	0.6	0.3	14.6	4.8	47.0	13.0
	uvr8	19.8	4.4	50.1	4.2	19.6	21.6	23.9	19.2	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	19.1	3.8	0.5	0.0	11.5	2.0	39.6	3.3
	uvr8-17D	62.3	11.6	48.8	11.3	33.5	26.0	29.9	15.6	0.1	0.3	0.4	0.3	0.0	0.0	0.0	0.0	88.4	29.7	49.0	27.8	123.8	57.4	110.5	31.7
	rup1 rup2	77.8	11.8	58.1	9.6	39.3	34.0	35.3	20.0	0.4	0.3	0.5	0.0	0.0	0.0	0.0	0.0	155.3	17.9	112.3	14.8	236.1	21.0	171.3	10.5
	tt4	39.3	4.6	63.0	7.6	28.6	27.8	31.7	23.0	0.1	0.3	0.5	0.0	0.1	0.3	0.0	0.0	0.9	0.5	0.9	0.5	2.0	1.1	1.5	0.7
	fah1	0.9	0.5	0.5	0.4	0.6	0.2	0.4	0.2	0.0	0.0	0.0	0.0	0.8	0.3	1.0	0.0	29.0	4.1	1.9	0.8	21.0	3.1	60.6	5.6
	fah1 tt4	0.4	0.3	0.3	0.3	0.3	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.4	0.3	1.0	0.0	0.5	0.0	0.3	0.3	0.6	0.3	1.1	0.6
	uvr8-17D fah1	1.9	3.8	0.6	1.3	1.9	1.4	1.3	0.5	0.1	0.3	0.3	0.5	3.3	1.5	1.4	0.8	69.4	37.9	54.3	25.8	117.8	57.3	92.5	46.7
	uvr8-17D tt4	91.8	27.4	53.8	22.1	48.7	31.9	39.1	14.7	1.5	1.1	1.9	1.2	0.9	0.5	0.3	0.3	1.1	0.9	0.8	0.9	2.0	1.8	1.5	1.4
	uvr8-17D fah1 tt4	1.0	0.7	0.5	0.4	0.7	0.3	0.5	0.2	0.6	0.5	1.3	0.9	10.9	8.4	3.4	2.3	0.5	0.0	0.1	0.3	0.9	0.3	0.6	0.3
	rup1 rup2 fah1	0.4	0.3	0.0	0.0	0.2	0.2	0.1	0.1	0.0	0.0	0.1	0.3	1.9	1.9	1.0	0.4	87.1	23.4	66.6	11.1	155.4	20.1	114.1	24.6
+ UV	Col	99.0	10.7	89.8	16.3	54.0	46.9	51.7	30.1	27.0	4.4	31.4	4.1	6.6	0.6	2.5	0.4	97.4	10.9	92.0	12.3	155.6	13.0	111.1	7.7
	uvr8	19.4	2.1	61.8	6.0	22.3	27.3	29.3	23.5	0.8	0.5	1.1	0.6	0.9	0.8	0.3	0.3	19.4	0.6	2.9	1.1	12.6	0.9	34.9	2.7
	uvr8-17D	121.6	7.9	79.4	6.8	53.9	56.5	49.1	30.5	32.4	6.8	38.6	5.8	9.5	1.8	3.1	0.5	134.8	11.4	134.5	8.8	238.5	9.6	136.3	7.5
	rup1 rup2	120.1	7.9	66.5	6.1	50.2	54.4	44.3	26.4	14.9	3.6	15.1	3.8	6.5	1.1	2.1	0.3	163.3	7.8	130.0	4.7	250.1	11.7	165.6	6.7
	tt4	168.6	14.6	99.4	3.8	71.6	77.5	63.1	41.3	108.9	6.8	124.4	7.6	21.0	2.3	9.5	1.2	3.8	0.3	2.9	0.9	4.4	1.1	2.4	0.5
	fah1	6.0	7.2	2.3	3.2	4.7	2.3	3.1	1.1	13.1	3.6	18.0	4.5	31.4	4.4	9.1	0.6	96.4	14.7	39.9	14.9	112.6	13.7	101.3	8.5
	fah1 tt4	0.5	0.0	0.1	0.3	0.2	0.2	0.2	0.1	27.1	2.8	39.5	4.9	60.9	10.8	20.4	1.7	1.6	0.8	1.9	1.4	3.0	1.8	2.0	0.9
	uvr8-17D fah1	1.5	2.0	0.5	0.4	1.1	0.8	0.7	0.3	38.9	3.6	43.5	3.9	74.0	3.5	16.8	1.4	144.9	8.2	130.3	7.0	227.1	8.6	130.5	3.8
	uvr8-17D tt4	208.9	9.7	89.0	6.8	78.6	94.8	67.3	40.9	163.1	28.8	179.1	26.6	26.0	4.4	11.1	1.8	8.8	5.7	9.4	8.9	15.3	12.8	7.5	6.2
	uvr8-17D fah1 tt4	2.6	1.9	1.3	0.9	1.7	0.8	1.1	0.4	137.1	3.7	159.6	5.0	170.0	6.1	53.8	3.2	2.5	2.1	3.9	3.6	5.9	4.7	3.0	2.5
	rup1 rup2 fah1	2.4	1.9	2.5	3.7	2.6	0.8	2.4	1.2	9.6	1.7	10.6	1.4	52.1	4.9	11.1	1.2	152.0	2.9	114.9	2.9	218.4	7.9	145.3	3.2

Average in µg equivalent of sinapate /g FW for HCA esters, in µg equivalent of rutin /g FW for flavonoids

SD= Standard Deviation of the mean (n=4)

K-3G-7R, kaempferol-3-O-glucoside-7-O-rhamnoside; Q-3G-7R, quercetin-3-O-glucoside-7-O-rhamnoside; K-3R-7R, kaempferol-3-O-rhamnoside-7-O-rhamnoside; K-3[G-R]-7R, kaempferol 3-O-[rhamnosyl-glucoside]-7-O-rhamnoside

Supplementary Table S4: Genotyping information

<i>GENE</i>	<i>mutant</i>	primer 1 (5'-3')	primer 2 (5'-3')	T°C	fragment length (bp)	
					WT	mutant
<i>HYH</i>	<i>hyh</i> (WiscDsLox253D10)	ACCCACACGCTCTGTGAATA	ACTCGCATAAGAACATGTGGG	55	1202	-
		ACCCACACGCTCTGTGAATA	AACGTCCGCAATGTGTTATTAAGTTG	55	-	500-800
<i>FAH1</i>	<i>fah1-101</i> (SAIL_80_C01)	CGTCGATACTAGTGTCTCCG	CTGAGTCATGGGCTTCAGTTC	55	1223	-
		CGTCGATACTAGTGTCTCCG	GCTTCCTATTATATCTTCCCAAATTACCAATACA	55	-	550
<i>TT4</i>	<i>tt4-11</i> (SALK_020583)	CTTCTCTGGACACCAGACAGC	TCGAATAGACCTGTCCAGCAC	55	1061	-
		CTTCTCTGGACACCAGACAGC	ATTTTGCCGATTTCGGAAC	55	-	500-800
<i>UVR8</i>	<i>uvr8-1</i>	ATGGGGCTGGGGAAGA	TAAATTATTGCCGACTCCTACT	55	1210	-
<i>CRY1</i>	<i>hy4-2.23N</i>	CAATTGGAATTTGGTACATTG	GCACGAGATTTGTGGGAAGC	55	524	-