SUPPLEMENTARY MATERIAL

UV regulates expression of phenylpropanoid biosynthesis genes in cucumber (*Cucumis sativus* L.) in an organ and spectrum dependent manner

Minjie Qian, Irina Kalbina, Eva Rosenqvist, Marcel A.K. Jansen, Yuanwen Teng, Åke Strid

Supplementary Table S1. Primers used for qPCR analysis.

Supplementary Table S2. Genes for cucumber phenylanine ammonia lyase (*CsPAL*), cinnamate 4-hydrolylase (*CsC4H*), and chalcone synthase (*CsCHS*) identified from Genbank accession ACHR00000000.

Supplementary Fig. S1. Three repeat experiments showing the expression of the *CsPAL1-12* genes in cucumber under normal greenhouse growth conditions in leaf, stem and root organ.

Supplementary Fig. S2. Three repeat experiments showing the expression of the *CsC4H1-3* genes in cucumber under normal greenhouse growth conditions in leaf, stem and root organ.

Supplementary Fig. S3. Three repeat experiments showing the expression of the *CsCHS1-3* genes in cucumber under normal greenhouse growth conditions in leaf, stem and root organ.

Supplementary Fig. S4. Expression of ten leaf-expressed *CsPAL* genes in cucumber plants in the UV-A- or UV-B-enriched exposure conditions.

Supplementary Fig. S5. Expression of two leaf-expressed genes of each of *CsC4H* and *CsCHS* in cucumber under UV-A- or UV-B-enriched exposure conditions.

Supplementary Fig. S6. Analysis of the 1000 bp promoter regions just up-stream of the 18 different *CsPAL*, *CsC4H*, and *CsCHS* genes for promoter elements.

Supplementary Table S1 Primers used for qPCR analysis.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
CsPAL1	GCTGCAGTCATGGAGTTCCT	TGTGGAGAGGTTCGAAGTGC
CsPAL2	GAGCGTGGTGAAAAGCACAG	GTCGAATTCAACGAATATCCATGC
CsPAL3	CTCTCTGTGCAGTACACTCTT	CCTCATCCAGATGGCTTCCC
CsPAL4	ATCACGGCTTCCGGTGATTT	GATCCAACACCCGTCCCATT
CsPAL5	ACGTCTCATCGGAGGACTGA	TACCATGGCAGCTCTTGTGG
CsPAL6	GGTTGAGGCGAGCAGTAAGT	CTCTTTAGTCCGGCGATGGG
CsPAL7	GAACCAAACAAGGTGGTGCTC	AGAACTTCCATCCAAAACCTGA
CsPAL8	CTCTTCGTGGAACCATTACTGC	GCCATTAACAAGTGCCAACCC
CsPAL9	TGCTTGCCTCTTCGTGGAAC	ATGGCCTTGGAGTTGGGTC
CsPAL10	CCACGTTGTCGATCCACTCA	CACCATCCGCTTCACCTCAT
CsPAL11	GCACTTGCAAATGGCGATGA	ATGGGTGCTTCTCCACTCTC
CsPAL12	TGGCCCTAGTCGAGACCATT	CTACCATGCGCTTCACCTCA
CsC4H1	AATCCTCCAGGTCCTCTCCC	AGCTCTGCATCTGACACCAC
CsC4H2	ATTCGAGAGGAGATTGCCAACG	CAATGGAATTGGGGTGTGCAA
CsC4H3	GACTCTCCGCCTTCGTATGG	TTCCAGTTGGCTGGGTTGTT
CsCHS1	CTCGTAGGCGTGGCACTATT	CCTTCCTCCGACAGTTTCCC
CsCHS2	CTACTTGCCTCTGTGTGCCA	AGCCTTGCGGATTTCAGACA
CsCHS3	CTTGCCAGACACCCAAAACG	AGGGTGAACTGCCCAAAACA
Cs18S	TCTGCCCGTTGCTCTGATG	TCACCCGTCACCACCATAG

Supplementary Table S2 Genes for cucumber phenylalanine ammonia lyase (*CsPAL*), cinnamate 4-hydrolylase (*CsC4H*), and chalcone synthase (*CsCHS*) identified from Genbank accession ACHR00000000. *CsPAL1A* and *CsPAL1B* are alternative splicing variants of the same gene.

Gene name	Gene ID	chromosome	CDS (bp)	peptide (aa)
CsPAL1A	LOC101205510	1	2136	711
CsPAL1B	LOC101205510	1	1779	592
CsPAL2	LOC101203846	4	1779	592
CsPAL3	LOC105434452	4	1941	646
CsPAL4	LOC101218156	6	2142	713
CsPAL5	LOC101206890	6	2142	713
CsPAL6	LOC101217907	6	2145	714
CsPAL7	LOC101218144	6	1677	558
CsPAL8	LOC101218382	6	2127	708
CsPAL9	LOC101218617	6	2142	713
CsPAL10	LOC101222092	6	2154	717
CsPAL11	LOC101218856	6	1842	613
CsPAL12	LOC101219091	6	2118	705
CsC4H1	LOC101205775	2	1608	535
CsC4H2	LOC101205537	2	1581	526
CsC4H3	LOC101221846	6	1518	505
CsCHS1	LOC101210994	1	1188	395
CsCHS2	LOC101205211	3	1203	400
CsCHS3	LOC101203006	4	1170	389

Supplementary Fig. S1 Three repeat experiments showing the expression of the *CsPAL1- 12* genes in cucumber under normal greenhouse growth conditions in leaf, stem and root tissue.



Plant tissues



Supplementary Fig. S2 Three repeat experiments showing the expression of the *CsC4H1- 3* genes in cucumber under normal greenhouse growth conditions in leaf, stem and root tissue.

Plant tissues





Experimental procedure for Supplementary Figs. S4 & S5: UV exposure setups and plant UV treatments

Open top, front and backside Perspex boxes (OTFB boxes) were used for UV irradiation. Perspex was used to cover the top, front and backside of the OTFB boxes for the exposure of the control plants in order to block out all UV. All UV measurements were carried out using a Gooch & Housego (Orlando, Florida) OL756 double monochromator spectroradiometer.

UV-A-enriched radiation (primarily UV-A but including some UV-B) was generated using UVA-340 lamps (Q-Lab, Cleveland, Ohio), which comprised 3.6 W UV-A m⁻². As the UV-A tubes generate a small amount of UV-B, the daily UV-B doses was calculated according to Yu and Björn (*J. Photochem. Photobiol. B Biol*, 1997, **37**, 212–218). 45.5 mW m⁻² plant-weighted UV-B was given during the four-hour UV-A exposure, giving a total irradiation of plant-weighted UV-B of 0.6 kJ m⁻² day⁻¹.

UV-B-enriched irradiation (primarily UV-B but including some UV-A) was obtained using fluorescent lamps (Phillips TL40/12 UV, Eindhoven, The Netherlands) filtered through 0.13 mm cellulose acetate sheets (Nordbergs Tekniska AB, Vallentuna, Sweden) covering the top, front and backside of the OTFB boxes to remove any UV-C radiation. For the calculation of daily UV-B doses, readings were normalized to 300 nm. 83.4 mW m⁻² plant-weighted UV-B was applied for the four-hour exposure giving a total irradiation of plant-weighted UV-B of 1.23 kJ m⁻² day⁻¹. This exposure also contained 0.34 W UV-A m⁻².

Cucumber plants with a first well-developing true leaf were exposed to either UV-Aenriched or UV-B-enriched irradiation and white light as described above. Control plants were exposed to white light in the same chamber as the corresponding UV-treated plants. Supplementary UV-A- or UV-B-enriched irradiation were given for 4 hours per day (from 10:00 h to 14:00 h) and white light was supplied for 16 hours per day (06:00 h to 22:00 h). UV-A- and UV-B-enriched treatments were carried out simultaneously in two separate chambers. In each chamber, three separate areas were used for growth of cucumber. Each separate area contained one UV-treatment OTFB box and one control OTFB box (see above), each of which could fit up to 48 cucumber plants. Plant leaves sampled from one separate area were regarded as one biological replicate, giving a total of three biological replicates for each of the two UV treatments. Plants were exposed to supplementary UV or VIS for 10 days, and the second true leaf was sampled at 14:00 h on day 0 (i.e. 20 h before commencement of the UV treatment), day 1, day 3, day 5, and day 10, for analysis of the effect of the different UV treatments on phenylpropanoid biosynthesis gene expression.

Supplementary Fig. S4. Expression of ten leaf-expressed *CsPAL* genes in cucumber plants in the UV-A- or UV-B-enriched exposure conditions. The expression of *CsPAL2* and *CsPAL7* could not be detected in leaves under the conditions used. Each value in the figures represents the mean \pm standard deviation of three biological replicates.



Supplementary Fig. S5. Expression of two leaf-expressed genes of each of *CsC4H* and *CsCHS* in cucumber under UV-A- or UV-B-enriched exposure conditions.



Days of UV exposure

Supplementary Fig. S6 Analysis of the 1000 bp promoter regions just up-stream of the 18 different *CsPAL*, *CsC4H*, and *CsCHS* genes for promoter elements. The most interesting cis-acting elements (ACE, MRE, and G-box) are denoted with red symbols. The names of the genes with expression kinetics corresponding to UV regulation pattern 2 are denoted in red (*CsPAL4*, *CsPAL10*, and *CsCHS2*).

0	GT1-motif [GGTTAA] 🛱 ch	ns-Unit 1 m1[ACCTACCACAC]	Box 4	[ATTAAT]	ACE [ACGTGG	A or AAAACGTTTA or GACACGTATG]
∇	Box I [TTTCAAA]	Box II [TGGTAATAA]	GAT	A-motif [AAC	GATAAGATT]	I-box [TATTATCTAGA]
•	Sp1 [CC(G/A)CCC]	TCT-motif [TCTTAC]	AE-bo	ox [AGAAAC	TT or AGAAACAA	as-2-box [GATAATGATG]
▼	LAMP-element [CTTTATCA]	GA-motif [ATAGAT.	AA]	MRE [AA	CCTAA]	GAG-motif [AGAGAGT]
۲	P-Box [CAACAAACCCCTT]	ATCT-motif [AATC]	[AATCT]	CATT-m	otif [GCATTC]	X H-box [ACCATTTTCACTC]
V	G-box [CACATGG or CACGT]	or CACGTA or CACGTC or CA	CGTG]	V L-box [.	ATCCCACCTAC]	* MBSII [AAAAGTTAGTTA]

Supplementary Fig. S6 continued p. 2(4)



11

Supplementary Fig. S6 continued p. 3(4)



Supplementary Fig. S6 continued p. 4(4)

