Supplementary Figures

Figure S1 (next page)

Phylogenetic analysis of VviUVR1 and UVR8-related proteins from different plant species. (A) Full-length protein sequence alignment of *Vitis vinifera* UVR1 and its closest homologs from *Arabidopsis thaliana, Thellungiella halophile, Populus trichocarpa, Ricinus communis* and *Physcomitrella patens*. Identical residues are shown in black and conserved residues in dark gray. Regulator of chromosome condensation (RCC1) domains are shown. Tryptophan (W) residues are indicated by asterisks. (B) Neighbor-joining phylogenetic tree of plant UV-B receptor proteins, using UVR8-like proteins from *P. trichocarpa, R. communis, Glycine max, A. thaliana, T. halophile, P. patens, Oryza sativa* and *Brassica rapa. A. thaliana* PHYA and PHYB proteins were used as out-groups. Bootstrap values are indicated at each branch. The scale bar represents 0.1 substitutions per site. GenBank accession numbers: VviUVR1 (AGG82479); AtUVR8 (Q9FN03); ThUVR8 (BAJ33982); PtUVR8 (EEE90389); RcUVR8 (EEF39472); PpUVR8 (EDQ78262); BrUVR8 (ABV89658); GmUVR8 (XP_003526878); OsUVR8 (ABA94217); AtPHYA (AAA21351); AtPHYB (AAR32737). Sequence alignments were performed using MEGA5 software (**Tamura** *et al.***, 2011**) and the MUSCLE algorithm (**Edgar, 2004**). Phylogenetic trees were constructed with the Neighbor-Joining method in MEGA5. The tree nodes were assessed by bootstrap values generated from 2000 retrials.



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Figure S2 (next page)

Phylogenetic relationships between VviHY5, VviHYH and their homologs. (A) Full-length protein sequence alignment with the closest homologs from A. thaliana, Malus domestica, B. rapa, Solanum lycopersicum, Morus notabilis, R. communis and P. trichocarpa. VviHY5 and VviHYH share 36% identity. The two grape proteins possess the conserved bZIP domain, which consists of a basic region mediating sequence-specific DNA-binding followed by a leucine zipper region required for dimerization. Identical residues are shown in black and conserved residues in dark gray. The COP1 interaction site, basic region and leucine zipper region are shown. The COP1-interaction site within motif 1 is preceded by an in vivo functional casein kinase II (CKII) phosphorylation site (Oyama et al., 1997; Hardtke et al., 2000). This sequence is formed by a cluster of 4-5 polar amino acids and is highly conserved in HY5 and HYH relatives. In Arabidopsis, these two sites are required for the physical interaction between AtHY5 and AtCOP1 (Holm et al., 2001), an interaction that negatively regulates AtHY5 activity in the absence of light (Osterlund et al., 2000). (B) Phylogenetic tree of different HY5 and HYH proteins from A. thaliana, M. domestica, B. rapa, S. lycopersicum, M. notabilis, R. communis, P. trichocarpa, G. max, Pisum sativum, O. sativa, Z. mays and V. vinifera. RUP1 and RUP2 proteins from A. thaliana were used as out-groups. Neighbor-joining tree with bootstrap values out of 2000 retrials. Scale bar represents 0.1 substitutions per site. GenBank accession numbers are: VviHY5 (AGX85877); VviHYH (AHX24181); AtHY5 (BAA21327); AtHYH (NP 850605); MdbZIP (BAM71071); BrHY5 (ABY83460); MnHY5 (EXC71566); SIHY5 (NP 001234820); RcHY5 (EEF43212); PtHY5 (EEF00511); GmSTF1 (AAC05017); PsLONG1 (ACP28171); OsHY5 (BAD35451); ZmHY5 (NP 001147637); AtRUP1 (Q9LTJ6); AtRUP2 (Q9FFA7).

۸		CC	OP1 interaction	n site
VvHY5 AtHY5 MdbZIP BrHY5 MnHY5 SIHY5	1 1 1 1	1 MQEQATSSLAAS LESSERSSSALQAEVKE	EIRRVPEIGSO EIRRVPEFG-O EIGRVPEIG-O EIRRVPEFG-O EIRRVPEFG-O EIRRVPEFG-O	; DPAGPSASGR ; EAVGKETSGR ; ESAGASASAS ; EATGKEISGS ; ESGGASASGR
VvHYH AtHYH RcHY5 PtHY5 OsHY5	1 1 1 1	MSLPNPTPSASR& VEQEGSSSSSPWRRHTADSFPPLNIHSDNKHK -WEDDU 1 WSLQR NGNSSSSS WRRHTADSFPPLNIHSDNKHK 1	DLFRVPDVEAC ELLMVPDMEAA DLFTVPDVETF DLFTVPDVEAF IVGRVPEFCLA)PPSDSTRTTT AGSTCVLSSSA }PPASSVVNPT }PPAEAAAAAA ALPGTSTSGRG
		Basic region	eucine zipper.	region
VvHY5 AtHY5 MdbZIP BrHY5 SIHY5 VvHYH AtHYH RcHY5 PtHY5 OsHY5	60 59 59 26 60 73 48 67 100 55	50 EAALVAGPD	$L = \mathbb{R}K \mathbb{N} \\ S = L = \mathbb{R}K \\ S = L = L \\ S = L \\$	LSTLQNENQM LSTLQNENQM LSTLQNENGM LSTLQNENHM LSTLQNENGM LSTLQNENGM (ISTLYNENTM (ISTLINENTM (ISTLINENTM KISTLINENTM KISTLQNENQM
VvHY5 Atthy5 MdbZIP BrHY5 MnHY5 SIHY5 VvHYH AtthyH RcHY5 PtHY5 OsHY5	144 143 141 110 142 160 134 147 199 154	44 LRHILKNTTASRRGGSSNNSNADGSL		
В		⁹⁹ AtHY5 ₆₅ BrHY5		





RT-PCR detection of the *VviUVR1* (A) and *VviHY5* (B) transgenes in Arabidopsis *uvr8-6* and *hy5-215* complemented lines, respectively. *AtACTIN2* was used as a control for cDNA integrity. Wt: wild type Col-0 plants. #: number of independent transgenic line.



Hypocotyl length and flavonol accumulation in *uvr8-6* and *hy5-215* complemented lines under dark conditions. (A-D) Hypocotyl length in Wt (Col-0), *uvr8-6* mutant, *hy5-215* mutant, and *UVR1* or *HY5* complemented lines. (E-F) Total flavonol accumulation in six-day-old seedlings grown under continuous dark. Error bars represent the standard deviation (SD, n=6 plates with 15 seedlings each). Statistical analysis didn't show any difference between genotypes.



Expression profiles of *UVR1, HY5* and *HYH* in grapevine organs of cv. Corvina. The expression profiles of *UVR1, HY5* and *HYH* were assessed in the global *V. vinifera* cv. Corvina (clone 48) gene expression atlas (Nimblegen platform) of different organs at various developmental stages. The fluorescence intensity values of each transcript in all organs were calculated as log2 and normalized, based on the mean expression value of each gene in all organs. The colour scale bar represents expression levels with respect to the mean value. Abbreviations after organ names indicate the developmental stage. FS, fruit set; PFS, post fruit set; V, veraison; MR, mid-ripening; R, ripening; PHWI, post-harvest withering (1st month); PHWII, post-harvest withering (2nd month); PHWIII, post-harvest withering (3rd month), Bud - L, latent bud; Bud - W, winter bud; Bud - S, bud swell; Bud - B, bud burst; Bud - AB, bud after burst; Inflorescence - Y, young inflorescence with single flowers separated; Inflorescence - WD, well developed inflorescence; Flower - FB, flowering begins; Flower - F, flowering; Tendril - Y, young tendril; Tendril - WD, well developed tendril; Tendril - FS, mature tendril; Leaf - Y, young leaf; Leaf - FS, mature leaf; Leaf - S, senescing leaf; Stem - G, green stem; Stem - W, woody stem.



Summary of enriched gene ontology (GO) biological process (BP) terms from *HY5* and *HYH* gene **co-expression networks**. Enrichments, expressed as $-\log 10$ (FDR) for each category are represented in a heatmap of colours in red (with varying intensities) and white, representing enriched and non-enriched categories for each GCN.

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	HY5		HYH	
	Atlas	Stress**	Atlas	Stress**
biological_process				
biosynthetic process				
carbohydrate metabolic process				
cellular component organization				
cellular homeostasis				
cellular process				
cellular protein modification process				
generation of metabolites and energy				
lipid metabolic process				
metabolic process				
multicellular organism development				
nucleobase metabolic process				
photosynthesis				
pollen-pistil interaction				
reproduction				
response to abiotic stimulus				
response to biotic stimulus				
response to external stimulus				
response to stress				
secondary metabolic process				
translation				
transport				

-log10(P-value)										
0	1	2	3	4	5	6	7	8	9	> 10

Genome-wide analysis of predicted HY5 and UV-B response cis-regulatory elements (CRE) in 29,839 random promoters sequences. (A) Distribution plots of HY5 and UV-B response CRE in random simulated promoter sequences. Each bin represents the total motif occurrence in 50 promoter bases, adjusted for the baseline occurrence. The baselines of C-box, C/A-box, C/G-box, E-box, and T/G-box in random simulated promoters are 316, 646, 313, 482, and 238, respectively. (B) Fold-enrichment of grapevine genes containing the CRE (Obs) compared to those identified in random simulated promoters (Exp) based on averages of 100 permutation observations.



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Relationships between grapevine photolyases and cryptochrome related flavoproteins. (A) The light-harvesting cofactor binding domain (cdd: pfam00875) was used in a search for all putative cryptochrome and photolyase flavoproteins in the grapevine 12Xv1 genome prediction. (B) Phylogenetic relationships between Arabidopsis and grapevine CRY-like flavoproteins. The blue-light photoreceptors PHOTOTROPIN 1 and 2 were used as outgroups.

A						
	Gene	Gene model 12X V1	Identities	ld %	Exp	Description
	CRY1	VIT_18s0001g05680	74/166 (160)	44	8e-30,	Cryptochrome 1
	CRY2	VIT_05s0049g00960	64/166 (160)	38	1e-25,	Cryptochrome 2
	FOT6-4	VIT_09s0002g05990	63/147 (160)	42	2e-24,	6-4 photolyase (UVR3-like)
	FOT2	VIT_07s0005g00590	52/128 (160)	40	6e-22,	Deoxyribodipyrimidine photolyase
	CRYD	VIT_04s0008g02670	47/120 (160)	39	8e-17,	Cryptochrome DASH
	FOT3	VIT_16s0022g01340	37/115 (160)	32	5e-11,	Deoxyribodipyrimidine photolyase
	FOT1	VIT_02s0241g00040	44/138 (160)	31	3e-05,	Cyclobutane pyrimidine dimer photolyase (PHR1/UVR2-like)



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Experimental setup of *in-vitro* **plantlets exposed to low fluence UV-B radiation (Cavallini** *et al.*, **2015).** (A) Tubes were covered with cellulose acetate filters that do not remove any UV-B radiation but exclude wavelengths lower than 280 nm. Percentage of PAR (B) and UV-B radiation (C) absorbed by 100 µm clear polyester film (PE film) (gray) used in -UV-B treatment.



Up-regulation of *HY5*, *HYH* and the flavonol related *MYBF1* and *FLS4* genes in UV-B treated grapevine stem cuttings. Stem cuttings with fully developed leaves were transferred to 4% UV-B and 30% UV-A light (18 W, 6500 K) radiation conditions (+). The control corresponds to cuttings not supplemented with UV-B (-). Vertical bars indicate the standard deviations (n=9) and asterisks refer to statistically significant expression changes.



Environmental parameters measured during the UV-B fruit exposure experiments. Temperature, relative humidity and photosynthetically active radiation (PAR) inside the UV-free greenhouse. Measurements taken during the month of January 2013 (A) and on the 15th January (around veraison) from 07.00 h to 23.00 h (B).



HPLC chromatograms of flavonol analysis for the low UV-B treatment at 9 WAV. Peaks were identified by absorbance pattern inspection, as seen in the box for peak No. 4.



Additional References

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research. **32**, 1792–1797.

Hardtke CS, Gohda K, Osterlund MT, Oyama T, Okada K, Deng XW. 2000. HY5 stability and activity in Arabidopsis is regulated by phosphorylation in its COP1 binding domain. The EMBO Journal 19, 4997-5006.

Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of Arabidopsis. Nature **405**, 462-466.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution. 30, 2725–2729.