1 **The photoreceptor UVR8 mediates the perception of both UV-B and UV-A wavelengths up to**

2 **350 nm of sunlight with responsivity moderated by cryptochromes**

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27

35

Abstract

36

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38 The photoreceptors UV RESISTANCE LOCUS 8 (UVR8) and CRYPTOCHROMES 1 and 2

39 (CRYs) play major roles in the perception of UV-B (280–315 nm) and UV-A/blue radiation (315–

40 500 nm), respectively. However, it is poorly understood how they function in sunlight. The roles of

41 UVR8 and CRYs were assessed in a factorial experiment with *Arabidopsis thaliana* wild-type and

42 photoreceptor mutants exposed to sunlight for 6 h or 12 h under five types of filters with cut-offs in

43 UV and blue-light regions. Transcriptome-wide responses triggered by UV-B and UV-A

44 wavelengths shorter than 350 nm (UV-A$_{sw}$) required UVR8 whereas those induced by blue and UV-

45 A wavelengths longer than 350 nm (UV-A$_{lw}$) required CRYs. UVR8 modulated gene expression in

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47 B and UV-A$_{sw}$. These results agree with our estimates of photons absorbed by these photoreceptors

48 in sunlight and with *in vitro* monomerization of UVR8 by wavelengths up to 335 nm. Motif

49 enrichment analysis predicted complex signaling downstream of UVR8 and CRYs. Our results

50 highlight that it is important to use UV waveband definitions specific to plants'

51 photomorphogenesis as is routinely done in the visible region.

52

Keywords

53

54

55 *Arabidopsis thaliana*, blue light, cryptochrome, gene expression, photoreceptor interaction, solar

56 radiation, ultraviolet radiation, UVR8.

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Conflict of Interest

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64 The authors declare no conflict of interests.

65 **Main text file**

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91

92 **1 INTRODUCTION**

93

94 Sunlight regulates plant growth, development and acclimation to the environment, while responses

95 to specific wavelengths are regulated by different photoreceptors. The contribution of different

96 wavelengths of sunlight to plant responses depends both on the optical properties of the

97 photoreceptors and on the spectrum and photon irradiance of the incident radiation. Research under

98 controlled conditions has shown that the photoreceptors UV RESISTANCE LOCUS 8 (UVR8) and

99   CRYPTOCHROMES 1 and 2 (CRYs) play major roles in the perception of UV-B (ground level

100   290–315 nm) and UV-A/blue radiation (315–500 nm), respectively (Ahmad & Cashmore, 1993;

101   Lin, 2000; Yu *et al.*, 2010; Rizzini *et al.*, 2011). However, in sunlight, the irradiances of

102   photosynthetically active radiation (PAR, 400–700 nm) and UV-A (315–400 nm) relative to UV-B

103   are much higher than those normally used in controlled environments making it necessary to assess

104   which wavelengths are effectively perceived by UVR8 and CRYs in sunlight.

105

106   Indoor and outdoor experiments with UV-B irradiances similar to those in sunlight have shown that

107   UVR8 can regulate transcript abundance of hundreds of genes, including those involved in UV

108   protection, photo-repair of UV-B-induced DNA damage, oxidative stress and several transcription

109   factors (TFs) shared with other signaling pathways (Brown *et al.*, 2005; Favory *et al.*, 2009;

110   Morales *et al.*, 2013). The first step in this response is the absorption of UV-B photons by UVR8

111   which triggers a change in conformation from homodimer to monomer enhancing its accumulation

112   in the nucleus (Brown *et al.*, 2005; Kaiserli & Jenkins, 2007; Rizzini *et al.*, 2011). Downstream

113   signaling depends on UVR8 monomers binding to CONSTITUTIVE PHOTOMORPHOGENIC 1

114   (COP1) (Favory *et al.*, 2009), thereby inactivating the E3 ubiquitin ligase activity of COP1. The

115   UVR8-COP1 association stabilizes the TF ELONGATED HYPOCOTYL 5 (HY5), a master

116   regulator of photomorphogenesis in plants (Favory *et al.*, 2009; Huang *et al.*, 2013; Gangappa &

117   Botto, 2016). Both HY5 and HY5 HOMOLOG (HYH) have been identified as key TFs regulating

118   the expression of most genes responding to UV-B through UVR8 signaling (Brown & Jenkins,

119   2008; Favory *et al.*, 2009). Moreover, UVR8 directly interacts with WRKY DNA-BINDING

120   PROTEIN 36 (WRKY36), BRI1-EMS-SUPPRESSOR1 (BES1) and BES1-INTERACTING MYC-

121   LIKE 1 (BIM1) TFs to regulate transcription (Liang *et al.*, 2018; Yang *et al.*, 2018). UV-B radiation

122   induces the expression of genes encoding REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1

123   (RUP1) and RUP2 proteins, which interact with UVR8 directly and convert the active monomers

124   into homodimers, thereby decreasing the abundance of UVR8 monomers through negative feedback

125   (Gruber *et al.*, 2010; Heijde & Ulm, 2013). Because indoor studies are often done using

126   unrealistically high UV-B:PAR and low UV-A:PAR ratios, the participation of UVR8 in the

127   regulation of the transcriptome in response to UV-B, UV-A and blue wavelengths of solar radiation

128   remains uncertain.

129

130   Our understanding of the molecular mechanisms underpinning CRY-mediated responses to blue

131   light is far better than for those to UV-A. In indoor experiments with blue light, CRYs have been

132   found to regulate photomorphogenesis and expression of genes involved in light signaling,

4

133 photosynthetic light reaction, the Calvin cycle, phenylpropanoid metabolic pathway and stress

134 response (Ohgishi *et al.*, 2004; Kleine *et al.*, 2007). Upon absorption of blue light photons, CRYs

135 alter conformation from monomers to homodimers and oligomers (Wang *et al.*, 2016). CRYs

136 homodimers or oligomers interact with COP1 and SUPPRESSOR OF PHY A (SPA) proteins

137 through various mechanisms (Yang *et al.*, 2001; Wang *et al.*, 2001; Lian *et al.*, 2011; Liu *et al.*,

138 2011b; Zuo *et al.*, 2011; Podolec & Ulm, 2018). These interactions stabilize HY5 abundance and

139 consequent transcriptional regulation (Liu *et al.*, 2011a; Yang *et al.*, 2017; Podolec & Ulm, 2018).

140 CRYs also interact directly with TFs such as BES1, BIM1, CRYPTOCHROME-INTERACTING

141 basic helix-loop-helix 1 (CIB1), PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) and PIF5

142 regulating transcription (Liu *et al.*, 2008; Pedmale *et al.*, 2016; Wang *et al.*, 2018). Thus, both

143 UVR8 and CRY signaling share some TFs such as HY5, HYH and BES1 suggesting the possibility

144 of crosstalk. Despite these studies under controlled conditions, the participation of CRYs in the

145 regulation of the transcriptome in sunlight remains poorly understood due to the unrealistic light

146 conditions used.

147

148 As photoreceptors have broad peaks of absorption, their absorption spectra partially overlap. Both

149 CRYs and UVR8 absorb in the UV-B region, while CRYs also absorb strongly at longer

150 wavelengths (Banerjee *et al.*, 2007; Christie *et al.*, 2012; Yang *et al.*, 2015). Given the overlapping

151 spectra of photoreceptors and the fact that plants are simultaneously exposed to all wavelengths of

152 sunlight, only research in sunlight can assess the roles of photoreceptors in nature. The available

153 absorption spectrum for the UVR8 molecule covers the UV-C and UV-B regions, extending only

154 15 nm into the UV-A (Christie *et al.*, 2012) making even speculations about the possible role of

155 UVR8 in the UV-A region uncertain. Furthermore, our previous studies indicated that different

156 regions within UV-A could trigger different responses to metabolite accumulation and transcript

157 abundance of selected genes (Siipola *et al.*, 2015; Rai *et al.*, 2019). Besides, results from an indoor

158 experiment suggested the participation of UVR8 in flavonoid accumulation in response to UV-A

159 from LEDs (Brelsford *et al.*, 2018). However, it is not yet clear which UV-A wavelengths are

160 perceived through UVR8 and which ones through CRYs.

161

162 To assess the roles of UVR8 and CRYs in the perception of solar UV-B, UV-A and blue radiation,

163 we measured transcriptome-wide responses in Arabidopsis plants exposed to sunlight. We also

164 measured the *in vitro* absorption spectrum of UVR8 in the UV-C, UV-B, UV-A and visible regions

165 (250–500 nm), the *in vitro* monomerization of UVR8 by different UV-A wavelengths, and

166 estimated numbers of sunlight photons absorbed by UVR8 and CRYs. We tested four hypotheses:

5

167 1) the perception of solar UV-B and UV-A wavelengths up to 350 nm (UV-A$_{sw}$) is through UVR8,

168 2) the perception of solar UV-A wavelengths above 350 nm (UV-A$_{lw}$) and blue light is through

169 CRYs, 3) crosstalk between UV-B/UV-A$_{sw}$ and UV-A$_{lw}$/blue light signaling is asymmetric in plants

170 exposed to sunlight, and 4) gene expression responses to different wavelengths of sunlight are

171 coordinated by multiple TFs resulting in multiple patterns of expression.

172

173 **2 MATERIALS AND METHODS**

174

175 **2.1 Plant material and treatments**

176

177 *Arabidopsis thaliana* ecotype Landsberg *erecta* (L*er*) and the photoreceptor mutants *uvr8-2* (Brown

178 *et al.*, 2005) and *cry1cry2* (Neff & Chory, 1998) were used. The *uvr8-2* genotype carries a mutation

179 in the C-terminus of the UVR8 protein that impairs signaling in response to UV-B (Cloix *et al.*,

180 2012). The *cry1cry2* genotype carries null mutations for CRY1 and CRY2 protein and consequently

181 is impaired in blue light perception through CRYs (Mazzella *et al.*, 2001). Seeds from different

182 genotypes used in the experiments were previously grown and harvested under the same growth

183 conditions. Seeds were sown in plastic pots (8 cm × 8 cm) containing a 1:1 mixture of peat and

184 vermiculite and kept in darkness at 4 °C for 3 d. Subsequently, the pots were transferred to

185 controlled-environment growth room at 23 °C:19 °C and 70%:90% relative humidity (light:dark)

186 under 12 h photoperiod with 280 µmol m^{-2} s^{-1} white light irradiance, 12 mol day^{-1} (Osram T8 L

187 36W/865 Lumilux). Rosco filter E-color 226 was used to block the small amount of UV-A radiation

188 emitted by the lamps. Four seedlings of the same genotype were transplanted into each plastic pot

189 (8 cm × 8 cm). After transplanting, plants were kept for 14 d in the same growth room and

190 conditions.

191

192 For exposure to sunlight, plants were moved to the field (Viikki campus, University of Helsinki,

193 60°13'N, 25°1'E) on 21 August 2014 between 07:30 and 08:15. Five treatments were created with

194 different plastic sheets (3 mm thick) and a film (0.12 mm thick) used as long-pass optical filters to

195 selectively exclude different wavebands of the UV and blue regions (Figure **1**, Methods S1). The

196 filters were kept 10–15 cm above the top of the plants, on their south and north edges, respectively.

197 Their transmittance was measured with a spectrophotometer (model 8453, Agilent, Waldbronn,

198 Germany, Figure **1**). Treatments were randomly assigned within four blocks (biological replicates).

199 One tray was kept under one filter and there was one filter of each type per block. Each tray

6

200   contained two pots per genotype, positioned at random within the trays, for sampling after 6 h and
201   12 h.

202

203   **2.2 Light conditions and sampling outdoors**

204

205   Hourly solar spectra at ground level were modeled for the 12 h of exposure period using a radiation

206   transfer model (libradtran, Emde *et al.*, 2016) and cloudiness estimates derived from global

207   radiation measurements (Lindfors *et al.*, 2009). Figure S1 shows the solar spectrum at different

208   times of the day when plants were moved outdoors. Figure S2 shows the hourly mean photon

209   irradiance of UV-B (290–315 nm), UV-A$_{sw}$ (315–350 nm), UV-A long-wavelength (UV-A$_{lw}$ 350–

210   400 nm), blue (400–500 nm) and PAR (400–700 nm), for the broader wavebands λ < 350 nm (290–

211   350 nm) and λ > 350 nm (350–500 nm). The hourly solar spectra were convoluted by the spectral

212   transmittance of each filter to estimate the spectrum the plants were exposed to. Then these spectra

213   were convoluted by the *in vitro* spectral absorptance of UVR8 (see Results) or of light-adapted

214   CRY2 (Banerjee *et al.*, 2007) to estimate the relative numbers of photons absorbed by UVR8 and

215   CRY2 in each treatment. Plotting and calculations on the simulated spectra were done in R (R Core

216   Team, 2018, Aphalo, 2015).

217

218   Samples were collected after 6 h and 12 h of exposure to sunlight (13:30–14:15, 19:30–20:20) by

219   block with treatments and genotypes in random order within each block. Each biological sample

220   consisted of leaves from four pooled rosettes from the same pot, which were immediately frozen in

221   liquid nitrogen and later stored at –80 °C. Each pooled sample was ground with mortar and pestle in

222   liquid nitrogen.

223

224   **2.3 RNA sequencing**

225

226   Total RNA was extracted from ground leaf samples with a GeneJET Plant RNA Purification Kit

227   following manufacturer's guidelines (Thermo Fisher Scientific, Vilnius, Lithuania). RNA quality

228   was checked with Agilent 2100 Bioanalyzer (Santa Clara, CA, USA) and RNA concentration was

229   measured with ND-1000 Spectrophotometer (NanoDrop Technologies, Thermo Fisher Scientific,

230   Waltham, MA, USA). For RNA-seq measurements, RNA extracts from two pairs of biological

231   replicates were combined into two pooled replicates. Libraries were constructed using TruSeq

232   Stranded mRNA Sample PrepKit (Illumina, San Diego, CA, USA) following manufacturer's

7

233   instructions. The library concentration was measured using Qubit Fluorometer (Life Technologies,

234   Carlsbad, CA, USA), and quality and size were checked by Fragment Analyzer (Agilent

235   Technologies). Libraries were sequenced on NextSeq 500 (Illumina) generating single end 75 bp

236   reads. RNA-seq raw data was deposited at Gene Expression Omnibus (accession number

237   GSE117199).

238

239   RNA-seq data analysis was done using the JAVA-based client-server system, Chipster (Kallio *et*

240   *al.*, 2011) and in R. The quality of raw reads was checked with FastQC (Andrews, 2014). Removal

241   of adapter sequences, trimming and cropping of the reads were done using Trimmomatic-0.33

242   (Bolger *et al.*, 2014) in single-end mode. The bases with a Phred score < 20 were trimmed from the

243   ends of the reads, and the reads shorter than 30 bases were removed from the analysis (-Phred33,

244   TRAILING:20, MINLEN:30).

245

246   Filtered reads were mapped to the Arabidopsis transcript reference database AtRTD2 (Zhang *et al.*,

247   2017) using Kallisto V-0.43.0 (CMD:quant) (Bray *et al.*, 2016) with 4000 bootstrap sets. The raw

248   count tables for the two pooled replicates were obtained as the mean of the bootstrap runs. Genes

249   with less than five counts in all 21 filter-treatment × genotype combinations were removed. The

250   count tables were analyzed for differential gene expression with edgeR 3.24.3 (Robinson *et al.*,

251   2010). The glmLRT (McCarthy *et al.*, 2012) method was used to fit the statistical model separately

252   to data from each genotype. Differentially expressed genes (DEGs) across treatments and

253   photoreceptor mutants under selected pairwise contrasts were assessed with method

254   decideTestsDGE using Benjamini-Hochberg FDR correction of *P*-values, with FDR ≤ 0.05. In a

255   separate step, $|logFC| > log_2(1.5)$ was used as threshold. Effects of wavebands were assessed by

256   comparing responses between pairs of filters as described above (Figure 1).

257

258   Function plotMDS from package limma 3.38.3 (Ritchie *et al.*, 2015) was used to carry out

259   dimensionality reduction to test for consistency between replicates. To compare RNA-seq and qRT-

260   PCR estimates of transcript abundance, estimates from RNA-seq were re-expressed relative to L*er*

261   UV0 to match qRT-PCR data and major axis regression applied (R package lmodel2 1.7-3,

262   Legendre, 2018).

263

264   Enrichment of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was assessed using

265   function topKEGG (edgeR 3.24.3) using pathway definitions downloaded from http://rest.kegg.jp

266   on 10 April 2019. Pathways whose definition included at least 15 but not more than 250 genes were

267   included in the analysis. The enrichments were tested for all lists of DEGs from the contrast tests

268   described above, and all the genes expressed in our experiment were used as background.

269   Conditions used to assess significance of pathway enrichment were P-value cut-off of 0.01 and at

270   least 1/3 of pathway genes differentially expressed. All pathways fulfilling both conditions in at

271   least one treatment contrast are reported.

272

273   **2.4 Cis-motif enrichment**

274

275   Transcription factor binding motifs were collected in the form of position-specific weight matrices

276   (PSWMs) from JASPAR 2018 (Khan *et al.*, 2018) and Cistrome (O'Malley *et al.*, 2016) databases.

277   In addition to PSWMs we analyzed a previously collected set of binding motifs (Blomster *et al.*,

278   2011). For each contrast within each genotype, the enrichment analyses were run on the lists of

279   upregulated and downregulated genes from RNA-seq analysis. The promoter sequences 1000 base

280   pairs upstream of transcription start sites of all Arabidopsis genes were scanned for binding motifs.

281   The regular expressions were matched in R, and PSWM hits were identified using MEME

282   (Ambrosini *et al.*, 2018) with default thresholds. The significance of the overlap between the gene

283   lists and motif occurrences was then tested with Fisher exact test, followed by FDR correction using

284   Benjamini-Hochberg correction. Based on the motifs enriched in each of the separate lists of up or

285   downregulated genes for the 12 waveband $\times$ genotype combinations, we identified putative TFs

286   which could regulate the expression of those genes. Clustering was done over the obtained lists of

287   TFs, based on the adjusted P-values for enrichment in each contrast and genotype combination. For

288   visualization, the P-values were first restricted to the range of 1 to 10^{-4} by converting all smaller

289   values to 10^{-4}, and subsequently applying a $\log_{10}$ transformation. Clustering and plotting of the

290   heatmaps was done with R package pheatmap 1.0.12 (Kolde, 2019). The cut point at 12 clusters was

291   subjectively chosen for plotting; cutting introduces visual breaks without changing the cluster tree

292   or the heatmap in any other way. The ordering of the tree was done with R package dendsort 0.3.3

293   (Sakai *et al.*, 2014).

294

295   **2.5 Quantitative real-time PCR and data analysis**

296

297   Transcript abundance of selected genes was measured with qRT-PCR from four biological

298   replicates and collected both at 6 h and 12 h. At 6 h the same RNA extracts were used for qRT-PCR

299   as for RNA-seq, but without pooling. The qRT-PCR was done according to Rai *et al.* (2019) using

300   primers listed in Table S1. In every run, normalized expression values were scaled to sample Ler λ

301   > 400 nm, $\log_{10}$ transformed, and exported from qbase^{PLUS} for statistical analyses in R. Linear

302   mixed-effect models with block as a random factor were fitted using function lme from package

303   'nlme' 3.1-137 (Pinheiro $et\ al.$, 2018). Factorial ANOVA was used to assess the significance of the

304   main effects (treatment, genotype and exposure time) and of the interactions (treatment $\times$ genotype,

305   treatment $\times$ exposure time, genotype $\times$ exposure time, and treatment $\times$ genotype $\times$ exposure time).

306   Function fit.contrast from package gmodels 2.18.1 (Warnes $et\ al.$, 2018) was used to fit pairwise

307   contrasts defined $a\ priori$ and P-values adjusted with function p.adjust in R (Holm, 1979). Figures

308   were plotted using R package ggplot2 3.1.0 (Wickham, 2009).

309

310   **2.6 In vitro absorption spectra of UVR8 protein**

311

312   Recombinant UVR8 was produced and isolated from $Escherichia\ coli$ with small variations from

313   the original procedure described by Wu $et\ al.$ (2012). The $E.\ coli$ codon-optimized gene for

314   Arabidopsis UVR8 was introduced into the pET11a expression vector generating a construct

315   carrying an N-terminal 6$\times$His-tag (Genscript). The construct was verified by DNA-sequencing and

316   transformed into the $E.\ coli$ expression host strain BL21. For the recombinant production of

317   functional UVR8, the N-terminal 6$\times$His-tagged UVR8 was overexpressed overnight at 18 °C using

318   0.2 mM β-d-thiogalactopyranoside for induction (Wu $et\ al.$, 2012). Following the overnight

319   induction, cells were harvested by centrifugation and flash-frozen in liquid nitrogen after which cell

320   pellets were stored at -80 °C. Upon purification of recombinant UVR8, cells were lysed by

321   sonication and soluble protein was separated from insoluble fractions by centrifugation. Further

322   isolation of His-tagged UVR8 was accomplished by immobilized metal-affinity chromatography

323   using a two segmented linear gradient of imidazole. Eluted fractions containing UVR8 was further

324   purified to homogeneity using size-exclusion chromatography as verified by SDS-PAGE.

325   Functionality was verified by electrophoresis assay and fluorescence quenching as described by Wu

326    $et\ al.$ (2012). The UV/Vis absorbance spectrum of UVR8 protein was measured by a standard

327   protocol using a spectrophotometer (Shimadzu UV-1800). To improve the signal-to-noise ratio for

328   spectral regions covering both long and short wavelengths, two separate data sets were recorded for

329   two concentrations of UVR8 protein, 57.0 µM and 4.5 µM, respectively. A 25 mM Tris (pH8),

330   150 mM NaCl buffer solution supplemented with 1 mM Beta-mercaptoethanol was used. Protein

331   concentrations were determined using a theoretical absorption coefficient of 91900 $M^{-1}cm^{-1}$ at

332   280 nm as determined by the ProtParam data server available through the SIB Swiss Institute of

333   Bioinformatics (Gasteiger $et\ al.$, 2005).

10

334

2.7 In vitro monomerization of purified UVR8 protein

336

His-tagged *Arabidopsis thaliana* full-length UVR8 was produced in *Nicotiana benthamiana* after *Agrobacterium tumefaciens* transfection using the pEAQ-HT plasmid (Sainsbury *et al.*, 2009) as expression vector. Purification of UVR8 was accomplished using Ni-NTA immobilized metal-affinity and size-exclusion chromatography. Purified UVR8 protein was exposed to UV wavelengths in a 50 µl cuvette using a pulsed Opolette 355+UV tunable laser (Opotek Inc. USA) with a thermostatic cuvette holder at 4 °C, as described in Díaz-Ramos *et al.* (2018), using doses between 1.9 and 6.2 µmol. After exposure, samples were added to 4×SDS sample buffer (250 mM Tris-HCl pH 6.8, 2% (w/v) SDS, 20% (v/v) β-mercaptoethanol, 40% (v/v) glycerol, 0.5% (w/v) bromophenol blue) (O'Hara & Jenkins, 2012) and were subsequently analyzed by SDS-PAGE without boiling (Rizzini *et al.,* 2011). Gels were stained with Coomassie blue to visualize the dimer and monomer bands.

348

3 RESULTS

350

3.1 Gene expression mediated by UVR8 and CRYs after 6 h of sunlight exposure

352

To get full assessment of changes in transcript abundances induced by sunlight, we performed RNA-seq from samples collected after 6 h of exposure of plants to filtered sunlight. RNA-seq libraries from the same genotype and treatment clustered together showing consistency among biological replicates (Figure S3). Furthermore, we validated the expression profiles obtained by RNA-seq with qRT-PCR using 11 genes responsive to UV radiation, and/or blue light, or involved in hormone responses (Table S1). The high positive correlation, $R^2 = 0.85$, between the two methods validates the RNA-seq data (Figure S4).

360

As shown in the Venn diagrams (Figure 2a), in wild type L*er*, out of 3741 DEGs, 2786 responded to blue light (868 UP, 1918 DOWN), 960 to UV-A (406 UP, 554 DOWN) and 653 to UV-B (343 UP, 310 DOWN). Only 101 DEGs responded to all three solar wavebands UV-B, UV-A and blue. Moreover, less than half of the DEGs responding to UV-B were specific, as the remaining ones responded also to UV-A, blue or both. In contrast, of the DEGs responding to UV-A or blue, more than half were specific (Figure 2a).

367

368   In *uvr8-2*, out of 2095 DEGs only 53 (35 UP, 18 DOWN) responded to UV-B (Figure **2b**). The

369   number of DEGs responding to UV-A was only 1/3 of that in L*er* (356 differentially expressed, DE;

370   125 UP, 231 DOWN) while also the number of DEGs responding to blue light was only 2/3 of that

371   in L*er* (1849 DE; 763 UP, 1086 DOWN) (Figure 2a,b). Furthermore, 1/3 of the DEGs responding to

372   blue in *uvr8-2* were unique and not shared by L*er* and *cry1cry2* (Figure S5). Thus, the results

373   confirm our hypothesis that UVR8 plays a role in UV-A perception and also indicate that functional

374   UVR8 modulates gene expression responses to solar blue wavelengths.

375

376   In *cry1cry2,* out of 2948 DEGs only 139 (87 UP, 52 DOWN) responded to solar blue light (Figure

377   2c). Surprisingly for this mutant, out of the 2948 DEGs, 1272 (425 UP, 847 DOWN) still responded

378   to UV-A (Figure 2c). Also, the number of DEGs responding to UV-B increased from 653 in L*er* to

379   2040 (784 UP, 1256 DOWN) in *cry1cry2* (Figure 2a,c).

380

381   To explore the UV-A signaling roles of UVR8 and CRYs in more detail, we next assessed the

382   effects of longer and shorter wavelength regions within UV-A by comparing responses between

383   pairs of filters: UV-A$_{sw}$ (>315 nm vs >350 nm) and UV-A$_{lw}$ (>350 nm vs >400 nm) (Figure 1). In

384   L*er*, out of 190 DEGs, 166 responded to UV-A$_{sw}$ (113 UP, 53 DOWN) and only 26 to UV-A$_{lw}$ (16

385   UP, 10 DOWN), with only 2 DEGs shared between the two treatments (Figure 3a). In *uvr8-2*, out of

386   77 DEGs, 7 (3 UP, 4 DOWN) responded to UV-A$_{sw}$, while 72 (17 UP, 55 DOWN) to UV-A$_{lw}$

387   (Figure 3b). In *cry1cry2*, out of 1057 DEGs, 1050 (340 UP, 710 DOWN) responded to UV-A$_{sw}$ and

388   only 16 (8 UP, 8 DOWN) to UV-A$_{lw}$ (Figure 3c). The number of DEGs responding to UV-A$_{sw}$ in

389   *cry1cry2* was more than six times those responding to UV-A$_{sw}$ in L*er,* a similar but stronger effect

390   to that of UV-B (Figure 3a,c). Furthermore, 3/4 and 9/10 of the DEGs responding to UV-B and UV-

391   A$_{sw}$, respectively, in *cry1cry2* were unique and not shared by L*er* or *uvr8-2* (Figure S5). Here, it

392   should be noted that the individual numbers of DEGs under UV-A$_{sw}$ and UV-A$_{lw}$ add up to a

393   smaller number than the number of DEGs for whole UV-A region (315–400 nm) (cf. Figures 3 and

394   2). This difference mainly arises from statistically comparing pairs of treatments that correspond to

395   smaller (UV-A$_{sw}$ and UV-A$_{lw}$) or larger (whole UV-A) amounts of sunlight attenuation.

396

397   We next tested whether the requirement of UVR8 vs CRYs observed within the UV-A remained

398   valid when including wavelengths in the UV-B and blue bands in the analysis. For this test, DEGs

399   responding to $\lambda < 350$ nm (contrast between >290 nm vs >350 nm) and $\lambda > 350$ nm (contrast

400   between >350 nm vs >500 nm) were quantified (Figure 4). The number of DEGs responding to $\lambda <$

401   350 nm in *uvr8-2* drastically decreased to 1/37 of those in L*er* while the number of those

402 responding in *cry1cry2* increased to 2.7 times of those in L*er*. The number of DEGs responding to λ

403 > 350 nm in *cry1cry2* decreased to 1/35 of those in L*er*, while the number of those responding in

404 *uvr8-2* also decreased but only to 4/5 of those in L*er* (Figure 4). This test indicates that in sunlight

405 functional UVR8 is required for transcriptome-wide response to λ < 350 nm and CRYs are required

406 for those to λ > 350 nm and that functional CRYs antagonize this transcriptome-wide response to λ

407 < 350 nm.

408

409 Our lists of DEGs for the12 waveband-contrast $\times$ genotype combinations were enriched for 45

410 KEGG metabolic pathways in total (Figure S6a-c). The analysis showed that UVR8 mediated the

411 expression of genes involved in all enriched metabolic processes induced by UV-B and UV-A$_{sw}$ in

412 L*er* and *cry1cry2*. Since the flavonoid biosynthesis pathway was still enriched in *uvr8-2* by UV-B,

413 dependence on UVR8 was partial (Figure S6a,b). Expression of genes involved in ribosome

414 biogenesis, protein processing and endocytosis under solar blue required functional UVR8 (Figure

415 S6a,b). CRYs mediated the expression of genes involved in most metabolic pathways regulated by

416 blue light in L*er* and *uvr8-2*, as these responses were missing in *cry1cry2*. The cases where the

417 response to blue light was not fully dependent on CRYs were flavonoid biosynthesis, diterpenoid

418 biosynthesis, phenylalanine metabolism, circadian rhythm, vitamin B6 metabolism,

419 phenylpropanoid biosynthesis. Strikingly, many pathways including photosynthesis, glucosinolates

420 biosynthesis, and plant hormone signal transduction were over-represented in *cry1cry2* compared to

421 L*er* in response to UV-B and UV-A$_{sw}$ (Figure S6a,c).

422

423 **3.2 Photon absorption and monomerization of UVR8**

424

425 To evaluate whether the actions of UVR8 and CRYs at λ < 350 nm and λ > 350 nm are consistent

426 with their absorption spectra, we estimated the relative number of photons absorbed by both

427 photoreceptors given the solar spectral irradiance on the sampling day under each filter (Figure 5).

428 For UVR8 we used a newly measured absorption spectrum extending into the visible region (Figure

429 S7), while for CRYs we used a published absorption spectrum for CRY2 (Banerjee *et al.*, 2007). As

430 a result of the shape of the solar spectrum, the estimates showed that UV-A$_{sw}$ was the band where

431 UVR8 was predicted to absorb most photons. Relative to these maxima, UVR8 was predicted to be

432 the main UV-A$_{sw}$ photoreceptor. In the UV-A$_{lw}$ our estimates showed that both CRYs and UVR8

433 absorbed a large number of photons (Figure **5**). While UVR8 absorbed a considerable number of

434 blue photons, CRY2 absorbed very few UV-B photons.

435

13

436 As monomerization of UVR8 is required for UV-B signaling (Rizzini *et al.*, 2011), we assessed *in*
437 *vitro* if laser radiation of different wavelengths within the UV-B and UV-A bands can monomerize
438 purified UVR8 protein. We found that UVR8 monomerized in response to UV wavelengths in the
439 range 300–335 nm but not in 340–350 nm (Figure 6).

440

441 **3.3 Cis-motif enrichment**

442

443 As enrichment of DNA binding motifs can inform about the putative involvement of TFs in the
444 observed gene-expression responses (McLeay & Bailey, 2010), we assessed *in silico* the enrichment
445 of known *cis*-regulatory elements in the promoter regions of the DEGs from each waveband-
446 genotype combination (Figures 7, S8). The analysis identified 187 putative regulatory TFs whose
447 binding motifs were significantly enriched among the DEGs for at least one waveband-genotype
448 combination (Figures 7, S8). The TFs grouped into 12 distinct clusters based on the similarity of
449 their enrichment patterns across four waveband contrasts and three genotypes (Figure 7). Out of
450 these TFs, 53 were themselves differentially expressed. Clusters A, C, D, F, H and I were
451 homogeneous with respect to TF family, whereas the rest were heterogeneous. Within
452 homogeneous clusters several promoter motifs were enriched (Figures 7, S8).

453

454 In cluster A, several MYB TFs including MYB111 were predicted to regulate the expression of
455 genes with increased transcript abundance in response to UV-B and blue in all genotypes, while in
456 response to UV-A$_{sw}$ significant enrichment was found only in L*er* and *cry1cry2* (cluster A, Figure
457 7). TFs in this group were the only ones enriched for genes responding to UV-B in *uvr8-2*. Out of
458 these seven TFs, four were themselves differentially expressed in response to the treatments.

459

460 HY5, PIF1, PIF3, PIF4, PIF7, BES1 were grouped in cluster B, and were predicted to regulate the
461 expression of genes with increased transcript abundance in response to solar UV-B in L*er* and
462 *cry1cry2* (Figure 7) and of genes with either increased or decreased transcript abundance in
463 response to solar blue in L*er* and *uvr8-2*. Most of these same TFs were enriched for two additional
464 responses only in *cry1cry2*: decreased transcript abundance by UV-B and increased transcript
465 abundance by UV-A$_{sw}$. Out of these 15 TFs, seven, including HY5, PIF1, PIF3, PIF4 and BES1,
466 were themselves differentially expressed. Cluster C which follows a response pattern similar to that
467 of cluster B groups 14 bZIP TFs of which three were differentially expressed.

468

14

469 Eleven members of HD-ZIP TFs including HAT5 grouped in cluster D and were enriched for

470 decreased transcript abundance only in response to blue light in L*er* and *uvr8-2* but in response to

471 UV-B and UV-A$_{sw}$ only in *cry1cry2* (Figure 7). Out of these 11 TFs, four including HAT5 were

472 differentially expressed. Cluster E which follows a response pattern similar to that of cluster D

473 contains TFs from a mix of families of which eight were differentially expressed.

474

475 Five GATA TFs were enriched for DEGs repressed by blue only in L*er* whereas in the other two

476 mutants these TFs were not enriched for any response (cluster F, Figure 7). WRKY TFs were

477 enriched only for DEGs with decreased expression in response to UV-A$_{sw}$ in *cry1cry2* (cluster G).

478 The number of differentially expressed TFs were 1 and 9 in clusters F and G, respectively. The

479 remaining clusters (H-L) showed multiple but poorly defined patterns of enrichment.

480

481 **3.4 Transcript abundance after 6 h and 12 h**

482

483 We used qRT-PCR to determine changes in transcript abundance after 6 h (mid-day) and after 12 h

484 (before sunset) of exposure to filtered solar radiation, allowing the assessment of transcript

485 abundance at two times of the day when solar UV-B:UV-A photon ratio was very different. We

486 tested 11 genes (Figures 8, S9). The three-way interaction, treatment × genotype × exposure time,

487 was significant for four genes: *RUP2* (involved in UVR8 signaling), *CHALCONE SYNTHASE*

488 (*CHS*) and *CHALCONE ISOMERASE* (*CHI*) (flavonoid biosynthesis); and *SOLANESYL*

489 *DIPHOSPHATE SYNTHASE 1* (*SPS1*) (ubiquinone biosynthesis) (Figure 8, Table S2). This

490 indicates that in sunlight the role of UVR8 and CRYs in the regulation of transcript abundance of

491 these genes changed in time. For these four genes across all genotypes, the response to filter

492 treatments at 6 h was stronger than at 12 h.

493

494 Solar UV-B at 6 h increased transcript abundance of *CHI* and *CHS* in all genotypes and of *RUP2* in

495 L*er* and *cry1cry2* but not in *uvr8-2* (Figure 8). This response to UV-B was stronger in *cry1cry2* than

496 in L*er* for all these genes. Solar UV-A$_{sw}$ at 6 h increased transcript abundance of *CHI*, *CHS*, *RUP2*

497 and *SPS1* in L*er* and *cry1cry2* but not in *uvr8-2*. As for UV-B, the transcript abundance response to

498 6 h of UV-A$_{sw}$ was stronger in *cry1cry2* than in L*er* for these four genes. Both solar UV-B and UV-

499 A$_{sw}$ at 12 h increased the abundance of *CHS*, but only in *cry1cry2*. These results support regulation

500 of transcript abundance by UVR8 in both UV-B and UV-A$_{sw}$, antagonized by CRYs.

501

15

502 Solar UV-A$_{lw}$ at both 6 h and 12 h decreased the transcript abundance of *CHS* in *cry1cry2* (Figure

503 8). UV-A$_{lw}$ at 12 h increased the transcript abundance of *RUP2* in L*er* and of *SPS1* in both L*er* and

504 *uvr8-2* but not in *cry1cry2*. Overall, transcript abundance was less responsive to UV-A$_{lw}$ than to

505 UV-A$_{sw}$. Solar blue light at 6 h increased transcript abundance of *CHI*, *CHS* and *RUP2* in all

506 genotypes whereas of *SPS1* in L*er* and *uvr8-2* but not in *cry1cry2*. Blue light at 12 h increased the

507 abundance of *CHI* and *RUP2* only in L*er* and *uvr8-2*, and of *CHS* in all three genotypes, but less in

508 *cry1cry2*. These results support regulation of transcript abundance by CRYs in UV-A$_{lw}$ and blue

509 light.

510

511 **4 DISCUSSION**

512

513 **4.1 Effective range of wavelengths for action of UVR8 and CRYs in sunlight**

514

515 In plant photobiology the consensus has been that UVR8 and CRYs function as UV-B and

516 blue/UV-A photoreceptors, respectively (Ahmad & Cashmore, 1993; Rizzini *et al.*, 2011).

517 However, here we show that UVR8 mediates transcriptome-wide changes in response to both solar

518 UV-A$_{sw}$ and UV-B (Figures 2–4). Assuming that UVR8 monomers are required for signaling and

519 response (Rizzini *et al.*, 2011), for UVR8 to mediate responses to UV-A$_{sw}$ it must absorb enough

520 photons at these longer wavelengths and monomerize. Our *in silico* estimates based on spectral

521 absorbance predict that UVR8 absorbs more UV-A$_{sw}$ photons than UV-B photons in sunlight

522 (Figure 5), because sunlight contains at least 30 times more UV-A$_{sw}$ photons than UV-B photons

523 (Aphalo, 2018). This explains why the role of UVR8 in UV-A$_{sw}$ perception has not been observed

524 in earlier studies using artificial light with unrealistically high UV-B:PAR and low UV-A:PAR

525 ratios. We also show that *in vitro* UVR8 dimers convert to monomers when exposed to radiation of

526 wavelengths between 300 nm and 335 nm but not in response to longer wavelengths (Figure 6). As

527 the UVR8 protein does absorb photons at wavelengths longer than 335 nm, a possible explanation

528 for this transition between 335 nm and 340 nm is a threshold in the energy per photon required for

529 monomerization. The dose we used at 335 nm was more than 3000 times the maximum used by

530 Díaz-Ramos *et al.* (2018), who observed *in vitro* almost complete monomerization at 310 nm, the

531 longest wavelength they investigated.

532

533 We also found that UVR8 affected blue light-induced gene expression, as fewer and in part

534 different genes responded to blue light in *uvr8-2* when compared to L*er* (Figures 2a,b, S5). This

535 effect was unexpected, as the effect of blue light was assessed in a background of strongly

16

536   attenuated UV-B and UV-A. Furthermore, KEGG pathway analysis indicates that functional UVR8

537   might be required for blue-light-dependent expression of genes involved in ribosome biogenesis,

538   protein processing and endocytosis (Figures S6a-c). Our estimates also predict that photon

539   absorption by UVR8 in sunlight extends as far as the blue region (Figure S7), where we observed

540   UVR8-dependent modulation of these specific responses to solar blue instead of a clear-cut

541   requirement as at shorter wavelengths. Although evidence for perception of blue light by UVR8 is

542   weak, the lack of monomerization in response to wavelengths longer than 335 nm suggests that

543   photoreception by UVR8 at longer wavelengths would have to depend on a different mechanism.

544

545   Our transcriptomic data at 6 h (solar noon) indicate that CRYs are the main photoreceptors

546   mediating gene expression responses to solar blue and UV-A$_{lw}$, but not to UV-A$_{sw}$. This contrasts

547   with the currently accepted role of CRYs in perception of the whole UV-A waveband (Yu *et al.*,

548   2010). Yet, the absence of CRYs increased the number of DEGs up to three times in response to

549   UV-B and up to six times in response to UV-A$_{sw}$ (Figures 2a,c and 3a,c), an unexpectedly large

550   effect affecting many metabolic processes (Figure S6). However, while CRYs absorb comparatively

551   fewer photons at these shorter wavelengths than in the blue, the effect of UV-B and UV-A$_{sw}$

552   exposure was assessed in a background of UV-A$_{lw}$ and blue radiation, a condition under which

553   CRY signaling was activated in the WT but not in *cry1cry2*. Thus, the previously described

554   negative regulation of four UVR8-mediated genes by CRYs in UV-B and UV-A$_{sw}$ (Rai *et al.*, 2019)

555   was now expanded to the whole transcriptome indicating an interaction between UVR8 and CRYs

556   leading to wide-ranging regulation of primary and secondary metabolism (Figures 2–4, S6).

557   Furthermore, this negative regulation was observed using qRT-PCR at both 6 h and 12 h for *CHS*

558   (Figure 8) indicating that this effect can persist until the end of the photoperiod even though UV-B

559   irradiance was very low at this time, suggesting a carry-over effect. The transcript abundance of

560   *RUP1* and *RUP2* was increased in response to UV-A$_{sw}$ in *cry1cry2* compared to L*er* (Figure 8),

561   indicating that the crosstalk between UV-B/UV-A$_{sw}$ and UV-A$_{lw}$/blue signaling pathways may

562   involve RUPs. In addition, a recent study demonstrated that both UVR8 and CRYs use VP motifs to

563   compete for binding the WD40 domain of COP1 (Lau *et al.*, 2019). Therefore, crosstalk between

564   the two signaling pathways could involve COP1. These earlier results together with our new

565   observations provide a good starting point for future studies on the molecular mechanism of

566   interaction between UVR8 and CRYs in sunlight and its relevance to plant adaptation and

567   acclimation to diurnal and seasonal variation in the solar spectrum.

568

569   **4.2 Putative TFs behind different patterns of gene expression response**

17

570

571 Our promoter enrichment analysis highlights the possible roles of several TFs in controlling the

572 observed gene expression responses downstream of UVR8 and CRYs. The analysis predicted that

573 MYB TFs, known regulators of flavonol accumulation (Stracke *et al.*, 2007), regulate the

574 expression of genes responding to UV-B partially independent of UVR8, and of those responding to

575 UV-A$_{sw}$ through UVR8 (Figure 7, cluster A). The data also show that HAT5 and PIF5 (clusters D,

576 E) are predicted to specifically regulate gene expression in response to solar blue through CRYs.

577 These results in sunlight agree with a previous report where PIF5 is shown to function downstream

578 of CRYs to mediate hypocotyl elongation in response to artificial blue light (Pedmale *et al.*, 2016).

579

580 Although earlier work has emphasized the role of HY5 as a master TF central to responses to UV

581 radiation and blue light (Brown & Jenkins, 2008; Favory *et al.*, 2009; Gangappa & Botto, 2016), the

582 array of response patterns of transcript abundance and motif enrichment observed here indicate that

583 several TFs play key roles in downstream signaling leading to gene expression. In addition to HY5,

584 the known regulators of UV and blue light signaling and photomorphogenesis including PIF1, PIF3,

585 PIF4, PIF7 and BES1 (Hayes *et al.*, 2014; Gangappa & Botto, 2016; Pedmale *et al.*, 2016; Liang *et*

586 *al.*, 2018; Wang *et al.*, 2018) were predicted to regulate gene expression in response to solar UV-B

587 through UVR8, and in response to solar blue through CRYs (Figure 7, cluster B). Our data also

588 indicate that HY5, PIFs, BES1, HAT5, WRKYs and many other TFs could regulate the expression

589 of genes responsive to UV-B or UV-A$_{sw}$ and require both UVR8 and CRYs (Figure 7, clusters B, C,

590 D and G). This shows that both UVR8 and CRY signaling employ some of the same TFs for gene

591 expression. However, the multiple points of interaction for crosstalk between UV-B, UV-A$_{sw}$, UV-

592 A$_{lw}$ and blue light signaling pathways downstream of UVR8, CRYs and other photoreceptors

593 remain to be explored.

594

595 **4.3 Implications and conclusions**

596

597 With few exceptions, gene expression in response to solar UV-B and UV-A$_{sw}$ depended on UVR8,

598 while that in response to UV-A$_{lw}$ and blue light depended on CRYs. Why the "UV-B

599 photoreceptor" UVR8 played a role in the perception of solar UV-A$_{sw}$ can be explained by the

600 numbers of solar UV-B and UV-A$_{sw}$ photons predicted to be absorbed by UVR8, a physico-

601 chemical mechanism. Our prediction of photons absorbed by UVR8 was made possible by the new

602 spectral absorbance data we report, demonstrating the usefulness of extending such measurements

603 far along the tails of absorption spectra. We also observed *in vitro* monomerization of UVR8 dimers

18

604 exposed to wavelengths between 300 and 335 nm but not when exposed to longer ones, extending

605 previous knowledge into longer wavelengths. This lack of monomerization may explain why UVR8

606 does not play an important role in the perception of UV-A$_{lw}$. Thus, we describe the mechanism by

607 which the steep slope of the solar spectrum in the UV region shifts perception of solar radiation by

608 UVR8 towards longer wavelengths than frequently assumed.

609

610 When considering both UVR8 and CRYs, we observed that the transcriptome-wide response

611 triggered by UV-B and UV-A$_{sw}$ exposure was very strongly and negatively regulated by CRYs. The

612 reverse effect, modulation by UVR8 of gene expression in response to blue light was also observed

613 although it was much weaker. These results demonstrate for the first time the extent of the effect of

614 interactions downstream of UVR8 and CRYs on the transcriptome. These data also allowed us to

615 putatively identify several metabolic pathways affected by the interaction.

616

617 Specific groups of TFs were predicted *in silico* to control cascades of gene expression

618 corresponding to different patterns of transcriptome response to wavelengths across genotypes,

619 patterns which can only arise as the result of a complex signaling network including multiple points

620 of interaction downstream of UVR8 and CRYs. This prediction highlights that current models of

621 signaling downstream of UVR8 and CRYs, rather unsurprisingly, describe only the top portion of a

622 much deeper and ramified signaling network. As our study demonstrates, experiments combining

623 the use of multiple light treatments and multiple mutants in a factorial design allow teasing out

624 some of the signaling complexity that is missing from current models. The *in-silico* predictions we

625 report can guide the development of hypotheses about the mechanisms and players involved in

626 signaling, hypotheses that will need to be tested in future experiments.

627

628 As the wavelength boundaries for effective sensitivity of UVR8- and CRY-mediated perception of

629 sunlight do not coincide with the definitions of UV-B and UV-A radiation in common use (Björn,

630 2015), we consider that quantification of solar radiation based on these definitions is only

631 marginally useful when studying sunlight perception by plants, i.e., photomorphogenesis rather than

632 stress damage. Even more important, is that in both irradiation- and waveband-attenuation

633 experiments different regions within UV-A will trigger responses through different photoreceptors,

634 possibly resulting in contradictory or confusing results. In the present study, splitting the UV-A

635 waveband at 350 nm into UV-A$_{sw}$ and UV-A$_{lw}$ was the key to revealing the effective roles of UVR8

636 and CRYs in the perception of UV radiation in sunlight. Thus, as we routinely do for red and far-red

637 light in the visible, it is also very profitable to use plant-photomorphogenesis-specific waveband

638 definitions to characterize radiation in the UV-A region.

639

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641

647

648 7 AUTHORSHIP

649

650 PJA and LOM planned the research. NR, MB, ÅS, PJA, and LOM designed experiments. NR,

651 AO'H, DF, KR, FW, AVL, and LOM performed experiments. NR, OS, JS, PJA, and LOM

652 analyzed data. NR, PJA and LOM wrote the paper with contributions from MB, JS, ÅS, GIJ, and

653 TL. All authors commented and approved the manuscript. PJA and LOM contributed equally as

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655

656

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849

850

851 **Figure legends:**

852

853 Figure 1. Transmittance of filters used in the outdoor experiment and the statistical contrasts
854 between pairs of filter treatments used to assess the effects of different ranges of wavelengths in
855 solar radiation.

856

857 Figure 2. Number of genes differentially expressed in response to 6 h of solar UV-B, UV-A and
858 blue radiation in (a) L*er*, (b) *uvr8-2*, (c) *cry1cry2*. The Venn diagrams show the unique genes for
859 each waveband contrast and the overlap of genes between the waveband contrasts in each genotype.
860 The stacked bar plots show total number of genes responding to the waveband contrasts in each
861 genotype. The red bar refers to genes with increased expression and the blue bar refers to genes
862 with decreased expression. See Figure 1 for the contrasts used to assess the effects of UV-B, UV-A
863 and blue radiation. FC > 1.5 and P_{adjust} < 0.05.

864

865 Figure 3. Number of genes differentially expressed in response to 6 h of solar UV-A$_{sw}$ and UV-A$_{lw}$
866 radiation in (a) L*er*, (b) *uvr8-2*, (c) *cry1cry2*. The Venn diagrams show the unique genes for each
867 waveband contrast and the overlap of genes between the waveband contrasts in each genotype. The
868 stacked bar plots show total number of genes responding to the waveband contrasts in each
869 genotype. The red bar refers to genes with increased expression and the blue bar refers to genes
870 with decreased expression. See Figure 1 for the contrasts used to assess the effects of UV-A$_{sw}$ and
871 UV-A$_{lw}$ radiation. FC > 1.5 and P_{adjust} < 0.05.

872

873 Figure 4. Volcano plots showing DEGs with significantly increased expression (in red), DEGs with
874 significantly decreased expression (in blue) and not significant (in grey) in response to 6 h of solar
875 radiation of λ < 350 nm (290–350 nm) and λ > 350 nm (350–500 nm), λ refers to wavelength, n
876 refers to number of differentially expressed genes. See Figure 1 for the contrasts used to assess the
877 effects of λ < 350 nm and λ > 350 nm. FC > 1.5 and P_{adjust} < 0.05.

878

879 Figure 5. Estimates of solar UV-B, UV-A$_{sw}$, UV-A$_{lw}$ and blue photons absorbed by UVR8 and
880 CRYs proteins throughout the day under the different filters used, expressed relative to their
881 respective daily maximum. The red horizontal lines show when the plants were moved outdoors
882 (0 h) or sampled (6 h and 12 h).

883

27

884   Figure 6. *In vitro* monomerization of purified UVR8 protein from *Nicotiana benthamiana* exposed
885   to UV radiation from a tunable laser. The time of exposure and photon doses at different
886   wavelengths were: 10 min and 1.9 µmol at 300 nm, 30 min and 5.1 µmol at 320 nm, 30 min and
887   4.5 µmol at 325 nm, 30 min and 3.8 µmol at 330 nm, 30 min and 3.4 µmol at 335 nm, 60 min and
888   6.2 µmol at 340 nm, 60 min and 5.8 µmol at 345 nm, 60 min and 5.3 µmol at 350 nm. The picture
889   shows the Coomassie stained gel. UVR8D refers to the dimer and UVR8M the monomer. The
890   figure is representative of three repeats.

891

892   Figure 7. The *in silico* enrichment of DNA-binding motifs in our RNA-seq data showing 187
893   putative regulatory transcription factors (TFs). See Figure S8 for the position weight matrix of the
894   enriched motifs. The enrichments were done in 1000 base pairs upstream of the coding regions of
895   the DEGs from each waveband contrast and genotype combination. TFs with $P_{adjust} < 0.01$ in at
896   least one contrast and genotype combination were included in the figure. Of the 187 enriched TFs,
897   those which were also differentially expressed in our experiment are presented in bold letters and
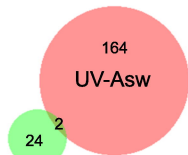898   shown as "DE" in the figure.

899

900   Figure 8. Transcript abundance ($log_{10}FC$) of four genes *CHI*, *CHS*, *RUP2* and *SPS1* after 6 h and
901   12 h of treatment outdoors. These genes showed a significant triple interaction (genotype ×
902   radiation treatment × exposure time). Mean ±1SE from four biological replicates. The horizontal
903   bars show P_{adjust} values for pair-wise comparisons between treatments within each genotype. P_{adjust}
904   values for pair-wise contrasts are shown only in those panels where the overall effect of filter
905   treatment within a genotype and time point was significant (see Table S2).

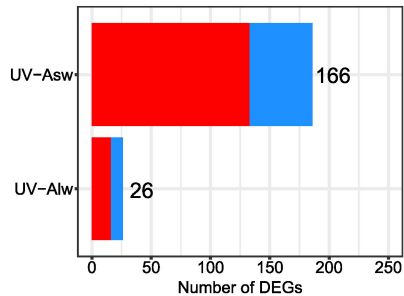| Contrast	Effect
>290 vs >350	$\lambda < 350$ nm
>350 vs >500	$\lambda > 350$ nm
>290 vs >315	UV−B
>315 vs >400	UV−A
>315 vs >350	UV−Asw (<350 nm)
>350 vs >400	UV−Alw (>350 nm)
>400 vs >500	Blue

(a) L*er*

(b) *uvr8-2*

(c) *cry1cry2*

(a) L*er*

(b) *uvr8-2*

(c) *cry1cry2*

CHI

| 6h | 6h | 6h | 12h | 12h | 12h |
| Ler | uvr8-2 | cry1cry2 | Ler | uvr8-2 | cry1cry2 |

CHS

| 6h | 6h | 6h | 12h | 12h | 12h |
| Ler | uvr8-2 | cry1cry2 | Ler | uvr8-2 | cry1cry2 |

RUP2

| 6h | 6h | 6h | 12h | 12h | 12h |
| Ler | uvr8-2 | cry1cry2 | Ler | uvr8-2 | cry1cry2 |

SPS1

| 6h | 6h | 6h | 12h | 12h | 12h |
| Ler | uvr8-2 | cry1cry2 | Ler | uvr8-2 | cry1cry2 |

Transcript abundance (log10 fold change)

Cut-off wavelengths of long-pass filters (nm)