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Induction of *ARI12* upon broad band UV-B radiation is suppressed by UVR8 and cryptochromes

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Abbreviations: ARI12, ARIADNE12; UVR8, UV RESISTANCE LOCUS; CRY1/2, CRYPTOCHROME 1/2; PHOT1/2, PHOTOTROPIN 1/2; PHYA/B, PHYTOCHROME A/B; GUS, beta-glucuronidase; COP1, CONSTITUTIVELY PHOTOMORPHOGENIC 1; Arabidopsis, Arabidopsis thaliana; CHS, CHALCONE SYNTHASE; HY5, ELONGATED HYPOCOTYL 5; HYH, HOMOLOG OF ELONGATED HYPOCOTYL; TUB9, TUBULIN BETA-9; HFR, high fluence rates

ARI12 belongs to a family of 16 potential E3 ligases in Arabidopsis and is strongly induced in leaves upon low and high fluence rates (HFR) of UV-B. We have shown that *ARI12* is a downstream target of the UV-B receptor, UVR8, and the transcription factors HY5 and HYH under low fluence rates. However under HFR of broad band UV-B *ARI12* expression was still downstream of HY5 and HYH but increased in *uvr8* mutants. To determine if other photoreceptors are responsible for the induction of ARI12 we quantified its expression in double mutants of the UV-A and blue light receptors, *CRY1/2* and *PHOT1/2*, and the red light receptors *PHYA/B*. While the expression of ARI12 was increased in cry1/2 it was unaffected in *phot1/2* and *phyA/B*. Therefore *ARI12* expression is suppressed by UVR8 and cryptochromes, and independent of phototropins and phytochromes A and B upon HFR of broad band UV-B.

Following the depletion of the stratospheric ozone layer increasing solar UV (UV)-B (280–315 nm) radiation will reach the earth. The increased UV-B radiation will have significant effects on natural and agricultural ecosystems.[1-3] While low doses of UV-B serve as signal to control growth and development, high doses inhibit growth and reduce yield.[4] Moreover high UV-B radiation causes DNA damages but also induces the production of UV-B protecting flavonoids.[4,5] While photoreceptors for UV-A, blue and red light have been known for decades the existence of an UV-B specific receptor has only recently been confirmed.[6,7] *UVR8* was identified in Arabidopsis because *uvr8* mutants were hypersensitive to UV-B, exhibit reduced UV-B-induced flavonoid biosynthesis and *CHS* expression.[8] Furthermore UVR8 mediates low fluence rates UV-B-dependent photomorphogenesis.[9] UVR8 is constantly expressed and present as inactive dimer in the cytoplasm. Upon UV-B radiation, UVR8 monomerises due to the disruption of salt bridges between arginines in the proximity of two tryptophanes that serve as UV-B chromophores and interact in the nucleus with the ubiquitin E3 ligase and central light regulator COP1.[7,10,11] Downstream of the UVR8-mediated signaling cascade are two transcription factors HY5 and HYH which have been proposed to regulate all UVR8 dependent genes.[12]

ARI12 is a member of a family of potential ubiquitin E3 ligases in Arabidopsis.[13] Under white light conditions, *ARI12* is expressed in roots and hypocotyls and hardly detectable in leaves.[14] We have recently shown that *ARI12* expression is strongly induced upon low and high fluence rates of UV-B radiation and under both conditions this expression depends on the transcription factors HY5 and its homolog HYH.[15]

While *ARI12* expression depended on UVR8 at low fluence rates, *ARI12* was higher expressed in *uvr8* mutants upon broad band HFR conditions. To determine if other photoreceptors are responsible for the induction of *ARI12* upon HFR we extended the expression analyses to mutants of the UV-A, blue light and the red light receptors. The receptors responsible for UV-A and blue light (320–500 nm) perception are cryptochromes and phototrophins.[16,17] The genome of Arabidopsis codes for two redundantly acting cryptochromes (CRY1 and 2) and phototropins (PHOT1 and 2) and a family of five phytochromes (PHYA-E) that perceive red and far-red light (600–700 nm).[18,19] PHYA and PHYB are the most prominent members[20,21] and both act through the transcription factor HY5.[22]

Similar to the previous analyses with *uvr8* and *hy5/hyh* mutants, double mutants of the photoreceptors *cry1/2*, *phot1/2* and *phyA/B* were cultivated under 140 μmol m^{-2} s^{-1} white

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light conditions and were exposed on day 25 for 90 min with 4.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ broad band UV-B. Leaves were harvested before and at different time points after UV-B exposure. *ARI12* expression was quantified by qRT-PCR as reported by Lang-Mladek et al.[15] While the expression of *ARI12* is very low before UV-B exposure in wildtype plants (**Fig. 1A**) it is significantly higher in *uvr8-6* mutants indicating that UVR8 might act as a suppressor of *ARI12* expression in white light and UV-B. However histochemical analyses of the *ARI12* promoter GUS reporter (*pARI12:GUS*) in *uvr8-6* does not support the qPCR results of the white light conditions (**Fig. 1B and C**). Since the expression of *ARI12* in the *uvr8-6* background is very low, the difference might be due to the lower sensitivity of the reporter construct compared with the qPCR quantification. Consistently, the difference of the *ARI12* expression between *uvr8-6* and wildtype is apparent with the histochemical staining after UV-B exposure probably because of its at least one magnitude higher expression (**Fig. 1D and E**). Independent of the mutant background the RNA abundance of *ARI12* peaked at about 2 h after UV-B exposure. The expression of *ARI12* in *phot1/2* and *phyA/B* was not significantly different from their wildtype backgrounds, indicating that these two photoreceptors are not involved in the UV-B specific induction of *ARI12*. That phototropins and phytochromes are not involved in *ARI12* expression agrees with our survey of the public available microarrays that have been explored with the Bio-Array Resource and the Genevestigator tool.[23,24] In these data sets *ARI12* was not significantly induced by blue, red nor high or low light conditions nor differently regulated in *phyA* or *phyB* mutants.[25-28]

In contrast *ARI12* was higher expressed in *uvr8-6* and the double mutant *cry1/2* at 2 h after UV-B exposure indicating that upon HFR of broad band UV-B radiation UVR8 and the CRYs are probably inhibiting *ARI12* expression.

In summary we present evidences that UVR8 and CRY1/2 are required to avoid excess of *ARI12* expression under HFR conditions. Thus *ARI12* is the first gene that is positively regulated by HY5/HYH and negatively by UVR8 at HFR of UV-B. The functional significance of this specific regulation however has to be determined yet.

Disclosure of Potential Conflicts of Interest

There were no potential conflicts of interest to expose.

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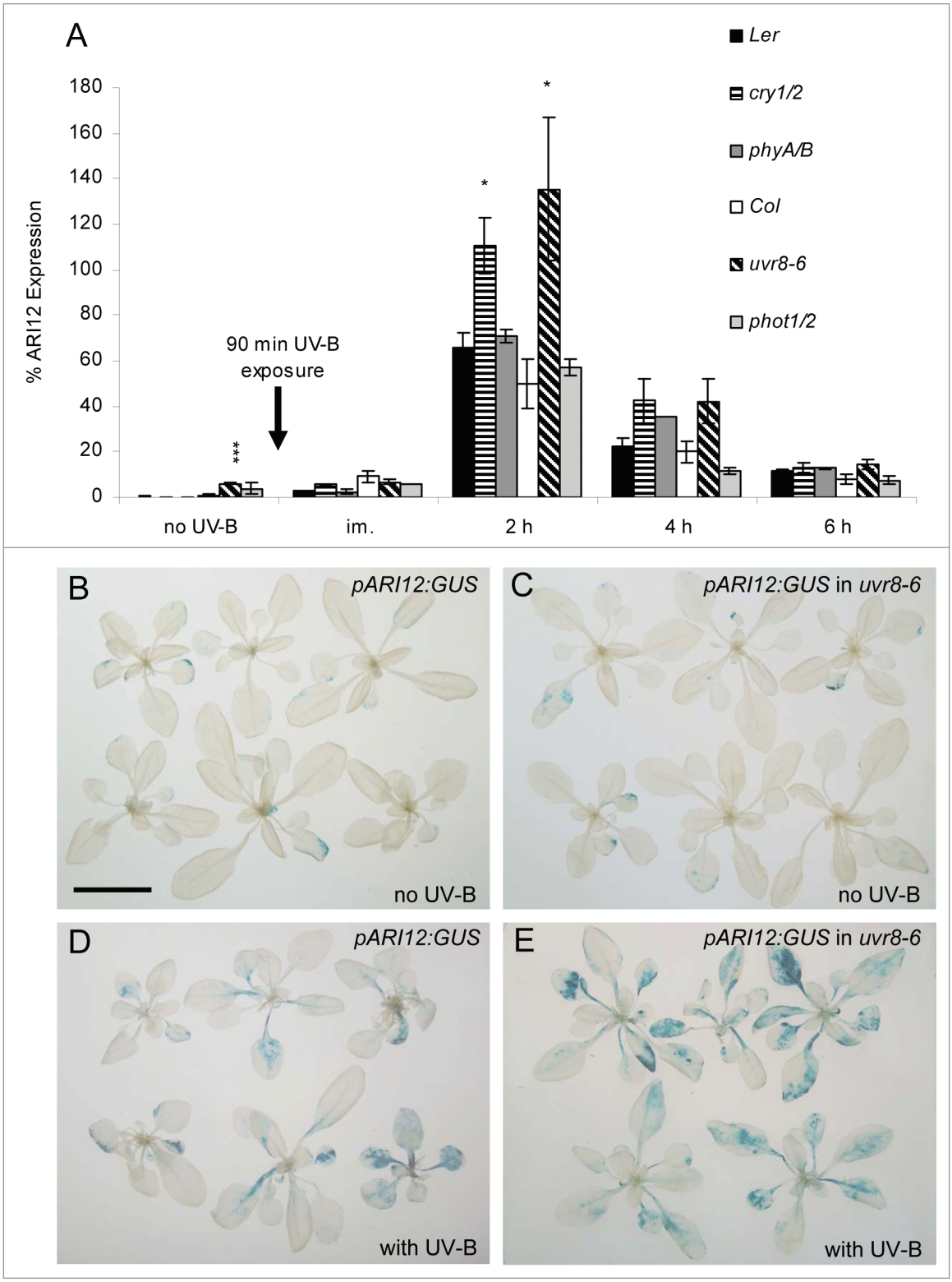

Figure 1. *ARI12* expression in *uvr8-6*, *cry1/2*, *phot1/2* and *phyA/B* single and double mutants upon high fluence rates of broad band UV-B radiation. (**A**) Time course of *ARI12* expression before (no UV-B, 140 μmol m^{-2} s^{-1} white light), immediately (im) and at different times after a 90 min addition of 4 μmol m^{-2} s^{-1} of UV-B. qRT-PCR data were normalized to the expression of the reference gene *TUB9*. Data represent means and standard errors of at least three independent biological replicates. Significant difference were calculated with Student's T-tests and * indicates p-values of ≤ 0.05, and *** of ≤ 0.001, respectively. (**B–E**) Histochemical staining of *pARI12:GUS* (**B,D**) and *pARI12:GUS* in *uvr8-6* mutants (**C,E**) before (**B,C**) and 6 h after UV-B exposure (**D,E**). Pictures were taken with the same magnification and the size bar in B corresponds to 20 mm.

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