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Transcriptome and metabolome analyses revealed that narrowband 280 and 310 nm UV-B induce distinctive responses in Arabidopsis

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SUMMARY (250 words)

In plants, the UV-B photoreceptor UV RESISTANCE LOCUS8 (UVR8) perceives UV-B and induces UV-B responses including synthesis of UV-B absorbing phenolic compounds such as anthocyanins. UVR8 absorbs a range of UV-B (260–335 nm). However, the responsiveness of plants to each UV-B wavelength has not been intensively studied so far. Here, we performed transcriptome and metabolome analyses of Arabidopsis using UV light emitting diodes (LEDs) with peak wavelengths of 280 and 310 nm to investigate the differences in the wavelength-specific UV-B responses. Irradiation with both UV-LEDs induced gene expression of the transcription factor ELONGATED HYPOCOTYL 5 (HY5), which has a central role in the UVR8 signaling pathway. However, the overall transcriptomic and metabolic responses to 280 and 310 nm UV-LED irradiation were different. Most of the known UV-B-responsive genes, such as salicylic acid, jasmonic acid, and defense-related genes, responded only to 280 nm UV-LED irradiation. Lipids, polyamines and organic acids were the metabolites most affected by 280 nm UV-LED irradiation, whereas the effect of 310 nm UV-LED irradiation on the metabolome was considerably less. Enzymatic genes involved in the phenylpropanoid pathway upstream in anthocyanin biosynthesis were up-regulated only by 280 nm UV-LED irradiation. On the other hand, no enzymatic genes downstream in anthocyanin biosynthesis were induced by the UV-LEDs, but rather, they were down-regulated by 310 nm UV-LED irradiation. These results revealed that the responsivenesses of Arabidopsis to 280 and 310 nm UV-B were significantly different, suggesting that UV-B signaling is mediated by more complex pathways than

the current model.

Keywords: UV-B, LED, metabolome, transcriptome, lipids, polyamines, phenylpropanoids, Arabidopsis.

INTRODUCTION

The sunlight that falls on the earth contains various wavelengths. Plants use the light not only as an energy source, but also as signals for optimizing growth and development. Light is classified according to the wavelengths it contains, and the shortest-wavelength component of the sunlight that reaches the ground is UV. UV is further divided into three bands, UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm). Since UV-C is absorbed by the stratospheric ozone, in the sunlight that reaches the ground, UV-B is the light that possesses the shortest wavelength and accordingly the highest energy. Because nucleic acids and proteins, have absorption peaks near 260 and 280 nm, respectively, UV-B directly causes damage to the molecules. UV-B also produces reactive oxygen species (ROS) that cause damage to DNAs, proteins, and lipids, and induce many processes like programmed cell death (PCD), abiotic stress responses and pathogen defense (Gill and Tuteja, 2010; Hideg *et al.*, 2013). Plants have adapted to this harmful UV-B during their evolution, acquiring plant-specific UV-B responses (Jenkins, 2009; Dotto and Casati, 2017; Yin and Ulm, 2017). As a typical UV-B response, the levels of phenolic compounds, such as anthocyanins, that absorb UV-B increase (Rozema *et al.*, 1997; Heijde and Ulm, 2012).

The UV-B receptor UV RESISTANCE LOCUS 8 (UVR8) mediates the UV-B response in plants (Rizzini *et al.*, 2011; Heijde and Ulm, 2012; Jenkins, 2017; Yin and Ulm, 2017). UV-B signaling requires monomerization of UVR8, which forms a homodimer in the ground state (Rizzini *et al.*, 2011). The absorption spectrum of UVR8 has a peak at 280 nm and extends from at least 250 nm to around 500 nm (Rai *et al.*, 2020). Monomerization of UVR8 occurs under UV in the range from 260 to 335 nm (Díaz-Ramos *et al.*, 2018; Rai *et al.*, 2020). UVR8 monomers interact with CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1), an E3 ubiquitin ligase, and inactivate COP1 activity, resulting in stabilization of ELONGATED HYPOCOTYL5 (HY5), a member of the bZIP transcription factor family, that inhibits hypocotyl growth and lateral root development, and promotes pigment accumulation (Heijde and Ulm, 2012; Gangappa and Botto, 2016; Jenkins, 2017; Yin and Ulm, 2017). HY5 has a central role in UV-B signaling, regulating the expression of about half of UVR8-induced genes, including defense-related and anthocyanin biosynthetic genes (Brown *et al.*, 2005; Vandenbussche *et al.*, 2018).

Metabolomic analysis of the UV-B response in Arabidopsis showed increased amounts of phenolic compounds, such as sinapoyl malate, and quercetin- and kaempferol-glycosides (Lake *et al.*, 2009). Reprogramming of both central and specialized metabolisms in response to UV-B was also

shown with increases in sugars, amino acids, and organic acids in the tricarboxylic acid (TCA) cycle, and phenolic compounds, including anthocyanins (Kusano *et al.*, 2011). Anthocyanins, which act as UV sunscreen in plants, are synthesized through the biosynthetic pathways of phenylpropanoids and flavonoids. The biosynthesis of phenylpropanoids starts from L-phenylalanine, which is synthesized by aroenate dehydratase (ADT) (Fraser and Chapple, 2011). *p*-Coumaroyl CoA, a precursor of flavonoids biosynthesis, is produced by L-phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL) in the phenylpropanoid pathway. Chalcone synthase (CHS), chalcone isomerase (CHI), flavonol 3-hydroxylase (F3H) and flavonol 3'-hydroxylase (F3'H) are main enzymes in flavonoid biosynthesis. Dihydroflavonol-4-reductase (DFR) and leucoanthocyanidin dioxygenase/anthocyanidin synthase (LDOX/ANS) are involved in synthesis of the basic skeleton of anthocyanin, and multiple anthocyanin glycosyltransferases (AGTs) modify the sugar moieties of anthocyanins. Expression of these enzymatic genes is regulated by several classes of transcription factors, such as MYB-bHLH-WDR (MBW) complexes (Broun 2005; Xu *et al.*, 2014, 2015; Ma and Constabel 2019), and coregulatory Mediator complexes (Bonawitz *et al.*, 2012; Wang *et al.*, 2020). Flavonoid biosynthesis is up-regulated under UV-B through regulation of these transcription factors mediated by UVR8 signaling (Jenkins 2014; Ma and Constabel 2019; Qian *et al.*, 2020).

Most of the previous studies on UV-B responses used broadband (BB) UV lamps with peaks around 310 nm and ranging from 280 to 380 nm (Brown *et al.*, 2005; Lake *et al.*, 2009; Kusano *et al.*, 2011; Vandenbussche *et al.*, 2018), although the maximal absorbance of UVR8 is at 280 nm. To our knowledge, the responsiveness of plants to each UV-B wavelength has not been intensively studied so far. Here, we performed transcriptome and metabolome analyses of *Arabidopsis* using UV light emitting diodes (LEDs) with peak wavelengths of 280 and 310 nm to investigate the UV-B responses under the narrowband (NB) UV-B light. Our results showed that the responsivenesses of *Arabidopsis* to 280 and 310 nm UV-B were significantly different.

RESULTS

Transcriptomic response to narrowband UV-B

In this study, 280 nm UV-LED with a peak wavelength of 280 nm, a half-width of 10 nm, and a range from 260 to 310 nm and 310 nm UV-LED with a peak wavelength of 310 nm, a half-width of 10 nm, and a range from 290 to 340 nm were used for irradiation (Figure 1). The photon flux density on the irradiated surface was adjusted to $2.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. *Arabidopsis* grown under long-day conditions (16-h light/8-h dark) for 14 days at 23°C was used for the irradiation experiments. The irradiation conditions were: (i) 45 min irradiation with the 280 nm UV-LED (280-0d); (ii) 45 min irradiation with the 310 nm UV-LED (310-0d); (iii) 45 min irradiation with the 280 nm UV-LED followed by incubation in the dark for two days (280-2d); and (iv) 45 min irradiation with the 310 nm UV-LED

109 followed by incubation in the dark for two days (310-2d). For the control, plants without UV-B
110 irradiation (C-0d) and plants incubated in the dark for two days without UV-B irradiation (C-2d) were
111 used.

112 To investigate the responses to NB UV-B, the transcriptome in Arabidopsis irradiated with the
113 280 or 310 nm UV-LED was analyzed using RNA-Seq. It was revealed that the expression of much
114 fewer genes was affected by 310 nm UV-LED irradiation, especially in 310-2d, compared with 280 nm
115 UV-LED irradiation. In comparison with the control, the numbers of differentially expressed genes
116 (DEGs) were 3386, 1277, 3948, and 96 in 280-0d, 310-0d, 280-2d, and 310-2d, respectively (Figure
117 2a). Moreover, DEGs in Arabidopsis under 280 and 310 nm UV-LED were not closely overlapped as
118 expected in terms of plant UV-B response. The numbers of commonly up-regulated DEGs between
119 280-0d and 310-0d and between 280-2d and 310-2d were 261 and 15, respectively, and the numbers of
120 commonly down-regulated DEGs between 280-0d and 310-0d and between 280-2d and 310-2d were
121 487 and 1, respectively (Figure 2b, Tables S1 and S2).

122 **Gene ontology term enrichment analysis of DEGs**

124 To investigate the underlying functions of the DEGs in the responses to 280 and 310 nm UV-LED
125 irradiation, gene ontology (GO) term enrichment analysis was conducted using the Database for
126 Annotation, Visualization and Integrated Discovery (DAVID), version 6.8 (Huang *et al.*, 2009a,
127 2009b). Enriched terms were totally different between the DEGs under 280 and 310 nm UV-LED
128 irradiation in accordance with their slight overlapping. Biotic or abiotic stress-related terms, such as
129 'response to chitin', 'defense response', 'defense response to bacterium', and 'response to salicylic
130 acid' were enriched in up-regulated DEGs in 280-0d and 280-2d. In contrast, the term 'response to
131 salicylic acid' was enriched in down-regulated DEGs in 310-0d, and other stress-related terms were
132 not enriched under 310 nm UV-LED irradiation. Auxin related genes in 'auxin-activated signaling
133 pathway' and 'response to auxin' were down-regulated under 280 nm UV-LED irradiation. Genes
134 related to photosynthesis and responses to light stimulus and far red light were down-regulated in
135 280-2d, whereas 'response to light stimulus' was enriched in DEGs in 310-0d. There was no enriched
136 term in DEGs in 310-2d (Figure 2c and Table S3).

137 In the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, up-regulated DEGs were
138 most enriched in 'plant-pathogen interaction' and 'biosynthesis of secondary metabolites' in 280-0d
139 and 280-2d, respectively. The term 'phenylpropanoid biosynthesis' was also enriched in DEGs in
140 280-2d. The terms related to lipid metabolism, such as ' α -linolenic acid metabolism', and 'fatty acid
141 degradation' were characteristically enriched under 280 nm UV-LED irradiation, whereas 'fatty acid
142 elongation' and 'cutin, suberine and wax biosynthesis' were down-regulated in 280-0d.
143 Down-regulated DEGs were most enriched in 'plant hormone signal transduction', 'ribosome
144 biogenesis in eukaryotes', and 'photosynthesis' in 280-0d, 310-0d, and 280-2d, respectively. There was

no enriched term in up-regulated DEGs in 310-0d and 310-2d, and in down-regulated DEGs in 310-2d (Figure 2c and Table S3).

Effect of narrowband UV-B on metabolome

The metabolome in 280-2d and 310-2d were analyzed using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) to investigate the effect of NB UV-B on the Arabidopsis metabolism. Overall, fewer metabolic changes were confirmed under 310 nm UV-LED irradiation as transcriptomic responses. GC-MS analysis showed that the amounts of 19 hydrophilic metabolites and seven fatty acids in 280-2d, and only two fatty acids in 310-2d, which were also increased in 280-2d, were increased in comparison with the control, whereas the amounts of two hydrophilic metabolites were decreased in 280-2d (Figure 3a,c and Table S3).

In LC-MS lipid analysis, positive and negative ion measurements were performed at the same time. Since some peaks appeared only in either ion mode, the positive and negative ions were separately analyzed. Increased numbers of hydrophilic metabolites were 3874 and 962, those of lipids in the positive ion mode (lipid-positive) were 625 and 105, and those of lipids in the negative ion mode (lipid-negative) were 172 and 41, in 280-2d and 310-2d, respectively. Decreased numbers of hydrophilic metabolites were 2189 and 1407, those of lipid-positive were 890 and 207, and those of lipid-negative were 263 and 85, in 280-2d and 310-2d, respectively (Figure 3b). Again, it was shown that changes in metabolic profiles under 280 and 310 nm UV-LED irradiation were not fully overlapped. The numbers of specifically increased hydrophilic metabolites were 3318 and 406, those of lipid-positive were 591 and 71, and those of lipid-negative were 163 and 32, in 280-2d and 310-2d, respectively, and numbers of specifically decreased hydrophilic metabolites were 1388 and 606, those of lipid-positive were 759 and 76, and those of lipid-negative were 214 and 36, in 280-2d and 310-2d, respectively (Figure 3c).

Among them, annotated metabolites registered in the metabolic pathway in Arabidopsis in KEGG are shown in the metabolic map in Figure 4. For example, metabolites in the TCA cycle and fatty acid biosynthesis, such as dodecanoic acid, decanoic acid, and octanoic acid, were increased more than two-fold in 280-2d in comparison with the control. On the other hand, metabolites in glucosinolate biosynthesis, such as glucoiberberin and glucoerucin, and glutathione metabolism, γ -L-glutamyl-L-cysteine, were decreased more than two-fold in 280-2d. Metabolites in glucosinolate biosynthesis, glucobrassicin, and phenylpropanoid biosynthesis, ferulate, were decreased more than two-fold in 310-2d. Identified lipids were further classified according to LIPID MAPS (Liebisch *et al.*, 2020) and the method described in Murphy (2015). It was shown that, in 280-2d, 17 lipids in ceramide (Cer) were characteristically increased, whereas 14 in phosphatidylethanolamine (PE), and 19 in triglyceride (TG) were decreased.

181 **280 nm specific activation of lipid, polyamine metabolism and TCA cycle**

182 Since the expression of genes related to lipid metabolism (Figure 2c) and the amounts of lipids were
 183 specifically increased or decreased under 280 nm UV-LED irradiation (Figure 4 and Table 1), the
 184 expression of genes involved in lipid metabolism was extracted from the transcriptomic data.
 185 Metabolome analysis showed that, in 280-2d, lipids in Cer were increased, whereas those in PE were
 186 decreased (Table 1). Cer is synthesized by Cer synthases catalyzing the condensation of sphingoid
 187 bases and coenzyme A (CoA)-activated fatty acids (Figure 5a). We assumed that the decreases of
 188 lipids in PE were due to their degradation. PE is hydrolyzed by phospholipase D (PLD) (Figure 5b)
 189 (Janda *et al.* 2013, Qin and Wang, 2002). Hence, gene expression of Cer synthases and PLDs was
 190 focused on. In addition, the relationship between the Cer metabolism and the hypersensitive response
 191 (HR)-type PCD has been shown (Ternes *et al.* 2011; Luttgeharm *et al.* 2015). Therefore, expression of
 192 HR-type PCD genes was also focused on. Gene expression of a member of Cer synthases, *LOH2*
 193 (Figure 5c), and four PLDs, *PLPζ2*, *PLDβ2*, *PLDγ3*, and *PLDγ1* (Figure 5d), were significantly
 194 increased in 280-2d together with five PCD markers, *FLAVIN-DEPENDENT MONO-OXYGENASE1*
 195 (*FMO1*), *peroxidase C (PRXc)*, *SENESCENCE-ASSOCIATED GENE 13 (SAG13)*, and
 196 *pathogenesis-related 2 and 3 (PR2, PR3)* (Figure 5e). This gene expression profile corresponds well
 197 with the metabolome data.

198 Spermidine (Spd) and γ -aminobutyric acid (GABA), which are produced from polyamine
 199 biosynthesis and catabolism, respectively, were also specifically increased in 280-2d. (Figure 4 and
 200 Table S3). In accordance with the metabolic change, genes involved in polyamine metabolism,
 201 polyamine oxidases, *PAO2*, *PAO3*, and *PAO4*, spermidine synthase, *SPDS3*, aldehyde dehydrogenases,
 202 *ALDH2B7* and *ALDH3H1*, and glutamic acid decarboxylase, *GAD1*, were up-regulated in 280-2d
 203 (Figure 6). Similarly, up-regulation of most of the genes involved in the TCA cycle, citrate synthase
 204 *CSY4*, isocitrate dehydrogenase, *IDH6*, the E₁ subunit of 2-oxoglutarate dehydrogenase complex,
 205 *E1-OGDH1*, the E₂ subunits of 2-oxoglutarate (α -ketoglutarate) dehydrogenase complex, *KGDHE2*
 206 and *At4g26910*, dihydrolipoyl dehydrogenase, *LPD2*, succinyl CoA ligase α -subunit, *At5g08300*,
 207 succinate dehydrogenases, *SDH1-1*, *SDH2-1*, and *SDH2-2*, fumarase, *FUM2*, and mitochondrial
 208 malate dehydrogenase, *mMDH2*, in 280-2d were confirmed (Figure 7).

210 **Effect of narrowband UV-B on flavonoid biosynthesis**

211 The amounts of shikimic acid and phenylalanine, precursors of flavonoids, were specifically increased
 212 in 280-2d (Figure 4). However, contrary to previous studies using BB UV-B lamps, flavonoids were
 213 not increased under NB UV-B. Moreover, ferulic acid, leucodelphinidin, and kaempferol 3-glucoside
 214 were decreased under NB UV-B instead of increasing (Figure 4). Since a GO term 'phenylpropanoid
 215 biosynthesis' was enriched in DEGs in 280-2d, we focused on the gene expression of typical enzymes
 216 involved in flavonoid biosynthesis, *ADT6*, *PAL1*, *C4H*, *CHS*, *CHI1*, *F3H*, *DFRA*, *LDOX* and

217 *UGT75C1*, which is one of AGTs (Figure 8a,b). We also focused on the gene expression of
 218 transcription factors such as HY5, which is a key component in the UVR8 signaling pathway, HYH, a
 219 homologue of HY5, MEDs, and members in MBW complexes (MYBs, EGL3, GL3, TTG1, and TT8),
 220 which are involved in flavonoid biosynthesis (Figure 8c). Unexpectedly, the expression of genes
 221 involved in flavonoid biosynthesis was down-regulated immediately after NB UV-B irradiation
 222 (Figure 8b). Then, expression of *ADT6*, *PAL1*, and *C4H*, involved in upstream phenylpropanoid
 223 biosynthesis, was specifically increased in 280-2d, whereas expression of *DFR*, *LDOX*, and *UGT75C1*,
 224 involved in downstream anthocyanin biosynthesis was specifically decreased in 310-2d (Figure 8b). A
 225 significant increase in gene expression of transcription factors, *HY5*, *ATMYBL2* and *TTG1*, and a
 226 decrease of *MED33A* and *MYB4* were observed in both 280-0d and 310-0d (Figure 8c). The
 227 expression patterns of other transcription factors, except for *MYB11* and *MYB111*, also showed similar
 228 tendencies in both 280-0d and 310-0d. However, after two days incubation in the dark, expression of
 229 *MYB13*, *GL3*, *MYB4*, *MYB7* were significantly up-regulated, and *MYBL2* expression was
 230 down-regulated only in 280-2d. On the other hand, no expression difference was confirmed in 310-2d
 231 as compared with the control C-2d.

232

233 **DISCUSSION**

234 **Effects of 280 and 310 nm UV-LED irradiation on gene expression and metabolic profile are** 235 **different**

236 Many studies have been conducted on the responses of plants to UV-B, but few studies have been
 237 conducted on the effect of each wavelength within the UV-B. Here, it was confirmed that the numbers
 238 of DEGs and increased or decreased metabolites were significantly lower under 310 nm UV-LED
 239 irradiation than under 280 nm UV-LED irradiation. HY5 regulates the expression of half of
 240 UVR8-regulated genes including defense-related and flavonoid biosynthetic ones (Brown *et al.*, 2005;
 241 Vandenbussche *et al.*, 2018). Here, we found that the responses to 280 and 310 nm UV-LED
 242 irradiation were inconsistent with each other even though HY5, a key transcription factor in UVR8
 243 signaling, was induced by both UV-LEDs (Figures 2–8). UVR8 is monomerized in the range of 260 to
 244 335 nm with the maximum response at 280 nm (Díaz-Ramos *et al.*, 2018). Under 310 nm irradiation,
 245 the monomerization efficiency was lower than under 280 nm irradiation, but *HY5* expression was
 246 comparable with that under 280 nm irradiation (Díaz-Ramos *et al.*, 2018; Rai *et al.*, 2020). Most of the
 247 studies on UV-B responses so far used BB UV-B lamps, which have a peak wavelength around 310 nm
 248 (Brown *et al.*, 2005; Lake *et al.*, 2009; Kusano *et al.*, 2011; Vandenbussche *et al.*, 2018). Since BB
 249 UV-B lamps contain a wide range of wavelengths, there is a possibility that the observed phenomena
 250 in the previous studies were caused by the interaction of multiple light signaling pathways.

251 One of the possible reasons for this may be that UVR8 is highly sensitive to 280 nm but less to
 252 310 nm as indicated by *in vitro* monomerization (Díaz-Ramos *et al.*, 2018), and the response to 310

nm is mediated by other photoreceptors. For example, in Arabidopsis seeds, phytochrome A (phyA) signaling has been shown to induce germination under a wide range of wavelengths from 300 to 780 nm (Shinomura *et al.*, 1996). It is known that phyA also inactivates the COP1/SUPPRESSOR OF PHYA-105 complex, leading to the rapid accumulation of transcription factors such as HY5 (Saijo *et al.*, 2003; Sheerin *et al.*, 2015). LONG HYPOCOTYL IN FAR-RED1 (HFR1), which acts in phyA signaling, has been shown to be stabilized under UV-B (Fairchild *et al.*, 2000; Tavridou *et al.*, 2020). In our experiment, only 310 nm UV-LEDs induced the expression of *HFR1* (Table S4). Therefore, phyA signaling may be involved in the induction of *HY5* by 310 nm UV-LED irradiation. It is also possible that other photoreceptors, such as phyB and CRYs, which absorb 310 nm, were activated by 310 nm UV-LED irradiation. Further research using each photoreceptor mutant is required to dissect the different responses under 280 and 310 nm UV-LED irradiation.

Stress response is induced by 280 nm UV-B

Irradiation of Arabidopsis with UV-B has been reported to induce salicylic acid and jasmonic acid associated and defense-related genes (Surplus *et al.*, 1998; A.-H.-Mackerness *et al.*, 1999; Vandenbussche *et al.*, 2018). Jasmonic acid is biosynthesized from α -linolenic acid (Vick and Zimmerman, 1984). Here, we confirmed that DEGs in Arabidopsis with 280 nm UV-LED irradiation showed enriched defense responses, including those to biotic and abiotic responses, responses to salicylic acid and jasmonic acid, and α -linolenic acid metabolic pathways. On the other hand, 310 nm UV-LED irradiation did not enrich these terms, but rather down-regulated 'response to salicylic acid' (Figure 2 and Table S5). The results indicate that stress responses are induced by short wavelengths in UV-B below 310 nm. Previously, it was shown that UVR8 dependent defense responses against *Botrytis cinerea* were retarded even in jasmonate signaling mutants, *jar1-1* and *P35S:JAZ10.4* (Demkura and Ballaré, 2012). On the other hand, mitogen-activated protein kinase (MAPK) signaling pathway involved in an environmental stress response was shown to be UVR8 independent (González Besteiro *et al.*, 2011). Accordingly, the signaling pathway that induced the 280 nm specific stress responses in this study remain to be uncovered.

Irradiation of peach skin with UV-B changed the amounts of certain lipids (Santin *et al.*, 2018). NB UV-B LED irradiation of Arabidopsis also changed the amounts of certain lipids, and 280 nm UV-LED irradiation induced a characteristic change of the lipid profile as an increase in Cer and decreases in PE and TG (Table 1). Cer is synthesized by ceramide synthase, LOH1, LOH2, and LOH3 in Arabidopsis (Markham *et al.*, 2011). Overexpression of *LOHs* affected Arabidopsis growth, and HR-type PCD marker genes have been shown to be up-regulated in the *LOH2* overexpressing strain of Arabidopsis (Luttgeharm *et al.* 2015). In this study, the expression of *LOH2* and HR type PCD marker genes, *FMO*, *PRXc*, *SAG13*, *PR2*, and *PR3*, were increased by 280 nm-UV LED irradiation (Figure 5c,e). PCD was induced in UV-B treated BY-2 tobacco cells (Lytvyn *et al.*, 2010). Our results might

289 explain one of the mechanisms involved in PCD induction through up-regulation of *LOH2* by UV-B.
290 Multiple photoreceptor signaling pathways including UVR8-dependent and -independent pathways
291 have also been proposed for PCD (Petrov *et al.*, 2015). Utilization of NB UV-LED in future molecular
292 genetic studies will promote our understanding of mechanisms in PCD induction by UV-B.

293 Phosphatidylethanolamine is a group of phospholipids, which is the major constituent of cell
294 membranes. Phosphatidylethanolamine is known to be hydrolyzed by phospholipase D (PLD) (Janda
295 *et al.* 2013; Takák, T., *et al.*, 2019). Our result showed that the gene expression of enzymes
296 hydrolyzing PE, *PLPζ2*, *PLDβ2*, *PLDγ3*, and *PLDγ1*, were increased by 280 nm UV-LED irradiation
297 (Figure 5d). This might be the reason for the specific decrease in PE by 280 nm UV-LED irradiation.
298 Phosphatidic acid produced by hydrolysis of PE plays an important role in the stress response of plants
299 (Yao and Xue, 2018). Decreased PE and up-regulation of the hydrolyzing enzyme gene are also stress
300 responses specifically induced by 280 nm UV-LED irradiation. The decreases of lipids were also
301 found in TG (Table 1). Triglyceride is used as an energy source through glycolysis and the TCA cycle
302 (Thompson *et al.*, 1998). The increase of organic acids in the TCA cycle discussed below and fatty
303 acid may be due to the degradation of TG (Figure 4).

304 Polyamines and GABA are induced in the responses to various stresses, including UV, and
305 exhibit cytoprotective effects (Rhee *et al.*, 2007; Kinnersley and Turano, 2000; Kusano *et al.*, 2011;
306 Chen *et al.*, 2019; Jiao and Gu, 2019). Here, we also found that 280 nm UV-LED irradiation
307 specifically increased the polyamine, SPD, and GABA (Figure 4 and Table S3). Homeostasis of
308 polyamines is regulated by the dynamic balance of biosynthesis and catabolism. Biosynthesis is
309 catalyzed by arginine decarboxylase (ADC), agmatine iminohydrolase (AIH), *N*-carbamoylputrescine
310 amidohydrolase (CPA), spermidine synthase (SPDS), spermine synthase (SPMS) and thermospermine
311 synthase (ACL5). Catabolism involves two types of enzymes. One is copper-dependent diamine
312 oxidase (DAO) and the other is flavin adenine diamine (FAD)-dependent PAO (Wang *et al.*, 2019; Yu
313 *et al.*, 2019). As a result of transcriptome analysis, expression of catabolic genes, *PAOs*, was induced
314 by 280 nm UV-LED irradiation (Figure 4b). It has been shown that polyamines of higher molecular
315 weight, including spermine and Spd, are subjected to PAO-mediated catabolism when their levels
316 increase beyond a threshold (Zhang *et al.* 2015). We found that *ADC1* was also up-regulated under 280
317 nm UV-LED irradiation (data not shown), so there is a possibility that polyamine biosynthesis was
318 activated under 280nm UV-LED irradiation, and the polyamine exceeded the threshold for induction
319 of *PAO* expression. Elucidation of precise mechanisms of Spd increase needs further investigation.
320 4-Aminobutanal generated by the terminal catabolism of polyamines is then converted to GABA by
321 ALDHs (Zarei *et al.*, 2015, 2016). GABA is also synthesized from glutamic acid by glutamate
322 decarboxylase (GAD) (Baum *et al.*, 1996; Kinnersley and Turano, 2000). As a result of transcriptome
323 analysis, both GAD and ALDH were increased (Figure 6b). Accordingly, it is considered that 280 nm
324 UV-B is a signal to increase GABA production, however, the involvement of UVR8 signaling in the

325 regulation of polyamine and GABA metabolism remains to be elucidated.

326

327 **Metabolites in the TCA cycle increase by 280 nm UV-B**

328 It was shown that UV-B irradiation increased the amounts of metabolites in the first half of the TCA
329 cycle and expression of most of the enzymatic genes in the TCA cycle (Kusano *et al.*, 2011).
330 Regarding metabolites, not only citrate, aconitate, and 2-oxoglutarate in the first half of the TCA cycle,
331 but also succinate, fumarate, and malate in the second half increased under 280 nm UV-LED
332 irradiation (Figure 4). As well as the metabolome, the transcriptome in 280-2d showed specific
333 changes (Figure 7b). On the other hand, our results showed that 310 nm UV-LED irradiation did not
334 affect the metabolites in the TCA cycle. The difference in the effects of 280 and 310 nm on the central
335 metabolism was also confirmed using NB UV-B LEDs.

336

337 **Narrowband UV-B irradiation does not induce flavonoid biosynthesis**

338 UV-B stimulates the expression of genes encoding enzymes involved in the anthocyanin biosynthetic
339 pathway in Arabidopsis (Tohge *et al.*, 2011). UV-B is sensed by the photoreceptor UVR8, which
340 activates the transcription factor HY5; then, HY5 up-regulates the expression of the transcription
341 factors that control the biosynthesis of flavonoids and anthocyanins (Brown *et al.*, 2005; Stracke *et al.*,
342 2010; Tohge *et al.*, 2011; Shin *et al.*, 2013). In addition, it has been shown that UVR8 binds directly to
343 the transcription factor MYB13 regulating flavonoid biosynthesis in a UV-B-dependent manner (Qian
344 *et al.*, 2020). Among the members in MBW complexes, at least *MYB12*, *MYB111*, *MYB75*, *MYB13* and
345 *GL3* have been shown to be induced by UV-B (Brown *et al.*, 2005; Stracke *et al.*, 2010; Tohge *et al.*,
346 2011; Yan *et al.*, 2012; Qian *et al.*, 2020). In this study *HY5*, *MYBL2*, and *TTG1* were significantly
347 up-regulated, and *HYH* and *MYB12* also tended to be up-regulated by both 280 and 310 nm UV-LED
348 irradiation. *MYB4*, a repressor of flavonoid biosynthesis, was also commonly down-regulated under
349 irradiation by both UV-LEDs (Figure 8c). However, other transcription factors were not affected by
350 NB UV-LED irradiation. *MYBL2* is also a repressor of flavonoid biosynthesis, which suppresses the
351 expression of *F3H*, *DFR*, *LDOX*, *GL3*, *TT8* and *MYB75*, and is negatively regulated by strong light
352 (Dubos *et al.*, 2008). The AtGenExpress global stress expression data show that *MYBL2* expression is
353 induced by abiotic stresses including UV-B (Kilian *et al.*, 2007; Winter *et al.*, 2007). Here, we
354 confirmed the up-regulation of *MYBL2* by NB UV-B irradiation (Figure 8c). The expression of *F3H*,
355 *DFR*, *LDOX*, *GL3*, and *TT8*, showed a decreasing tendency, which is considered to be due to the
356 influence of *MYBL2* (Figure 8b,c). *MYB4* suppresses the expression of *C4H* (Jin *et al.* 2000). In our
357 experiments, expression of *MYB4* was reduced by both 280 and 310 nm UV-LED irradiation, but
358 expression of *C4H*, together with *ADT6* and *PAL*, was increased only by 280 nm irradiation (Figure
359 8b,c). There was no significant difference in the expression of *MYB13*, *MYB11* and *MYB111*, but
360 interestingly they showed the opposite behavior under 280 and 310 nm UV-LED irradiation (Figure

8c). After all, NB UV-LED irradiation could induce only upstream phenylpropanoid biosynthesis but not downstream flavonoid and anthocyanin biosynthesis. The phenomena reported in previous studies with BB UV-B lamps may have been induced by multiple signal transductions, as shown by the inconsistent gene expression profiles of members in MBW complexes under 280 and 310 nm UV-LED irradiation in this study.

In conclusion, our study revealed that the responsivenesses of *Arabidopsis* to 280 and 310 nm UV-B were significantly different, and *Arabidopsis* distinguished 280 and 310 nm UV-B. It is considered that the phenomena confirmed in the previous experiments using BB UV-B lamps were induced by the multiple signal transductions generated by several wavelengths in UV-B. Our results obtained with NB UV-B LEDs indicate that UV-B signal transduction is mediated through more complex pathways than the current model in which HY5 plays a central role. Utilization of NB UV-LEDs will lead to new insights into the plant UV-B responses.

EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

Seeds of *Arabidopsis thaliana* Columbia (Col-0) were sterilized with 0.5% sodium hypochlorite and 0.02% (w/v) Triton-X100 and cultured on half strength Murashige and Skoog medium (pH 6.0) containing 100 mg l⁻¹ myo-inositol, 0.1 mg l⁻¹ thiamin hydrochloride, 0.5 mg l⁻¹ nicotinic acid, 0.5 mg l⁻¹ pyridoxine, 2 mg l⁻¹ glycine, 1% sucrose and 0.8% agar in a growth chamber at 23°C with a 16-h light/8-h dark photoperiod for 14 days.

UV-B treatment

Fourteen-day-old *Arabidopsis* seedlings were kept under LED light with a peak wavelength of 280 nm and a half-bandwidth of 10 nm (NCSU234BU280, Nichia, Tokushima, Japan) or LED light with a peak wavelength of 310 nm and a half-bandwidth of 10 nm (NCSU234BU310, Nichia) for 45 min at 2.5 μmol m⁻² s⁻¹, and were kept at 23°C for two days in the dark.

RNA-Sequencing

RNA extraction and transcriptome analysis were conducted at Takara Bio, Shiga, Japan. Briefly, total RNA was extracted from approximately 100 mg fresh weight (FW) of *Arabidopsis* shoots using NucleoSpin RNA Plant (Takara Bio) according to the manufacturer's instructions. Three biological replicates for each treatment were used for analysis. RNA amplification was done using SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Illumina, San Diego, CA, USA). A DNA library was prepared using the Nextera XT DNA Sample Preparation Kit (Illumina). RNA-Seq was performed using NovaSeq system (Illumina), and the obtained nucleotide sequences were mapped to the

397 Arabidopsis genome sequence (TAIR 10.46) using STAR version 2.6.0c (Dobin *et al.*, 2013). Then,
398 gene expression levels were estimated as transcripts per million (TPM) using Genedata Profiler
399 Genome version 13.0.11 (Genedata, Basel, Switzerland).

400

Identification and enrichment analysis of DEGs

402 A statistical comparison of TPM was performed using Microsoft Excel to select DEGs between
403 control and UV-LED irradiated samples. The data were presented as means ($n = 3$), and Welch's *t*-test
404 was applied to detect significant differences. A gene with a *P*-value less than 0.05 and a difference
405 more than two-fold was considered as a DEG. To identify the most significant gene sets associated
406 with GO and KEGG pathways, enrichment analysis of DEGs was performed using DAVID
407 (<http://david.abcc.ncifcrf.gov>) (Huang *et al.*, 2009a; 2009b).

408

Profiling of hydrophilic metabolites using GC-MS

410 Metabolite extraction and metabolome analysis were conducted at Kazusa DNA Research Institute,
411 Chiba, Japan. Briefly, 50 mg FW of Arabidopsis shoots were extracted with 75–80% methanol, loaded
412 on a MonoSpin C18 column (GL Science, Tokyo, Japan), and eluted with 70% methanol.
413 Methoxyamine and pyridine were added to the obtained fraction for methoximation, and then
414 *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide was added for trimethylsilylation. Three biological
415 replicates for each treatment were used for analysis. The analysis was performed using a gas
416 chromatograph–quadrupole mass spectrometer, QP2010 Ultra (Shimadzu, Kyoto, Japan), with an auto
417 sampler AOC-5000 Plus (Shimadzu). Chromatographic separation was achieved using a DB-5
418 column (inner diameter, 0.25 mm $\times$ 30 m and film thickness, 1.00 μ m, Agilent Technologies,
419 Wilmington, NC, USA). The carrier gas was helium at a flow rate of 1.1 ml min⁻¹. The injection
420 temperature was 280°C, and the injection volume was 0.5 μ l. The temperature program was
421 isothermal for 4 min at 100°C, and was then raised at a rate of 4°C min⁻¹ to 320°C and held for 8 min.
422 The transfer line temperature, ion source temperature and scan speed were set to 280°C, 200°C and
423 2000 unit s⁻¹, respectively. Data acquisition was performed in the mass range of 45 to 600 *m/z*. The
424 obtained data was analyzed using the GCMSsolution software (Shimadzu) and the GC/MS metabolic
425 component database Ver.2 (Shimadzu).

426

Profiling of fatty acids using GC-MS

428 Metabolite extraction and metabolome analysis were conducted at Kazusa DNA Research Institute.
429 Briefly, 50 mg FW of Arabidopsis shoots were extracted with 650 μ l of methanol/methyl *tert*-butyl
430 ether, 3:10, 125 μ l of ultrapure water was added, and the methyl *tert*-butyl ether fraction was collected.
431 After adding 10% boron trifluoride methanol to the obtained fraction for methyl esterification,
432 ultrapure water and hexane were added, and then a hexane fraction was analyzed. Three biological

433 replicates for each treatment were used for analysis. The analysis was performed using a QP2010 Ultra
434 (Shimadzu) with an auto sampler AOC-5000 Plus (Shimadzu). Chromatographic separation was
435 achieved using a DB-5ms column (inner diameter, 0.25 mm $\times$ 30 m and film thickness, 0.25 μ m,
436 Agilent Technologies). The carrier gas was helium at a flow rate of 1.1 ml min⁻¹. The injection
437 temperature was 280°C, and the injection volume was 0.5 μ l. The temperature program was
438 isothermal for 2 min at 40°C, and was then raised at a rate of 6°C min⁻¹ to 320°C and held for 5 min.
439 The transfer line temperature, ion source temperature and scan speed were set to 280°C, 200°C and
440 2500 unit sec⁻¹, respectively. Data acquisition was performed in the mass range of 45 to 500 *m/z*. The
441 analysis of the obtained data is same as described above.

442

443 **Profiling of hydrophilic metabolites using LC-MS**

444 Metabolite extraction and metabolome analysis were conducted at Kazusa DNA Research Institute.
445 Briefly, 100 mg FW of Arabidopsis shoots were extracted with 75% methanol, loaded on a MonoSpin
446 C18 column (GL Science), and eluted with 75% methanol. Three biological replicates for each
447 treatment were used for analysis. The analysis was performed using a high-performance liquid
448 chromatography (HPLC) Ultimate 3000 RSLC (Thermo Fisher Scientific, Waltham, MA, USA)
449 coupled with a high-resolution mass spectrometer Q Exactive (Thermo Fisher Scientific) with
450 electrospray ionization (ESI) in the positive mode. Chromatographic separation was achieved using an
451 Inert Sustain AQ-C18 column (2.1 mm $\times$ 150 mm, 3 μ m-particle, GL Science). The column was kept
452 at 40°C, and the flow rate was 0.2 ml min⁻¹. The mobile phase solutions were water with 0.1% formic
453 acid (eluent A) and acetonitrile (eluent B) and were implemented in the following gradient: 0–3 min,
454 2% B; and 3–30 min, 2–98% B. The injection volume was 2 μ l. Mass spectrometry conditions were as
455 follows: the scan range was set at *m/z* 80–1200. The full scan resolution was 70,000. The MS/MS scan
456 resolution was 17,500. The obtained data was analyzed using a ProteoWizard
457 (<http://proteowizard.sourceforge.net>) and a PowerGetBatch (Kazusa DNA Research Inst.). Then, the
458 KEGG database (<http://www.genome.jp/kegg/>) was used to annotate the metabolites.

459

460 **Profiling of lipids using LC-MS**

461 Metabolite extraction and metabolome analysis were conducted at Kazusa DNA Research Institute.
462 Briefly, 100 mg FW of Arabidopsis shoots were extracted with 650 μ l of methanol/methyl *tert*-butyl
463 ether, 3:10, 125 μ l of ultrapure water was added, and the methyl *tert*-butyl ether fraction was collected.
464 Three biological replicates for each treatment were used for analysis. The analysis was performed
465 using an Ultimate 3000 RSLC (Thermo Fisher Scientific) coupled with a Q Exactive (Thermo Fisher
466 Scientific) with ESI in the positive or negative mode. Chromatographic separation was achieved using
467 a SunShell C18 column (2.1 mm $\times$ 150 mm, 2.6 μ m-particle, ChromaNik Technologies, Osaka, Japan).
468 The column was kept at 40°C, and the flow rate was 0.2 ml min⁻¹. The mobile phase solutions were

acetonitrile/water (60:40 v/v) (eluent A) and 2-propanol/acetonitrile (90:10 v/v) (eluent B), both containing 0.1% formic acid and 10 mM ammonium formate and were implemented in the following gradients: 0–10 min, 30–35% B; 10–20 min, 35–55% B; 20–35 min, 55–65% B; 35–45 min, 65–100% B; and 45–50 min, 100% B. The injection volume was 2 µl. Mass spectrometry conditions were the same as described above. The obtained data was analyzed using a ProteoWizard, a PowerGetBatch (Kazusa DNA Research Inst.) and a Lipid Search (Mitsui Knowledge Industry Co., Ltd., Tokyo, Japan). Lipid classification was performed by Lipid Search according to the LIPID MAPS (<https://www.lipidmaps.org/>, Liebisch *et al.*, 2020) and the method of Murphy (2015) described previously (Yamada *et al.*, 2013), and the MS/MS spectrum was compared with the lipid spectrum registered in the Lipid Search database. Some of the detected peaks had the same retention time in the positive and negative modes. In that case, the one with the larger peak area was selected.

Statistical analysis for metabolite profiling

Differences between the relative quantity of metabolites were evaluated using Welch's *t*-test. The data were presented as the means ($n = 3$), and *P*-values less than 0.05 were considered statistically significant.

DATA AVAILABILITY STATEMENT

RNA-seq data were deposited in the DDBJ Sequence Read Archive (<https://www.ddbj.nig.ac.jp/dra/index.html>), under accession number DRA011512. Metabolomics data were submitted to MetaboLights (<https://www.ebi.ac.uk/metabolights/>, Haug *et al.*, 2013), under study ID MTBLS2461.

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CONFLICT OF INTEREST

Tomohiro Tsurumoto and Yasuo Fujikawa are employees of Nichia Corporation. The other authors declare no conflicts of interests associated with this manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Common DEGs in 280-0d and 310-0d.

Table S2. Common DEGs in 280-2d and 310-2d.

Table S3. Enriched terms of KEGG pathway and gene ontology (GO) biological process for

505 sample-specific DEGs

506 **Table S4.** List of metabolites whose amounts were significantly changed by UV-LED irradiation.

507 **Table S5.** DEGs specifically up-regulated in 310-0d.

508

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Table 1. Classification of identified lipids whose amounts were significantly changed by UV-LED irradiation. Welch's *t*-test was performed to determine significant differences ($P < 0.05$) between the control and UV-LED irradiated Arabidopsis.

Group	Lipid class	Increased			Decreased		
		280-2d specific	310-2d specific	common	280-2d specific	310-2d specific	common
Neutral lipids	AcHexChE	1	-	-	-	-	-
	AcHexCmE	6	-	-	-	-	-
	AcHexSiE	2	-	-	-	-	-
	AcHexZyE	-	-	-	1	-	-
	CmE	-	-	-	2	-	-
	DG	2	-	-	-	-	-
	TG	13	1	1	19	4	7
Phospholipids	PA	-	-	-	-	-	1
	PC	-	-	-	1	-	-
	PE	4	-	-	14	-	1
	PG	3	1	-	3	-	-
	PI	2	-	-	3	-	-
	PIP2	-	-	-	-	-	1
	PS	2	1	-	9	-	-
Sphingolipids	Cer	17	-	1	3	-	-
	Hex1Cer	2	-	-	4	-	-
Derivatized lipids	BisMePA	-	-	-	1	2	2
Glycoglycerolipid	MGDG	-	-	-	3	-	-
Fatty acyl and other lipids	Co	1	-	-	-	-	-

AcHexChE, AcylGlcCholesterol ester; AcHexCmE, AcylGlcCampesterol ester; AcHexSiE, AcylGlcSitosterol ester; AcHexZyE, AcylGlcZymosterol ester; CmE, campesterol ester; DG, diglyceride; TG, triglyceride; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIP2, phosphatidylinositol; PS, phosphatidylserine; Cer, Ceramides; Hex1Cer, simple Glc series; BisMePA, bis-methyl phosphatidic acid; MGDG, monogalactosyldiacylglycerol; Co, coenzyme

720 **FIGURE LEGENDS**

721 **Figure 1.** Relative emission spectra of 280 and 310 nm UV light emitting diodes (LEDs) used in the
722 experiments.

723 The 280 nm UV-LED has a peak wavelength of 280 nm, a half-width of 10 nm, and a range from 260
724 to 310 nm. The 310 nm UV-LED has a peak wavelength of 310 nm, a half-width of 10 nm, and a range
725 from 290 to 340 nm.

726
727 **Figure 2.** Overall description of transcriptome data.

728 (a) Comparison of up-regulated and down-regulated numbers of DEGs under 280 and 310 nm UV.
729 Welch's *t*-test was applied for comparison of UV-irradiated samples with control, and a gene with a
730 *P*-value less than 0.05 and a two-fold change was considered as a DEG.

731 (b) Venn diagrams illustrating the shared and unique up-regulated and down-regulated DEGs between
732 samples under 280 and 310 nm UV-B.

733 (c) Gene ontology enrichment analysis of up-regulated and down-regulated DEGs in biological
734 processes and Kyoto Encyclopedia of Genes and Genomes pathway using DAVID ver. 6.8. Top five
735 terms were selected according to the *P*-values when more than five terms were enriched.

736
737 **Figure 3.** Overall description of metabolome data

738 (a) Comparison of increased and decreased numbers of metabolites under 280 and 310 nm UV
739 annotated by GC-MS. Welch's *t*-test was applied for comparison of UV-irradiated samples with
740 control, and a metabolite with a *P*-value less than 0.05 was considered significantly changed.

741 (b) Comparison of increased and decreased numbers of metabolites under 280 and 310 nm UV
742 annotated by LC-MS. Welch's *t*-test was applied for comparison of UV-irradiated samples with
743 control, and metabolite with a *P*-value less than 0.05 was considered significantly changed.
744 lipid-positive, lipids annotated in the positive ion mode; lipid-negative, lipids annotated in the
745 negative ion mode.

746 (c) Venn diagrams illustrating the shared and unique increased and decreased metabolites between
747 samples under 280 and 310 nm UV-B.

748
749 **Figure 4.** Metabolic responses in Arabidopsis to 280 and 310 nm UV- LED irradiation.

750 Welch's *t*-test was applied for comparison of UV-irradiated samples with control, and metabolite with
751 a *P*-value less than 0.05 was considered significantly changed. The annotated metabolites are shown in
752 squares and the degrees of changes are visualized by the colors: red, increased by UV-LED irradiation;
753 blue, decreased by UV-LED irradiation; gray, not significantly changed ($P \geq 0.05$).

754
755 **Figure 5.** Gene expression profiles of Cer synthase, PLDs, and markers in HR-type PCD under 280

756 and 310 nm UV-LEDs
 757 (a) Metabolic map of Cer biosynthesis. LCB, long-chain base; FA, Fatty acid, VLC, very-long-chain.
 758 (b) Metabolic map of PE hydrolysis.
 759 (c) Heat map showing the expression level of *LOH2*.
 760 (d) Heat map showing the expression level of the *PLDs*.
 761 (e) Heat map showing the expression level of HR-type PCD marker genes.
 762 Changes in gene expression levels relative to the control are expressed as $\log_2$ (fold change) values. As
 763 shown on the color scale, blue indicates down-regulation and red indicates up-regulation. Asterisks
 764 indicate significant differences between the control and UV-LED irradiation using Welch's *t*-test ($*P <$
 765 0.05).
 766

767 **Figure 6.** Gene expression profiles of enzymes in polyamine metabolism and GABA biosynthesis
 768 under 280 and 310 nm UV-LEDs

769 (a) Metabolic map of polyamine metabolism and GABA biosynthesis.
 770 (b) Heat map showing the expression level of enzymatic genes in polyamine metabolism and GABA
 771 biosynthesis.
 772 Changes in gene expression levels relative to the control are expressed as $\log_2$ (fold change) values. As
 773 shown on the color scale, blue indicates down-regulation and red indicates up-regulation. Asterisks
 774 indicate significant differences between the control and UV-LED irradiation using Welch's *t*-test ($*P <$
 775 0.05).
 776

777 **Figure 7.** Gene expression profiles of enzymes in TCA cycle under 280 and 310 nm UV-LEDs

778 (a) Metabolic map of TCA cycle.
 779 (b) Heat map showing the expression level of enzymatic genes in TCA cycle.
 780 Changes in gene expression levels relative to the control are expressed as $\log_2$ (fold change) values. As
 781 shown on the color scale, blue indicates down-regulation and red indicates up-regulation. Asterisks
 782 indicate significant differences between the control and UV-LED irradiation using Welch's *t*-test ($*P <$
 783 0.05).
 784

785 **Figure 8.** Gene expression profiles of enzymes and transcription factors involved in flavonoid
 786 biosynthesis under 280 and 310 nm UV-LEDs

787 (a) Metabolic map of flavonoid GABA biosynthesis.
 788 (b) Heat map showing the expression level of enzymatic genes in flavonoid biosynthesis.
 789 (c) Heat map showing the expression level of transcription factors in flavonoid biosynthesis.
 790 Changes in gene expression levels relative to the control are expressed as $\log_2$ (fold change) values. As
 791 shown on the color scale, blue indicates down-regulation and red indicates up-regulation. Asterisks

792 indicate significant differences between the control and UV-LED irradiation using Welch's *t*-test (**P* <
793 0.05). ND, not detected.

Figure 1

Figure 2

Figure 3

Figure 4

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Figure 5

Figure 6

Figure 7

Figure 8