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Metabolic changes in cucumber leaves are enhanced by blue light but differentially affected by UV interactions with light signalling pathways in the visible spectrum.

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ABSTRACT

Ultraviolet radiation (UV, 280–400 nm) as an environmental signal triggers metabolic acclimatory responses. However, how different light qualities affect UV acclimation during growth is poorly understood. Here, cucumber plants (*Cucumis sativus*) were grown under blue, green, red, or white light in combination with UV. Their effects on leaf metabolites were determined using untargeted metabolomics. Blue and white growth light triggered increased levels of compounds related to primary and secondary metabolism, including amino acids, phenolics, hormones, and compounds related to sugar metabolism and the TCA cycle. In contrast, supplementary UV in a blue or white light background decreased leaf content of amino acids, phenolics, sugars, and TCA-related compounds, without affecting abscisic acid, auxin, zeatin, or jasmonic acid levels. However, in plants grown under green light, UV induced increased levels of phenolics, hormones (auxin, zeatin, dihydrozeatin-7-N-dihydrozeatin, jasmonic acid), amino acids, sugars, and TCA cycle-related compounds. Plants grown under red light with UV mainly showed decreased sugar content. These findings highlight the importance of the blue light component for metabolite accumulation. Also, data on interactions of UV with green light on the one hand, and blue or white light on the other, further contributes to our understanding of light quality regulation of plant metabolism.

1. Introduction

Plants respond to fluctuations in the light environment through multiple mechanisms that enable them to acclimate to the new environment. The perception of light quantity, quality, and duration (Fankhauser and Chory J, 1997) induces diverse plant responses, including changes in metabolism. By sensing light through several photoreceptors, plants can detect and respond to a wide and complex range of light signals (Fankhauser and Chory J, 1997; Heijde and Ulm, 2012; Rai et al., 2021). Within the visible region of the spectrum, red (600–700 nm) and far-red radiation (700–800 nm) are perceived by phytochromes (Sharrock and Quail PH, 1989), whereas blue (400–500 nm) radiation is absorbed by cryptochromes and phototropins (Briggs and Huala, 1999; Briggs and Christie, 2002). Moreover, both phytochromes and cryptochromes are sensitive to green light (500–600 nm) but trigger weaker responses upon its absorption (Folta and Maruhnich, 2007). Outside the visible spectra, ultraviolet (UV) radiation B (280–315 nm) is perceived by the UV RESISTANCE LOCUS 8 (UVR8) photoreceptor (Rizzini et al., 2011; Wu et al., 2011), whilst UV-A (315–400 nm) can be perceived by UVR8 (UV-A_{SW}, 315–350 nm), cryptochromes and phototropins (UV-A_{LW}, 350–400 nm) (Rai et al., 2021; Ahmad and Cashmore, 1993; Christie et al., 1998).

Light greatly impacts plant metabolism (Ohashi-Kaneko et al., 2007; Li and Kubota, 2009; Mizuno et al., 2011; Zhao et al., 2020). In response to increased UV radiation, plants accumulate a plethora of UV-absorbing compounds with strong antioxidant capacity that protect the plant from potential UV damage (Lattanzio et al., 2006; Agati and Tattini M, 2010). Flavonoids, in particular anthocyanins and hydroxy-cinnamic acids,

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accumulate in the vacuoles of epidermal cells in response to UV radiation and act both as antioxidants and as sunscreens, attenuating the penetration of UV radiation into the deeper cell layers without affecting the absorption of visible light (Lattanzio et al., 2006; Agati and Tattini M, 2010; Day et al., 1993; Hideg and Strid, 2017). Both under artificial and natural conditions, flavonoid glycosides and hydroxycinnamic acids are accumulated in response to low doses of UV radiation (Krizek et al., 1997; Tegelberg et al., 2004; Morales et al., 2013; Qian et al., 2019; Rai et al., 2019; Palma et al., 2021).

Compared to the number of studies focusing on the effects of the spectral composition of light on post-harvest quality (Jensen et al., 2018; Li et al., 2019; Wu et al., 2020; Lafuente et al., 2021), the effects of light quality on plant primary metabolism have been studied to a lesser extent. In recent years, we have advanced our understanding of how UV regulates metabolic responses in plants grown under broadband white light background and solar photosynthetic active radiation (PAR, 400–700 nm) (Lattanzio et al., 2006; Agati and Tattini M, 2010; Kotilainen et al., 2008; Morales et al., 2010; Morales et al., 2011; Siipola et al., 2015; Castro-Alves et al., 2021). However, how different monochromatic light backgrounds affect the metabolic responses remains poorly understood. Furthermore, it is unclear how different light qualities interact with UV radiation to regulate the metabolite composition.

Plant responses to UV radiation are dependent on dosage, prior acclimation to UV, surrounding light environment, and other abiotic factors (Rai et al., 2021; Hideg and Strid, 2017; Huché-Thélier et al., 2016; Jenkins, 2017). In addition, UV-induced responses are also dependent on the levels of PAR and the UV:PAR ratio (Hideg and Strid, 2017; Jenkins, 2017; Krizek, 2004; Lidon et al., 2012). While different PAR intensities provided by artificial light or natural sunlight have been used to assess the effects of UV radiation on plant metabolism, how individual wavebands within the PAR region affect UV acclimatory responses at the metabolic level is still unexplored.

Metabolomics approaches allow for a qualitative and quantitative analysis of the dynamic changes of all metabolites in a single cell, tissue, or whole organism under specific conditions (Yan et al., 2019). In plant metabolomics studies, ultra-performance liquid chromatography (UHPLC) and quadrupole-time of flight (Q-TOF) mass spectrometry (MS) has been widely used due to its high quality and reproducibility, high sensitivity, good chromatographic resolution, and handling capacity.

In this study, cucumber (*Cucumis sativus* L.) was selected as a model plant due to its sensitivity to light quality and fast growth rate, as well as its importance as a food crop. Cucumber plants have thus been used in studies of regulation of plant development and physiology by UV radiation in a number of laboratories for almost four decades (Krizek et al., 1997; Qian et al., 2019; Palma et al., 2021; Qian et al., 2020; Qian et al., 2021; Palma et al., 2021; Murali and Teramura, 1986; Adamse and Britz, 1992; Fukuda et al., 2008; Shinkle et al., 2010; Yamasaki et al., 2014). The effects of different monochromatic lights and supplementary UV radiation on metabolite profiles in cucumber leaves were investigated using untargeted metabolomics. We hypothesised that: (I) different monochromatic wavelengths of light trigger differential metabolite changes in cucumber leaves, and (II) the response of cucumber to UV radiation is highly dependent on the monochromatic light backgrounds.

2. Materials and methods

2.1. Plant material and growing conditions

Cucumber seeds (*cv.* 'Lausanna RZ F1', Semenco, Asmundtorp, Sweden) were individually germinated in plastic pots (0.24 L) containing peat substrate (Grön Torvmull 50-liter, SW Horto, Hasselfors Garden, Örebro, Sweden). Upon cotyledon expansion, the seedlings were transferred to a room without natural light at 22 ± 1/18 ± 1 °C day/night temperature and 60 ± 5% relative humidity and randomly placed in four custom-made trolleys containing four different LED assemblies (blue, green, red, and broadband white) as described in Palma et al. (2021a). After a period of acclimation to the light environment of 9 days, 72 plants in each trolley were randomly divided into two treatments: (i) light background alone and (ii) light background supplemented with UV radiation. Fresh leaf tissue samples of four cucumber seedlings grown under each light quality were harvested after 14 days of UV exposure. The samples were from the first fully expanded leaf counted from the top of each plant. One leaf was harvested per plant and there were 6 replicates per treatment. The leaves were harvested right after the UV exposure ended in the afternoon (15:00 h), frozen in liquid nitrogen, and stored at − 80 °C until further use.

2.2. Light treatments

Seedlings were grown with 200–212 μmol m^{-2} s^{-1} PAR under four different growth light regimens: (1) blue (wavelength peak at 448 nm); (2) green (528 nm); (3) red (660 nm); and (4) broadband white (33% blue [400–500 nm], 40% green [500–600 nm] and 27% red [600–700 nm]) created with FL300 Sunlight LED luminaires (Senmatic, Søndersø, Denmark) (Palma et al., 2021a).

Two open top, front and backside Perspex boxes (OTFB boxes; *c.f.* Qian et al., (2019)) were placed in each trolley to filter the UV radiation provided by fluorescent tubes (Philips TL20/12 UV, Eindhoven, The Netherlands). For the control plants, all UV radiation was blocked by covering the OTFB boxes with sheets of Perspex, whilst for the UV-treated plants, the UV-C radiation (< 292 nm) emitted by the UV lamps was blocked from reaching the plants by using 0.13 mm cellulose diacetate (CA) sheets (Nordbergs Tekniska AB, Vallentuna, Sweden).

An OL756 double monochromator spectroradiometer (Optronic Laboratories, Orlando, FL, USA) was used to determine the spectral composition and photoirradiance inside the OTFB boxes (Palma et al., 2021a). The plant-weighted UV normalized to 300 nm (Thimijan et al., 1978; Yu and Björn, 1997; Kalbina et al., 2008) was quantified to 42.4 ± 3.4 mW m^{-2}, corresponding to 0.912 ± 0.074 kJ m^{-2} day^{-1} obtained by a 6 h daily UV exposure (Palma et al., 2021).

2.3. Non-target metabolite profiling

2.3.1. Metabolite extraction

Frozen material (35 mg) was extracted with 1 mL of cold methanol containing 0.1% of formic acid and 1 μg/mL of each internal standard (IS; ferulic acid-d3, glutamic acid-d5, and succinic acid-d4) for normalization purposes. Samples were then vortex-mixed and ultrasonicated for 10 min. After centrifugation (11,000 g, 10 min), 400 μL of supernatant was collected and vortex-mixed with 220 μL of cold chloroform and 440 μL of water for clean-up. The upper phase was transferred to LC-vials after another round of centrifugation (2200 g, 15 min). Equal aliquots from the upper phase of samples were also mixed to prepare a pooled quality control (QC) sample. A total of 12 QC injections were performed along the sample worklist to further select features based on their stability (%RSD in QC injections < 20%). Extraction blanks were also prepared. All samples were kept at − 80 °C until analysis.

2.3.2. Ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS)

A UHPLC 1290 Infinity system (Agilent Technologies, Santa Clara, CA) was used for analysis. A mixed-mode (reversed-phase/anion-exchange) Atlantis Premier BEH C18 AX column (2.1 ×100 mm, 1.7 μm; Water, Milford, USA) was used for analysis. Mobile phases were composed of 0.2% (v/v) formic acid and 10 mM ammonium formate either in (A) water or (B) acetonitrile (95% v/v in water). The isocratic flow (0.4 mL/min) started with 0% B (0 – 2 min), 0 – 100% B (2 – 4 min), 100% B (4 – 8 min), and re-equilibration with 0% B for 5 min (13 min/sample). The column and auto sampler temperatures were maintained at 50 °C and 10 °C, respectively. The injection volume was 5 μL. The

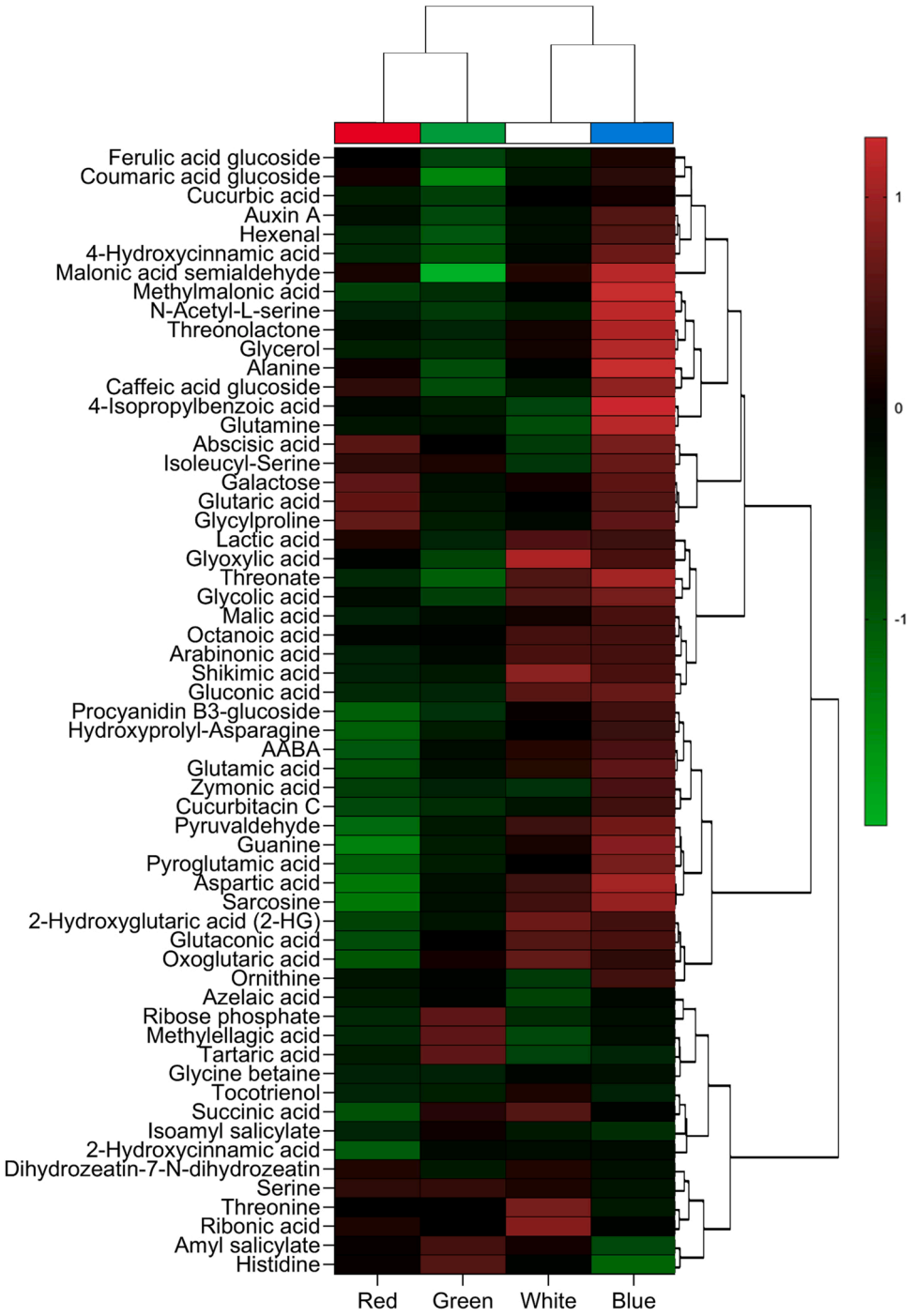

Fig. 1. Heat map of hierarchical clustering analysis of metabolic changes in cucumber leaves developed under the four different growth light backgrounds without UV supplementation, 14 days after the start of the UV experiment. Data are mean values of three independent experiments (n = 3 replicates, each containing four individual leaves collected from six different plants).

UHPLC system was interfaced with a dual ESI source to a 6545 QTOFMS system (Agilent Technologies). The QTOFMS system was programmed as follows: acquisition in negative ion mode (2 spectra/s) with *m/z* range 50 – 1200, collision energy 0 V, capillary voltage 3.6 kV, nozzle voltage 1500 V, and N_2 pressure at nebulizer, flow rate and temperature as sheath gas set at 21 psi, 10 L/min, and 379 °C, respectively. Pooled samples were also analysed using collision energies of 10, 20, and 40 V for confirmation of fragmentation patterns. Calibration curves (100, 500, 1000, 2500, and 5000 ng/mL) using 69 analytical standards were also prepared to evaluate analysis performance. MassHunter B.06.01 software (Agilent Technologies) was used for data acquisition.

UHPLC-QTOFMS data pre-processing and analysis performance: Data pre-processing was performed using the MZmine 2.53 software (Du et al., 2020). The pre-processing steps included (1) mass detection, (2) chromatogram building, (3) chromatogram deconvolution, (4) isotopic peak grouper, (5) alignment, (6) filtering, and (7) gap filling. The following parameters were applied: (1) mass detection on centroid mode with a noise level 500; (2) ADAP chromatogram builder with a minimum of 4 scans, minimum span count 100 group intensity threshold 100, and *m/z* tolerance 0.009 *m/z*; (3) chromatogram deconvolution using the local minimum search algorithm with 70% of chromatographic threshold, minimum RT in range 0.07 and minimum ratio of peak/top

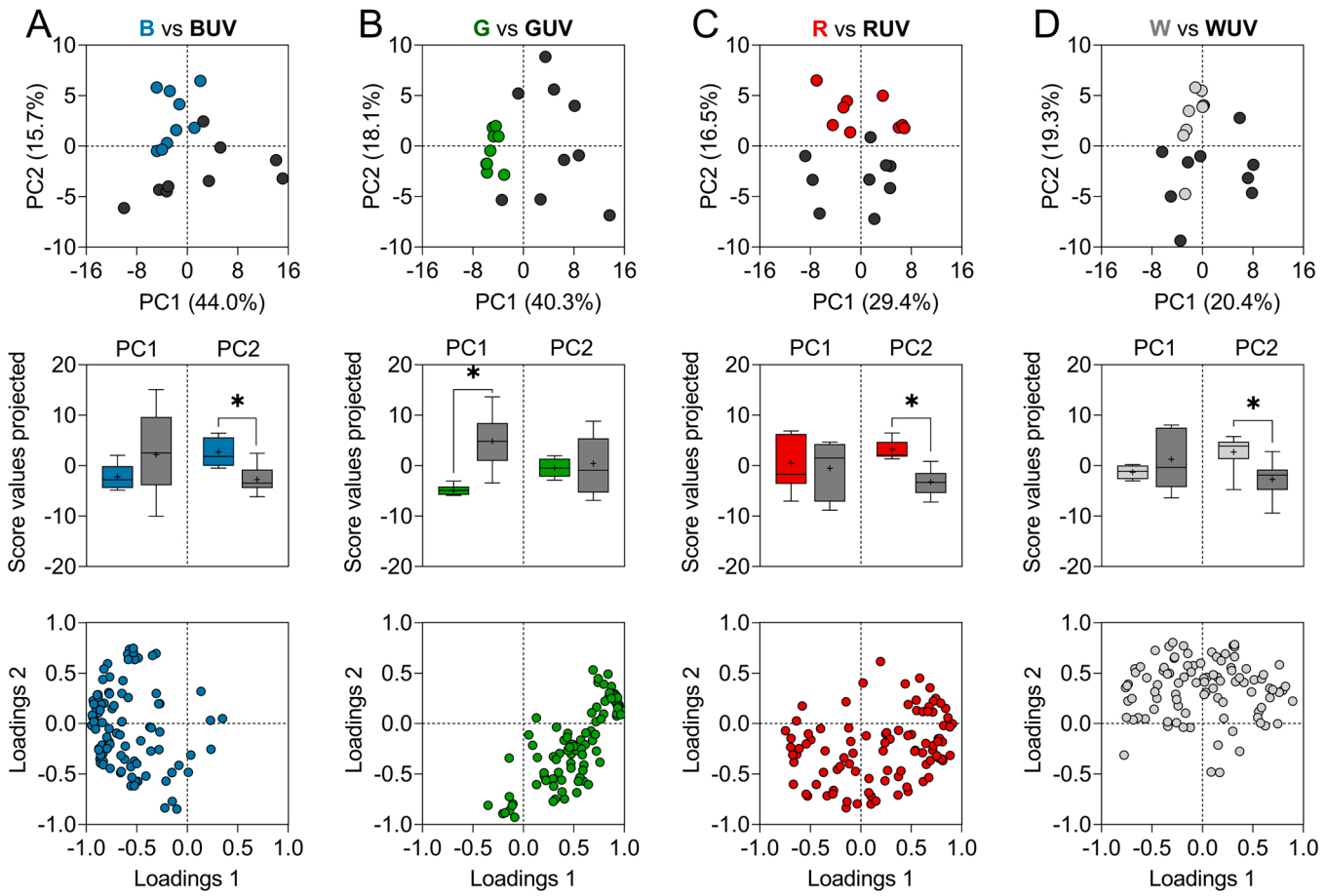

Fig. 2. PCA plots (upper row), score values projected (middle row) and loadings (bottom row) for samples from cucumber leaves grown under (A) blue, (B) green, (C) red, or (D) white background light and with or without 14 days of exposure to UV light.

edge 1.3; (4) isotopic peak grouper with maximum charge of 2 and the most intense isotope set as the representative isotope; (5) alignment with 0.01 (or 10 ppm) *m/z* tolerance (weight 2) and an RT tolerance of 0.15 min (weight 1), with comparison of spectra similarity and no requirement of charge state or ID and isotope pattern; (6) peak list row filters to maintain peaks found on at least 74 samples (20% of the samples), (7) gap-filling with intensity, *m/z* and RT tolerance of 4%, 0.01 *m/z* (or 12 ppm) and 0.2 min, respectively.

The peak list was exported as.mgp file for spectral information and as.csv for metadata (RT, *m/z*, and base peak area). Samples were normalized by weight and by internal class-specific standards (valine-d8 [amino acids], succinic acid-d4 [organic acids], and ferulic acid-d3 [phenolic acids]) for identified compounds and closest internal standard RT for other compounds. Only features with QC:blank ratio > 5 and %RSD $< 20\%$ (in QC samples) were maintained in the final peak list. Imputation of missing values was calculated as one-fifth of the feature's minimum observed value.

Three levels of identification were defined for the features: metabolites with the same RT, precursor *m/z* and fragmentation pattern of in-house standards (level 1), metabolites with the same precursor *m/z* and fragmentation pattern to that of compounds available in METLIN database (level 2) and metabolites with same precursor *m/z* to that of compounds available either in METLIN or FoodDB (level 3).

2.4. Statistical analysis

The data were collected from three independent experiments, log-transformed and auto scaled before statistical analysis. Analysis of variance (ANOVA) was performed in R (version 3.3.1., R Core Development Team, 2017) to identify differences in metabolite levels between the treatments. Linear mixed-effects models were fitted using the nlme package (Pinheiro et al., 2022) with experimental replicate as random-grouping factors. Significant main effects of the light background were identified by fitting contrasts between the four light backgrounds using the function fit.contrasts from the gmodels package (Warnes et al., 2015). The function p.adjust (Holm, 1979) was used to adjust all the p-values from individual contrasts. For each annotated compound, the effects of the different light backgrounds on metabolite accumulation were tested for significance ($p < 0.05$). Hierarchical Cluster Analysis (HCA) for different light backgrounds was performed in Metaboanalyst 5.0 using the Euclidean distances. The effect of UV radiation was tested solely within the same light background. Principal Component Analysis (PCA) was applied for dimension reduction and significance tests were performed on the score values projected onto PC1 and PC2 to validate differences. Linear mixed-effects models with experimental replicate as a random factor were used to test differences between control and UV-treated plants. Differences were assessed using ANOVA and the output of the statistical analysis is described in the Supplemental Material.

3. Results

The effects of four different growth light backgrounds on the metabolite profiles were assessed both in plants exposed to UV-enriched supplementary radiation and in non-UV-treated plants 14 days after the commencement of UV exposure.

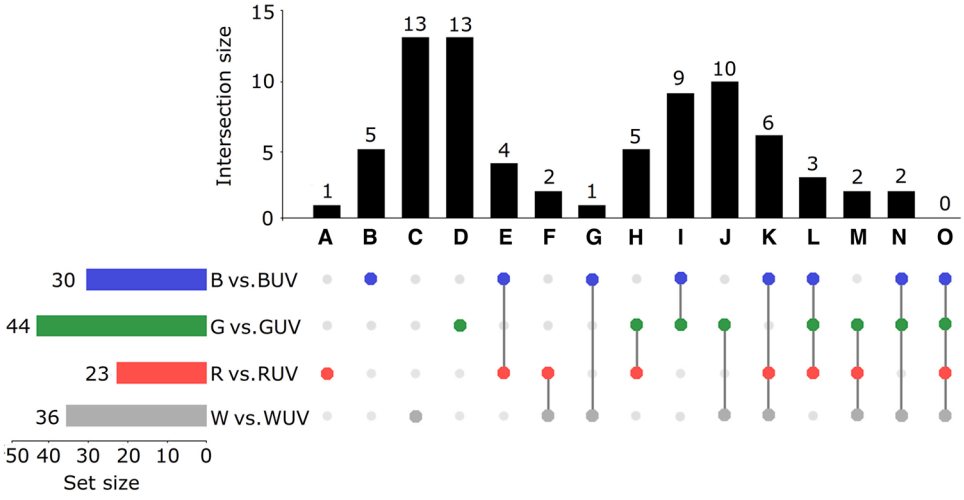

Fig. 3. UpSet plot for metabolic changes (increases and decreases) of cucumber plants grown under the four different growth light qualities. The number of metabolites regulated by UV light under only one of the four growth light qualities is shown in the first four columns (A-D). The number of metabolites regulated by UV light under two of the four growth light qualities, i.e. six possible permutations, are shown in columns (E-J). The number of metabolites regulated by UV light under three of the four growth light qualities, i.e. four possible permutations, are shown in columns (K-N). Finally, the number of metabolites regulated by UV light under all four growth light qualities, i.e. 0 metabolites, are shown in column O. The coloured bars on the left indicate the number of metabolites that were significantly affected by supplemental UV light in plants grown under blue, green, red or white light.

3.1. Metabolic changes in cucumbers grown under different growth light qualities

A total of 90 metabolites were annotated after data processing and filtering (Table S1), out of which 59 were affected by the different growth light backgrounds (Table S3). Cluster analysis was performed using these 59 compounds to assess similarities between metabolite profiles induced by the different light growth qualities. As shown in Fig. 1, blue-enriched light backgrounds (white and blue light) had distinct effects on metabolite changes compared to blue-depleted light environments (green and red light).

The growth light background affected the concentration of amino acids involved in protein synthesis (Fig. 1, Table S3). Glutamine, alanine, and aspartic acid levels were highest in plants grown under blue light in comparison with the other light treatments, whereas glutamic acid concentrations were higher in plants grown under white and blue light. In contrast, the levels of histidine and serine were lowest in plants grown under blue light, whereas threonine was significantly higher in plants grown under white light compared to blue or green-light-grown plants.

Compounds related to the sugar metabolism were also affected by the growth light quality (Fig. 1, Table S3). Plants grown under blue light showed increased content of threonolactone and white-light grown plants had higher ribonic acid and arabinonic acid content compared to those grown under green or red light. Gluconic acid content was significantly higher in plants grown under white or blue light compared to green or red light-grown plants.

The spectral distribution of the growth light background affected compounds related to the TCA cycle (Fig. 1, Table S3). Plants grown under white or blue light had higher 2-Hydroxyglutaric acid, glycolic acid, and oxoglutaric acid content compared to those grown under green or red light. Malonic acid semialdehyde, methylmalonic acid, and glycerol contents were highest in plants grown under blue light. Plants grown under blue light showed higher content of glutaric acid and malic acid compared to green or red light-grown plants, respectively. Plants grown under white light had higher glyoxylic acid content than those grown under green or red light and showed higher succinic acid content compared with red light-grown plants.

The light quality also had an impact on the content of hormones including abscisic acid, auxin, and dihydrozeatin-7-N-dihydrozeatin (Fig. 1, Table S3). Plants grown under blue light showed higher levels of auxin compared to plants grown under green light, and the abscisic acid content was higher in plants grown under blue or red light compared with those grown under white light.

The growth light quality also affected pathways leading to biosynthesis of different phenolic compounds including aromatic amino acids, hydroxycinnamates, and flavonoids and their glycosylated derivatives, such as anthocyanins (Fig. 1, Table S3). Plants grown under white light had the highest levels of shikimic acid, a precursor for the synthesis of aromatic amino acids, while 4-hydroxycinnamic acid was found at higher levels in plants grown under blue light. Caffeic acid content was highest in plants grown under blue or red light and coumaric acid glucoside showed increased accumulation in blue light-grown plants compared to plants grown under green or white light. Plants grown under blue light showed higher ferulic acid glucoside content than green-light-grown plants, and higher levels of procyanidin B3-glucoside compared to plants grown under green or red light. The content of 2-hydroxycinnamic acid was higher in blue- or white-light-grown plants compared to those grown under red light, whereas green light-grown plants had the highest methylellagic acid concentration.

3.2. UV regulation of plant metabolite profiles is dependent on growth light quality

We assessed the effects of UV radiation on metabolite profiles in plants grown under different monochromatic light backgrounds. The PCA in Fig. 2 shows a clear separation of metabolite composition in leaves from non-UV-treated and UV-treated plants under all four growth light qualities. In plants grown under blue, red, or white light, UV treated plants are separated from non-UV treated mainly in PC 2 which explains 19.3%, 15.7%, and 16.5% of the total variability (see upper row in Fig. 2A, C, and D), respectively. In contrast, plants grown under green light and exposed to UV radiation were clearly separated from the non-UV-treated plants along PC1, which explained 40.3% of the total variance (Fig. 2B).

Scattering of data was most pronounced in PC1 in UV-treated samples (Fig. 2, middle row), where the scattering was larger than in non-UV-treated samples. However, in red light, PC1 scattering was also substantial in plants devoid of UV treatment. In PC2, noteworthy scattering was only found in the UV-treated plants grown under green light.

The number of metabolites affected by UV exposure differs among plants grown under different light backgrounds: 30 under blue, 44 under green, 23 under red, and 36 under white light (Fig. 3, Tables 1 and S2).

Overall, supplemental UV radiation triggered changes in amino acid content depending on the growth light quality (Table 1). The content of amino acids for protein synthesis increased in green- and red-light-grown plants after exposure to UV light but decreased in plants grown under blue or white light as compared to non-UV-exposed plants.

Notably, supplemental UV radiation given to plants grown under a green light background led to increased levels of metabolites related to sugar metabolism, when compared to plants that were not exposed to UV radiation, whilst supplemental UV decreased the levels of these

Table 1

Metabolic changes in plants grown under blue, green, red, or white light backgrounds after 14 days of UV exposure. Arrows represent decreased (↓) or increased (↑) levels of metabolites after UV exposure compared with the UV-free-control.

Metabolic pathway	Compound	Treatments			
		B vs BUV	G vs GUV	R vs RUV	W vs WUV
Sugars	Ribonic acid	↓	-	↓	-
	Arabinonic acid	↓	↑	-	-
	Erythrose	↓	-	↓	↓
	Galactose phosphate	-	-	-	↓
	Gluconic acid	-	↑	-	↓
	Galactose	↓	-	↓	↓
	Threonolactone	-	-	-	↓
Amino acids for protein synthesis	Glutamine	↓	-	↑	↓
	Glycine	-	-	-	↓
	Alanine	↓	↑	-	-
	Asparagine	-	↑	-	↓
	Glutamic acid	-	↑	-	↓
	Histidine	-	↑	-	-
	Serine	-	-	-	↓
	Threonine	-	-	-	↓
	GABA	↓	-	-	-
	Aspartic acid	-	-	↑	↓
Tricarboxylic acid cycle (TCA)	Glyceraldehyde	-	-	-	↓
	Glutaric acid	↓	↑	↓	-
	Glyoxylic acid	↓	↑	-	↓
	Malic acid	-	-	-	↓
	Malonic acid semialdehyde	↓	↑	-	-
	Oxalosuccinic acid	-	↑	↑	-
	Fumaric acid	-	-	-	↓
	Succinic acid semialdehyde	-	↑	-	-
Hormones	Abscisic acid	-	-	-	-
	Auxin A	-	↑	-	-
	Zeatin	-	↑	-	↓
	Dihydrozeatin-7-N-dihydrozeatin	↓	↑	-	-
	Jasmonic acid	-	↑	-	-
Phenolics					
Flavonids and anthocyanins:	Tetrahydroxyflavanone	-	↑	-	↑
	Procyanidin B3-glucoside	-	↑	-	↑
	Cyanidin diferuloylsophoroside 5-glucoside	-	↑	-	-
Hydroxy-cinnamates and their glucosides:	4-Hydroxycinnamic acid	-	↑	-	-
	Caffeic acid glucoside	↓	↑	-	↓
	Caffeic acid sulfate	-	↑	-	-
	Ferulic acid glucoside	↓	↑	-	-
	2-Hydroxycinnamic acid	-	↑	↑	-
	Coumaric acid glucoside	-	↑	-	↓
Precursors and other branches:	Shikimic acid	-	-	-	↓
	Gallic acid sulfate	↓	↑	-	-
	Syringic acid	↑	↑	-	-
	Methylellagic acid	↑	-	-	-

metabolites in plants grown under the other three light qualities (Table 1). The levels of TCA cycle-related metabolites also increased in plants grown under green light after UV exposure and decreased in plants grown under white or blue light.

Hormone accumulation (auxin A, zeatin, dihydrozeatin-7-N-dihydrozeatin, jasmonic acid) was also triggered by UV light in plants grown under green light (Table 1). In contrast, supplemental UV decreased the content of cytokinins in blue- or white-light-grown plants. UV did not influence the levels of abscisic acid in the plants, irrespectively of under what light quality they had been grown.

Among the 14 annotated metabolites related to the phenylpropanoid pathway, 11 metabolites changed in response to UV radiation in plants grown under green light (Table 1). Compared to plants receiving only green light, UV light induced the accumulation of seven hydroxycinnamic acids and their glucosides and sulfates (4-hydroxycinnamic acid, caffeic acid glucoside, caffeic acid sulfate, ferulic acid glucoside, ferulic acid sulfate, 2-hydroxycinnamic acid, and coumaric acid glucoside), two phenolic acids (gallic acid 3-sulfate, syringic acid), and three flavonoids and anthocyanins (tetrahydroxyflavanone, cyanidin 3-(diferuloyl-sphoroside) 5-glucoside, and procyanidin B3-glucoside). In contrast, plants exposed to UV under blue light background showed a decrease in all metabolites related to the hydrocinnamate or flavonoid pathway but

increased the content of the phenolics syringic acid and methylellagic acid. Meanwhile, plants grown under white light showed a metabolite-specific response to UV light, leading to increased content of tetrahydroxyflavanone and procyanidin B3-glucoside and a decrease in caffeic acid glucoside and coumaric acid glucoside levels, i.e. a down-regulation of the coumaric branch of the pathway but an up-regulation of the flavonoid/anthocyanin branch. Furthermore, red light-grown plants showed no effect of UV light in the accumulation of compounds related to the phenylpropanoid pathway, except for an increase in 2-hydroxycinnamic acid content (Table 1).

4. Discussion

Plants synthesize a panoply of compounds in response to changes in the light environment. Specific wavelengths, such as blue light and UV radiation, play a key role in regulating several plant metabolic responses in natural sunlight (Kotilainen et al., 2008; Morales et al., 2010; Morales et al., 2011; Siipola et al., 2015). However, how monochromatic light backgrounds alone or in combination with UV radiation affect plant metabolic responses is still unclear. Here, we show that the metabolite profile is differentially affected by the spectral composition of the growth light background alone or in combination with UV.

The different light qualities within the visible spectrum differentially affected the levels of metabolites related to both primary and secondary metabolism. Higher levels of amino acids for protein synthesis and metabolites related to the sugar metabolism and the TCA cycle were observed in plants grown under blue or white light compared with under green or red light. Similarly, plants grown under blue light showed an increased content of compounds related to the phenylpropanoid pathway. The role of blue light in regulating free amino acid or sugar metabolism has been previously described in tomato (Dhakal and Bae, 2014), Chinese kale (Li et al., 2019), barley (Koga et al., 2013), and wheat (Toldi et al., 2019).

Our data also demonstrate that hormone levels are affected by the spectral distribution of light. Plants grown under blue light showed increased auxin (IAA) levels compared to plants grown under green light, while abscisic acid content was higher in plants grown under blue or red light compared to white light. It was previously demonstrated that blue light stimulates the expression of abscisic acid biosynthetic genes in barley embryos and cut carnation flowers (Gubler et al., 2008; Aalifar et al., 2020), as well as the expression of IAA biosynthetic genes in rosemary (Gil et al., 2021). Additionally, a higher abscisic acid content was observed in barley grain in response to blue light compared with grain kept in darkness or treated with red or far-red light (Gubler et al., 2008), whereas an increased endogenous free auxin content was observed in CRY1-overexpressing transgenic *Arabidopsis* seedlings compared with the wild type (Zeng et al., 2010). Thus, our metabolite data, combined with earlier reports from other species, indicate that blue light plays a fundamental role in the regulation of both primary and secondary metabolism. Plants grown under broadband white light showed similar responses to those of blue-light-grown plants, possibly due to the fraction of blue light (33%) emitted by these lamps, while the lower levels of some metabolites that were observed in plants grown under green and red light could potentially be associated with a blue-light-depleted environment.

We also found that UV-acclimatory responses in cucumber are compound-specific and dependent on the quality of the growth light. After 14 days of UV exposure, plants grown under white and blue light showed decreased levels of sugars, amino acids for protein synthesis, phenolics, and hormones. In our experiment, the UV treatment used primarily activated UVR8 (Rai et al., 2019; Rai et al., 2020) as it is enriched in wavelengths below 350 nm (Palma et al., 2021). As such, the UV-induced changes in metabolite profiles in cucumber leaves are expected to be largely mediated by UVR8. A role for UVR8 in triggering metabolite changes, through the induction of the phenylpropanoid metabolism and flavonoid accumulation, has been shown (Morales et al., 2013; Favory et al., 2009; Demkura and Ballaré, 2012; Liu et al., 2018). Previous research has also demonstrated that cryptochromes mediate blue light repression of UVR8 signalling through REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP) proteins (Tissot and Ulm, 2020). Thus, it is possible that suppression of UVR8 signalling by cryptochromes mediated the decreased levels of metabolites observed in plants exposed to UV under the blue light background. However, further experiments will be required to rule out this hypothesis.

Interestingly, plants grown under green light and exposed to UV showed an overall increased concentration of sugars, amino acids, phenolics, and hormones, indicating a positive interaction between UV and green light perception. The green light-induced responses are most likely mediated by cryptochromes or an unknown photoreceptor (Palma et al., 2021). While blue light absorption induces cryptochrome activation, exposure to green light returns the cryptochrome to a biologically inactive state by triggering a different redox state of the active flavin chromophore (Talbott et al., 2006; Smith et al., 2017). Although, our results show that blue light alone (or as a component of white light) in the absence of UV triggers more metabolic changes than that of green light alone, adding the UV component leads to an overall decrease in the metabolite levels of leaves exposed to blue or white growth light, in contrast to the case of plants grown under green light. The effect of UV leading to increased metabolite content in green-light-grown plants could result from a lower metabolite level prior to UV exposure, while plants grown under blue light already had a higher content of many of these compounds.

There is an almost uniform increase in the metabolites studied in plants grown under green light and treated with 14 days UV and the corresponding almost monotonous decrease in metabolites in UV-treated plants grown under blue or white light (Table 1). This raises the question of whether the observed differences might arise from a systematic error. The only error we could anticipate is if the UV treatments would have led to substantial changes in fresh or dry weights of the plants (since 35 mg fresh weight material was used for each sample; see the Materials and Methods section). However, in the first paper in our series of studies on the same plants (see Fig. 2c in Palma et al., 2021), it is obvious that the UV treatment led to a small but statistically significant decrease in dry matter which was similar in size independently of what colour of light the plants had been grown under. In turn, this rules out any systematic error caused by differences in the amounts of material used.

In response to UV, red-light grown plants showed lower accumulation of compounds related to sugar metabolism while the levels of amino acids, phenolics, and hormones were generally not affected, apart from isolated changes in a few compounds. However, our data do indicate a negative crosstalk between UV and red light perception that may specifically regulate sugar metabolism. Furthermore, the lack of metabolic changes in response to red light alone and in combination with UV suggests that cryptochrome blue light receptors are the ones that play an essential role in regulating overall plant metabolite levels.

The scattering of data in Principal Component Analysis (Fig. 2) seems to be confined to PC1 and UV-treated plants, although red-light-grown control plants also showed substantial data scattering. Since there was much less scattering in PC2, except for green-light-grown plants that had also been subjected to UV treatment, and we have no indication of any discrimination in handling of the UV-treated and control plants, we do not consider this result to be due to any systematic difference in how the experiments have been performed.

Further studies using photoreceptor mutants are required to unravel detailed mechanisms mediating metabolite accumulation of plants exposed to UV during growth under different monochromatic light backgrounds. Also, metabolic responses may be highly species-specific and subjected to diurnal changes, both being factors that need to be considered.

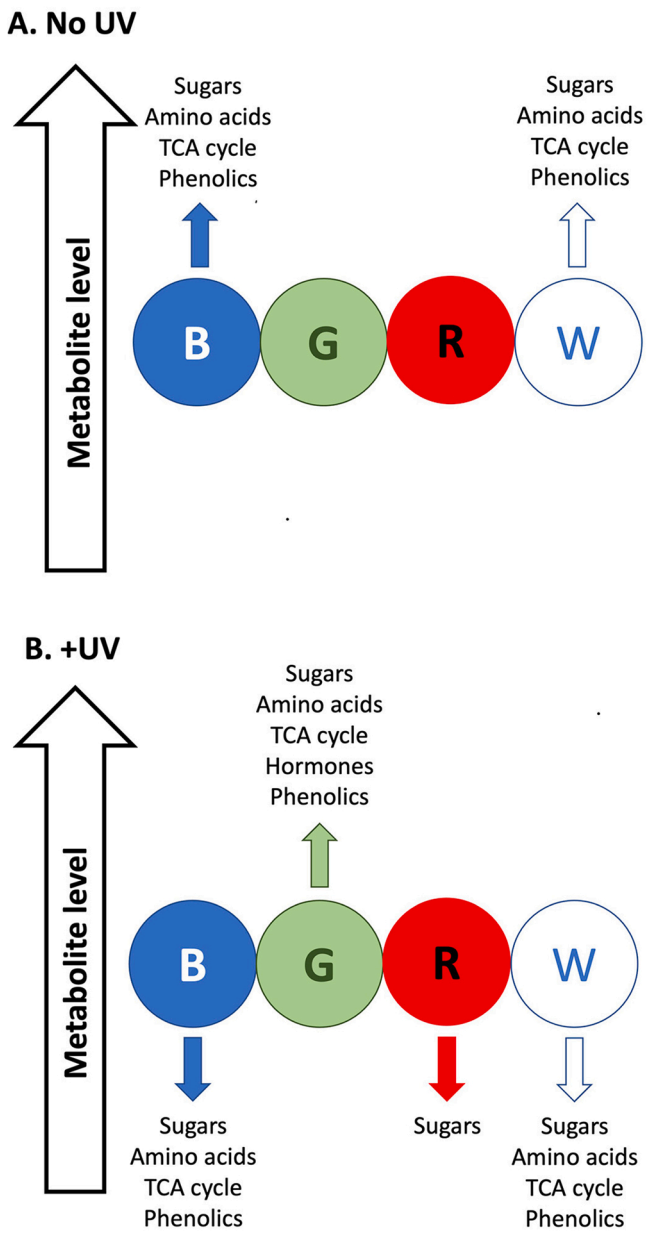

Fig. 4. A summary of the overall pattern of regulation of the accumulation of selected classes of metabolites in cucumber leaves as a function of both different growth light qualities and supplementary UV.

5. Conclusions

The growth light background influences the metabolite profile of cucumber leaves in a wavelength-dependent manner, confirming our first hypothesis. Generally, plants grown under blue and white growth light had higher metabolite concentrations compared to plants grown under green and red light backgrounds (Fig. 4A), indicating the importance of the blue light component for regulation of metabolite levels. In agreement with our second hypothesis, in cucumber leaves, UV responses are highly dependent on the colour of the light background. Supplemental UV antagonistically decreased the content of many metabolites in plants grown under white or blue light, while plants grown under green light showed increased metabolite levels when exposed to UV radiation (Fig. 4B). Thus, both monochromatic light backgrounds alone and in combination with UV radiation triggered light-dependent acclimatory responses in cucumber. Furthermore, these acclimatory responses likely resulted from crosstalk between different receptors, highlighting the importance of considering the spectral composition of light when assessing UV-induced metabolic changes in controlled environments. Our findings would be a valuable contribution to the design of light recipes targeting the production of specific compounds to improve crop quality in horticultural production settings.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Author contributions

CFFP designed the experiment with contributions by LM, ER, C-OO, and ÅS. CFFP performed the experiment and analysed the data with contributions by TH, VC-A, and LM. VC-A performed metabolite analysis and prepared the figures. CFFP wrote the manuscript with contributions from VC-A, LM, TH, ER, C-OO, and ÅS. All authors contributed to the article and approved the submitted version.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.plantsci.2022.111326.

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