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ORIGINAL ARTICLE

Blue light is a key factor in regulating stomatal movement, transpiration rate and ABA metabolism under varying vapour pressure

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Abstract

Vapour pressure deficit (VPD) and blue light (BL) are import signals for stomatal regulation and highly relevant during cultivation of economically important crops such as cucumber in controlled environments. However, the information about combined effects of VPD and BL and the regulation of abscisic acid (ABA) in this respect, is limited and seems to vary between species. This study investigated how moderate (1.12 kPa) or low (0.28 kPa) VPD combined with 5% or 30% BL affect the foliar ABA metabolism, transpiration rate, nutrient uptake, and growth in well-watered cucumber plants (*Cucumis sativus* 'Quatro'). Additional BL, regardless of VPD, resulted in a decrease in the nighttime ABA content (52%), as well as increased adaxial stomatal density (8–19%), transpiration rate during the day (40–50%) and foliar nitrogen content (30%). ABA regulation was unaffected by VPD, but the BL levels during the day influenced the ABA inactivation at night; both the transpiration rate and the ABA-glucose ester (ABA-GE) content then increased in 30% BL compared to 5% BL. An increase in starch degradation due to additional BL led to higher content of the transport carbohydrate raffinose in source leaves and increased the fruit appearance rate (fruit day^{-1}) in low VPD but reduced it in moderate VPD. This study provides novel insight into the regulation of ABA under different VPDs and BL proportions in cucumber, and reveals that under well-watered conditions, BL is a stronger determinant of stomatal aperture regulation than ABA. Also, a combination of high VPD and high BL can act as a weak stressor and affect cucumber growth and yield.

Keywords Blue light · Morphology · Foliar nitrogen · Carbohydrate metabolism · Stomata · Transpiration rate · Vapour pressure deficit

Introduction

Plant transpiration depends mainly on the stomatal opening, which is controlled by a pair of specialised epidermal guard cells located on the adaxial and/or abaxial leaf surfaces (species-dependent). Stomata respond to the surrounding environment, balancing water loss through stomata with the need for CO_2 uptake in photosynthesis (Tallman 2004). Changes in leaf-to-air vapour pressure deficit (VPD) is one of the main force driving transpiration in plants, and the rate decreases with decreasing difference in VPD (Armstrong and Kirkby 1979; Grange and Hand 1987). The ideal VPD range for plant growth and development is typically from 0.3–1.0 kPa. However, high air humidity (VPD < 0.4 kPa) is common in controlled environment agriculture (CEA) (Shamshiri et al. 2018), and particularly within the micto-climates of dense plant crown or canopy, creating VPD gradients between the canopy and surrounding air (Fanourakis

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et al. 2020). The hormone abscisic acid (ABA) is an important regulator of water status of plants and plays a significant role in signals controlling transpiration and growth in both stressed and unstressed plants (Yoshida et al. 2019). Lower ABA content has been measured in plants grown in lower compared to higher VPD (Aliniaeifard and van Meeteren 2013; Arve et al. 2013; Fanourakis et al. 2016). In addition, an ABA-deficient tomato mutant ('*flacca*') showed a more pronounced increase in biomass in response to low VPD compared to the corresponding wild type (WT) (Innes et al. 2021). In low VPD, '*flacca*' had a higher transpiration rate during both day and night than the WT (Innes et al. 2021). Therefore, differences in transpiration and growth in low VPD seen in different species and genotypes can be linked to differences in ABA content and/or ABA metabolism.

In leaves, the amount of ABA is determined by the balance between biosynthesis and inactivation via catabolism or conjugation (Cutler and Krochko 1999; Nambara and Marion-Poll 2005). Whilst stomatal closure in response to high VPD (low air humidity) involves rapid changes in expression of ABA biosynthesis genes (McAdam and Brodribb 2016), exposure to low VPD was reported to result in inactivation of ABA rather than changes in ABA biosynthesis (Okamoto et al. 2009). ABA can be inactivated by one of two pathways; the oxidative catabolic pathway that converts ABA to phaseic acid (PA) by the enzyme (+)-ABA 8′-hydroxylase (Nambara and Marion-Poll 2005), and the conjugation with glucose to form ABA-glucose ester (ABA-GE) which is considered a storage and transport form of ABA (Cutler and Krochko 1999). ABA-GE can later be cleaved by β-D-glucosidase (BG) and ABA can be released when needed (Lee et al. 2006). Increased activity of the CYP707A genes that encode ABA 8′-hydroxylases, and accordingly oxidation of ABA into PA, was found at night in *Arabidopsis thaliana* growing at low VPD and this correlated with reduced dark-induced stomatal closure (Arve et al. 2015). However, in the woody species *Rosa* x *hybrida*, more ABA was conjugated into ABA-GE in low VPD, and not released during night like in higher VPD, resulting in lower active ABA content and more open stomata. Clearly, different ABA inactivation pathways are apparently mobilised in different species in response to low VPD, and this differs in light and darkness (Arve 2013; Arve et al. 2015; Arve and Torre 2015; Innes et al. 2021).

In addition to VPD, radiation has a strong impact on stomatal opening and transpiration, and both light irradiance and -quality affect the transpiration rate substantially. Stomata normally open in response to blue light (BL) (400–500 nm), when plants are grown under well-watered conditions. Even low levels of BL can induce stomatal opening in isolated stomatal guard cells as well as intact leaf tissue (Matthews et al. 2020). Amongst photoreceptors involved in BL sensing, cryptochromes (CRY) and phototropins (PHOT) are the best known, but also phytochromes sense BL in addition to being red and far-red light sensor. In addition to regulating the stomatal opening, such photoreceptors control a range of different plant processes such as photomorphogenic responses, vegetative growth, flowering and branching (Briggs and Huala 1999). Moreover, BL triggers specific biochemical and physiological processes leading to increased degradation of starch in leaves and guard cells and increased biosynthesis of secondary metabolites, such as the phenolic compounds flavonoids as well as phytohormone synthesis and signalling (Dong et al. 2021; Flütsch et al. 2020; Kubasek et al. 1992; Sharma et al. 2014; Terfa et al. 2020). Whilst stomatal responses to BL are modulated by VPD, it is less known how whole plant transpiration rate and growth are affected under combinations of different VPDs and BL proportions (Assmann and Grantz 1990; Terfa et al. 2020).

The effect of BL on stomatal opening is important, because gas exchange is necessary not only for photosynthesis but also for water and nutrient uptake from the roots. In many plant species, including cucumber, a low level of BL and low VPD reduce uptake and translocation of nitrogen (N) (Bakker and Sonneveld 1988; Clavijo-Herrera et al. 2018; Gislerod et al. 1987; Jiao et al. 2022; Lihavainen et al. 2016; Xu et al. 2021). N is essential for photosynthesis and growth as it is a key component of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites (Wang et al. 2018). It has been found that the photosynthetic rates and/or the spectral distribution of the light during daytime can influence nighttime transpiration. In *Rosa* x *hybrida*, increased irradiance during the day resulted in faster stomatal closure in response to darkness (Blomzandstra et al. 1995). Furthermore, in orange light a low irradiance (25 µmol m^{-2} s^{-1}) of BL prevented stomatal closure 2.5 h into the night compared to white light (control) (Blomzandstra et al. 1995). On the other hand, cultivation of *Rosa* x *hybrida* with additional BL (30%) resulted in improved dark-induced stomatal closure (Terfa et al. 2020). The signals involved in dark-induced stomatal closure are unclear, but N status may play a role. The pattern of nighttime water use has been found to be related to foliar N content in different woody species (Rohula et al. 2014). Some studies have also linked ABA signalling to dark-induced stomatal closure, but the exact involvement is unclear. However, recent research suggested that whilst ABA signalling and metabolism are not essential for dark-induced closure, these do affect the speed of the response (Pridgeon and Hetherington 2021).

Cucumber is an economically important crop produced in controlled environment and responds strongly to VPD and BL (Bakker et al. 1987; Liang et al. 2021). Given the importance of stomatal regulation, there is a need to improve the understanding of ABA metabolism in response to VPD and

BL. Photomorphogenesis signalling pathways interact with the ABA signalling pathway at several points (Mukherjee et al. 2023). For example, a *cry*-mutant showed increased level of ABA in the leaves, indicating that diminished BL-sensing by CRY results in increased ABA content (Boccalandro et al. 2012). Therefore, it is reasonable to assume that VPD and the proportion of BL affect the foliar content of ABA and its metabolites. Indeed, studies of *Araidopsis thaliana* have shown that under drought, the BL-stimulation of stomata opening is effectively suppressed by ABA, resulting in stomatal closure and minimised water loss (Inoue and Kinoshita 2017). Yet, the inverse occurs under well-watered conditions. BL then suppresses ABA signalling to promote stomatal opening. To the best of our knowledge, ABA biosynthesis and the catabolic/conjugation pathways of ABA in cucumber leaves in response to VPD and BL are not known. In this study, the experimental set-up was designed to investigate interactions between VPD and BL on transpiration and growth. In addition, we aimed to clarify the inactivation pathways of ABA in cucumber in response to VPD and BL during day and night and to test the hypothesis that BL can increase transpiration and improve the foliar N-content and growth of cucumber in low VPD.

Materials and methods

Pre-cultivation and experimental set-up

Seeds of snack cucumber (*Cucumis sativus* L. cultivar Quarto, from LOG, Norway) were sown directly into pots (12 cm) filled with sphagnum peat (Degernes Torvstrøfabbrikk AS, Degernes, Norway) in a greenhouse compartment at 23 °C, 70% relative air humidity (VPD of 0.84 kPa) and ambient CO_2 (400 ppm) at the Norwegian University of Life Sciences (NMBU). When the first true leaves were expanding, plants were transferred to growth chambers at 23 °C with either a VPD of 1.12 kPa or 0.28 kPa (corresponding to 60% and 90% relative air humidity, respectively) and ambient CO_2 levels (400 ppm). In the growth chambers, the experiments were set up in a 2x2 factorial design with the two VPD levels and two light environments containing different amounts of blue light, giving a total of four growth treatments. All plants received 20 h of photosynthetically active radiation (PAR; 400–700 nm) at a photon flux density of 200 µmol m^{-2} s^{-1} from either high-pressure sodium lamps (HPS, Gavita Superagro, Norway), or a combination of HPS + BL, and four hours of darkness per day (Tables 1, S1). Chambers with BL received 50 µmol m^{-2} s^{-1} from light emitting diodes (LEDs) (400–500 nm, peak at 460 nm, Philips GreenPower LED module HF Blue) and 150 µmol m^{-2} s^{-1} from 400 W HPS lamps as measured with a Li-COR LI 190 SA quantum sensor (LI-COR Inc. USA). The spectra (S1) for both light treatments were measured with an OceanOptics SD2000 spectrometer (model SD2000, OceanOptics, Eerbeek, The Netherlands). The amount of BL was 5% and 30%, calculated by adding the intensity of all wavelengths between 400–500 nm and then calculating the percentage of the total intensity between 400–700 nm (Table 1). The plants were watered daily at the same time right after the light was turned on with a 50/50 mixture of YaraLiva® Calcinit™ calcium nitrate solution (14.4% NO_3, 1.1% NH_4, 19.0% Ca, Yara Norge AS, Oslo, Norway) and Kristalon™ Indigo (7.5% NO_3, 1% NH_4, 4.9% P, 24.7% K, 4.2% Mg, 5.7% S, 0.027% B, 0.004% Cu, 0.06% Mn, 0.2% Fe, 0.004% Mo, 0.027% Zn, Yara, Oslo, Norway). The EC level was 1.7–2.0 mS cm^{-1}. Trays were placed under the pots and runoff was removed 30 minutes after the watering. The climate in the greenhouse compartment and the growth chambers was controlled by a PRIVA system (Priva, De Lier, The Netherlands).

Table 1 Description of the light quality treatments in the growth chambers used in the study of cucumber cultivated with moderate and low VPD (1.12 kPa or 0.28 kPa)

Blue light[a]	Photosynthetic Active Radiation (µmol m^{-2} s^{-1})		Total PAR	R/FR	PSS[b]	Plant temperature (°C)[c]
	HPS	Blue LED				
5%	200	–	200	3.5	0.70	23
30%	150	50	200	3.5	0.70	23

The main light source was High Pressure Sodium (HPS) lamps (Osram NAV T-400W): 5% blue light (BL) and the 30% BL treatment (HPS + BL) was supplemented with blue light emitting diodes (Philips GreenPower LED module HF Blue, Philips, Eindhoven, The Netherlands). See S1 for the spectral distribution

PAR photosynthetically active radiation, *R/FR* red/far red ratio

[a]The amount (%) of BL was calculated by adding the intensity of all wavelengths between 400–500 nm and then calculating the percentage of the total intensity between 400–700 nm

[b]The phytochrome photostationary state (PSS) was calculated according to Sager et al. (1988)

[c]Plant temperature was measured with an infra-red sensor in the shoot apical region of the seedlings

Growth, chlorophyll and measurements of nutrients

A main focus of this study was to understand effects of VPD and BL on the transpiration rate of whole plants (see below). Hence, plants were harvested after two weeks of growth when they had 10–12 leaves. The total plant height was measured from the base of the shoot to the shoot apical meristem. The number of leaves and cucumbers > 1 cm were recorded. Fresh and dry weights (DW) of the leaves, stems and side shoots were measured at harvest. Dry weight was determined after drying the plant material for 5 days at 56 °C. Leaf area was measured with a leaf area meter (LI-3100, Li-Cor Inc.) at the end of the experiment for each treatment, and specific leaf area (SLA) was calculated as the leaf area/leaf DW ratio: SLA = total leaf area/total leaf DW. The relative chlorophyll content (dimensionless), determined using dual-wavelength optical absorbance, was measured with a handheld chlorophyll content meter on each side of the mid-vein of one expanded leaf per plant (Hansatech instrument model CL-01 Instruments Ltd, UK). For analyses of macro- and micronutrients, expanded leaves from the middle part of the plants from four random plants in each treatment were harvested in the middle of the day (8 h after light turned on) and used for nutrient analysis. The leaves were dried at 56 °C prior to grinding. Element analysis of macro- and micronutrients were performed using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). Total nitrogen content (N_{tot}) and carbon was measured using the Dumas method at LabTek, NMBU.

Transpiration rate

To analyse transpiration from whole plants, the pots were covered with plastic bags to prevent water loss through evaporation from the soil. The pots were weighed right before dark (EOD) and right after the dark period (EON) for three consecutive days. After the measurements, the leaf area was determined with a leaf area meter (LI-COR, LI-3100). The rate of water loss per leaf area per second (transpiration rate) was calculated with the following equation:

$$\text{Transpiration rate } \left(\mu\text{mol } H_2O\ m^{-2}s^{-1}\right) = \frac{\Delta W}{t * A * 3600 * 10,000 * M_{H_2O}} * 10,00,000$$

ΔW is the weight difference between EOD or/EON and the previous period, t the duration of the dark- or light period (h), A the total leaf area (cm^2), and M_{H_2O} the molar mass of water (18.01528 g mol^{-1}).

Microscopy analysis of leaves and stomatal imprints

Stomatal imprints for aperture measurements

To measure stomatal apertures of the plants under different growth conditions, epidermal imprints were made of the third leaf from the base of the plants. This was done using Suzuki's Universal Micro-Printing (SUMP) method consisting of SUMP liquid and SUMP plate B (SUMP Laboratory, Tokyo, Japan) as described previously (Tanaka et al. 2005). Imprints were taken in the middle of the light and dark period in all treatments after 2 weeks of cultivation. The SUMP imprints were observed under a light microscope (Leitz, Labolux K, Type 0.2, Wetzlar, Germany) and stomata images were obtained with a Leica camera (Leica DC200, Heerbrugg, Switzerland). Stomatal aperture was measured using Fiji (Fiji Is Just ImageJ, http://fiji.sc/). Cucumber has stomata on both sides of the leaves but due to the high number of trichomes it was not possible to take good imprints on the adaxial side of the leaves.

Scanning electron microscopy for stomatal density and length calculations

To count the number of stomata (stomatal density), scanning electron micrographs (SEM) were taken on both abaxial and adaxial leaf side. Source leaves from four random plants were selected and more than 20 small pieces (3 × 3 mm^2) from the middle of the leaf, close to the mid-vein were cut and fixed in 2% paraformaldehyde and 1.25% glutaraldehyde in 50 mmol/L L-piperazine-N-N′-bis(2-ethane sulfonic) acid buffer (pH 7.2) for 24 h at room temperature. Fixed tissue was rinsed with the same buffer and dehydrated in an ethanol series (70-80-90-96-4× 100%) before being dried using a critical point dryer (BAL-TEC CPD 030, BAL-TEC AG). The dried samples were mounted onto studs and sputter coated with a gold–palladium mix using a Polaron SC 7640 Sputter Coater (Quorum Technologies, Ltd.). Random pieces ($n = 5$) were taken for further analysis and at least five areas per piece were analysed for stomatal density and stomatal length. For each treatment the length of at least 150 stomata were measured. The samples were analysed using a Zeiss EVO 50 Scanning Electron Microscope.

Light microscopy for leaf anatomy and carbohydrate visualisation

For light microscopy (LM), leaf samples were dehydrated in a graded ethanol series before being infiltrated with L.R. White acrylic resin (TAAB Laboratories, Aldermaston,

Berkshire, England), and polymerised at 60 °C for 24 h. For LM study, semi-thin (1–2 µm) sections were cut with a glass knife. The sections were dried on slanted slides and stained with Stevenel`s blue, mounted with immersion oil, and imaged in a Leitz Aristoplan light microscope (Ernst Leitz Wetzlar, Wetzlar, Germany). For carbohydrate staining, the periodic acid/Schiffs (PAS) method was used.LM sections were oxidised with periodic acid (1%) for 30 min, then washed with distilled water, and treated with Schiffs reagent (Sigma Chemical Co.) for 2 h in darkness. Control slides were incubated in distilled water prior to treatment with Schiffs reagent. After several washes, the sections were dried, mounted, and imaged as above.

ABA and ABA metabolite quantification

Fully developed source leaves were sampled five hours after the beginning of the light period and in the middle of the night, immediately flash frozen in liquid nitrogen and stored at −80 °C. Samples from three individual plants were harvested per treatment after two weeks of cultivation.

Chemicals and calibration curves

DPA, ABA-GE, PA, 7′-OH-ABA, *neo*PA, and *trans*-ABA as well as deuterated forms of these substances, which were used as internal standards; d3-DPA, d5-ABAGE, d3-PA, d4-7′-OH-ABA, d3-*neo*PA, d4-ABA and d4-*trans*-ABA, as well as two deuterated compounds used as recovery (external) standards; d6-ABA and d2-ABA-GE, were synthesised and prepared at the National Research Council of Canada, Saskatoon, SK, Canada (NRCC SK) (Abrams et al. 2003; Zaharia et al. 2005). Calibration curves were created for all compounds of interest.

Instrumentation

Analysis was performed on a UPLC/ESI-MS/MS utilising a Waters ACQUITY UPLC system, equipped with a binary solvent delivery manager and a sample manager coupled to a Waters Micromass Quattro Premier XE quadrupole tandem mass spectrometer via a Z-spray interface. MassLynx™ and QuanLynx™ (Micromass, Manchester, UK) were used for data acquisition and data analysis.

Extraction and purification

An aliquot (100 µL) containing the deuterated internal standards, each at a concentration of 0.2 ng μL^{-1}, was added to weighed homogenised plant tissue; 3 mL of isopropanol:water:glacial acetic acid (80:19:1, v/v/v) was then added, and the samples were agitated in the dark for 24 h at 4 °C. The samples were then centrifuged, and the supernatant was isolated and dried on a Büchi Syncore Polyvap (Büchi, Switzerland). The samples were reconstituted in 100-µL acidified methanol, adjusted to 1 mL with acidified water, and then partitioned against 2-mL hexane. After 30 min, the aqueous layer was isolated and dried as above. Dry samples were reconstituted in 100 µL acidified methanol and adjusted to 1 mL with acidified water. The reconstituted samples were loaded onto equilibrated Oasis HLB cartridges (Waters, Mississauga, ON, Canada), washed with acidified water, and eluted with acetonitrile:water:glacial acetic acid (30:69:1, v/v/v). The eluate was then dried on a LABCONCO centrivap concentrator (Labconco Corporation, Kansas City, MO, USA). An internal standard blank was prepared with 100 µL of the deuterated internal standards mixture. Quality control standards (QCs) were prepared by adding 100 µL and 30 µL (separately) of a mixture containing the analytes of interest, each at a concentration of 0.2 ng μL^{-1}, to 100 µL of the internal standard mix. Finally, the samples, blanks, and QCs were reconstituted in an aqueous solution of 40% methanol (v/v), containing 0.5% acetic acid and 0.1 ng μL^{-1} of each of the recovery standards.

Hormone and hormone metabolite quantification by UPLC-ESI-MS/MS

The samples were subjected to UPLC-ES-MS/MS analysis and quantification (Ross et al. 2004). The samples were injected onto an ACQUITY UPLC® HSS C18 column (2.1 × 100 mm, 1.8 µm) with an ACQUITY HSS C18 VanGuard Pre-column (2.1 × 5 mm, 1.8 µm) and separated by a gradient elution of water containing 0.025% acetic acid against an increasing percentage of acetonitrile containing 0.025% acetic acid. Briefly, the analysis utilised the Multiple Reaction Monitoring (MRM) function of the MassLynx v4.1 (Waters Inc) control software. The resulting chromatographic traces were quantified off-line by the QuanLynx v4.1 software (Waters Inc) wherein each trace was integrated, and the resulting ratio of signals (non-deuterated/internal standard) was compared with a previously constructed calibration curve to yield the amount of analyte present (ng per DW sample). Calibration curves were generated from the MRM signals obtained from standard solutions based on the ratio of the chromatographic peak area for each analyte to that of the corresponding internal standard (Ross et al. 2004). The QC samples, internal standard blanks and solvent blanks were also prepared and analysed along each batch of tissue samples.

Carbohydrate and flavonoid measurements

Analyses of carbohydrate and flavonoids were performed on fully developed source leaves harvested in the middle of

the day, flash frozen in liquid nitrogen and stored at −80 °C prior to extraction.

Flavonoid analysis

Freeze dried crushed plant material was extracted five times with methanol (MeOH) before the MeOH was evaporated under vacuum and the dried residue was frozen. The residue was redissolved in MeOH and water (200 + 200 µl) before being centrifuged, poured through syringe filters and sealed into High-Performance liquid Chromatography (HPLC) vials. Flavonoids were analysed by HPLC (Agilent, Series 1100, Germany), which consisted of a binary pump (G1312A), a thermostated autosampler (G1329A), a thermostated column oven (G1316A) and a diode array detector (G1315B). The metabolites were separated using an ODS Hypersil C18 (4.6 × 50 mm) HPLC column (Thermo Scientific, Waltham, Massachusetts, USA). The samples were eluted (flow rate 2 ml min^{-1}) using a MeOH:water gradient (Nybakken et al. 2012). The auto-injection volume was 20 µl and all runs were performed at 30 °C. Identification of metabolites was completed by comparison of retention times. Standard curves for certain compounds were obtained from a previously reported study by Da Graça Campos and Markham (2007).

Carbohydrate analysis

The analyses of the contents of soluble carbohydrates, including stachyose, raffinose, glucose, sucrose, and fructose in cucumber leaves, were conducted according to the principle outlined by Pharr et al. (1985) and details described in Sonsteby et al. (2016). Carbohydrates were extracted by heating the samples of approximately 100 mg in 1.5 ml of 80% ethanol at 70 °C for 30 min using an ultrasonic bath. Ethanol was evaporated from the supernatant at 60 °C using a vacuum desiccator (Eppendorf AG 22331, Hamburg, Germany). Subsequently, 1 ml of water was added to the dried extract, and the mixture was heated at 60 °C for 30 min before being centrifuged at 15,000 rpm for 3 min. The resulting supernatant was collected separately and filtered through a 0.45 µm GHP membrane filter (Millipore) in preparation for HPLC analysis.

For analyses of starch, the soluble carbohydrates were first extracted as described above from samples of approximately 200 mg, and the supernatant with the soluble sugars was discarded. Starch in the precipitate were solubilised by adding 2 ml dimethylsulfoxide (DMSO) and placing the tube in a boiling water bath for 5 min. Immediately, 2.9 ml MOPS buffer (pH 7) and 0.1 ml thermostable amylase (*B. licheniformis*, Megazyme) was added, and the tube was incubated 6 min in the boiling water bath. For hydrolysis of the starch, the tube was then placed in a 50 °C water bath and 4 ml sodium acetate buffer (pH 4.5) and 0.1 ml (20 units) amyloglucosidase (*A. niger*, Megazyme) was added and the tube was incubated for 30 min at 50 °C. After the hydrolysis of starch, the glucose content was analysed by HPLC. Standards of pure starch hydrolysed together with the plant samples.

Both soluble carbohydrates and glucose after hydrolysis of starch were analysed with HPLC (Series 1200 HPLC, Agilent Technologies, Waldbronn, Germany) using an Agilent Hi-Plex Ca USP L19 column (4.0 × 250 mm). The mobile phase was water with a flow rate of 0.3 ml min^{-1}, and the column temperature was maintained at 80 °C. 10 µl of the extracted sample was injected to the HPLC column and eluted carbohydrates were detected with a refractive index detector. They were identified and quantified by comparison of retention times and areas of standard sugars and starch.

Statistical analysis

The growth experiment was repeated three times. The trends in growth and transpiration rate were similar in all three repeated experiments, and the data was accordingly merged in the statistical analysis. Samples for ABA ($n = 3$), nutrients ($n = 4$), carbohydrate ($n = 5$) and flavonoid ($n = 5$) analysis were taken from the first and third experiment. The results were analysed using a normally distributed general linear model (GLM) and Tukey's HSD post-hoc test. Differences with $p \leq 0.05$ were considered significantly different. All statistical analyses were performed in Minitab 16.1.1 (Minitab 16.1.1, windows version, State College, Pennsylvania, USA).

Results

Growth and morphology

Total plant height was significantly shorter when plants were exposed to 30% BL compared to 5% BL, but the response was dependent on VPD ($p < 0.05$), with a 40% and 23% reduction in moderate and low VPD, respectively (Table 1). A similar trend was found for leaf expansion. The average leaf area per plant was reduced by 10% in low VPD but by almost 20% in moderate VPD in response to 30% BL (Table 2, Fig. 1). A significant interaction between VPD and BL was found for the leaf unfolding rate and fruit appearance rate ($p < 0.05$). In low VPD, 30% BL increased the leaf unfolding rate (leaf day^{-1}) and fruit appearance (cucumber day^{-1}), but an opposite response was found in moderate VPD (Table 2). The leaf unfolding rate and fruit appearance rate were similar for plants grown in low VPD + 30% BL and moderate VPD + 5% BL (Table 2, Fig. 1). The dry weight (leaves + stem) was significantly reduced in plants

Table 2 Effect of VPD and proportion of blue light (BL) on foliar C/N ratio, growth, morphology, size and fruit appearance, and relative chlorophyll content of cucumber plants (*Cucumis sativus* 'Quarto')

	Moderate VPD		Low VPD	
	30% BL	5% BL	30% BL	5% BL
Foliar C/N ratio	9.8 ± 0.22^{c}	13.2 ± 0.74^{b}	12.1 ± 0.69^{bc}	16.2 ± 0.94^{a}
Plant height (cm)	35.0 ± 1.16^{c}	59.38 ± 1.27^{a}	47.81 ± 1.84^{b}	62.81 ± 1.86^{a}
Leaf area/leaf (cm^2)	128.4 ± 4.7^{bc}	159.2 ± 4.1^{a}	126.2 ± 1.9^{c}	137.3 ± 5.7^{b}
Specific leaf area ($cm^{-2}\ g^{-1}$)	299.8 ± 12.3^{a}	333.4 ± 6.9^{a}	314.5 ± 7.2^{a}	319.0 ± 21.9^{a}
Leaf unfolding rate (leaf day^{-1})	0.90 ± 0.01^{c}	0.95 ± 0.02^{b}	1.00 ± 0.1^{a}	0.95 ± 0.02^{b}
Dry weight, stem + leaves (g)	6.31 ± 0.12^{c}	7.84 ± 0.26^{a}	6.86 ± 0.21^{bc}	7.02 ± 0.29^{b}
Developing fruits day^{-1}	0.52 ± 0.02^{b}	0.64 ± 0.02^{a}	0.64 ± 0.02^{a}	0.51 ± 0.04^{b}
Fruit length/thickness ratio	2.45 ± 0.07^{b}	2.60 ± 0.15^{ab}	3.10 ± 0.19^{a}	1.30 ± 0.04^{c}
Relative chlorophyll content*	28.26 ± 1.99^{a}	20.55 ± 0.75^{b}	25.93 ± 0.96^{a}	17.55 ± 0.80^{b}

Plants were cultivated in moderate (1.12 kPa) and low VPD (0.28 kPa) in combination with 5% and 30% BL for 14 days in growth chambers with 23 °C and a PAR of 200 µmol $m^{-2}\ s^{-1}$. Data are means ± SE, and different letters in a line indicate significant differences (Tukey HSD, $p < 0.05$), ($n = 8$)

*Dimensionless measurement, provided as a relative unit ranging from 0–2000 units. Absorbance ratios between red (660 nm) and near-infrared (940 nm) regions are used as a proxy for the amount of chlorophyll in the leaf

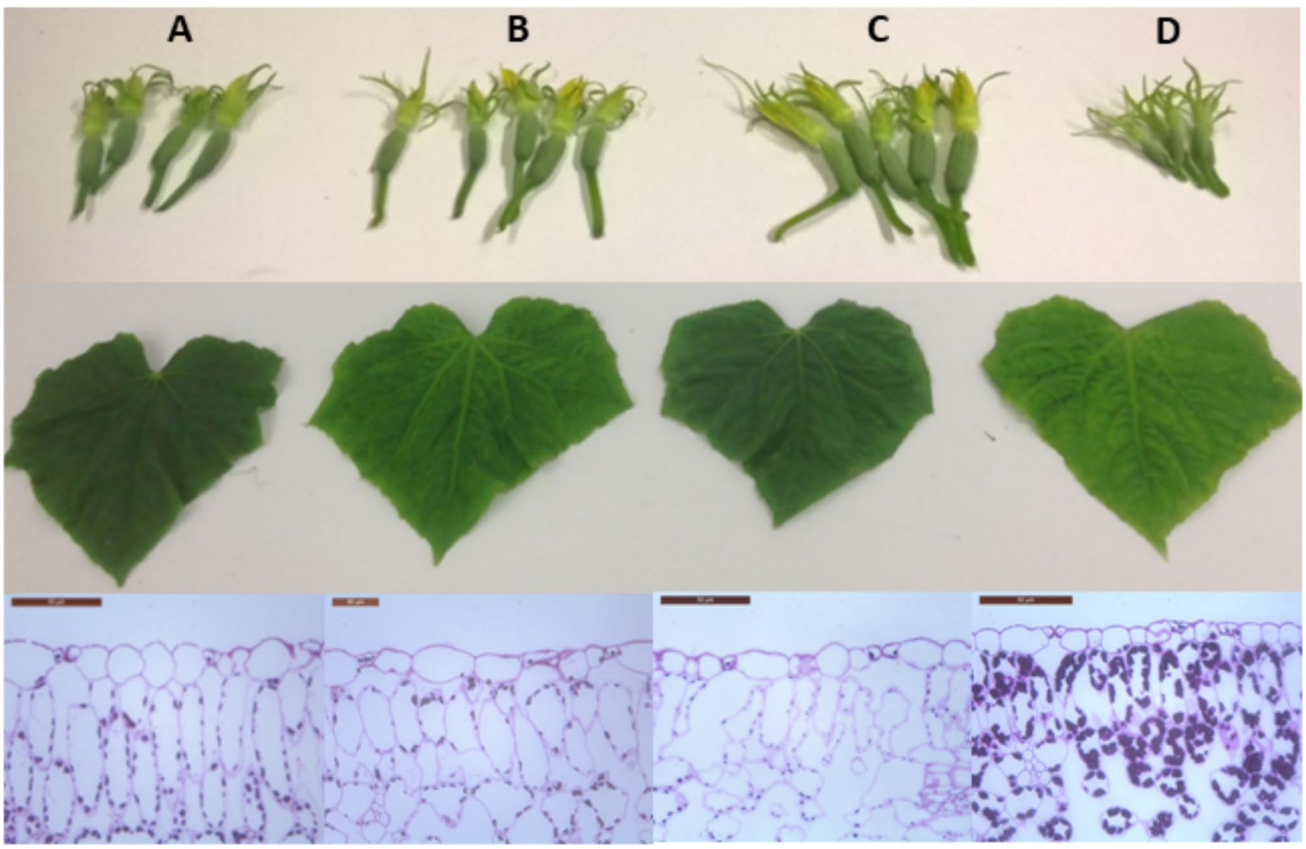


Fig. 1 The size of developing cucumber fruits and fully expanded leaves taken from cucumber plants (*Cucumis sativus* 'Quarto') grown in moderate (1.12 kPa) and low VPD (0.28 kPa) in combination with 5% and 30% blue light (BL) for 14 days in growth chambers with 23 °C and a PAR of 200 µmol $m^{-2}\ s^{-1}$. Column A: Moderate VPD + 30% BL, Column B: Moderate VPD + 5% BL, Column C: Low VPD + 30% BL, Column D: Low VPD + 5% BL. The images at the bottom show LM cross sections from the middle part of the leaves, stained with carbohydrate staining (PAS) (LM bar = 50 µm). See whole cross sections from the same leaves stained with Stevenels blue in S2

exposed to 30% BL in moderate VPD, but no significant effect of additional BL was found in low VPD (Table 2).

The leaf anatomy changed in response to 30% BL in moderate VPD, and the palisade cells were shorter and tended to be organised in a double layer in moderate VPD + 30% BL compared with the other treatments (Figs. 1, S2). Leaves exposed to 5% BL and low VPD contained a high amount of starch grains (Figs. 1, S2), but the samples for microscopy was taken in the end of the day, when the leaves are expected to have the highest starch content. 30 % BL reduced the ratio between carbon and nitrogen (C/N significantly) in both moderate and low VPD (Table 2).

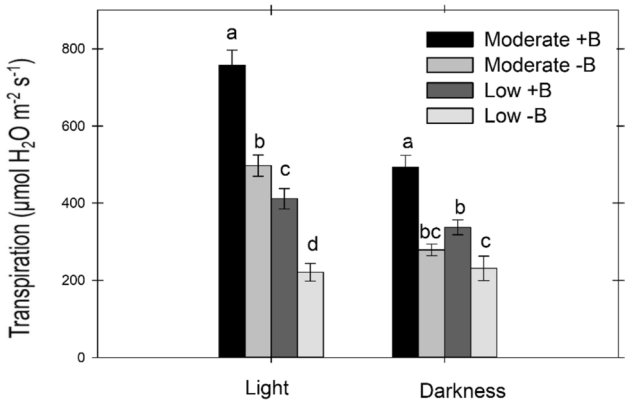


Fig. 2 Effect of VPD and proportion of blue light (BL) on transpiration rate during day and night from cucumber plants (*Cucumis sativus* 'Quarto') cultivated in moderate (1.12 kPa) and low VPD (0.28 kPa) in combination with 5% (−B) and 30% (+B) BL for 14 days. The temperature in growth chambers was 23 °C and the PAR was 200 µmol m^{-2} s^{-1}. Data are means ± SE, and different letters indicate significant differences between treatments for a given time of day (light/dark) as determined by post-hoc Tukey HSD analyses ($p < 0.05$), ($n = 8$)

The transpiration rate of whole plants during day and night

No significant interaction between VPD and BL on daytime transpiration rate was found ($p > 0.05$). However, the daytime transpiration rate was significantly higher in moderate VPD compared with low VPD ($p < 0.05$) and significantly higher with 30% BL compared with 5% BL ($p < 0.05$) (Fig. 2). During darkness, no significant effect of VPD was found ($p > 0.05$) but 30% BL during the day increased transpiration rate at night significantly ($p < 0.05$) irrespective of VPD. The day/night transpiration ratio was significantly higher for plants exposed to moderate VPD (1.6) compared with low VPD (1.1), irrespective of light quality, indicating improved nighttime closure in moderate VPD (data not shown).

Stomatal characteristics

Cucumber has amphistomatous leaves with stomata on both adaxial and abaxial sides. Low VPD, compared to moderate VPD, significantly increased the density on the abaxial side only.

Stomatal length was not affected by any of the treatments, but 30% BL resulted in significantly higher stomatal density on both leaf surfaces, irrespective of VPD (Table 3). A significant effect of VPD on stomatal aperture was found, and low VPD increased aperture size compared with moderate VPD in light and darkness ($p < 0.05$). A significant interaction between VPD and BL was found on stomatal aperture during darkness ($p < 0.05$). In moderate VPD, no significant effect of additional BL was found, but in low VPD, 30% BL resulted in stomata with a wider aperture (Table 3).

Table 3 Effect of VPD and proportion of blue light (BL) on stomatal density, -morphology and -aperture on the abaxial side of cucumber leaves during light and darkness

	Moderate VPD		Low VPD	
	30% BL	5% BL	30% BL	5% BL
Stomatal density, adaxial (50 µm^{-2})	111 ± 3.8^{a}	102 ± 4.3^{b}	112 ± 3.4^{a}	91 ± 4.1^{c}
Stomatal density, abaxial (50 µm^{-2})	172 ± 7.4^{b}	153 ± 5.1^{c}	196 ± 5.8^{a}	183 ± 2.8^{a}
Stomatal length, adaxial (µm)	9.8 ± 0.12^{a}	10.5 ± 0.26^{a}	11.1 ± 0.21^{a}	9.4 ± 0.29^{a}
Stomatal length, abaxial (µm)	10.0 ± 0.25^{a}	10.7 ± 0.32^{a}	10.9 ± 0.32^{a}	9.1 ± 0.59^{a}
Abaxial stomatal aperture, light (µm)	4.69 ± 0.16^{b}	4.77 ± 0.15^{b}	5.32 ± 0.17^{a}	5.21 ± 0.19^{ab}
Abaxial stomatal aperture, darkness (µm)	4.66 ± 0.09^{c}	4.27 ± 0.11^{c}	5.72 ± 0.13^{a}	5.11 ± 0.13^{b}

Aperture was measured in the beginning of the day (1 h after start of photoperiod) and in the middle of the night. Plants were cultivated in moderate (1.12 kPa) and low VPD (0.28 kPa) in combination with 5% and 30% and BL of PAR for 14 days, in growth chambers with 23 °C and a PAR of 200 µmol m^{-2} s^{-1}. Data are means ± SE, and different letters in a line indicate significant differences (Tukey HSD, $p < 0.05$), (stomatal density: $n = 25$; stomatal length: $n \geq 150$; stomatal aperture, $n \geq 100$ stomata per light treatment per VPD per timepoint)

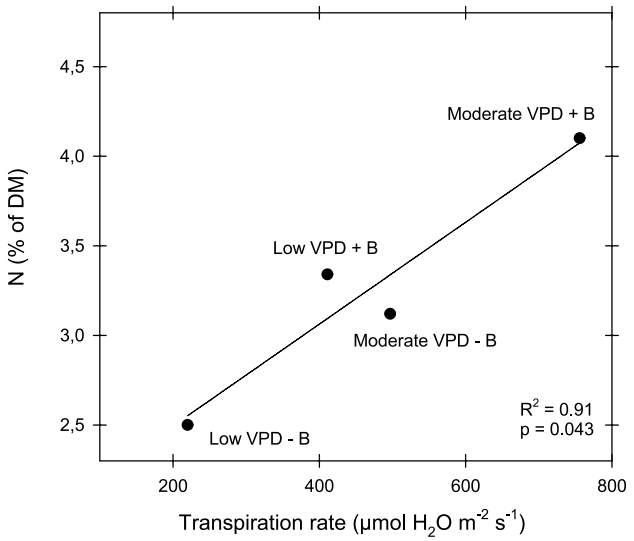


Fig. 3 Linear relationship between the mean foliar N content and daytime transpiration rate of cucumber plants developed under four treatments of different VPDs and blue light (BL) proportions ($n = 4$ per treatment): moderate (1.12 kPa) and low VPD (0.28 kPa) in combination with 5% (−B) and 30% (+B) BL. Plants were cultivated for 14 days in growth chambers with 23 °C and a PAR of 200 µmol m^{-2} s^{-1}

Relationship between the foliar N content and the transpiration rate during the day

The content of N increased significantly in response to 30% BL compared to 5% BL ($p < 0.05$), with N being affected by VPD (Fig. 3). A positive linear relationship was found between daytime transpiration rate and the foliar N content of source leaves ($p = 0.043$, $R^2 = 0.91$, Fig. 3).

Changes in ABA and its metabolites during day and night

The content of ABA is a result of the biosynthesis and conjugation/catabolism. ABA and its conjugates/catabolites (ABA-GE, PA, DPA, 7′OH-ABA, *neo*PA, *trans*-ABA and *cis*-ABA) were quantified in fully developed leaves. In all treatments, the levels of 7′OH-ABA, *neo*PA, and *trans*-ABA were very low (data not shown), and they have only been included (when quantification was possible) in the calculation of total ABA (= ABA + conjugates/catabolites). During the day, VPD did not significantly affect the content of *cis*-ABA ($p > 0.05$) or ABA-GE ($p > 0.05$), but low VPD increased the content of PA ($p < 0.05$) and DPA ($p < 0.05$) significantly compared to moderate VPD (Fig. 4). Total ABA was significantly affected by VPD ($p < 0.05$) during daytime and was 37% higher in low VPD compared with moderate VPD. Additional BL reduced the concentrations of *cis*-ABA, PA, and DPA (all $p < 0.05$) during daytime but had no significant effect on the content of ABA-GE ($p > 0.05$). Similarly to at daytime, the *cis*-ABA content was not significantly affected by VPD during darkness ($p > 0.05$). VPD did not affect the content of ABA-GE, PA or DPA during nighttime but total ABA was significantly increased (23%) in leaves grown with low VPD compared with moderate VPD ($p < 0.05$).

Similarly to VPD, additional BL did not affect the content of *cis*-ABA during darkness. However, the content of the different ABA conjugates/catabolites was significantly affected by the light conditions. Additional BL during daytime doubled the content of ABA-GE during darkness ($p < 0.05$) but reduced the content of PA by 50% ($p < 0.05$) and DPA by 66% ($p < 0.05$) compared to treatment with 5% BL during daytime. Total ABA was significantly affected by additional BL ($p < 0.05$) during darkness and was 52% reduced in leaves exposed to 30% BL compared to 5% BL.

Carbohydrates and flavonoids are mainly affected by BL

No significant effect of VPD were found for any of the carbohydrates measured ($p > 0.05$) (Table 4). However, the starch content was significantly reduced and raffinose increased in plants exposed to 30% BL compared with 5% BL (Table 4). Stachyose was also increased in 30% BL (significant in low VPD, trend in moderate VPD) (Table 4). Three different flavonoids (apigenin, luteolin and resveratrol) were detected in cucumber leaves (Table 4). Apigenin was significantly affected by both VPD and BL ($p > 0.05$). The content of apigenin was lower in low VPD compared with moderate VPD (significant for 30% BL, trend for 5% BL), but additional BL induced a higher content in both VPDs. A similar response as for apigenin was found for resveratrol, with a significant effect of both VPD and BL, with lower content in low VPD compared with moderate VPD and in 5% BL compared to 30% BL (significant in low VPD, trend in moderate VPD). On the other hand, luteolin was not significantly affected by VPD or BL (Table 4). µmol H_2O m^{-2} s^{-1}.

Discussion

Photoreceptors such as CRY and PHOT are the best-known BL sensors, but also phytochromes detect BL in addition to being red and far-red light sensors. The relative amount of active phytochrome (Pfr) expressed as the phytochrome photostationary state (PSS), was the same (0.88) under the two light treatments (Table 1). Therefore, the observed responses can be attributed to BL detection

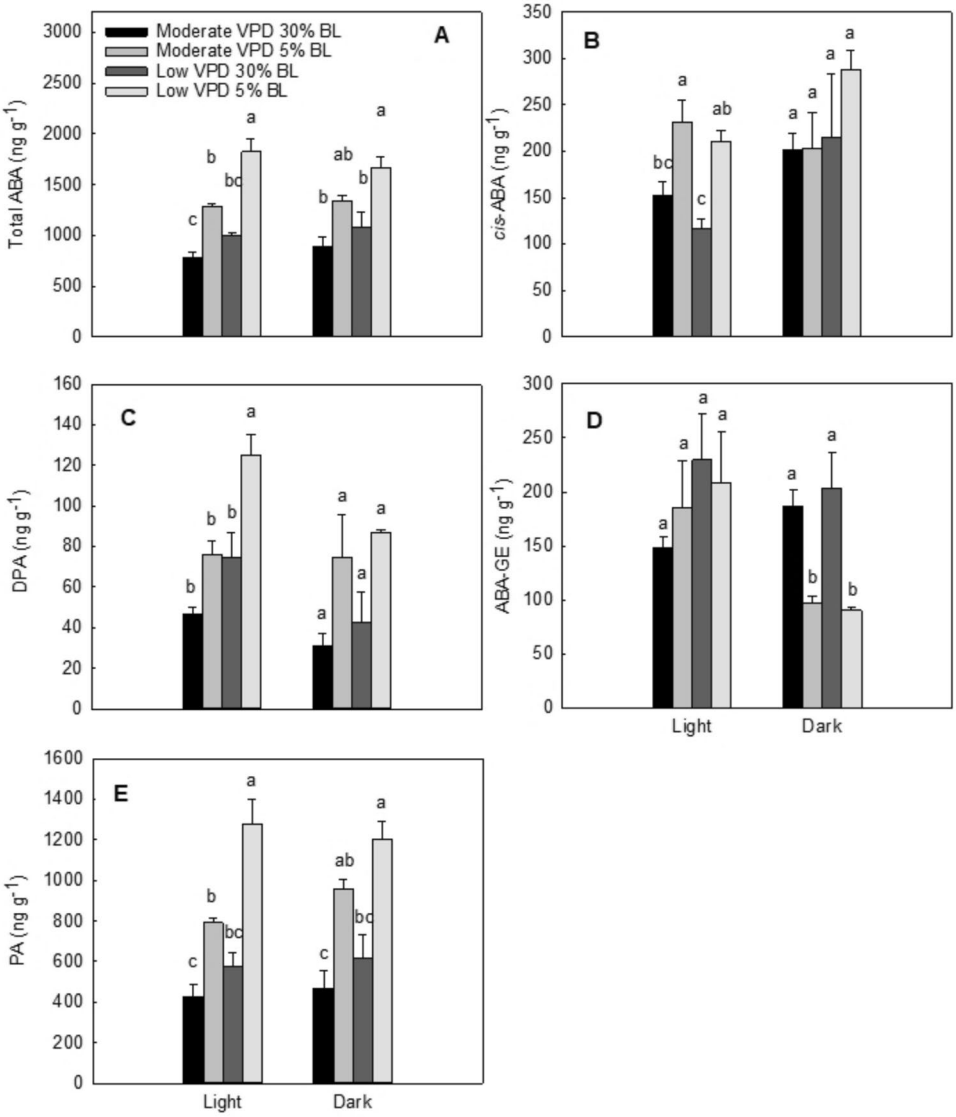


Fig. 4 Total foliar ABA and ABA conjugate/catabolite concentrations (ng g^{-1} DW) in cucumber plants sampled during light and darkness. Plants were grown in moderate and low VPD with HPS lamps (5% blue light) and extra blue light (BL) (30%) from LED lamps. Means ± SE are shown. Different letters indicate significant differences between treatments within a given time of the day (light/dark), as determined by ANOVA and post-hoc Tukey HSD analyses ($p < 0.05$), ($n = 3$)

by BL sensors such as CRY and/or PHOT. Control of transpiration is crucial to ensure sufficient water and nutrient uptake, optimal growth, and high yield of plants produced in controlled environment (Ding et al. 2022; Jones et al. 2022). Here we demonstrate that a higher proportion of BL increased the transpiration rate and foliar N uptake in cucumber plants regardless of VPD, with the highest transpiration rate occurring at moderate VPD and a high BL portion. On the other hand, growth expressed as fruits day^{-1} and fruit length/thickness ratio, responded differently to BL in low and moderate VPD. Additional BL improved fruit growth in low VPD, but in moderate VPD it seemed to act as a mild stressor, reducing fruit growth likely due to excessive transpiration.

VPD did not affect ABA, but BL reduced ABA and its metabolites and increased transpiration rate during day and night

ABA plays a key role in regulating transpiration by influencing the behaviour of stomata. When plants experience drought, increased ABA levels normally induce stomata closure (Wilkinson and Davies 2002). It is well known that ABA plays a role in the control of transpiration also at non-stress conditions but under well-watered conditions, ABA signalling in guard cells may be suppressed by other signals (Inoue and Kinoshita 2017; Yoshida et al. 2019). In this study, the plants were well-watered, and a significantly lower content of ABA and catabolites were found in plants exposed to additional BL along with increased transpiration. Under low VPD, the *cis*-ABA content was reduced by 50% in 30% BL compared to 5% BL (Fig. 4). Previous studies

Table 4 Effect of VPD and proportion of blue light (BL) on the content of starch and soluble carbohydrates and three different flavonoids detected in cucumber leaves with HPLC

	Moderate VPD		Low VPD	
	30% BL	5% BL	30% BL	5% BL
Carbohydrates (% of DW)				
Starch	28.6 ± 9.54^{b}	52.5 ± 3.94^{a}	33.9 ± 7.79^{b}	58.9 ± 4.41^{a}
Sucrose	0.70 ± 0.23^{a}	0.59 ± 0.05^{a}	0.52 ± 0.01^{a}	0.49 ± 0.04^{a}
Glucose	1.39 ± 0.31^{a}	0.91 ± 0.12^{ab}	1.10 ± 0.16^{ab}	0.82 ± 0.03^{b}
Fructose	0.54 ± 0.18^{a}	0.53 ± 0.14^{a}	0.73 ± 0.21^{a}	0.41 ± 0.07^{a}
Stachyose	0.87 ± 0.08^{ab}	0.55 ± 0.02^{bc}	0.95 ± 0.27^{a}	0.48 ± 0.19^{c}
Raffinose	0.92 ± 0.06^{a}	0.63 ± 0.02^{b}	0.80 ± 0.06^{a}	0.56 ± 0.02^{b}
Total soluble carbohydrates	4.43 ± 0.88^{a}	3.22 ± 0.29^{ab}	4.07 ± 0.64^{ab}	2.80 ± 0.07^{b}
Flavonoids (mg g^{-1} DW)				
Apigenin	3.10 ± 0.20^{a}	1.52 ± 0.13^{bc}	2.20 ± 0.10^{b}	0.93 ± 0.19^{c}
Luteolin	8.23 ± 0.90^{a}	3.76 ± 0.84^{ab}	6.37 ± 0.34^{ab}	2.37 ± 1.03^{b}
Resveratrol	8.99 ± 0.74^{a}	6.41 ± 1.23^{ab}	5.76 ± 0.49^{b}	2.68 ± 0.70^{c}

Plants were cultivated in moderate (1.12 kPa) and low VPD (0.28 kPa) in combination with 5% and 30% and BL of PAR for 14 days, in growth chambers with 23 °C and a PAR of 200 µmol m^{-2} s^{-1}. Data are means ± SE, and different letters in lines indicate significant differences (Tukey HSD, $p < 0.05$), ($n = 5$)

have also shown a relationship between BL and ABA content. In a well-watered *Arabidopsis cry*-mutant increased level of ABA in the leaves was found, indicating that lack of BL-sensing by CRY leads to higher ABA content and lower transpiration (Boccalandro et al. 2012). Other studies have reported that light signalling pathways interact with ABA regulation at several points (Movahedi et al. 2021; Mukherjee et al. 2023). ABA may have different roles and does not only regulate stomatal aperture, but whole plant hydraulics, activity of aquaporins, xylem differentiation and stomatal density (Yoshida et al. 2019). For example, higher stomatal density and bigger stomata were reported in the ABA deficient mutant '*flacca*' (Innes et al. 2021). In addition, ABA and BL have opposite effects on xylem sap pH, which in turn affects the leaf hydraulic conductance through up- or down-regulation (by BL and ABA, respectively) of bundle sheath cell H^{+}-ATPase (AHA2) and autonomous signalling cascades dependent on the environmental conditions and stress status in the plant (Torne-Srivastava et al. 2024).

The length of the stomata was not affected by VPD and BL in this study, but the stomatal density was higher in plants exposed to additional BL (Table 3). Previous reports have shown an universal increase in stomatal size across several species exposed to low VPD during growth (reviewed in Fanourakis et al. 2020), and this has been attributed to mediation by ABA concentration (Giday et al. 2014). The higher transpiration rate in plants grown in additional BL might at least partly be due to the increased number of stomata. This is consistent with previous work on cucumber, which showed that a higher proportion of BL increases transpiration and stomatal development (Li et al. 2023), and increases stomatal conductance partly due to increased density and size (Savvides et al. 2012). Hogewoning et al. (2010) also found that the stomatal conductance in cucumber increased proportionally with an increased proportion of BL, and this was related to both a higher number and increased opening of stomata.

BL promotes stomata opening and synthesis more than other light wavelengths (Inoue and Kinoshita 2017). However, in this study we found that increased BL during the day increased the stomatal aperture during night mainly in low VPD, and not in moderate VPD, suggesting that stomata developed in low VPD are more responsive to increased BL (Table 3). It should be kept in mind that growth conditions, such as VPD may alter the water status of the plant, and therefore may impact stomatal sensitivity to BL (Lanoue et al. 2018; Wang et al. 2011; Aasamaa and Aphalo 2016). In addition, it is important to note that in our study, the stomatal aperture was measured on the lower (abaxial) side only, and not on the upper (adaxial) side due to technical difficulties with high density of trichomes. It is known that the sensitivity to light is generally higher in the abaxial than adaxial stomata (Wang et al. 2011). However, in the study of Hogewoning et al. (2010) the number of stomata on the adaxial leaf surface increased significantly with increasing BL percentage, whilst no significant changes were found on the abaxial leaf surface.

Previous studies have shown that leaves of *Tradescantia virginiana, Rosa* x *hybrida* and *A. thaliana* developed in low VPD have lower ABA concentrations compared with those developed in moderate VPD (Arve et al. 2013, 2015; Nejad and van Meeteren 2005). However, cucumber responded differently, showing increased total ABA, PA and DPA content in low VPD combined with low BL

(5%). The higher PA and DPA levels in plants exposed to low VPD and 5% BL could possibly be due to higher ABA biosynthesis and a constant inactivation rate of ABA to PA and DPA. A recent study found that low VPD (95% RH) combined with low temperature resulted in increased ABA concentration compared to a control treatment (80% RH) (Amin et al. 2023). The nutritional status of the plants was not reported in Amin et al. (2023) but other studies suggest that N deficiency can increase ABA concentration in leaves (de Oliveira et al. 2022). This can accelerate the initiation and progression of leaf senescence and reduce the photosynthetic activity and chlorophyll content of the leaves (de Oliveira et al. 2022; Gepstein and Thimann 1980; Zdunek-Zastocka and Lips 2003). In our study, no significant effect of VPD or BL was found on the foliar content of S, K, Ca, P, Mo, Na or Mn (S3). By contrast, the content of N, Fe and Mg increased significantly in response to BL ($p < 0.05$), with N being more affected by VPD (Figs. 3, S3). However, we found low N and lower chlorophyll content, along with higher ABA content, in plants exposed to low VPD and 5% BL compared with other treatments. Whether this is connected to the high ABA content will need further investigation.

The BL fraction during the day affects ABA regulation at night

Similar to daytime transpiration, nighttime transpiration was higher in plants exposed to additional BL in both moderate and low VPD (Fig. 3). Incomplete stomatal closure and transpiration in darkness are widespread. Previous studies estimate that 10–30% of leaf and plant water losses occur at night, depending on plant species and the VPD between leaves and the air (Chowdhury et al. 2022; de Dios et al. 2016). It has been suggested that photosynthetic rates or the spectral distribution of the light during daytime can influence nighttime transpiration, although the exact mechanism is unclear and different species seem to respond differently. For example, in *Rosa* x *hybrida*, increasing BL increased foliar ABA content, improved stomatal closure and reduced nighttime transpiration in darkness (Terfa et al. 2020). The background light quality in this study contained more green light compared to the study by Terfa et al. (2020). Although the receptors and exact mechanism of stomatal response to green light are not fully identified, green light is known to deactivate the BL-sensing CRY photoreceptors (Bouly et al. 2007).

In our study, the total contents of ABA and conjugate/catabolites were lower in darkness when BL was added during the day, and the regulation was different (Fig. 4). The amount of BL affected the conversion rates of ABA to PA and ABA-GE during the night, showing that the amount of BL during the day can change ABA metabolism and direct ABA inactivation pathways at night. The amount of the catabolite PA was significantly lower in plants developed with additional BL (Fig. 4). The lower PA levels during night in plants exposed to 30% BL were most likely a result of the lower ABA levels and a lower inactivation rate of ABA to PA, as well as an increase in the conjugation of ABA to ABA-GE (Fig. 4). Several studies have hypothesised that ABA-GE is a storage form of ABA and can be converted back to ABA when needed (Dietz et al. 2000; Sauter et al. 2002). ABA-GE is generally present at low levels in young seedlings, but is present at higher levels in stressed plants, old plants, and fruits (Zeevaart and Creelman 1988). We speculate that an environment with a higher proportion of BL promotes ABA storage into ABA-GE (Seki et al. 2007), which can then be easily released when needed, i.e. during drought, and/or high irradiance.

High BL fractions combined with moderate VPD result in more compact morphology and reduced growth

In cucumber grown under moderate VPD, a high proportion of BL had negative effects on growth and increased water usage due to excessive transpiration. By contrast, improved growth under a high proportion of BL was found in low VPD. This indicates that the amount of BL beneficial for cucumber growth depends on the background VPD. High BL proportions may be perceived as a stressor by the plants (Roeber et al. 2021). However, our findings show that plants have a greater tolerance to BL when grown in low VPD environments. This tolerance may be because humid air reduces the risk of dehydration and allows plants to maintain turgidity even when stomata are wide open.

Inhibition of stem and leaf expansion is a common response to BL (Folta et al. 2003). Additional BL significantly reduced plant height, with a stronger effect observed in moderate VPD compared to low VPD (Table 2). A similar trend was observed for leaf area. BL reduced leaf area by 10% more in moderate VPD than in high VPD (Table 2). These findings align with other studies indicating that morphological changes in response to light quality, such as ultraviolet (UV) radiation and far-red (FR) light, can be influenced by the background VPD (Innes et al. 2018, 2019; Shibuya et al. 2019). The high fruit appearance rate observed in low VPD conditions with 30% BL may be attributed to an increased amount of photoassimilates (Table 4). In addition, increased BL has been reported to enhance photosynthesis in cucumber plants (Hogewoning et al. 2010; Wang et al. 2009). The lack of growth improvement observed in additional BL in moderate VPD may be due to weak stress caused by the excessive transpiration measured during both day and night (Fig. 2). Reduced growth was confirmed by

lower dry weight accumulation (leaves + stems) in plants exposed to moderate VPD + 30% BL (Table 2). Furthermore, plants from moderate VPD + 30% BL contained significantly higher levels of raffinose and apigenin compared to other treatments (Table 4). Raffinose family oligosaccharides (raffinose and stachyose) are known to accumulate and act as stress protectants in cucumber during stress conditions like drought and cold (Sui et al. 2012). Flavonoids are known to act as antioxidants involved in scavenging reactive oxygen species (ROS) under abiotic stress conditions (Ma et al. 2014; Shomali et al. 2022). The higher content of flavonoids in plants grown with higher BL is consistent with other reports (Almaghrabi and Almulaiky 2021; Palma et al. 2021). Interestingly, although the growth patterns and secondary metabolite content suggest mild stress in plants exposed to moderate VPD and 30% BL, there was no increase in ABA levels under these conditions. This observation contrasts with the widely accepted paradigm that ABA biosynthesis is typically induced during stress (Vishwakarma et al. 2017). However, ABA-independent responses to abiotic stress are well-documented (Roychoudhury et al. 2013). We hypothesise that whilst plants under moderate VPD and 30% BL exhibited some signs of a stress response, reflected in altered growth and secondary metabolite accumulation, the stress was insufficient to trigger significant upregulation of ABA biosynthesis.

In summary, our findings suggest that VPD influences the regulation of ABA and its conjugates/catabolites in response to BL, with BL signals being the stronger driver of stomatal aperture regulation under well-watered conditions compared to ABA. Furthermore, the combination of drier air and a high proportion of BL appears to serve as a mild stressor, impacting various aspects of cucumber growth and yield. Further research on the interaction between light conditions and other environmental factors across different crop species will be invaluable for optimising the light environment in controlled environment agriculture.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11738-025-03818-0.

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Author contributions S.T., S.N.I., J.E.O. and S.B.J. designed the growth experiments and the sampling times. They also did most of the growth analysis of cucumber plants. S.I. and S.B.J. measured stomata imprints, counting stomata and calculating the transpiration rate. H.A., R.I.R., and K.A.S. developed the methods for carbohydrate and flavonoid measurements in cucumber leaves, sampled at different time points and carried out all the HPLC analysis. R.B.R made the micrographs with images from light microscopy. S.I., R.B.R. and S.T. analysed most of the data and wrote the manuscript, with contribution from all authors.

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Data availability The authors confirm that the datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare they have no known conflict of interests and no financial interests to disclose.

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