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Effects of Blue Light on Assimilation, Growth, Morphology, and Water Usage in Cucumber Seedlings

Effekter av blått lys på assimilasjon, vekst, morfologi og vannforbruk i småplanter av agurk

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MSc Plant Sciences – Plant Biotechnology

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Forord

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Sammendrag

Blått lys (BL) kan påvirke plantevekst og -utvikling på flere måter, og agurk (*Cucumis sativus*) er spesielt sensitiv for lyskvalitet. Formålet med denne studien var å undersøke hvordan ulike andeler BL påvirker assimilasjon, vekst, morfologi og vannforbruk i småplanter av agurk. Sorten 'Hi Light' ble eksponert for ulike andeler BL, enten 10% eller 30% av fotosyntetisk aktiv stråling (PAR) i forsøkskammere med 23℃, 60% relativ luftfuktighet og en irradians på 235 μmol m^{-2} s^{-1} PAR i seksten dager. Assimilasjon ble studert med målinger på bladnivå av fotosyntesehastighet, innhold av fotosyntesepigmenter og karbohydrathusholdning – mer spesifikt konsentrasjon av stivelse og løselig sukker i eldre blader. Biomasse og morfologi ble målt og benyttet i vekstkomponentanalyse. Vannforbruk av hele planter ble målt, og spalteåpningstetthet ble undersøkt for å bedre forstå årsaken til ulikt vannforbruk. Effektiviteten av vannforbruk ble estimert basert på fotosyntesehastighet og konduktans på bladnivå.

Planter dyrket med 30% BL hadde høyere fotosyntesekapasitet, høyere konsentrasjon av klorofyll *a* and *b*, samt betraktelig lavere stivelseskonsentrasjon sammenliknet med planter dyrket med 10% BL. Det var også en tendens til høyere konsentrasjon av løselig sukker i planter dyrket med 30% BL, men det ble kun statistisk signifikant utslag på konsentrasjonen av raffinose. Det var små forskjeller i plantehøyde, totalt bladareal og relativ veksthastighet mellom plantene fra de to lysbehandlingene. Vekstkomponentanalysen viste at sistnevnte var forårsaket av motsatte effekter på assimilasjonshastighet og spesifikt bladareal (SLA) i de to gruppene. En lavere assimilasjonshastighet ble forklart med høyere mørkerespirasjon og potensielt mer rotvekst og/eller kortere petioler i plantene dyrket med 30% sammenliknet med 10% BL, og tilsvarende kunne høyere SLA forklares med tynnere blader og/eller med lavere tetthet. Til slutt, det høyeste vannforbruket ble målt i plantene dyrket under 30% BL, noe som antakelig kun skyldtes en direkte effekt på spalteåpningene. Som en konsekvens hadde disse plantene også den laveste vannforbrukseffektiviteten, men kun ved lave lysnivåer på grunn av en høy fotosyntese ved høye lysnivåer.

Med en bedre forståelse av fysiologiske og morfologiske effekter av BL kan resultatene i denne oppgaven brukes som et grunnlag for videre forskning for å kartlegge den optimale andelen BL i ulike produksjonsstadier, fra produksjon av frøplanter til fruktbærende planter, og i ulike produksjonssystemer som topp- og mellombelysning.

Abstract

Blue light (BL) affects plant growth and development in many ways, and cucumber (*Cucumis sativus*) is a species known to be particularly sensitive to light quality. The objective of this thesis was to better understand how different proportions of BL affect assimilation, growth, morphology, and water usage in cucumber seedlings. The cultivar 'Hi Light' was exposed to two fractions of BL, either 10% or 30% (of photosynthetic active radiation), in controlled environment chambers with 23℃, 60% relative humidity and a photosynthetic photon flux density of 235 μmol m^{-2} s^{-1}. The duration of the experiment was sixteen days. The effects on assimilation were investigated with single-leaf measurements of photosynthetic rate, photosynthetic pigments, and carbohydrate status – specifically the concentration of starch and soluble sugars in source leaves. Biomass and morphological traits were measured and used in an analysis of growth components. Water usage was measured on the whole-plant level, its underlying mechanism investigated by calculating the stomatal density, and the water-use efficiency was estimated based on photosynthetic rate and stomatal conductance on a single-leaf level.

Plants grown with 30% BL had a higher photosynthetic capacity, higher concentrations of chlorophyll *a* and *b*, and a considerably lower concentration of starch than plants grown with 10% BL. There was also a trend for higher concentrations of soluble sugars in plants acclimated to 30% BL, but only that of raffinose was statistically significant. Shoot length, total leaf area and relative growth rate (RGR) were similar between both treatments. Growth analysis revealed that the latter was due to opposite effects on net assimilation rate (NAR) and specific leaf area (SLA) between treatments. In plants grown with 30% compared to 10% BL, lower NAR was explained by a higher dark respiration and possibly enhanced root growth and/or shorter petioles, while higher SLA was due to thinner and/or less dense leaves. Finally, 30% BL resulted in the highest water usage, and this was likely due to instantaneous rather than acclimatory effects on stomata. Consequently, the 30% BL treatment resulted in the lowest water-use efficiency, but only at low irradiances due to high photosynthesis at high irradiances.

With a better understanding of the physiological and morphological responses to BL, the results presented here can be used as a foundation for further research to find the optimal fraction of BL in different stages of the crop, from seedlings to fruiting crops of cucumber, and in different production systems such as top- and interlighting.

Abbreviations

A	net CO_2 assimilation rate
A_{max}	maximum net photosynthesis rate
BL	blue light
DLI	daily light integral
DW	dry weight
FR	far-red
g_s	stomatal conductance to water vapor
LAR	leaf area ratio
LED	light-emitting diode
LMR	leaf mass ratio
NAR	net assimilation rate
PAR	photosynthetic active radiation
PPFD	photosynthetic photon flux density (400-700 nm)
RFO	raffinose family oligosaccharides
RGR	relative growth rate
RH	relative humidity
R/FR	proportion of red to far-red light
SLA	specific leaf area

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1 Introduction

Plants absorb light to provide energy for assimilation of CO_2 to organic carbon via photosynthesis. The organic carbon is further used as building blocks or an energy source for growth, reproduction, protection, and defense. Light is also used as a signal, and different light qualities can affect developmental, physiological, and morphological processes through photomorphogenesis. There is growing evidence for species-specific responses to spectral quality, with cucumber often being reported as more sensitive than other crops, particularly to blue light (BL) (Snowden et al., 2016; Graham et al., 2019; Johnson et al., 2020; Spaninks et al., 2020; Zou et al., 2020; Liang et al., 2021).

Supplemental light is required for year-round greenhouse production in northern latitudes to provide enough light to the crops when solar radiation is limited. Artificial light can be the sole light source in parts of the year, while indoor farming, as in plant factories, completely relies on it. More traditional lamps like high-pressure sodium (HPS) are increasingly being replaced by light-emitting diodes (LEDs) as supplemental light in greenhouses. LEDs offer several advantages, mainly a high efficacy (μmol J^{-1}), possibilities for choosing spectral distribution, and less heat radiation (Massa et al., 2008). LEDs have also made vertical farming more profitable and opened the possibility for interlighting in dense canopies (Massa et al., 2008; Al Murad et al., 2021).

BL makes up about 20-30% of the visible light in the solar radiation reaching Earth's surface (de Gálvez et al., 2022), while the blue fraction in HPS lamps is 5% and usually between 5-30% in horticultural LEDs (Kusuma et al., 2020). Choosing a light spectrum offers opportunities for growers to manipulate crop growth and development. However, with opportunities come choices, and spectra different than the sun’s can cause unpredictable responses. It is therefore important to understand the plant responses to different wavelengths.

Signals from BL are closely connected to the plants’ water- and carbon status through effects on stomata and the photosynthetic apparatus (Wang et al., 2009; Li et al., 2020b; Matthews et al., 2020). BL seems to be required to avoid excessive stem elongation and low dry mass (Hernández and Kubota, 2016), and increasing blue fractions are often reported to further reduce plant height, hypocotyl length, and leaf expansion in several species (Islam et al., 2012; Cope and Bugbee, 2013; Snowden et al., 2016; Chen et al., 2021; Liang et al., 2021). Morphological effects of BL can also depend on spectral background, daily light integral

(DLI), air climate, and possibly even cultivar (Hernández and Kubota, 2014; Snowden et al., 2016).

Cucumber (*Cucumis sativus*) is an important greenhouse crop in Norway, making up 10% of the Norwegian vegetable production (SSB, 2023). Of the total cucumber turnover, 60-70% is produced domestically (Landbruksdirektoratet, 2020). The crop requires high temperatures (~24℃) and high light conditions (30 mol m^{-2} d^{-1}) (Bævre et al., 2006), which makes Norwegian cucumber production energy intensive, and strategies for optimizing production are sought after. Many different cultivars are used in production. The cultivar 'Hi Light' is adapted to cultivation using the high wire method and supplemental lighting (Nunhems BV, 2023). It grows well under LEDs and is therefore preferred by some growers (Norwegian growers, pers. comm.).

Given its many effects, BL can be a powerful, yet relatively cheap and simple tool to manipulate growth and development. The aim of this study was to better understand how different proportions of BL affect physiological and morphological traits in the cultivar 'Hi Light'. To investigate this, seedlings were grown in controlled environment chambers for sixteen days under two proportions of BL (10% and 30% of PAR) to a warm-white background spectrum. Specific aims were to compare the response to the two proportions of BL in regard to:

1. Assimilation – with single-leaf measurements of photosynthetic rate, the content of photosynthetic pigments and carbohydrates in source leaves, as well as whole-plant net assimilation rates
2. Growth and morphology on whole plant level – by analyzing growth rates, dry matter distribution and measurements of morphological traits
3. Water usage – measured on the whole-plant level, along with single-leaf measurements of water-use efficiency and analysis of stomatal density.

2 Background

2.1 Light as an energy source

Light is the small part of the electromagnetic spectrum that is visible to the human eye, between wavelengths of 400 and 700 nm photons (Visser and Rolinski, 2014). This spectral range is also what McCree (1971) defined as photosynthetically active radiation (PAR), which consists of the most efficient wavelengths to drive photosynthesis. Electromagnetic waves deliver energy in "packets" called photons, which are harvested through pigments such as chlorophylls and carotenoids. Subsequentially, light energy is converted through a series of reactions, into chemical energy used to make sugars from CO_2 (Visser and Rolinski, 2014).

Photosynthetic efficiency is wavelength-dependent. The energy of a photon is inversely proportional to its wavelength, with blue photons (400-500 nm) containing more energy than longer-wave radiation such as green (500-600 nm) and red (600-700 nm) light. However, the photosynthetic rate is highly influenced by different absorption efficiencies of photosynthetic and nonphotosynthetic pigments (McCree, 1971). As illustrated in Figure 2.1, this results in different quantum yields – defined as the number of photochemical products that is formed per total number of photons absorbed (Taiz et al., 2018).

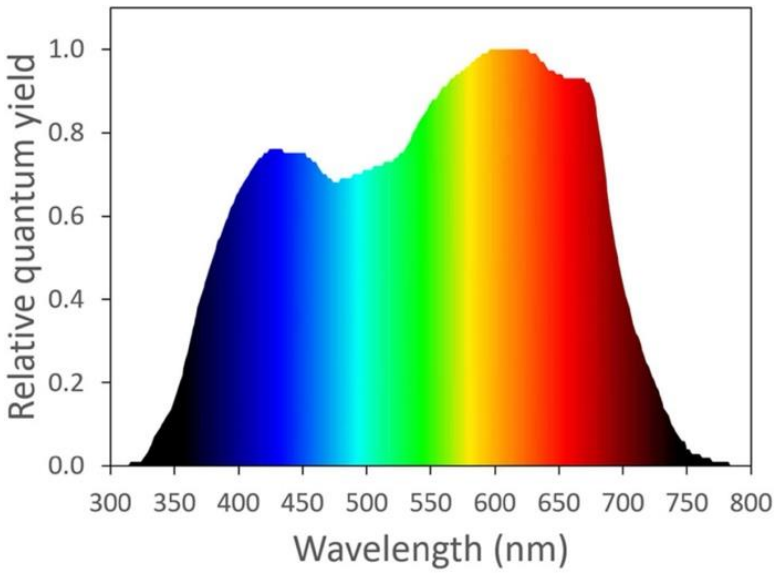

Figure 2.1: The efficiency of photons in different spectral regions to drive photosynthesis, here illustrated as the relative quantum yield of CO_2 assimilation (Liu and van Iersel, 2021).

When photons hit a leaf, they are either reflected, absorbed, or transmitted. The main photosynthetic pigments of higher plants, called chlorophyll *a* and *b*, absorb mostly red and blue wavelengths with a high efficiency in transferring energy to the photosystems, resulting in a high quantum yield of red and blue photons compared to photons of other spectral regions. Still, red light has higher quantum yield than blue photons. This is because a higher proportion of blue photons are absorbed by other pigments such as photosynthetic carotenoids and various non-photosynthetic pigments. The energy transfer efficiency of carotenoids is relatively low, and zero for non-photosynthetic pigments (McCree, 1971; Hogewoning et al., 2012).

Furthermore, photosynthesis can also be influenced indirectly by different wavelengths through changes in plant morphology and physiology, which can affect light interception and processing (Zhu et al., 2010).

2.2 Light as a signal

Light is also used by plants as a signal, to adapt to their surroundings by response to the quantity (fluence), quality (wavelength), duration (photoperiod) and direction of light (Jiao et al., 2007). A broader span of the electromagnetic spectrum is used for signaling than for energy harvesting, from the UV- to the far-red (FR) region. The different light qualities affect plant growth, development, morphology, and physiology through absorption and activation of various photoreceptors. The main groups of photoreceptors that have been identified are phytochromes, cryptochromes and phototropins. Phytochromes absorb mainly red and FR wavebands, while cryptochromes and phototropins are more sensitive to the UV and blue regions (Figure 2.2).

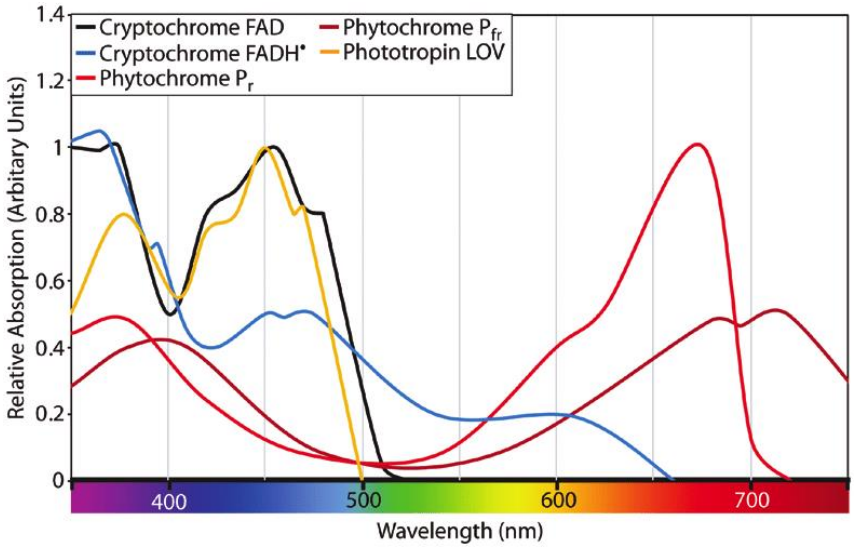

Figure 2.2: Absorption spectra of the main groups of photoreceptors cryptochromes, phytochromes and phototropins (Battle et al., 2020).

Some responses are mediated through one specific photoreceptor. For example, in *Arabidopsis thaliana,* activation of cryptochrome is required for normal development of the photosynthetic apparatus through the expression of genes encoding photosystem II (PSII) components (Li et al., 2020b) and activation of phototropins in guard cell chloroplasts regulates the stomatal aperture (Horrer et al., 2016). However, other responses such as the phytochrome- and cryptochrome mediated suppression of hypocotyl elongation is a result of coaction between the two photoreceptors (Taiz et al., 2018). Further, the various photoreceptors absorb a broad range of wavelengths, and in some cases their absorption spectra overlap. For example, BL can activate both cryptochromes and phytochromes, while cryptochromes also absorb well in green wavebands (Figure 2.2). Assigning specific responses to certain wavelengths can therefore be misleading.

Studying plant responses to light quality is not straightforward. To understand the fundamental effects of specific wavebands, plant responses are often studied under monochromatic light in isolation, but this will disturb the normal interaction between photoreceptors, making generalization difficult (Graham et al., 2019). Therefore, in practical studies, it makes more sense to compare broader spectral distributions. However, one cannot avoid changing one waveband without affecting their proportion to others and potentially induce unintended effects.

2.3 Measuring light

The quantity of light received by a plant is measured as the irradiance, which is the number of photons received per unit area and time (e.g., μmol m^{-2} s^{-1}). Photosynthetic photon flux density (PPFD) describes the irradiance within the PAR range, and daily light integral (DLI) describes the PPFD integrated over the whole photoperiod. DLI is expressed as mol m^{-2} d^{-1} and is particularly useful for describing the light environment a plant experiences in horticultural settings. Since both PPFD and photoperiod can be manipulated with supplemental lighting, different light environments used by growers and in experimental research setups are easily compared by using DLI.

2.4 Carbohydrate metabolism

The energy from the light that is absorbed by a leaf is either lost as heat, fluoresence or used in photorespiration. The rest is used to assimilate CO_2 into organic carbon molecules, which is the gross photosynthesis and where the dynamic process of carbon metabolism begins. The organic carbon can provide building blocks for biomass production or be converted into various sugars, either used immediately as an energy source, stored as starch for later use or transported to other plant parts (sinks) (MacNeill et al., 2017).

When used as an energy source, the carbon is lost as CO_2 through cellular respiration. Net photosynthesis describes the difference between the CO_2 uptake in gross photosynthesis minus the loss through respiration (Taiz et al., 2018). The energy provided from respiration can be used for growth or maintainance. The former leads to biomass accumulation, while the latter is used to replace old proteins, in protection mechanisms against various stresses, for transport or repair from damage (Zhu et al., 2010).

Starch is a polymer of glucose and the main storage carbohydrate in higher plants. Soluble sugars accumulate during the day when photosynthesis is fueled by light, and 25-30% of these sugars are stored as transient starch in leaves (MacNeill et al., 2017). The energy storage can be rapidly mobilized when carbon demand exceeds the production at night or when the demand from strong sink tissues is high, e.g. during flowering (Hu et al., 2009). Abiotic stress can also enhance starch degradation, where it may serve as a source of energy or to provide osmoprotectants (Krasensky and Jonak, 2012; Zanella et al., 2016).

Plant organs that produce more sugars than they consume are described as a ‘source’ organ, while ‘sink’ organs consume more than they produce. Sink organs can be flowers, fruits, non-photosynthetic organs (roots, seeds) or developing photosynthetic organs (meristems and younger leaves). The largest sinks during vegetative growth are roots and immature leaves, but flowers, seeds and fruits are normally the largest sinks during reproductive growth (MacNeill et al., 2017). Sugars originating from a source leaf are transported to the sink in the vascular tissues, usually as sucrose. During the day, photosynthates can be directly used for sucrose synthesis prior to export, while during the night, the transient starch is used as a carbon source for sucrose synthesis (MacNeill et al., 2017).

As a leaf is growing, it is gradually transforming from sink to source. Turgeon and Webb (1973) showed that the whole leaf acts as a sink (i.e., imports carbon entirely) until it is 10% expanded, and thereafter gradually starts carbon export. The process starts at the leaf tip, followed by the outer edges, and ends at the base when the leaf is about 45% expanded. From then on, it acts as the main carbon source for sink organs. In squash (*Cucurbita pepo*), the whole transition from a complete sink to source takes about two days, starting when the leaf is ∼5 cm long and finishing at ∼10 cm length, and the progressive loss of import capacity happens in only one leaf at a time.

Other sugars than sucrose can also be used for long-distance transport. Raffinose family oligosaccharides (RFOs) are derivatives of sucrose and include raffinose (tri-), stachyose (tetra-), verbascose (penta-) and ajugose (hexasaccharide) (Sanyal et al., 2023). Cucumber is a so-called RFO-transporting species, which mainly transports sucrose and stachyose, but raffinose may also be used as a transport sugar (Hendrix, 1982; Pharr et al., 1985; Hu et al., 2009). Prior to transport, the sugars must be loaded from source cells and eventually unloaded into sink cells. RFO-transporting species use polymer trapping as a loading mechanism into the phloem, allowing symplastic loading though plasmodesmata (Turgeon, 1996). RFO metabolism is a complex network, involving many enzymes, intermediates and sugars serving many functions. Besides their role in phloem transport, RFOs can be used as carbon storage and in protection against abiotic stress (Sanyal et al., 2023).

2.5 Growth analysis

Plant growth is influenced by its physiology, morphology, and biomass allocation. Classical growth analysis allows us to separate plant growth into various components in order to analyze the drivers behind plant morphological development (Hunt, 1990). Relative growth rate (RGR) is the rate of biomass increase during growth period per unit biomass already present. It is influenced by net assimilation rate per leaf area (NAR), leaf mass ratio (LMR) and specific leaf area (SLA).

Variation in NAR is determined by the balance between gross photosynthesis and the carbon lost through respiration (Lambers et al., 2008a). SLA depends on leaf thickness, area and/or mass density and LMR depends on assimilate partitioning to leaves. Together SLA and LMR indicate how efficiently the leaves produce biomass, collectively expressed as the leaf area ratio (LAR).

Variation in relative growth rate can therefore be attributed to variation in photosynthesis and/or respiration (NAR), morphology (SLA), or leaf allocation (LMR) (Lambers et al., 2008a). All of these components seem to be influenced by both light quantity and quality (Snowden et al., 2016).

A lot of research has been conducted to find which of the components best explain differences in RGR (Lambers et al., 2008a). A meta-study which analyzed over 600 plant species, of which 153 were herbaceous dicots, found that for herbaceous species, SLA is the most important predictor of RGR at daily light integrals (DLI) of 15 mol m^{-2} d^{-1}, while NAR is more important at higher DLIs (> 25 mol m^{-2} d^{-1}). Between these DLIs, NAR and SLA seem to be about equally important and RGR are never strongly related to LMR (Shipley, 2006).

2.6 Transpiration and water usage

Most of the water taken up by plant roots is lost by evaporation from the leaves in the process of transpiration, while the remaining water is used to support growth and consumed in chemical reactions (Taiz et al., 2018). Important functions of transpiration are the uptake and distribution of water and nutrients throughout the plant, and for regulating the energy balance through evaporative cooling (von Caemmerer and Baker, 2007). The evaporation from leaf surfaces is driven by the difference between the water vapor concentration of the air outside and inside the leaf but is also largely influenced by various resistances, mainly the stomatal pores (Taiz

et al., 2018). The transpiration rate has a major impact on the climate in controlled environments, through the increase in air humidity, which in turn affects the transpiration rate (Stanghellini et al., 2018).

Stomata, being important regulators of transpiration, are important regulators for a plants' water usage. In addition, they are the only way for CO_2 influx to the leaves, thus playing a vital role in carbon assimilation. Plants must balance carbon gain and water loss to provide enough substrate for photosynthesis without dehydrating. Photosynthesis and transpiration are also connected through the uptake of nutrients, nitrogen in particular, since a large fraction of leaf nitrogen is used by chlorophyll and the enzyme RuBisCO (Evans, 1989).

Light availability and the plants' water status are major regulators of stomata (Taiz et al., 2018). Light signaling through photoreceptors regulates their aperture, where BL has been shown to activate starch degradation in guard cells, providing osmolytes and energy for stomatal opening (Matthews et al., 2020). BL can therefore regulate plant productivity and water usage through the effect on stomata.

The relationship between carbon gain to water loss is described as the water-use efficiency (WUE). It can be described on various scales, from single leaves to whole canopies, and a common way of expressing the former is the intrinsic water-use efficiency (iWUE) – the rate of photosynthesis to stomatal conductance to water vapor (Lawson and Blatt, 2014).

3 Materials and methods

In two replicate experiments, cucumber seedlings were grown in enclosed chambers where they were subjected to different light quality treatments. The experimental period took place between August 26th and November 3rd (2022) at the Norwegian University of Life Sciences in Ås, Norway.

3.1 Plant material and pre-cultivation

Seeds of cucumber (*Cucumis sativus* L., var. 'Hi Light', Nunhems Netherlands BV, Haelen, the Netherlands) were sown in 12 cm pots containing fertilized *Sphagnum* peat media (Norgro AS, Hamar, Norway) with a pH of 5.0-6.0 and an electrical conductivity (EC) of 1.0-1.5 dS m^{-1}. The plants were kept in a greenhouse compartment with glass roof and polycarbonate walls during a pre-cultivation period of 10 days (first experiment) and 11 days (second experiment) at the Centre for Plant Research in Controlled Climate, NMBU (59°40'05.8"N 10°46'17.2"E). The cultivation climate was controlled through a PRIVA system (Priva, De Lier, The Netherlands), and the room was kept at a constant temperature of 22℃ (SD=2) and a relative humidity (RH) of 66% (SD=5), with ambient CO_2 and a photoperiod of 18h. Supplementary light from both high-pressure sodium (HPS) lamps (GAN 4-550 AL 400W, Gavita International, Rozenburg, The Netherlands) and metal halide lamps (Powerstar HQI-BT, Ledvance GmbH, Garching, Germany) provided 200 μmol m^{-2} s^{-1} PAR when the solar radiation went below 250 Wm^{-2}. The plants were watered with tap water as needed.

3.2 Experimental design and growth conditions

At the start of the experiment, 10-11 day-old seedlings were distributed between two growth chambers where they were exposed to different fractions of BL for 16 days. When placed in the chambers, the first true leaf had already expanded and measured about 3 cm in length. Each chamber contained nine plants (n=9). Additionally, six plants were sown together with the rest and harvested for growth analysis (section 3.3.1) on the same day as experimental start.

In both chambers the climate was kept at a constant temperature of 22.9℃ (SD=0.4), ambient level of CO_2, RH of 62.3% (SD=3.7) and the photoperiod was set to 18h followed by 6-hour dark periods (9AM-3PM). The climate was controlled through a PRIVA system (Priva, De Lier, The Netherlands).

The light spectra were measured at the top of the canopy with a handheld spectrometer (LI-180, LI-COR Biosciences, NE, USA) at 13 different locations in the chambers in the beginning and end of each experiment. At the end of the experimental period, no light parameters had changed more than 10%.

The plants were subjected to a PPFD of approximately 235 μmol m^{-2} s^{-1} – either provided by white LEDs only (EPX FS 2021, Evolys, Oslo Norway; 400-750 nm; 10%B, 20%G, 70%R; peak 660nm), or in combination with blue LEDs (Philips GreenPower LED module HF blue, Signify, Eindhoven, The Netherlands; 400-500 nm, peak 455 nm). The chamber with white light only (henceforth referred to as "WL") had a blue fraction of about 10% of total PAR, while the chamber with additional blue light ("WL+B") had a blue fraction of about 30%. The spectral distribution is shown in Figure 3.1, and the light environment, described as the PPFD, blue fraction, red to far-red ratio (R/FR), and DLI in Table 3.1.

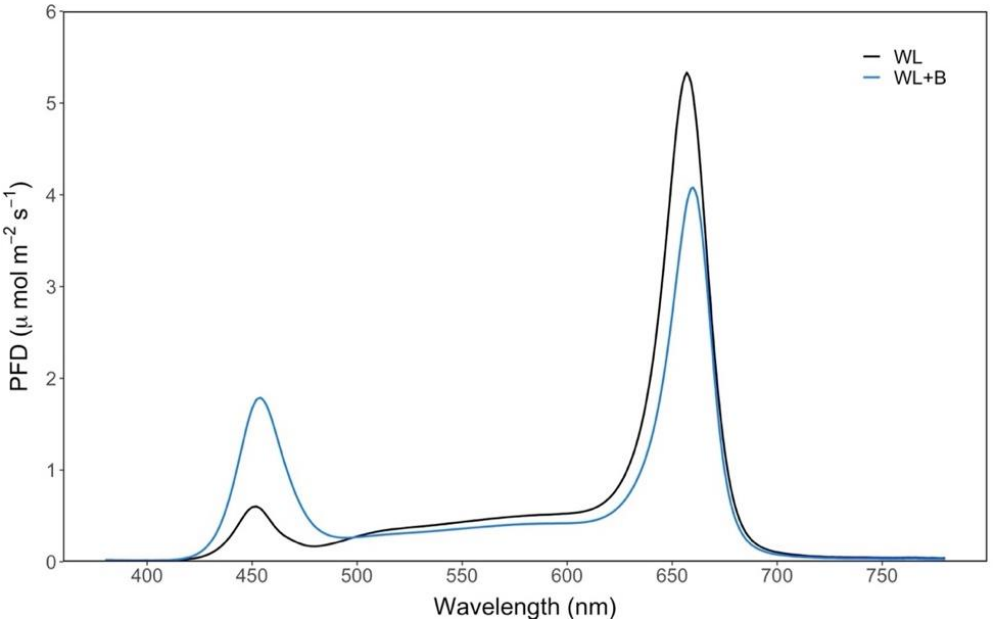

Figure 3.1: The spectral distribution (PFD nm^{-1}) of the two light quality treatments applied to seedlings of *C. sativus* for 16 days with a photoperiod of 18h.

Table 3.1: Description of the two light quality treatments for both replicate experiments as the mean (SD) of PPFD, blue fraction, R/FR and DLI (n=13).

Experimental repeat	Chamber	PPFD (μmol m^{-2} s^{-1})	Blue fraction[1] (%)	R/FR[2]	DLI[3] (mol m^{-2} d^{-1})
1	WL	234 (10)	9 (0)	57 (1)	15 (1)
	WL+B	224 (16)	28 (3)	57 (1)	15 (1)
2	WL	241 (9)	9 (0)	71 (1)	16 (1)
	WL+B	238 (20)	28 (4)	71 (1)	15 (1)

[1] Percentage blue light (400-500nm) of PAR (400-700nm), with SD measured in percentage points.
[2] Ratio between red light (600-700nm) and FR light (700-750nm).
[3] Estimated by multiplying the measured PPFD with the photoperiod of 18h.

The pots were watered daily with a nutrient solution (EC 1.5 dS m^{-1}), composed of a 50/50% solution of YaraTera Calcinit (14.4% NO3, 1.1% NH4, 19.0% Ca) and Kristalon Indigo (7.5% NO3, 1.0% NH4, 4.9% P, 24.7% K, 4.2% Mg, 5.7% S, 0.027% B, 0.004% Cu, 0.2% Fe, 0.06% Mn, 0.004% Mo, 0.027% Zn) (Yara Norge, Oslo, Norway). The plant positions were rotated daily to reduce border effects.

3.3 Measurements

Several measurements were performed during the 16-day experimental period. Measurements of growth parameters, gas exchange, pigments and water usage were performed on the same plants, while the remaining plants were used for destructive leaf sampling. All measurements were performed on both experimental repeats after the same number of days after experimental start, except for carbohydrate analysis and stomatal density where samples were taken only from the first experiment.

3.3.1 Growth

During the whole experiment, leaf length on one leaf from three plants per treatment was measured at the beginning of each photoperiod (n=6 with both experiments). The measurement was taken on the first visible leaf appearing after experimental start (the second oldest true leaf), along the midrib from base to apex (or until a width ≥1 mm).

Growth component analysis was performed by harvesting six plants before the start of the experimental period (t_1), and 2-3 plants per treatment at the end (t_2), all randomly selected. For each plant, leaf area (including cotyledons if still viable at t_2) was measured using an area meter (LI-3100, LI-COR Biosciences, NE, USA), height was measured from the base of the stem to the shoot apical meristem using a ruler, and the number of true leaves were counted. The first unfolded leaf at t_1 was approximately 3 cm in length, so 3 cm was used as a limit for what would count as the number of true leaves at t_2 (until a width ≥1 mm). All above-ground biomass was dried at 55 ℃ for at least seven days, with the leaf blades separated from the rest of the shoot biomass.

Dry weight (DW) and leaf area were used to calculate the relative growth rate (RGR, d^{-1}) and its components; net assimilation rate (NAR, mg d^{-1} cm^{-2}), leaf area ratio (LAR, cm^2 g^{-1}), specific leaf area (SLA, cm^2 g^{-1}) and leaf mass ratio (LMR g g^{-1}). RGR was calculated as described by Hoffmann and Poorter (2002) using the mean natural logarithm-transformed weights, with the following equation:

$$\mathrm{RGR} = \frac{\left(\overline{\ln W_2} - \overline{\ln W_1}\right)}{t_2 - t_1} \qquad \textbf{(3.1)}$$

where W_1 and W_2 are the total above-ground DW at times t_1 and t_2.

The growth components making up RGR are defined by the following equations (Hunt, 1990):

$$\mathrm{RGR} = \mathrm{NAR} \times \mathrm{SLA} \times \mathrm{LMR} = \mathrm{NAR} \times \mathrm{LAR} \quad \textbf{(3.2)}$$

$$\mathrm{NAR} = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{\ln A_2 - \ln A_1}{A_2 - A_1} \quad \textbf{(3.3)}$$

$$\mathrm{LAR} = \frac{A_2}{W_2} = \mathrm{SLA} \times \mathrm{LMR} \quad \textbf{(3.4)}$$

$$\mathrm{SLA} = \frac{A_2}{WL_2} \quad \textbf{(3.5)}$$

$$\mathrm{LMR} = \frac{WL_2}{W_2} \quad \textbf{(3.6)}$$

where A_1 and A_2 are the total leaf areas at times t_1 and t_2, and WL_2 is the total leaf DW at time t_2. All replicates from both harvests (at t_1 and t_2) were paired with each other before computing RGR and NAR. With both experiments combined, the number of replicates per treatment were 6 (WL) and 5 (WL+B).

3.3.2 Leaf gas exchange

To estimate photosynthesis (A) and stomatal conductance (g_s), gas exchange was measured at seven levels of irradiance with an infrared gas analyzer (IRGA, LI-6400XT Portable Photosynthesis System). The IRGA was connected to a leaf chamber fluorometer (LI-COR 6400-40) (LI-COR Biosciences, NE, USA) providing 90% red light and 10% blue light (with center wavelengths at 630 and 470 nm, respectively).

The auto-program LightCurve2 was used with decremental light intensities of 1000, 600, 300, 150, 100, 50 and 0 μmol m^{-2} s^{-1}, stability wait time from 90 to 150 seconds and IRGA matching before logging from each light intensity. Before each new measurement the leaf was exposed to a light intensity of 1000 μmol m^{-2} s^{-1} in the leaf chamber until reaching stability.

As for the environmental controls, the flow rate was set to 300 μmol s^{-1}, block temperature to 23℃ and reference CO_2 to 415 μmol mol^{-1}. RH was attempted to be kept at chamber conditions ranging from 56-71% with an average of 61%. The whole system was placed inside the growth chamber during measurements.

Measurements were performed after 10 and 11 days of treatment, 1-2 hours after the beginning of each photoperiod. The second oldest leaf (youngest fully expanded true leaf at that time) of 3-4 randomly selected plants per treatment were chosen for analysis in each experiment (n=7).

Light response curves were fitted as A/I, where A is the net photosynthesis (μmol CO_2 m^{-2} s^{-1}) and I the irradiance (μmol photons m^{-2} s^{-1}). Curves were fitted for all replicates with the Excel spreadsheet provided by de Lobo et al. (2013), using the non-rectangular hyperbola-based model (Eqn. 6) by Prioul and Chartier (1977).

Water-use efficiency at leaf level was calculated as intrinsic water-use efficiency (iWUE), using parameters from the gas exchange measurements, with the formula:

$$\mathrm{iWUE} = \frac{\mathrm{A}}{\mathrm{g_s}} \qquad \textbf{(3.7)}$$

where g_s is the stomatal conductance to water vapor (mol H_2O m^{-2} s^{-1}).

3.3.3 Photosynthetic pigments

At the end of each experiment, pigment extraction was performed during harvesting for growth analysis (section 3.3.1), from the same leaf as gas exchange was measured (section 3.3.2). Two discs were collected from each leaf using a 9mm cork borer (0.64 cm^2) – one was taken next to the midrib near the base, and the other from the vein next to the midrib at the same height (higher if injured). The discs were collected in separate 1.5 ml Eppendorf tubes containing DMSO solution saturated with $MgCO_3$ immediately after cutting. They were then placed in an ultrasonic water bath (USC200TH, VWR International, PA, USA) containing 60℃ deionized water for 40 min. All the work was performed away from sunlight and the samples were kept in darkness between steps.

The absorbance was measured on extracts of 1 ml with a scanning spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at wavelengths 665, 649, 480 and 750 nm (for chlorophyll *a*, chlorophyll *b,* combined carotenoids, and background noise, respectively). The same solution of DMSO that was used for extraction was used as a reference.

The concentrations of chlorophyll *a* (chl *a*) chlorophyll *b* (chl *b*) and total carotenoids (car) were calculated according to Wellburn (1994), using the absorbance values (A) at the different wavelengths:

$$\text{Chl } a = 12.19(A_{665} - A_{750}) - 3.45(A_{649} - A_{750}) \quad \textbf{(3.8)}$$

$$\text{Chl } b = 21.99(A_{649} - A_{750}) - 5.32(A_{665} - A_{750}) \quad \textbf{(3.9)}$$

$$\text{Car} = \frac{1000(A_{480} - A_{750}) - 2.14C_a - 70.16C_b}{220} \quad \textbf{(3.10)}$$

The calculated concentrations from both discs of the same leaf were averaged before the statistical analysis, resulting in *n*=6 (WL) and *n*=5 (WL+B) with both experiments combined.

3.3.4 Leaf samples

Leaf samples were collected from the first experiment to determine the content of carbohydrates and the stomatal density after 15 days of treatment. Leaves from the same plant were harvested for both analyses simultaneously. The second oldest leaf was used for counting stomata and the third oldest was used for carbohydrate analysis. Both leaves were developed under experimental conditions and considered source leaves.

3.3.5 Carbohydrate analysis

Leaves from three plants were sampled 1-2 hours after the beginning of the dark period and the following light period (*n*=3 per time period).

During sampling, leaf blades were detached from the petiole, collected in centrifuge tubes, and then immediately frozen in liquid N_2. They were then kept at -80 ℃ until further use. The frozen samples were freeze-dried with a Telstar LyoQuest-55 benchtop freeze-dryer (Azbil Telstar, Barcelona, Spain) and crushed to fine powder using pestle and mortar with some liquid N_2. The powder was then divided in smaller portions and kept in Eppendorf tubes at -80 ℃ until analysis.

Due to non-significant differences between the two time periods, the data is presented as pooled means from both time periods (*n*=6) for both the starch- and sugar analysis.

3.3.5.1 Starch

Starch content was determined enzymatically using the assay kit Total Starch HK (AMG/α-amylase/HK method, Megazyme, 2018), using approximately 100 mg freeze-dried material, weighed accurately. The samples were assumed to contain D-glucose and/or maltodextrins and resistant starch, and the protocol followed accordingly. After starch digestion and dilution (following normal procedure at step a.6 with a total volume of 100ml), the concentration of D-glucose was determined using a UV-Visible Spectrophotometer at 340nm (Helios Alpha 9423, Thermo Scientific, MA, USA). The starch concentration (c_{starch}, g l^{-1}) was calculated with the formula provided in the manual, without further dilution and with a sample volume of 0.05 ml. This value was eventually used to calculate the starch content (g mg^{-1} sample DW) as:

$$\frac{c_{\text{starch}}}{c_{\text{sample}}} \times 1000 \qquad \textbf{(3.11)}$$

3.3.5.2 Soluble sugars

The concentration of soluble sugars (fructose, glucose, raffinose, stachyose and sucrose) were determined with high pressure liquid chromatography (HPLC).

Sugar extraction was performed by adding 1.5 ml 80% EtOH to approximately 100mg freeze dried material (weighed accurately), vortexing and heating in a 70℃ ultrasonic water bath (USC200TH, VWR International, PA, USA) for 15 min, followed by centrifuging at 15000 rpm for 3 min (5417C, Eppendorf AG, Hamburg, Germany). The extractions were performed four times, using only 0.5 ml EtOH for the last extraction. The supernatant from all extractions were combined in vacuum centrifuge tubes and the ethanol completely evaporated in a vacuum centrifuge with heating (Savant SC210A SpeedVac Concentrator, Thermo Scientific, MA, USA). The pellet was dissolved in 1 ml distilled water, vortexed, heated at 70℃ in an ultrasonic water bath for 15 min, vortexed and centrifuged at 15000 rpm for 3 min. The supernatant was transferred to clear 1.5 ml HPLC glass vials (VWR, PA, USA) using a sterile 2 ml BD Emerald^{TM} syringe (BD Switzerland Sarl, Vaud, Switzerland) and syringe filter (PTFE membrane, Acrodisc 13mm minispiker) with 0.45 μm pore size (Pall Corporation, Pall Biotech Oslo, Norway).

Separation was performed with an Agilent 1200 HPLC (Agilent Technologies, CA, USA), using an Agilent Zorbax Carbohydrate column (4.6 mm ID x 150 mm, 5µm, Agilent Technologies, CA, USA) combined with guard columns (4.6 mm ID x 12.5 mm, Agilent Technologies, CA, USA). Acetonitrile (67.5%) was used as the mobile phase and silica as

stationary phase. The flow rate was set to 1.4 ml min^{-1} and column temperature 30℃. A Refractive Index Detector (RID) was used for detection of peaks. Peak areas were analyzed using Agilent Chemstation software (version B.04.02 SP1, Agilent Technologies, CA, USA) and determination of sample concentrations were based on peak areas of external standards of stachyose, raffinose, glucose, sucrose, and fructose with concentrations (c) of 0.125, 0.25, and 0.5% (w/v). Sample concentrations were calculated using the concentration area (A) with the following equation:

$$c_{\text{standard}} \times \frac{A_{\text{sample}}}{A_{\text{standard}}} \quad \textbf{(3.12)}$$

and adjusted according to sample weights.

3.3.6 Stomatal density

Leaves were sampled 1-2 hours after the beginning of the light period. During sampling, small pieces were cut with a scalpel along the midrib near the base, immediately fixed in 4% paraformaldehyde and stored at 4℃ until further use. To prepare for imaging, the pieces were washed in 0.05M PIPES buffer, then dehydrated in a graded series of ethanol, ending with 100% EtOH. Critical point dehydration was then performed with Bal-Tec CPD 030 (Bal-Tec AG, Pfäffikon, Switzerland) before finally coating the samples with platinum, both performed by the Imaging Centre, NMBU. Stomata were counted using micrographs (0.123 mm^2) taken with a scanning electron microscope (SEM, Zeiss Evo 50, ZEISS, Oberkochen, Germany) at 700x magnification. Stomata were counted on both the abaxial and adaxial surface, on two pieces per treatment on three different areas per piece (n=6 per treatment and leaf side) using the Multi-point Tool in ImageJ (Schneider et al., 2012).

3.3.7 Whole-plant water usage

To estimate transpiration at plant level, water loss was measured gravimetrically during the last three days of the experiment (after 13-16 days of treatment).

Two or three plants per treatment were thoroughly watered with the same nutrient solution as before and the pots were covered with plastic bags to minimize soil evaporation. The plants were weighed at the end of night (EON) and at the end of day (EOD) during three subsequent dark- and light periods. With both experiments combined, the number of replicates per treatment and time were 6 (WL) and 5 (WL+B).

Water usage (μmol m^{-2} s^{-1}) for each period was calculated as:

$$\text{Water usage} = \frac{\Delta W}{t \times A_2} \times M_{H2O} \quad \textbf{(3.13)}$$

where ΔW is the weight difference between EOD/EON and the previous period, t is the time of the dark- or light period, A_2 is the total leaf area (as measured and described in section 3.3.1), and M_{H2O} is the molar mass of water (= 18.01528 $gmol^{-1}$). The average from the three periods was used in the statistical analysis. Transpiration ratio was calculated as the average water usage during the light period divided by that of the dark period.

3.4 Data analysis

The statistical analyses and graphical outputs were performed with R software v4.2.2 (R Core Team, 2022). Figures were produced using ggplot2 package (Wickham, 2016). All analyses were performed using linear models (ANOVA) with post-hoc tests (Tukey's HSD) using the Tukey-Kramer method for groupwise comparisons of means, both with a significance level (α) of 5% (p<0.05).

All data were averaged from both experimental repeats (except carbohydrates and stomatal density), and several parameters differed significantly (p<0.001) between experiments. Both experiments were still averaged since no reasonable arguments could be found to exclude any data. The repeats were instead used as a blocking variable to account for nuisance factors (i.e., variation from other factors than the light treatment).

The number of plants measured in some analyses were slightly unbalanced due to plant damage during the first experiment, and all data was analyzed using type III sums of squares as a first measure to correct for this. Model assumptions were examined graphically in addition to a Shapiro-Wilk normality test for distribution of residuals and Levene's Test to check for homogeneity of variance.

Violations to assumption of normality were assessed by transforming the response with the natural logarithm before averaging (height and number of leaves). In cases with normal residuals and homoscedasticity, but with significant violations to assumption of equal variance, Welch's t-test was performed (chl *b*).

4 Results

4.1 Growth

The development of plants grown under 10% BL (treatment WL) between day 1 and 15 are shown in Figure 4.1. Yellow arrows indicate the leaf on which length was measured (second oldest) and shows that it was fully expanded after 7 days of treatment.

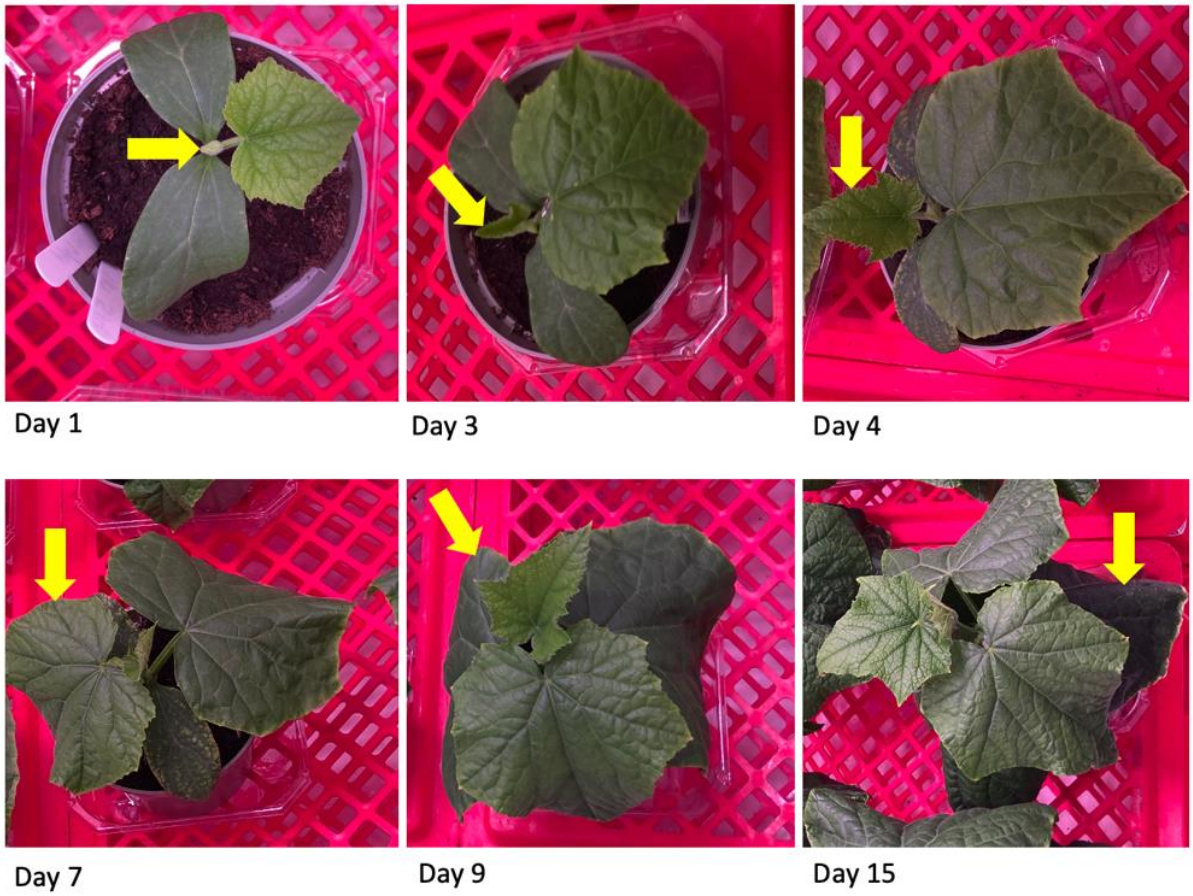

Figure 4.1 The development of plants in treatment WL during the experiment, from day 1 to 15. Leaf length was measured daily on the second oldest leaf, developed under experimental conditions (shown with yellow arrow).

Additional blue light (treatment WL+B) did not substantially affect leaf length compared to WL (Figure 4.2A). Up to day 10, the leaves were slightly shorter under treatment WL+B compared to treatment WL, whereas the trend turned after 10 days. No significant difference between treatments was found on the last day (p=0.121, one-way ANOVA). Figure 4.2B shows the daily change in leaf length during the experimental period, with a clear peak after six days.

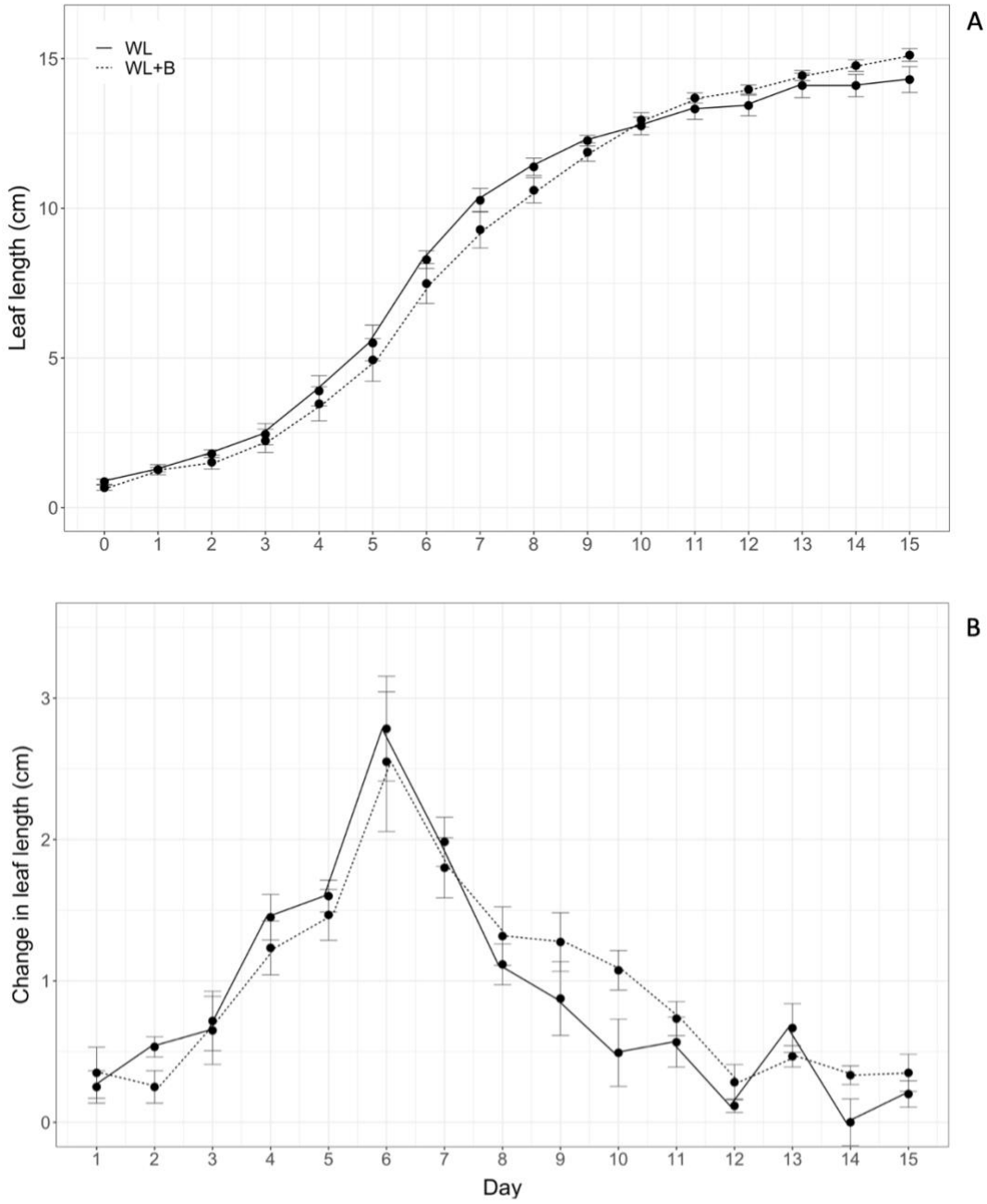

Figure 4.2: Leaf length (A) and daily change in leaf length (B) during the experimental period of 16 days under white light (WL) and with additional blue (WL+B). Leaf length was measured on the first visible leaf (second oldest true leaf) after experimental start (day 0). Values are means from both experiments combined ± SE (n=6).

Total leaf area did not differ significantly between treatments (p=0.790, one-way ANOVA, Table 4.1) nor did the number of true leaves (p=0.467). There was a trend for shorter shoot length, and lower leaf- and shoot DW under WL+B compared to WL, though the means were not significantly different (p=0.069, 0.058 and 0.091 respectively).

Table 4.1: Morphological responses under white light (WL) and with additional blue (WL+B), measured at the end of each experiment. Values are means from both experiments combined ± SE, with n=6 (WL) and n=5 (WL+B). Significance codes[1] from one-way ANOVA (α=5%) are also shown.

	WL	WL+B	ANOVA
Total leaf area (cm^2)	975.8 ± 74.5	974.1 ± 50.4	ns
Number of true leaves[2]	7.5 ± 0.4	7.6 ± 0.6	ns
Shoot length (cm)[2]	16.3 ± 1.0	14.4 ± 1.0	.
Leaves DW (g)	4.2 ± 0.2	3.6 ± 0.2	.
Shoot DW (g)	4.9 ± 0.3	4.3 ± 0.3	.

[1] Signif. codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 'ns'.
[2] Mean of natural logarithm-transformed response due to non-normal residuals.

As for the growth analysis, RGR did not differ significantly between treatments (p=0.097, one-way ANOVA), though it was slightly lower in treatment WL+B compared to WL (Table 4.2). However, there was a significant decrease in NAR and LMR (p<0.001 and p=0.004, respectively), and an increase in LAR and SLA (p<0.001) in WL+B compared to WL.

Table 4.2: Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf mass ratio (LMR) under white light (WL) and with additional blue (WL+B). Values are means from both experiments combined ± SE, with n=6 (WL) and n=5 (WL+B). The relative change in all components compared to WL are also shown, along with significance codes[1] from one-way ANOVA (α=5%).

	WL	WL+B	Relative change (%)	ANOVA
RGR (d^{-1})	0.22 ± 0.00	0.21 ± 0.00	-4.5	.
NAR (mg $m^{-2}d^{-1}$)	0.98 ± 0.01	0.85 ± 0.03	-13.3	***
LAR ($cm^2\ g^{-1}$)	199.6 ± 4.4	228.5 ± 2.6	+14.5	***
SLA ($cm^2\ g^{-1}$)	230.5 ± 5.5	268.4 ± 2.7	+16.4	***
LMR ($g\ g^{-1}$)	0.87 ± 0.00	0.85 ± 0.00	-2.3	**

[1] Signif. codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 'ns'.

4.2 Photosynthesis

Leaf net photosynthesis in treatment WL+B was 42-61% higher compared to WL at the three highest levels of irradiance (300-1000 μmol m^{-2} s^{-1}, respectively) (p<0.001, one-way ANOVA, Figure 4.3). At the lowest irradiances (50-150 μmol m^{-2} s^{-1}), the treatment did not significantly affect net photosynthesis (p=0.078, 0.763 and 0.240 respectively). Dark respiration was 39% higher in WL+B compared to WL (p=0.014).

Based on values from the fitted curve, net photosynthesis at chamber irradiance (235 μmol m^{-2} s^{-1}) was estimated to be 7.8±0.4 and 10.2±0.5 μmol CO_2 m^{-2} s^{-1} under treatment WL and WL+B respectively (Supplementary Materials, Table 9.1). This corresponds to 31% greater photosynthesis in WL+B compared to treatment WL (p=0.003, one-way ANOVA).

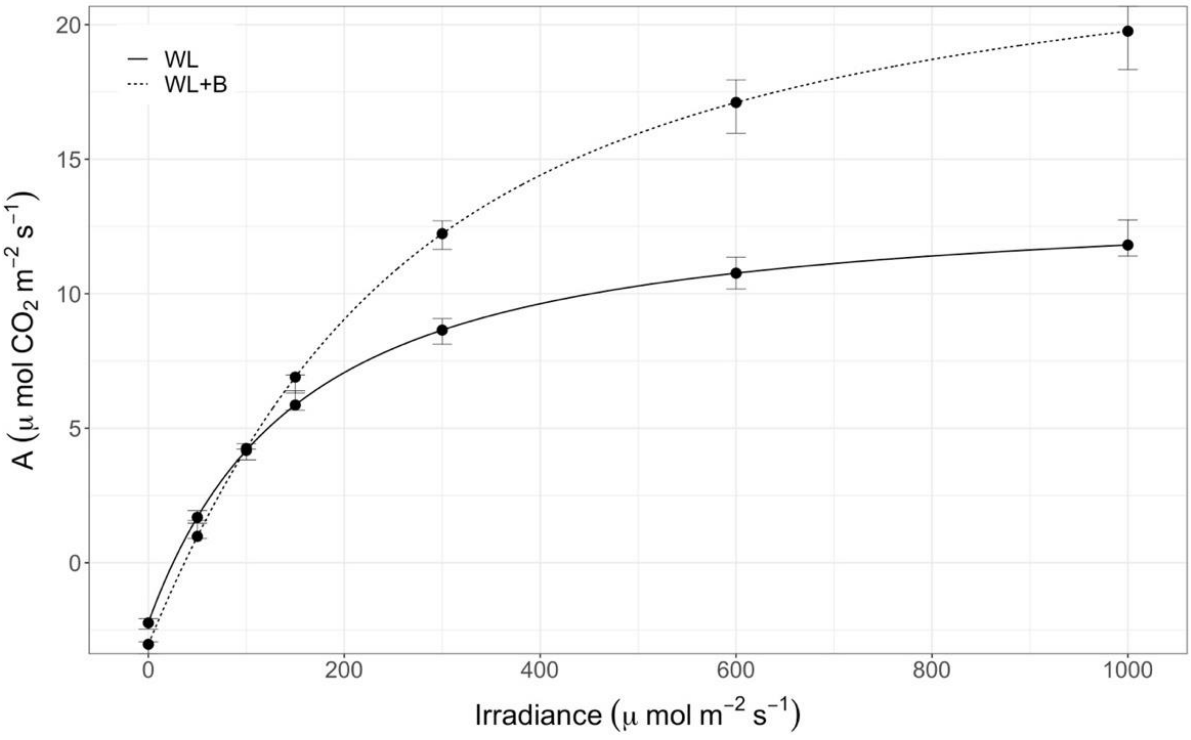

Figure 4.3: Leaf photosynthetic light response curve under white light (WL) and with additional blue (WL+B). Net photosynthesis (A) was measured on the youngest fully expanded true leaf (second oldest) at seven levels of irradiance after 10-11 days of treatment, with a fluorometer providing red-blue light. Chamber irradiance was ~235 μmol m^{-2} s^{-1}. Curve fitting was performed with the Excel spreadsheet provided by de Lobo et al., using the non-rectangular hyperbola-based model (Eqn.6) from Prioul and Chartier (de Lobo et al., 2013; Prioul and Chartier, 1977). Values are means from both experiments combined ± SE (n=7).

4.3 Photosynthetic pigments

The concentrations of chl *a* and *b* were significantly higher under treatment WL+B (p=0.004 and p=0.013 respectively, one-way ANOVA, Table 4.3). Combined, the chlorophyll concentration was 37% higher in WL+B compared to WL. The ratio of chl *a*:*b* and concentration of carotenoids did not differ significantly between treatments (p=0.366 and p=0.052, respectively).

Table 4.3: Concentration of chlorophyll *a* and *b*, total chlorophylls, chlorophyll *a*:*b* ratio, and carotenoid concentration under white light (WL) and with additional blue (WL+B), measured at the end of each experiment on the second oldest true leaf. Values are means from both experiments combined ± SE, with n=6 (WL) and n=5 (WL+B). Significant codes[1] from one-way ANOVA ($\boldsymbol{\alpha}$=5%) are also shown.

	WL	WL+B	ANOVA
Chl *a* (μg cm^{-2})	31.4 ± 0.7	41.2 ± 2.3	**
Chl *b* (μg cm^{-2})[2]	10.0 ± 0.2	13.4 ± 0.9	*
Total chlorophylls (μg cm^{-2})	41.3 ± 1.0	54.6 ± 3.2	**
Chl *a*:*b*-ratio	3.2 ± 0.0	3.1 ± 0.1	ns
Carotenoids (μg cm^{-2})	5.2 ± 0.2	6.3 ± 0.4	.

[1] Signif. codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 'ns'.
[2] Significance tested with Welch's t-test due to unequal variances

4.4 Carbohydrates

Additional BL had a large effect on the starch content, but not on the content of soluble sugars (Figure 4.4). The starch content was significantly lower (57%) in leaves exposed to treatment WL+B compared to WL (p<0.001, Tukey's HSD test). There was a trend for higher concentration of soluble sugars in WL+B than in WL, though the difference was not significant (p=0.975, Tukey's HSD test).

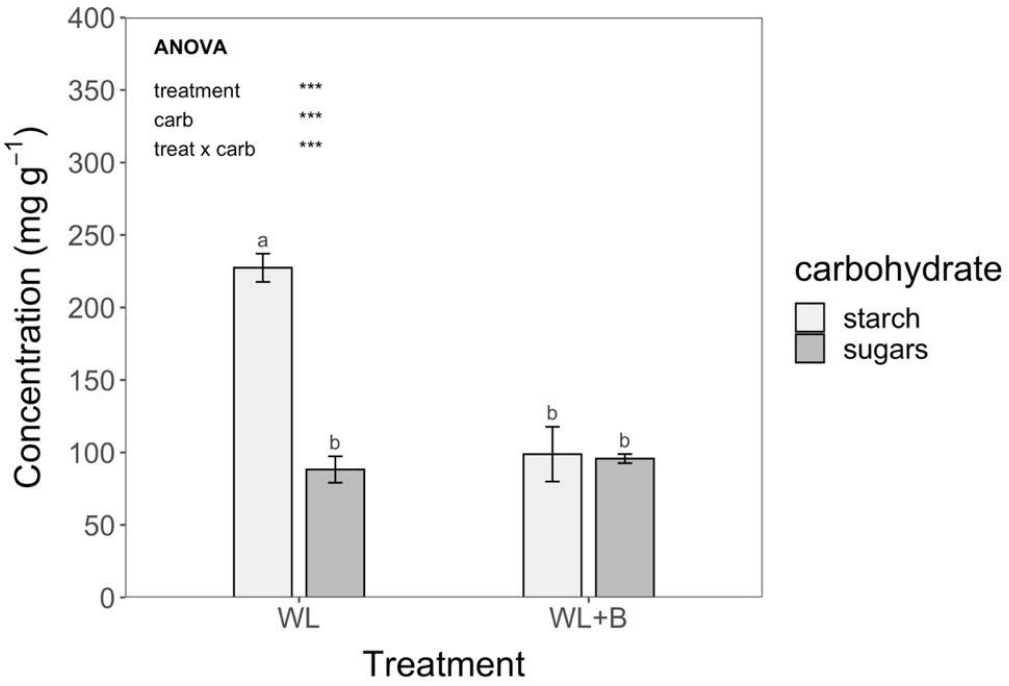

Figure 4.4: Concentrations of starch and soluble sugars (mg g^{-1} DW) in leaves exposed to white light (WL) and with additional blue (WL+B). Samples were taken from the third oldest leaf towards the end of the first experimental repeat at day and night. Data are means with day- and night samples combined ± SE, with n=6 per treatment and carbohydrate. Means with different letters are significantly different (Tukey's HSD test; $\boldsymbol{\alpha}$=5%). Results from two-way ANOVA with interaction is presented with asterisks ($\boldsymbol{\alpha}$=5%). Signif. codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 'ns'.

Similar to the concentration of total sugars, there was also a trend for higher concentration of each individual sugar in WL+B compared to WL (Figure 4.5), though the difference between treatments was only significant for raffinose (p=0.007, one-way ANOVA), where leaves exposed to WL+B had 76% higher concentrations compared to WL. Among the soluble sugars in both treatments, the concentration of sucrose was highest, followed by stachyose, fructose, glucose, and with raffinose as the least common sugar.

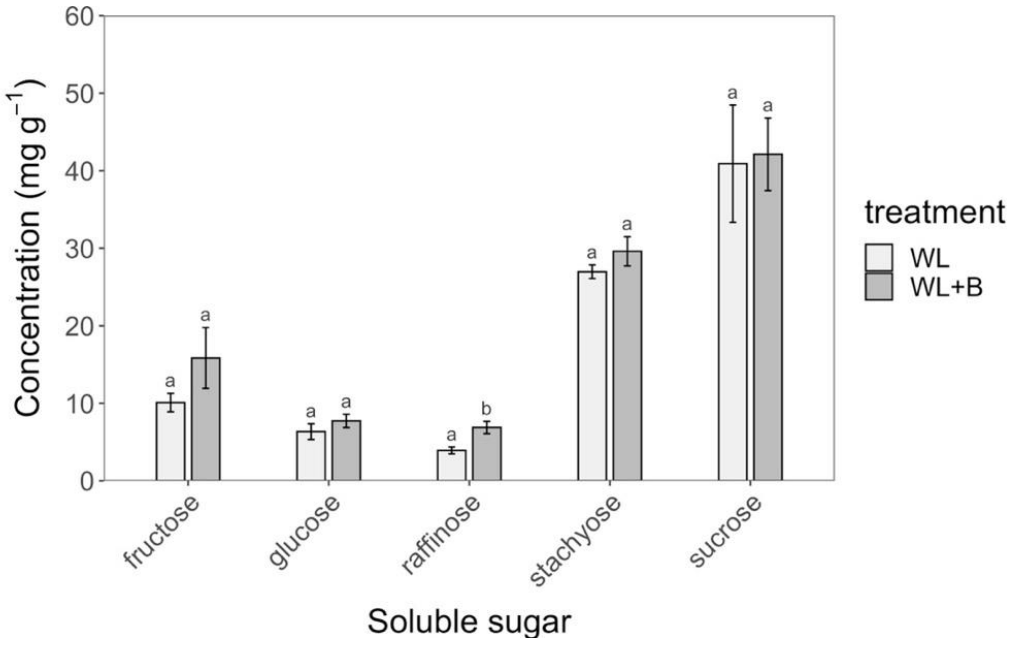

Figure 4.5 Concentration (mg g^{-1} DW) of fructose, glucose, raffinose, stachyose and sucrose under white light (WL) and with additional blue (WL+B). Samples were taken from the third oldest leaf towards the end of the first experimental repeat at day and night. Data are means with day- and night samples combined ± SE with n=6 per treatment, except for fructose (n=4) and raffinose (n =5) in WL+B. Means with different letters within each sugar are significantly different (one-way ANOVA; $\boldsymbol{\alpha}$=5%).

4.5 Water usage

The whole-plant water usage was 36% higher in treatment WL+B compared to WL, independent of time of day (p<0.005, Tukey's HSD test, Figure 4.6). The water usage was significantly lower at night compared to the day, irrespective of light treatment (p<0.001, Tukey's HSD test) with similar transpiration ratios (water usage day/night) of 1.73±0.04 in treatment WL and 1.75±0.13 in WL+B p=0.764, one-way ANOVA).

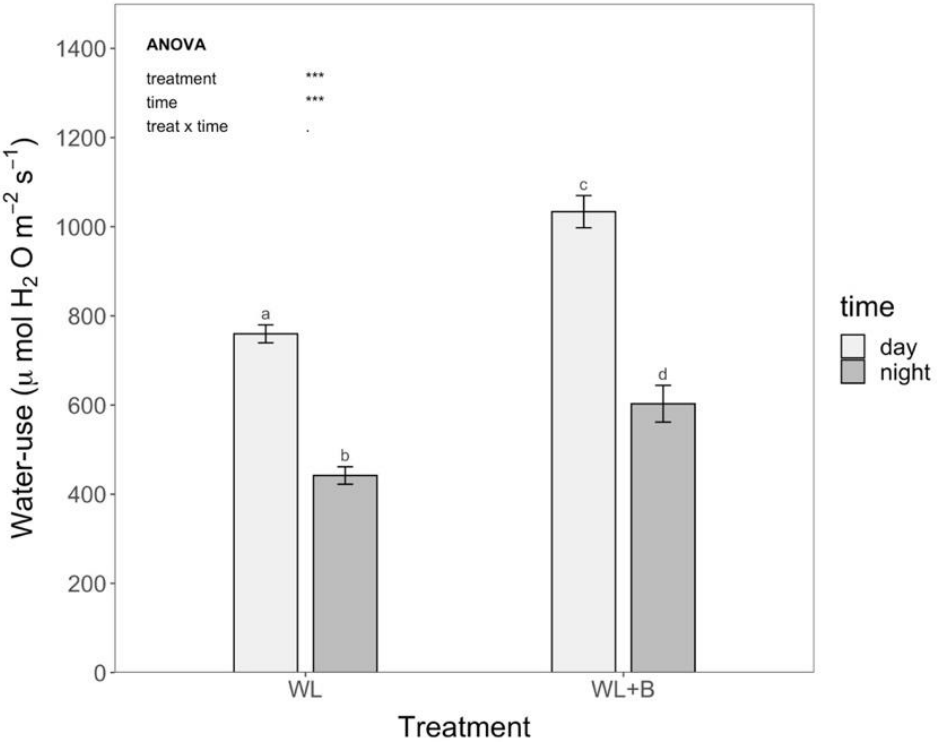

Figure 4.6: Whole-plant water usage during light period (day) and dark period (night) under white light (WL) and with additional blue (WL+B), measured after 13-16 days of treatment. Values are means from both experiments combined ± SE with n=6 (WL) and n=5 (WL+B) per treatment and time. Means with different letters are significantly different (Tukey's HSD test; α=5%). Results from two-way ANOVA with interaction is presented with asterisks (α=5%). Signif. codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 'ns'.

Leaf stomatal conductance to water vapor (g_s) increased with increasing irradiance and was higher under WL+B than WL (Figure 4.7), in accordance with results from whole-plant water usage. The differences were significant for all levels of irradiance (p<0.001 for 0-150 μmol m^{-2} s^{-1} and p<0.01 for 300-1000 μmol m^{-2} s^{-1}, one-way ANOVA). By using g_s between 150 and 300 μmol m^{-2} s^{-1} as a visual estimate for the stomatal conductance under chamber irradiance (235 μmol m^{-2} s^{-1}), treatment WL+B relative to WL led to 75-78% higher conductance.

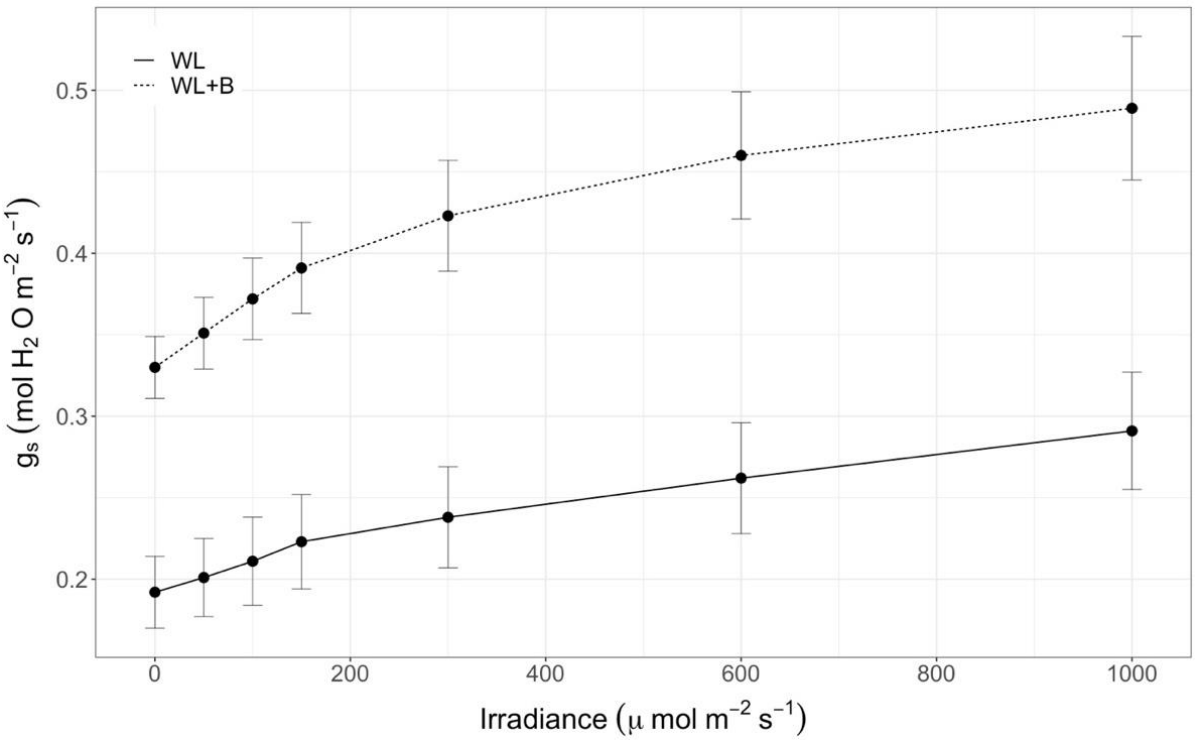

Figure 4.7 Single-leaf stomatal conductance to water vapor (g_s) over seven levels of irradiance, under white light (WL) and with additional blue (WL+B). Measurements were performed with IRGA under red-blue light. They were taken 1-2 hours after start of photoperiod on the youngest fully expanded true leaf (second oldest) after 10-11 days of treatment. Values are means from both experiments combined (n=7).

In general, the intrinsic water-use efficiency (iWUE) was lower for WL+B compared to WL (Figure 4.8). The difference was significant for the lowest irradiances, 50-150 μmol m^{-2} s^{-1} ($p<0.01$, one-way ANOVA), but not for the highest irradiances, 300-1000 μmol m^{-2} s^{-1} (p=0.064, 0.208 and 0.527 respectively).

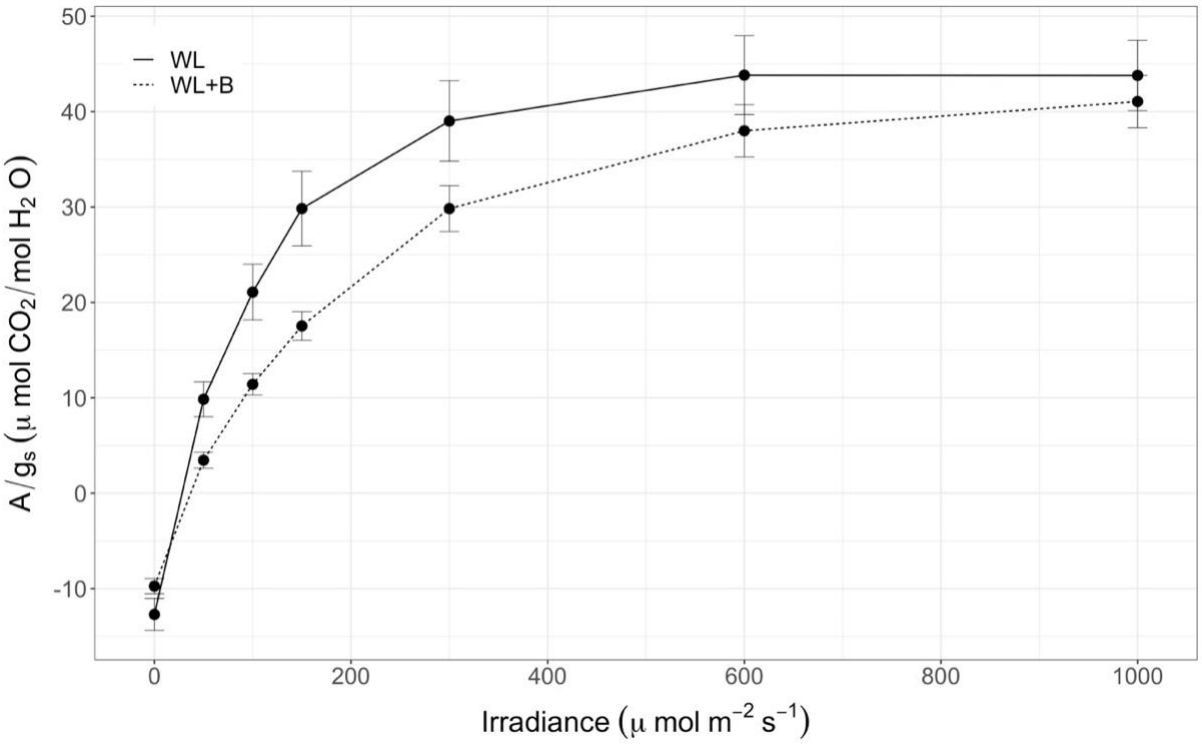

Figure 4.8: Intrinsic water-use efficiency (iWUE) over seven levels of irradiance, under white light (WL) and with additional blue (WL+B). Measurements were performed with IRGA under red-blue light. They were taken 1-2 hours after start of photoperiod on the youngest fully expanded true leaf (second oldest) after 10-11 days of treatment. Values are means from both experiments combined (n=7).

4.6 Stomatal density

The stomatal density varied significantly between treatments (p=0.037, two-way ANOVA, Figure 4.9), with plants in treatment WL+B having a density of -17% on the adaxial (upper) and -5% on the abaxial (lower) side of the leaf, compared to WL. However, the significant difference in means ceased with the post-hoc analysis (p= 0.149 and 0.878 for adaxial and abaxial side respectively, Tukey's HSD test).

The abaxial leaf side had a significantly higher density than the adaxial side (p= 0.016, two-way ANOVA), with a stomatal ratio (abaxial/adaxial) of 1.2 (WL) and 1.4 (WL+B). A significant difference remained only for WL+B when performing groupwise comparisons of means (p=0.071 and 0.003 for WL+B and WL respectively, Tukey's HSD test). Overall, the interaction between treatment and leaf side was not significant (p= 0.305, two-way ANOVA).

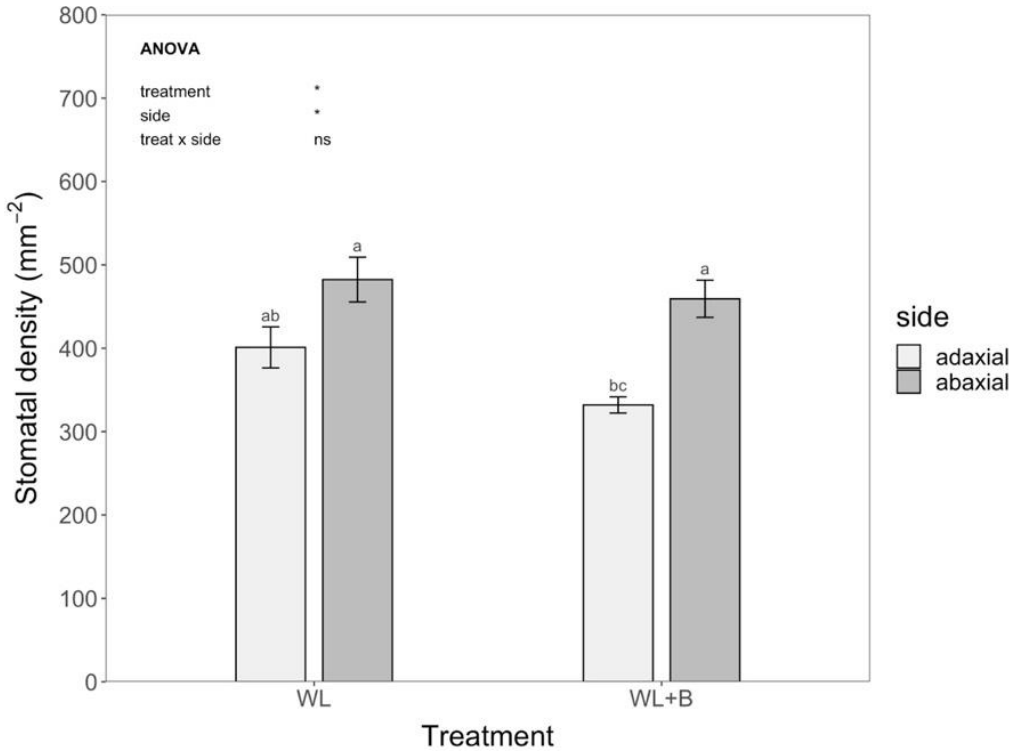

Figure 4.9 Stomatal density on upper (adaxial) and lower (abaxial) side of the leaf under white light (WL) and with additional blue (WL+B). Samples were taken from the second oldest leaf towards the end of the first experimental repeat. Data are means from three different areas on two different leaves ± SE (n=6 per treatment and side). Means with no letter in common are significantly different (Tukey's HSD test; α=5%). Results from two-way ANOVA with interaction is presented with asterisks (α=5%). Signif. codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 'ns'.

5 Discussion

5.1 Additional BL increased photosynthetic capacity, chlorophyll concentration and stomatal conductance

In line with expectations, additional BL resulted in higher photosynthetic rate (A) at high light intensities (Figure 4.3), meaning that the photosynthetic capacity of plants acclimated to 30% BL was improved compared to plants grown under 10% BL. The WL+B curve also seems to continue to increase above 1000 μmol m^{-2} s^{-1}, suggesting that the maximum photosynthetic rate (A_{max}) had not yet been reached by leaves acclimated to 30% BL, and would likely continue to increase CO_2 assimilation at even higher light intensities. Indeed, Kang et al. (2021) found A_{max} to increase above the highest level measured here (20 μmol CO_2 m^{-2} s^{-1}) in leaves acclimated to 72% BL. Leaves acclimated to high light intensities have been found to have higher chl *a:b* ratios and carotenoid concentrations (Lichtenthaler et al., 1981). Nevertheless, despite plants grown under WL+B being better acclimated to high light intensities, chl *a:b* ratio and carotenoid concentrations were not affected by the treatment.

The estimated A at chamber irradiance was also significantly greater in WL+B (+31% compared to WL). Since the measurements were performed with the same light source, the difference in photosynthetic rates between treatments reflects different acclimations rather than direct effects on photosynthesis. Due to the relatively low quantum yield of blue photons compared to red photons (McCree, 1971) this is unsurprising.

Acclimation to higher fractions of BL can induce several photomorphogenic responses related to photosynthesis. Higher photosynthetic capacity has previously been correlated with a higher stomatal conductance (g_s), concentration of chlorophylls and nitrogen (Hogewoning et al., 2010b; Kang et al., 2021), and the results presented here show agreement with this (Figure 4.7 and Table 4.3). In theory, higher chlorophyll content should increase the capacity to capture light (Lambers et al., 2008b), and higher g_s is strongly correlated to A due to increased CO_2 uptake, at least under steady state conditions (Farquhar and Sharkey, 1982). Furthermore, BL is also important for transcription of RuBisCO and other key enzymes involved in CO_2 fixation and thus photosynthesis (Wang et al., 2009). Kang et al. (2021) found that leaves acclimated to a high fraction of BL under a very low DLI could maintain a stable and high photosynthesis after exposure to high and variable light intensities. On the other hand, plants acclimated to lower blue fractions got more stressed in these conditions, which resulted in relatively lower

photosynthesis. They found that BL-acclimation caused enhanced photoprotective capacity, preventing damage caused by high irradiances. After one week of growth under solar radiation, these plants had obtained the highest increase in biomass. This may have consequences for plant performance in cucumber production, and it would be interesting to investigate how BL-acclimation affects growth in the long term, and the potential effects on yield.

5.2 Allocation and assimilation affected by BL, but not growth rate or leaf development

Despite shoot DW and RGR being less affected by the additional BL, the growth analysis revealed differences in integrated net assimilation, leaf morphology and partitioning (Table 4.1 and 4.2).

LAR was highest in WL+B, which indicates that 30% BL enhanced the leaves' efficiency to produce biomass. This was caused by a higher SLA since the other component, LMR, was lower. The latter suggests that WL+B plants allocated less resources to the leaves compared to WL plants, although the treatment effect was quite small. Higher SLA is normally associated with higher radiation capture due to increased leaf area (Lambers et al., 2008a), but leaf area was virtually unaffected by treatment and can therefore not explain the higher SLA in WL+B compared to WL. The leaves must therefore have been thinner or less dense, and how this may affect growth rate is discussed in section 5.4.

Despite higher LAR in treatment WL+B, RGR was not affected due to a lower NAR, proportional to the increased SLA. Lower NAR can be explained by reduced photosynthesis, increased respiration or a combination of both (Lambers et al., 2008a). The seeming contradiction with leaf net photosynthetic rate (A) is discussed further in section 5.3. The similar magnitude of change in NAR and SLA fits well with the meta study conducted by Shipley (2006), who reported equal importance of SLA and NAR as predictors for RGR at DLIs between 15 and 25 mol m^{-2} d^{-1}, with SLA as most important at lower DLIs. Here, the DLI was about 15 m^{-2} d^{-1}.

The leaf growth curve (Figure 4.2A) revealed that the cumulative growth in leaf length was not affected by light treatment. Although leaf width was not measured, the lengths developed similarly throughout the growth period, indicating that additional BL did not affect leaf expansion at any stage during the growth period of sixteen days. Based on the source-sink development of squash leaves (Turgeon and Webb, 1973), the growth curve suggests that all

leaf measurements were performed on leaves having transitioned to sources entirely. The curve showing change in leaf length (Figure 4.2B) also revealed a peak in growth rate after six days of treatment, followed by a sharp decrease followed by a relatively low daily increase. Compared with the aforementioned study on squash leaves and own observations, the leaves were fully expanded after 5-6 days.

Leaf area and shoot length were similar between treatments (Table 4.1). Although they tended to be lower in WL+B compared to WL, the effect was small and the difference not significant. This, together with increased SLA was unexpected, since numerous studies report strong effects of BL on compactness in cucumber (reduced shoot growth, leaf expansion and increased thickness), even with similar conditions as here (Hogewoning et al., 2010b; Hernández and Kubota, 2016; Kang et al., 2021; Liang et al., 2021). However, these contradictions can be explained by comparing the experimental conditions in the different studies more carefully. All studies used different cultivars. Kang et al. (2021) and Liang et al. (2021) compared 30% BL with higher blue fractions, and Hogewoning et al. (2010b) and Hernández and Kubota (2016) used very low DLIs and RB background spectrum (∼6 mol m^{-2} d^{-1}). Snowden et al. (2016) and Hernández and Kubota (2014) used similar experimental conditions as here and studied responses to increasing blue fractions under very low, low and high DLIs (5, 12-16 and 30 mol m^{-2} d^{-1}, respectively). Shoot length and leaf area were not affected at low DLIs, and SLA was unaffected at high DLI. It seems that the compact morphology typically associated with high blue fractions only happens at very low DLIs, and the cultivar used here, 'Hi Light', may also respond differently to BL than other genotypes. Moreover, the amount of FR light used in this study was very low. In many species, such as cucumber, a high proportion of red to FR (R/FR) light induces a compact morphology (Demotes-Mainard et al., 2016). It is therefore conceivable that the high R/FR ratio used here induced a compact morphology in both treatments. Consequently, the effect of BL on compactness might have been outweighed.

To summarize, only small morphological differences were found between plants in the different treatments, and minor differences between the relative growth rates. This was unexpected, but explained by the light conditions used, such as the DLI and spectral background. Nevertheless, 30% BL resulted in lower biomass accumulation per leaf area compared to plants grown under 10% BL, but this was compensated for by leaf morphology, resulting in similar RGR. Additional BL may therefore alter the 'route' taken to the same growth rate though effects on growth components.

5.3 Single-leaf contra whole-plant net assimilation rate

Leaf photosynthetic rate does not necessarily correlate well with growth rate because the latter depends on both the net assimilation and resource allocation. Net assimilation was measured on single leaves with gas exchange (A) and on whole plants with growth component analysis (NAR). One would expect them to be equal when irradiance is constant over the growth period because both measure CO_2 uptake minus CO_2 loss through respiration, with the same units (mass per unit area per unit time). However, treatment WL+B resulted in a lower NAR and higher A compared to treatment WL. Measurements of photosynthesis on a single leaf will in many cases not be representative for the whole canopy due to microclimate variations affecting conductance and gas exchange (Lambers et al., 2008c).

Additionally, one important difference between these two parameters is that A is an instantaneous measurement of a single leaf, while NAR integrates gain and losses over the whole growth period. The relatively high dark respiration in WL+B (Figure 4.3) could therefore explain the unexpectedly low NAR, and BL have also been shown to stimulate respiration (Hogewoning et al., 2010b; Taiz et al., 2018). NAR measures the actual respiration experienced by the plants during the whole growth period of 16 photo- and dark periods. If respiration exceeds photosynthesis the energy is used for maintenance rather than growth, the whole growth period could integrate into negative net assimilation.

A further explanation that would lead to a mismatch between photosynthesis on whole-plant and leaf level, is the possible treatment effect on petioles (not measured). BL has been shown to decrease petiole length at similar DLI as here (Snowden et al., 2016). If so, light interception may decrease due to more leaves overlapping each other, leading to less assimilation.

Finally, it is possible that NAR was indeed higher in WL+B than WL, but the extra assimilated carbon was used for something other than building shoot biomass. Root biomass was not measured in these experiments, but enhanced root growth would underestimate NAR. Root DW has been shown to increase with increasing blue fractions from 30-50% (Yan et al., 2022). One can only speculate whether this would increase NAR enough to increase RGR, as would be expected based on the single-leaf measurements.

In short, discrepancies between net assimilation rates on leaf- and whole-plant level can have been caused by higher respiration rates, shorter petioles and/or enhanced root growth in WL+B compared to WL.

5.4 How leaf density and thickness can affect growth rate

SLA is the ratio between total leaf area and total leaf weight, and changes in SLA reflect changes in leaf morphology which can affect radiation capture and thereby growth rate. Variation in growth rate is in fact frequently attributed to changes in radiation capture, and often through changes in leaf area (Hogewoning et al., 2010a; Hernández and Kubota, 2014; Snowden et al., 2016). In these experiments, SLA was higher in WL+B compared to WL, but it was not caused by increased leaf area. This suggests that the leaves in WL+B were thinner and/or less dense than the leaves in WL.

A higher SLA and associated traits (larger, thinner leaves) are typical for acclimation to shade, where growth rate is enhanced by investing a larger proportion of photosynthates into leaf area compared to plant mass to compensate for radiation limitation (Lambers et al., 2008a). Light absorptance by shade leaves is enhanced by a higher proportion of mesophyll cells compared to palisade cells, allowing enhanced light scattering (Lambers et al., 2008a). Increased SLA can also decrease the mesophyll resistance of CO_2 diffusion into the leaf (Flexas et al., 2008), and decreased resistance can increase photosynthetic rate (Lambers et al., 2008a). Thinner, less dense leaves could also improve whole-plant radiation capture due to enhanced light penetration to lower leaves.

Even though thinner leaves with lower density can enhance growth rate, it remains a mystery why treatment WL+B resulted in higher SLA. Acclimation to BL has previously been shown to induce some of the same traits typical for sun leaves, e.g. lower SLA due to increased leaf thickness (Kang et al., 2021). Leaf thickening caused by acclimation to high irradiances may lead to longer (and sometimes multiple layers of) palisade cells and a lower proportion of spongy mesophyll (Lambers et al., 2008a) and this may also be the case for BL-acclimated leaves (Terfa et al., 2013). It would therefore be of great interest to study transverse sections of the leaf tissues in subsequent studies to reveal the internal structures.

5.5 Elevated water usage and decreased water-use efficiency at low irradiance under additional BL, but not due to stomatal density

As expected, plants grown under WL+B had a higher water usage and stomatal conductance (g_s) than the plants exposed to WL (Figure 4.6 and 4.7 respectively), indicating that plants grown under WL+B had the highest transpiration rates. The underlying mechanism for this was investigated by analysis of stomatal density.

The stomatal conductance is influenced by the stomatal density, size (total area) and aperture (pore area). Changes in density and size are long-term responses, while the aperture is an instantaneous response (Hernández and Kubota, 2014). It is well known that BL stimulates stomatal opening (Horrer et al., 2016; Matthews et al., 2020), leading to increased aperture. The long-term responses are however less obvious. The results presented here show that the stomatal density was similar in both treatments, both on the adaxial (upper) and abaxial (lower) leaf surface (Figure 4.9), leaving only increased aperture and possibly larger size as the cause for higher transpiration in WL+B. Previous studies have shown that cucumber leaves exposed to blue fractions from 15 to 50% can increase both the stomatal aperture and density on the adaxial side (Hogewoning et al., 2010b), while size seem only to be increased with higher blue fractions, as shown when raising the blue fraction from 26 to 72% (Kang et al., 2021). This suggests that the stomatal size was not different between leaves grown under WL+B and WL, and that the higher transpiration in WL+B was only caused by increased aperture.

It is generally believed that increased transpiration rate enhances the uptake and long-distance transport of nutrients (Tanner and Beevers, 2001), and Jakobsen (2016) found higher levels of N, Fe and Mg in cucumber leaves exposed to 30% compared to 5% BL. Increased chlorophyll content and higher photosynthesis in WL+B compared to WL indicates a higher N uptake, since a large fraction of leaf nitrogen is used in the synthesis of chlorophyll and RuBisCO (Evans, 1989).

The intrinsic water-use efficiency (iWUE) is the ratio of leaf net assimilation to stomatal conductance to water vapor, and thus estimates the balance between CO_2 uptake and water loss. iWUE was significantly lower in WL+B than in WL at the lowest irradiances (Figure 4.8). This means that at irradiances below 150 μmol m^{-2} s^{-1}, water loss from WL+B plants was unnecessary high compared to the carbon gained. At the highest irradiances however, plants grown under WL+B could maintain a high net photosynthesis without compromising with water loss. The ratio between transpiration in day and night (transpiration ratio) was slightly higher under WL+B than WL, although not significant – indicating that there was no difference in the dark-induced stomatal closure.

To sum up, a blue fraction of 30% compared to 10% led to higher stomatal conductance and transpiration, likely due only to instantaneous stomatal effects. Stomatal closure in darkness was not improved. The higher transpiration with additional BL can only be justified at high irradiances, where photosynthesis is increased to a similar extent.

5.6 Less starch with additional BL, but same total sugars

The starch concentration in leaves exposed to treatment WL+B was remarkably lower than in WL leaves (Figure 4.4). Given the dynamic nature of starch metabolism, it is not obvious whether WL+B caused more degradation or less accumulation compared to WL. The measurements were just snapshots, and the situation may look different at another time point. The poor correlation between A and NAR further complicates the discussion because it is not clear if net assimilation was higher or not during the growth period. Nevertheless, there seems to be more evidence for enhanced degradation, which will be discussed in this section.

Starch metabolism is coordinated with sink strength and photosynthesis (Ainsworth and Bush, 2011). High sink demands, such as growing fruits, have been shown to enhance starch degradation (Hu et al., 2009) and stimulate photosynthetic rate (Pharr et al., 1985) in mature cucumber leaves. In treatment WL+B, the relatively high photosynthesis and low starch concentrations in source leaves can therefore indicate that photosynthesis and starch degradation was stimulated by a higher demand for soluble sugars. This demand may come from a high dark respiration and/or enhanced root growth, resulting in no net shoot biomass gain. Both high dark respiration and enhanced root growth would act as sinks, and photosynthesis can be up-regulated by increased sink demand (Pharr et al., 1985). The apical bud will also act as a sink until the young leaves have unfolded and expanded 10% (Turgeon and Webb, 1973). An increased sink strength in the apical bud would ‘pull’ on the sugars and thus enhance starch degradation. At last, BL has been shown to up-regulate transcription of key genes related to starch and sucrose metabolism in cucumber (Zhou et al., 2018) and suggested to promote starch degradation in tomato leaves, rather than influencing its synthesis (Dong et al., 2021).

Starch degradation releases glucose (through maltose), where it is combined with fructose to yield sucrose, and sucrose is one of many substrates in RFO biosynthesis, yielding raffinose and stachyose, among others. Sucrose and stachyose are the main transport sugars in cucumber, while stachyose and raffinose can also accumulate in response to stress (e.g. Ma et al. (2021)). These sugars, or their precursors glucose and fructose, are therefore likely to be the main products from starch degradation. Hu et al. (2009) found an increase in all these sugars in cucumber leaves during fruit development, together with an equivalent decrease in starch levels.

Total sugar levels were however almost identical in both treatments (Figure 4.4). Although raffinose levels were higher in WL+B than in WL (Figure 4.5), it is not enough to account for the difference in starch (-129 mg g^{-1} starch and +8 mg g^{-1} raffinose in WL+B compared to WL). This could make the case for a lower starch accumulation in WL+B. On the other hand, there was a consistent trend for higher levels of all soluble sugars in WL+B compared to WL (Figure 4.5), and different methods was used to measure starch and sugars, possibly with different sensitivities. Further, RFOs include other sugars as well, and their complex metabolism involves many intermediates (Sanyal et al., 2023). Other, unmeasured metabolites could, at least partly, be an explanation for the 'missing' sugars. *myo*-inositol and galactinol are both involved in RFO metabolism, as both substrates for biosynthesis and products of hydrolysis (Sanyal et al., 2023). They can both accumulate during osmotic stress (Li et al., 2020a). Other sugars such as the glucose precursor maltose also accumulates during starch degradation (Dong et al., 2021), and proline have been suggested to derive directly from degraded starch in response to osmotic stress (Zanella et al., 2016). In abiotic stress tolerance, proline is likely to protect cells from damage though acting as an antioxidant, osmoprotectant and molecular chaperone in protein stabilization (Krasensky and Jonak, 2012). The relatively high dark respiration in WL+B (Figure 4.3) could also lead to direct consumption of glucose in cellular respiration for energy production and for production of other compounds. BL has indeed been shown to induce a wide variety of secondary metabolites in cucumber (Palma et al., 2022).

Although the exact role of raffinose in stress tolerance is poorly characterized, it has often been shown to accumulate during osmotic stress in several species, cucumber included (e.g. Ma et al. (2021)). Cucumber is a drought-sensitive plant, and WL+B increased transpiration. Treatment WL+B might therefore have induced a mild drought stress response. This could explain the suggested increase in root growth, which would be necessary to support the higher water usage. Drought is a strong signal for stomatal closure, to prevent detrimental water loss. However, BL is a strong signal for stomatal opening. It could seem that BL increases the stomatal conductance, and drought stress is induced as a consequence, although not enough for it to close stomata. The high transpiration is allowed to continue, and root growth is enhanced to support the high water uptake.

The experiments were conducted with a moderate RH (60%), and on top of this, the pots were watered manually. If the experiment was to be conducted with higher RH and unlimited water supply, the effect of BL could be tested without a possible confounding drought effect. This would also better represent the situation in cucumber production. Further, to get a clearer

picture on the relationship between starch and sugar metabolism in response to BL, consecutive experiments could measure root growth, sugar content in phloem and shoot apex, and measure a wider range of metabolites.

In summary, it seems that the addition of BL enhanced starch degradation, but the degradation products were not detected – either due to accumulation of unmeasured metabolites (maltose, proline and/or other RFOs), glucose consumption for energy production or for methodological reasons. Raffinose accumulation indicates that a stress response was induced, which could lead to enhanced root growth and explain why NAR was lower in treatment WL+B compared to WL.

5.7 Suggestions for further research and practical implications

All results considered, a fraction of 30% BL compared to 10% increased stomatal opening and chlorophyll content. The former increased transpiration, while both may have contributed to enhanced assimilation. A higher photosynthesis at high light intensities together with high transpiration in WL+B resulted in similar water-use efficiency in both treatments. At low light intensities however, WL+B resulted in high water usage compared to the carbon gain. Despite higher photosynthesis estimated for the chamber irradiance, shoot growth rate was unaffected, due to altered leaf morphology to compensate for altered shoot net assimilation rate. The latter may be due to higher dark respiration, and possibly shorter petioles. The proportion of BL also seems to affect the mobilization of starch reserves.

A typical situation in greenhouse production in northern latitudes is light limitation during the winter months and excess light during summer months. Supplemental light is necessary half of the year, while the other half often requires shading. Irradiance may reach 2000 μmol m^{-2} s^{-1} on a clear summer day, and it was found that photosynthesis was not inhibited in WL+B at 1000 μmol m^{-2} s^{-1}, the highest irradiance measured. The higher photosynthetic capacity of plants acclimated to 30% BL may benefit production since the plants can utilize a larger proportion of the available light energy during spring and summer. It may also be applied only during winter or transition to spring, where the need for acclimatization to high light intensities is highest. For the same reasons, seedling production in plant factories can also benefit from using a higher blue fraction to acclimate plants for transplanting, but with the additional benefit of allowing low light intensities. High irradiances in indoor farming leads to excessive heating and energy use. BL may therefore be used instead, to “prepare” the seedlings for the light

conditions meeting them in a greenhouse. These 'transition effects' of BL would be interesting to further investigate. To extend the findings of previous studies, experiments should be conducted until fruiting to evaluate potential effects on yield.

The morphological responses to BL may depend on both the DLI and the R/FR ratio, and the benefit of a compact morphology depends on purpose. Comparing the results presented here with previous studies, a DLI below 10 mol m^{-2} d^{-1} may be necessary to induce a more compact morphology with additional BL, and a lower R/FR ratio than used here may reverse it. A more compact morphology would save space in a vertical farm producing seedlings, whereas an open morphology is convenient in fruit production due to enhanced light penetration to fruits and easier management of the canopy. A smaller leaf area could also reduce the light use efficiency. How BL interacts with both DLI and R/FR ratio could be further investigated to find the optimal combinations in different stages of the crop, depending on the desired morphology to go with the other effects of BL.

Enhanced starch degradation should release more soluble sugars, potentially available for growing flowers and fruits which could affect the yield. This would be particularly interesting to study further, and should be performed in longer experiments, with samples taken from phloem sap and the shoot apex in addition to source leaves, and preferably until fruiting.

The results indicate that 30% BL may have induced a mild drought stress response without reducing stomatal conductance. Rather, it may have enhanced root growth to support high transpiration rates. A strong root system is important for nutrient uptake and anchoring, and could produce stronger seedlings in production of transplants (Yan et al., 2022). However, drought stress can consume valuable energy for other processes than root growth as well and should be further investigated. Root growth is difficult to study, which is probably why BL effects on root growth is lacking in the literature.

Increased water usage must be carefully considered against the potential benefits of applying high fractions of BL, namely increased photosynthetic rate, enhanced starch mobilization, and possibly enhanced root growth and nutrient uptake. In a closed system with water recirculation, a high water usage would not necessarily be a problem in itself. However, unless high-investment air circulation systems are installed, it would increase air humidity and thus energy use and disease pressure. Greenhouses that depend on ventilation of excess humidity by windows, and in countries with cold winters and nights, the benefits will probably not outweigh the high energy costs that would follow. Moreover, transpiration regulates the leaf temperature,

which already is negatively affected by LEDs compared to HPS lamps (Nelson and Bugbee, 2015), so that a high transpiration could amplify this effect. However, only applying higher fractions of BL to limited parts of the canopy could tip the scale towards the beneficial effects. For example, applying additional BL with a total irradiance >150 μmol m^{-2} d^{-1} to source leaves solely could limit the elevated water usage only to parts of the canopy and still enhance CO_2 uptake. With a photoperiod of 18h, this would correspond to a DLI > 10 mol m^{-2} d^{-1}. Another important consideration that should be weighed against the benefits is the implication for the working environment since BL could be exhausting to work with and make plant disorders harder to detect. Consecutive experiments could therefore apply BL in different production systems, such as the use of interlighting, as well as different blue fractions to find the 'sweet spot' where the benefits outweigh the losses. At last, since transpiration can be too low under the high air humidity often experienced in a greenhouse, it is possible that the use of BL would bring more advantages in this scenario. Experiments should therefore be performed with a RH higher than 60%.

6 Conclusion

The objective of this study was to better understand how different proportions of BL affect assimilation, growth, morphology, and water usage in seedlings of 'Hi Light'. The results indicate that assimilation and water usage were strongly affected by the proportion of BL, while growth rate and morphology were not.

The highest photosynthetic capacity, concentrations of chl *a* and *b*, and the lowest concentration of starch were observed in leaves of plants grown with 30% BL. The latter was likely due to enhanced degradation. There was also a trend for higher content of total soluble sugars with 30% BL compared to 10% BL, and a particularly high raffinose content indicated that a mild drought stress response was induced in plants grown with 30% BL. The whole-plant assimilation rate was lower in leaves grown with 30% BL than in those with 10%, despite photosynthetic rate being higher in 30%.

Growth rate and morphology were less affected by different blue fractions. Despite relatively low assimilation rates in plants exposed to 30% BL, decreased leaf thickness and/or -density resulted in similar growth rates between treatments. Additional BL therefore seems to alter the 'route' taken to the same growth rate.

The highest water usage was observed in plants grown with 30% BL. This was likely due to instantaneous stomatal effects such as increased aperture, rather than acclimatory effects such as stomatal density, and stomatal closure in darkness were similar between treatments. The higher water usage with additional BL is easier justified at high irradiances due to the high photosynthetic capacity in these conditions, leading to similar water-use efficiencies.

Overall, the results confirmed that BL can induce a variety of physiological effects on cucumber plants, while morphological effects seem to be conditional. The results can be used as a foundation for further research to find the optimal fraction of BL in different production systems and stages of the crop, particularly for the cultivar 'Hi Light'. Climate factors such as DLI, R/FR ratio and RH should be carefully considered in consecutive experiments in respect to the aim of study.

7 Comments on variation between the experimental repeats and sources of error

There was a large variation in most measurements between the experimental repeats (Supplementary Materials, Figures 9.1-9.5), which was likely caused by challenges with the climate control, most notably the humidity- and light control during the first experiment. Additionally, the pots were drier during the first experiment compared to the second. Together, this probably contributed to the large variation in the data, with the following potential consequences:

1. Non-significant trends such as more compact morphology and higher sugar levels in WL+B might have been significantly different from WL with a larger sample size.
2. Transpiration ratio and chl *a:b* ratio varied in opposite directions between experiments. Confounding factors introduced by an unstable climate in the first experiment could make these measurements unreliable. After analyzing the experiments separately, the chl *a:b* ratio turned out to be significantly lower in WL+B in the second experiment (p=0.002, one-way ANOVA). This would be interesting to investigate further, since it is a typical shade-acclimation to enhance radiation capture, just like the unexpectedly high SLA in WL+B.
3. Lastly, leaf samples (carbohydrates and stomatal density) were for practical reasons only analyzed for the first experimental repeat. The unstable climate and possible drought stress can have affected the results.

Unpredictable events must be expected, and they are part of the reason why large sample sizes and replicate experiments are important. Although practically not possible for this thesis, it would have helped with larger sample sizes and additional experimental repeats to minimize the consequences of sources of error such as a variable climate.

8 References

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9 Supplementary Materials

9.1 Descriptive statistics of leaf net photosynthesis

Table 9.1: Leaf net photosynthesis (A) at eight levels of irradiance after 10-11 days of treatment under white light (WL) and with additional blue (WL+B). Values are means from both experimental repeats ± SE (n=7). The first seven rows show the measurements on the youngest fully expanded true leaf (second oldest) under red-blue light from the fluorometer, while the final row is the estimated net photosynthesis at chamber irradiance, based on values from the fitted curve. Significance codes[1] from one-way ANOVA ($\boldsymbol{\alpha}$=5%) are also shown.

Irradiance (μmol m^{-2} s^{-1})	A (μmol CO_2 m^{-2} s^{-1})		ANOVA
	WL	WL+B	
0	-2.3±0.2	-3.2±0.2	*
50	1.8±0.2	1.2±0.3	.
100	4.0±0.2	4.1±0.3	ns
150	6.0±0.4	6.6±0.3	ns
300	8.6±0.5	12.2±0.5	***
600	10.8±0.6	17.0±1.0	***
1000	12.1±0.7	19.5±1.2	***
235[2]	7.8±0.4	10.2±0.5	**

[1] Signif. codes: 0 '***', 0.001 '**', 0.01 '*', 0.05 '.', 0.1 'ns'.
[2] The average irradiance in growth chambers.

9.2 Raw data and descriptive statistics from both experimental repeats

9.2.1 Morphology

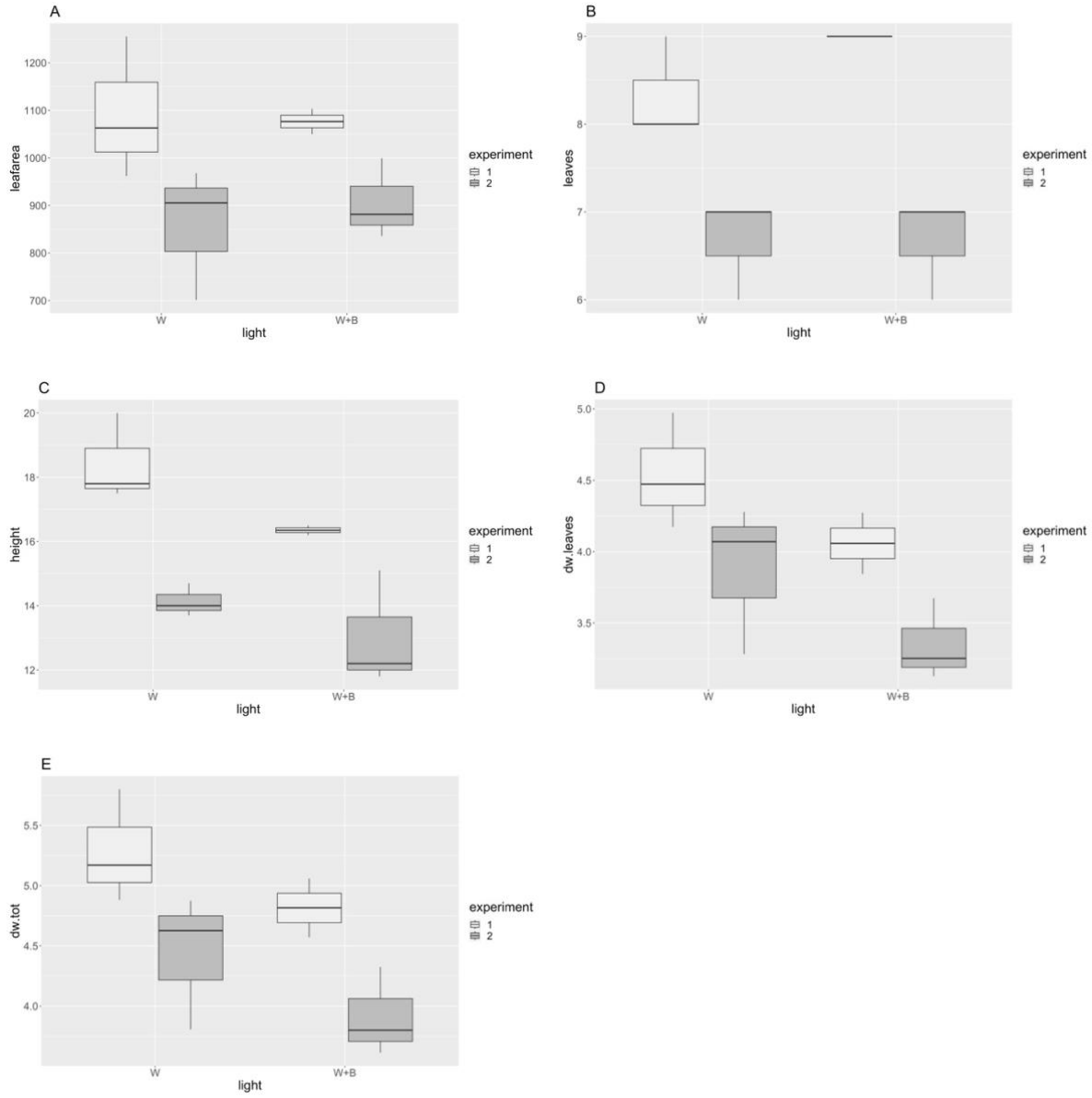

Figure 9.1: Leaf area (A), number of true leaves (B), shoot length (C), DW of leaves (D) and DW of total shoot biomass (E) under white light (WL) and with additional blue (WL+B), from the first (light grey) and second (dark grey) experimental repeat.

9.2.2 Growth components

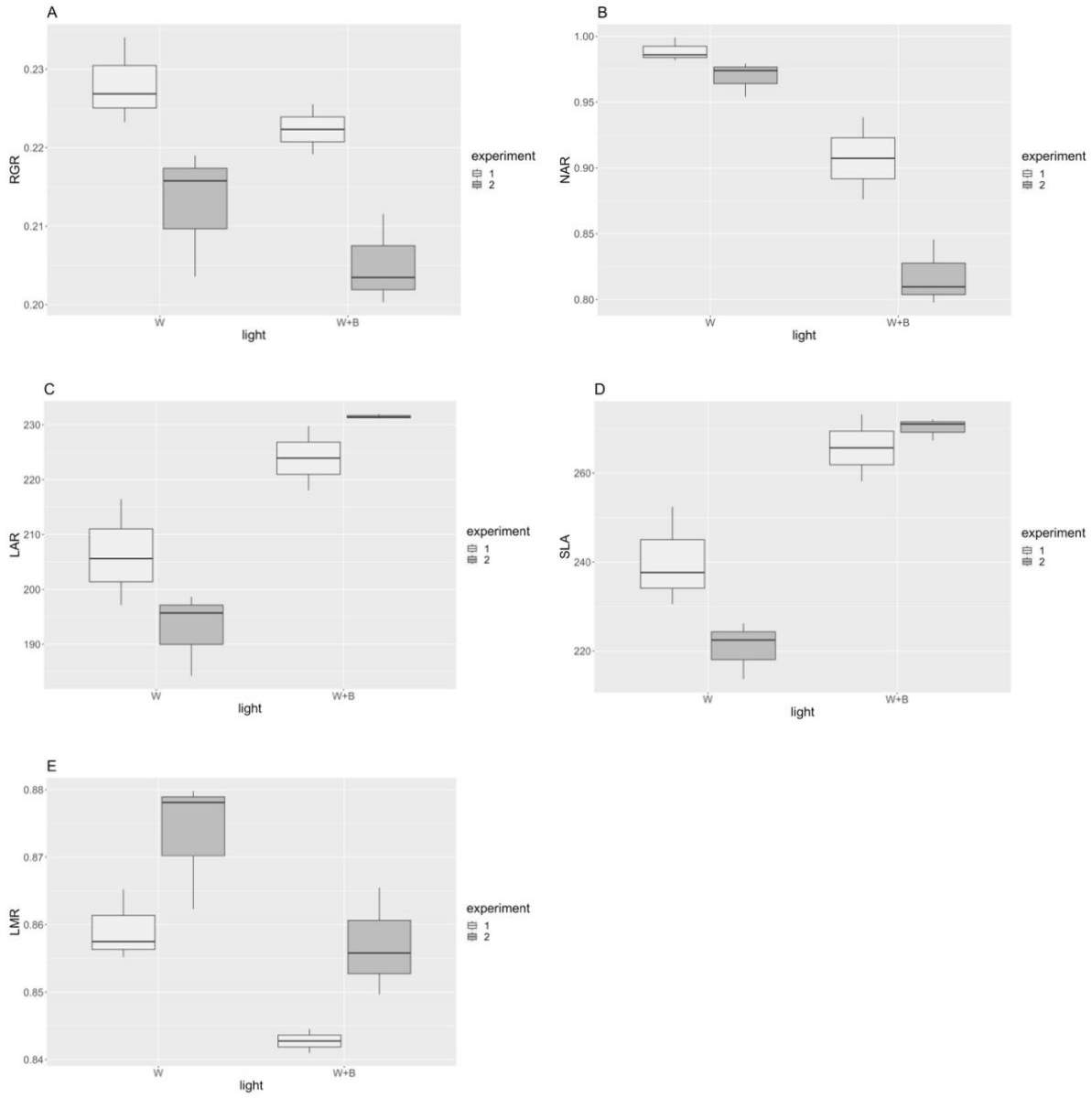

Figure 9.2: RGR (A), NAR (B), LAR (C), SLA (D) and LMR (E) under white light (WL) and with additional blue (WL+B), from the first (light grey) and second (dark grey) experimental repeat.

9.2.3 Leaf gas exchange

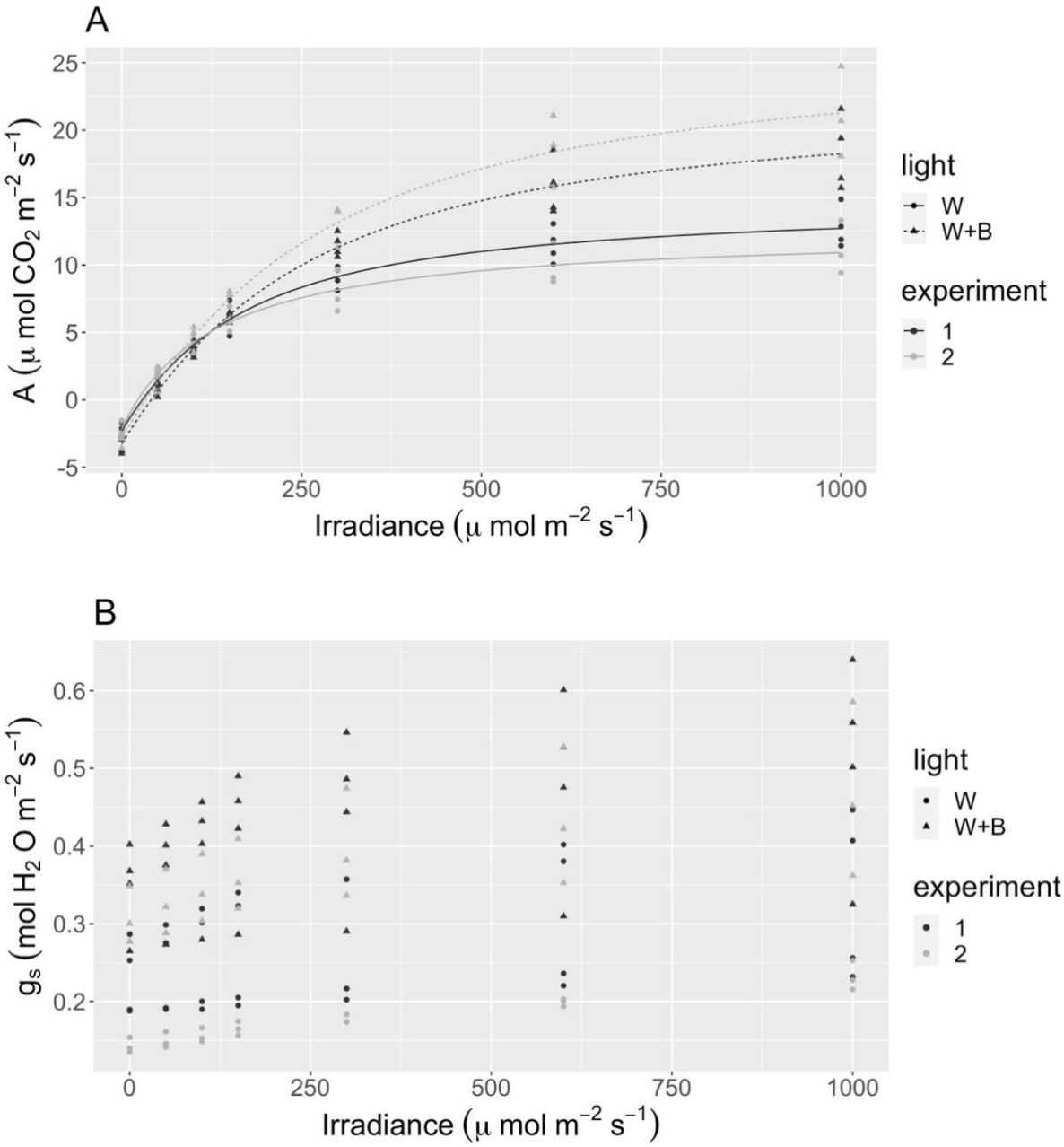

Figure 9.3 Net photosynthesis (A) and stomatal conductance (B) under white light (WL, solid line) and with additional blue (WL+B, dashed line), from the first (light grey) and second (dark grey) experimental repeat.

9.2.4 Photosynthetic pigments

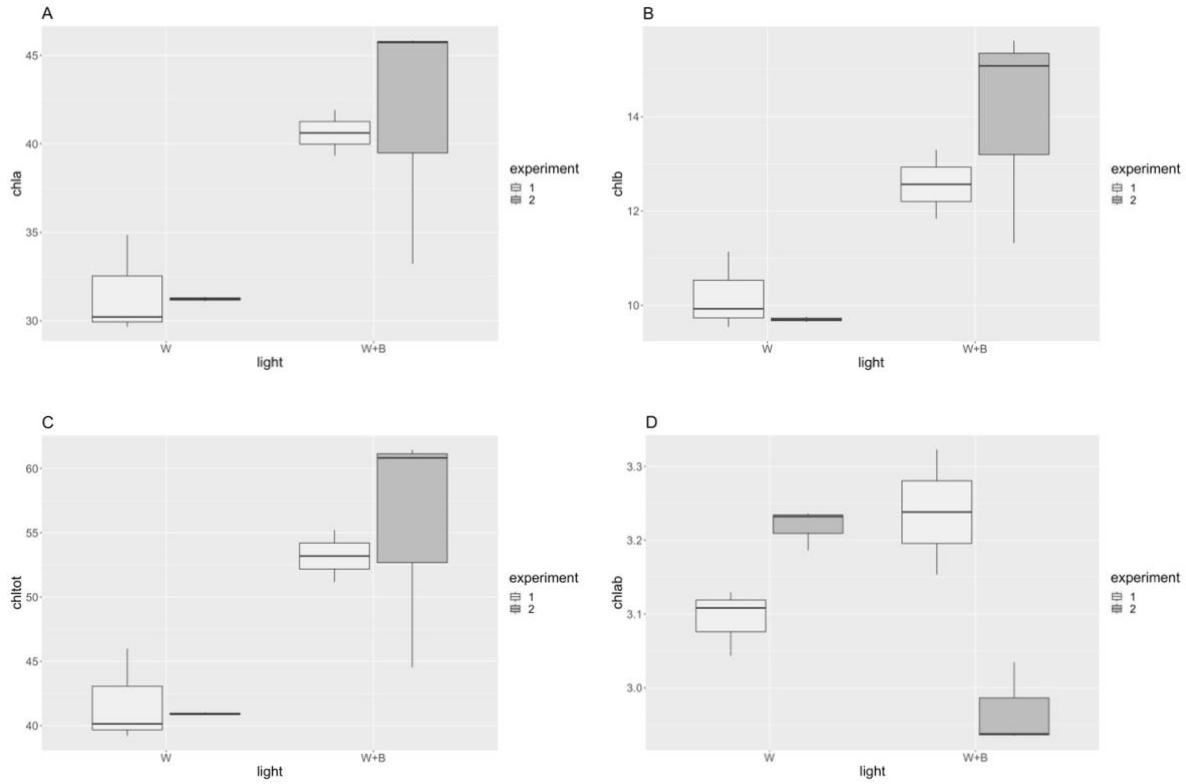

Figure 9.4 Concentration of chlorophyll *a* (A), *b* (B), total chlorophylls (C) and chlorophyll *a*:*b* ratio (D) under white light (WL) and with additional blue (WL+B), from the first (light grey) and second (dark grey) experimental repeat.

9.2.5 Water usage

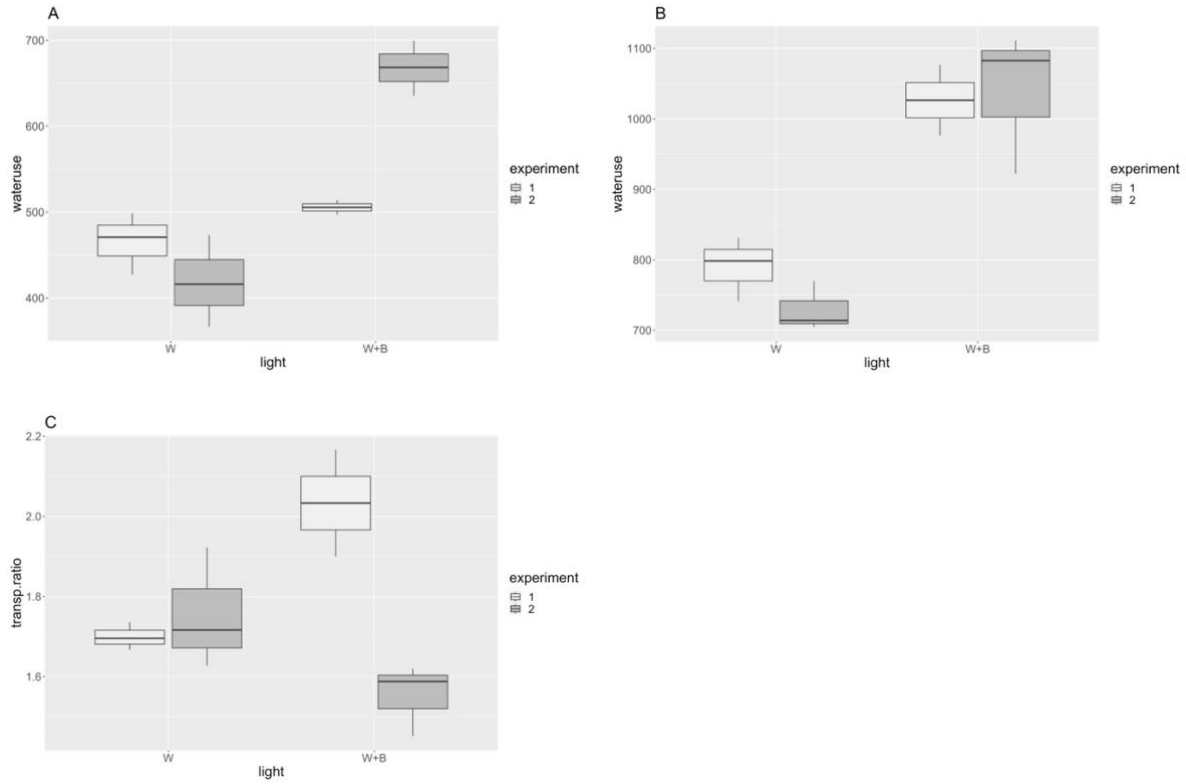

Figure 9.5 Water usage during dark period (A), water usage during photoperiod (B) and transpiration ratio (C) under white light (WL) and with additional blue (WL+B), from the first (light grey) and second (dark grey) experimental repeat.

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