The effect of different light spectra on photosynthetic rates in three leafy plant species

The role of green light for photosynthesis, stomatal conductance and chlorophyll fluorescence

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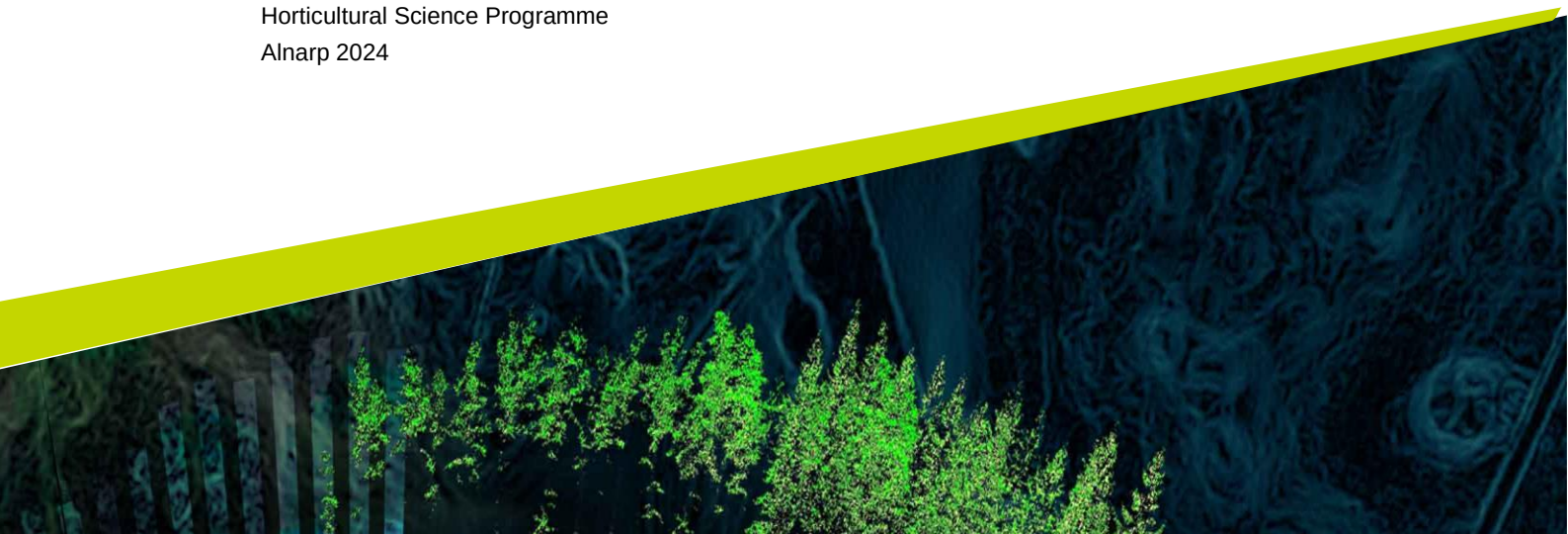

The effect of different light spectra on photosynthetic rates in three leafy plant species

The role of green light for photosynthesis, stomatal conductance and chlorophyll fluorescence

Effekten av olika ljusspektra och hur de påverkar fotosyntesen hos tre bladgrönsaker och dess påverkan på fotosyntes, stomatakonduktans och klorofyllfluorescens

Linda Golobov

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Keywords: *A_{max}, Bell pepper, Brassica rapa subsp. chinensis, Capsicum annuum, Chlorophyll fluorescence, Greenhouse, Green light, IRGA, LED light, Pak Choi, Plant factory, Photosynthesis, RB, RGB, Swiss Chard*

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Abstract

By year 2050 and beyond, the global population is projected to increase significantly, accompanied by climate changes. In this context indoor plant production, or plant factories, holds good promise for enhancing food security. Previous research highlights that green light can penetrate deeper into leaf tissues when compared to blue and red light, influencing photosynthesis. Recent studies have highlighted the importance of adding green light spectra to other light spectra. The previous research raised the hypothesis that supplementing green light with red-blue light possibly can enhance photosynthesis in higher plants.

The main hypothesis in this study was that the addition of green light would enhance the photosynthetic rates. Hence, this study's aim is to optimise photosynthesis performance of plants produced in greenhouses or plant factories utilizing artificial light. The three different plant species were all separately analysed in photosynthesis measurements, the plant species bell pepper (*Capsicum annuum*), Pak Choi (*Brassica rapa* subsp. *Chinensis*) and Swiss chard (*Beta vulgaris* var. *cicla*) were used in this study that aimed to examine the potential of different wavelength of light and to optimise photosynthesis. The photosynthetic capacity, A_{max} and the g_s, the stomatal conductance was together measured by the Infra-Red Gas Analyser (IRGA).

The analysation of the results of the photosynthesis measurements indicates that the addition of green light to a combined red-blue light spectra did not enhance the photosynthetic rates in the three plant species under given circumstances. The results from the chlorophyll fluorescence trials and the response from these specific plants show variability and were difficult to interpret.

In conclusion, comprehensive research can possibly identify patterns and trends, which in the future can lead to new practical recommendations for horticultural practices and provide insight into practical implications of light treatments.

Keywords: A_{max}, Bell pepper, Beta vulgaris var. cicla, Brassica rapa subsp. chinensis, Capsicum annuum, Chlorophyll fluorescence, Photosynthesis, Greenhouse, Green light, IRGA, LED light, Pak Choi, Plant factory, Photosynthesis, RB, RGB, Swiss Chard

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Abbreviations

A_{max}	Photosynthetic capacity (the maximum rate at which leaves are able to fix carbon during photosynthesis)
B	Blue (light)
CEA	Controlled Environment Agriculture
Chl a	Chlorophyll a
Chl b	Chlorophyll b
CL	Continuous light
DLI	Day Light Integral
G	Green (light)
HPS	High pressure sodium lamps
F_0	Minimum fluorescence yield in dark-adapted state
F_m	Maximum fluorescence yield in dark-adapted state
F_v	Variable fluorescence
F_t	Steady-state fluorescence
F_v/F_m	Ratio of variable to maximum fluorescence after dark-adaptation. Quantum yield of PS II photochemistry in the dark-adapted state
g_s	Stomata conductance
IRGA	Infra-Red Gas Analyser
LED	Light emitting diodes
P_n	Photosynthetic net rate
PAR	Photosynthetic active radiation
PFAL	Plant factories with artificial lighting
PPF	Photosynthetic photon flux
PPFD	Photosynthetic photon flux density
PPE	Photosynthetic photon efficiency
PSI	Photosystem I
PS II	Photosystem II
Q_A	Primary Stable Quinone Acceptor
Q_B	Secondary (loosely bound) Quinone Acceptor

R	Red (light)
RB	Red and blue light
RGB	Red, green, and blue light
SLU	Sveriges Lantbruksuniversitet (Swedish University of Agricultural Sciences)
UV	Ultra-violet

1 Introduction

1.1 Plant factories, greenhouses and food security

The global population is projected to increase to 9.7 billion people by 2050, and 10.4 billion by 2100, according to the United Nations. (United Nations 2022). In a world with an increasing population and environmental changes, ensuring food security becomes increasingly crucial.

Photosynthesis serves as the foundation of plant growth, and improving photosynthesis can contribute to enhanced food security in the light of the increasing world population (Evans 2013). Additionally, controlled plant production or controlled environment agriculture (CEA), offers an alternative approach to secure food production (Ramin Shamshiri *et al*. 2018; Cowan *et al*. 2022). Plant production in greenhouses and indoor facilities, known as plant factories with artificial lighting (PFALs) is a way to control the environment for the plants, thereby increasing their productivity. Other terms for plant factories are vertical farms or indoor farms. These closed systems were invented in Japan in the 1980s (Goto 2012).

Common crops in these systems are a range of lettuces and other leafy greens, used as fresh market products and ready-to use vegetables (Goto 2012). These leafy green products became positively evaluated by the food service industry in the 1990s. PFALs or indoor farms is increasing as production technologies especially in urban areas with limited access to arable land or in types of production where the plants have special requirements (Kozai 2018).

Some crops are difficult to grow in fields outdoors due to weather conditions or sunlight limitation especially in cooler climates like in Scandinavia or Northern Europe. Production in plant factories, where production can run all year around, with little or no limitations of the weather conditions, results in a higher yield (Zou *et al.* 2024). Plant production in closed systems with artificial light potentially have benefits such as higher quality of transplants, shorter production period and a smaller amount of needed resources in comparison with conventional systems (Kang *et al*. 2013).

Greenhouses or PFALs have evolved systems to reduce water use and compared to plant production in fields the indoor productions use less water (Barbosa *et al*. 2015; Majid *et al*. 2021). A study by Graamans *et al*. (2018) analysed energy, water, carbon dioxide (CO_2), land use in lettuce production to compare resource efficiency of plant factories and greenhouses across diverse latitudes: Sweden, the Netherlands, and the United Arab Emirates. The results of the study indicated that plant factories used all four resources more efficiently, though greenhouses required less purchased energy due to solar energy benefits, even in extreme climates like Kiruna and Abu Dhabi. This indicates that greenhouses remain more energy efficient than plant factories even in challenging climates like where one might expect plant factories to have an advantage.

Additionally, Kozai & Niu (2016) found that plant factories growing plants using hydroponic systems, can possibly recover for water lost for transpiration and can result up to 97 % water savings as compared to conventional agriculture.

In hydroponic systems, water and nutrients are for the most part recirculated with minimal or no losses (Massa *et al*. 2020). Different cultivation system, technical solutions, nutrient and water management methods can importantly impact emissions outcomes. Closed systems further enhance sustainability by enabling collection and reuse of drainage, and by those minimising water and nutrients losses to the environment. Plant factories are a controlled environment which can benefit some pests, but overall pesticides are less frequently used in indoor production (Kozai 2018).

Proper management of closed cultivation facilitation minimizes the contact of domestic animals, birds and insects, which are common carriers of foodborne pathogens in conventional agriculture (Sela Saldinger *et al*. 2023).

Additionally, indoor cultivation protects plants from soilborne microorganisms in dust particles, which can pose contamination risks under field conditions. The higher hygiene standards in hydroponic systems will help to prevent diseases when it comes to pathogens humans. The harvested part of the produce rarely comes in contact with the nutrient solution or the substrate, reducing the risk of cross-contamination. Plant factories are a controlled environment which can benefit some pests, but overall pesticides are less frequently used in indoor production (Kozai 2018). The hydroponic produce is mostly safe for consumption and is considered to carry minimal risk for harmful microbes (Dankwa *et al*. 2020; Sela Saldinger *et al*. 2023).

To achieve global food security and environmental sustainability, it is necessary to cease the expansion of agriculture, particularly at the cost of tropical forests, close yield gaps in underperforming landscapes are currently below average, increase agricultural resource efficiency of water, nutrients, and other agricultural measures, increase food delivery by changing diets and reducing waste (United Nations 2022).

Locally grown products reduce transportation costs and the dependence on imports, thereby ensuring a more stable food supply chain (Ramin Shamshiri *et al*. 2018). With an increased amount of plant factories in urban areas lowering carbon emissions and transportation costs can be a future reality. Plant factories, vertical farms or similar production systems can possibly provide healthy fresh food where access to fresh foods is limited (Kozai 2013). Urban indoor facilities for growing vegetables and herbs can also offer a possible way to strengthen local retail markets (Liaros *et al*. 2016).

Plant factories could possibly offer a sustainable solution for the challenges of feeding a growing population while preserving natural resources and with minimal impact on the environment (Kozai 2013; Liaros *et al*. 2016).

The drawback is the large amount of electricity and energy that is needed to operate greenhouses and plant factories (Graamans *et al*. 2018; Ramin Shamshiri *et al*. 2018).

1.2 Photosynthesis

Photosynthesis is an essential process which sustains life on Earth by enabling organisms to derive solar energy and convert it into chemical energy (Taiz & Zeiger 2010). Photosynthetic organisms, such as plants, algae, and some bacteria, derives solar energy from sunlight to convert CO_2 and water into organic compounds like glucose. Glucose providing the fundamentals of our food supply and sustains the majority of organisms, serving as an important energy source either directly or indirectly. The releasing oxygen into the atmosphere as a byproduct of these processes. This oxygen is vital for the respiration of nearly all living organisms.

The research of McCree (1972) indicates that photosynthetic activity is wavelength dependent. Wavelengths within the 400-700 nm range effectively drives photosynthesis (figure 1), termed Photosynthetically Active Radiation (PAR) (McCree 1972; Wientjes *et al*. 2017; Liu *et al*. 2020). Light with wavelengths shorter than 400 nm or longer than 700 nm was thought to be unimportant for photosynthesis, because of its low quantum yield of CO_2 assimilation (McCree 1972; Liu & Van Iersel 2021).

Wavelengths outside this PAR range, while inefficient for photosynthesis, may still influence plant morphology and growth (Smith *et al*. 2017; Paradiso & Proietti 2022).

In plants, oxygenic photosynthesis is driven by Photosystem I (PSI) and Photosystem II (PSII) (Wientjes *et al*. 2017). Photosynthesis is a complex process, that can be divided in two stages, the light-dependent reactions and the Calvin cycle, or the light-independent reactions, often referred to as the dark reactions. During the light-dependent reactions, photosynthetic pigments within PSI and PSII absorbs photons, initiating electron transfer passing through different protein complexes embedded in the thylakoid membrane. These electron flow produces adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NADPH), which are then utilized in the dark reactions or Calvin cycle to fix CO_2 into glucose within the chloroplast stroma (Wientjes *et al*. 2017; Baslam *et al*. 2020; Liu *et al*. 2020).

Harvesting photons is the initial step in photosynthesis, transforming solar energy into chemical energy (Caffarri *et al*. 2014). Photosynthetic membranes in plants and algae are rich in protein complexes that bind chlorophyll and carotenoid pigments, forming functional units known as PSI and PSII (Wientjes *et al*. 2017).
The amount of chlorophyll of PSI and PSII is adjusted to a light irradiance spectrum, which is regulated by the plants. PSI and II are pigment protein complexes located in the thylakoid membrane of higher plants, algae, and cyanobacteria, which all uses solar energy for their photosynthetic water splitting reaction. Photosynthesis efficiency is affected by light quality, light quantity (light intensity and photoperiod), temperature and CO_2 concentration, and better knowledge in those areas can limit or enhance photosynthetic rates (Evans 2013; Liu & Van Iersel 2021; Paradiso & Proietti 2022).
Moreover, photosynthesis is also important for climate conditions on Earth by removing CO_2 from the atmosphere and storing it in plant biomass (Tkemaladze & Makhashvili 2016).

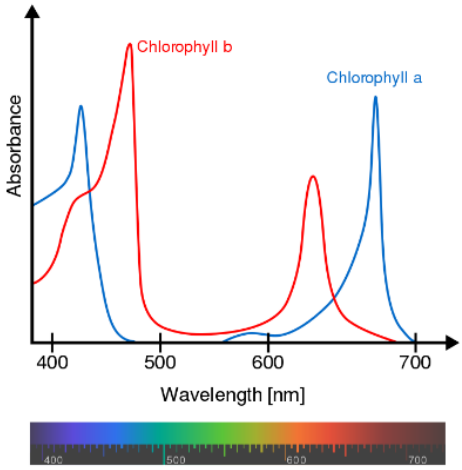

Figure 1. A graph illustrating the visible light spectra 400-700 nm including Chlorophyll a and b. Creative commons.

1.3 Artificial light and light intensity

The solar radiation encompasses a broad spectrum of wavelengths, but photosynthesis utilizes only a narrow range within that spectrum known as Photosynthetic Active Radiation (PAR), which spans from 400-700 nm (Goto 2012; Poorter *et al*. 2012) and is roughly the same spectrum as light visible to the human eye.

Photosynthetic Photon Efficacy (PPE) is an important metric to measure the luminaire efficiency of a fixture or plant lighting. PPE is the PAR photon output of the light source divided by the input power. The accurate metric unit used to measure PPE is $\mu mol \cdot J^{-1}$ (Park & Runkle 2018).

Two important factors when it comes to plants and light intensity is the Photosynthetic Photon Flux (PPF), Photosynthetic Photon Flux Density (PPFD) is another factor for LED plant lighting, and PPF is the amount of PAR produced per second, while PPFD is the amount of PAR per second affecting an area, with the $\mu mol\ m^{-2}\ s^{-1}$.

The PPFD is often referred to as the light intensity, or representing the number of equally weighted photons striking a square metre per second, and the accurate metric for light intensity is $\mu mol\ m^{-2}\ s^{-1}$ (Poorter *et al*. 2012; Wang *et al*. 2013; He *et al*. 2022).

1.3.1 Artificial light sources and energy demand

The two most commonly used artificial light sources in greenhouses or plant factories are Light-emitting diodes (LED) and High-Pressure Sodium (HPS) lamps (Paucek *et al*. 2020; Katzin *et al*. 2021; Trivellini *et al*. 2023). In the late 2000s LEDs were introduced in plant factories as a more effective light source (Goto 2012). LEDs is a promising light source for greenhouses, which can be applied as the main or as supplementary source of light (Zhang *et al*. 2017).

LEDs are solid-state semiconductors that produce light through electroluminescence, making them fundamentally different from traditional plant lighting. They are the first light source that enables precise control over both spectral composition and light intensity (Paradiso & Proietti 2022). LEDs are efficient and have long operating time, low thermal emission, and can be tailored for different crops depending on their requirements (Gómez *et al*. 2013; Shaw *et al*. 2016).

Linear LEDs, which are long narrow LED light sources in a straight line, are currently often used in the horticulture market, in indoor production and for supplemental lighting in greenhouses (Paucek *et al*. 2020).

Greenhouses, especially in high latitudes, consume large amounts of energy required for heating and supplemental lighting (Gómez *et al*. 2013; Shaw *et al*. 2016; Katzin *et al.* 2021). As LEDs are more efficient in converting electricity to light than older lighting technologies based on electric discharge, one consequence is that they emit less heat. This might lead to increased need for heating when LED technology is used (Gómez *et al*. 2013; Shaw *et al*. 2016; Katzin *et al*. 2021). Therefore, the amount of energy saved by LEDs is somewhat reduced by the increased utilization of heating systems.

In a study published by Katzin *et al*. (2021), the energy demands for greenhouses are analysed transitioning from HPS lamps, by providing a quantification of the total energy savings achieved when transitioning to LEDs. The study used the open-source model Green light, to examine a wide range of climates from subtropical China to arctic Sweden, and used multiple settings for temperature, lamp intensity, lighting duration, and insulation. For the most part, the total energy saving by switching to LED was 10-15%. LEDs reduced the energy needed for lighting but increased the demand for heating.

Earlier research showed similar results; a study by Gómez *et al*. (2013), showed that that the electrical conversion of LED light into biomass was 75 % higher than the HPS lighting. A life cycle assessment by Zhang *et al*. (2017) indicates similar results. All these studies strongly indicate that LEDs lamps lower the energy costs significantly.

1.3.2 Light recipes and light quality

Through dedicated research efforts, growers can tailor light recipes to optimise photosynthetic efficiency (Mickens *et al*. 2019; Paucek *et al*. 2020; Trivellini *et al*. 2023; Naveen *et al*. 2024). This customization involves understanding that plants respond differently to light spectra and can benefit specific plants or crops differently. McCree (1972) demonstrated that, at a given light intensity, photosynthetic efficiency varies with wavelength composition. In most species, the blue and red wavelengths are the most effective for photosynthesis, following a pattern closely aligned with the absorption spectrum of photosynthetic pigments (Paradiso & Proietti 2022). By adjusting light intensities, specific wavelengths and the proportion between them, ensuring the plant receives the most suitable lighting conditions for photosynthesis, optimal growth, and better yields.
Different LED technologies are available in a wide range of configurations, making it possible to be adaptable to many different plant producing environments (Paucek *et al*. 2020). LED technology offers the ability to select specific wavelengths, enabling the possibility to develop tailored light recipes, resulting in a higher efficiency in plant production (Mickens *et al*. 2019; Paucek *et al*. 2020; Trivellini *et al*. 2023). Different light recipes depending on crop and light conditions in that region need to be considered. Selecting the right LED lamp holds great importance due to the range of wavelengths they offer. There are options for green wavelengths in LEDs like 510 nm, 520 nm and 530 nm on the market. According to a study by Johkan *et al*. (2012) the green wavelength 510 nm demonstrated the best effect on plant growth when evaluating lettuce (*Lactuca sativa L*.).

The varieties of different light recipes can manipulate specific plant traits like plant architecture, branching, rooting, and leaf expansion, which are influenced by the spectral composition of LEDs (Paucek *et al*. 2020; Katzin *et al*. 2021; Trivellini *et al*. 2023). Commercial plant producers often choose LED systems that combine red and blue wavelengths, since the absorption spectra of photosynthetic pigments mainly focus on blue (400-500 nm) and red (600-700 nm) light.

Blue and red LEDs, which are widely employed for plant production, are particularly effective since chlorophyll a (chl a) and chlorophyll b (chl b) exhibit high absorption rates for blue and red wavelengths (Bantis *et al*. 2018). Specifically, the absorption maxima for chl a are at 430 and 663 nm, while chl b's absorption peaks at 453 nm and 642 nm (Bantis *et al*. 2018).

Naznin *et al*. (2019) compared different light qualities and their effect on various plants. A 100 % red (691 nm) LED light treatment was compared to a combination

of red and blue (449 nm) LED light, where the addition of blue ranged from 5% to 9 %. The solely red LED light treatment stimulated plant height in lettuce, kale, and pepper, but produced less favourable results in spinach and basil. The most optimal combination was found with an addition of 5% to 9% blue to red light, which enhanced biomass production and total chlorophyll accumulation in tested plants. Increasing blue light 9% to 17% also stimulated carotenoid contents and antioxidant levels. This is one of many studies that indicates that finding the right combination of red and blue LED light, and possibly other wavelengths can enhance plant growth, pigments contents, and antioxidant activity in plants (Claypool & Lieth 2020).

1.3.3 Red, green, and blue, light spectra and their effect on plants

The principle of spectral quantum yield was established by McCree (1972), showing some photons to be more efficient for photosynthesis than others (Mickens *et al*. 2019). Light affects not only photosynthesis directly, but also the phytochemical content in the tissues of the plant (Zheng *et al*. 2018; He *et al*. 2022).

Red and blue light is often considered the most effective for plant development and growth (Zheng *et al*. 2018; Liu & Van Iersel 2021; He *et al*. 2022).

Red LEDs as a light source can drive photosynthesis, but the plants require more than red light to develop (Naznin *et al*. 2019). Blue light is needed to regulate other important processes than photosynthesis and plant growth, and has been proven to affect stomatal opening, secondary metabolism, photomorphogenesis, chlorophyll synthesis, and vegetative growth (Goto 2012). According to Kopsell & Sams (2013) blue light applications resulted in significant increases in nutritional important carotenoids, glucosinolates and mineral element in broccoli microgreens. Most significant increases were in β-carotene, glucraphanin, K, Mg and Fe. the potential benefits of increasing concentrate of primary and secondary metabolites can be of great importance. Phytochromes are photoreceptors that are highly sensitive to red and far-red radiation (Samuolienė *et al*. 2020; Trojak *et al*. 2022). They play a crucial role in regulating various aspects of plant growth and development, including seed germination, de-etiolation, when plants transition from darkness to light, shade avoidance responses, circadian clock radiation, and flowering (Samuolienė *et al*. 2020; Trojak *et al*. 2022). Cryptochromes are photoreceptors that respond to UV-A, blue and green light. They are also involved in regulating de-etiolation, entrainment of the circadian clock which are biological rhythms with light-dark cycles (Samuolienė *et al*. 2020; Trojak *et al*. 2022).

Green light is the least absorbed wavelength by leaves and provides them with their green appearance (McCree 1972; Nishio 2000). Green light is often considered

to be the least efficient wavelength for plants and their photosynthesis (Sun *et al*. 1998; Nishio 2000; Terashima *et al*. 2009; Smith *et al*. 2017; Liu & Van Iersel 2021; Trojak *et al*. 2022). One common misconception is that plants only reflect the green light and does not absorb it.

Still, green light is important in photosynthesis, as it help plants to better adapt to different light intensities (Liu & Van Iersel 2021). The absorbance of chlorophylls channels green light deeper into leaves, providing more uniform light absorption and energy to cells further from the adaxial side of the leaf. Plants also use green light to regulate plant architecture (Smith *et al*. 2017). Green light has received less attention in research than red and blue light, primarily due to its lower absorptivity coefficient in the absorptivity spectra of chlorophyll absorption when compared to blue and red light (Nishio 2000; Terashima *et al*. 2009; Brodersen & Vogelmann 2010; Wang *et al*. 2013; Dou *et al*. 2019; Meng *et al*. 2019).

In the research of Terashima *et al*. (2009) it was found that, in high-intensity white light environments, supplemental green light was more efficient at driving photosynthetic activities compared to red light. Their observation implies that green light penetrates deeper into leaf tissues and additionally functions effectively under conditions of intense illumination.

Its high transmittance and reflectance allows green light to penetrate more deeply into the mesophyll layers at a single-leaf level and the lower leaves at a canopy level, into the plant canopy, and may potentially increase whole canopy photosynthesis and light interception (Sun *et al*. 1998; Kim *et al*. 2004).

The research by Kim *et al*. (2004) showed how supplementing green light could enhance increased light penetration, increasing photosynthesis in lower-level leaves in the canopy. Similar results was found in research by Sun *et al*. (1998). In contrast, red and blue wavelengths are mostly absorbed by the upper leaves. Green light induces shade avoidance responses and plays a crucial role in regulating secondary metabolites in plants (Zhang & Folta 2012; Smith *et al*. 2017; Dou *et al*. 2019).

Green light (500-600 nm) wavelengths are less efficient than red (600-700 nm) and is a radiation required for photosynthesis, whereas the blue light (400-500 nm) is the main light source for photosynthesis which regulates many physiological responses in plants through photoreceptors (Folta & Maruhnich 2007; Liu & Van Iersel 2021; He *et al*. 2022; Trojak *et al*. 2022). Green light was shown to improve the growth of lettuce when added to red-blue light spectra, due to its deeper transmission through leaf tissue and canopies.

The low absorption of green light explains its lower efficiency in CO_2 assimilation. In a study conducted by Liu & Van Iersel (2021) exploring light spectra involving red, blue, and green wavelengths, it was discovered that green light also shows varying photosynthetic efficiency at different light intensities. In that same study, green light exhibited the lowest photosynthetic efficiency at a lower PPFD 200

µmol m^{-2} s^{-1}. However, in contrast, at higher PPFD levels 1000 µm^{-2} s^{-1} green light demonstrated a notable increase in quantum yield.

1.3.4 Artificial light in Scandinavia

In Scandinavia, The Day Light Integral (DLI) expressed in (mol·m^{-2}·d^{-1}) remains relatively low during the winter months. In November, December and January, it ranges from 0-5 mol·m^{-2}·d^{-1}, increases slightly in February 6-10 mol·m^{-2} ·d^{-1}, and March 10-15 mol·m^{-2}·d^{-1}, according to Hernandez Velasco (2021). To maintain a year-round plant production in this region, supplemental light is of great importance. Depending on the type of crop and type of greenhouse, the need for supplementary light differs in greenhouses. During the winter season, the amount of supplemental lighting needed can surge to as much as 80-90 %, depending on the type of greenhouse and choice of crop (Hernandez Velasco 2021; Bergstrand 2023).

1.4 Stomatal conductance

Environmental factors like plant water status, CO_2 concentration and light quality and intensity can affect the stomata and the stomatal movements (Kim 2004; Taiz & Zeiger 2010; Wang *et al*. 2011; Matthews *et al*. 2020).
A stoma consists of a pair of specialized epidermal cells known as guard cells. (Taiz & Zeiger 2010; Wang *et al*. 2011; Suetsugu *et al*. 2014).

Stomata, the plural form of stoma, are small openings found on the surfaces of plant leaves and stems that play a crucial role in regulating gas exchange and water loss in plants (Kim 2004; Taiz & Zeiger 2010; Wang *et al*. 2011; Driesen *et al*. 2020; Haworth *et al*. 2021). Each individual stoma is composed of two specialized epidermal cells known as guard cells (Taiz & Zeiger 2010; Wang *et al*. 2011; Suetsugu *et al*. 2014). The opening of each stoma is finely regulated through the complex management of ion transport and solute production within the guard cells.

Photoreceptors like phytochromes and cryptochromes are involved in stomatal movements (opening and closing), and chlorophyll formation. Phytochromes and cryptochromes are also green light receptors, which makes it harder to characterize true green responses (Folta & Maruhnich 2007).

The intensity of light plays a role in stomatal behaviour (Driesen *et al*. 2020; Matthews *et al*. 2020). Bright light generally promotes stomatal opening, while low levels of light tend to keep them closed. This phenomenon is caused by the photosynthetic activity of plants, resulting in lower CO_2 concentration within the leaf, which in turn triggers stomatal opening.

The CO_2 concentration in the surrounding environment directly affects stomatal movement (Taiz & Zeiger 2010; Driesen *et al*. 2020). A lower concentration of CO_2 stimulates stomatal opening, whereas high concentrations together with bright light can lead to stomatal closure. This closure is a protective mechanism to prevent water loss when CO_2 is abundant.

Light affects stomatal behaviour depending on the different wavelengths (McCree 1972; Sharkey & Raschke 1981; Wang *et al*. 2011; Vialet-Chabrand *et al*. 2021). Blue light (430-460 nm) is approximately ten times more effective in inducing stomatal conductivity compared to red light (630-680 nm), indicating that blue light has more potent influence on stomatal regulation (Kim 2004). The stomatal reactions to red light correspond to the process of CO_2 assimilation during photosynthesis (Matthews *et al. 2020)*. However, when photosynthetic electron transport is inhibited, the stomatal response to red light is eliminated (Sharkey & Raschke 1981; Matthews *et al. 2020)*. This may suggest that the red-light response is primarily triggered by light absorption by chlorophyll and closely tied to photosynthesis. Blue-light response on the other hand seems to be independent of photosynthesis (Wang *et al*. 2011; Matthews *et al*. 2020). Guard cells, the specialised cells that control stomatal openings, respond to blue light even when other photosynthetic processes are inactive. This shows that blue light has a particularly strong influence on stomatal conductance, and this understanding of these factors is crucial for plant physiology and to optimise conditions for production and plant growth (Matthews *et al*. 2020).

1.4.1 Chlorophyll fluorescence

Chlorophyll fluorescence occurs during photosynthesis when chlorophyll molecules emit light as they return from an excited state to their ground state (Maxwell & Johnson 2000; Cessna *et al*. 2010). Chlorophyll (a) fluorescence emitted by green plants reflects photosynthetic activities in a complex manner (Krause & Weis 1991). The chlorophyll fluorescence comes from the photosystem II since the phytochemicals are initiated here.

The mechanisms of chlorophyll fluorescence involve the excitation of chlorophyll molecules by absorbed light, when a molecule absorbs a photon of light, an electron within the molecule is promoted to a higher energy level, resulting in an excited state (Maxwell & Johnson 2000; Cessna *et al*. 2010; Porcar-Castell *et al*. 2014). The excited stage is short-lived and unstable, the excited molecule returns to its ground state, releasing excess energy in the form of fluorescence, consisting of light at longer wavelength than the absorbed light.

The observation of changes in chlorophyll fluorescence yield were first done by Kautsky & Hirsch (1931) (Maxwell & Johnson 2000; Baker 2008). Kautsky & Hirsch (1931) discovered that when photosynthetic material was transferred from

dark to light, there was an increase of yield of chlorophyll fluorescence within approximately one second. This increase was later explained as a consequence of reduction of electron acceptors in the photosynthetic pathway, downstream of PSII, notably plastoquinone and in a particular Q_A, the primary stable quinone electron acceptor in PS II. It serves as the primary electron acceptor, accepting an electron from the primary electron donor, pheophytin, after light energy excites chlorophyll in the PSII complex.

During the functioning of PSII and when light is absorbed and Q_A has accepted an electron, it cannot accept another electron until it has passed the first onto a subsequent electron carrier Q_B, which is the secondary binding site.

The emitted fluorescence can be measured with specialized equipment such as a Pulse-Amplified-Modulated (PAM) fluorometer. These measurements provide information about the efficiency of photosynthesis and the health of the plant, indicating the level of stress the plant experienced. It also indicates different parameters that can affect the health and quality of the plant. Changes in fluorescence intensity can reflect alterations in the photosynthetic apparatus. Chlorophyll fluorescence is a frequently used method in applied plant physiology research, which gives better insight into mechanisms of fluorescence emission and also measures the photosynthetic performance in a plant (Krause & Weis 1991; Maxwell & Johnson 2000; Baker 2008; Murchie & Lawson 2013).

Chlorophyll fluorescence is a non-invasive measurement for understanding the photosynthetic PSII activity (Krause & Weis 1991; Baker 2008; Murchie & Lawson 2013). The measurements are mostly used for crop improvement purposes in greenhouse facilities or in the field. The use of measuring chlorophyll fluorescence from intact plant leaves has increased as an intrusive method of monitoring photosynthetic rates and measuring the physiological state of the plant. It is an indicator of how plants respond to environmental change and different sorts of stress and an important technique to understand the sensitivity of PSII activity to abiotic and biotic factors.

The maximum fluorescence (F_m) is achieved when no photochemical quenching occurs, serving as a reference point for comparing other fluorescent measurements (Maxwell & Johnson 2000). By comparing this value with the yield of steady-state fluorescence ($F_{t,}$) in the presence of light and the fluorescence yield without actinic photosynthetic light, basic fluorescence (F_0) insight is gained of the efficiency of photochemical quenching. And by extension, information on the performance of PSII.

In addition to changes in photochemical efficiency, the effectiveness of heat dissipation, (referred to as non-photochemical quenching) can also vary based on a range of internal and external factors. These changes can be seen as variations in the level of F_m. Variable fluorescence (F_v) is used in the ratio F_v/F_m, which is the ratio of variable to maximum fluorescence after dark-adaptation. It is utilized as to measure the quantum yield of PS II photochemistry in the dark-adapted state. F_v/F_m

represent the proportion of light energy utilized for photosynthesis in relation to the amount of light absorbed by the leaf. It is a valuable parameter that is also described in the literature in these different terms such as light-harvesting efficiency, maximal quantum yield, potential quantum efficiency, photosynthetic light use efficiency, or PSII efficiency (Evans 2013). The values of F_v/F_m of an unstressed plant is mostly consistent, with values of ~0.83(Murchie & Lawson 2013). Chlorophyll fluorescence can be crucial to gain a deeper understanding of the photosynthetic activity of plants, serving a valuable tool for monitoring plant health, studying the impact of environmental factors, and improving crop productivity (Murchie & Lawson 2013).

2 Aim and hypothesis

2.1 Aim

The aim for this study is to optimise the photosynthetic performance of plants grown in a greenhouse, plant factory or climate chambers with artificial light.

2.1.1 Research questions

The following research questions were constructed and addressed in the current study.

- How does different light spectra affect photosynthesis rates and stomatal conductance in Bell pepper, Swiss chard, and Pak Choi in the shorter treatments performed in a climate chamber using a leaf chamber equipment? Can the green light improve the performance?
- Can the addition of green light to a spectrum of red and blue light improve chlorophyll fluorescence in the five days trial of Swiss Chard, Bell pepper and Pak Choi?
- How does the presence of green light in the spectrum affect photosynthesis differently than a spectrum with only red and blue light? Do the three plant species respond differently to different spectra of light?

2.1.2 Hypothesis

The hypothesis suggests that the addition of green light plays an important role for photosynthesis and chlorophyll fluorescence. This response can be similar between different plant species.

3 Material and methods

3.1 Plant material and experimental design

The plants for this study were cultivated in the greenhouse facilities of the department of Biosystems and Technology, at the Swedish University of Agricultural Science, Alnarp, Sweden, starting from October 2022 continuing until March 2023.

Three different plant species were included in the study: Pak Choi (*Brassica rapa* subsp. *chinesis)* F_1 'Joi Choi' (Impecta Fröhandel AB, Julita, Sweden), Swiss Chard (*Beta vulgaris* var. *cicla*)'Perpetual Spinach' (Nelson Garden AB, Tingsryd, Sweden), and for bell pepper (*Capsicum anuum)* 'Balconi F_1' (Olssons Frö AB, Helsingborg, Sweden). The temperature in the greenhouse chamber was set at 20 ^{o}C.

The cultivation process involved sowing the plants in trays using Såjord, peat-based growing media (Hasselfors AB, Örebro, Sweden), on October 11th and then transplanted into 13 cm pots on October 20th. In October, the first set of plants consisted of 12 Pak Choi, and 12 Swiss chards. On November 14th, 2022, an additional set of 15 pots each for Pak Choi, Swiss Chard were planted resulting in a total of 69 plants. These pots were arranged in rows on a table within a chamber in the greenhouse.

For the Bell pepper, the initial planting took place on November 23rd, in a sowing tray using Såjord, peat-based growing media (Hasselfors AB, Örebro, Sweden). Later, these were transplanted to 13 cm pots filled with K-jord, another peat-based growing media (Hasselfors Garden AB, Örebro, Sweden). K-jord was enriched with a pelletized fertilizer, Basacote plus 3M (16-8-12) (COMPO, Münster, Germany), following the recommended guidelines of 5g/1L.

3.1.1 Climate conditions for the plants

The plants were watered using tap water used for irrigation in the greenhouse. In the beginning of the trial, manual irrigation was done every third day and increased to once a day as the plants became bigger.

HPS lamps (Philips Green Power, 400 W), were used as a supplementary light source to the natural sunlight. The photoperiod in the greenhouse was set at 12:12 hours, which means that the HPS lamps were lit from 6am to 6pm. The average temperature in the greenhouse chamber was set at 20 ºC, with a mean humidity of 64.5 %. The average intensity of light, or PFFD provided with HPS lamps were 60 µmol m^{-2} s^{-1}. The plants were randomly positioned in the chamber and rearranged after each irrigation, for the plants to receive equal access to sunlight and to the supplemental light from the HPS lamps, as well to avoid shading. The plants were used in the measurements when they were developed enough, from about 8 weeks old until they were 15 weeks old.

To prevent Scaridae flies, a biological control treatment using nematodes of the species *Steinernema feltiae* (Entonem, Koppert) was applied to the plants on January 12th, 2023.

3.2 Light treatments

Two different trials involving the two alternate LED light treatments, Red and Blue (RB) and Red, Green and Blue (RGB) were conducted during this study. The fractions (or ratios) of light in this study were for RB treatment: red 50 %, and blue 50%, and for the RGB treatment: Red 33%, green 33%, and blue 33%.

3.2.1 Light treatment for the photosynthetic measurements

The plants were first grown in a greenhouse and then transferred to climate chamber for the measurements, the measurement was performed on the plants individually.
The photosynthetic capacity, the maximum rate at which leaves are able to fix carbon during photosynthesis (A_{max}) and stomatal conductance (g_s) was measured in these part-trials.
For the photosynthetic measurement each plant species was exposed separately to the two different light spectrums using a LED light lamp (Heliospectra DYNA, Heliospectra AB, Gothenburg, Sweden) installed in a climate chamber. The plants were treated for 20 minutes nine times for each plant. Each plant underwent nine sessions of 20-minutes exposure to their respective light treatment, resulting in a total of 18 measurements (nine for each treatment). The RGB LED light treatment,

which involved three colours (wavelengths) of light from LED light lamps: blue (460 nm), green (525 nm) and red (620 nm). The fractions of light (or ratios) in this study were for RB treatment: Red 50 %, and Blue 50%, for the RGB treatment: Red 33%, Blue 33%, Green 33%. During these trials the PPFD, was set at 100 µmol m^{-2} s^{-1}, using a light quantum sensor (Skye PAR Quantum Sensor, Skye Instruments, Llandrindod Wells, UK). The quantum sensor was used continuously during the whole study to keep the PPFD at the same intensity.

3.2.2 Light treatment for chlorophyll fluorescence measurements

The photoperiod was set at 16:8 in the climate chamber where light treatments for the chlorophyll fluorescence measurements took place. During the chlorophyll fluorescence measurements, the PPFD in the chamber was set at approximately 100 ± 10 µmol m^{-2} s^{-1}. Measured once per day during five consecutive days.

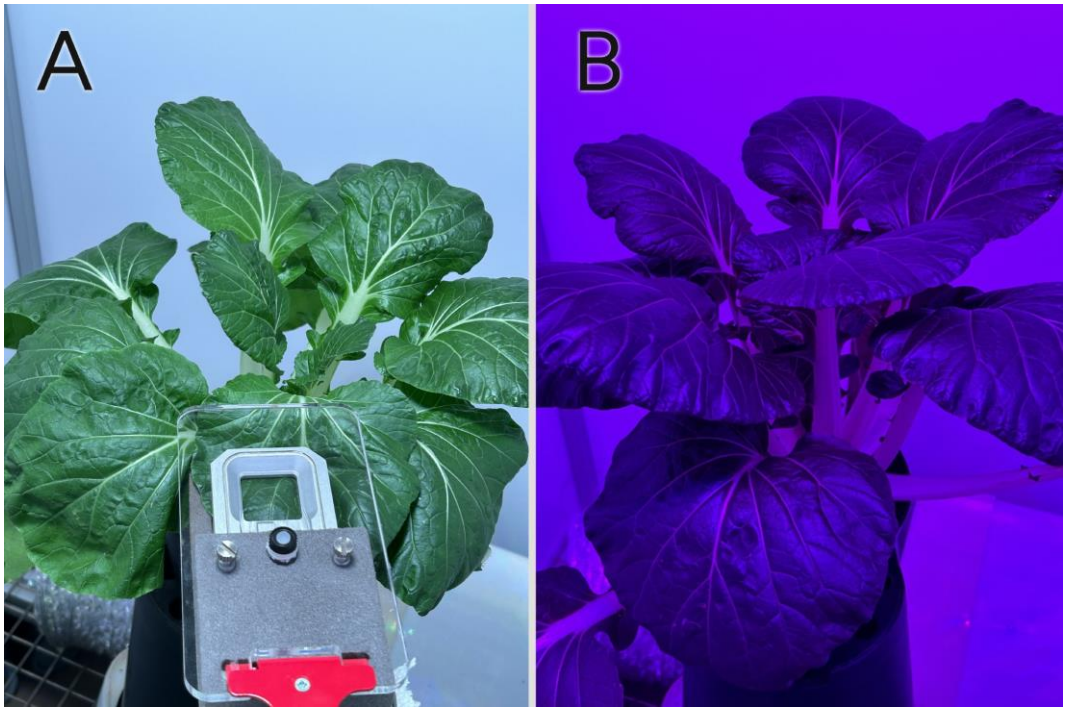

Figure 2. The RGB LED light treatment to the left (A), and the RB LED light treatment to the right (B), both performed on Pak choi plants, measuring photosynthetic rates, A_{max} and stomatal conductance, g_s.

3.3 Photosynthetic measurements

Plants previously grown in the greenhouse were individually transferred to a controlled climate chamber when the second leaf of the plant were big enough to be measured with the IRGA equipment, which occurred when the plants were around five weeks. The trials focused on assessing A_{max} and g_s using an Infra-red Gas Analyser (IRGA), LCpro (ADC Bioscientific, Hoddesdon, UK). A_{max} was measured in µmol CO_2 m^{-2} s^{-1}, while g_s was measured in µmol H_2O m^{-2} s^{-1}.

To minimise the stress in the plant, the first measurement was started approximately 30 minutes after relocating the plant to the climate chamber.
A second youngest leaf was selected for the measurements, and the readings were taken on the adaxial side, the upper side of the leaf.
During the trials a consistent light treatment was applied continuously for the nine measurements of that plant before transitioning to the alternate light treatment.
Each plant underwent nine sessions of 20-minutes exposure to their respective light treatment, resulting in a total of 18 measurements (nine for each treatment). The order of two light treatments, RGB or RB, was randomized to ensure accuracy.
The 20-minutes exposure was also a resting interval between each measurement to allow for photosynthesis to reset. The Heliospectra DYNA software Systemassistent, linked to the LED lamp (Heliospectra AB, Gothenburg, Sweden) was utilized to adjust settings for the experiments. No control plants were used in this part of the trial.

3.3.1 Chlorophyll fluorescence measurements

The Chlorophyll fluorescence measurements trials were conducted using six randomized plants from the same pool of 15 plants grown in the greenhouse, representing the three plant species Pak Choi, Bell pepper and Swiss chard. The plants were positioned differently in the chamber and were rearranged after each day's measurements and watering. The plants required continuous measurements with a quantum sensor to adjust the PPFD to 100 ± 10 µmol m^{-2} s^{-1}. The chlorophyll fluorescence measurements were performed using an imaging-pulse amplitude-modulated (chlorophyll fluorescence) meter PAM-2500 (Heinz Walz GmbH, Effeltrich, Germany) on leaves that had been dark-adapted for 20 minutes. The measurements were performed on one of the second fully expanded leaf below the apex.

This part-trial spanned for five days, where the first day was a control day measuring the chlorophyll fluorescence rates before the treatments. The

measurements for the six plants were conducted at the same time consistently before lunchtime, day one to five, Monday to Friday.

The six plants were treated the first week with one of the mentioned treatments, randomly, the RGB treatment which involved red, blue, and green light, or the RB treatment which only involved red, and blue light. Then the following week the same six plants were treated with the other light treatment and measured the same way. It is important to note that on the first day, measurements were taken before the treatment began, making it serve as a control.

The effects of RGB and RB treatments and the mean values for five days for each treatment was examined and compared. The measurements of chlorophyll fluorescence were also conducted to determine if the inclusion of green light enhances plant responses compared to a light spectrum involving only red and blue light.

Several key parameters were analysed to assess the impact of the two light treatments on six plants from each species, examined for five days of experiments separately for each treatment. The parameters assessed were F_0 (basic fluorescence), F_m (maximal fluorescence), and F_v/F_m which represents the maximum quantum yield of photosystem II (PSII) following dark adaptation.

3.4 Statistics and data analysis

The data was documented and prepared in the software Excel, and then later statistically analysed in R studio, graphs and plots were constructed in R studio (R studio IDE, Posit PBC).

A total of 24 plants from three different plant species, Bell pepper, Swiss chard, and Pak Choi (eight from each plant species), were included in the photosynthesis measurement part of the study. These plants were subjected to two treatments, red, green, and blue LED light (RGB) and red and blue LED light (RB). The plants were randomly assigned to receive either the RGB treatment at first or RB treatment as the first treatment.

To assess the photosynthetic rates, an analysis of deviance (Type II Wald F test with Kenward-Roger degrees of freedom) was performed using a linear mixed-effects model. The analysis of deviance, similar to an analysis of variance (ANOVA), provided the statistical results. The statistical significance was evaluated at a 0.05 significance level.

For the chlorophyll fluorescence measurements 18 plants were used, six from each plant species. Analysing the results for the two treatments and the plant response of F_0, F_m, and F_v/F_m, paired t-tests were performed using R studio.

3.4.1 Literature study

Google Scholar, and ScienceDirect, was mainly utilized for finding relevant literature. Zotero was used as a reference program.

4 Results

4.1 Photosynthetic rates, A_{max}

The results indicate that there was a significant difference between the two treatments. The analysis of deviance (Type II Wald F tests with Kenward-Roger df), a similar analysis to ANOVA for A_{max} for all three plant species showed statistical significance for the two treatments, at 0.05* significance level (0.03121*). The species and the interaction between plants was also analysed, which showed that species didn't show any statistical significance in the same analysis.

The light treatments were the factor that affected the plant species at a significant level. The ANOVA indicates statistical significance for the treatments. The red-blue RB LED light treatments that excluded the green light, demonstrated the best A_{max} results. Therefore, in these short-term trials, green light did not improve the overall photosynthetic performance.

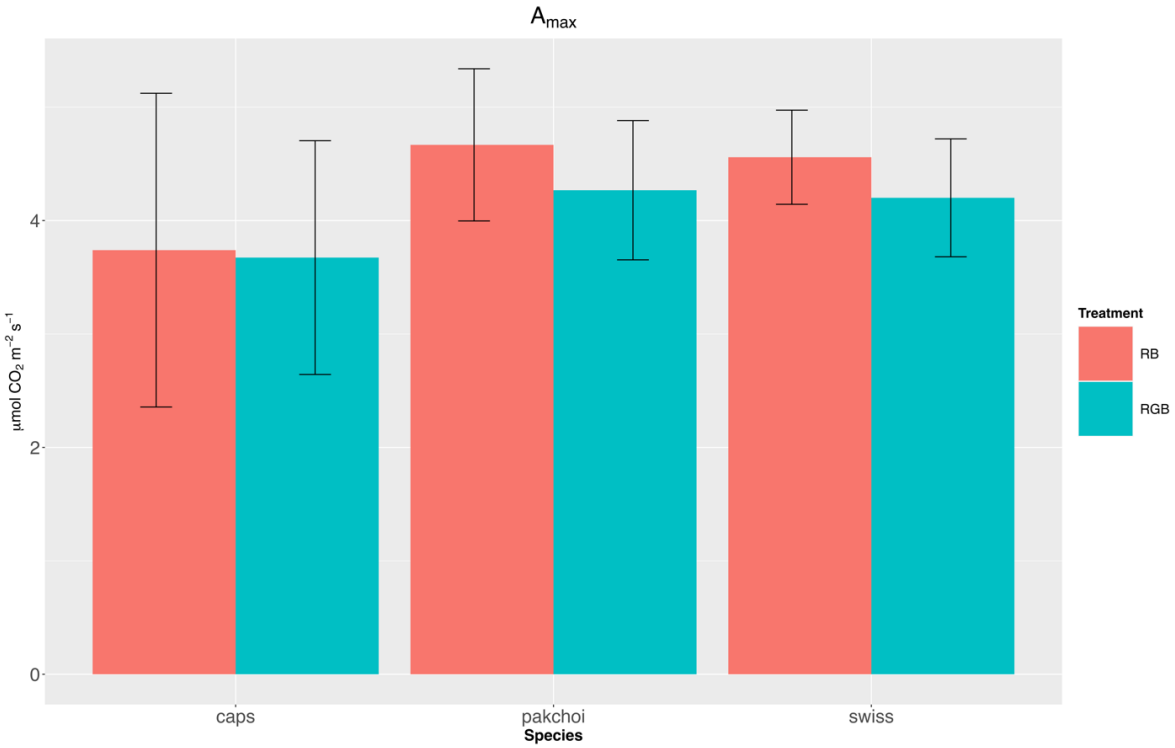

Figure 3. Mean value for photosynthetic capacity (A_{max}) for bell pepper (Caps annuum), Pak Choi (Brassica rapa subsp. *chinesis) and Swiss Chard (Beta vulgaris var. cicla). Under RB= Red (620 nm) Blue (460 nm) treatment. Under RGB= Red (620 nm), Green (525 nm), Blue (460 nm) treatment. Chart shows mean values for nine measurements per plant N=8.*

4.2 Stomatal conductance measurements

The stomatal conductance, g_s, which was measured with the IRGA as for all three plant species. The analysis of deviance (ANOVA) conducted for g_s showed that the p-value for the treatments was 0.07108, which is not statistically significant. The species and the interaction species: treatment neither was not statistically significant in the same analysis.

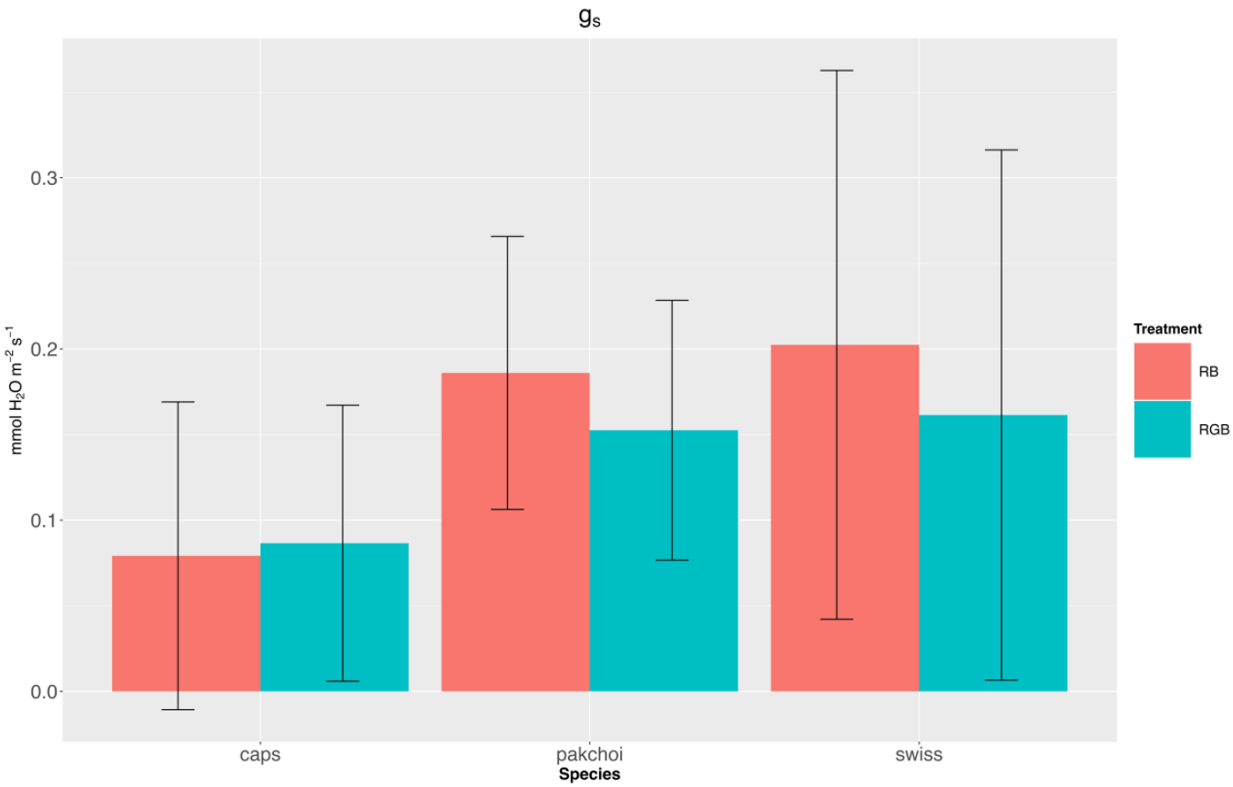

Figure 4. Mean values for stomatal conductance, g_s for three plant species Bell pepper (Caps annuum), Pak Choi (Brassica rapa subsp. chinesis) and Swiss Chard (Beta vulgaris var. cicla). Red, Green, Blue (RGB) treatment in blue/turquoise bars, while the Red and Blue (RB) treatment is shown in red bars. Under RB= Red (620 nm) blue (460 nm) treatment. Under RGB =Red (620 nm), Green (525 nm), Blue (460 nm) treatment. Chart shows mean values for nine repetitions per plant N=8.

4.3 Chlorophyll fluorescence

The result of the chlorophyll fluorescence rates, F_0, F_m, and F_v/F_m was compared with paired t-tests for the two treatments and are presented in tables below.

Table 1. This table presents paired t-test for the mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), F_m (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Swiss Chard treated under RB= Red (620 nm) blue (460 nm) treatment.). The measurements were performed on six plants over a period of five days. A paired t-test was conducted to compare the chlorophyll fluorescence parameters (F_v/F_m, F_m, and F_0) between the two treatments RGB and RB for Swiss Chard. The significance level was *set at* a *0.05 level*, (0.01**, 0.001***)*

Day	**F_v/F_m P-value**	**F_m P-value**	**F_0 P-value**
1	0.05303*	0.001957***	0.07106
2	0.02695*	0.08618	0.02585*
3	0.01629*	0.2031	0.01584*
4	0.5366	0.0001677***	0.5387
5	0.6086	0.0009167***	0.641

For Swiss chard, the mean values for F_v/F_m, were higher for the RGB treatment, and likewise for F_m, while the F_0 exhibited higher values under the RB treatment (figure 4-6, and tables 1-2 in appendix 1). The paired t-test showed significance for the F_v/F_m in favour of the RGB treatment on day one, two and three, and significant differences in F_m on day one, four, and five. Significant differences were observed in day two and three, but for this parameter the RB were in favour.

Significant differences were observed in F_v/F_m between the two treatments during the first three days of the part-trial. This can suggest that the RGB treatment had a notable effect on this parameter during the initial phase of the experiment.

F_m statistically significant differences were observed in F_m on the first, fourth and fifth days of the experiment. These findings suggest that the RGB treatment had a significant impact on F_m at these time points.

The data showed statistical significance in F_0 on the second and third day of the trial. This indicates that the RB treatment influenced F_0 during these days.

In summary, the RGB treatment positively influenced F_v/F_m on the days one, two and three, additionally it also affected F_m positively on the first, fourth and fifth day. The RB treatment on the other hand, had a notable effect for the F_0 on the second and third day. The findings of this study provide valuable insights into the

differential impacts of the two light treatments on Swiss Chard, across various parameters and days.

Table 2. This table presents paired t-test for *the mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), F_m (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Bell Pepper (caps) treated with red, blue, and green light from a LED light source (Heliospectra Dyna). The measurements were performed on six plants over a period of five days. A paired t-test was conducted to compare the chlorophyll fluorescence parameters (F_v/F_m, F_m, and F_0) between the two light treatments RGB and RB for Bell pepper (capsicum annuum). The significance level* was *set at a 0.05 level*, (0.01**, 0.001***)*

Day	**F_v/F_m P-value**	**F_m P-value**	**F_0 P-value**
1	0.9047	0.7015	0.873
2	0.02113*	0.007078**	0.06131
3	0.03436*	0.07559	0.03506*
4	0.3241	0.001457*	0.328
5	0.9351	0.2729	0.9546

A similar five-day treatment with RB light was conducted and compared with a five-day RGB treatment on six bell pepper plants.

The first measurements were conducted on bell pepper plants before any treatments so they can be regarded as a control day. The mean values of F_v/F_m and F_m, had higher mean values observed for the RB (figure 4-6, and tables 3-4 in appendix 1). However, the mean values for F_0 were higher for the RGB treatment. Paired t-tests were conducted to compare the two treatments of the experiment and to examine if there were statistical significance between the two.

The results of the paired t-test showed statistically significant differences in F_v/F_m, on the second and third day.

There were statistically significant differences in F_m noted on the second and fourth days in favour of the RB treatment. Although there is no clear pattern in the results, the significance for both F_v/F_m and F_m, indicates that the RB treatment influenced these parameters.

On the third day statistical significance was observed for F_0, suggesting a specific impact of the RGB treatment on this parameter.

In summary, the RB treatment has more impact on the Bell pepper for the F_v/F_m and F_m on the second day, and similarly on a few of the other days of the week. The RGB influenced the F_0 positively on the third day.

Table 3. This table presents the mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), F_m (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Pak Choi treated with red, green, and blue light from a LED light source (Heliospectra Dyna). The measurements were performed on six plants over a period of five days. A paired t-test was conducted to compare the chlorophyll fluorescence parameters (F_v/F_m, F_m, and F_0) between the two light treatments RGB and RB for Pak Choi. The significance level was *set a 0.05 level*, (0.01**, 0.001***).*

Day	F_v/F_m P-value	F_m P-value	F_0 P-value
1	0.01231*	0.004747**	0.01054*
2	0.2098	0.8194	0.2194
3	0.4125	0.0009167***	0.3792
4	0.466	1.737e-05***	0.4657
5	0.2703	0.6012	0.2759

The mean values of the Pak Choi plants responded differently to the RGB treatment, resulting in overall higher mean values for F_v/F_m but lower mean values for F_m and F_0 (table 1, and tables 5-6 in Appendix 1).

Surprisingly, the paired t-test unexpectedly revealed statistical significance for all three parameters (F_v/F_m, F_m and F_0) on the first days, in favour of the RB treatment for F_m and F_0, while the RGB treatment had a positive influence on F_v/F_m. Apart from this observation, significant differences between the treatments were shown on the third and fourth day for F_m, specifically the RB treatment had a positive impact.

The paired t-test compared the first day for two weeks with both treatments. This may suggest the possibility of prior treatment effects from the week before or the influence of other factors that have affected the six Pak Choi plants. Statistically significant differences in F_m were observed on the third and fourth days of the study, positively affected by the RB treatment.

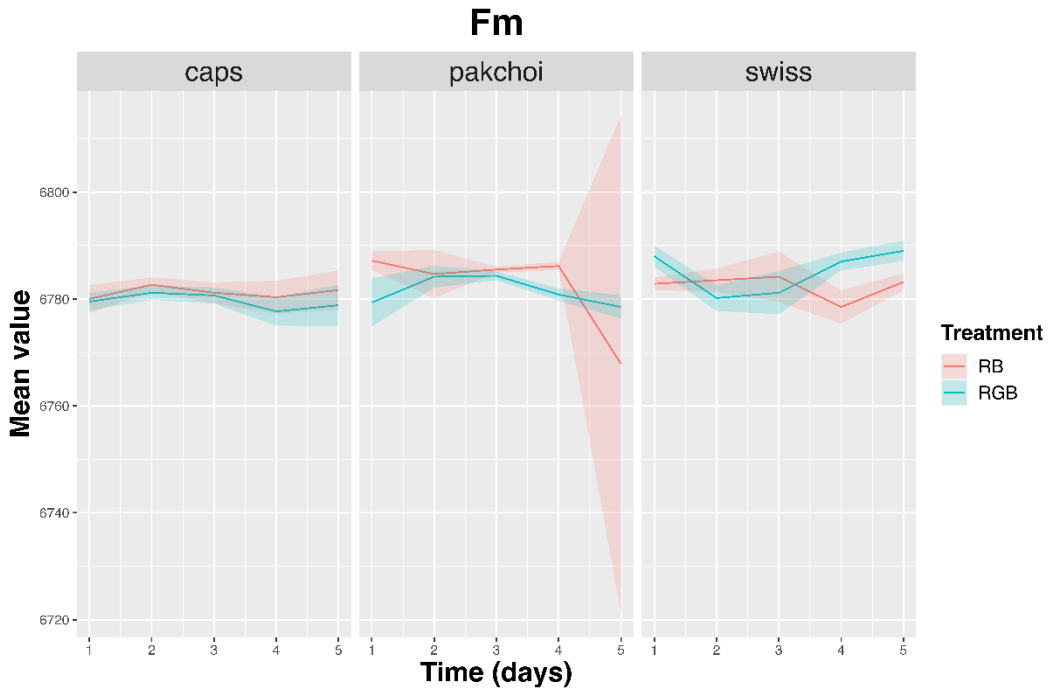

Figure 5. Mean changes in the chlorophyll fluorescence parameters of maximal fluorescence F_m in darkness for six randomly chosen plants of each species, with separate measurements conducted for each species and for the two light treatments. Three plant species: bell pepper (Caps annuum), Pak Choi (Brassica rapa subsp. chinesis) and Swiss Chard (Beta vulgaris var. cicla) were examined twice in five-day trial in a controlled climate chamber for the two light treatments RB and RGB. Under RB = red (620 nm) blue (460 nm) treatment. Under RGB = red (620 nm), green (525 nm), blue (460 nm) treatment. F_m is a dimensionless quantity.

When examining the chart line for F_m for all plant species, they have all responded differently during these 5 x 2 days (the trial was preceding for 10 workdays during a period for two weeks). For Bell pepper (*Capsicum annuum*), it is evident that the mean values for the RB and RGB treatment are quite similar, with a slight tendency towards higher mean values for the RB treatment, when comparing the end of the five-days periods.

For Pak Choi, the initial mean values favour the RB treatment (figure 5-7). However, as the trials progress, the results become difficult to interpret due to fluctuations, especially towards the end of the trial where the RGB treatment exhibits higher mean values.

When examining the Swiss chard, the RGB treatment initially leads to higher mean values during the first few days and towards the end of the five day-trial. This pattern makes it difficult to draw a conclusion regarding which treatment had a more positive impact on the F_m parameter, and which one provided benefit to the plants. In summary, the results of the F_m parameter are harder to interpret and vary among the different plant species.

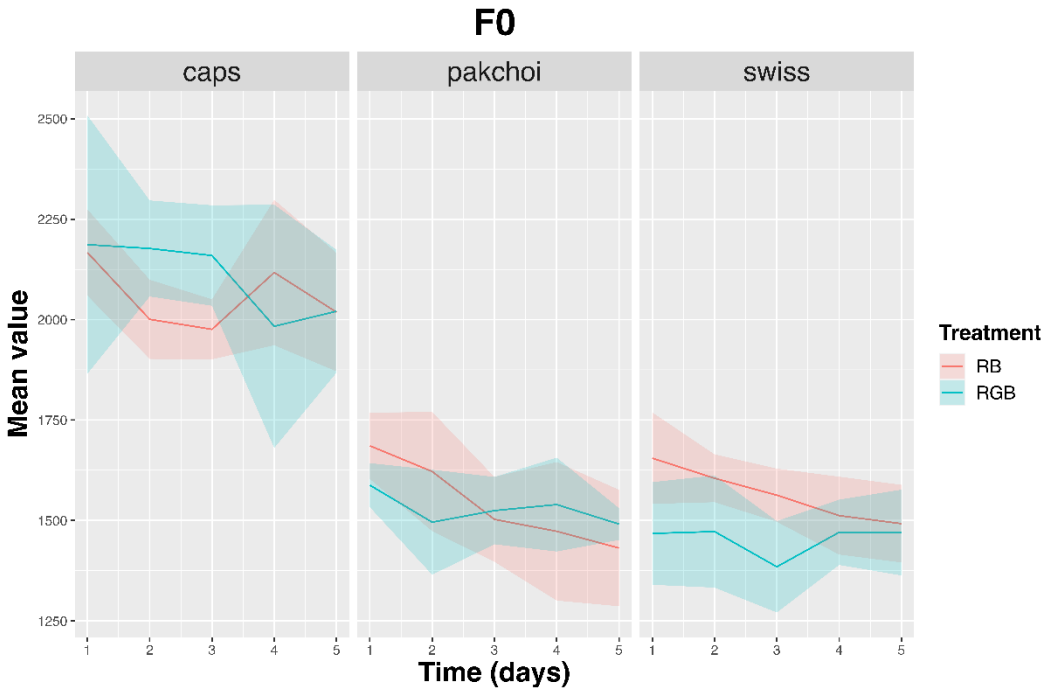

Figure 6. Mean changes in the chlorophyll fluorescence parameters of basic fluorescence F_0 for three plant species bell pepper (Caps annuum), Pak Choi (Brassica rapa subsp. chinesis) and Swiss Chard (Beta vulgaris var. cicla), were examined twice in five-day trials in a controlled climate chamber for the two light treatments, RB and RGB. Under RB = red (620 nm) blue (460 nm) treatment. Under RGB = red (620 nm), green (525 nm), blue (460 nm) treatment. F_0 is a dimensionless quantity.

This line chart provides a visual representation of the mean F_0 values for Swiss chard, bell pepper, and Pak Choi. These three plant species exhibited dissimilar responses to the two light treatments. For bell pepper, F_0 initially showed a positive response to RGB treatment, but later as the five-day trial progressed, there was a shift towards higher mean values under the RB treatment.

When observing the results for Pak Choi, a similar pattern with fluctuations is found, making it difficult to draw any conclusion.

For Swiss chard, it was displayed a more consistent response where the RB treatment had a positive throughout the experimental period.

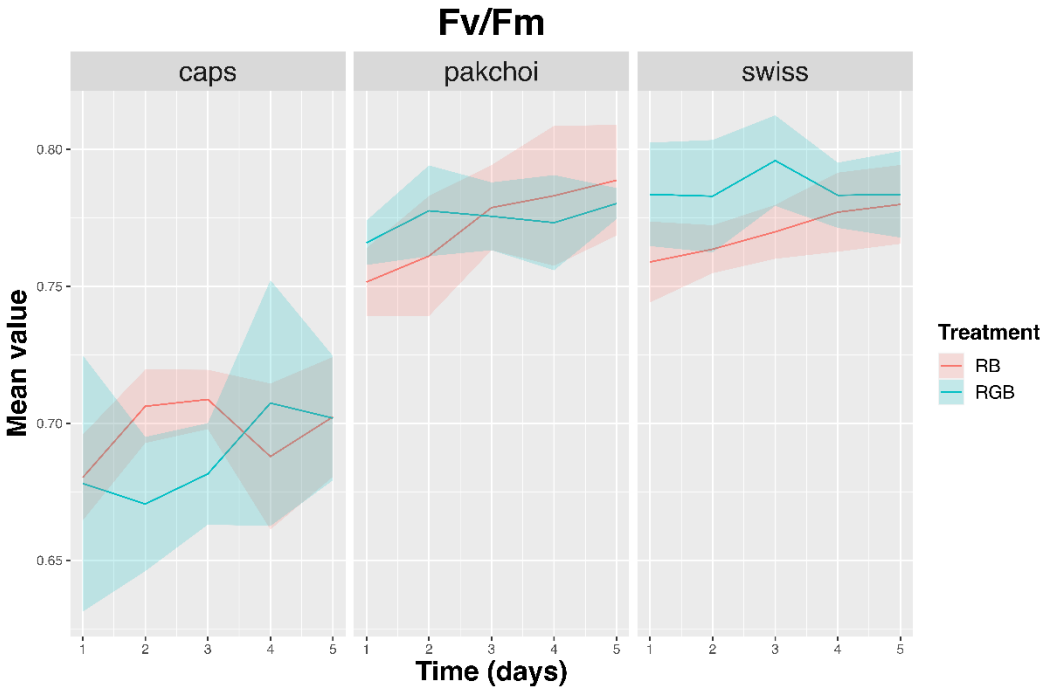

Figure 7. Mean changes in the chlorophyll fluorescence parameters of maximal fluorescence of yield F_v/F_m for three plant species bell pepper (Caps annuum), Pak Choi (Brassica rapa subsp. chinesis) and Swiss Chard (Beta vulgaris var. cicla), were examined twice in five-day trials in a controlled climate chamber for the two light treatments, RB and RGB. Under RB = red (620 nm) blue (460 nm) treatment. Under RGB = red (620 nm), green (525 nm), blue (460 nm) treatment. F_v/F_m is a dimensionless quantity.

According to (Murchie & Lawson 2013) the values of F_v/F_m of an unstressed plant is mostly consistent, with values of ~ 0.83. By looking at values of the results the Swiss chard and Pak Choi both looks healthy, but the Bell pepper has a lower F_v/F_m value, which indicate some stress. The stress may impact how well they respond to the different light treatments.

The line chart provides a comparative view of the mean values of F_v/F_m for Bell pepper, Pak Choi, and Swiss chard. The responses of these three plant species to the RB and RGB light treatments differ significantly for all examined parameters. Here we look at the F_v/F_m parameter.

In the case for bell pepper, the RB light treatment consistently showed a positive influence for most of the days, except on the fourth day, where a deviation is observed.

For Pak Choi, the pattern is more inconsistent. Initially, the RGB light treatment appears more beneficial, with higher mean values observed during the first two days. This trend shifts as the trial progresses, and the RB light treatment becomes

more influential during the last three days. In contrast, Swiss chard exhibits a more consistent and clearer trend. Throughout the experimental period, the RGB light treatment continuously has a positive influence on the mean values for F_v/F_m. In summary, the bell pepper demonstrates mostly positive effects from the RB light treatment, Pak Choi's response shifts over time, and Swiss chard consistently benefited from the RGB light treatment.

5 Discussion

It is a well-known fact that red and blue wavelengths can be readily absorbed and utilized by plant leaves, and are most efficient for photosynthesis (McCree 1972). It has been generally recommended to use a combination of red and blue light to optimise plant biomass production and photosynthesis (Bergstrand & Schüssler 2012; Izzo *et al*. 2020).

Most LED lighting have been designed without incorporating green wavelengths (Smith *et al*. 2017; Claypool & Lieth 2020).

As highlighted in one of the previous sections, historically much of the plant physiological research has focused on red, far-red and blue light and their effect on plant growth and photosynthetic activity, and signal responses in plants (Claypool & Lieth 2020). However, recent studies suggest that other wavelengths may also play a significant role in these processes.

Research has shown that numerous other benefits from green LED light, like enhanced stress tolerance, drought tolerance, increased dried weight, and plant growth (Zhang & Folta 2012; Kang *et al*. 2016; Bian *et al*. 2019; Kaiser *et al*. 2019). Previous research that explored the effects of different light spectra, which included green light, and how it enhanced photosynthesis and other vital processes in plants served as the basis for this study. The present study focuses here primarily on the addition of green light in light treatments to assess whether it can improve photosynthesis or not.

5.1 The photosynthetic rates, A_{max} and g_s, and the results

An optimisation of photosynthesis can lead to better growth rates, healthier plants and more nutrition-dense better produce in greenhouses, or other CEA to make advances in plant production (Cessna *et al*. 2010; Evans 2013). Moreover, optimising the light spectra also contributes to better energy efficiency, reduce an overall carbon footprint, decrease waste, and minimize resource usage and

promotes a more sustainable environmental approach within horticultural practices (Evans 2013; Ramin Shamshiri *et al*. 2018).

In the present study the various light spectra affected the three plant species; Bell pepper, Swiss chard and Pak Choi, in a way that proved that the LED light treatment excluding green light (RB) were more beneficial for the stomatal conductance and photosynthetic rates. All three plant species exhibited similar responses to the two light treatments.

The RGB treatment did not enhance any photosynthetic rates or stomatal conductance in the experiment, where the plants were exposed for the 20 minutes treatments. The results shows that the presence of green light was not beneficial in this study under the given conditions.

In contrast, many researchers have found that adding green light to other wavelengths had a positive effect on the photosynthetic rates (Sun *et al*. 1998; Kim *et al*. 2004; Terashima *et al*. 2009). The experimental conditions of these studies are all different to the present study, and which is important to consider when drawing conclusions.

In other similar comprehensive studies the examined plants were cultivated in an environmentally controlled climate chamber and followed up with trials with different PPFDs (Fu *et al*. 2012; Johkan *et al*. 2012; Muneer *et al*. 2014; Kang *et al*. 2016). But there are also examples of similar studies were an average PPFD is used (Kim *et al*. 2004; Bian *et al*. 2018).

The results in this present study can potentially be attributed to several factors, including the experimental design, growing conditions of the plants, data gathering. In the present study, regarding the sample size, it can be a factor in this case, but not the most likely factor as eight plants from different plant species were measured nine times, which means that there were 72 measurements for each plant species and each treatment respectively (144 measurements for both RB and RGB). However, it cannot be ruled out that a larger sample size may have shown some differences in the results.

Regarding the experimental setup, the plants of all three species were cultivated in a greenhouse using sunlight as the primary light source, followed by 12 hours of supplemental light provided by HPS lamps, and later treated with RGB or RB LED light treatment in a control climate chamber.

The ambition for the experimental design aimed to cultivate two sets of plants under two different light spectra in solely a climate chamber and subsequently measure their photosynthetic rates. Due to research constraints, the plants were first grown in a greenhouse and then treated and measured in the climate chamber. These aspects of the experimental setup and growing conditions can possibly have influenced the outcomes and the obtained results in this study. As of now, this could also be considered as a research gap since a similar experimental design has not been found in the literature study.

5.1.1 Duration of light exposure

Bian *et al*. (2018) studied Lettuce (*L. sativa L.*) with exposure to different continuous light (CL) conditions by different combinations or red and blue LEDs supplemented with or without green LEDs. Longer photoperiods or longer durations of light exposure including green light improved the photosynthetic rates in that study. The results from that study demonstrated that the positive effect of green light on photosynthesis may depend on longer exposure durations. The photosynthetic capacity trials included in this present study consisted of 20 minutes of light exposure repeated nine times.

5.1.2 Light intensity and wavelength

In previous comprehensive studies, involving green light in multiple PPFDs were included to examine the impacts of LED light treatments. Johkan *et al*. (2012) was evaluating various light intensities and different peak wavelength of green light performed on Lettuce (*L. sativa L.)* The researchers found that the leaf photosynthetic rate (Pn) of plants irradiated with green LED light at PPFD 200 $\mu m^{-2} s^{-1}$ was dramatically higher compared to PPFD at 100 $\mu m^{-2} s^{-1}$. The plants irradiated with green light with 510 nm had the best growth rates. These results indicate that higher light intensity of green LED light was effective to promote the plants. Moreover, that study revealed that the most beneficial green light wavelength was 510 nm, but more studies is needed to investigate further.

Both the PPFD set at 100 $\mu m^{-2} s^{-1}$ and the green light wavelength (525 nm) are factors that could have altered the results of this present study. Further studies are needed to investigate other variations in PPFD and wavelength.

5.1.3 Ratio of light

Different fractions of light are often included in other studies of light spectra including green light to assess the differences in plant responses (Klimek-Szczykutowicz *et al*. 2022; Trojak *et al*. 2022).

The National Aeronautics and Space Administration's (NASA) Biological science research group (Kim *et al*. 2006) at Kennedy Space Center conducted several experiments with lettuce (*L. Sativa L.*) to evaluate the effects of green light in a controlled environment. The plant growth was significantly reduced when the proportion of green light fraction increased to more than 50%. In treatments with 24% supplemental green light enhanced the plant growth.

One configuration of light ratios (or combinations of light) was used in this present study. The RGB treatment consisted of red 33%, green 33%, blue 33%, while the RB treatment consisted of 50 %, and 50% of red and blue light respectively. That is a significant decrease of red and blue light, which most likely had a direct effect on the photosynthetic rates. The same ratio of lights in RB and RGB treatments was used in a study by (Chow 2020). That study was conducted to assess how plant growth in Lettuce (*L. sativa L.*) was affected by green light in the presence of blue and red light. A significant difference between Chow (2020) and this present study, is the use of a higher PPFD for the RGB treatment as compared to the RB treatment, 26-47 (RB), 85-87 (RGB). The results of that study indicated that the treatment without green light had better rates, similarly to this present study. The mentioned studies indicate that a green light fraction above 33 % was not beneficial for the plant growth or photosynthetic rates (Kim *et al*. 2006; Johkan *et al*. 2012; Chow 2020).

A suggestion for further studies is to compensate for the loss of red and blue light in RGB by using additional LEDs for investigating another ratios of RGB, or to increase the PPFD for the light treatment which includes green light, due to its lower absorption (Chow 2020). Further studies are necessary, to further investigate the impact of various fractions of light, different wavelengths of green light, various PPFDs, and a variation of (longer) light exposure durations involving RGB and RB (and possibly other light qualities) to further investigate photosynthetic rates and other plant responses.

5.2 The Chlorophyll fluorescence trials

Analysing chlorophyll fluorescence allows researchers and growers to assess plant stress responses and evaluate a plant's ability to tolerate environmental stresses (Krause & Weis 1991; Maxwell & Johnson 2000; Cessna *et al*. 2010; Murchie & Lawson 2013). Specifically, this technique provides insights into the extent of stress inducing damage to the photosynthetic apparatus, the efficiency of photosynthesis, and the plant's overall physiological state.

In this analysis the most important parameter F_v/F_m, is a good metric to evaluate the health of the plant, reflecting the maximal photochemical efficiency of the active center of PS-II in the dark.

Different light quantities (light intensity and photoperiod) can affect photosynthetic efficiency different depending on given conditions (Paradiso & Proietti 2022). However, it is crucial to further examine if light also can explain chlorophyll fluorescence parameter like F_v/F_m which indicates health of the plants.

A study by Joshi *et al*. (2019) conducted on bell pepper shows a very good response of green light in photosynthetic rates with intercanopy LEDs. The study found no significance differences in F_v/F_m values when comparing the treated plants with the control plants. However, the results of yield were significantly higher for the intercanopy RGB LED light treatment.

Comparing this with a study by Nie *et al*. (2024) where the photosynthetic physiological characteristics of seedlings of Namnu (*Phoebe bournei*) were analysed under five types of light qualities; red, white, blue, green, and red and blue, all with the same PPFD levels; 100 μm^{-2} s^{-1}. The only green light treatment revealed the highest F_v/F_m values but the lowest photosynthetic rates, Pn. The red-light treatment exhibited the highest rates of initial fluorescence F_0 and had the lowest rates of maximal photochemical efficiency, F_v/F_m. These results indicates that green light can improve F_v/F_m, but that it did not improve photosynthesis as a single light treatment (Nie *et al*. 2024).

In the chlorophyll fluorescence trials in this present study, it was examined whether the RGB or the RB treatment was improving any of the chlorophyll fluorescence rates. The results exhibit a consistent positive influence of the RB treatment on F_v/F_m mean values in bell pepper. When further examining the results, which was longer trials (five days) six plants, the results are more conflicting.

The results reveals that the three plant species responded differently in the chlorophyll fluorescence parameters. This difference in plant responses was expected, considering the three plant species unique prerequisites and backgrounds, plant physiology as they all belong to unrelated plant families.

Specific patterns or trends in the results were difficult to detect, and more knowledge is crucial.

5.2.1 Limitations of the study

The limitations of the study are possibly the experimental design, time constraints, solely using one light intensity, and the ratio of light used. The photoperiod used could be considered too short as compared to other studies. Additionally, only one cultivar from each plant species were used throughout the trials and the number of plants for each species were limited to an area of space in the climate chamber.

5.3 Outlook and conclusion

This study acknowledges the previous research that suggested the positive (and negative) effects of green light on photosynthesis and plant growth, which served as the scientific foundation for this study.

The primary finding in this study is the RGB light spectrum treatments did not improve photosynthetic rates or stomatal conductance in the plants that were assessed in the IRGA trials. Photosynthetic rates were not improved in this study under the given conditions and limitations. This indicates that the benefit of green light was not found in this specific trial. The findings from the chlorophyll fluorescence trials indicated similar results but with more variations.

There were several potential factors that might have contributed to the results, including the experimental design, growing conditions, and possibly a too short duration of green light exposure. One LED light source was utilized during the trials, with the green light 525 nm wavelength. To compare wavelengths of green light would be of great value in further comprehensive studies. Additional research in light treatments is necessary to understand the complex interactions between light quality, fractions of light, light intensity, different wavelength, duration of light exposure and the given plant responses. The possible synergistic effect of the wavelengths, the different PPFDs, and fractions of green, red and blue light also needs to be further analysed.

Emerging technologies, and advances in lighting systems will possibly support the optimisation of photosynthesis. It is difficult to overview so divergent results and understand which areas that affected the results, to understand the complex interactions between light, light treatments, and plant responses. It is hard to pin-point the mechanisms that causes the results. Future innovations can possibly contribute to a more precise and efficient control of light for plant growth and enhancement of photosynthesis.

Comprehensive research can lead to new practical recommendations for horticultural practices and provide insight into different settings of LED light treatments that includes green light.

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Popular science summary

Plant production, agriculture, greenhouses and plant factories plays an important role in securing our food supply, optimising production and increasing yield is therefore of great importance.

It will be even more important in the future when the population is expected to increase greatly. Plant production can be improved in many ways like plant breeding, optimal conditions for that specific plant cultivar, and preventing outbreaks of diseases.

One way to improve and to make a production more efficient is to optimise the photosynthesis, which is done with the right settings of light wavelength and light intensity. By optimising light conditions, it is possible to enhance plant growth and improve productivity, and by that also food security.

The ability to grow plants in controlled environments allows for year-round production, which also mitigates the effects of seasonal variations and potential crop losses. Moreover, closed systems provide a cleaner environment for the plants, mitigating hazards of dangerous pathogens and the need for pesticides, making plant production safer for consumers and the environment. The aim of this study was to find out if green light could possibly enhance the photosynthetic activity in the plants involved. The results from the study show that a light spectrum with green light didn't improve the photosynthetic activity.

In this present study, the potential effects of green light on photosynthesis were investigated in three different plant species, Bell pepper, Swiss Chard, and Pak Choi, and explored the potential of optimising photosynthesis. Based on literature and new research insights, green light could possibly enhance the photosynthetic rates, A_{max} in that part of the photosynthetic measurements. A_{max} represents the maximum rate of carbon fixation during photosynthesis. The study utilized Light Emitting Diodes (LEDs) with different light spectra in two different treatments, one with red, green, and blue light, (1:1:1) and another treatment conducted with red and blue light (1:1). The results showed that the combination of red and blue light from the LED light sources improved the photosynthetic rates, A_{max} as compared to red, blue, and green light.

The study also examined the effect of green LED light treatment on chlorophyll fluorescence. Chlorophyll fluorescence is a technique used to measure the light

emitted by chlorophyll molecules during photosynthesis. This emitted light, or fluorescence, provides valuable insights into the health and functionality of plant photosystems. Plants and responds to changes in their environment, also depending on the plant species or cultivar. This non-destructive method helps growers and researchers in optimising growth conditions, diagnosing plant stress, and understanding stress tolerance, and the impact of environmental conditions on plant health.

Insights from this study can possibly contribute to LED lighting strategies that can optimise plant production in the future. More research and other extensive studies are also needed to understand the mechanisms even more profoundly.

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Linda Golobov, January 2024

Appendix 1

Table 1. Mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), Fm (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Swiss Chard treated with Red, green and Blue (RGB) light from a a Dyna LED light source. The measurements were performed on six plants over a period of five days.

Day and treatment	**F_v/F_m mean value**	**F_m**	**F_0**
1 RGB	0.784	6788	1468
2 RGB	0.783	6780	1472
3 RGB	0.796	6781	1385
4 RGB	0.783	6787	1471
5 RGB	0.784	6789	1470

Table 2. Mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), F_m (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Swiss Chard treated with Red and Blue (RB) LED light from a Dyna LED light source. The measurements were performed on six plants over a period of five days.

Day and treatment	**F_v/F_m mean value**	**F_m**	**F_0**
1 RB	0.759	6783	1655
2 RB	0.764	6784	1605
3 RB	0.770	6784	1563
4 RB	0.777	6778	1512
5 RB	0.780	6783	1492

Table 3. Mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), F_m (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Bell pepper (caps) treated with Red, Blue, and Green (RGB) light from a Dyna LED light source. The measurements were performed on six plants over a period of five days.

Day and treatment	F_v/F_m	F_m	F_0
1 RGB	0.678	6780	2187
2 RGB	0.670	6781	2177
3 RGB	0.682	6781	2160
4 RGB	0.707	6778	1983
5 RGB	0.702	6779	2021

Table 4. Mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), F_m (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Bell pepper (caps) treated with Red and Blue (RB) light from a Dyna LED light source. The measurements were performed on six plants over a period of five days.

Day and treatement	F_v/F_m	F_m	F_0
1 RB	0.680	6780	2167
2 RB	0.706	6783	2001
3 RB	0.709	6781	1976
4 RB	0.688	6780	2117
5 RB	0.702	6782	2018

Table 5. Mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), F_m (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Pak Choi treated with Red, Blue, and Green (RGB) light from a Dyna LED light source. The measurements were performed on six plants over a period of five days.

Day and treatment	F_v/F_m	F_m	F_0
1 RGB	0.766	6779	1588
2 RGB	0.778	6784	1496
3 RGB	0.776	6784	1525
4 RGB	0.773	6781	1540
5 RGB	0.780	6778	1491

Table 6. Mean values of chlorophyll fluorescence measurements parameters, including F_v/F_m (maximal quantum yield of PSII), F_m (maximal fluorescence in darkness) and F_0 (basic fluorescence) for Pak choi treated with Red and Blue (RB) light from a Dyna LED light source. The measurements were performed on six plants over a period of five days.

Day and treatment	**F_v/F_m**	**F_m**	**F_0**
1 RB	0.752	6787	1686
2 RB	0.761	6785	1622
3 RB	0.779	6786	1503
4 RB	0.783	6786	1473
5 RB	0.789	6768	1432

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