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Effect of Red to Far-red ratio on assimilation, growth, and morphology of two cucumber cultivars (*Cucumis sativus* cv. Hi light and Imea)

Shailaja Thapa
MSc. in Plant Sciences – Plant Production Systems

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Supervised by Prof. Sissel Torre and Dr. Sheona Noemi Innes

Norwegian University of Life Sciences (NMBU)

Faculty of Biosciences

Department of Plant Sciences

P.O. Box 5003

1432, Ås,

Norway

Preface and Acknowledgements

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Abstract

Far-red (FR) light (700-800nm) affects the assimilation, morphological development, and growth of plants. However, FR light is either excluded or used in limited quantity in lamps used for plant production, including light emitting diodes (LEDs). Additionally, the effect of FR light varies with species and cultivars. In this study, the effect of three different red (R) to far-red (R:FR) ratios; 10.0, 5.0 and 1.7 on two cultivars of cucumber; 'Hi Light' and 'Imea' were investigated in a controlled environment in a growing period of 30 days. The results on photosynthetic efficiency, morphological characteristics, growth, and carbohydrate accumulation in leaves and fruits were analyzed by comparing three R:FR ratios in both cultivars. In addition to R:FR ratio and cultivars, carbohydrate accumulation was observed at two different time points; at the end of the day, and end of the night.

Decreased R:FR ratio increased the assimilation of carbon-dioxide (CO_2) even though a decrease in chlorophyll pigment concentration was found. Relative growth rate (RGR) also increased with an increase in FR proportion, and this was attributed to an increase in net assimilation rate (NAR), though decreased leaf area ratio (LAR), specific leaf area (SLA) and leaf mass ratio (LMR) was observed. Increased extension growth of plant height, internode length, petiole length, and individual leaf area were evident in treatments with lower R:FR ratios. No significant difference was found in leaf number per day and fruit number due to variation in R:FR ratio. The assimilates like soluble sugars (sucrose, raffinose and stachyose) and starch were also found to increase with a decrease in R:FR ratio and were higher at the end of the day than at the end of the night in leaves and fruits. The 'Hi Light' and 'Imea' cultivars responded similarly to one another, except for some inconsistency in chlorophyll pigment, and soluble sugars like raffinose and stachyose in leaves and fruit. Overall, addition of FR and lowering R:FR ratio to a value close to natural light (R:FR ratio in natural sun light ≈1.0-1.2) improved light capturing capacity by increasing leaf area and promoted photosynthesis by maintaining the excitement level between two photosystems and accumulation of more assimilates in the source and fruit. In conclusion, adding FR-light improves the production potential of cucumber.

***Key words**: cucumber, R:FR ratio, controlled environment, net assimilation, morphology, growth, carbohydrate metabolism.*

List of Abbreviations

A_n	Net assimilation of CO_2
ANOVA	Analysis of Variance
Chl a/b	Ratio of Chlorophyll a to Chlorophyll b
C_i	Intercellular CO_2
CO_2	Carbon-dioxide
EOD	End of the Day
FR	Far-red
HPLC	High Pressure Liquid Chromatography
HPS	High Pressure Sodium
IRGA	Infra-red gas analyzer
LAR	Leaf Area Ratio
LEDs	Light Emitting Diodes
LMR	Leaf Mass Ratio
NAR	Net Assimilation Rate
PAR	Photosynthetically Active Radiation
PFD	Photon Flux Density
PPE	Phytochrome Photoequilibrium
PPFD	Photosynthetic Photon Flux Density
PSI	Photosystem I
PSII	Photosystem II
R	Red
R:FR	Red : Far-red
RGR	Relative Growth Rate
RH	Relative Humidity
SLA	Specific Leaf Area
SOD	Start of the Day

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1. Introduction

Plants convert light energy to chemical energy through photosynthesis, and photosynthesis is not possible without light. Thus, light is the basis of the food chain in plants. Additionally, light factors like light intensity, light quality and light duration (photoperiod) play a crucial role for regulating the development of the plant (Bhatla et al., 2018). Light intensity affects photosynthesis (Singh et al., 2015), and determines the light saturation points for different plant species (Moe et al., 2005). Flowering, among other developmental factors, is influenced by the photoperiod (Singh et al., 2015). Light quality, or spectral distribution, plays a vital role on morphology as well as metabolite accumulation in the plant (Kozai, 2016). Green light is less absorbed in comparison to red and blue light (Terashima et al., 2009), but the assimilation of CO_2 per quantum of light absorbed, called quantum yield, (Evans, 1987) of red light is higher followed by green and then blue light (Liu & Van Iersel, 2021). Blue light is essential for chlorophyll and chloroplast development, and circadian cycle activation (Ménard et al., 2005), while red and FR light stimulates the phytochrome status (Sager et al., 1988), influencing the development of stem and leaf (Procko et al., 2014; Tan et al., 2022), germination (Contreras et al., 2009), and flower initiation or development (Runkle & Heins, 2001). However, the responses to light quality, and specifically R:FR ratio, are species and genotype-dependent. Therefore, it is essential to have light recipes specific to the crop and cultivars.

In Northern latitudes, there is limited natural light especially during autumn, winter, and early spring (Gajc-Wolska et al., 2021). In Norway, there is huge variation of daylength across season and location, and it is not possible to produce plants year-round under natural light (Moe et al., 2005). To avoid these limitations, artificial light sources are being used. Yet, it is also essential that the artificial light used should be economically feasible for production and environmentally friendly to minimize the carbon footprint (Särkkä et al., 2017). Amongst artificial lighting sources, light-emitting diodes (LEDs) is gaining popularity above fluorescent, high-pressure sodium (HPS) lamps and other type of lamps because of comparatively higher electricity to light conversion feature, cost effectiveness and longer lifespan (Kozai, 2016; Lee et al., 2015). More importantly, the luminous efficiency of LEDs is high, and the spectral distribution of LEDs can be customized based on the requirement of the plant (Dutta Gupta & Agarwal, 2017; Kozai, 2016). The suitability of LEDs over other artificial light lamps has caused scientists to consider whether they can be used solely or as an additional source of light for survivability of life such as crop production in space as well (Massa et al., 2008).

Cucumber is grown worldwide and has great economic value (Ji, F. et al., 2020). Because of high demand, off-season production in greenhouses and controlled environments is also expanding in many countries including Norway. Cucumber requires high light intensity (Badgery-Parker et al., 2015). Among conventional lamps, HPS lamps have more luminous efficiency (Dutta Gupta & Agarwal, 2017) and provide intensity requirements for cucumber production. Therefore, at Northern latitudes, HPS lamps are often being used as supplemental light for greenhouse cucumber production when the daily light integral from sun is low (Särkkä et al., 2017). Over time, LEDs are also being used for cucumber production either as a sole or supplemental source of light and as interlighting.

Yet, the LEDs used in horticultural production mainly use the red and blue spectrum which increases the photosynthetic efficiency but differs greatly from the spectrum of natural light (Van Ieperen et al., 2012), or mostly with 75-85% of red light (Runkle, 2016). LEDs, especially white LEDs used in commercial production, have a high R:FR ratio (approximately 10.0), HPS lamps have a R:FR ratio of 3.8, whereas natural sunlight only has around 1.1-1.2 R:FR ratio during daytime. This shows the gap between the natural and controlled conditions for production.

Differences were found in the growth component of tomato genotypes in response to FR, (Ji et al., 2021) which suggests that response to R:FR ratio might vary with genotype. 'Hi Light' is a new cucumber cultivar developed to be more responsive to artificial lighting, especially LEDs whereas 'Imea' is a well-recognized, older cultivar preferred for commercial greenhouse production. Screening of different cucumber cultivars at Særheim research station (NIBIO, Norway) showed that 'Hi Light' and 'Imea' responded differently in different lamp types (pers. com. Henk Maessen). More importantly, the effect of R:FR ratio across different cucumber genotypes is less studied.

The objective of this thesis was to study the effect of different R:FR ratios: 10.0, 5.0 and 1.7 on photosynthetic efficiency, changes in morphology and growth components, and assimilates of two important commercial cucumber cultivars ('Hi Light' and 'Imea') in a controlled environment. The specific aims were to compare:

1. The effect of different R:FR ratio on photosynthetic efficiency, and content of pigments in leaves and fruits

2. The effect of different R:FR ratios on morphological characteristics and fruit development
3. The effect of different R:FR ratios on relative growth rates, other growth components and dry matter distribution
4. How different R:FR ratios affect the accumulation of soluble sugars and starch in leaves and fruits at the start and end of the photoperiod.

2. Background

2.1 Light, a basis for life

Light has a property of particles and wavelength. The light energy is delivered in a particle called photons, and the energy of a photon (E) equals to h*ν where h is Planck's constant (6.62 * 10^{-34} J s) and ν is the frequency of light. The frequency (ν) equals to c/λ, where c is the speed of light (3.0 * 10^{8} m s^{-1}) and λ is wavelength of light. Because of this relation, the longer the wavelength the lower the energy (Taiz et al., 2015).

The energy from light is used to take electrons from water to produce Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and Adenosine Triphosphate (ATP) (**Figure 2.1**). Oxygen (O_2) is produced as a byproduct and released into the environment. To be more detailed, photosynthetic pigments like chlorophylls, and carotenoids are found in the light harvesting complexes in reaction centers of photosystem I (PSI) and photosystem II (PSII). The light energy absorbed by photosynthetic pigments in PSII are used for splitting water which releases electrons. The electron passes into the electron transfer chain where ATP is generated. The light energy absorbed in PSI, in addition to light energy absorbed in PSII and passed through the Cytochrome b6f complex (Cytb6f) to PSI reduces ferredoxin (Fd, **Figure 2.1**), ultimately producing NADPH (Asmelash, 2021; Bhatla et al., 2018). NADPH is a highly reducing compound and ATP is a high energy compound, and they are further used in the Calvin- Benson cycle for fixing carbon-dioxide (CO_2) and production of carbohydrate (Willey, 2015) as shown in **Figure 2.1**. Plants can survive and reproduce with this accumulated carbohydrate which is also the source of food for humans and others. Thus, the lives of most living beings on earth depend on this process either for breathing or for eating.

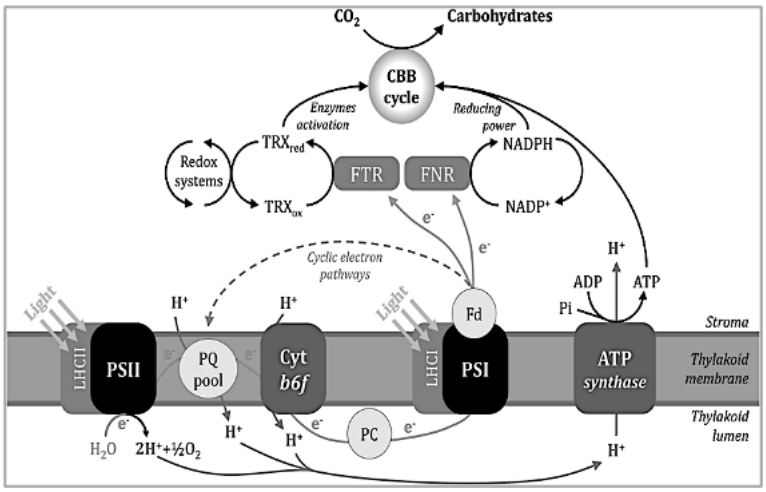

Figure 2.1. Photosynthetic electron transfer chain in thylakoid membrane and fixation of CO_2 in Calvin-Benson cycle for carbohydrate production (Lima-Melo et al., 2021).

2.2 Source of light

2.2.1 Natural source

The sun emits solar radiation over a wide range of spectral illumination, called the electromagnetic spectrum. However, the part of the spectrum from the sun that reaches the Earth's atmosphere (**Figure 2.2 (A)**) is mainly categorized into three ranges; a) ultraviolet (UV), b) visible, and c) infra-red (Singh et al., 2015). These three ranges are further classified into ultraviolet C (UVC) from 200-280 nm, ultraviolet B (UVB) from 280-320 nm, and ultraviolet A (UVA) from 320-400 nm (Moan, 2001), blue light from 400-500 nm, green light from 500-600 nm, red light from 600-700 nm, far-red light from 700-800 nm (Särkkä et al., 2017) and infrared from 800 nm onwards (Holmes et al., 1986).

Not all solar radiation reaches the Earth's surface, as shown in **Figure 2.2(B)**. Around 30% of solar radiation is reflected back to space whereas 70% reaches the earth. Of this proportion, UV radiation is around 6%, visible radiation is around 50% and infra-red radiation is around 40% at sea level (Moan, 2001). Most of the infra-red is absorbed by the water and oxygen molecules in the atmosphere and UVC and most of the UVB is absorbed by the ozone layer (Franklin & Whitelam, 2007). In normal days, the R:FR ratio (proportion of photon fluence rate in a 10 nm band centered on 660 to photon fluence rate in a 10 nm band centered on 730nm) from direct or diffused sunlight is 1.15. However, during dusk and dawn, the elevation of light beam decreases less than 10°C resulting in a lower R:FR ratio (0.7) (Smith, 1982; Smth & Holmes, 1977).

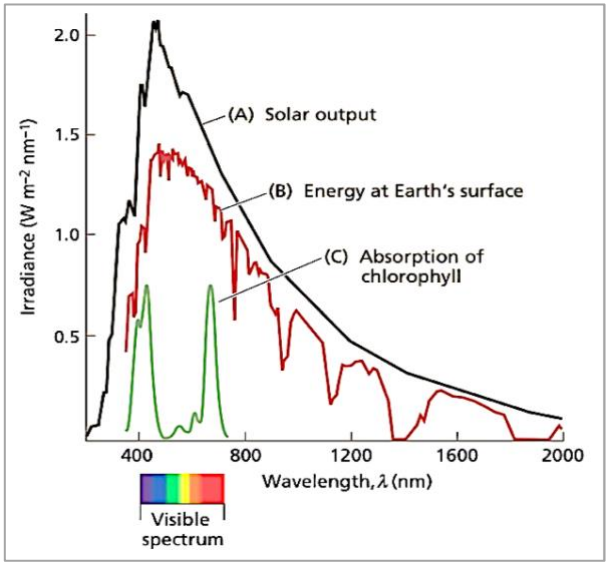

Figure 2.2. Distribution of spectrum from sun (A), energy at the earth's surface (B) and wavelength absorbed by plant (C) (Taiz et al., 2015).

Fluctuation in intensity and spectrum of natural light over seasons directly affects the absorption of photosynthetic pigments and causes an unbalancing of the photosystem excitation, resulting in lower photosynthetic quantum yield (Hogewoning et al., 2012). This

shortcoming of natural light in the commercial production of plants is being overcome by greenhouse and controlled environment production with artificial lighting.

2.2.2 Artificial sources of light

The use of artificial lighting for plant growth was recorded in the 1860s, but commercially started for production in the early twentieth century (Pinho & Halonen, 2017). The lamps used for the plant lighting included incandescent types like incandescent lamps (ILs), and gas discharge types (GDLs) like fluorescent lamps (FLs), high pressure mercury lamps (HPMLs), high pressure sodium (HPSLs) and metal-halide lamps (MHLs). However, the use of more electric energy, high release of heat energy and comparatively short lifespan made GDLs less cost effective in controlled environment production (Dutta Gupta & Agarwal, 2017). Among GDLs, HPS lamps are considered electrically more efficient and have wider spectrum of emission (Dutta Gupta & Agarwal, 2017).

In recent years, the use of LEDs solely or in combination with other types of light has been used in crop production. One of the key features of LEDs is low production of radiant heat. Most of the LED's armatures are designed in a way to transmit the light downwards to the plant canopy, whereas the heat radiation is passed upwards (Särkkä et al., 2017). Because of this, LEDs can be used closely to the plant canopy, saving energy as well as space. Other advantages of LEDs are their flexibility and controllability. The light spectra can be manipulated based on the absorption spectra of the plants, and the light intensity can be accurately changed (van Iersel, 2017). Therefore, manipulating the spectra of LEDs based on plant specific requirement of light spectra will aid production both in quantity and quality (Kim et al., 2005).

2.3 Plant responses to light

Light is either used as a source of energy or for signaling in the plant. The responses to light in plants are photosynthesis, photoperiodism, phototropism and photomorphogenesis. This thesis will mainly focus on photosynthesis and photomorphogenesis.

2.3.1 Photosynthetic efficiency

For photosynthesis mechanisms, light in the range of 400-700 nm wavelengths is known to be effective in plants, algae, and cyanobacteria. Hence, this range is known as the

photosynthetically active radiation (PAR) range (Zhen & Bugbee, 2020). In addition, the quantum yield of photosynthesis decreases rapidly with wavelengths shorter than 400 nm and longer than 680 nm, defining the range between 400 nm and 700 nm as the photosynthetic range (Evans, 1987).

Previously, Theodor Wilhelm Engelmann in 1883 conducted an experiment to test the photosynthetically efficient wavelengths of light by placing bacteria around algae. Here, his basic principle was the evolution of oxygen when a specific type of light drives photosynthesis. He found that with the use of red and violet light the bacteria moved toward the algae for oxygen (*Theodor Wilhelm Engelmann*, 2023). Because of this, it was believed that the most efficient light for photosynthesis belongs to only blue and red wavelengths.

Reflectance and transmittance from green wavelengths are higher with PAR range (Franklin & Whitelam, 2007) as shown in **Figure 2.3**. Though the absorptance is low with green wavelengths in comparison to blue and red, the photosynthetic quantum yield of green light is higher than blue light and comparable to red light if compared based on the absorbed light (Terashima et al., 2009). At low light levels, the photosynthetic efficiency of green light is lower. However, at higher light levels, blue and red light as a preferred light is immediately absorbed in the upper layer of the leaf whereas green light penetrates deep into the leaf tissue and increases photosynthetic efficiency by fixing more carbon (Sun et al., 1998). Additionally, green light also transmits to the leaves in lower canopy (Gitelson et al., 2022) which is again important for carbon fixation when the upper layer of leaves is saturated with the blue and red range of light.

Blue light is only 70-75% photosynthetically efficient in comparison to red light (Singh et al., 2015). The lower quantum yield of blue light might be because it is absorbed by the flavonoids, carotenoids, or both. The energy absorbed by carotenoids is transferred to either chlorophyll a or chlorophyll b, and the quantum yield of energy transferred varies greatly based on which excitation stage of carotenoid is involved or its location in photosynthetic apparatus (Croce et al., 2001; de Weerd et al., 2003a; de Weerd et al., 2003b). In addition, the light absorbed in blue light and green light range by non-photosynthetic pigments like flavonoids and carotenoids are not passed to the reaction center (Hogewoning et al., 2012). These findings indicate that spectrum from PAR range including green wavelengths is essential for photosynthesis though some difference in photosynthetic efficiency is evident.

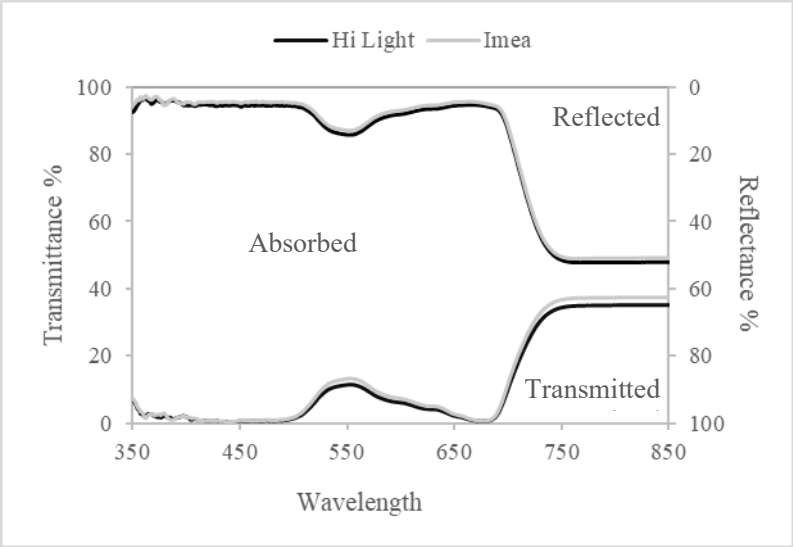

Figure 2.3. Reflectance, absorptance and transmittance recorded on fully grown young cucumber leaves of three weeks old 'Hi Light' and 'Imea' (Solhaug et al., 2023).

2.3.2 Photosynthesis measurement

Portable infra-red gas analyzers (IRGAs) are available and feasible to use for measuring the instantaneous assimilation of CO_2 (A), intercellular CO_2 (C_i), stomatal conductance of leaf (g_s), transpiration rate (E) and many other parameters (Long et al., 1996). The main objective of observing gas exchange in the leaf will always be the photosynthetic efficiency, and, the assimilation of CO_2 is mostly studied against the light incidence or intercellular CO_2 (Long & Bernacchi, 2003). By measuring across a range of CO_2 concentrations, the relationship between A and C_i can be identified based on mesophyll processes, eliminating the effect of boundary layer and stomata (Long & Bernacchi, 2003).

2.3.3 The red drop and Emerson enhancement effect

Emerson and Lewis (1943) measured the quantum yield of light absorbed which was constant throughout the photosynthetic range but decreased drastically from wavelength 680nm as shown in **Figure 2.4A**. This red drop is not because of the low chlorophyll absorption but because of the less efficient longer wavelengths. However, with the combined beam of R and FR, the photosynthetic efficiency was found to be higher than under the individual R or FR beams. This is known as the Emerson enhancement effect (Emerson & Rabinowitch, 1960) as shown in **Figure 2.4B**.

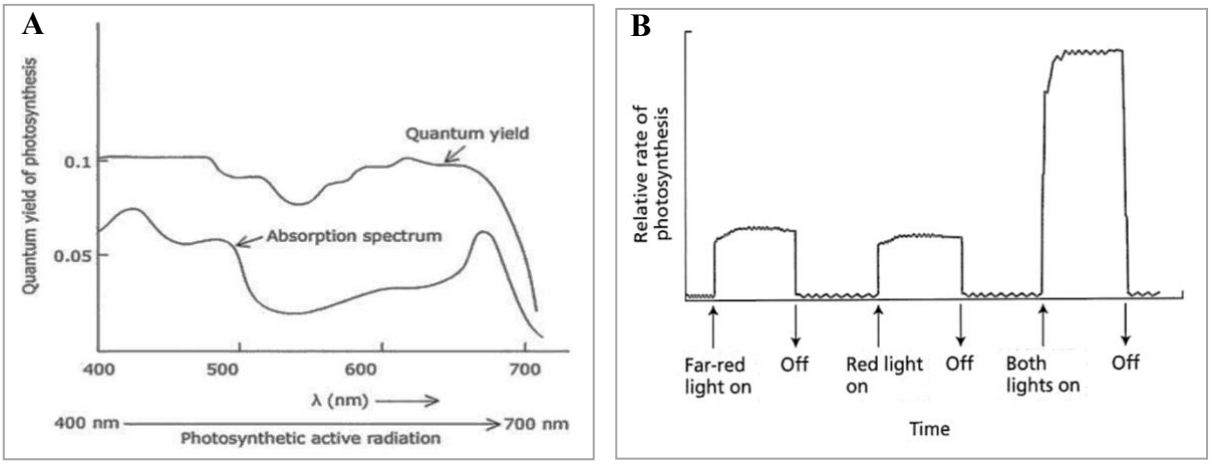

Figure 2.4. Emerson red drop effect (A) (Bhatla et al., 2018) and enhancement effect on photosynthesis (B) (Asmelash, 2021).

2.3.4 Far-red light and photosynthesis

PSI and PSII mainly absorb light in approximately 700 and 680 nm, respectively, which is why they are known as P700 and P680 (Barber & Archer, 2001; Taiz et al., 2015). So, PSII prefers shorter wavelength and PSI absorbs from comparatively longer wavelength. When these photosystems receive energy from light, they are excited based on acclimation to light quality, and over-excitement of one photosystem limits the photochemical reactions (Zhen et al., 2019).

The coefficient of canopy absorption of the FR range is 7-10% of green or red region's coefficient of absorption (Gitelson et al., 2022) which is not negligible. Addition of FR to red/blue light increased the quantum yield of photosystem II in lettuce slightly more than the addition of FR to warm-white light in lettuce, so Zhen and Van Iersel (2017) thought this might be because of some FR light present in white light. Moreover, light from the range of red/blue or warm-white might have caused PSI to be under-excited resulting in less photochemical reaction and eventually lower fixation of CO_2 (Zhen & Van Iersel, 2017). Zhen and Bugbee (2020) also mentioned that the photosynthetic range from solar radiation excites mainly PSII, so availability of FR light is most likely to stabilize the excitation level between PSI and PSII.

When FR light was added to the red/blue and warm-white light, the average increase in net photosynthesis was 4% and 3% respectively per 1% PPFD increment (Zhen & Van Iersel, 2017). Similarly, in Waldmann´s dark green cultivar of lettuce, adding FR light as 10-35% of white light (400 µmol $m^{-2}s^{-1}$; 400-700 nm) increased gross photosynthesis by 6.7-20% while

adding the same percentage to white light also improved gross photosynthesis by 6.7-21%, suggesting the efficiency of photons of FR and white wavelength are similar (Zhen & Bugbee, 2020). In the same study, the consistency of effect of FR light across 16 cultivars of 12 C_3 species, including cucumber cv. Straight eight, and two C_4 species was found. These results from different studies show that addition of FR light to other light adds photosynthetic values.

2.3.5 Photomorphogenesis

Changes in spectral distribution, direction and duration of light are detected via the photoreceptors in plants (Franklin & Whitelam, 2007; Teixeira, 2020). Receptors are specific to wavelengths *i.e.*, UVB radiation receptor (UVR8) in 290-320nm, blue light receptors (cryptochromes, phototropins, zeitlupe) in 350-500nm, red light receptor (phytochrome) in 620-700nm and far-red light receptor (phytochrome) in 710-850nm (Taiz et al., 2015). These receptors help plants to respond to different light quality from UV-B to the far-red range (Kong & Okajima, 2016). Cryptochromes are UVA and blue light receptors (Mishra & Khurana, 2017), whereas UVB is perceived by UV resistance locus 8 (UVR8) in the plant (Wang et al., 2017). The exposure to UVB light suppressed the hypocotyl length in cucumber and tomato seedling (Barnes et al., 1996), and inhibited apical dominance by destructing auxin in cassava (Ziska et al., 1993). In addition to cryptochromes, phototropins are also photoreceptors of blue and UVA light (Jedynak et al., 2013). Blue light reduced internode length in cucumber and tomato (Ménard et al., 2005), and reduced leaf width and length and size of lettuce plant (Kang et al., 2016). No specific green light receptors are known but cytochrome, carotenoids, some chlorophylls, and other pigments can absorb the green light (Golovatskaya & Karnachuk, 2015). The proportion of 24% green light with red and blue light increased growth of lettuce, and the production of lettuce in combination with red, blue and green light seemed more aesthetic (Kang et al., 2016). These studies illuminate that, with reception of different light types, plants develop differently.

2.3.6 Photo-reversibility and phytochrome photoequilibrium

The major feature of phytochrome is photo-reversibility, which is also known as photoconversion or photochromism. The active and FR absorbing isomer P_{fr} and inactive R light absorbing isomer is P_r (Bhatla et al., 2018; Lee et al., 2015). However, only 88% of P_r can be converted to P_{fr} when provided with saturating R light as there is overlapping of

absorption spectra of P_r and P_{fr}. Not all P_{fr} can be converted back to P_r as some FR light is absorbed by P_{fr}, which creates an equilibrium known as the photostationary state (Bhatla et al., 2018). Availability of R to FR ratio to the plants is assessed with photo-reversible phytochrome (Bhatla et al., 2018; Franklin & Whitelam, 2007), and the active phytochrome to total phytochrome ratio is known as phytochrome photostationary state (PPS) or phytochrome photoequilibrium (PPE) (Kusuma & Bugbee, 2021).

When the light is absorbed by the leaf, it is reflected, refracted, and diffracted inside the leaf, causing a higher intensity of photons in the epidermis layer (Seyfried & Fukshansky, 1983) and the scattering of photons within a leaf is shown in **Figure 2.5** (Kusuma & Bugbee, 2021). So, multiplication of spectral distortion (**Figure 2.6A**) with the phytochrome conversion (**Figure 2.6B**) enhances the prediction of PPE by assuming equal distribution of photons in the whole leaf.

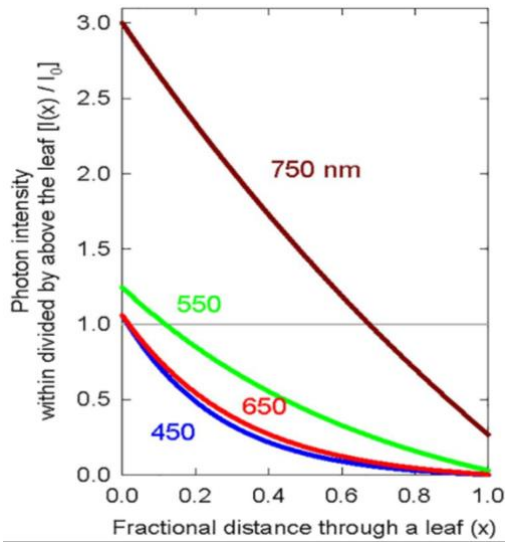

Figure 2.5. Photon intensity at the depth of leaf from blue, green, red and far-red range (Kusuma & Bugbee, 2021).

In addition, the lighting in a controlled environment has specific wavelength ratios which strengthens the predictive ability of PPE (Kusuma & Bugbee, 2021). With the variation in photons from R and FR wavelengths, the response of morphology is predicted effectively (Kusuma & Bugbee, 2021) and usually in inverse linear relationship (Kalaitzoglou et al., 2019) whereas other plant developmental responses to R:FR vary based on regulated phytochrome and its responses, making the prediction unreliable (Park & Runkle, 2017).

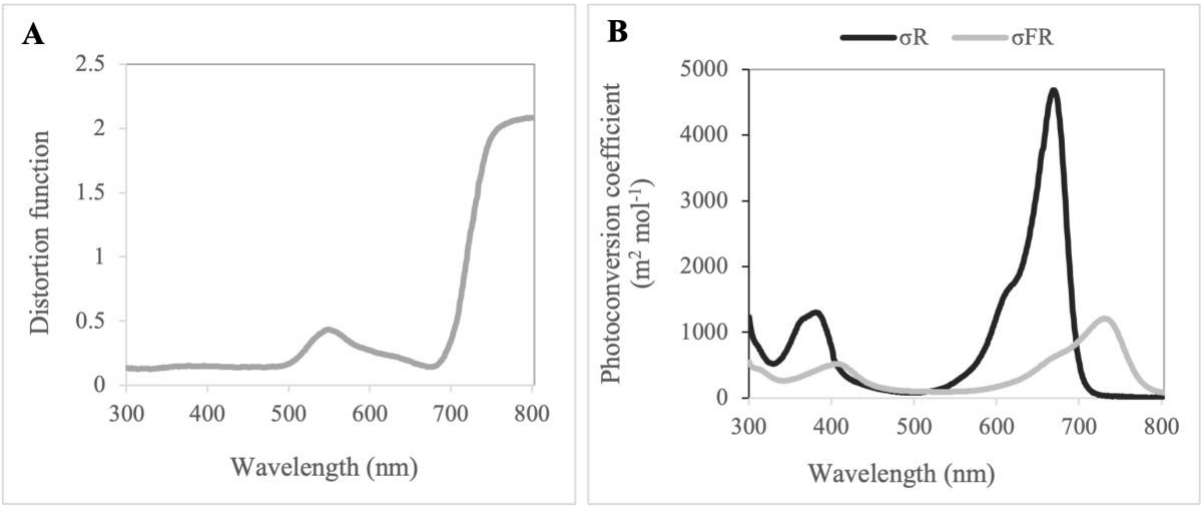

Figure 2.6. Distortion function to assume homogeneous distribution of phytochrome (A) from Kazarinova-Fukshansky et al. (1985) and photoconversion coefficient (B) from Lagarias et al. (1987) used in Kusuma and Bugbee (2021). In figure B, σR are photoconversion coefficient for converting P_r to P_{fr} and σFR are photoconversion coefficient for converting P_{fr} to P_r.

2.3.7 Far-red light and photomorphogenesis

R and FR light are sensed by phytochrome, so changes in R and FR light changes the phytochrome status. Photomorphogenesis is regulated by the active form of phytochromes, by their translocation from cytoplasm to nucleus and regulation of related genes (Paradiso & Proietti, 2022). Phytochrome is involved in controlling germination, flowering, and photosynthetic apparatus development-related phenomena in the plant (Sager et al., 1988). With an increase in R:FR ratio the active state of phytochrome P_{fr} also increases (Franklin & Whitelam, 2007), repressing the extension of shoot as well as decreasing the dry biomass of plant as mentioned in Shibuya et al. (2012). Phytochromes available in internodes as well as leaves can perceive FR light, and in white mustard, the FR light absorbed in stem led to immediate response (Morgan et al., 1980).

High-density planting or proximity of neighbor plants decreases the R:FR which warns plants about the closeness of competitors (Franklin & Whitelam, 2007) and to outcompete the competitors for harvesting sufficient light, the stem and hypocotyl are extended. This phenomenon is also known as shade avoidance syndrome (Procko et al., 2014; Tao et al., 2008). Studies have found shade avoidance syndrome is expressed as a elongation growth with biosynthesis of auxin through TAA1 pathways in *Arabidopsis* (Tao et al., 2008). Supplementary FR light increased fresh biomass, dry biomass, plant height, length of leaf and

width of leaf by 28%, 15%, 14%, 44% and 15% respectively in comparison to white light in baby leaf lettuce (Li & Kubota, 2009).

FR light in addition to red light promotes flowering in many species belonging to the long day group (Runkle, 2016). Deficiency of FR delayed visible bud initiation in *C. grandiflora* by 14 days, delayed flowering in *V. xwittrockiana* by 21 days, but increased flowering by 44% in *L. speciosa* (Runkle & Heins, 2001). Lettuce seeds produced under low R:FR ratio were found 5% heavier than those produced under higher R:FR ratio, but seeds from both light treatments had similar germination percentage under normal conditions (Contreras et al., 2009). However, in sub-optimal conditions, the germination of seed produced under high red-light proportion was found higher (Contreras et al., 2009). Yet, with high density planting, low R:FR ratio initiates secondary dormancy in seeds as a shade response regulated by phytochrome, (Smith & Whitelam, 1997). FR light influences the morphology, germination, and flowering in the plants, but the effects might vary based on the species.

2.4 Nutritional values and production of cucumber

2.4.1 Nutritional values of cucumber fruit

Cucumber is one of the most popular vegetable fruits and normally consumed fresh. With more than 95% water in fruit, it helps to maintain hydration in the body. Additionally, cucumber fruit contains fiber, vitamin A, C, and K, and vitamin K promotes the absorption of calcium (Mallick, 2022). The cucurbitacin in fruit helps to reduce high blood pressure by reducing sodium and promoting potassium intake, maintain blood sugar, prevent cancer, and have other anti-inflammatory values (Mallick, 2022). Some of the important nutrients present in cucumber are provided in **Table 2.1**.

Table 2.1. Nutrient content in 100 g raw cucumber fruit with peel, *Source. FoodData Central, USDA, Agricultural Research Service, 2022.*

Contents	Amount	Contents	Amount
Water	95.9 g	**Magnesium**	10.1 mg
Energy	14-16 kcal	**Phosphorus**	23 mg
Ash	0.38 g	**Potassium**	170 mg
Protein	0.62 g	**Sodium**	2 mg
Nitrogen	0.1 g	**Zinc**	0.2 mg
Total Fat	0.62 g	**Copper**	0.063 mg
Carbohydrates	2.95 g	**Manganese**	0.085 mg
Calcium	16 mg	**Molybdenum**	0.085 mg
Iron	<0.25 mg	**Biotin**	0.962 µg

2.4.2 Growing conditions

The cucumber is believed to have originated from the warm and humid area of southern Asia and grows well when combined with high temperature, relative humidity, light intensity and optimal nutrient and water supply. As a warmth-loving crop, cucumber is susceptible to low temperature stress and thrives well in temperatures higher than 20°C, however, exposure of plants to heat stress during reproductive stage makes the fruits bitter (Singh et al., 2017).

Yet, yield potential in year-round cultivation in open fields is not guaranteed and in some cold locations the plants will not even survive to yield. With a protective cultivation system, like plastic tunnels, greenhouses, or controlled environments, the favorable environment for year-round production can be maintained (Singh et al., 2017). The first greenhouse built for cucumber production was found evident during ancient Roman times (Badgery-Parker et al., 2015).

For greenhouse production, Moe et al. (2005) recommended 250 µmol m^{-2} d^{-1} photosynthetic photon flux (PPF) with a photoperiod of 20 h d^{-1} for propagation and 300 µmol m^{-2} d^{-1} with 20-22 h d^{-1} for cultivation. A temperature between 27-28 °C is optimum for germination of seeds, and after germination, it can be decreased by 3-4 °C (Badgery-Parker et al., 2015). The optimal relative humidity is 75-80%. Increase in CO_2 concentration increases the cucumber production by increasing the fruit number and size in comparison to ambient levels (Kläring et al., 2007).

The yield of cucumber grown with 700 µmol m^{-1} CO_2 increased first cycle yield by 18.4% in comparison to growth conditions with 345 µmol m^{-1} CO_2 (Parra et al., 2000). For germination, the salinity and pH should be maintained around 2.0-2.5 and 5.0-5.5 mS/cm, whereas for production it can be maintained at 3.0 and 5.5-6.0 mS/cm respectively.

2.4.3 Response to different light source and light quality

The spectral distribution affects the growth and developmental process in cucumber plants. The addition of green light by 24% to red and blue light increased the height of cucumber hybrid 'Mandy F1', whereas UV light decreased the height (Brazaitytė et al., 2009). Increase in proportion of red to blue (R:B) light increased the plant height, leaf length, leaf width, stem diameter, and weight of shoot when compared between 9:1, 7:3, 5:5, 3:7 and 1:9 R:B ratios in cucumber (Jin et al., 2023). Addition of blue light to HPS for daily light integral increased the yield of cucumber by 30%, but decreased the internode length, (Ménard et al., 2005). Combination or supplementation of different spectral range affects the development of plants and production of fruits.

The use of HPS lamps in greenhouses is very common in Nordic countries when the daily light integral from sun is low in unclear summer days, fall and winter, (Särkkä et al., 2017). LEDs are also being used in cucumber production for top or interlighting. The fruit fresh weight per mol PAR (g mol^{-1}) was found to be higher under a combination of LED-LED as a top and inter-light in comparison to combination of HPS-LED and HPS-HPS but during mid-winter, lower leaf temperature and light level led to extension of leaf and stem, causing low flowering and increased fruit abortion. However, in the same study, the combination of HPS as top light and LED as inter-light lamp was found to produce the highest yield in cucumber cv. Toploader (Särkkä et al., 2017). Use of green LEDs in addition to HPS lamps as a source of green photons enhanced the inflorescence development (Novickovas et al., 2010). By using LEDs as supplementary lighting to HPS lamps, the shortcomings of HPS lamps are being attenuated.

2.4.4 Assimilates and translocation in cucumber

The assimilates production in the leaf of cucumber is stimulated by the sink strength in cucumber fruit, resulting in more carbon assimilation in the fruiting plant (Barrett & Amling, 1978). Carbohydrates are mainly translocated in the form of stachyose in cucumber but might also be as a sucrose or raffinose (Weidner, 1964). Galactose is the precursor of stachyose as

shown in **Figure 2.7**. However, stachyose can also be converted back to raffinose or galactose and galactose can convert to glucose or sucrose (Gross & Pharr, 1982). In pickling cucumber, sucrose was found in large amount in peduncle extracts, stachyose and an unknown compound between stachyose and raffinose was found in midget-sized fruit samples, whereas in all fruit samples, glucose and fructose were strongly detected (Pharr et al., 1977).

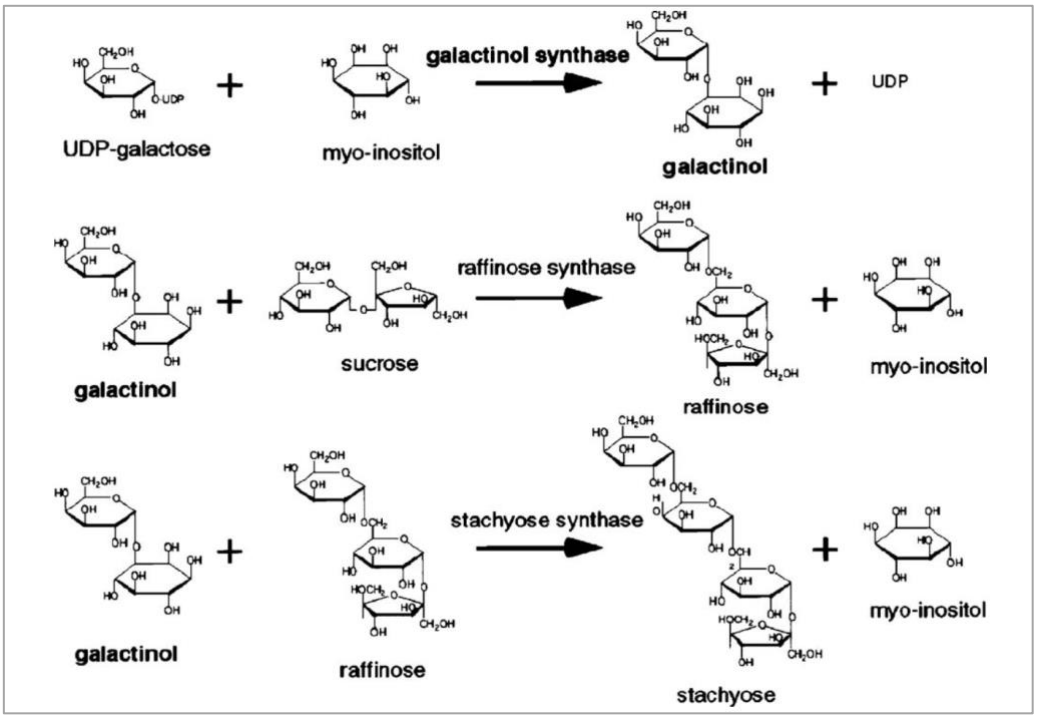

Figure 2.7. Pathway for biosynthesis of raffinose and stachyose (Taji et al., 2002).

Additionally, cucumber fruit is also able to produce assimilates for themselves. The green coloration in fruit of cucumber contains photosynthetic pigments. Darker skin color contains more chlorophyll available mainly in exocarp (Sui et al., 2017). Maximum and steady-state quantum yield of PSII in the exocarp of fruits was found to be similar to a leaf up to nine days after anthesis, which suggests that the structure photochemical reaction might have established in fruit at the young stage (Sui et al., 2017). The intercellular space is tightly arranged in fruit which helps to prevent losing CO_2 during respiration, as well as refixing the internal CO_2, resulting in more accumulation of organic acid (Sui et al., 2017). Therefore, cucumber fruits are also able to contribute for photochemical activities.

3. Materials and Methods

3.1 Plant materials and nursery management

The cucumber (*Cucumis sativus* L.) seedlings were grown in the greenhouse at the Centre for Plant Research in Controlled Climate (SKP), at the Norwegian University of Life Sciences (NMBU, Ås, Norway). The seeds from two different cultivars 'Imea' and 'Hi Light' were sown in 3-liter pots filled with fertilized sphagnum peat medium (Veksttorv, Norgro AS, Lier, Norway). The electrical conductivity (EC) level and pH level of the growth media were 1.0 - 1.5 dSm^{-1} and 5.0 - 6.0 respectively. The growth conditions were maintained at 20°C and 65% relative air humidity (RH) by controlling the climate with PRIVA system (Priva, De Lier, Netherlands). The greenhouse had a glass ceiling and acrylic walls. A photoperiod of 18-hours was maintained with High Pressure Sodium (HPS, GAN 4-550 AL 400W, Gavita International, Rozenburg, The Netherland) and Quartz Metal Halide (HPI, Powerstar HQI-BT metal halide lamps, Ledvance GmbH, Garching, Germany) lamps. The seedlings were regularly watered with tap water. Ten days after seed sowing, 15 uniform plants of each cultivar were selected and transferred to the growth chamber.

3.2 Growth chamber conditions and management

A modified CONVIRON chamber (CONVIRON EUROPE LTD., Unit 1 Hall Barn Road Industrial Estate, UK) with controlled light, temperature and RH was used for growing plants in all experiments. The chamber was equipped with white LED lamps (EAX130 5000K WWW, EVOLYS®, Norway, **Figure 3.1A**), red LED lamps (EVOLYS®, Norway, **Figure 3.1B**) and far-red LED lamps (EVOLYS®, Norway, **Figure 3.1C** and LUMITRONIX® High-Performance LED- Technologies & Solutions, Germany, **Figure 3.2D**).

The growth chamber was set with a photosynthetic photon flux density (PPFD) of 400 µmol m^{-2} s^{-1} measured by a LI-COR spectrometer (LI-180, LI-COR Biosciences, NE, USA). A Skye SKR 110 Red/Far-Red Sensor with SKR100 Display Meter (Skye Instruments Ltd., Llandrindod Wells, Powys Wales, UK) was used to measure the red (660nm): far-red (730nm) (R:FR) ratio before transferring the plants into the growth chamber. Afterwards, a LI-COR 180 spectrometer was used to record the values for all light parameters: photon flux density (PFD, 380-780 nm), photosynthetic photon flux density (PPFD, 400-700 nm), photon flux density of UV (PFD-UV, 380-400 nm), photon flux density of blue light (PFD-B, 400-500 nm), photon

flux density of green light (PFD-G, 500-600 nm), photon flux density of red light (PFD-R, 600-700 nm), photon flux density of far-red light (PFD-FR, 700-780 nm) and red to far-red ratio (R:FR ratio, 600-700 : 700-780 nm) as shown in **Table 3.2.**

Figure 3.1. Light integrated in the CONVIRON growth chamber; white LEDs (A), red LED (B), and far-red LEDs (C and D) in the growth chamber.

The photoperiod was maintained at 16 hours day length and 8 hours of darkness. The climate inside the growth chamber was controlled using a PRIVA system. Average daily temperature and RH were maintained at 23°C and 75% respectively along with ambient CO_2 (400 ppm). The growth chamber settings were started a few days before transferring the plants to stabilize the chamber climate. Three experiments with different R:FR were conducted in the controlled growth chamber: 1) R:FR ratio 10.0, 2) R:FR ratio 5.0, and 3) R:FR ratio 1.7. The light spectrum for all experiments is shown in **Figure 3.3**.

The internal height of the growth chamber was 2.08 m with an area of 3.4 m^2 (2.46*1.38 m^2). Initially, 30 plants were placed in the chamber, with spacing of 0.11 m^2 per plant. After a week, 10 plants were harvested for initial growth analysis (see below) increasing space to 0.17 m^2. The plants were spaced so they never touched neighboring plants. A few plants were removed during the experimental period to main the distance between plants (**Table 3.1**).

Table 3.1. Plant spacing in the growth chamber throughout the experiment. DAT means days after light treatment. The values provided are in m^2.

Experiments	1-7 DAT	8-14 DAT	15-21 DAT	21-30 DAT
Exp. 1 (R:FR 10)	0.11	0.17	0.17	0.24
Exp. 2 (R:FR 5.0)	0.11	0.17	0.24	0.24
Exp. 3 (R:FR 1.7)	0.11	0.17	0.24	0.28

The fertigation was done with an equal mixture of YaraTera® Calcinit™ (15.5% N-14.4% NO_3 and 1.1% NH_4 and 19.0% Ca, Yara Norge AS, Oslo, Norway) and Kristalon™ Indigo (8.5% N-7.5% NO_3 and 1.0% NH_4, 4.95 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 AS, Oslo). The EC level of the fertilizer was maintained at 2.5 dSm^{-1} with 5.0 – 6-0 pH.

3.2.1 Experiment 1 (R:FR ratio 10.0)

In experiment 1, white LED lamps from EVOLYS® were used as top light in the chamber. The total PPFD was measured to be approximately 400 µmol m^{-2} s^{-1} and the R:FR ratio was measured to be 10.0. After the plants were moved to the growth chamber, the light was measured on the first day and every week (total five times) at fifteen spots over the growing period (**Figure 3.2**). The light was measured at the top of the canopy. Therefore, with the increase in the plant height the light intercepted by the plant canopy also increased. The mean distribution of light spectrum per wavelength is shown in **Figure 3.3**A and **Table 3.2**. The average temperature and RH is shown in **Figure 3.4** and **Table 3.2**.

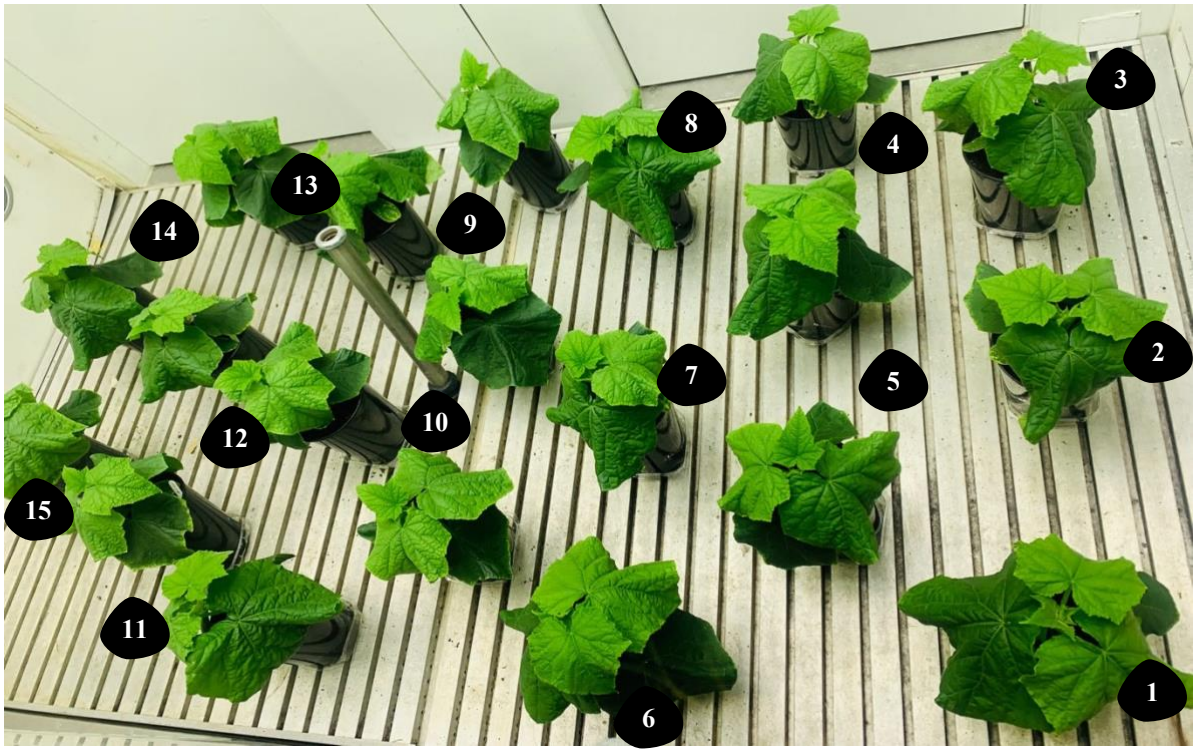

Figure 3.2. General layout of spots for measurement of light for all three experiments.

The PPE was also calculated based on all five light measurements and fifteen positions by modifying the wavelength range in equation 3.1 and using the method as mentioned in Kusuma and Bugbee (2021), the photoconversion coefficient from Lagarias et al. (1987), and spectral distortion from Kazarinova-Fukshansky et al. (1985);

$$PPE = \frac{P_{fr}}{P_{total}} = \frac{\sum_{\lambda=380nm}^{\lambda=7800nm} I_\lambda \sigma R,\lambda}{\sum_{\lambda=380nm}^{\lambda=780nm} I_\lambda \sigma R,\lambda + \sum_{\lambda=380nm}^{\lambda=780nm} I_\lambda \sigma FR,\lambda} \quad (3.1)$$

where, PPE is phytochrome photoequilibrium,

P_{fr} is the active form of phytochrome absorbing the far-red light,

P_{total} is sum of P_{fr} and P_r and P_r is inactive form of phytochrome absorbing red light,

I_λ is photon flux density at wavelength λ and multiplied by the spectral distortion factor before using in formula,

σR, λ is photoconversion coefficient for converting P_r to P_{fr} at wavelength λ and σR,

λ is photoconversion coefficient for converting P_{fr} to P_r at wavelength λ.

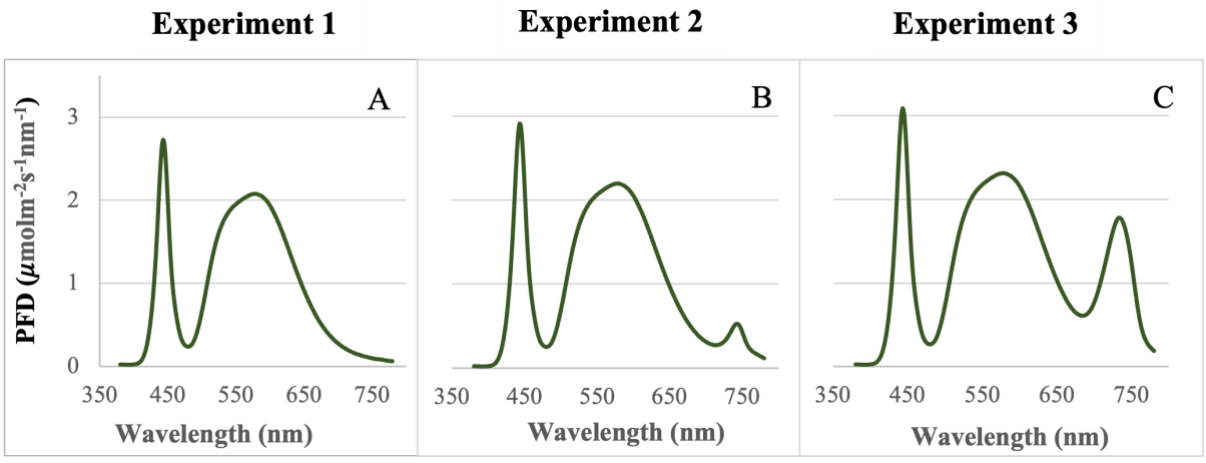

Figure 3.3. The light constituent at the top of plant canopy measured in three experiments. The wavelength and PFD per wavelength are provided in x-axis and y-axis respectively. A. spectral distribution of white LED used in experiment 1 (R:FR 10.0), B. spectral distribution of FR LED in combination with white LED from experiment 2 (R:FR 5.0), and C. spectral distribution of extra FR LED in addition to far-red and white LED from experiment 3 (R:FR 1.7).

Table 3.2. Light, temperature and humidity setup in experiment 1, experiment 2, and experiment 3. The value of average temperature. RH, photosynthetic photon flux density (PPFD), photon flux density (PFD, 380-780nm), photon flux density of UV (PFD-UV, 380-400nm), photon flux density of blue light (PFD-B, 400-500nm), photon flux density of green light (PFD-G, 500-600nm), photon flux density of red light (PFD-R, 600-700nm), and photon flux density of far-red light (PFD-FR, 600-780nm), daily light integral (DLI), ratio of red and far-red (R:FR ratio), and phytochrome photoequilibrium (PPE) measured at the top of plant canopy in all three experiments. Values are mean ± SEM.

Parameters	Experiment 1 (R:FR 10)	Experiment 2 (R:FR 5)	Experiment 3 (R:FR 1.7)
Temperature (°C)	23.1 ± 0.0	23.1 ± 0.0	22.9 ± 0.0
RH (%)	75.5 ± 0.0%	70.8 ± 0.1	76.0 ± 0.0
PFD (µmol m^{-2} s^{-1})	364.1 ± 18.6	401.0 ± 24.3	485 ± 50.7
PPFD (µmol m^{-2} s^{-1})	353.2 ± 18.0	375.6 ± 22.1	399.8 ± 38.2
PFD-UV (µmol m^{-2} s^{-1})	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1
PFD-B (µmol m^{-2} s^{-1})	78.0 ± 4.0	83.7 ± 5.0	87.7 ± 8.5
PFD-G (µmol m^{-2} s^{-1})	175.9 ± 9.0	186.5± 10.9	195.5± 18.4
PFD-R (µmol m^{-2} s^{-1})	99.3 ± 5.0	105.4 ± 6.1	116.6±11.4
PFD-FR (µmol m^{-2} s^{-1})	10.5 ± 0.6	25.0 ± 2.2	85.3 ± 12.5
DLI[*](µmol m^{-2} d^{-1})	20.35 ± 1.0	21.6 ± 1.3	23.0 ± 2.2
R:FR ratio[**]	9.5 ± 0.2	4.3 ±0.3	1.4 ± 0.1
PPE	0.72 ± 0.0	0.61 ± 0.0	0.39 ± 0.0

[*] *DLI was calculated as a mean of photosynthetic photon flux density (400-700nm) from different positions and times and light duration of 16 hours.*

[**] *R/FR value is recorded using the LI-COR 180 spectrometer which records the range of red from 600-700nm and far-red from 700-780nm.*

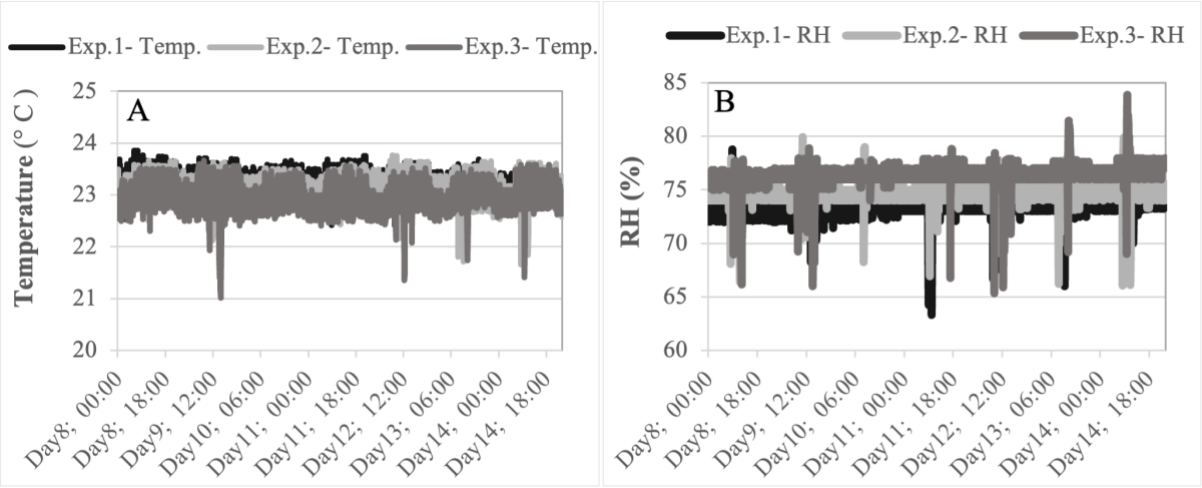

Figure 3.4. The relative air humidity (RH, %) (A) and Temperature (°C) (B) from growth chamber of experiment 1 (Exp.1), experiment 2 (Exp.2) and experiment 3 (Exp.3). The time interval is provided in day; hour: minute in x-axis and in y-axis, RH and temperature is plotted. The graph represents the data of second week, Day 8 (00:00) to Day 14 (24:55), recorded at 5-minute intervals.

3.2.2 Experiment 2 (R: FR ratio 5.0)

In experiment 2, the far-red LEDs (EVOLYS®, Norway) fitted to the growth chamber in addition to the white LEDs used in experiment 1 were turned on and used. The total PPFD was measured nearly approximately 400 µmol m^{-2} s^{-1} and R:FR ratio was measured as 5.0 in the chamber. After transferring the plants, the light was measured as described in experiment 1. The mean distribution of light spectrum per wavelength is shown in **Figure 3.3B** and **Table 3.2**. The average temperature and RH is shown in **Figure 3.4** and **Table 3.2**.

3.2.3 Experiment 3 (R:FR ratio 1.7)

In experiment 3, far-red LED from LUMITRONIX® were added to the existing white and far-red LED used in experiment 2. In the same way as experiments 1 and 2, total PPFD was measured approximately 400 µmol m^{-2} s^{-1} and R:FR ratio was measured as 1.7 in the chamber. After moving the plants into the chamber, the light was measured in the same way as the first and second experiments. The mean distribution of light spectrum per wavelength is shown in **Figure 3.3C** and **Table 3.2**. The average temperature and RH is shown in **Figure 3.4** and **Table 3.2**.

3.3 Leaf gas exchange measurement

After 20 days from transfer of the cucumber seedlings, the photosynthetic assimilation of CO_2 (A) as a function of the intercellular CO_2 (C_i) in the leaf was measured on the seventh or eighth leaf in experiment 1 and sixth or seventh leaf in experiment 2 and 3 in four plants per cultivar using a portable gas analyzer (LI-6400 XT Portable Photosynthesis System, LI-COR Biosciences, Lincoln, NE, USA). The leaf cuvette with transparent window connected to the gas analyzer used the light source from growth chamber. The flow rate and block temperature in the cuvette were maintained at 500 µmol s^{-1}, and 23°C. The measurements were recorded on leaf areas of 6 cm^2 at eight different levels of CO_2 concentration which was scripted firstly in decreasing and then increasing order as; 400, 300, 200, 100, 50, 400, 700, 1000, 1300 ppm. The average value of data recorded in 400 ppm was used for further calculations.

The measurement was started after the CO_2 level stabilized after placing the leaf in the cuvette. The minimum and maximum wait time was set 60 and 240 seconds, respectively followed by matching IRGAs before each change in concentration of CO_2. The measurement of values was

done with auto-program 'A-C_iCurve2'. For all measurements, the entire gas analyzer machine was placed inside the growth chamber maintaining average relative humidity at 65 ± 12% while taking the readings. The measurements were taken for two days regularly between six to eleven hours after the starting of the light period.

3.4 Chlorophyll and carotenoid extraction and calculations

On the last day of the experiment, two leaf discs from the seventh/eight leaf of three plants per cultivar were taken with a cork borer. The diameter of cork borer was 9 mm which extracted a leaf disc of approx. 0.64 cm^2. The same cork borer was used to take out two chunks of fruit developed on sixth/seventh node of three plants per cultivar. The skin of the fruit was sliced as thin as possible from the extracted chunk using a scalpel. The area of the fruit skin is assumed to be the same as the leaf.

The extracted leaf disks and fruit skins were immediately placed in 2 ml Eppendorf tubes with 1.5 ml of dimethyl-sulfoxide magnesium carbonate (DMSO $MgCO_3$) solution. The Eppendorf tube was placed in an ultrasonic water bath (VWR Ultrasonic Cleaner USC200TH, VWR International, Malaysia) at 60°C for 30 mins for leaf samples and additional 25 mins for fruits samples. Around 1 ml of solution was taken out and transferred to a cuvette (UV-Cuvette semi-micro, BRAND GMBH + CO KG, Germany). The cuvette was then placed in a spectrophotometer (UV-1800, Shimadzu, UV spectrophotometer) and the absorbance was read at 480, 649, 665 and 750 nm for carotenoids, chlorophyll b, chlorophyll a, and background noise respectively, using DMSO as a reference.

The background noise was deducted from the wavelength *i.e.,* 480, 649 and 665 nm, and chlorophyll a, chlorophyll b and carotenoid concentrations were calculated using the following equations (Wellburn, 1994),

$$C_a = 12.19A_{665} - 3.45A_{649} \quad (3.2)$$

$$C_b = 21.99A_{649} - 5.32A_{665} \quad (3.3)$$

$$C_{x+c} = \frac{(1000A_{480} - 2.14C_a - 70.16C_b}{220} \quad (3.4)$$

where, A_{665}, A_{649} and A_{480} are the absorbances at 665, 649 and 480 nm wavelength respectively after deducting the background noise (absorbance at 750 nm),

C_a is the concentration of Chlorophyll a,

C_b is the concentration of chlorophyll b, and

C_{x+c} is the concentration of carotenoids.

3.5 Growth analysis

The plant height (cm) and true leaf number were recorded on the first day after transferring the plant to the growth chamber and every seventh day afterwards. The plant height was measured from the soil surface to the shoot apical meristem. Only the unfolded leaf longer than 3 cm was counted as a true leaf as shown in **Figure 3.5**. After one week of light treatment, five plants from each cultivar were selected randomly and data were collected on plant height and leaf number. Leaves of the plants were separated without petiole and area was measured with an area meter (LI-3100 Area Meter, LI-COR Biosciences, Lincoln, NE, USA). Afterwards, fresh weight of the stem with petiole and leaves were measured separately and dried at 60°C for seven days. The dry weight of shoot and leaves were recorded.

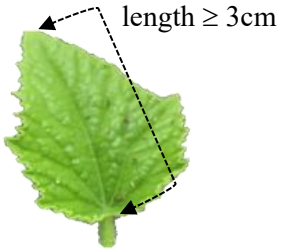

Figure 3.5. Measurement of leaf length

In addition, the date of first visible fruit and first flowers were noted. The number of visible fruits (fertilized and unfertilized fruits of all sizes) and number of fruits (fertilized and unfertilized fruits ≥1cm) and length of fruits from third internode were recorded every third day. The side-shoots were detached from the plants every third day and both dry and fresh weight were recorded. At the end of the experiment, three plants were randomly selected for each cultivar, and data were recorded on the plant height, leaf number, and leaf area. Individual leaf area was calculated by dividing total leaf area with number of leaves. The petiole length of 12 petioles from bottom of the stem was also measured and average petiole length was calculated. Fresh weight of stem, leaves, leaf petioles, fruits, fruit petioles were recorded and dried at 60°C for 10 days before measuring the dry weight.

Afterwards, the dried weight of five plants harvested after one week against dried weight of all three plants harvested at the end of the experiment were used for calculating the relative growth

rate (RGR, d^{-1}), net assimilation rate (NAR, g m^{-2} d^{-1}), leaf area ratio (LAR, cm^{-2} g^{-1}), specific leaf area (SLA, cm^{-2} g^{-1}) and leaf mass ratio (LMR, g g^{-1}) with the following equations by Shibuya et al. (2016) and Radford (1967) respectively;

$$RGR = \frac{InW_2 - InW_1}{t_2 - t_1} = NAR \times LAR \quad (3.5)$$

$$NAR = \frac{W_2 - W_1}{A_2 - A_1} \times \frac{InA_2 - InA_1}{t_2 - t_1} \quad (3.6)$$

$$LAR = \frac{A_2 - A_1}{InA_2 - InA_1} \times \frac{InW_2 - InW_1}{W_2 - W_1} = SLA \times LWR \quad (3.7)$$

$$SLA = \frac{A_2 - A_1}{InA_2 - InA_1} \times \frac{InL_2 - InL_1}{L_2 - L_1} \quad (3.8)$$

$$LMR = \frac{L_2 - L_1}{InL_2 - InL_1} \times \frac{InW_2 - InW_1}{W_2 - W_1} \quad (3.9)$$

where, W_1 and W_2 are the total dry mass of plant above soil surface at t_1 and t_2 which is seven days after the transfer of plant to the growth chamber and end of the experiment respectively,

A_1 is the initial leaf area measured after seven days in the growth chamber and A_2 is the final leaf area measured at the end of the experiment,

L_1 and L_2 are the leaf mass at seven days after light treatment and last day of the experiment.

3.6 Determination of carbohydrates

At the end of the experiment, the source leaf and fruits were collected from three plants of each cultivar. Single, fully matured leaves from the middle part of the plant (seventh to twelfth nodes) were collected at start of the day (SOD, 45 minutes after the starting of light period), and end of the day (EOD, 45 minutes before the starting of dark period). All fruits longer than 1 cm were collected at EOD. The collected leaves and fruit were immediately placed in liquid nitrogen to avoid carbohydrate breakdown. Afterwards, the samples were stored at -80°C.

The frozen samples were freeze dried using a freeze drier (LyoQuest -55 NO PLUS, Telstar LyoQuest Laboratory Freeze Drier, Spain). The fruit and leaf samples were kept for 9 days and 6 days respectively in the freeze drier with the temperature below -30°C and vacuum pressure ranging 0.2-0.3 mbar. After drying, the samples were ground as fine as possible using a mortar and pestle and stored at -80°C.

3.6.1 Analysis of soluble sugars by HPLC

Approximately 100 mg of ground dried leaf and fruit samples were weighed and placed in a 2ml Eppendorf tube. 1.5 ml of 80% ethanol was added to the tube followed by vortexing until the sample was homogenous. Then, the sample solution was placed in an ultrasonic bath at 70°C for 20 mins followed by centrifuging (Centrifuge 5417C, Eppendorf AG 22331, Hamburg, Germany) at 1500 rpm for 3 minutes. Afterwards, the supernatants were carefully collected into a separate centrifuge tube. This process was repeated twice more using 1.5 ml, followed by a final time using 0.5 ml to avoid any loss of sugars. Overall, 5 ml of supernatant was collected and placed in vacuum desiccator (Concentrator Plus, Eppendorf AG 22331, Hamburg, Germany) at 60°C until the ethanol was completely evaporated.

After the ethanol was completely evaporated, 1 ml of distilled water was added to the dried extract and the tubes were placed in an ultrasound water bath at 70°C for 20 mins followed by vortexing. Then the solution was transferred to new Eppendorf tubes and centrifuged at 1500 rpm for 3 minutes. The supernatant was then diluted with distilled water at 1:1 ratio (0.4 ml distilled water and 0.4 ml supernatant). The diluted solution was pipetted out using sterile 2 ml BD Emerald™ syringes (Becton, Dickinson and Company, Fraga, Spain) and transferred to clear High Pressure Liquid chromatography (HPLC) glass vials (VWR, PA, USA) through syringe filters (Acrodisc 13 mm minispike with 0.45 µm pore size, PTFE, Pall Corporation, Puerto Rico).

Vials with combined standard solution of glucose, sucrose, fructose, raffinose and stachyose were also prepared at 0.5%, 0.25% and 0.125% (w/v) concentrations. All standards with one concentration were placed before and in between the sample vials in the HPLC (Agilent 1200 Series HPLC Agilent Technologies, Waldbronn, Germany). The samples and standards were analyzed using a ZORBAX Carbohydrate Analysis Column (4.6mm ID × 150mm, 5µm, Agilent Technologies, USA) and guard column (ZORBAX C8, Avantor™, VWR International,

LLC). The mobile phase used was 67.5% acetonitrile with 32.5% distilled water. The flow rate was set at 1.4ml/min. The sugar peaks were detected using a refractive index detector (Agilent Technologies 1200 Series, G1362A RID) from 20 µl sample volume at 30°C temperature. Sugar peaks were identified using the external standards (known) retention times, against which the cucumber sample peaks were analyzed (unknown) as shown in **Figure 3.6**.

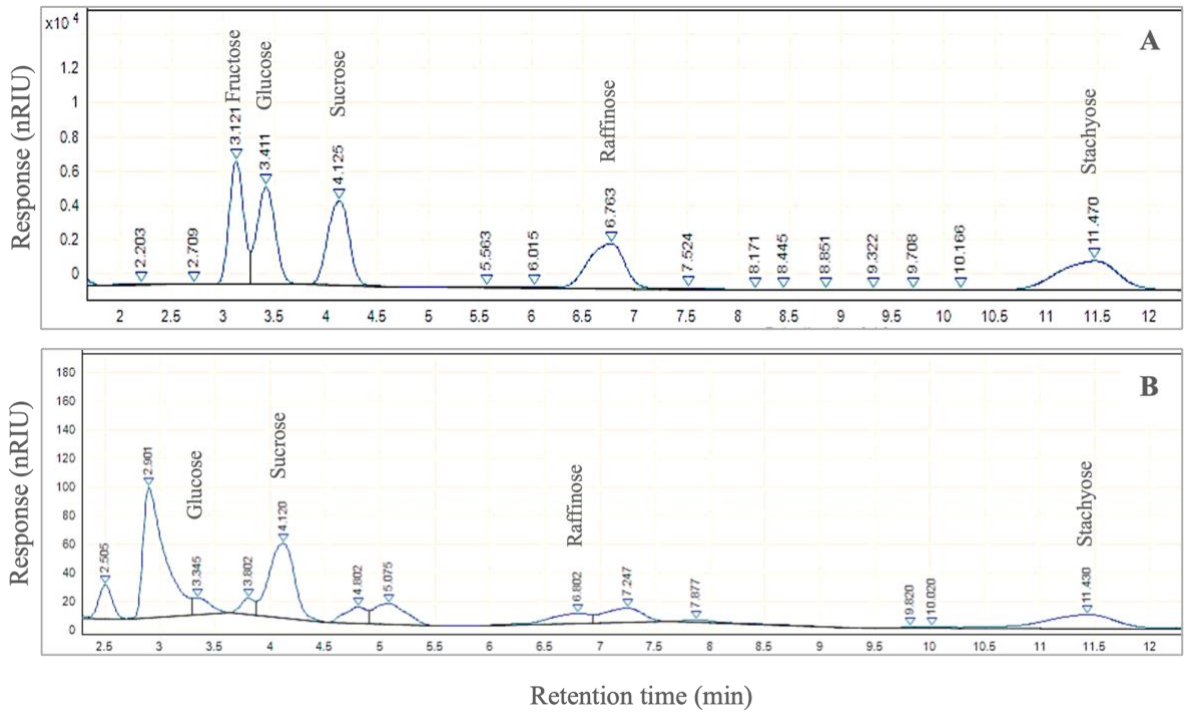

Figure 3.6. Chromatographs showing separation of different sugars in combination of sugar standards of fructose, glucose, sucrose, raffinose and stachyose with known concentration (A) and sample of cucumber leaf collected at the end of the day (B) in chromatograph by HPLC.

Sugar concentrations were calculated using the formula for external standard from Kupiec (2004) provided in equation 3.9, and afterwards concentrations were adjusted according to sample weights to determine precise concentrations. All HPLC data was analyzed using Agilent Chemstation software (version B.04.02 SP1, Agilent Technologies, CA, USA).

$$\text{Conc.}_{\text{unkonwn}} = \frac{\text{Area}_{\text{unkown}}}{\text{Area}_{\text{known}}} \times \text{conc.}_{\text{known}} \qquad (3.10)$$

where, $\text{conc.}_{\text{unkonwn}}$ is different sugar concentration from the sample,

$\text{conc.}_{\text{known}}$ is concentration of standard sugar,

$\text{Area}_{\text{unkown}}$ is area of sugars from sample derived from HPLC chromatograph, and

$Area_{known}$ is area of sugars from standard.

3.6.2 Analysis of starch

The starch analysis was done with Megazyme K-TSHK Assay Kit (Megazyme, www.megazyme.com). The kit contained; Bottle 1: α-amylase, Bottle 2: Amyloglucosidase, Bottle 3: Buffer and sodium azide, Bottle 4: $NADP^+$ and ATP, Bottle 5: Hexokinase and glucose-6-phosphate dehydrogenase suspension, Bottle 6: D-Glucose standard solution and Bottle 7: Standardized regular maize starch control and they were used as provided, except Bottle 4, which was dissolved in 12 ml of distilled water. In addition to the kit, two more reagents; Reagent 1; Potassium hydroxide solution (2 M) and Regent 2; Sodium acetate buffer (1.2 M, pH 3.8) were prepared and analysis of starch in the sample were done as described in the Megazyme TSHK Assay Procedure (AMG/α-amylase/HK method).

Following the protocol supplied with the kit, approximately 100 mg of ground sample of dried leaves and fruits, as well as standard maize starch provided in the kit were weighed and kept in glass centrifuge tubes. To remove D-glucose and possible maltodextrins, firstly, steps 1-5 of sample preparation example 'e' were followed. Secondly, as there was the possibility of resistant starch in the sample, steps 4-6 of sample preparation example 'c' were applied. After following the previous two steps it was assumed that there was no resistant starch, D-glucose or maltodextrins in the sample. Step 6 from sample preparation example 'a' was followed and 4 ml of sample assay was taken in a tube.

During the final steps, 3ml of distilled water was pipetted into a cuvette (VWR Cuvettes PMMA macro, VWR, Germany) with 1 cm light path to be used as a reference in the spectrophotometer (UNICAM, Thermo Spectronic Helios Alpha 9423 UVA 1002E, England). Then, in each cuvette, 1.85 ml of distilled water, and 0.2 ml of sample assay was added except in one cuvette where an additional 0.2 ml of distilled water was added instead of sample assay to have a blank sample. Afterwards, 0.1 ml of solution from Bottle 3 and Bottle 4 were added continuously and gently mixed by covering the opening with parafilm and left for 3 mins. Then absorbance, A_1 was recorded. The solution from Bottle 5 was then added to each cuvette and mixed well and left for 5 mins. The absorbance A_2 was recorded and recorded again after 2 mins until there was stability in the value.

Finally, the absorbance value from the blank sample, and from other samples were calculated using the Mega-Calc™ Data Calculator (Megazyme, www.megazyme.com) and total starch was determined per 100 g of sample. The value of standard maize starch was used to verify the authenticity of the process.

3.7 Data Analysis

Experiment one ended after 30 days of light treatment whereas experiments two and three ended after 28 days of light treatment. So, for growth analysis, the time (days) were adjusted accordingly. The plants used for experiment 3 were smaller and had no true leaves whereas the plants used for experiment 1 and 2 had one leaf which was deducted and leaf number per day was analyzed based on total days with light treatment.

The data were analyzed using R (R version 4.2.2, The R Project for Statistical Computing and R-studio 2022.12.0 Build 353, Posit Software, PBC) for macOS. The data was checked for homogeneity of variance with LeveneTest and normal distribution with QQ-norm and Shapiro-Wilk test. Two-way ANOVA was used to analyze average petiole length, individual leaf area, number of fruits, sugar concentration, and starch content in leaves followed by post-hoc Tukey HSD test in the case of significance difference. One-way ANOVA was used with post-hoc Tukey HSD test in net assimilation rate, internode length, chlorophyll and carotenoids contents, plant height, and starch content in fruits to analyze the difference by light as there was no significant difference found in cultivar when two-way ANOVA was done. Significance level (alpha) was set to 5% ($p = 0.05$) for all tests performed.

Data recorded for CO_2 level of 50 ppm and chlorophyll a in fruit of 'Hi Light' and leaf number per day for both cultivars did not meet the assumption of normal distribution, and data at CO_2 level 200 and 300 ppm in 'Hi Light', did not meet assumption of homogeneity of variance, while conducting one-way ANOVA. Similarly, SLA did not meet the assumption of homogeneity of variance, LMR did not meet the assumption of normal distribution and NAR, LAR and RGR did not meet both assumptions to conduct two-way ANOVA. So, the Kruskal-Wallis test was done followed by post-hoc DunnTest to analyze the result by light treatment.

4. Results

4.1 Leaf gas exchange response

Net assimilation (A_n) rate increased with decreased R:FR ratio in both 'Hi Light' and 'Imea'. A significant difference in A_n was found between the light treatments at intercellular CO_2 (Ci) concentrations of 100, 200, 300, 400, and 700 ppm in 'Hi Light' and 400, 700, 1000 and 1300 ppm in 'Imea' (**Figure 4.1 & Table 8.1**).

Furthermore, the value of A_n dropped to CO_2 compensation point when provided with 50 ppm CO_2 in both cultivars. Both cultivars showed a linear increase in A_n rate up to 400 ppm, following which it gradually increased (**Figure 4.1**).

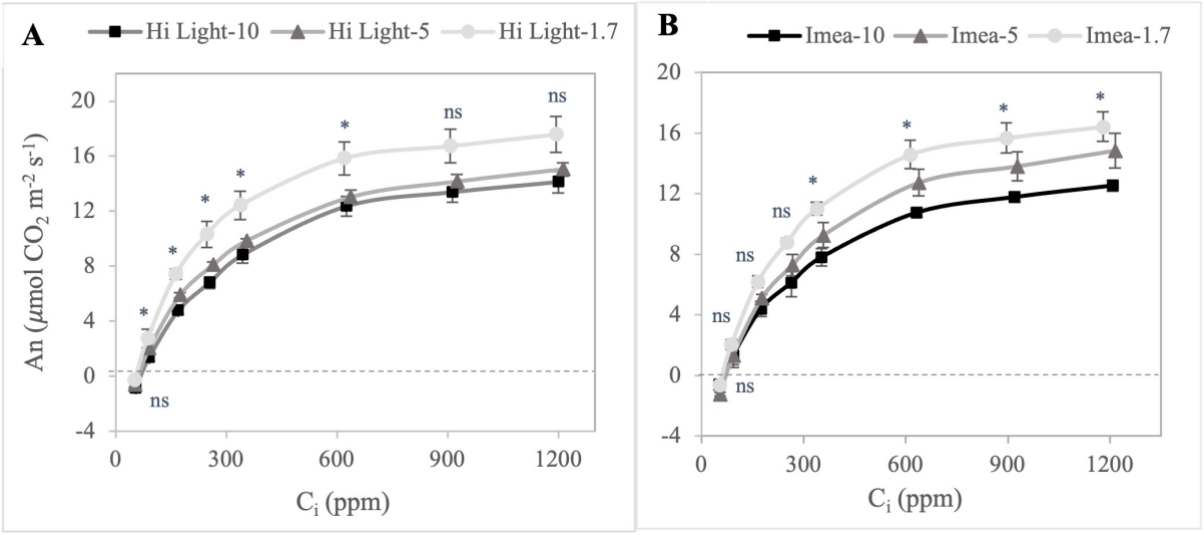

Figure 4.1. Effect of R:FR ratio on net assimilation (A_n) rate by intercellular CO_2 (C_i) concentration on 'Hi Light' (A) and 'Imea' leaves (B) measured with the light condition as in the growth chamber. The measurement was done on a leaf from the sixth or seventh node. Data were analyzed using one-way ANOVA except where assumptions for ANOVA was not met, Kruskal-Wallis test was done '*' Asterisks means significant difference in the values when tested with one-way ANOVA or Kruskal-Wallis test, * means p <0.05, ** means p <0.01, *** means p <0.001 and 'ns' means no significant difference. Value = mean ± SEM (n=4). Dot line represents the CO_2 compensation point.

The transpiration rate also increased with addition of FR in both cultivars (**Figure 4.2 & Table 8.2**). Significant difference was found at all CO_2 levels in 'Hi Light' (**Figure 4.2A**) whereas only in higher CO_2 levels (≥700 ppm) in 'Imea' (**Figure 4.2B**). Transpiration rate increased slightly with the increase in C_i, but started to decrease after C_i reached 400 or 700 ppm for both 'Hi Light' and 'Imea' (**Figure 4.2A&B**). The stomatal conductance also followed the same trend as transpiration rate (not shown).

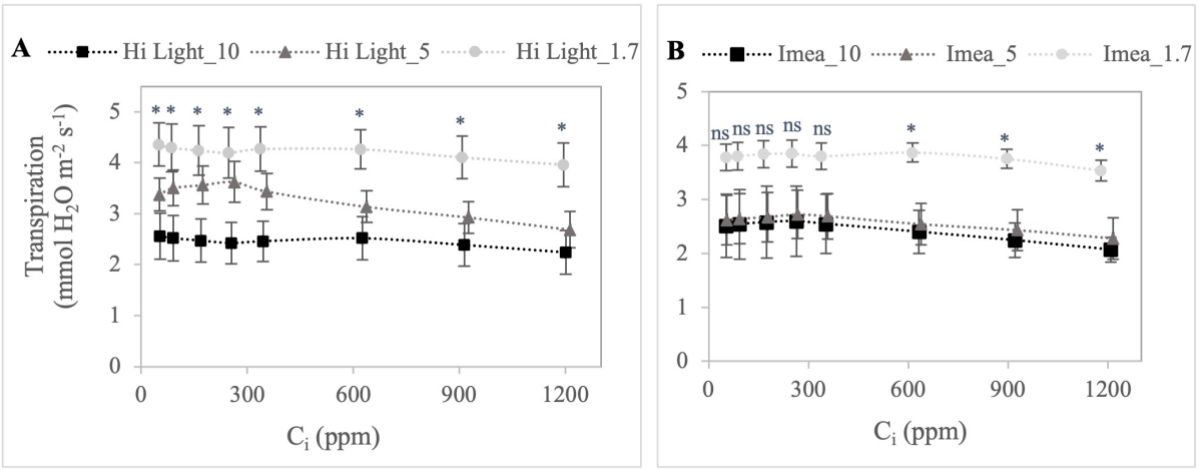

Figure 4.2. Transpiration rate in Hi Light (A) & Imea (B) recorded on eight different intercellular CO_2 level (C_i) on the sixth or seventh leaf using the light source from growth chamber. '*' Asterisks means significant difference in the values when tested with one-way ANOVA, * means p <0.05, ** means p <0.01, *** means p <0.001 and 'ns' means no significant difference. Value = mean ± SEM (n=4).

4.2 Total chlorophyll, ratio of chlorophyll a/b, and total carotenoid

In leaves, there was no significant difference in chlorophyll a, chlorophyll b, total chlorophyll, and ratio of chlorophyll a to chlorophyll b (Chl a/b) between cultivars or light treatments. However, a significant difference was found in carotenoid content (p = 0.048) between light treatments. Though there was no significant difference in other values between light treatment, the mean value shows that the chlorophylls and carotenoids decreased with a decrease in R:FR ratio (**Table 4.1**).

In fruits, no significant difference was found in chlorophyll a, and carotenoids between cultivars or light treatments. However, there was a significant decrease in chlorophyll b (p = 0.009) and total chlorophyll (p = 0.008), and a significant increase in the ratio of Chl a/b (p = 0.009) with decreasing R:FR ratios in 'Hi Light'. A similar trend was found for 'Imea', but the data was not significant (**Table 4.1**).

Table 4.1. The value of foliar and fruit chlorophyll a, chlorophyll b, total chlorophyll, ratio of chlorophyll a to chlorophyll b (Chl a/b) and total carotenoids in 'Hi Light' and 'Imea' treated with three different ratios of red and far-red light (R:FR). Values are means ± SEM (n=6).

Cultivar	Light (R:FR)	Chlorophyll a	Chlorophyll b	Total chlorophylls (g cm^{-2})	Chl a/b	Total carotenoids (g cm^{-2})
Hi Light (Leaves)	10	46.2 ± 3.8	13.5 ± 1.4	59.7 ± 5.2	3.4 ± 0.1	7.1 ± 0.7
	5	38.9 ± 3.7	11.7 ± 0.8	50.5 ± 4.4	3.3 ± 0.1	5.6 ± 1.0
	1.7	35.8 ± 2.2	10.2 ± 0.8	45.9 ± 3.0	3.5 ± 0.1	5.6 ± 0.4
	***p*-value**	**0.154**	**0.153**	**0.152**	**0.306**	**0.316**
Imea (Leaves)	10	39.8 ± 2.1	11.4 ± 0.8	51.2 ± 2.9	3.5 ± 0.1	6.3 ± 0.3[a]
	5	33.8 ± 2.1	9.9 ± 0.5	43.7 ± 2.5	3.4 ± 0.1	4.9 ± 0.4[a]
	1.7	36.1 ± 1.3	10.5 ± 0.3	46.6 ± 1.4	3.4 ± 0.1	4.7 ± 0.4[a]
	***p*-value**	**0.154**	**0.239**	**0.155**	**0.862**	**0.048**
Hi Light (Fruits)	10	37.4 ± 0.7♣	16.1 ± 1.2[a]	53.5 ± 1.9[a]	2.3 ± 0.1[b]	4.9 ± 0.8
	5	33.9 ± 0.9♣	12.4 ± 0.4[b]	46.4 ± 1.3[b]	2.7 ± 0.0[ab]	5.0 ± 0.3
	1.7	32.2 ± 0.9♣	10.9 ± 0.5[b]	43.1 ± 1.4[b]	2.9 ± 0.1[a]	5.4 ± 0.3
	***p*-value**	**0.051**	**0.009**	**0.008**	**0.009**	**0.759**
Imea (Fruits)	10	38.4 ± 3.8	15.9 ± 2.0	54.4 ± 5.8	2.4 ± 0.1	5.6 ± 1.3
	5	37.5 ± 0.7	13.4 ± 0.3	50.9 ± 0.9	2.8 ± 0.0	5.8 ± 0.3
	1.7	34.2 ± 2.6	10.9 ± 0.5	45.7 ± 2.9	2.9 ± 0.2	5.8 ± 0.7
	***p*-value**	**0.545**	**0.102**	**0.324**	**0.071**	**0.99**

The data was first analyzed with two-way ANOVA, but results showed no significant difference by cultivar. So, one-way ANOVA was performed within each cultivar.

The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with $p<0.05$.

'♣' denotes that data are tested for significancy with Kruskal-Wallis test as the assumption of normal distribution for ANOVA was not met.

4.3 Growth and development

4.3.1 Morphological characteristics

The plant height at the start of experiment was found significantly higher in R:FR ratio 5.0 however, at the end of the experiment, the stem length was found significantly higher R:FR ratio 1.7 in both cultivars. The stem length was found longer by 8.7% and 9.4% in 'Hi Light' and 16.4% and 17.1% in 'Imea' with R:FR ratio 5.0 and 1.7 respectively (**Figure 4.3 A&B**).

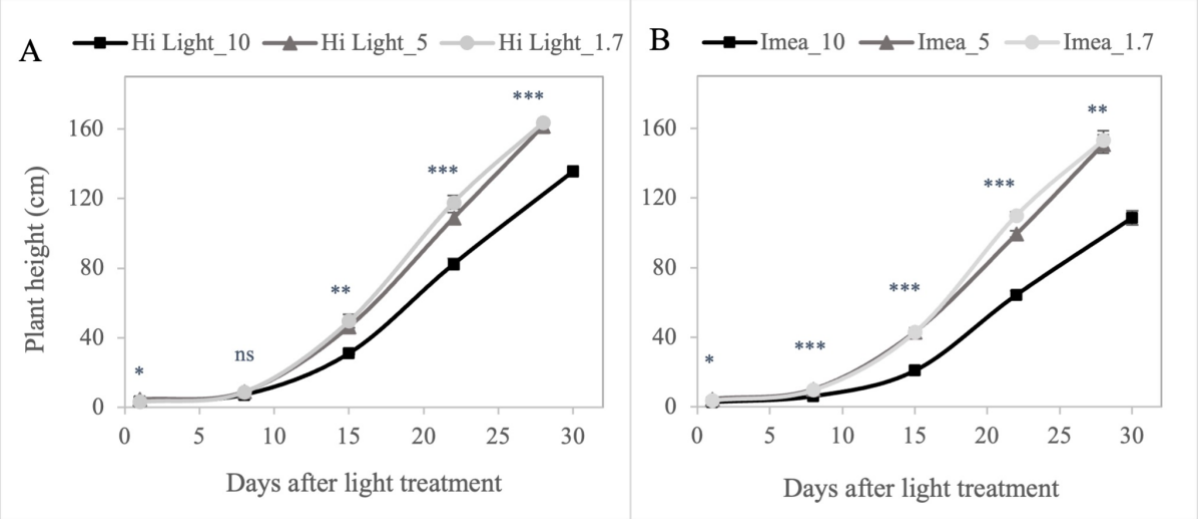

Figure 4.3. The plant height recorded in Hi Light (A) and Imea (B) every week in three different light treatments. '*' Asterisks means significant difference in the values when tested with one-way ANOVA, * means p <0.05, ** means p <0.01, *** means p <0.001 and 'ns' means no significant difference. Value = mean ± SEM (n=3).

A significant difference was found in internode length by light treatment in 'Hi Light' (p = 0.007) and 'Imea' ($p < 0.001$). The internode length increased with a decrease in R:FR ratio by 9.7% and 14.1% in 'Hi Light' and 16.3% and 23.8% in 'Imea' from R:FR ratio 10.0 to R:FR ratio 5.0 and 1.7 respectively (**Figure 4.4A**). Additionally, internode length increased linearly in 'Hi Light' (R^2 = 0.87) and 'Imea' (R^2 = 0.89) with decrease in PPE from 0.72 to 0.39 (not shown).

Similarly, the length of petiole also increased with a decrease in R:FR ratio in both 'Hi Light' and 'Imea'. There was a significant difference in petiole length by cultivar ($p < 0.001$), light ($p < 0.001$) and interaction of cultivar and light (p = 0.013) (**Figure 4.4B**). The petiole length increased by 18.8% and 34.6% in 'Hi Light' and 22.8% and 33.3% in 'Imea' when R:FR ratio was decreased to 5.0 and 1.7 respectively.

The number of leaves per day showed no significant difference by light in both 'Hi Light' and 'Imea' (**Figure 4.4C**). The individual leaf area of 'Imea' was significantly higher ($p < 0.001$) than 'Hi Light'. In addition, significant difference by light treatment ($p < 0.001$) and the interaction of cultivar and light treatment ($p = 0.002$) as shown in **Figure 4.4D**.

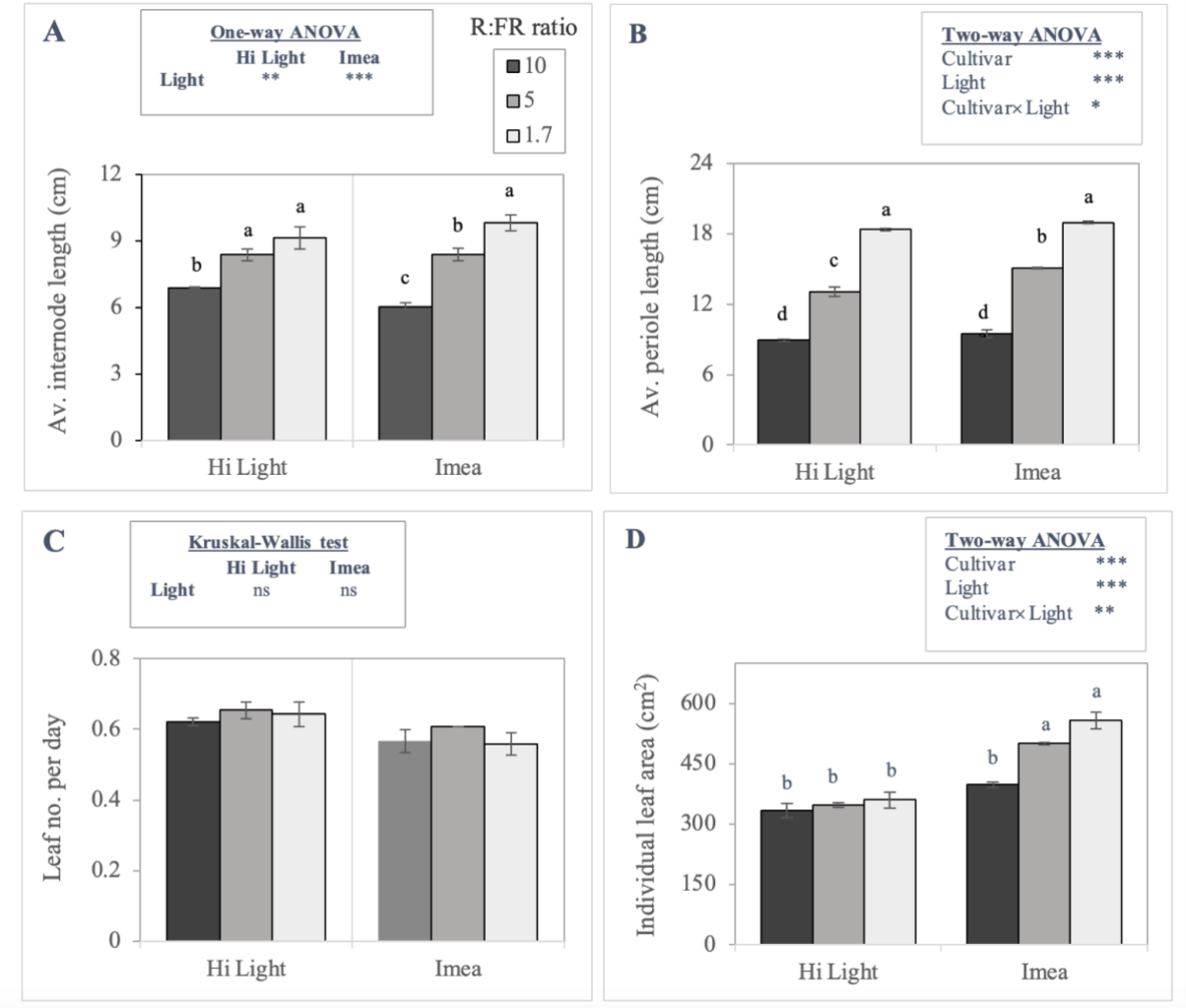

Figure 4.4. The internode length (A), petiole length (B), no. of leaves ≥ 3cm (C), and individual leaf area (D), recorded in 'Hi Light' and 'Imea' with three different light treatments. No significant difference was found in internode length by cultivar so one-way ANOVA was done. Values of leaf number per day did not meet the homogeneity of variance assumption for two-way ANOVA, so Kruskal-Wallis test was done. '*' Asterisks means significant difference in the values when tested with one-way, two-way ANOVA or Kruskal-Wallis test, * means p <0.05, ** means p <0.01, *** means p <0.001 and 'ns' means no significant difference. The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with p <0.05. Value = mean ± SEM (n=3).

4.3.2 Flowering and development of fruits

The days taken to first visible fruit were the same in 'Hi Light' and 'Imea'. In R:FR 10.0, 5.0 and 1.7, the days taken to first visible fruits after light treatment were 12, 10 and 11 days respectively. The first flowering in 'Hi Light' was observed 22 days after the start of the light treatment in R:FR ratio 10.0 and 5.0 and 23 days after the start of the light treatment in R:FR ratio 1.7. Flowering in 'Imea' was observed one day after 'Hi Light' cultivar in all experiments.

The number of fruits ≥1 cm length showed significant difference by cultivar (p = 0.025) but not by light treatment and interaction of cultivar and light treatment (**Figure 4.5**). The number of overall fruits was slightly higher in R:FR ratio 10.0 in comparison to R:FR ratio 5.0 and 1.7. No significant difference was found in visible fruit number (not shown). Result from fruit length measurement from 19-28 days after the start of the light treatment in 'Hi Light' showed no significant difference by light but showed a trend of longer fruits in R:FR ratio 1.7 followed by R:FR ratio 5.0 and 10.0 respectively. However, in 'Imea', a significant difference was found up to 22 days after light treatment. Fruit was longer in R:FR ratio 5.0 whereas the fruit length was similar in R:FR 10.0 and 1.7 (not shown). Still, at the end of experiment, fruit dry weight was slightly higher in high R:FR ratio even though no significant difference was found in dry and fresh weight of fruit (not shown).

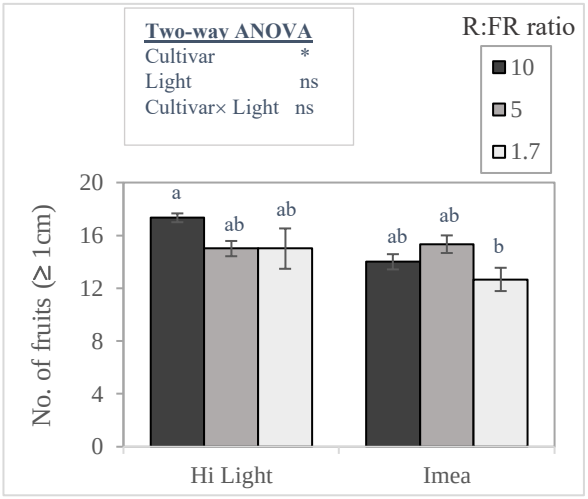

Figure 4.5. Number of fruits (≥1 cm) at end of experiments. '*' Asterisks means significant difference in the values when tested with two-way ANOVA, * means p <0.05, ** means p <0.01, *** means p <0.001 and 'ns' means no significant difference. The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with p<0.05. Value = mean ± SEM (n=3)

4.3.3 Growth Components

There was a significant difference in RGR, NAR, LAR, LMR and SLA by light (p < 0.001) within each cultivar as shown in **Figure 4.6**. The RGR increased by 4.4% and 7.0% in 'HiLight' and 7.1% and 14.7% in 'Imea' with a decrease in R:FR ratio to 5.0 and 1.7 respectively (**Figure**

4.6A). Similarly, NAR also increased by 9.5% and 20.7% in 'HiLight' and 12.2% and 25.7% in 'Imea' with R:FR ratio of 5.0 and 1.7 respectively (**Figure 4.6B**).

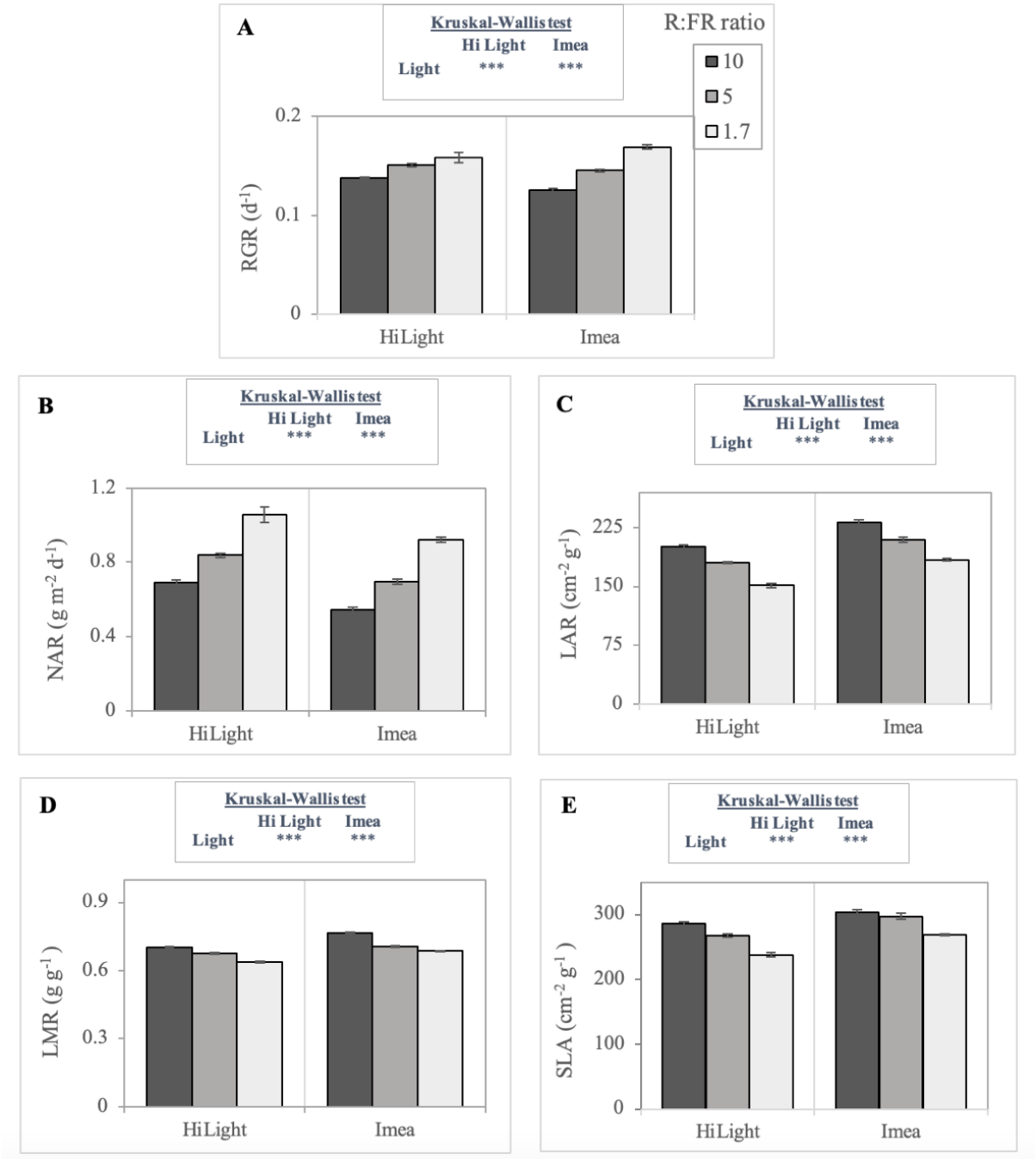

Figure 4.6. The relative growth rate (A), net assimilation rate (B), leaf area ratio (C), leaf mass ratio (D), and specific leaf area (E) calculated based on the dry weight. Kruskal-Wallis test was done for checking significancy as SLA did not meet the assumptions homogeneity, LMR did not meet assumption of normal distribution and NAR, LAR and RGR did not meet assumption of homogeneity and normal distribution for ANOVA. '*' Asterisks means significant difference in the values when tested with Kruskal-Wallis test, * means $p<0.05$, ** means $p<0.01$, *** means $p<0.001$ and 'ns' means no significant difference. Value = mean ± SEM (n=15).

In contrast, the LAR, LMR and SLA decreased with decrease in R:FR ratio. The LAR decreased by 5.3% and 13.8% in 'HiLight' and 5.0% and 11.5% in 'Imea' when R:FR was decreased to 5.0 and 1.7 respectively from R:FR ratio 10.0 (**Figure 4.6C**). Similarly, the LMR decreased by 1.9% and 4.8% in 'HiLight' and 4.0% and 5.5% in 'Imea' (**Figure 4.6D**) and SLA decreased by 3.3% and 9.2% in 'HiLight' and 1.0% and 6.1% in 'Imea' (**Figure 4.6E**) with decrease in R:FR ratio from 10.0 to R:FR ratio 5.0 and 1.7, respectively.

4.3.4 Partitioning of dry matter

As shown in **Figure 4.7**, the dry weight of leaf was higher than other parts of plant in both 'Hi Light' and 'Imea' in all three experiments. With increase in FR proportion, the dry mass in leaf, fruits, and side shoots decreased whereas the dry weight of stem, and petioles increased. Overall, the dry biomass of plants and fruit increased by 7.8% and 13.4% in 'Hi Light' and 17.6% and 22.4% in 'Imea' when R:FR ratio decreased to 5.0 and 1.7 from R:FR ratio 10.0.

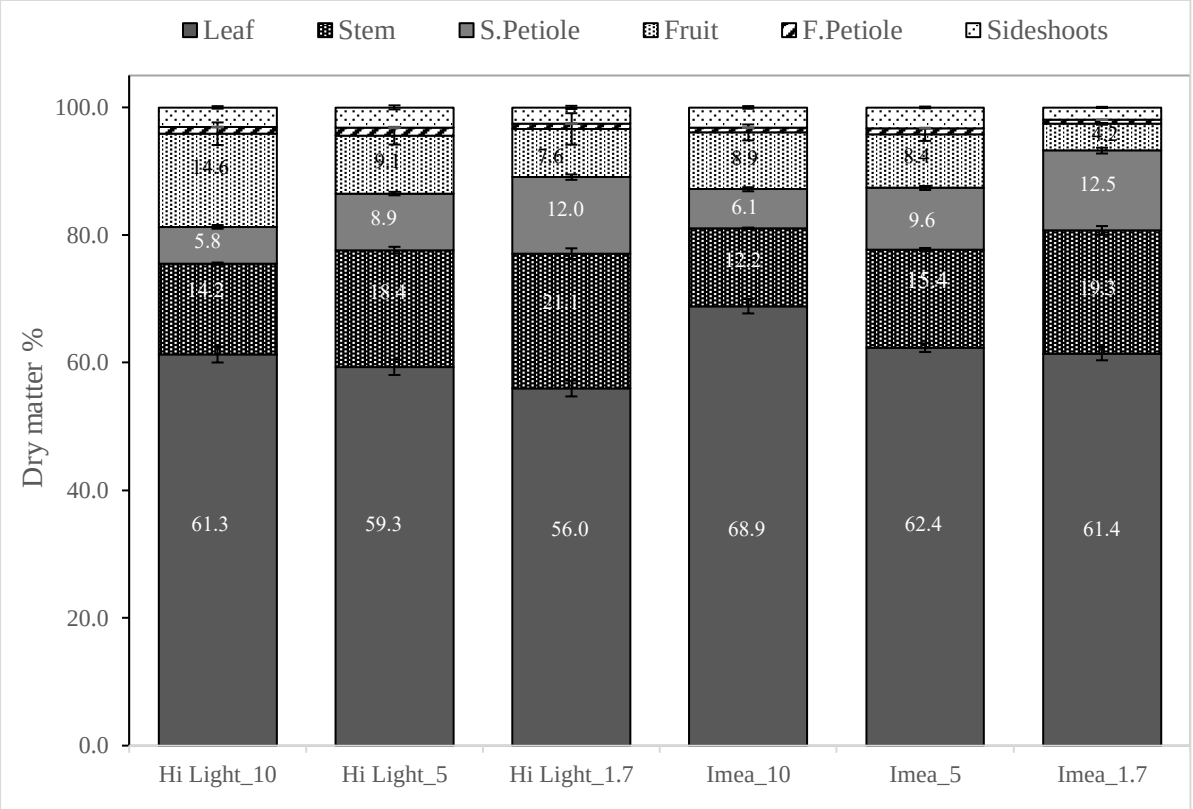

Figure 4.7. Percentage value of dry matter calculated at the end of experiments and dried for 10 days in 60°C for different plant parts of cucumber cultivars HiLight and Imea. S. Petiole means the leaf petioles and F.Petiole means the petioles of fruit. Values within the bar represent the percentage of partitioning. Value = mean ± SEM (n=3).

4.4 Determination of carbohydrate

4.4.1 Sugar Analysis

The fructose and glucose from both leaf and fruit samples were difficult to identify as they were overlapping with unknown peaks or present as a shoulder of the peak. Additionally, the same problem was observed in the peak of raffinose in fruit samples. To avoid any miscalculation, these data were not included.

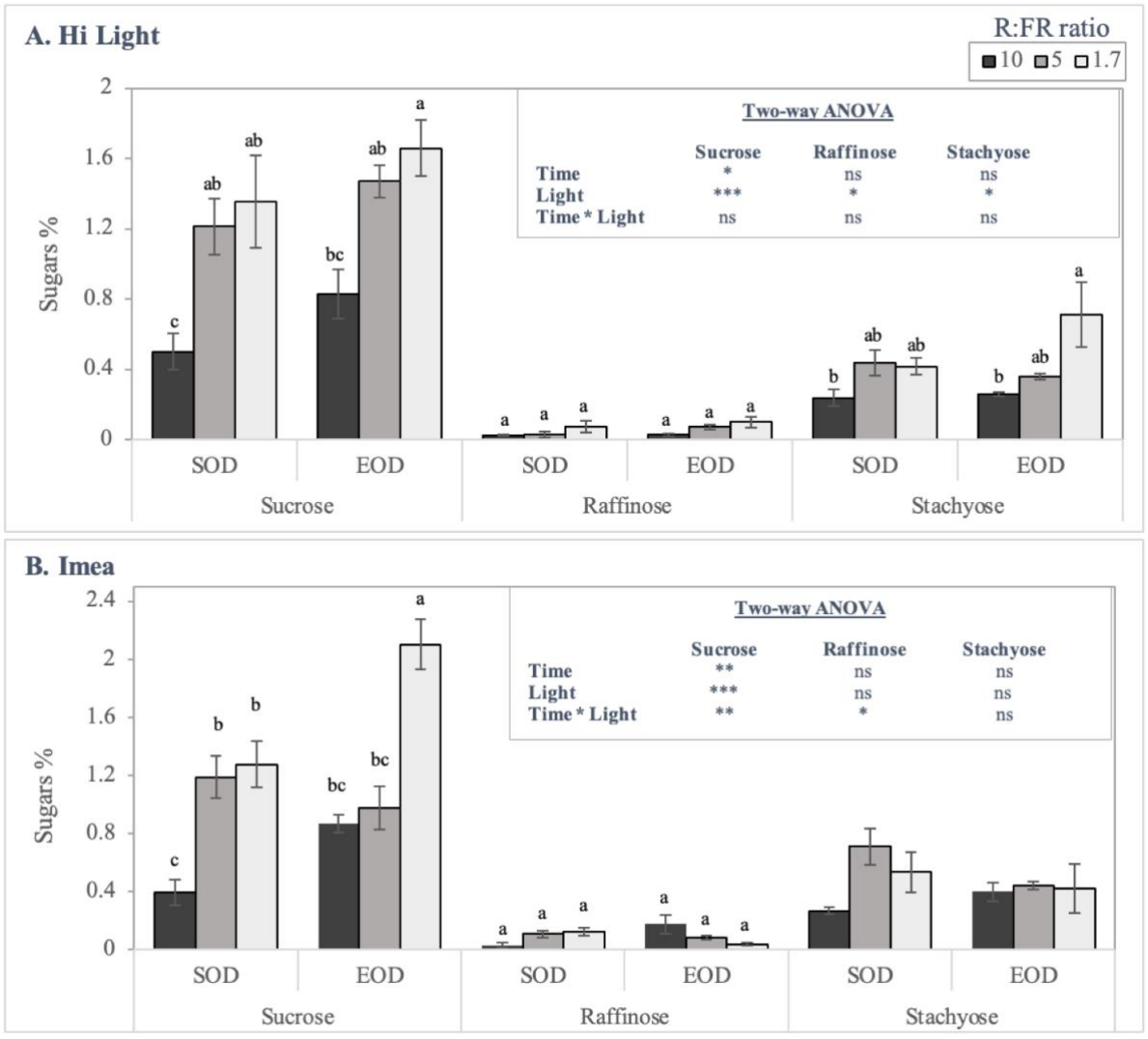

Figure 4.8. Sucrose, Raffinose and Stachyose in leaf of 'Hi Light' (A) and 'Imea' (B). SOD means start of the day and EOD means end of the day. Analysis for each sugar was done separately in both cultivars. '*' Asterisks means significant difference in the values when tested with two-way ANOVA, * means $p<0.05$, ** means $p<0.01$, *** means $p<0.001$ and 'ns' means no significant difference. The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with $p<0.05$. Value = mean ± SEM (n=3).

In foliar sample of 'Hi Light', an increase in sucrose, raffinose and stachyose concentration with decrease in R:FR and at the EOD was found (**Figure 4.8A**). A significant difference was found in sucrose by light ($p < 0.001$) and by time of day ($p = 0.046$), and by light in raffinose ($p = 0.040$) and stachyose ($p = 0.011$) as shown in (**Figure 4.8A**).

In foliar sample of 'Imea', there was a significant increase in sucrose concentration with a decrease in R:FR ratio. This was greater at the EOD. A significant difference in sucrose was found by light ($p < 0.001$) and time of day ($p = 0.007$), in raffinose by interaction of light and time of day ($p = 0.008$), and no significant difference was found in stachyose. Inconsistency was found in raffinose and stachyose concentrations across R:FR ratio and time of day (**Figure 4.8B**).

In fruits, no significant difference was found in sugars from 'Hi Light' by light but the sucrose and raffinose concentration increased as a trend with increase in FR light (**Figure 4.9A**). In 'Imea', a significant difference was found by light in sucrose ($p < 0.001$) and stachyose ($p = 0.018$) as shown in **Figure 4.9B**. Similar to 'Hi Light', there was an increase in sucrose and raffinose percentage with addition of FR light but not in stachyose.

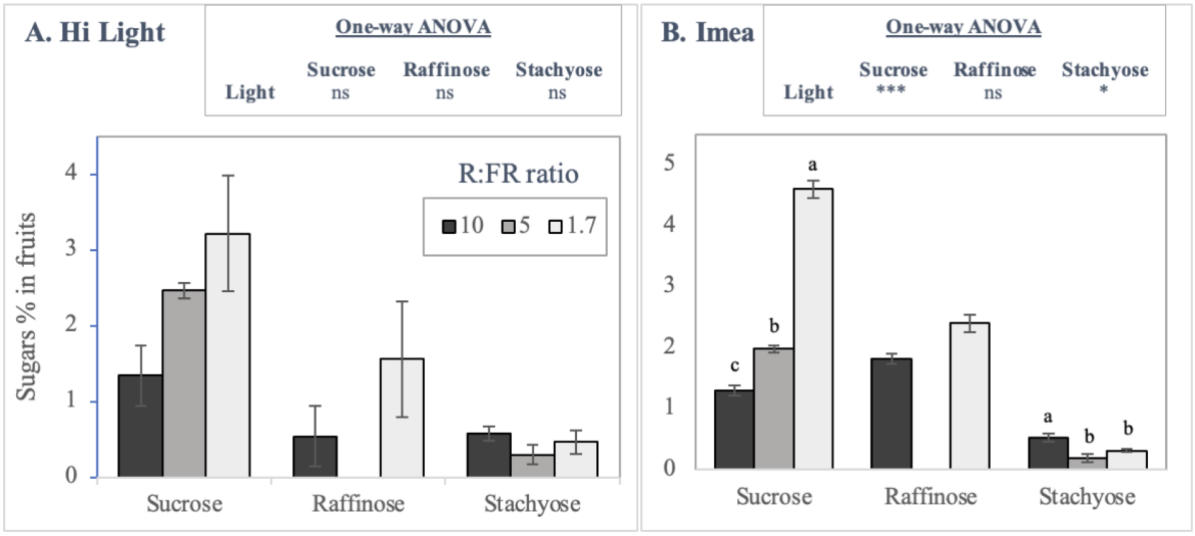

Figure 4.9. Sucrose, Raffinose and Stachyose in leaf of 'Hi Light' (A) and 'Imea' (B) at end of the day. Analysis for each sugar was done separately in both cultivars. Significance difference was not found in sucrose and stachyose so one-way ANOVA was done. '*' Asterisks means significant difference in the values when tested with one-way ANOVA, * means $p<0.05$, ** means $p<0.01$, *** means $p<0.001$ and 'ns' means no significant difference. The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with $p<0.05$. Value = mean ± SEM (n=3).

4.4.2 Starch Analysis

In 'Hi Light' leaves, starch content showed significant difference by time of day ($p < 0.001$), light treatment ($p < 0.001$), and interaction between time of day and light treatment ($p = 0.035$) as shown in **Figure 4.10A**. In 'Imea' leaves, the difference was significant by time of day ($p = 0.003$) and light treatment ($p = 0.036$) as shown in **Figure 4.10B**.

At the SOD, the starch content of leaves from 'Hi Light' increased by 10.5% and 50.8% in R:FR ratio 5.0 and 1.7 respectively compared with R:FR ratio 10. In EOD sample, the starch content decreased by 25.3% and increased by 28.9% in the R:FR ratio 5.0 and 1.7 respectively (**Figure 4.10A**). But, when the data was analyzed by time of day, the starch content was increased by 64.2%, 37.8% and 46.1% from SOD to EOD in R:FR 10.0, 5.0, and 1.7 respectively.

In the foliar sample of 'Imea', the starch content in the SOD was increased by 4.2% and 39.7% whereas in EOD, the starch is decreased by 16.7% and increased by 4.7% in the R:FR ratio 5.0 and 1.7 respectively (**Figure 4.10B**). However, the starch content was increased by 47.8%, 30.0%, and 14.6% from SOD to EOD in R:FR ratio 10.0, 5.0 and 1.7 continuously. Overall, the starch content in leaf of both cultivars was found higher in lower R:FR ratio and at the EOD.

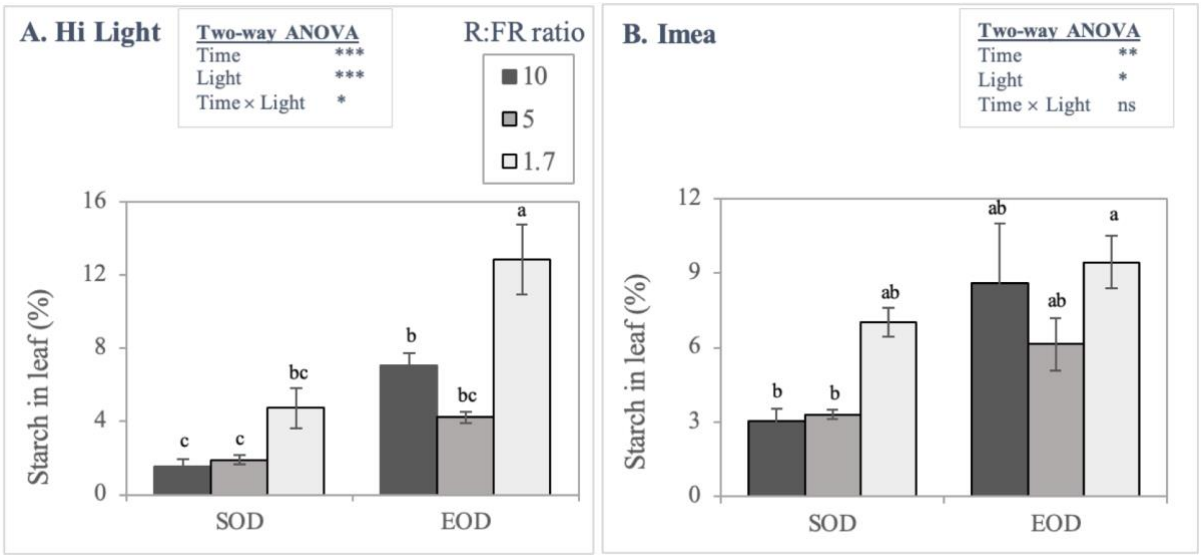

Figure 4.10. Starch content in leaf of 'Hi Light' (A) and 'Imea' (B). SOD means start of the day and EOD means end of the day. '*' Asterisks means significant difference in the values when tested with two-way ANOVA, * means $p<0.05$, ** means $p<0.01$, *** means $p<0.001$ and 'ns' means no significant difference. The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with $p<0.05$. Value = mean ± 1 SEM (n=3).

The starch content in fruit increased with a decrease in R:FR ratio. There was no significant difference by light treatment in 'Hi Light', but there was a significant difference in 'Imea' ($p < 0.001$). In 'Hi Light', there was an increase in starch content by 19.2% and 40.6% and in 'Imea'. The starch content increased by 22.9% and 56.8% in R:FR ratio 5.0 and 1.7 respectively in comparison to R:FR ratio 10.0 (**Figure 4.11**).

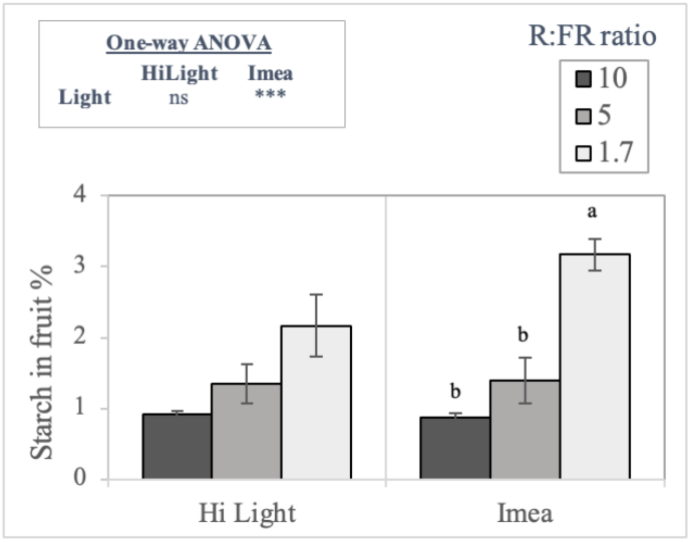

Figure 4.11. Starch content in fruits samples of 'Hi Light' and 'Imea' taken at the end of the day. Cultivar did not show significant difference so, one-way ANOVA was done within each cultivar. '*' Asterisks means significant difference in the values when tested with one-way ANOVA, * means $p<0.05$, ** means $p<0.01$, *** means $p<0.001$ and 'ns' means no significant difference. The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with $p<0.05$. Value = mean ± 1 SEM (n=3).

5. Discussion

5.1 Photosynthetic pigments and leaf gas exchange response

5.1.1 Photosynthetic pigments

Content of pigments are normally found to be affected by light quality. Addition of FR light decreased the chlorophyll content in clover (Heraut-Bron et al., 2000) and in cucumber (Kusuma & Bugbee, 2021), as well as decreased carotenoids in leaf of tomato (Dorokhov et al., 2021). Similarly, in this study, the chlorophyll content in leaf and fruit skin, and carotenoids in leaf of both cultivars was found lower in low R:FR ratio. However, the carotenoids in fruit increased with decrease in R:FR ratio (**Table 4.1**) though the specific reason is unknown. Additionally, Kasperbauer and Hamilton (1984) found that application of FR light increased chl a/b ratio in tobacco. This result was evident in this study as well. The chl a/b in leaf increased with decrease in R:FR ratio (**Table 4.1**).

5.1.2 Leaf gas exchange response

Though there was a decrease in photosynthetic pigments, *i.e.*, chlorophyll in the leaf, the A_n in both 'Hi Light' and 'Imea' was found increasing with a decrease in R:FR ratio (**Figure 4.1A&B**). Zhen and Van Iersel (2017) found that addition of FR to red/blue light provided an enhancement effect on net photosynthesis in lettuce. The study by Zhen and Bugbee (2020) showed that in Marshall cultivar of lettuce, addition of FR to white light increased net photosynthesis. In the same study, the efficiency of additional FR or white light was found similar as the net photosynthesis under 400 µmol m^{-2} s^{-1} white light with addition of 60 µmol m^{-2} s^{-1} FR was comparable to 460 µmol m^{-2} s^{-1} white light. A slight increase in net assimilation was found in clover with addition of FR light under both high and low irradiance despite a decrease in chlorophyll content (Heraut-Bron et al., 2000).

To elaborate, short wavelengths overexcite PSII, causing a reduction in the proportion of active PSII reaction centers, whereas addition of FR light particularly excites PSI. This balances the excitement level between the two photosystems, resulting in enhanced photosynthesis as well as increased quantum yield of PSII by reallocating the light harvesting complexes back to PSII (Hogewoning et al., 2012; Zhen & Van Iersel, 2017). Additionally, the more open framework in plants with extended internodes as a response to increased FR aids in capturing more light (Ji et al., 2021). For example, Sarlikioti et al. (2011) found an increase in photosynthesis by 5-

6% in canopy level with 10 cm increment in internode length. Thus, growth of plants with increased FR aids in capturing more light as well as balanced photochemistry results more net assimilation of CO_2. Kalaitzoglou et al. (2019) found a higher rate of photosynthesis in leaves of Komeett cultivar of tomato at PPE level 0.80 than 0.87, despite decreased photosynthetic pigment content. Yet no additional increase in photosynthesis was found when PPE was decreased to 0.70, suggesting the response might vary based on the species or cultivar.

The single leaf gas exchange measurement data shows that net assimilation was negative when CO_2 was decreased to 50 ppm (**Figure 4.1A&B**). This is because at lower level of CO_2, the CO_2 released with respiration is equivalent to CO_2 used in photosynthesis, known as CO_2 compensation point (Smith et al., 1976), also referred as threshold since there is cessation of assimilation below this level (Bravdo, 1971). In addition, decrease in CO_2 level below ambient level lowers the pools of intermediates in Calvin cycle resulting decrease in photosynthesis (Long & Bernacchi, 2003). And, elevation in CO_2 level increase photosynthesis by increasing the carboxylation of Ribulose 1,5-biphosphate (RuBP) however, limitations of Rubisco and RuBP regeneration start around 200 and 300 ppm CO_2 respectively (Sharkey et al., 2007). Furthermore, Long and Bernacchi (2003) mentioned that higher CO_2 level plateaus the assimilation rate because of limitations in Rubisco, RuBP regeneration or trios-phosphate (TPU) utilization. Though these limitations are not graphed in this paper, and it is unknown which of these limitations are the cause, but the initial rapid incline in net assimilation with increase in CO_2 concentration above compensation point and steady state of net assimilation curve in higher CO_2 range is evident in both cultivars (**Figure 4.1A&B**).

The transpiration rate in both cultivars increased with increase in FR light (**Figure 4.2A&B**). Stomatal conductance showed the same trend as transpiration rate (not shown). Holmes et al. (1986) found that FR light in addition to white light increased the transpiration rate as well as maintained the steady state of stomatal conductance in *Phaseolus vulgaris* L. However, stomatal conductance by addition of white and red light were more efficient than FR, which showed FR light was not stimulating the stomatal conductance directly, instead increased photosynthesis with addition of FR light might be the reason for stomatal response (Zhen & Bugbee, 2020).

5.2 Growth and development

5.2.1 Morphological characteristics

Low R:FR ratio extended the stem of soyabean (Yang et al., 2020), and cucumber (Shibuya et al., 2016). This was also evident in this study. With increase in FR light, the stem length was elongated in both 'Hi Light' and 'Imea' (**Figure 4.3**). However, no significant difference was found in leaf number by light (**Figure 4.4C**) (which also means there is similar internode number. Again, in this experiment, the length of average internode significantly increased with decreasing R:FR ratio (**Figure 4.4A**) indicating the extension of stem is related to elongation of internodes rather than an increase in internode number. Additionally, Garrison and Briggs (1975) found increase in internode length of *Helianthus annuus* L. with FR light by increasing the cell number and length. Similarly, with addition of FR to white light, increase in internode length of bean was also observed up to three times because of cell elongation, and also cell division as internode elongation proportion was higher than cell elongation proportion (Beall et al., 1996). In the same study, the active Gibberellin acids (GAs), GA_1 and G_{20} were found higher in the internode exposed to FR suggesting elongation growth is related to GA concentration and metabolism. This finding suggests that FR regulates the endogenous hormones resulting elongation growth which might also be the reason for extension in internode length with low R:FR ratio in this study.

Kusuma and Bugbee (2021) also found an increase in plant height with a decrease in PPE or increase in FR percentage in several plant species. Additionally, the plant height in Komeett cultivar of tomato increased with decrease in PPE and internode length followed the same trend as height (Kalaitzoglou et al., 2019). A similar relationship was found in this experiment between internode and PPE in both cultivars (not shown). However, the relationship was not strongly linear as the height difference in plants between R:FR 5.0 and 1.7 was unexpectedly similar (**Figure 4.3**). Shibuya et al. (2016) showed that plant height of cucumber cv. Hokushin with R:FR ratio 1.4 was significantly higher than R:FR ratio 4.3. But it is unsure if the results between the two studies are comparable because of the difference in other climatic conditions and lamp types used as source of light.

With a decrease in R:FR ratio, an increase in petiole length was observed in both cultivars (**Figure 4.4B**). This is supported by Smith and Whitelam (1997), where reduced R:FR ratio strongly caused elongation of petiole along with the internode elongation. Similarly, the

individual leaf area also increased with a decrease in R:FR ratio in both cultivars, but individual leaves of 'Imea' expanded more in comparison to 'Hi Light' (**Figure 4.4D**). A study by Yang et al. (2020) showed that leaf area of soyabean increased with addition of FR. A low R:FR ratio resulted in higher individual leaf area during late growth stages of lettuce (Lee et al., 2015). Increase in both petiole length and leaf area seems to be shade avoidance response with addition of FR light to capture more light and outcompete neighbor plants.

5.2.2 Flowering and fruiting.

No considerable difference was found on days taken to visible fruits and flowering in either cultivar (not shown). Similarly, the time taken to flowering shifted by one or two days earlier with addition of FR light in tomato, but no significant difference was found by Ji, Y. et al. (2020). No significant difference was found in the number of fruits with different light treatments in this experiment, however, it seemed slightly higher in high R:FR ratio (**Figure 4.5**). In contrast, Kalaitzoglou et al. (2019) stated that fruit set in tomato was stimulated along with individual fruit growth because of increase in source strength with addition of FR. This was evident in this study as well. Increase in FR increased the length of cucumber fruits when compared at ten days, though no significant difference was found (not shown). Yet, the result was different between cultivars, so it is difficult to be sure that lowering R:FR ratio rapidly increases the fruit size. In addition, the experimental period was short to observe the potential of FR in fruit development.

5.2.3 Dry Matter Partitioning

Decreased R:FR ratio decreased the dry matter partitioning towards leaf and increased the dry biomass of stem and petioles (**Figure 4.7**). Similar result was found in study of Kusuma and Bugbee (2021) where increase in FR light increased the mass of stem and decreased the leaf mass in cucumber. Kasperbauer and Hamilton (1984) also stated that partitioning of dry matter to stem increases with exposure to FR light in comparison to red light. Furthermore, the addition of FR light in tomato production increased the partitioning of dry matter to the fruit and stem while reduced the fraction to leaves by strengthening the sink strength in fruits (Ji, Y. et al., 2020).

Tucker (1975) found that, the introduction of FR at the end of day decreased branching in tomato plants, without affecting the branch related to flowering and fruiting. FR light induced

the apical dominance by regulating auxin for elongation growth of plant resulting suppression in development of branches of Arabidopsis (Holalu et al., 2021). So, suppression in development of lateral branches might also reduce their biomass. Similar result was observed in this study. A slight decrease in dry weight of side shoots with more FR light was observed in this study (**Figure 4.7**). However, side-shoots were removed from the plants every third day, so it was difficult to observe clear differences.

The partitioning of dry matter to fruit was slightly higher with high R:FR ratio (**Figure 4.7**), though no significant difference was found in dry weight between light treatments (not shown). Franklin and Whitelam (2007) mentioned that on dicotyledon plants a decrease in R:FR ratio extended the petiole and stem and caused apical dominance at the cost of development of storage organs. On the contrary, supplemental FR light shifted allocation of dry-matter fractioning to the tomato fruit by increasing photosynthetic and water use efficiency (Kim et al., 2019). Ji, Y. et al. (2020) also found increased sink strength in tomato fruit with a significant increase in dry biomass of individual fruit with addition of FR light to red and blue light. However, studies by Kim et al. (2019) and Ji, Y. et al. (2020) are both done with long term experimental setup. So, longer study period is required for better understanding the sink effect and increase in fruit yield with FR light in cucumber as well.

In general, increased dry biomass of whole plant including fruit was found with low R:FR ratio. This was also observed in an experiment by Yang et al. (2020), where the biomass of soyabean plants increased with low R:FR ratio under both normal and low light intensity. Increase in plant dry mass was observed in tomato with addition of FR mainly because of increased light absorption with increase in leaf areas (Kalaitzoglou et al., 2019). Similarly, Legendre and Van Iersel (2021) also mentioned that, increase in FR increases projected canopy size by expanding leaf area which provides more light incident to the plant resulting increase in dry matter accumulation. This might also be the reason for increased dry biomass in this study with lower R:FR ratio.

5.2.4 Relative growth of plant

Leaf thickness is reduced with increased FR light (Kasperbauer & Hamilton, 1984) or reduction in R:FR ratio (Lee et al., 2015; Smith & Whitelam, 1997). Similarly, in this study SLA was decreased with decrease in R:FR (**Figure 4.6E**). With the addition of FR light, the plant showed elongation growth and prioritized partitioning towards the stem at the expense of leaf

partitioning, which decreased the proportion of leaf mass to total plant mass, resulting decreased LMR (**Figure 4.6D**). As LAR is the multiplication value of SLA and LMR, a decrease in both values resulted in a decrease in LAR (**Figure 4.6C**).

NAR was increased in lower R:FR ratio in growth component analysis (**Figure 4.6B**). This agrees with the increase in net assimilation rate that was found in gas exchange measurement with addition of FR (**Figure 4.1**). FR light promoted the light capturing ability in clover leaf by extension of leaf area (Heraut-Bron et al., 2000). Because of the increment in light capturing ability and photosynthetic efficiency as mentioned above, FR light increases the NAR. And increase in NAR increased the RGR (**Figure 4.6A**), despite the decrease in LAR. Ji et al. (2021) also found similar results in tomato genotypes reacting highly to increased FR. According to the same study, not all genotypes responded positively to FR, which is why cultivars were categorized into strongly, moderately, and weekly responding groups. In moderately and weekly responding group of tomato genotypes, the LAR increased because of an increase in SLA with FR light, (Ji et al., 2021). However, in this study, both 'Hi Light' and 'Imea' responded similarly and strongly to the addition of FR.

5.3 Sugars and starch accumulation

5.3.1 Sugars accumulation

Low R:FR ratio in addition to normal light intensity increased sucrose content significantly in soyabean (Yang et al., 2020). Similarly, the sugar concentration was also higher with increased FR light treatment in tobacco leaves in comparison to R light treatment (Kasperbauer & Hamilton, 1984). Driesen et al. (2023) indicated, addition of FR to blue and white light enhanced the photosynthesis in sweet basil resulting in an increase in sugar concentration. As addition of FR increased the photosynthetic efficiency in both cultivars, increase in sugars like sucrose, raffinose and stachyose concentrations with a decrease in R:FR ratio is also reasonable in this study, though some inconsistency in raffinose and stachyose concentrations was observed in 'Imea' (**Figure 4.8A&B**).

According to the time point of sample collection, different sugars were found in higher concentrations at the end of the photoperiod (**Figure 4.8A&B**). Similar result was found in other studies where increased sucrose level was found consistently during the light period in cucumber cv. Calypso (Pharr et al., 1985) and concentration of sugar in leaves of tobacco was

found lower in the night (Kasperbauer & Hamilton, 1984). As there is no photosynthesis during night, the plant metabolism and night growth depends on stored carbohydrate resulting sugar starvation at the beginning of the day (Driesen et al., 2023). So, this might also be the cause of lower sugar concentration at the SOD in this study even though sugar content in 'Imea' seems inconsistent in comparison to 'Hi Light' for unknown reasons.

Genes related to sugar transportation and metabolism are regulated by FR light, increasing sugar concentration in tomato fruit (Ji, Y. et al., 2020). This might be the reason for increased sugars concentration in cucumber fruit with decrease in R:FR ratio (**Figure 4.9A&B**) as well as in comparison to leaf sugar level observed in this study. Additionally, Pharr et al. (1985) also found that, the sucrose, raffinose and stachyose concentration in fruiting plants is normally lower in leaves. Similarly, the sugar concentration was found high in fruit in comparison to leaf of both 'Hi Light' and 'Imea' in this study However, some unknown peaks were also visible in chromatographs (**Figure 3.6B**) of foliar and fruit samples, and are suspected to belong to unknown sugar components, sugar alcohols or their precursors. Weidner (1964) also detected verbascose, manninotriose, serine, aspartic acids, and malate in addition to sucrose, stachyose and raffinose in source leaf of cucumber. However, it is not possible to confirm if the unknown peaks in samples of this study belong to these compounds without running standards for the extra sugars.

5.3.2 Starch accumulation

When foliar starch content was compared between different light treatments, it was found to have high concentrations in low R:FR ratios in both cultivars (**Figure 4.10A&B**). The study of Yang et al. (2020) also found that low R:FR ratio in normal as well as low light intensity increased the starch percentage in soyabean suggesting increase in starch content might be related to increased photosynthetic efficiency with increased FR light. In contrast, tobacco leaves had higher concentrations and larger starch granules when provided with red light at the end of the light period in comparison to FR light (Kasperbauer & Hamilton, 1984) and this indicates that starch accumulation in response to FR might vary in species. However, the overall trend is confusing as the starch content in R:FR ratio 5.0 was lower than R:FR ratio 10.0 and 1.7, which is difficult to explain. In terms of time of day, the starch level in both cultivars was found to be lower at the SOD in comparison to the EOD (**Figure 4.10A&B**). This

might be because the starch accumulated during the daytime might have been used during the nighttime, resulting in low starch in the morning (Pharr et al., 1985).

FR accelerated the accumulation and degradation of starch in tomato fruit according to Ji, Y. et al. (2020). However, Driesen et al. (2023) found increased starch level in sweet basil leaf with FR added to blue and white light. Similarly, in this study, starch content increased with addition of FR in both cultivars (**Figure 4.11**). Besides, Sui et al. (2017) found that in 'ZN16' cultivar of cucumber the photosynthetic rate of fruit was 13.8-15.8% per unit area of leaf photosynthesis during six to nine days after anthesis. In the same experiment, when the fruits were bagged and photosynthesis only relied on the leaves, the yield of fruit decreased. Furthermore, they found that 2.4-22.1% of the total carbohydrates required for growth of fruit is contributed by photosynthesis in the fruit itself, but the contribution might vary based on leaf to fruit ratio. This indicates that fruit are also able to assimilate and might be the reason for different trend of starch concentration in fruit from the leaves found in this study with light treatment.

5.4 Practical implications of far-red light

In recent years, the importance of FR light for enhancement effect has been a subject of interest. Several studies, including this one, have shown that an increase in FR light increases the leaf area and extensional growth of internodes which helps to capture more light. FR light with a background of white light helps to balance the energy absorption level between photosystems I and II, resulting in increased photosynthetic efficiency and promoting growth and development of the plants. Additionally, an increase in soluble sugars and starch content in both leaves and fruits with an increase in FR light, which might be helpful for fruit growth and improve nutritional value. Though no increase in fruit yield was found with increased FR light because of the short growing period in this study, Ji, Y. et al. (2020) and Kim et al. (2019) found increased partitioning towards the fruit by additional FR light.

In addition, the results were found consistent between 'Hi Light' and 'Imea' in this study though Særheim research station (NIBIO, Norway) found different response with the use of different lamp types. In this study, both cultivars followed the trend of either increase or decrease similarly except some variation in chlorophyll pigments and raffinose and stachyose concentration. So, it implies that, even if these cultivars responded differently with lamp types,

the effect of different proportion of FR photon with the addition of FR LEDs to white LEDs is similar.

Furthermore, the addition of FR as a light source means addition of electric usage. Though there was an increase in electricity consumption with addition of FR to light spectrum, the energy was well converted to plant biomass of sweet basil (Driesen et al., 2023). Same study explored that, average leaf and stem biomass per electricity consumed (W m^{-2}) increased by 43% and 42% on basis of fresh and dry weight respectively when added FR to blue and white light. This indicates addition of FR for commercial production is practically worthy even with increase in energy use. However, most of the cucumber production is done in the greenhouse and it is difficult to know what proportion of FR should be provided in addition to the natural light.

Color pigments like chlorophylls are not only involved in photosynthesis, but also related to post harvest quality of cucumber fruit. Ménard et al. (2005) stated that a higher R:FR ratio helps in fruit coloration and conservation of fruit life after harvest. The addition of R light in greenhouse production during spring increased the post-harvest life of English cucumber cv. Mustang in comparison to supplementary FR, even though no difference was found in chlorophyll content during harvest (Lin & Jolliffe, 1996). Thus, reduced chlorophyll content in fruit with lower R:FR might affect the fruit quality as consumers prefer greener fruit. Moreover, longer time required for degradation of more chlorophyll (Lin & Jolliffe, 1996) with high R:FR ratio might be the reason for longer self-life. Mitigation of this issue might be the subject area for another study.

Despite some postharvest issues in fruits and difficulty in estimation of FR proportion under natural light condition, FR as a supplementary source of light during production seems to have many positive impacts on quality as well as quantity of produce by enhancing the photochemistry, and translocation of assimilates to the fruit. Additionally, energy use efficiency is also good in response to fresh and dry plant biomass. Therefore, it can be recommended as an additional source along with white LEDs for commercial production.

6. Conclusion

Use of far-red LEDs together with white LEDs during the whole light period enhanced the photosynthetic efficiency of cucumber plants despite a decrease in photosynthetic pigment. Extension in morphological structure like plant height, internode length, petiole length and leaf area were found with a low R:FR ratio. Not much difference was found in the number of leaves, days taken to fruiting and flowering, and number of fruits. However, biomass partitioning was more allocated to the stem and petiole with decrease in R:FR ratio. Relative growth rate was higher in lower R:FR with increased net assimilation rate and decreased leaf area ratio. Assimilates like sugars and starch in leaves and fruits was higher in lower R:FR ratio. A similar trend was observed in both cultivars, except for a few contradictory results in photosynthetic pigment and some soluble sugars like raffinose and stachyose.

To conclude, addition of FR to the background of white light was found to be beneficial in both cultivars. However, further detailed investigation is also recommended regarding fruit development and yield, as the study period was short compared with commercial cucumber production in Norway.

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8. Supplementary data

8.1 Analyzed data of net assimilation rate

Table 8.1. CO_2 assimilation values at different levels of CO_2 concentration in two different cultivars of cucumber; 'Hi Light' and 'Imea' after 20 days of light treatment. The measurement was done on a sixth or seventh leaf with infrared gas analyzer using the light source from the growth chamber. Values are means ± 1 SEM (n=4).

Cultivar	**Light (R/FR)**	**A (μmolCO$_2$ m^{-2} s^{-1})**							
CO_2 (μmol)		**50**	**100**	**200**	**300**	**400**	**700**	**1000**	**1300**
Hi Light	10	-0.9 ± 0.3♣	1.3 ± 0.3^{b}	4.8 ± 0.3♠	6.8 ± 0.4♠	8.8 ± 0.6^{b}	12.4 ± 0.7^{b}	13.4 ± 0.7	14.1 ± 0.8
	5	-0.6 ± 0.2♣	2.0 ± 0.2^{ab}	5.9 ± 0.2♠	8.1 ± 0.2♠	9.8 ± 0.2^{ab}	13.0 ± 0.5^{ab}	14.2 ± 0.5	15.0 ± 0.5
	1.7	-0.3 ± 0.2♣	2.7 ± 0.4^{a}	7.4 ± 0.7♠	10.3 ± 0.9♠	12.4 ± 1.0^{a}	15.8 ± 1.2^{a}	16.7 ± 1.2	17.6 ± 1.3
	***p*-value**	**0.334**	**0.023**	**0.013**	**0.013**	**0.013**	**0.039**	**0.056**	**0.062**
Imea	10	-0.6 ± 0.4	1.3 ± 0.5	4.4 ± 0.8	6.1 ± 0.9	7.8 ± 0.6^{b}	10.8 ± 0.3^{b}	11.9 ± 0.3^{b}	12.5 ± 0.4^{b}
	5	-1.2 ± 0.1	1.4 ± 0.2	5.1 ± 0.6	7.3 ± 0.7	9.3 ± 0.8^{ab}	12.7 ± 0.9^{ab}	13.8 ± 0.9^{ab}	14.9 ± 1.1^{ab}
	1.7	-0.6 ± 0.3	2.1 ± 0.4	6.2 ± 0.3	8.8 ± 0.3	11.0 ± 0.4^{a}	14.6 ± 0.9^{a}	15.7 ± 0.9^{a}	16.4 ± 0.9^{a}
	***p*-value**	**0.102**	**0.37**	**0.171**	**0.067**	**0.018**	**0.019**	**0.025**	**0.037**

The data was first analyzed with two-way ANOVA, but the result showed no significant difference by cultivar. So, one-way ANOVA was performed within each cultivar.

The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with $p<0.05$

'♣' and '♠' denotes that data did not meet assumption of normal distribution and homogeneity of variance for ANOVA respectively so Kruskal-Wallis test was used to find significant difference between light treatment.

8.2 Analyzed data of transpiration rate

Table 8.2. Transpiration rate at different levels of CO_2 concentration in two different cultivars of cucumber; 'Hi Light' and 'Imea' after 20 days of light treatment. The measurement was done on a sixth or seventh leaf with infrared gas analyzer using the light source from the growth chamber. Values are means ± 1 SEM (n=4).

Cultivar	**Light (R/FR)**	**Transpiration rate (mmol H_2O m^{-2} s^{-1})**							
CO_2 (μmol)		**50**	**100**	**200**	**300**	**400**	**700**	**1000**	**1300**
Hi Light	10	2.6 ±0.5^{b}	2.5 ± 0.5^{b}	2.5 ± 0.4^{b}	2.4 ± 0.4^{b}	2.5 ± 0.4^{b}	2.5 ± 0.4^{b}	2.4 ± 0.4^{b}	2.4 ± 0.4^{b}
	5	3.4 ± 0.3^{ab}	3.5 ± 04^{ab}	3.6 ± 0.4^{ab}	3.6 ± 0.4^{ab}	3.4 ± 0.4^{ab}	3.1 ± 0.3^{ab}	2.9 ± 0.3^{ab}	3.6 ± 0.4^{ab}
	1.7	4.4 ± 0.4^{a}	4.3 ± 0.5^{a}	4.2 ± 0.5^{a}	4.2 ± 0.5^{a}	4.3 ± 0.4^{b}	4.3 ± 0.4^{a}	4.1 ± 0.4^{b}	4.2 ± 0.5^{a}
	***p*-value**	**0.035**	**0.047**	**0.049**	**0.049**	**0.032**	**0.027**	**0.031**	**0.038**
Imea	10	2.5 ± 0.6	2.5 ± 0.6	2.6 ± 0.7	2.6 ± 0.7	2.6 ± 0.2	2.4 ±0.4^{b}	2.2 ± 0.3^{b}	2.6 ± 0.7^{b}
	5	2.6 ± 0.5	2.6 ± 0.5	2.7 ± 0.5	2.7 ± 0.5	2.7 ± 0.4	2.5 ± 0.4^{ab}	2.4 ± 0.4^{ab}	2.7 ± 0.5^{ab}
	1.7	3.8 ± 0.3	3.8 ± 0.3	0.38 ± 0.3	3.8 ± 0.3	3.9 ± 0.2	3.8 ± 0.2^{a}	3.5 ± 0.2^{a}	3.8 ± 0.3^{a}
	***p*-value**	**0.139**	**0.186**	**0.198**	**0.185**	**0.104**	**0.038**	**0.029**	**0.022**

The data was first analyzed with two-way ANOVA, but result showed no significant difference by cultivar. So, one-way ANOVA was performed within each cultivar.
The superscript letter represents the significant differences tested by posthoc Tukey's HSD test with $p<0.05$.

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway