THE EFFECTS OF LED LIGHTING ON THE COMMERCIAL CULTIVATION OF STEVIA REBAUDIANA

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ABSTRACT

Stevia rebaudiana is a perennial plant from the Asteraceae family, native to the highlands of Brazil and Paraguay. It is a high-value crop due to the strong commercial demand for its metabolites (steviol glycosides, SG) as an organic low-caloric sweetener with up to 300 times the sweetness of conventional sugar. *Stevia rebaudiana* a short-day plant, has a tendency for a shortened vegetative stage and early flowering when grown under a photoperiod of 12 h or less. The amount of SG in the leaves reduces by up to 50% after flowering. Given the strong commercial demand for Stevia products, and the limited supply available domestically, intense cultivation in controlled environment agriculture (CEA) systems is a viable option. Lighting energy can account for more than 70% of the total electrical energy used in a CEA system while the CEA system itself can consume up to 100% more electrical energy compared to a conventional greenhouse. This study included three experimental set-ups, using four different lighting strategies. All artificial lighting systems used high powered light emitting diodes (LED). In the first experiment, the plants were grown under Red + Blue light with photoperiods of 8, 12 or 16 hours (8H, 12H, 16H) and an intermittent photoperiod of 5 hours 20 minutes (16HI) per 8 hours (for a total of 16 hours each day). A control sample was grown under natural sunlight and photoperiod (12 hours) in the climate-controlled greenhouse (GH). In the second experiment, the plants were grown under 6 different spectral compositions that had a base Red + Blue (RB) spectra. The control plants were grown under pure RB spectra while others were grown under RB supplemented with Far Red (FR), Ultraviolet A (UVA), Blue (BR), Green (GR), FR+UVA+GR (FS). In the third experiment, varying fractions of UV-A and green light in addition to the base RB was used. Two treatments with green fractions (GR1 & GR2), two treatments with UVA (UV1 & UV2) and two treatments that had both (UVGR1, UVGR2). A separate set of plants were grown under RB and natural sunlight before being transferred to GR2, UV2, UVGR2, and monochromatic light

treatments of blue, green and UVA, for 3 and 10 days before harvest. Plants grown under the UVGR1 had the highest dry leaf biomass accumulation of 4.75 g plant^{-1} ($P<.05$). UVA had the highest metabolite (ST + Reb A) concentration of 27% ($P<.05$) while plants grown under sunlight had a mean SG concentration of 15%. UVGR1 had the highest metabolite yields and energy use efficacy of 1.05 g plant^{-1} and 30.24 mg kWh^{-1} ($P<.05$) respectively. In terms of productivity, the GR1 spectral composition was the most productive, producing 18.7 ($P<.05$) milligrams of ST + Reb A compounds for every mol of light used. Overall, this study demonstrated the effects of different lighting strategies on the productivity and energy use efficacy of indoor grown *Stevia rebaudiana*. It was observed that strategies that used spectral composition with green and UV-A were more productive and had higher efficacies compared to photoperiod manipulation, or the use of pre-harvest lighting.

Keywords : Stevia, horticulture, lighting, photoperiod, photobiology

ABSTRAK

Stevia rebaudiana, sejenis tumbuhan saka daripada keluarga Asteraceae yang berasal dari tanah tinggi Brazil dan Paraguay, mempunyai nilai tinggi kerana permintaan komersil yang kukuh untuk metabolitnya (steviol glycosides, SG) sebagai pemanis organik rendah kalori dengan kemanisan sehingga 300 kali ganda berbanding gula semula jadi. *Stevia rebaudiana* merupakan tumbuhan siang pendek, dan mempunyai kecenderungan untuk berbunga awal sambil mengalami peringkat pertumbuhan vegetatif yang pendek ditanam di kawasan yang mempunyai tempoh waktu siang yang kurang daripada 12 jam sehari. Jumlah kandungan SG dalam daun pokok Stevia akan berkurangan sehingga 50% apabila ia mula berbunga. Memandangkan permintaan komersial yang tinggi untuk produk Stevia, dan bekalan tersedia yang terhad di dalam negara, penanaman intensif dalam sistem pertanian persekitaran terkawal (CEA) adalah pilihan yang berdaya maju. Tenaga pencahayaan boleh menrangkum lebih daripada 70% daripada jumlah tenaga elektrik yang digunakan dalam sistem CEA, manakala sistem CEA sendiri boleh menggunakan sehingga 100% lebih tenaga elektrik berbanding rumah hijau konvensional. Kajian ini dibahagikan kepada 3 eksperimen, yang secara kesuluruhanya menggunakan empat strategi pencahayaan yang berbeza. Semua sistem pencahayaan yang digunapakai dalam kajian ini menggunakan teknologi diod pemancar cahaya (LED) yang berkuasa tinggi. Dalam eksperimen pertama, tumbuhan telah ditanam di bawah cahaya Merah + Biru dengan tempoh pencahayaan selama 8, 12 atau 16 jam (8H, 12H, 16H) dan tempoh pencahayaan terputus-putus selama 5 jam 20 minit (16HI) setiap 8 jam (untuk sejumlah 16 jam setiap hari). Sampel kawalan ditanam di bawah cahaya matahari dan tempoh pencahayaan semula jadi (12 jam) didalam rumah hijau yang dikawal iklim (GH). Dalam eksperimen kedua, tumbuhan telah ditanam di bawah 6 komposisi spektrum berbeza yang mempunyai spektrum asas merah + biru (RB). Tumbuhan kawalan untuk experiment kedua ditanam di bawah spektrum RB tulen manakala tumbuhan yang lain ditanam di

bawah cahaya RB yang ditambah dengan spektrum merah jauh (FR), ultraviolet A (UVA), biru (BR), hijau (GR), dan gabungan kesemua spektra FR+UVA+GR (FS). Dalam eksperimen ketiga, pelbagai pecahan UV-A dan spektrum hijau sebagai tambahan kepada spektrum asas RB telah digunakan. Dua rawatan pencahayaan dengan pecahan hijau berbeza (GR1 & GR2), dua rawatan dengan bahagian UVA berbeza (UV1 & UV2) dan dua system pencahayaan yang mempunyai kedua-dua spektrum hijau dan UVA (UVGR1, UVGR2) telah digunakan. Satu set tumbuhan yang berasingan ditanam di bawah RB dan cahaya matahari semula jadi sebelum dipindahkan ke GR2, UV2, UVGR2, dan rawatan cahaya monokromatik biru, hijau dan UVA, untuk selama 3 atau 10 hari sebelum penuaian. Tumbuhan yang ditanam di bawah UVGR1 menghasilkan pengumpulan biojisim daun kering tertinggi sebanyak 4.75 g tumbuhan^{-1}. UVA mempunyai kepekatan metabolit (ST + Reb A) tertinggi sebanyak 27% berbanding tumbuhan yang ditanam di bawah cahaya matahari yang mempunyai purata kepekatan SG sebanyak 15%. UVGR1 mempunyai hasil metabolit dan keberkesanan penggunaan tenaga tertinggi iaitu 1.05 g tumbuhan^{-1} dan 30.24 mg kWh^{-1} masing-masing. Dari segi produktiviti, komposisi spektrum GR1 adalah yang paling produktif, menghasilkan 18.7 miligram sebatian ST + Reb A untuk setiap mol cahaya yang digunakan. Secara keseluruhannya, kajian ini menunjukkan kesan strategi pencahayaan yang berbeza ke atas produktiviti dan keberkesanan penggunaan tenaga *Stevia rebaudiana* yang ditanam dalam system CEA. Diperhatikan bahawa strategi yang menggunakan komposisi spektrum hijau dan UV-A adalah lebih produktif dan mempunyai keberkesanan yang lebih tinggi berbanding dengan manipulasi tempoh pencahayaan atau penggunaan pencahayaan pra-penuaian.

Kata kunci: Stevia, hortikultur, pencahayaan, fotokala, fotobiologi

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LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
AI	Artificial Intelligence
ANOVA	Analysis of Variance
ANSI	American National Standards Institute
ASABE	American Society of Agricultural and Biological Engineers
BR	Blue Red
BTU	British Thermal Unit
C	Celsius
CAGR	Compound Annual Growth Rate
CCT	Correlated Colour Temperature
CEA	Controlled Environment Agriculture
CIE	International Commission on Illumination
CO_2	Carbon Dioxide
COAG	The Council of Australian Governments
CPD	Cooling Power Density
CRI	Colour Rendering Index
CRY	Cryptochromes
DAP	Days After Planting
DC	Direct Current
DLC	Design Lights Consortium
DLI	Daily Light Integral
DMAPP	Dimethylallyl diphosphate
DW	Dry Weight
EC	European Commission
EFSA	European Food Safety Authority

EMO	Environmentally Modified Organisms
EU	European Union
EUE	Energy Use Efficacy
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
FR	Far Red
FS	Full Spectrum
FSANZ	Food Standards Australia New Zealand
FTL	Fluorescent Tubular Lamps
FW	Fresh Weight
FWHM	Full Width Half Maximum
GA	Gibberellic Acid
GH	Greenhouse
GHG	Green House Gases
GR	Green
GRAS	Generally Recognised as Safe
HDL	High Density Lipoprotein
HPLC	High Performance Liquid Chromatography
HPS	High Pressure Sodium
HSD	Highly Significant Difference
HVAC	Heating, Ventilation and Air-Conditioning
IBM	The International Business Machines Corporation
IEC	International Electrotechnical Commission
IEEE	Institution of Electrical and Electronics Engineers
IES	Illuminating Engineering Society
IOT	Internet of Things

IPP	Isopentenyl diphosphate
JECFA	Joint Expert Committee on Food Additives
JTC	Joint Technical Committee
KA	Kaurene Acid
KA13H	Kaurenoic Acid 13-hydroxylase
KO	Kaurene 19-oxidase
LCMS	Liquid Chromatography Mass Spectrometry
LCMSMS	Liquid Chromatography Tandem Mass Spectrometry
LD	Long Day
LDL	Low Density Lipoprotein
LED	Light Emitting Diode
LLC	Limited Liability Company
LM	Lighting Measurement and Testing
LPD	Lighting Power Density
LUE	Light Use Efficacy
MEP	Methylerythritol 4-phosphate
MH	Metal Halide
NKEA	National Key Economic Area
NPRA	National Pharmaceutical Regulatory Authority
NREL	National Renewable Energy Laboratory
PAR	Photosynthetically Active Radiation
PBAR	Plant Biological Active Radiation
PC	Phosphor Converted
PCE	Photon Conversion Efficacy
PES	Polyethersulfone
PF	Plant Factory

PFD	Photon Flux Density
PH	Pre-harvest
PHOT	Phototropins
PHY	Phytochromes
PPE	Photosynthetic Photon Efficacy
PPF	Photosynthetic Photon Flux
PPFD	Photosynthetic Photon Flux Density
PSS	Phytochrome Photostationary State
PWI	Preliminary Work Items
PWM	Pulse Width Modulation
QPL	Qualified Product List
QTOF	Quadrupole Time of Flight
RB	Red Blue
SD	Standard Deviation
SG	Steviol glycosides
SPSS	Statistical Package for the Social Sciences
SREE	Stevia rebaudiana Ethanolic Extract
SS	Spectrum Strategy
SSL	Solid State Lighting
ST	Stevioside
STZ	Streptozotocin
TC	Technical Committee
TG	Triacylglycerol
TM	Technical Memorandum
TW	Chinese Taipei
UAE	United Arab Emirates

UDP	Uridine diphosphate
UGT	Uridine diphosphate dependent glycosyltransferase
UK	United Kingdom
UM	University of Malaya
USA	United States of America
USD	United States Dollar
UV	Ultraviolet
UVA	Ultraviolet A
W	Watt
WHO	World Health Organisation
YPF	Yield Photon Flux

CHAPTER 1 : INTRODUCTION

1.1 Background

The global market for herbal products was valued at USD70 billion in 2020 and is projected to grow at a compounded annual growth rate (CAGR) of 6.5% to USD124 billion in 2028 (Vantage Market Research, 2022). The World Health Organisation (WHO) stated that between 75% to 80% of the global population depend on herbal medication for their healthcare needs and more than 25 out of the 250 drugs classified as essential are botanically derived (Bareetseng, 2020). In Malaysia, the herbal industry was identified in 2011 as a potential area of focus under the New Key Economic Areas (NKEA) with a market that is projected to grow up to RM28 billion in 2028 (Malaysian Investment Development Authority, 2021). More than 50% of total products registered by the Malaysian National Pharmaceutical Regulatory Agency (NPRA) in 2019 were natural products (Malaysian Investment Development Authority, 2021). According to the Malaysian National Traditional and Complementary Medicine (T&CM) Blueprint (2018-2027), the use of plant based medicine could reduce costs of modern healthcare by up to RM13 billion in 2027 (Malaysian Investment Development Authority, 2021).

Stevia rebaudiana is an herb that is gaining popularity globally as a non-calorific sweetener with a global market of USD650 million in 2021. The demand for Stevia is expected to grow, resulting in a CAGR of 8.9% globally, reaching a market value of USD1.4 billion in the year 2030 (Straits Research, 2022). In Malaysia, products derived from Stevia are not only used as a non-calorific sweetener but also as an herbal supplement (Rengasamy et al., 2022a). The occurrence of overweight and obesity among adults in Malaysia rose to 30.0% and 17.7% respectively in 2015 from 16.6% and 4.4% respectively in 1996, while the rate of childhood obesity stood at 11.9%. Hypertension (30.3%), diabetes (17.5%) and, hypercholesterolemia (47.7%) are other diseases ravaging Malaysians (Saharudin et al., 2020a). Most of these issues are associated with the

overconsumption of sugar and high calorie foods. This has driven the need for a safe and healthy option, and Stevia is an ideal candidate. Besides having no known side effects, unlike artificial sweeteners, Stevia is also reported to have other therapeutic properties that can assist in insulin regulation, and managing obesity and hypertension, among others (Peteliuk et al., 2021).

At present, Stevia leaves and products available in Malaysia are sourced from China, India, South America and other locations as large-scale cultivation of Stevia for commercial purposes is still not popular. Being a short-day plant, Stevia would flower early under Malaysian environmental conditions, reducing the overall yield quality and quantity (Othman et al., 2018; Tan et al., 2008) . Availability of resources such as land and manpower, while not unique to Stevia, is another factor limiting its commercial cultivation in Malaysia. The use of controlled environment agriculture (CEA) systems has been promoted as a possible option to address the issues associated with traditional farming. The most common CEA is a climate-controlled greenhouse (GH) that has all environmental parameters such as temperature, humidity and nutrient content controlled, with sunlight being the only external factor. Meanwhile, plant factories (PF) where artificial light is used as the primary light source. In PFs, all elements that is best suited for the cultivation of the plant can be controlled facilitating the highly optimized yields (An et al., 2021; Shaari et al., 2021).

1.2 Research Scope

The scope of this research was limited to the use of artificial lighting as the primary light source for the photosynthetic and photo morphological activities of *Stevia rebaudiana*. The laboratory analysis of the steviol glycosides of the plants, the preparation of special medium, the design and construction of specialized lighting facilities and phenotyping facilities are not within the scope of this research. The variant of the *Stevia rebaudiana* used for this research is of the standard commercially available type. No specific genotype

was selected. While is it acknowledged that other cultivation parameters such as soil, fertilisers, temperature, humidity, and other non-lighting parameters can have a positive effect on indoor cultivation of Stevia, this study was focused only on the lighting aspects. As such, all other parameters were not evaluated, and care was taken to ensure that similar parameters and materials was used for all experiments and replications.

This research was focused towards identifying the effects of the light quality, quantity, and photo-period on the growth, biomass, and steviol glycoside content of the *Stevia rebaudiana* plant. Only currently available lighting technology was considered for this research. Future and experimental technologies were not considered.

1.3 Research Problem

The growing demand for herbal products and the increasing occurrence of diabetes and obesity among adolescents globally is driving overall demand for a sustainable and reliable supply of the *Stevia rebaudiana* herb. This surging demand underlines the need for a higher biomass yield from each plant to meet downstream activities in the local and global herbal industry. Malaysia's unpredictable weather patterns with extended drought and higher than usual rainfall (Malaysian Meteorological Department, 2019) experienced in the past years has taken a toll on conventional open field farming techniques resulting in a loss of yield and a disruption in the overall supply chain to produce herbal products. To support the local herbal industry in a sustainable manner, the quality of the raw materials supplied has to be maintained with minimum variation from batch to batch. The nature of the plant that is sensitive to its environmental conditions, resulting in different quality levels of its chemical composition, makes it difficult for producers to be able to source materials with equivalent chemical contents from different sources and locations. At present, most local herbal companies obtain their supply of raw materials from overseas sources. The increasing demand with limited supply and the fluctuating currency and economic situation poses a risk of endangering the supply of the required raw

material. The increasing logistical and transportation costs is affecting the cost of raw materials. To sustain and drive the local herbal industry, a secure source of materials is required domestically. The relatively short day in Malaysia is not conducive for large scale commercial cultivation of *Stevia rebaudiana* due to the lower yield per acre and lower steviol content of locally grown plants. The short-day results in early harvests of locally field grown plants with a yield of approximately 2.8 tonnes per hectare of dried leaf biomass locally as compared to an average of 5-8 tonnes per hectare from China and India (Othman et al., 2018; Tan et al., 2008). The current practice of open field farming using either traditional or modern industrial techniques is land resource intensive. With a planting density of between 50,000 to 75,000 plants per hectare (Munz et al., 2018; Parris et al., 2016), the growing demand for this plant would result in the need for new land to be cleared for its cultivation. While it is a common misconception that Malaysia has sufficient agricultural land available, with the current rampant illegal and unplanned clearing and exploration of land in the name of agriculture, a higher and more efficient land utilization is required to support the new commercial scale herb cultivation to avoid the past mistakes made during other cash crop booms.

While the use of artificial lighting as a supplemental lighting is not new, the large-scale commercial adoption of artificial light source as either a primary or supplemental light source has been relatively low especially in the Southeast Asian region. The high capital cost and the perceived technical complexities associated with the SSL technology have been a key factor for this. The current horticultural lighting systems that employ high end expensive ceramic based high powered LEDs are not only expensive, but their implementation is also made difficult due to its technical requirements for the system's cooling, driving and control. Another key challenge with regards to the use of artificial lighting as a primary light source has been the overall energy consumption of the system. In previous systems, less efficient fluorescent and high intensity discharge systems were

used that flooded the plants with an unweighted light spectrum. These systems were typically high-powered systems consuming hundreds of watts of power per lamp and generating a huge amount of heat as a by-product. The need for additional cooling in addition to extra electrical energy has been a detrimental factor discouraging the use of artificial light as the primary light source. This research addressed the challenges of cultivating Stevia in Malaysia using a PF, employing artificial lighting strategies to not only improve the overall yields of SG metabolites but to also improve the overall electrical energy use efficacy.

1.4 Research Objectives

This research has the following objectives:

1. To identify the effects of artificial light intensity and photoperiod on the biomass and metabolite yields of indoor cultivated *Stevia rebaudiana.*
2. To ascertain the effects of varying spectral compositions on the biomass and metabolite yields of indoor cultivated *Stevia rebaudiana.*
3. To investigate the effects of varying Green and Ultraviolet A (UVA) spectral fractions, and the use of Green and UVA pre-harvest treatments on the biomass and metabolite yields of indoor cultivated *Stevia rebaudiana.*
4. To determine the lighting strategy that has the highest productivity and energy use efficacy for the indoor cultivation of *Stevia rebaudiana*.

1.5 Importance and Relevance of Research

This is the first study to evaluate the full cycle of *Stevia rebaudiana* cultivation under full artificial light from seed germination to harvest. All experimental cycles in this study were started with seed germination, unlike previous studies that used plantlets, seedlings, or cuttings as a base. The present study also employed a 175-day planting cycle that was replicated 3 times over the experimental period. Comparable, past studies on the effects

of artificial light in Stevia had shorter experimental periods, with some reporting this shorter period to be the reason for a lack of findings (de Andrade et al., 2021; Yoneda et al., 2017b). In this study, the biomass and metabolite yields were quantified. From a commercial perspective, the final realizable metabolite yields in terms g plant^{-1} are of utmost importance. While past studies on the effect of light on Stevia declared the overall biomass yields, metabolite concentrations or both, not many studies published the final realizable metabolite yields, specifically the full cycle yields (Ceunen et al., 2012a; Esra et al., 2016a; Yoneda et al., 2017a).

In the current study, multiple lighting strategies and treatments were used to identify the most productive and effective strategy. This study used light emitting diodes (LED) from amsOSRAM (amsOSRAM, 2021), Cree (Cree, 2021), Edison (Corporation, 2023), that had a narrow waveband. The spectral purity of these type of high-powered LEDs is significantly higher compared to phosphor converted (PC) LEDs used in past studies (Evans et al., 2015; Shulgina et al., 2021; Yoneda et al., 2017b). Past studies used PC LEDs to provide white light, while many studies also used standard fluorescent lamps. The spectral information was often not declared for these studies. In the present study, each light treatment was specially curated with its spectral composition carefully measured. This enabled a more precise measurement to be made. This was also the first study to use varying fractions of green and ultraviolet-A (UV-A) light to elicit higher productivity of Stevia, besides being the first study to use pre-harvest treatments to improve the quality of artificial and natural lighted Stevia plants.

The use of alternative measurement approaches to define the productivity and energy use efficacies of the different light strategies on Stevia in this study was also a first. Past studies on Stevia measured productivity in terms of yields. In this study the use of photon conversion efficacy (PCE) that represented the effectiveness of the full light spectrum to produce SG yields was used extensively. The PCE gave a true representation of the

spectral productivity, and it also highlighted the limitation of the current industry definitions of photosynthetically active radiation (PAR) and photosynthetic photon efficacy (PPE). This was also the first study to evaluate the energy consumption and energy use efficacy (EUE) for indoor cultivation of Stevia under artificial and natural light.

1.6 Thesis Structure

This thesis is structured in the article type format following University of Malaya's Guidelines for the Preparation of Research Reports, Dissertation and Thesis 2021. Each chapter with results (Chapters 3-6) has its own subdivision of introduction, brief literature review, results, discussion, and conclusion. The overall structure of the thesis is as follows:

- Chapter 2: Literature Review
    - This chapter is divided into two sections. The first section presents a detailed description on the history and current status of LEDs in horticulture, highlighting the challenges and opportunities that LEDs bring to the horticulture lighting application. The second section presents an overview of Stevia together with an overview of past studies on the effect of light quality, quantity and intensity on *Stevia rebaudiana.*
- Chapter 3: The Effect of Photoperiod on *Stevia rebaudiana*
    - In this chapter, Stevia seeds were germinated under different photoperiods and intensities while maintaining an identical daily light integral (DLI) and spectral composition. The rate of flowering, yields, PCE and EUE values were evaluated.
    - Methodology : Fixed spectrum, varying intensity and photoperiod to achieve similar DLI across all treatments
- Chapter 4: The Effect of Light Quality on *Stevia rebaudiana*

- Here, Stevia seeds were germinated under different spectral compositions with the same DLI and photoperiod. The overall rate of germination, flowering and yields were measured.
- Methodology : Fixed intensity, photoperiod, and DLI. Varying spectrum content across different light treatments.

- Chapter 5: The Effect of Green and UV-A Fractions, and Pre-Harvest Treatments on *Stevia rebaudiana*
  - Two different experiments were done. In experiment 1, Stevia seeds were germinated under treatments that had different fractions of green, UV-A or both. In the second experiments, Stevia plants that had grown under natural sunlight or a base red and blue LED light were then subjected to either a 3-day or 10-day pre-harvest treatment. The yields for both experiments were measured, while the germination and flowering rate was measured for the first experiment.
  - Methodology :
    - Experiment 1 : Fixed intensity, photoperiod and DLI, with varying fractions of Green and UVA spectrum
    - Experiment 2 : Plants cultivated under sunlight or red-blue (RB) spectrum are then subjected to either 3 or 10 days pre-harvest light treatment under Green, Blue, UVA monochromatic light and RB+UVA, RB+B, RB+GR multispectral light.
- Chapter 6: Overall Energy and Photon Conversion Efficacy Analysis
  - In this chapter, the overall electrical energy profile of both the GH and PF were measured. Subsequently, the overall PCE and EUE for all strategies from Chapters 3, 4 and 5 were evaluated.
- Chapter 7: Conclusion

- This chapter provides a general conclusion of the thesis based on the results and discussion sections from chapters 3-6 and reflects its fulfilment of the respective research objectives and problems. The potential future work on the topic is also discussed in this chapter.

CHAPTER 2 : LITERATURE REVIEW

2.1 LEDs in Horticulture

2.1.1 Introduction

The use of artificial lighting for plant growth has been explored since the mid-19th century (Mangon, 1861; McCree, 1971). The use of artificial light with continuous wide spectral range from 350 to 750 nm, was previously confined mostly to greenhouses situated in latitudes where seasons with short days were present (Paucek et al., 2020a; Viršilė et al., 2017). Supplemental light was provided to enhance and extend the photoperiod in these areas, extending the growth cycles of selected crops into seasons that would otherwise be not suitable for cultivation (Katzin et al., 2021; Shailesh, 2019). Artificial light is also used as a primary light source for indoor tissue cultivation (Bantis et al., 2018). Incandescent lamps were the first types used in greenhouses before it was replaced with the more efficient high pressure discharge lamps (Gupta & Agarwal, 2017). Florescent lamps (FTL) were however the light source of choice for tissue cultivation as it emitted lower radiant heat and could be placed closer to the samples. At present, the High-Pressure Sodium (HPS) lamps, typically used for street and area lighting are still the most commonly used light source in greenhouses (Wu et al., 2020a). The HPS and FTL lamps currently used are however not spectrally optimised for photosynthetic and plant development activity, and is optimised for general lighting applications, prioritising human visual acuity (Paucek et al., 2020a). Light emitting diodes (LED) are increasingly being adapted to replace traditional technologies in general lighting applications (Paucek et al., 2020a). As the technological advancement of general lighting LEDs progress, it provides opportunities for a major evolution within horticultural lighting (Viršilė et al., 2017; Wu et al., 2020a).

With the increasing use of LEDs in new horticulture applications such as vertical farms, controlled environment agriculture (CEA) systems or as a replacement for traditional light sources in existing installations, there is a need for careful consideration and understanding of the challenges, opportunities and characteristics of LEDs that was not common to previous technology. Unlike previous technologies that were adapted from general lighting applications, LED based horticulture light sources are specifically designed for optimum plant growth and development (Kusuma et al., 2020; Paucek et al., 2020a; Viršilė et al., 2017). Past reviews focused on the application and effects of the different wavelengths on plant development and growth, on the global horticulture lighting market, and the energy efficiency and efficacy of horticulture lighting system.

This chapter presents a comprehensive review covering the critical elements that are unique to LED based horticulture lighting systems, different from traditional lighting technologies, as well as the current challenges and future direction with regards to the application and adoption of LEDs in horticulture lighting. In this study, LEDs refers to the LED package and does not refer to the chip housed within the package, the complete luminaire, or the bulb.

2.1.2 Plants and Light

Plants use light energy, harvested via a series of photo pigments, to synthesis energy via photosynthesis. (Demotes-Mainard et al., 2016; Macchia et al., 2007; Yadav et al., 2020). The quality, quantity and photoperiod of light also affects the development of plants, from germination to reproduction, seedling de-etiolation, stem elongation, phototropism, movement of stomata, triggering shade avoidance response, maintenance of circadian rhythms, synthesis of metabolites and regulation of flowering time in plants (Deng & Quail, 1999; Lazzarin et al., 2020; Liu et al., 2020). The primary photosynthetic pigments have peak absorption wavelengths of 430 nm and 665nm for chlorophyll a, and 453 nm and 642nm for chlorophyll b (Figure 2.1). Most plants also have other photoreceptors,

each with its own function and wavelength sensitivity (Ouzounis et al., 2015; Zheng et al., 2019b). The phytochromes (PHY), absorbs light in the red and far-red (FR) light spectrum from 600 to 800 nm and influences the plant's developmental performance, including gravitropism, phototropism, and shade avoidance response (Brouwer et al., 2014; Shafiq et al., 2020; Shinomura et al., 1996) while the cryptochrome (CRY), with an absorption spectra in the 350 nm to 500 nm ultraviolet A (UV-A) and blue range, regulates the physiological and developmental processes, including photomorphogenesis, flowering, circadian clock regulation and stress response (Sullivan & Deng, 2003; Wang et al., 2014). The phototropins (PHOT) with an absorption spectra similar to CRY, are photoreceptors that control a wide range of responses such as the stomatal opening, phototropism, movement of chloroplast, de-etiolation and leaf flattening (Ballaré & Casal, 2000; Kasahara et al., 2002; Li & Mathews, 2016; Mawphlang & Kharshiing, 2017). The ultraviolet B (UV-B) sensitive UVR8 photoreceptor, with sensitivities in the range of 280 nm to 315 nm, is responsible for initiating plant stress responses that includes the accumulation secondary metabolites (Jenkins, 2017; Rizzini et al., 2011; Zheng et al., 2019b).

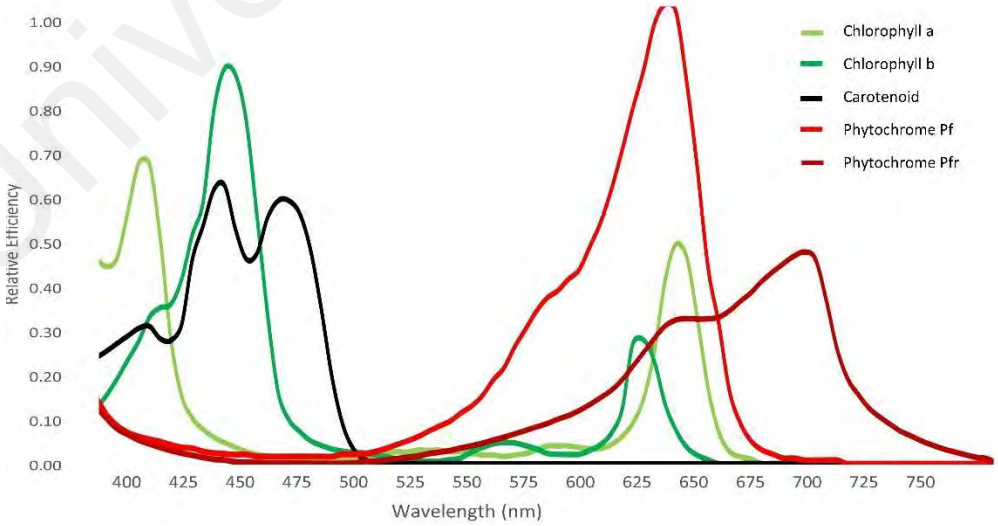

Figure 2.1 : Photosynthetic Pigments Absorption Spectra. Sourced from amsOSRAM (2020)

2.1.3 Photometric, Radiometric, and Photosynthetic Properties of Light

Early studies on the relationship between light and plants used photometric measurements and terminology to describe and illustrate the light quality, intensity, and efficiency (Ashdown, 2019a; Burns, 1933; Gilewski, 2019; Hoover, 1937). In most of these studies, artificial light sources used were of the traditional incandescent and discharge type, with broadband spectral emissions (Kumari et al., 2014; Sipos et al., 2020). As photometric measurements were based on the human eye sensitivity curve (Runkle & Bugbee, 2013; Sipos et al., 2020) and is not a representation of the radiant energy emitted, subsequent studies moved towards the use of radiometric values. Total luminous flux, represented in lumens was replaced with total radian flux, expressed in Watts (Table 2.1). The photosynthetic active radiation (PAR) range, where photosynthetic organisms can synthesize carbohydrates from the carbon in carbon dioxide (CO_2), was defined to be between 400 nm to 700 nm with an equal weightage of all photons, regardless of the difference in energy between the wavelengths (Bugbee, 2016). The PAR range assumes that radiation within this range complies to the Stark-Einstein law, that states every photon or quantum absorbed will excite exactly one electron, regardless of the photon's energy (Ashdown, 2019a; Kusuma et al., 2020; Mashkov et al., 2017).

Multiple studies in the early 20th century have shown that in single leaves, the assumption that radiation within the PAR range complies to the Stark-Einstein law may not be entirely accurate (Burns, 1933; Hoover, 1937). McCree (1971) revisited the topic, measuring the spectral absorptance and quantum yield for the leaves of 22 different plant species. Taking the measurements at 25 nm intervals under low intensity and a short photoperiod, McCree (1971) developed the absorption spectra curve that illustrates the relative amount of light absorbed by the leaves at the various wavelengths, and the relative quantum yield that represents the relative rate of photosynthesis per absorbed photon (μmol s^{-1}), of the plants studied (Figure 2.2). A third curve, the action spectrum, referring to the relative rate of

CO_2 uptake as a function of energy received (J s^{-1},W) was also developed for each wavelength. The action spectrum is often referred to as the yield photon flux (YPF) while the quantum yield spectrum is referred as the photosynthetic photon flux (PPF) (Ashdown, 2019a). The relationship between these 2 terms is given by the Planck – Einstein's relation (Kusuma et al., 2020; Sipos et al., 2020) :

$$E = hc/\lambda$$

Where,

E = Energy of a photon (quantum energy)

h = Planck's constant (6.626 x 10-34 J s^{-1})

c = Speed of light in vacuum (2.998 x 10^8 m s^{-1})

λ = Wavelength in meters

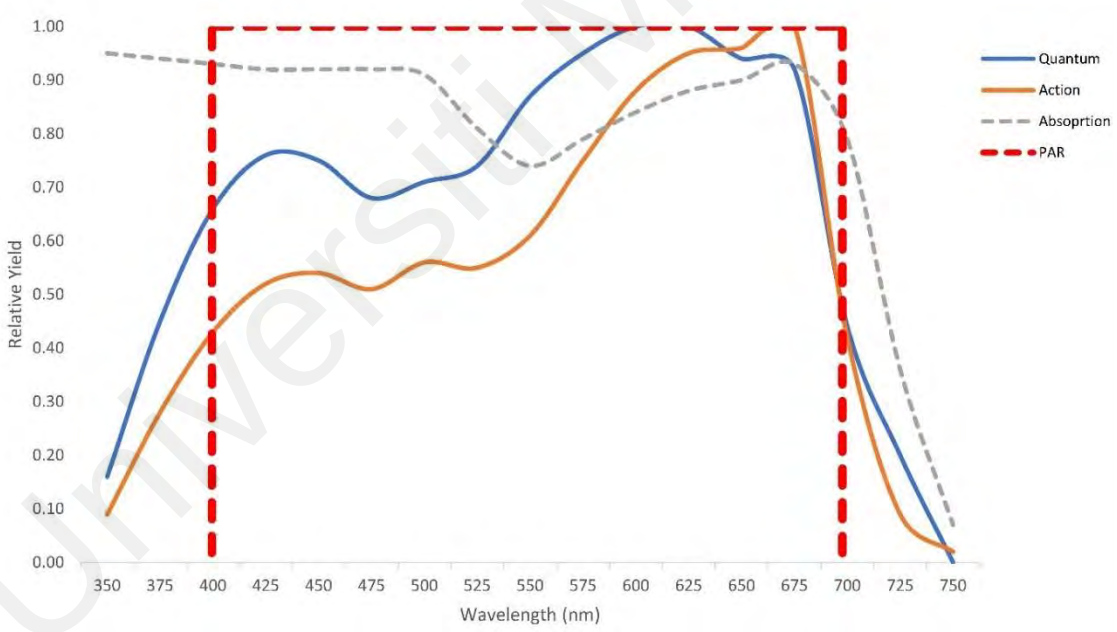

Figure 2.2 : McCree Curves and PAR. Drawn from data obtained from McCree (1971) chamber grown plants.

The PPF is still the preferred term for horticulturist while YPF is used mainly for energy balance calculation of photosynthetic organisms (Ashdown, 2019a; Bugbee, 2016). Although McCree (1972) stated PPF was a better predictor of photosynthetic activity over YPF, especially under conditions where different spectral power distributions are

considered, he went on to state that neither PPF nor YPF are ideal representations of PAR, as effectiveness of blue relative to red wavelengths are overestimated in both (Ashdown, 2019a; McCree, 1972).

The action and quantum spectra curves put forward by McCree (1971) is still one of the most used references within the horticulture lighting world. However, as the curves are representative in relative to each other with the highest value set arbitrarily at 100% or 1, it does not mean wavelengths of values close to 100% or 1 are absorbed entirely during photosynthesis and converted to energy. In reality, only 4% to 6% of absorbed radiation is converted to chemical energy (Zhu et al., 2010). The curves are also not a specific indicator of plants lighting needs nor is it the ideal plant photosynthetic lighting profile. The McCree curves were based on individual leaf photosynthetic efficiency under individual wavelengths and does not indicate whole plant photosynthetic response or the effects of multiple wavelengths working in tandem (Viršilė et al., 2017; Wu et al., 2019).

Table 2.1 : Photometric, Radiometric and Photosynthetic Terminology

	Photometric	**Radiometric**	**Photosynthetic**
Range / Limits	Evaluation based on specific response curves	Unweighted evaluation from 1nm to 1mm	Unweighted evaluation from 400 nm to 700nm
Total output from a source	Total luminous flux (lumens), lm	Radiant Power (Watts), W	Photosynthetic Photon Flux (PPF), µmol s^{-1}
Incident energy per unit area	Illuminance (lux), lm m^{-2}	Irradiance, W m^{-2}	Photosynthetic Photon Flux Density (PPFD), µmol $m^{-2}s^{-1}$
Efficiency / Efficacy	Lumens per watt, lm/W	Efficiency, %	Photosynthetic Photon Efficiency (PPE), µmol J^{-1}

2.1.4 Horticulture Lighting Market

The global horticulture lighting market has experienced phenomenal growth over the past years and is expected to continue the trend, growing from USD2.3 billion in 2020 to USD6 billion in 2025, at a compound annual growth rate (CAGR) of 21.40% during the period (Paucek et al., 2020a). LED based technology is expected to dominate the growth

with an expected growth from USD576 million in 2016 to USD5.1 billion by 2022 (Paucek et al., 2020a). In the United States of America (USA), Elliott et al. (2020) reported that LED based horticulture lighting is dominating the growth in indoor sole sourced, non-supplemental application, growing from 66% adoption in 2017 to 100% in 2019 for vertical farms, and from 4% to 11% adoption in not stacked indoor farms. Discharge technologies such as HPS, continue to dominate the greenhouse supplemental lighting in the USA, with LED adoption at a consistent 2% from 2017 to 2019 (Elliott, 2019; Elliott et al., 2020).

The adoption of LEDs in horticulture lighting is driven by several technical, commercial, and regulatory factors. Significant improvements in LED package efficiencies have led to the increased proliferation of the technology in general lighting applications. LED based lighting systems accounted for approximately 44% of global lighting sales in 2020 and continues to grow annually (Elliott, 2019). This has resulted in an increase of both front end and back-end LED processing facilities and capacities resulting in reduced costs. As horticulture LEDs use similar technologies and manufacturing processes as general lighting LEDs, it has benefitted from these improvements, making horticulture specific LEDs more efficient and cost effective compared to conventional lighting technologies (Bantis et al., 2018; Pattison et al., 2016). This has reduced the overall cost of adoption for new installations and retrofits (Pattison et al., 2016).

The increase in global energy costs and the push for a more environmentally friendly agriculture practise has also contributed significantly to the use of LEDs in horticulture. In CEA systems, lighting accounts for a major energy cost, often more than 60% of total energy needs, while in greenhouses supplemented with artificial lighting, the energy consumption is up to twice that of unlighted greenhouses (Elliott et al., 2020; Graamans et al., 2018; Katzin et al., 2021). HPS lamps converts approximately 30% (Paucek et al., 2020a) of its consumed energy into light with the remaining 70% converted into heat,

while a monochromatic blue LED has a conversion efficiency of up to 93% (Kusuma et al., 2020). Besides being able to realise a savings in lighting energy costs, the higher efficiencies of LED based horticulture lighting product allows growers to increase the lighting intensity or expand their growing space without increasing their total energy demand (Elliott et al., 2020). This is especially crucial is areas where electricity supply is limited and an increase in required maximum demand would result in costly upgrades of the overall electrical energy supply system (Elliott et al., 2020).

Subsidies and incentives from the government and private sectors, promoting the use of energy efficient practises and products such as LED lamps and luminaires, are also a key catalyst, increasing market adoption and acceptance of LED lighting in general and horticultural lighting applications (Elliott, 2019). In the USA alone there are 376 incentives and policies available with regards to energy efficient lighting and LEDs (N.C. State University, 2021). The Indian government subsidizes the distribution of LED bulbs to promote their use over traditional halogen and incandescent lighting products (Sipos et al., 2020) while the Malaysian government has implemented several tax incentives for investments and use of green and energy efficient products including LED lighting systems (Malaysian Green Technology And Climate Change Centre, 2021). Besides fiscal and not fiscal incentives, the introduction of multiple regulatory restrictions on the manufacture, sale and use of lower efficient lighting products has accelerated the adoption of LEDs in these regions.

In 2009 the European Union (EU), via Regulation (EC) No. 244/2009 phased out incandescent lamps, aiming at reducing energy and resource consumption in Europe. In December 2019, the EU issued the new “Single Lighting Regulation” that combined all relevant past regulations and takes into consideration of all available lighting technologies including LEDs (Fligge, 2020). These regulations also spelled out minimum energy performance and lifetime requirements that need to be met by lighting products intended

for sales and use in the EU. The high efficiency requirements not only pushed out traditional incandescent lamps but also low efficient discharge and LED ones. This left a vacuum in the market as replacement lamps were not widely available, pushing for the retrofits with LEDs to be used in place. Similar regulations have been passed in the USA, Russia, Brazil, China, and South Korea (Sipos et al., 2020). The Council of Australian Governments (COAG) Energy Ministers, in April 2018 agreed to the phasing out of inefficient halogen lamps in Australia and to the introduction of minimum standards for LED lamps in Australia and New Zealand in line with EU directive, effectively phasing out the remaining incandescent and halogen lamps where an equivalent LED light bulb is available, from the Australian market (Energy Rating, n.d.). These regulations have resulted in the widespread distribution and use of LED based systems that has made its way from general lighting to horticulture lighting applications.

Indoor vertical farms and CEAs are being touted as a complementary solution to the global food crisis, being able to support the food supply chain with lower transportation costs and energy usage (Graamans et al., 2020). While it may not be able to entirely replace commercial agriculture practises, the use of a controlled environment system that includes artificial lighting, can lead to year-round production and improved land usage with vertical stacking of the growth areas (Graamans et al., 2020; Paucek et al., 2020a; Xu, 2019). Water and use of chemicals can also be reduced drastically, providing a safer food supply. The indoor vertical farming industry is expected to experience an exponential growth from 2022 to 2027, reaching a global market size of more than USD 17 billion by the year 2027 (Paucek et al., 2020a). Vertical farm start-ups continue to attract investors with investments increasing from approximately USD38.1 million in 2016 to USD406.54 million in 2020 (i3, 2021a). As vertical farms use LED based horticulture systems primarily due to its low radiant heat, the adoption of LEDs in vertical farm lighting systems is expected to continue to grow in line with the expansion of new

facilities. Besides the boom in vertical farms, a major driver in horticulture lighting has been the expansion and introduction of new cannabis growing facilities (Paucek et al., 2020a). The legalization of cannabis cultivation and sale in several states of the USA in 2012, and the Canadian government to allow the recreational use and production of cannabis in 2018, boosted the cannabis cultivation industry (Hammond et al., 2020). Due to the limitation in licensed cultivation area, and due to security concerns, cannabis is typically grown in single layered CEA systems with high light intensities. Early cultivation of cannabis used HPS and other high powered discharge lamps as light sources. However, with the improvement in LED system efficiencies and the positive effect of LEDs on final yields, the use of LEDs lighting has increased (Magagnini et al., 2018; Namdar et al., 2019; Paucek et al., 2020a). As cannabis is a crop with high commercial value, the higher initial capital requirements of LED based systems were not a significant barrier to entry for this market segment, as evident by the high number of cannabis specific LED horticulture lighting system available in the market (Paucek et al., 2020a).

2.1.5 Specific Consideration for LEDs in Horticulture

2.1.5.1 LED operating principle

Discharge lamps and LEDs both emit radiation due to release of excess energy from electrons however, unlike discharge lamps where electrons are impelled into a higher state resulting in thermionic excitation due to electric arcing, LEDs comply to the principle of electroluminescent, emitting radiation as the electrons pass on to a lower energy orbital (Gupta & Agarwal, 2017). This reaction occurs in the chip housed within the LED package. The p-n junction within the chip is doped with chemical impurities. As an electron meets a hole in the depletion region, the drops to the valence band from the conduction band, resulting in the emission of a narrow bandwidth irradiation (Gupta & Agarwal, 2017; Wu et al., 2020a). The energy and wavelength of the photons released is

determined by the material used in the p-n junction (Wu et al., 2020a). Indium Gallium Nitride (InGaN) and Gallium Nitride (GaN) are the most commonly used material for blue LEDs and as the base for white LEDs, while red LEDs are typically made from Aluminium Indium Gallium Phosphide (AlInGaP) materials (Gupta & Agarwal, 2017). Coloured LEDs are produced either by using the different chip materials where light emitted from the chip within the package is desired colour, or by using a blue LED that is used to excite one or more phosphors to achieve the required colour (Zhuo et al., 2018). The phosphor converted (PC) types typically have a wider spectrum spread compared to the direct emitted LEDs and is also the main conversion method used in white LEDs (Lin et al., 2017; Zhuo et al., 2018).

The unique characteristics of LEDs present a host of new opportunities for horticultural applications. Likewise, as LEDs operate on a different principle and its design and parameters can vary by type, manufacturer and application, careful evaluation and understanding on areas not previous considered under traditional technologies are needed. Discharge lamps have been in use for more than a century and is a product that is highly regulated and standardised. The standards available for traditional lighting spell out the operating requirements, from the driving current and voltage through to the physical dimensions and the maximum allowed operating temperatures for the products (International Electrotechnical Commission, 2011). When evaluating and considering the use of LEDs in horticultural applications, users will have to pay attention to 4 areas where use of LEDs has resulted in a major difference compared to traditional lighting technologies. The 4 specific areas are 1) the spectra, 2) efficiency, 3) reliability, and 4) standards.

2.1.5.2 Spectral composition, distribution, and purity

It has been established that light quality, intensity, and photoperiod have significant effects on the growth and development of plants. However, the light quality or spectral

aspect of the lighting system, is not a parameter that can be easily rectified or altered during applications, unlike intensity that can be adjusted by simple dimmers or by adjusting the distance between the luminaire and the plant canopy, and the photoperiod that can be altered by a simple flick of the switch. Traditional technologies had limited single colour waveband selections and no narrow bandwidth option. These lamps typically had fixed broadband emission spectra even in single colour varieties. LEDs have more than 100 options for narrow band spectra commercially available in the market although at present most horticulture lighting systems only use approximately 10 of it (Wu et al., 2020a). LEDs offer the option to optimise the light recipe by highly customising the spectral composition needed for the plant by combining several narrow band LEDs or even having a combination of narrow and broad band LEDs in a single system.

2.1.5.3 Photosynthesis and Photosignaling

In LED based horticulture systems, the selection of the appropriate light recipe or spectral composition is important to elicit the optimum intended plant response. The optimised spectral composition varies with the plant species, lifecycle stage, and the desired outcome (Zheng et al., 2019b). Most horticulture lighting products emit light in the blue and red combined range as these lights have been deemed to be best suited for photosynthetic activities (Zhang et al., 2020b). Although the photosynthetic response among plants does not vary significantly (Kusuma et al., 2020) and plants can take advantage of available spectra within the PAR range (Ashdown, 2019b), plants also perceive and use light for photosignaling, getting the right light recipe has a major impact on the overall plant development and secondary metabolite accumulation (Kusuma et al., 2020).

It is important to consider the effects of the selected spectra on the photoreceptors within the plants and not just the photosynthetic pigments. The overall effect of the combined

wavelengths should be considered as plants response is highly influenced by the relative spectral composition; what other wavelengths are in the spectra and the fraction of these wavelengths. While a plant's response to photosynthetic radiation is integral in nature, with every photon within the PAR range resulting in photosynthesis at different energy levels, a plant's photomorphogenesis response is not. Studies have also shown that plant's response in terms of growth and biomass accumulation to a combination of 2 or more monochromatic light sources is significantly improved as compared to the response under monochromatic sources. For example, plants grown under red and blue light had higher biomass accumulation and yields compared to plants grown under either red or blue with the same intensity (Demotes-Mainard et al., 2016; Huché-Thélier et al., 2016; Ouzounis et al., 2015; Zheng et al., 2019b). Similarly, plants irradiated with a red+blue light resulted in lower yields compared to plant grown under red+blue+green and white+red lights (Mickens et al., 2019; Zheng et al., 2019b).

2.1.5.4 Alternative spectra and fractions

The introduction of a 3rd spectral component from the non-PAR and low-PAR range, such as green, UVA or FR has also been reported to effect yield compared to monochromatic or dichromatic spectral composition (Lin et al., 2021; Mickens et al., 2019). Besides affecting plant development, recent studies have also shown that supplemental UVA and FR spectra component, though not within the PAR range, had a positive effect in enhancing photosynthetic activities in some plants (He et al., 2020b; Zhen & Bugbee, 2020c). Small amounts of FR spectrum together with red light results in a significantly higher photosynthetic levels compared to red or FR alone, in what is described as the Emerson effect (Hwang et al., 2020; Zhen & van Iersel, 2017).

A plant's response to a wavelength or colour varies with the intensity and the fraction with relation to the total radiated light spectrum (Demotes-Mainard et al., 2016; Huché-Thélier et al., 2016; Ying et al., 2020). Blue irradiation has been shown to have a stem

elongation promoting effect at lower intensities levels that convert to an inhibitory response at higher intensities (Huché-Thélier et al., 2016; Zheng & Van Labeke, 2018). Similarly, the effect of green light spectra in photosynthesis increases exponentially at higher intensities (Samuoliene et al., 2020a). The red to blue ratio (R:B) and red to far red (R:FR) ratios are the most used spectral component fractions in LED horticulture lighting systems (Ajdanian et al., 2020; Kusuma & Bugbee, 2020; Kusuma et al., 2020). These relative ratios have a significant effect on plant morphological characteristics. R:FR ratio influences flowering in many plants and is also involved in the shade avoidance response while the R:B ratio influences biomass and metabolite accumulation in plants (Demotes-Mainard et al., 2016; He et al., 2020a; Huché-Thélier et al., 2016; Kusuma & Bugbee, 2020).

2.1.5.5 Complexity of White LEDs

The use of white LEDs in horticulture lighting applications are becoming increasingly popular due to its lower cost, higher availability, and continuous spectral distribution (Mickens et al., 2019; Park & Runkle, 2018). However, as most white LEDs are designed and optimised for general lighting applications, its colour characteristics are often described in photometric terms (Sipos et al., 2020; Wu et al., 2020a). The corelated colour temperature (CCT) that describes how warm or cool a light appears, and the colour rendering index (CRI) that defines how well colours are precepted under the light in relation to an ideal light source, are the most common terms used (International Commission on Illumination, 2021b). These parameters are optimised for the human eye and has no significance to plants. The spectral distribution of white LEDs, is highly dependent on its chip design and the phosphor used (Swan & Bugbee, 2017; Wu et al., 2020a). Both elements not only vary by manufacturer but also by the CRI (Kusuma et al., 2020).

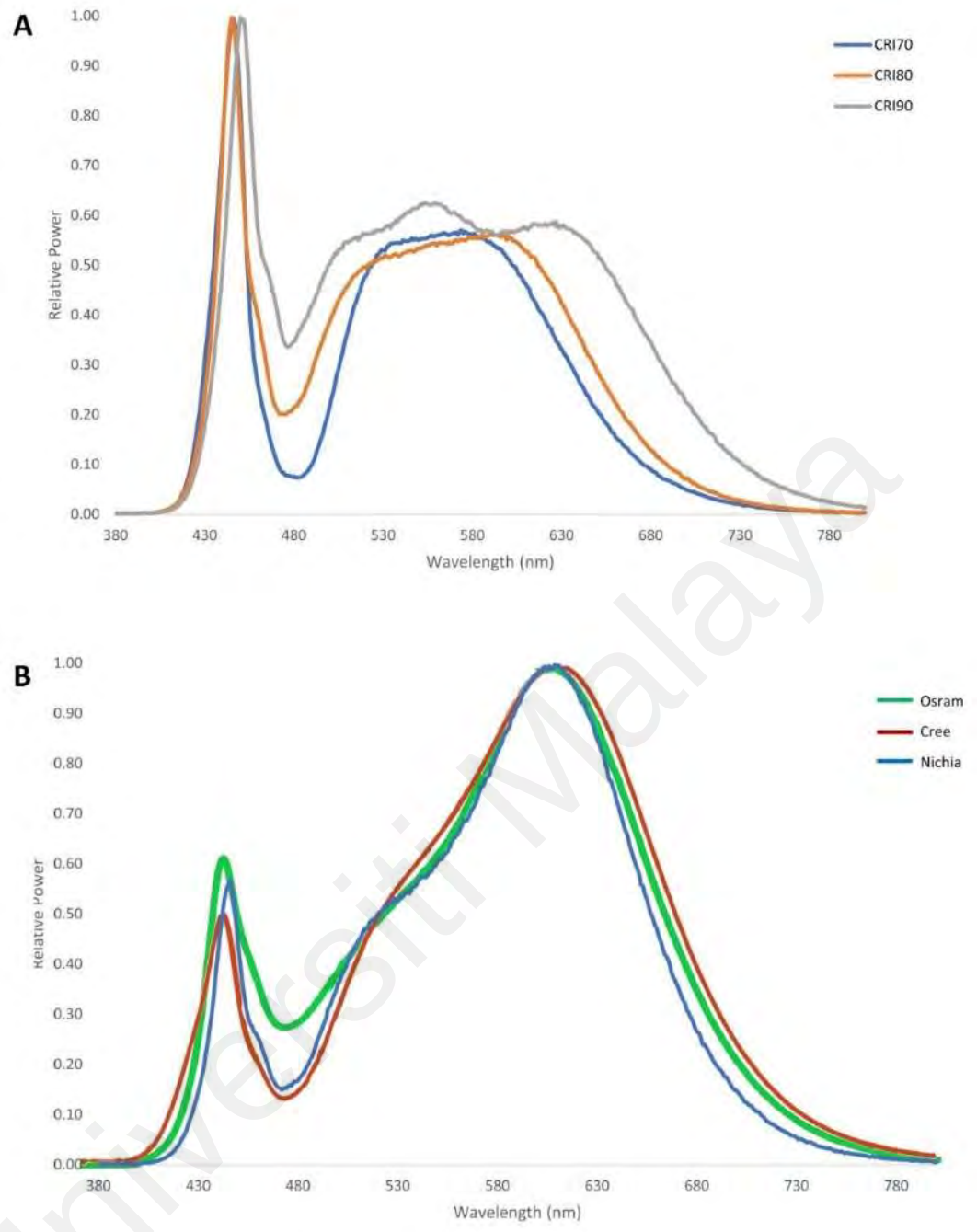

Figure 2.3: Spectral Power Distribution of White LEDs. A)CCT 3000K LEDs driven at 700mA with different CRI from the same manufacturer (amsOSRAM, 2021). B) CCT 5000K CRI80 White LEDs driven at 700mA from different manufacturers (amsOSRAM, 2021; Cree, 2021; Nichia, 2021)

A white LED with an identical CCT but different CRI can have very different spectral distribution as highlighted in Figure 2.3A. All products are identical to the naked eye, radiating a warm white light (3000K), driven at the same currents (700mA), and all 3 LEDs are also made by the same manufacturer. However, the spectral distribution, especially in the blue and red regions, critical regions for plants, vary significantly

between the different CRIs. Similarly, when comparing white LEDs from different manufacturers but with the same CCT, CRI and driving currents, there is still a difference, specifically at the blue region (Figure 2.3B). As such, it is critical for users to evaluate the spectral distribution data of white LEDs intended for use and not just to rely on photometric or colorimetric data.

2.1.5.6 Spectral Purity

While PC LEDs are relatively cost effective compared to the direct emitter type and are the primary technology used in white LEDs, PC colour LEDs have a drawback as its full width at half maximum (FWHM) is wider than those of the direct emitter variant (Zhuo et al., 2018) (Figure 2.4). For this reason, the PC colour LEDs are deemed to have less 'pure' radiation. The spectral purity among monochromatic lighting sources has been a topic of discussion and investigation for more than a century. Early studies by Hoover (1937); McCree (1971) highlighted the concerns with spectral purity on the accuracy of photosynthetic action spectra measurements. Recent studies have shown the effect of FWHM on plant growth, with results contradicting previous studies that used blue light with a wider bandwidth, highlighting the importance of spectral purity especially when using monochromatic light sources (Johnson et al., 2020; Kong et al., 2019b). The effect of the spectral purity on photosynthesis was observed in past studies. Studies that had a FWHM larger than 25 nm did not notice distinct peaks in the blue region while studies that had a FWHM lower than 20 nm observed a distinct blue peak (Wu et al., 2019). It is expected that if a spectral response to photosynthesis study is done with LEDs with narrower FWHMs the curves in Figure 2.2 would have a different shape (Wu et al., 2019).

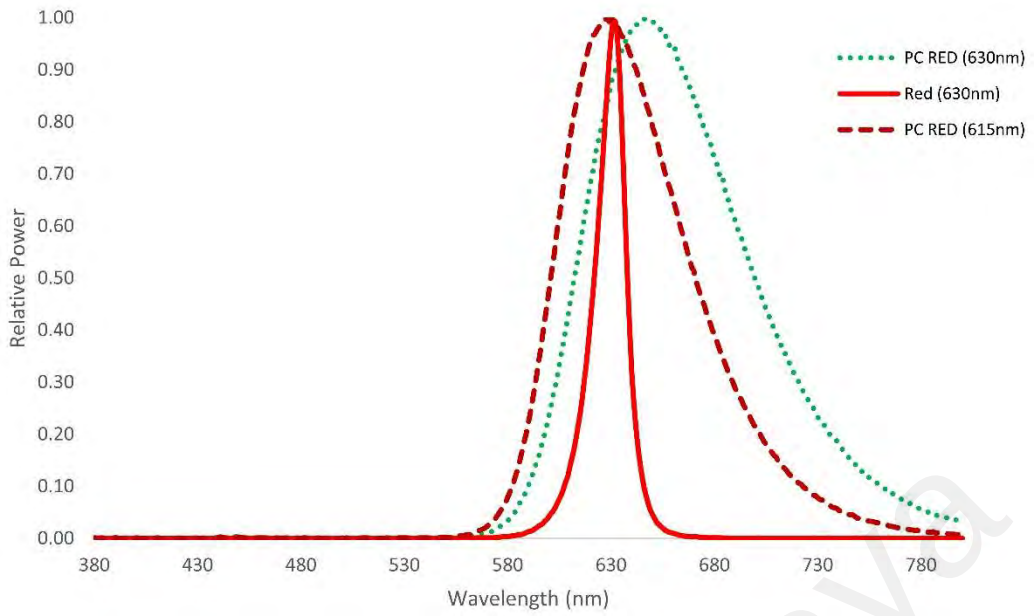

Figure 2.4: Spectral Purity (FWHM) of Different Red LEDs. Data obtained from Osram GmbH.

2.1.5.7 Efficiency and Efficacy

There are several terms used to describe efficiency and efficacy of LEDs. The luminous efficacy (lmW^{-1}) is typically used for white LEDs, especially for general lighting applications whereas radiometric efficiency, described in % (W_{Output}/W_{Input}) is commonly used in colour LEDs. However, these terms do not provide any important information for horticulture applications. The efficacy of horticulture specific LEDs components and products are described as the Photosynthetic Photon Efficacy ($\mu mol\ J^{-1}$), defined as the photon output within the PAR range emitted per unit of energy (Joule) consumed (Bugbee, 2016; Runkle & Bugbee, 2013). The PPE values are not only determined by the radiometric energy output but also by the wavelength of the light as per Planck-Einstein relationship. Hence, even though blue LEDs have a higher radian efficiency compared to red, it has a lower PPE (Kusuma et al., 2020) (Table 2.2).

Table 2.2: Efficiency and efficacy of some common LEDs. Data obtained from amsOSRAM (2020); Kusuma et al. (2020)

Colour	Wavelength / CCT (nm/ K)	Radiant Efficiency (W/W)	Photon Efficacy (µmol J^{-1}) (280nm-800nm)	Photosynthetic Photon Efficacy, PPE (µmol J^{-1}) (400 nm -700 nm)
Blue	450	0.93	3.5	3.5
Green	530	0.42	1.9	1.9
Red	660	0.81	4.5	4.5
Far Red	730	0.77	4.7	0.5
Cool White	6500K	0.76	2.9	2.8
Warm White	2700K	0.69	2.6	2.5

The PPE values are representative of how much photons within the PAR range are produced by the LED package or total luminaire, a luminaire or package performance measure, and in no way represents the photosynthetic effectiveness. It does not mean plants grown under a luminaire with a PPE of 4.0 µmol J^{-1} will perform better than plants grown under a luminaire a PPE of 2.5 µmol J^{-1}. Another drawback of the PPE is that it does not include non-PAR radiation such as UV-A and FR that have been proven to enhance and play a role in plant photosynthesis. Instead, PPE values tend to penalise LED luminaires and packages that contain these wavelengths. As observed in Table 2.2, the FR LED package, although having the highest photon efficacy has the lowest PPE value as most of its radiated spectrum lies beyond the PAR range. As the PPE does not provide any method to evaluate the effectiveness of the lighting used on the final yields, the photon conversion efficacy (PCE) has been used to measure the economics efficacy of artificial lighting in horticulture (Kubota et al., 2016; Nemali & Langenhoven, 2018). The PCE, also often referred to as the light use efficiency, is defined as the amount of biomass (g) that can be produced by 1 mol of light and is a relatively new term that has yet to gain popularity (Slattery & Ort, 2015). The PCE is however dependent on several variables

such as the plant species, growing methods and other environmental factors and hence is not a value that can be defined by the equipment manufacturer and needs specific detailed studies to be quantified. It is a representation on the effectiveness of the specific light recipe or spectra with regards to the intended final quantitative yields and is not a measure of the equipment's overall efficiency or efficacy.

2.1.5.8 Reliability

The reliability of LEDs is affected by the junction temperature and driving current. The increase in driving current and junction temperature leads to a reduction in LEDs radiant efficiencies (Davis et al., 2019) and lifetime (Vaskuri et al., 2018), and leads to a shift in spectral properties (Wu et al., 2020a). Unlike traditional discharge lamps, LEDs operate at lower temperatures and driving currents. Although LEDs do not radiate heat, substantial amount of heat is still generated and would need to be conducted away from the chip to maintain a reasonable junction temperature (Wu et al., 2020a). The drop in efficiency within LEDs under high current and high temperature conditions is due to the droop effect. Current droop is a well-studied phenomena that occurs within the chip of the LED package and is known to be caused by various reasons that includes electron leakage, caused by energetic carriers escaping from the active region resulting in a leakage current across the p-n junction (David & Grundmann, 2010) (Tanner et al., 2015), Auger recombination, a non-radiative carrier recombination process (Lin et al., 2017), and extended defect recombination (Zhao et al., 2018). In blue LEDs, Auger recombination typically occurs at higher junction temperatures and electron leakage at lower temperatures, while in AlInGaP based red LEDs, the electron leakage is the main cause of droop due to the unfavourable band structure within the AlInGaP material itself (Lin et al., 2017; Pattison et al., 2016; Zhao et al., 2018). The current driven droop causes an increase in junction temperature. As the junction temperature increases, the non-radiative recombination process is further enhanced with more carriers participating in it

(Lin et al., 2017), releasing excited energy as heat that results in self-heating and a further increase in the junction temperature (Wu et al., 2020a). This increase in junction temperature also causes the radiant intensity to decrease, and the peak and dominant wavelengths emitted to shift towards longer wavelengths (Figure 2.5) due to the decrease in bandgap energy experienced. The temperature induced spectral shift is more prevalent in narrow band LEDs, with up to 20 nm shift in red and 10 nm shift in blue LEDs, compared to white LEDs that had small degree of chromaticity shifts (Wu et al., 2020a) although, in conditions of extended operation under high junction temperature, the phosphor and silicon based materials within the white LEDs will begin to degrade, causing a permanent spectral shift (Pattison et al., 2016).

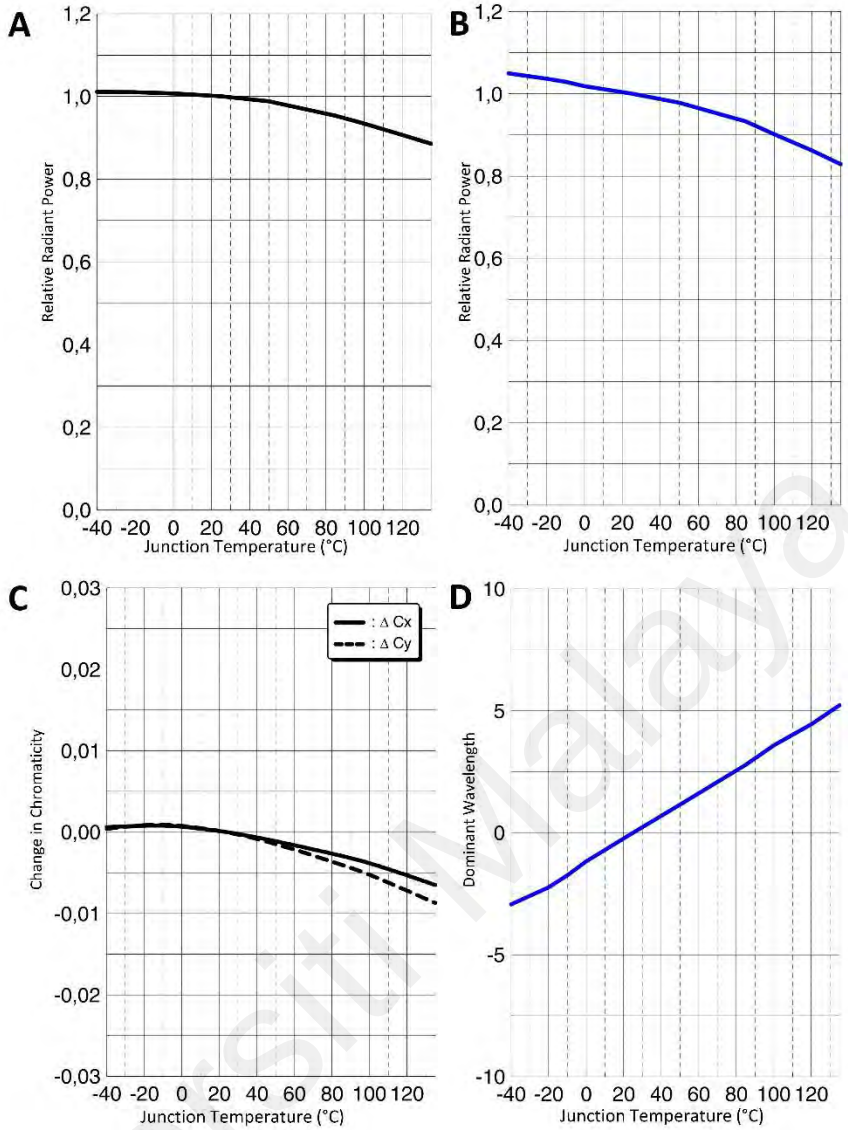

Figure 2.5: Effect of Junction Temperature. A) Radiant power of White LED. B) Radiant power of Blue LED C) Change in chromaticity of White LED D) Change in dominant wavelength of Blue LED. All LEDs operating at 700mA. Figures extracted from amsOSRAM (2020)

2.1.5.9 Standards

Due to the absence of horticulture specific standards for lighting products, most products in the market were either not tested or were tested according to the available general lighting standards. The IEC62471 and IEC60598 series standards were used for photobiological safety and general requirements respectively, while the IEC62722 series were used for the general performance of LED specific luminaires (International Electrotechnical Commission, 2021). The measurement methods of photometric

characteristics of LED packages and luminaires were based on IES LM-80 and IES LM-79 respectively while the lifetime projection was typically based on IES TM-21 standards (Illuminating Engineering Society, 2019, 2020; UL, 2019a). However, with the increase in the usage of LEDs in horticulture lighting application, there was a need for the development of horticulture specific standards (Runkle, 2017).

In 2017, the American Society of Agricultural and Biological Engineers (ASABE) introduced the first of 3-horticulture lighting specific standards. The ASABE S640 standard defined 33 electromagnetic radiation types, including the spectral ranges for ultraviolet (280nm – 400nm), photosynthetic (400nm-700nm), and far-red radiation (700n m-800nm)(Wright, 2017). While ASABE S640 did not redefine the PAR range nor did it provide a definition for spectral range of specific colours such as blue, green, and red, it did define a new plant biologically active radiation (PBAR) range, from the 280nm to 800nm (ASABE, 2017; Ashdown, 2017; Wright, 2017). The ASABE S642 standard released in 2018, guides manufacturers on the methods for measurement and testing of LED products designed for horticulture applications (American Society of Agricultural and Biological Engineers, 2018; Wright, 2018). This standard specifically targets the 280nm to 800nm range and spectral power and quantum measurements be made at a minimum of 5nm intervals across the entire range (Wright, 2018). The ASABE S642 also requires changes in radiant and photon flux be documented and allows for the projection of performance and life of the product, based on prevailing IES TM-21 methodologies (Society, 2011; Wright, 2018). Both standards however did not address any safety requirements or performance specifications.

To address the specific safety concerns of horticulture lighting products, the UL8800 Standard for Safety for Horticultural Lighting Equipment was released by UL LLC in 2018 (UL, 2019a, 2019b). This standard brings in some key changes compared to the UL1598 and IEC60598. Besides having a scope that specifically covers horticulture

lighting products and systems that are dynamic in nature, the UL8800 standards incorporates the photobiological safety requirements, consistent with IEC62471 (UL, 2019b). The products will also be evaluated for its suitability to high humidity and elevated ambient temperature environmental conditions. To further simulate the operations in horticulture applications such as greenhouses, UL8800 also sets out requirements to ensure polymeric materials used in these products do not get brittle or damaged by UV that is typically present in greenhouses (UL, 2019b). Another major difference in the UL8800 standards is that it considers the dynamic nature of horticulture lighting installations. In greenhouses and CEAs, the lighting systems are often lowered, raised and repositioned, hence there is a need for safe specialized wiring and connection methods that can support the required flexibility and UL8800 address that concern (UL, 2019b).

The Design Light Consortium (DLC) has established a set of testing and reporting requirements for LED based horticultural lighting to comply to in order to be registered in its Qualified Product List (QPL). The Technical Requirements for LED-Based Horticulture Lighting (Design Light Consortium, 2022) not only lists out the testing methods and reference standards (ASABE,IEC,IES,UL) to be used, but also spells out specific qualitative technical requirements that need to be met. The documents provide an option for manufacturers to also report photobiological active radiation (PBAR) flux and efficacy in addition to PAR range values (Sparks, 2020). It further requires the values within the PAR region to be split into 100nm bins and the flux and efficacy information to also be provided for these individual bins. The document requires the flux and efficacy of the FR bin from 700nm to 800nm to be included besides providing the spectral quantum distribution, the $\mu mol\ s^{-1}\ nm^{-1}$ for each wavelength (Design Light Consortium, 2022). Products are required to have a minimum PPE of 2.30 $\mu mol\ J^{-1}$ and to have a maximum photosynthetic photon flux (PPF) output depreciation of 10% after 36,000

hours of operation (Design Light Consortium, 2022). The requirements set forth by DLC is to ensure that only qualified lighting products that can withstand the greenhouse ambient conditions are listed in the QPL while at the same time providing transparent, unbiased technical information to user, enabling an informed decision to be made (Sparks, 2020).

In 2021, the Illumination Engineering Society (IES) published the ANSI/IES RP-45-21 document titled Recommended Practice : Horticultural Lighting (Illuminating Engineering Society, 2021) that describes the difference between horticultural and architectural lighting design. The ANSI/IES RP-45-21 document was intended to act as a reference for both lighting designers and botanists. It covers a wide range of topics that include overviews of horticulture lighting definitions, daily light integral requirements of popular plant species, plant botany, and light sources. While design considerations for vertical farms and greenhouses are included in ANSI/IES RP-45-2, unlike the Technical Requirements for LED-Based Horticulture Lighting issued by DLC, it does not state any minimum qualitative value that must be met (Design Light Consortium, 2022; Illuminating Engineering Society, 2021).

2.1.6 Challenges and Issues

The use of LEDs with higher intensities and narrow bandwidth has raised concerns of photobiological eye safety especially with regards to the use of blue and blue-based white LEDs. This was a primary area of concern in general lighting and multiple manufacturers, professional bodies, and standardisation agencies have issued technical documents, guidelines and standards (Wu et al., 2020a). The IEC co-developed the IEC62471:2006 standards on the photobiological safety of lamps and lamp systems together with the CIE (International Electrotechnical Commission, 2006). This standard provides guidance on the evaluation of photobiological safety of light sources, luminaires, and system. It also specifies the exposure limits, reference measurement methodologies and classification

schemes (International Commission on Illumination, 2019; International Electrotechnical Commission, 2006). The CIE highlighted that while there has been claims that blue light hazard may lead to age-related macular degeneration, these claims are speculative in nature and have yet to be supported by substantial peer-reviewed literature (International Commission on Illumination, 2019). However, these standards and guidelines are based on general lighting applications that typically use white LEDs, under relatively lower intensities, and positioned away from the users, unlike in CEAs where lighting used typically consists of high intensity narrow band blue light optimised for plant growth (Wu et al., 2020a). Hence, it is important that while LED based lighting systems are designed to optimise plant growth and development, adequate care and consideration is also given to limit the user's exposure to potential high levels of blue.

Traditional discharge-based lighting technology such as HPS and FTL rely on mercury content as a catalyst to initiate the excitation of electrons for generation of light. This leads to residual mercury being present in these lamp types at the end of its lifecycle, leading to potential environmental concerns in the event of improper disposal. LEDs, being a semiconductor does not experience this problem as it does not content any mercury. However, as with any major semiconductor manufacturing operations, LEDs require a significant amount of organic solvent and clean water for production. Volatile organic compounds (VOC) and waste water are the by-products released during this process, raising concerns of possible environmental contamination if not managed effectively (Wu et al., 2020a). Studies have reported that while LEDs do not contain mercury, it had a high concentration of aluminium, up to 22%, followed by iron, copper, and zinc besides having heavy metals such as lead, arsenic, and antimony present (Kumar et al., 2019). Kumar et al. (2019) reported that although most LED based products end up in the landfill due to the lack of a proper recycling process, the landfill leachate

concentration was found to be lower than the maximum permissible levels by local standards.

One of the key drivers for adoption in LEDs is the low radiated heat generated and its higher overall efficacy and efficiency. While it may seem as a positive trait, the lack of radiated heat generated is actually a key factor impeding the adoption of LEDs as supplemental lighting in greenhouses (Katzin et al., 2021). The reports by Stober et al. (2017) and Elliott et al. (2020) shows the use of LEDs in greenhouses to remain constant at 2% over the years with HPS and other discharge lamps dominating the balance 98% of the applications. One key factor contributing to this slow rate of adoption is the lack of radiated heat generated by LED based lighting systems (Katzin et al., 2021). HPS lamps radiate a high amount of heat that is used to supplement the heating requirements of the green house (Katzin et al., 2021; Katzin et al., 2020). As these lamps are replaced with LED based systems, there is a need for the additional heating requirements. This often leads to a reduction in the total greenhouse energy savings realised. Most studies focused on quantifying the energy saving potentials of LED based horticulture lighting systems without considering the overall effect LEDs has on the complete energy demand. This has led to situations where overall energy savings obtained from replacement of HPS with LEDs being disappointing and not being able to justify the high costs. Dieleman et al. (2016) obtained a savings of 37% in lighting energy but had to use most of the saving in additional heating load, resulting in a final total energy savings of 11% while Ouzounis et al. (2018) realised a 60% savings in lighting energy needs by converting to LEDs but had a total energy demand reduction of only 6.5% as heating requirements increased significantly. This is however not applicable to greenhouses that use geothermal heating sources or those that use co-generation plants that generate heat, CO_2, and power.

2.1.7 Future Trends and Direction

2.1.7.1 Standards and labelling

The development and release of new standards are expected to continue with the release of ASABE S644 expected in the very near future (Alsop, 2020). This standard will investigate establishing appropriate performance criteria of luminaires and systems designed specifically for horticultural applications. It is also expected that this standard will provide recommendations on minimum and advanced criteria options, including specific values where suitable, with regards to electromagnetic output and efficacy parameters, and also methods to compare anticipated plant spectral response and energy performance (American Society of Agricultural and Biological Engineers, 2019; Wright, 2018). On the international front, the CIE and IEC have initiated exploratory work to identify the needs and to lay out the foundation for future standards development activities specific to horticulture lighting applications. CIE have set up their first joint technical committee (JTC) between divisions 6, that investigates photobiological aspects of lighting, and division 2, that focuses on the metrology aspects of lighting within the organisation. The resulting JTC19 aims to develop an international standard for terms and definitions used in horticultural lighting, incorporating existing national and regional publications such as the ASABE S640 (International Commission on Illumination, 2021c). The CIE division 2 also has a reportership, DR 2-83 established to characterize the spatial light distribution of horticultural applications by identifying quantities and indicators based on key lighting factors that influence plant growth (International Commission on Illumination, 2021a). The resulting technical note is expected to also discuss possible measurements methodology and for these quantities and indicators, and will provide a basis for future standard development activities (International Commission on Illumination, 2021a).

The IEC via technical committee (TC) 34 that focuses on topics regarding light and lighting, has set up 5 preliminary work items (PWI) specific to horticulture lighting applications. 3 of the PWIs looks into the specification sheet, characterisation methods and safety aspects of LED packages and light sources while the remaining 2 PWI concerns the safety of horticulture luminaires and the performance of both luminaire and light sources (International Electrotechnical Commission, 2021). These PWIs were initiated in November 2020 and were expected to move to the next stage where the proposal for the new work on standards will be prepared, in December 2022 (International Electrotechnical Commission, 2021).

With the added complexity introduced by LEDs in horticulture lighting and the need of additional standardised information for users to be able to setup precision horticulture lighting systems, a standardised product label was proposed by Both et al. (2017). The proposed label (Figure 2.6) would enable users to evaluate products on a comparative basis, having similar metrics and methodologies used in obtaining the data.

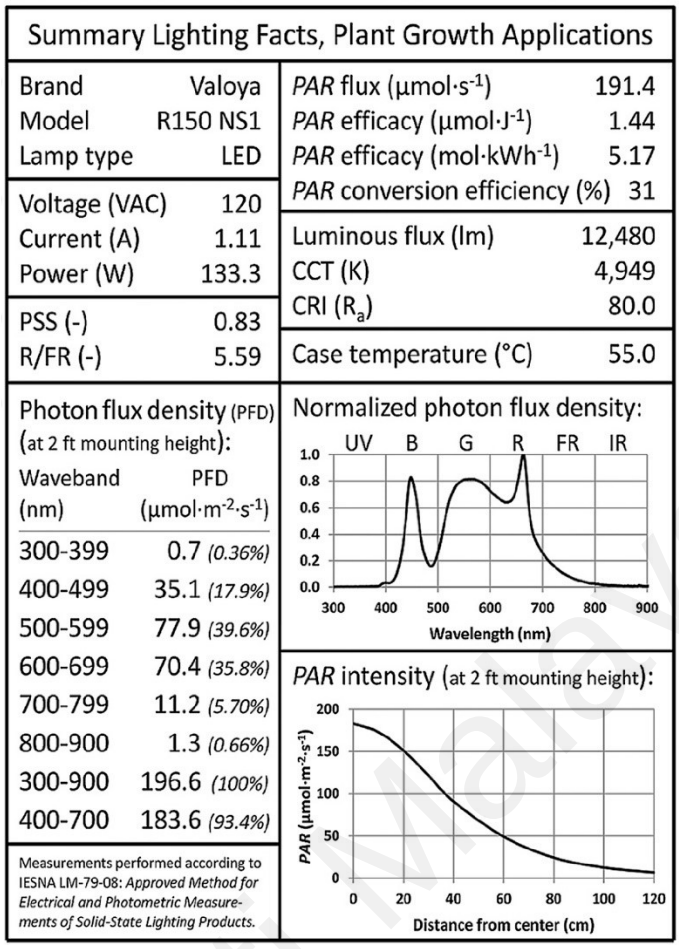

Figure 2.6: Proposed horticulture lighting product label by Both et al. (2017)

The PAR efficacy, PPFD output in selected wave bands at a fixed height, as well as the phytochrome photostationary state (PSS), red to FR ratios, and the normalised graphs of photon flux density across the 300 nm to 900 nm range are some of the information proposed to be included in the label. The label would also include the PAR conversion efficiency, expressed in % (W/W) that represented the radiometric energy within the PAR region emitted from the luminaire with regards to the electrical energy consumed. This standardized product label however, in its current state, can only be applied to horticulture lighting systems that do not have spectral and intensity tunability functionalities (Both et al., 2017). Dynamic lighting systems would require a much sophisticated methodology

and labelling requirements due to the multiple variables within its design (Wu et al., 2020a).

2.1.7.2 Growing importance of Far-red

Recent research interest with regards to FR application in horticulture lighting has shifted to its effects on the photosynthetic activities. FR is increasingly being used to regulate flowering and morphological developments in plants successfully (Demotes-Mainard et al., 2016). It is well established that FR spectrum, when used with longer wavelength red light, results in enhanced photosynthetic activity due to the Emerson effect (Legendre & van Iersel, 2021; Zhang et al., 2019). There has been calls to redefine the PAR range to include FR irradiation (Zhen & Bugbee, 2020c; Zhen et al., 2021). Zhen and Bugbee (2020c) and Zhen and van Iersel (2017) reported that FR radiation are not only synergistic with longer wavelengths but also with shorter wavelengths, prompting calls for FR to be reconsidered for its role in photosynthesis (Kusuma et al., 2020). However, FR radiation on its own, results in a significantly lower photosynthetic efficiency (Zhen & Bugbee, 2020c). As described by the Emerson effect, the effect of FR on photosynthesis is highly depended on the other wavelengths within the spectra, and this was a blocking point for FR to be included in the PAR region (Zhen et al., 2021). All other wavelengths within the PAR region are assumed to contribute equally to the photosynthetic activity and its contribution is not depended on other wavelengths, which was not the case with FR irradiation. The findings from Zhen et al. (2021) however prompts this assumption to be revisited and have proposed the use of a new extended PAR (ePAR) range that covers from 400 nm to 750 nm. Although FR irradiation may not have been included into the PAR region by ASABE or DLC at present, its growing importance on plant growth and development is not ignored. ASABE has included a specific definition of FR range in its ASABE S640 standard, while the DLC has included FR specific technical requirements that include output depreciation requirements and inclusion of the FR range in efficacy

and output calculation, in the latest version of its technical documents, highlighting the important role of FR spectral range (ASABE, 2017; Design Light Consortium, 2022).

The adoption of LEDs in horticulture lighting is set to increase exponentially and with it comes a new set of concerns. Unlike traditional light technologies, LEDs allows for the design of highly specialised spectral content catering to different plant species and at every stage of its growth and development. As plants do not perceive light as humans, it is important for the design of horticulture lighting products and system to prioritise plant response over human visual acuity or comfort, using the appropriate metrics, to maximise the potential benefits of LEDs in horticulture. Although there are no major environmental concerns with regards to the use of LEDs, there is a need to explore further on the proper methods of disposal and recycling of LED based products.

2.2 *Stevia rebaudiana* Bertoni

2.2.1 Introduction

Stevia rebaudiana Bertoni, a member of the Asteraceae family, is small perennial shrub that grows between 30 cm to 85 cm tall though some species can reach heights of up to 120 cm (Amarakoon, 2021; Olas, 2022; Rai & Han, 2022). Native to north-eastern Paraguay, it is the one of only two plants from the 154 recognised members of the genus Stevia that could accumulate steviol glycosides (SG), with *Stevia phlebophylla* being the other (Basharat et al., 2021; Olas, 2022). However, studies have reported that unlike *Stevia rebaudiana*, the concentration of SG in *Stevia phlebophylla* is very low, making it commercially not viable (Gunasena et al., 2021). The SG, accumulated mostly in the plant's leaves has been used over the years as an artificial sweetener and for medicinal purposes by the indigenous people of South America (Ahmad et al., 2020; de Andrade et al., 2021). As the popularity of Stevia as a source of non-calorific organic sweetener grew globally, so did large scale commercial cultivation in other countries such as Brazil,

Canada, China, USA, Korea, Japan, and the United Kingdom (Ciriminna et al., 2019; Rengasamy et al., 2021). Stevia's leaves and purified SG are being studied for their potential therapeutic benefits, which include anti-diabetic, anti-carcinogenic, anti-hypertensive, antioxidant, anti-tuberculosis, anti-inflammatory, neuroprotective, vasodilator, and non-toxic effects on the reproductive system (Talevi, 2022). There are in-depth reports covering the plant's functional characteristics. Aqueous extracts of Stevia have recently been used to assess the effects on wound healing. It reduced the number of macrophages and lymphocytes present on the wound surface, while the extract increased the number of blood vessels and fibrocytes (Abbasi et al., 2021). Because their leaves have the highest concentration of SG, Stevia plants with a high leaf-to-stem ratio are preferred. It has a small, lanceolate, oblong leaf shape with an alternate leaf arrangement while its shoot is pubescent and sub ligneous (Libik-Konieczny et al., 2021). It is a short-day plant, with a critical photoperiod of between 12 to 13 hours and had a three-month flowering period (de Andrade et al., 2021). The tiny white flowers are arranged in clusters of 2 to 6 florets in corymbs that are arranged in loose panicles. The Stevia seeds are in achenes that are 3 mm long. Although the viability of the seeds is very low and varies, reproduction still occurs through them (Libik-Konieczny et al., 2021; Rai & Han, 2022). It is a self-incompatible plant that is heavily cross-pollinated and pollinated by insects (Basharat et al., 2021). This results in plants grown from seed to vary in their growth, quality and quantity of secondary metabolite accumulation(Hernández et al., 2022).

2.2.2 Steviol glycosides

The extracts and metabolites taken from the leaves of Stevia plants are known as steviol. It is a calorie-free, naturally sweet-tasting chemical that is also a renewable raw food ingredient that can be utilized as an alternative to artificial sweeteners and a sugar substitute on the global market (Libik-Konieczny et al., 2021; Srivastava & Chaturvedi, 2022). Steviol glycosides (SG) are tetracyclic diterpenoids, which are a subclass of the

diterpenoid family of secondary metabolites found in plants (Rai & Han, 2022). SGs are highly sweet, non-mutagenic, non-toxic diterpenoids having significant commercial applications in the pharmaceutical, food, and beverage industries. These glycosides can be converted into sugar and a non-sugar component via hydrolytic cleavage (Libik-Konieczny et al., 2021; Olas, 2022) and consist of a non-sugar component linked to a sugar molecule from carbohydrates (glycone) (aglycone) (Basharat et al., 2021). Steviobioside, Stevioside (ST), Rebaudioside A (Reb A), Rebaudioside B (Reb B), Rebaudioside C (Reb C), Rebaudioside D (Reb D), Rebaudioside E (Reb E), Rebaudioside F (Reb F), Rebaudioside I (Reb I), Rebaudioside M (Reb M), Rubusoside, and Dulcoside are the eleven most prevalent diterpenoids that have been found in the leaf tissues of Stevia plants (Rai & Han, 2022). The first SG to be isolated from Stevia leaves was ST. ST and Rebaudioside make up 95% of the total metabolite composition of SGs (Basharat et al., 2021). These SGs have been shown to be non-genotoxic, non-carcinogenic, and safe (Yang et al., 2022). ST and Reb A are found in higher concentrations compared to the other glycosides with reported concentrations of between 4-13% for ST, and 2-4% Reb A on dry leaf weight basis (Kurek et al., 2022) . ST is reported to be 110–270 times sweeter than sucrose, while Reb A is 150–320 times sweeter. However, at high concentrations, ST has a metallic bitter aftertaste, conversely Reb A's higher concentration lessens this aftertaste and enhances flavour (Libik-Konieczny et al., 2021). Hence, leaves with higher Rebaudioside concentration over ST is highly preferred. These glycosides are dissolved and extracted in aqueous solution, where they exhibit improved stability at high pH levels of between 2 and 10, as well as strong thermostability of up to 200°C (Rai & Han, 2022). When combined with other sweeteners or tastes, these glycosides have a synergistic effect that improves the taste, sweetness, and flavour of its sweetener. Reb M has been promoted as a suitable option as a sweetener to make sweet products like candy, where the sweet taste is desired, as it

tastes like sugar. However, despite having a flavour profile that is noticeably less bitter than Reb A, Reb M currently isn't economically practical for mass production due to its extremely low extraction rate of less than 1% (Tao & Cho, 2020).

2.2.3 Biosynthesis Pathway

The biosynthesis pathway of steviol glycosides within the leaves of the Stevia plant has been of interest to researchers, as it is not only complex but also shares a common foundation compound ent-kaurenoic acid that is also involved in the gibberellic acid (GA) pathway (Libik-Konieczny et al., 2021). Although the SG biosynthesis pathway has been described by past researches, most of these studies reported a need for more detailed research to done in order to better understand the regulation of this complex process (Rai & Han, 2022; Wu et al., 2020b). Studies have described the SG biosynthetic pathway as a 3 stage process (Li et al., 2021; Libik-Konieczny et al., 2021).

The first stage of SGs biosynthesis is carried out in the plastids using a multi-step mechanism starting with methylerythritol 4-phosphate (MEP), which yields isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the building blocks needed to create ent-kaurene (Hernández et al., 2022). In second stage, the ent-kaurene is transferred to the endoplasmic reticulum where it is oxidised to produce ent-kaurene acid (ent-KA) by the ent-kaurene 19-oxidase (KO) enzyme. The ent-KA is the final shared substrate for the SG and GA synthesis pathway in Stevia plants (Libik-Konieczny et al., 2021). Diverting from the common pathway, the kaurenoic acid 13-hydroxylase (KA13H) then catalyses the formation of steviol from the hydroxylation of ent-KA in the endoplasmic reticulum (Rai & Han, 2022). The sequence of glycosylation, which is catalysed in the cytosol, is the third step in the biosynthetic route. The Uridine diphosphate (UDP) dependent glycosyltransferase (UGT) family controls these transformations. Past studies have identified three primary UGTs to be involved at this stage, namely UGT85C2, UGT74G1, and UGT76G1 (Hernández et al., 2022; Srivastava

& Chaturvedi, 2022; Yoneda et al., 2017a). UGT85C2 acts as a primary regulator in the production of steviolmonoside from steviol. This is a critical step as steviolmonoside is subsequently converted to Stevioside (ST). Steviolbioside is converted from the steviolmonoside, although the UGT involved remains unknown (Hernández et al., 2022; Srivastava & Chaturvedi, 2022). Stevioside (ST) is then produced from the glycosylation of steviolbioside, catalysed by UGT74G1. Subsequently, Reb A is synthesized from ST by UGT76G1(Libik-Konieczny et al., 2021). Recent studies have determined that UGT91D2 is also crucial within the SG biosynthesis pathway as it is associated with the synthesis of several rebaudiosides, specifically the synthesis of Reb D from Reb A (Rai & Han, 2022). Likewise, Reb M produced via three separate crossways, with Reb A serving as the biosynthesis's main support structure (Rai & Han, 2022). Reb T, Reb U, and Reb Q were recently isolated from Stevia leaves, albeit their characterisation and sweetness concentration are unclear (Zhang et al., 2020a).

2.2.4 Cultivation of Stevia

Very few nutrients are needed for Stevia. The ideal amount of nitrogen and potassium per hectare for Stevia growth is typically 100–120 kg of nitrogen and 50–60 kg of potassium (Hossain et al., 2017). The optimal temperature for seed germination is 24 C, and seed germination is temperature dependent. Wind disperses seeds, which have a small endosperm, from plants. Stevia seeds are typically dark in colour while infertile ones are typically light in colour (Aghighi Shahverdi et al., 2019). Stevia has a large root system that consists of fine roots near the soil's surface and deeper, thicker roots. The roots are the only area of Stevia where there is little to no accumulation of Stevioside (Rai & Han, 2022).

2.2.5 Industrial and Medical Application and Benefits

2.2.5.1 Industrial Application

The USA Food and Drug Administration (FDA) has since 2008, approved the use of Reb A in foods and beverages as generally recognised as safe (GRAS) (Basharat et al., 2021) . In 2011, the European Union allowed Stevia extracts to include up to 75% of ST or Reb A as food additives. This was further increased in 2016, where 11 SGs were allowed as combinations or ratios in the extract in amounts up to 95%. The eleven SG approved were ST, Reb A, Reb B to F, Reb M, dulcoside A, steviolbioside, and rubudioside that were permitted to be used in commercial preparations (Ilias et al., 2021). The use of Stevia and its extracts are more prominent in beverages as compared to food. Stevia has been used in the production of dairy products such as yogurt and ice creams, breakfast cereals and even baked goods (Schiatti-Sisó et al., 2022). Stevia is also of great importance to the health food industry as it contains a range of therapeutic properties that can assist in addressing issues related to diabetes, hypertension, obesity and cancer (Lemus-Mondaca et al., 2018). Due to the availability of biologically active components such polyphenols, chlorophylls, carotenoids, and tannins, Stevia can be used to produce nutraceuticals and functional meals such as natural sweeteners and oral hygiene products (Kovačević et al., 2018). Stevia has also been used extensively as an alternative to sucrose due to the global demand for natural non calorific sweeteners (Ciriminna et al., 2019; Peteliuk et al., 2021). An early study by Kulthe et al. (2014) reported higher sensory quality aspects of low-calorific and high protein cookies that was prepared by replacing 20% of sucrose and wheat flour with Stevia and soy flour respectively, while Karp et al. (2016) observed no negative effect on the quality and consumer acceptance of muffin that had 20% of its sucrose content replaced with Stevia and were made with cocoa dietary fibres. Suckling et al. (2023) reported the lower environmental effect of Stevia compared to conventional sugar. The life cycle analysis of Stevia cultivated in Europe reported lower effect on four

impact categories; global warming potential, land use, water eutrophication and water use, highlighting the potential environmental benefits that can be realised with Stevia consumption as compared to conventional sugar (Suckling et al., 2023) . This positive environmental effect was attributed in part to the fact that Stevia is significantly sweeter than sugar and as such very low quantities of Stevia would be needed to achieve a sweetness level similar to 1 kilogram of sugar (Suckling et al., 2023).

2.2.5.2 Medical and Health Application

Stevia extracts may be helpful in the prevention and treatment of atherosclerosis, according to a recent study (Ilias et al., 2021). Past studies have reported the hypolipidemic effects of consuming 20 ml of Stevia extract that has the potential of reducing the risk of cardiovascular diseases (Olas, 2022). The Stevia extract was reported to increase the levels of high density lipoprotein (HDL) concentration while reducing the low-density lipoprotein (LDL), and triacylglycerol (TG) concentrations (Olas, 2022). Brijesh and Kamath (2016) reported the effect of ST in increasing the excretion of bile acid, and on the activities of cholesterol 7 a-hydroxylase. This facilitates the reduction of cholesterol levels by improving its conversion to bile acid in the liver (Olas, 2022).

Multiple studies have reported the beneficial effect of ST in reducing and regulating hypertension. An early study by Melis and Sainati (1991) found that ST induced diuresis, natriuresis and hypotension in rats and attributed this to possible changes in the prostaglandin activity. Early studies by Kinghorn and Soejarto (1985) reported ST to cause hypotension in humans while Chan et al. (1998) and Chan et al. (2000) reported the effectiveness of ST on the reducing blood pressure of rats when administered intravenously. Past literature also suggests that Stevia lowers blood pressure by inhibiting the influx of calcium (Ca2+) (Olas, 2022). Liu et al. (2003) and Lee et al. (2001) found that ST lowers blood pressure in different strains of hypertensive rats, and that this effect was mediated by inhibition of Ca2+ influx. More recently, Wang and Wu (2019) observed

that SGs isolated from the ethanol extract and Stevia leaf protein hydrolysates inhibited 26.60%, 59.56% and 74.38% of angiotensin-converting enzyme activities, respectively (Ahmad et al., 2020).

The rising occurrence of obesity among adult and children are of major concern in Malaysia and globally (Saharudin et al., 2020a). Although there may be many factors causing this, the increased intake of calories due to the over consumption of sugar in food and beverages has been reported to be one of the main reasons (Peteliuk et al., 2021). Past studies suggest that Stevia may help reduce calorie intake by reducing appetite. Farhat et al. (2020) found that Stevia does not lead to an increase in hunger and energy intake, while Stamataki et al. (2020) observed that total energy intake was significantly lower in participants after consuming a Stevia beverage compared with water. While Farhat et al. (2019) reported that Stevia lowers the sensation of appetite and does not further increase food intake, Ajami et al. (2020) reported that Stevia had no effects on blood glucose, HbA1C, insulin and lipid levels. In a different recent study, it was discovered that consuming Stevia leaf powder-infused cookies reduced appetite compared to eating control cookies composed entirely of wheat flour (Ahmad et al., 2018).

A study by Samakkarnthai et al. (2018) reported that Stevia does not affect blood glucose levels in obese patients. Ajami et al. (2020) found that Stevia does not influence blood sugar levels, insulin levels, or glycosylated haemoglobin levels in type 2 diabetic patients however, Ritu and Nandini (2016) reported that Stevia can safely be used as an anti-diabetic herb, and it significantly lowered fasting and post-prandial blood glucose levels in patients with type 2 diabetes. In a 2013 study, rats pre-fed with powdered Stevia leaves before receiving an injection streptozotocin (STZ), a type of diabetogen, displayed less severe symptoms of diabetes, such as polyphagia and weight loss, and their hyperglycaemia was less elevated than the untreated diabetic rats. This study reported that Stevia leaf powder and its polyphenol extract boosted insulin production from

pancreatic islet cells in type-1 diabetic rats and improved glucose tolerance and cellular insulin sensitivity in rats with type-2 diabetes (Ahmad et al., 2020). Chang et al. (2005) found that ST improves insulin sensitivity in rats, and Piovan et al. (2018) observed that the non-sweetener fraction of Stevia rebaudiana has an insulinotropic effect, meanwhile Mohd-Radzman et al. (2013) found that Stevia rebaudiana may be effective in abrogating insulin resistance and diabetes. The inhibition of the activities of α-amylase and α-glucosidase, significant enzymes used in the digestion of dietary carbohydrates, is another potential mechanism by which Stevia can lower blood glucose levels. This property makes Stevia useful in the management of blood glucose level in diabetic patients. Recently, it was discovered that Stevia leaf extract suppressed the activity of the enzymes α-amylase and α-glucosidase in vitro (Ahmad et al., 2020).

When compared to a commercial antibiotic, a study by Abdel-Fattah et al. (2018) found that wild Stevia extracts (aqueous, ethanolic, and alcoholic) had antimicrobial effects against four pathogenic bacteria, including *Enterococcus facium*, *Pseudomonas aeruginosa, Bacillus cereus*, and *Klebsiella poneumoniae* (Chloramphenicol). Alcoholic extract of Stevia displayed greater antibacterial potential among the three extracts that were examined (Ahmad et al., 2020). Ortiz-Viedma et al. (2017) suggested the potential use of Stevia extracts as a preservative for salmon paste and other seafood items due to the herb's antibacterial and antioxidant qualities. The antibacterial ability of Stevia extracts against many types of bacteria has also been confirmed by other investigations (Gupta et al., 2012; Puri et al., 2011; Yadav et al., 2011). It was reported that effectiveness of Stevia as an antibacterial agent is also dose-dependent in all the species tested (Ahmad et al., 2020).

The presence of a high concentration of bioactive components, including phenolic compounds, tannins, flavonoids, and vitamin C, among others, gives rise to the discovery that the Stevia plant possesses antioxidant capabilities. The presence of a high

concentration of bioactive components, including phenolic compounds, tannins, flavonoids, and vitamin C, among others, gives rise to the discovery that the Stevia plant possesses antioxidant capabilities (Lemus-Mondaca et al., 2018; Lemus-Mondaca et al., 2012).

An early study by Toyoda et al. (1997) reported a reduction in adenomas of the mammary gland in female rats treated with ST compared to the controls (Talevi, 2018). In a more recent study, it was shown that steviol, a component contained in Stevia leaves, significantly inhibited the growth of human gastrointestinal cancer cells (Chen et al., 2018). Several papers have suggested that Stevia may have anticancer effects. Iatridis et al. (2022) reported that Stevia had various benefits to human health, including anticancer effects while Martínez-Rojo et al. (2020) found that Stevia extracts significantly reduce the viability and migration of prostate cancer cells. Meanwhile López et al. (2016) reported that a Stevia rebaudiana ethanolic extract (SREE) was able to scavenge free radicals and induced cell death in the three cancer cell lines tested while Deshmukh and Kedari (2014) found that ST was able to inhibit cancer cell growth. Consequently, Stevia may have potential as a means of cancer prevention and therapy.

2.2.6 Risks and Safety of Stevia

Numerous regulatory agencies and scientific organisations from around the world have examined and considered the use and safety of steviol glycosides. More than 150 nations and regions have allowed or adopted the use of high-purity Stevia leaf extracts in meals and beverages (Ahmad et al., 2020). In Japan, steviosides have been consumed often for more than 20 years without any negative side effects being noted. SG are not well absorbed in the stomach and upper intestine of both humans and rats, contributing significantly to the safety of Stevia (Mathur et al., 2017). Instead, SGs are metabolized by the cecal microflora producing steviol from steviolbioside, partially absorbing the steviol. This also holds true for the later conjugated steviol that the bile excretes into the

gastrointestinal tract (Olas, 2022). Past studies come to the conclusion that high-purity Stevia leaf extract sweeteners, including steviol glycosides, are safe for adults, children, nursing women, and diabetics when used in appropriate doses in food products (Abbas Momtazi-Borojeni et al., 2017; Magnuson et al., 2016). The Food Standards Australia New Zealand (Foods Standards Australia New Zealand, 2008) and the European Food Safety Authority (Authority, 2011) both established the ADI of SG and equivalents to be at 4mg per kilogram of body weight per day (4mg kg^{-1}bw day^{-1}). The World Health Organisation (WHO) Joint Expert Committee on Food Additives (JECFA) issued a recommendation in 2015 with identical ADI values (Olas, 2022).

2.2.7 Factors Affecting SG and Stevia Growth

As most of SG within the Stevia plant is located within its leaves, the time of harvest, cultivar type , and post-harvest storage conditions are some of the factors that influence the accumulation of Reb A, ST, and the ratio of Reb A to ST (Zeng et al., 2013). Growing circumstances and crop management practices, have also been reported to have an impact on the SGs content of Stevia plants (Díaz-Gutiérrez et al., 2020; Khiraoui et al., 2021) . A soil that is sandy, well-drained, and organically rich in potassium and phosphorus rather than nitrogen is ideal for the growth of Stevia. Soil that is sandy, well-drained, and organically rich in potassium and phosphorus rather than nitrogen is ideal for the growth of Stevia (Vozhehova et al., 2021). Potassium (K) deficiency was reported to cause of the downregulation of expression in key genes associated with SG, reducing the concentration of SG with no effect on the productivity of leaf biomass (SUN et al., 2021). Although it is classified as a short-day plant, Stevia grows best in a sunny setting with partial shade, and it has been found that growth in a long day photoperiod favours the generation of SGs (Jarma-Orozco et al., 2020). There is still debate over the ideal light range for the growth of Stevia (Hernández et al., 2022). Numerous studies have been done on how light affects Stevia's ability to regulate its genes, and both the quality and quantity

of light have a significant impact on both the basic and complex processes of metabolism (Yoneda et al., 2017a; Yoneda et al., 2017b). In some situations, it has been demonstrated that this leads to variations in the amounts of bioactive GA (Vishal & Kumar, 2018). Studies have shown that light conditions control the genes encoding the enzymes involved in the manufacture of GAs (Yang et al., 2018).

2.2.8 Past studies on effect of Light on Stevia

Light is an essential element that influences the growth and development of plants. Multiple studies have been published on the effects of different lighting approaches and conditions on the growth and yields of *Stevia rebaudiana* (Table 2.3). Studies have reported the effect of light intensity on the concentration of ST and Reb A, with higher intensities resulting in higher total SG accumulation and higher transcription levels of the KA13H, UGT74G1 and UGT76G1 genes (Hernández et al., 2022). (Ceunen & Geuns, 2013b) and Ceunen and Geuns (2012) reported an increase in SG yields when Stevia plants are cultivated under long day conditions as prolonged vegetative stage resulted in higher biomass accumulation. It was also noted that while the SG quantities improved, the overall ratio between ST and Reb A did not vary between the short- and long-day conditions. de Andrade et al. (2021) reported similar findings, with a 16-hour photoperiod under fluorescent lamps to be most effective for the production of SG and antioxidants, while a 15-hour photoperiod under the same treatment was the most beneficial in terms antimicrobial activity. Yoneda et al. (2017b) studied the effects of light intensity and photoperiod on the growth and gene expression levels of indoor grown Stevia plants irradiated with white fluorescent lamps. In this study, it was observed that higher light intensities positively influenced the accumulation of biomass, but it did not have any effect on the transcription levels of KO, UGT85C2 and UGT74G1 genes . A study by Evans et al. (2015) explored the effect of daily light integral (DLI) on SG in field and greenhouse grown Stevia plants. The SG content of the plants was observed to increase

as the DLI increased to 10 mol m^{-2} day^{-1} remaining constant at higher DLIs. Meanwhile a change in the ratio of ST and Reb A was reported. Reb A concentrations increased with the increase in DLI up to 8.53 mol m^{-2} day^{-1}, remaining constant with further increase in DLI. While higher DLIs did not have any effect on Reb A, ST concentrations declined by up to 22% as the DLI increased from 10 to 39.7 mol m^{-2} day^{-1} (Evans et al., 2015).

Stevia rebaudiana is a short-day plant, having a critical photoperiod of between 12 to 13 hours that induces flowering. Early studies by Valio and Rocha (1977) and Zaidan et al. (1980) reported the use incandescent lamps to provide a 1-hour night interruption to be beneficial in delaying flowering of Stevia plants under short day conditions. Ceunen et al. (2012a) realized an increase in SG yields by up to 300% in plants subjected to a 1 hour night break with a low level monochromatic red light. Rivera-Avilez et al. (2021) reported an increase of ST and Reb A concentrations by up to 17% and 24% respectively under a 20-minute night interruption employing a white fluorescent lamp with an intensity of 250 µmol m^{-2} s^{-1}. It was reported that this improvement was dependent on both the cultivar and the duration of the interruption. Meanwhile Yoneda et al. (2017b) reported an increase of almost 200% in terms of biomass for plants under a 4-hour night break using a fluorescent light source with an intensity of 50 µmol m^{-2} s^{-1}. No significant improvement in yields over the 8-hour control were observed in night break treatments with white, red, and far-red light that had a lower intensity of 20 µmol m^{-2} s^{-1}, indicating a possible influence of light intensity in the effectiveness of the night interruption approach.

Besides the photoperiod and light intensity, the spectral composition or light quality has also been reported to affect the growth and the accumulation of biomass and secondary metabolites in Stevia. Simlat et al. (2016) found that blue LED light increased seed germination and produced the largest number of leaves and roots in 4-week-old Stevia plantlets while Abdullateef et al. (2015b) reported the monochromatic red to be optimum to improve the rate of germination of Stevia seeds. Yoneda et al. (2017a) found that blue

light, and a combination of red and far-red light (R-FR) to have higher ST and Reb A concentrations compared to white fluorescent lamps with that had identical intensities and photoperiod. The expression of the UGT85C2 was enhanced under both the blue and R-FR light treatment. However, plants under blue light were shorter and had a more compact morphology (Yoneda et al., 2017a). A recent study by Melviana et al. (2021) reported a similar finding with far-red supplemental lighting resulting in significant increase in biomass accumulation and expression levels of all genes associated with the synthesis of SG. An increase in ST and Reb A concentrations by 37.15% and 2.99% respectively was obtained in plants supplemented with far-red compared to the control (Melviana et al., 2021). The activation of photoreceptors, which activate signalling pathways and alter gene expression, was put forward as a possible reason for the enhanced production of secondary metabolites under the different wavelengths (Rai & Han, 2022). Shulgina et al. (2021) who observed higher ST concentrations in plants grown under light treatments that did not have blue spectral content, suggested the production of secondary metabolites in Stevia is regulated by the red-light spectrum instead.

Table 2.3 : Previous Studies on the Effects of Photoperiod, Light Quality and Night Interruption on Stevia

Aspect	References	Light Treatment	Light Specification	Outcome	Gaps
Photoperiod	1. Ceunen and Geuns (2013b) 2. de Andrade et al. (2021) 3. Yoneda et al. (2017b) 4. Valio and Rocha (1977) 5. Zaidan et al. (1980) 6. Abdulameer et al. (2018) 7. Evans et al. (2015) 8. Yang et al. (2015) 9. Nakonechnaya et al. (2019)	1. 8-hour and 16-hour photoperiod, both with same intensity 2. 12-15 & 16-hour photoperiod 3. Photoperiod from 8 to 24-hours 4. Extended photoperiod 5. Extended photoperiod 6. Sunlight vs 14-hour 7. Sunlight with photperiod & intensity extension 8. 8,10,12 & 16-hour photoperiod 9. Varying intensities	1. High Pressure Sodium Lamp 2. Fluorescent Lamp 3. Fluorescent Lamp 4. Incandescent Lamp 5. Incandescent Lamp 6. Standard White Lamp 7. High Pressure Sodium Lamp 8. No information 9. LED with spectral composition similar to sunlight	1. Increased biomass & metabolite under LD 2. Higher biomass under 15 & 16-hour 3. Increased biomass at higher intensity and longer photoperiod 4. Delayed flowering & increased biomass under long-day 5. Delayed flowering & increased biomass under long-day 6. Long day improved biomass and delayed flowering. 7. Metabolite increased with DLI until 10 mol m^{-2} day^{-1}. 8. Long day improved gene transcription 9. High intensity improved biomass	1. DLI not considered. 2. DLI, Intensity, Spectra information not available /considered. 3. DLI, spectra not considered. 4. DLI, spectra, PPFD not considered. 5. DLI, spectra, PPFD not considered. 6. DLI, PPFD, Spectra not considered/ information not available. 7. Spectra not considered. 8. No Information on artificial lighting system used. 9. DLI not considered.
Light Quality	1. Yoneda et al. (2017a) 2. Melviana et al. (2021) 3. Shulgina et al. (2021) 4. Esra et al. (2016b) 5. Simlat et al. (2016)	1. Red, blue and Far red for constant photoperiod and intensity 2. 1 hour far red light	1. LEDs 2. Far Red LEDs 3. LEDs 4. Fluorescent Light 5. LEDs	1. Red+ Far red & blue improved gene expression and SG accumulation 2. FR improved biomass and SG accumulation	1. Energy data not available 2. No energy data and information on base spectra composition

	6. Ramírez-Mosqueda et al. (2016) 7. Aghighi Shahverdi et al. (2019)	3. White, red+blue, monochrome red, blue, green, and varying combination of red, blue, far red 4. White light 5. Red+white, Red +blue 6. White, red, blue, red+blue 7. Light and Darkness	6. Fluorescent white, LEDs 7. No information	3. Red+blue inhibited shoot growth, red encouraged shoot growth, blue stunted, green same as control 4. Highest rate of germination in darkness 5. Blue light improved germination, red increased height, red+white had highest biomass, blue improved phenolics. 6. Lower shoot length under red, highest under red+blue 7. Highest germination with light	3. No clear information on intensity and photoperiod, energy not considered. 4. No spectral or photoperiod consideration 5. No spectral information 6. Experiment on plantlet, no spectral distribution information 7. No information on spectrum, intensity, photoperiod
Night Interruption	1. Yoneda et al. (2017b) 2. Rivera-Avilez et al. (2021) 3. Ceunen et al. (2012a)	1. Night interruption with red, far red and white for 4 hours. 2. Up to 20 minutes of white night interruption 3. Short night interruption with low level red light	1. LEDs and fluorescent 2. Fluorescent lamps 3. Red LED	1. Higher intensity night interruption improved biomass. 2. Night interruption increased biomass and metabolite yields and delayed flowering. 3. Delayed flowering and improvement in biomass yields	1. No spectral information 2. No information on spectrum and energy 3. No energy or spectral information.

Note : DLI = Daily Light Integral, PPFD = Photosynthetic Photon Flux Density, LD = Long Day, SD = Short Day, SG = Steviol glycoside

CHAPTER 3 : THE EFFECT OF PHOTOPERIOD ON *STEVIA REBAUDIANA*

In this chapter, Stevia plants were germinated from seeds under different photoperiods ranging from 8 to 16 hours. All light treatments had identical daily light integrals and spectral composition. The biomass and metabolite yields, as well as the photon and energy use efficacies for the different photoperiods were obtained. This chapter has been published in 2022 under the title "*Artificial Lighting Photoperiod Manipulation Approach to Improve Productivity and Energy Use Efficacies of Plant Factory Cultivated Stevia rebaudiana*" in *Agronomy* with N Rengasamy, RY Othman, HS Che and JA Harikrishna as the authors.

3.1 Introduction

The quality, intensity and the period of photon activity, or photoperiod, are factors known to regulate the morphogenesis, growth and differentiation of plant cells and tissues (An et al., 2021). The photoperiod, or duration of light, plays a key role in establishing and regulating the plant's internal biological clock for phenological events (de Andrade et al., 2021; Palmer & van Iersel, 2020). Except for day-neutral plants, photoperiod also affects flowering time, with flowering induced under a short photoperiod for short day plants and under a long photoperiod for long day plants (Jones, 2018).

3.2 Literature Review

Early studies on the effects of artificial light on plants utilised wide band light sources and filters (Hernández et al., 2016). The advent of Light Emitting Diodes (LED) paved the way for very narrow beam monochromatic light to be made available for use in horticulture, as supplemental lighting or as a sole source of light, and its use has grown exponentially over the past years. Typically, red, and blue LEDs are used together to

provide the most efficient photosynthetically active radiation (PAR), and Far-red (FR) is commonly added to induce flowering (Morgan Pattison et al., 2018; Zhang et al., 2020b; Zheng et al., 2019b). Horticulture lighting systems design are often determined by the optimal daily light integral (DLI) of the plants to be cultivated. The DLI, described in mol m^{-2} day^{-1}, represents the cumulated photon number of moles that falls on a surface of 1 m^2 over a 24-h period (American Society of Agricultural and Biological Engineers, 2017; Samuoliene et al., 2020a). The optimal DLI values for plants, the amount of light needed by the plant to produce the best results, are often based on natural sunlight and do not consider light quality, intensity or photoperiod (Palmer & van Iersel, 2020). DLI is related to the intensity and photoperiod and has significant impact on the electrical energy usage associated with artificial lighting in indoor cultivation. As the lighting load typically accounts for between 40% to 80% of artificially lighted controlled environment agriculture (CEA) system, it provides the greatest opportunity for optimisation that can lead to an overall reduction in cultivation costs (Graamans et al., 2018; Shaari et al., 2021). The proliferation of LEDs in horticulture has presented an opportunity for manipulation of light intensity and photoperiod to improve plant productivity and energy efficacies. Unlike conventional discharge lamps, LEDs can easily be dimmed without affecting their life span (Palmer & van Iersel, 2020). Studies have also shown improved photosynthetic activity in certain plants that were cultivated under conditions with lower intensities and longer photoperiods compared to those under higher intensities and shorter photoperiod, under the same DLI (Elkins & van Iersel, 2020a, 2020b).

Stevia rebaudiana Bertoni is a perennial plant from the Asteraceae family, native to the highlands of Brazil and Paraguay (Geuns, 2010). The steviol glycoside (SG) compounds found in its leaves, stems and flowers form the basis of zero calorie sweeteners that have become highly popular globally due to the lack of harmful side effects commonly associated with alternative synthetic artificial sweeteners (Libik-Konieczny et al., 2018;

Wojewoda et al., 2018). At present, most countries import Stevia as unprocessed leaves and as processed products from China, India and South America with little local cultivation due to the non-ideal photoperiodic conditions (Abdulameer et al., 2018; Tan et al., 2008). To address the over-reliance of imported products, while catering to the expanding local demand, there is a need to improve the productivity of *Stevia rebaudiana* outside its normal range of latitude, by optimisation of the cultivation environment in controlled environment agriculture (CEA) systems (Abdulameer et al., 2018; Tan et al., 2008).

Past studies on the productivity of Stevia under artificial lighting did not consider the effects of DLI (Ceunen & Geuns, 2013a; Nakonechnaya et al., 2019; Yoneda et al., 2017b). Studies typically focused on the effects of light intensity or photoperiod without considering the total DLI, while studies with constant DLI employed varying spectral compositions (de Andrade et al., 2021; Rengasamy et al., 2021; Yoneda et al., 2017a; Yoneda et al., 2017b). When experiments are conducted using light sources with similar intensity but different photoperiods, the overall DLI can vary significantly, as reported in experiments comparing a 12-h photoperiod with a 16-h photoperiod under identical intensities, where the difference in DLI varied by as much as 33% (Evans et al., 2015; Yoneda et al., 2017b). There is also no available literature reporting evaluation of the lighting energy requirements and efficacies for indoor cultivation of Stevia. Other studies on energy requirements of indoor cultivation focused on lettuce (Chen et al., 2021), spinach (Hardanto & Sumarni, 2021), pepper (Olvera-Gonzalez et al., 2021a) and cucumber (An et al., 2021), either under fully artificially lighted or supplemental lighted conditions. While the use of LEDs has proven to be more energy efficient compared to traditional lighting technologies such as the high-pressure sodium (HPS) lamps, and presents the possibility to enhance the overall yields by employing the use of selected wavelengths, these specialised spectral content systems come at a price: The cost of green,

Far-red (FR), and Ultraviolet (UV) LEDs are often far higher than that of the red and blue LEDs that are common in standard horticulture lighting products. Hence, for an existing facility, it would be beneficial to consider lighting strategies that manipulate the photoperiod and light intensity, to optimise the productivity and overall energy efficacy of the current installation, before resorting to a change of its total lighting system.

This study explored the use of photoperiod and light intensity manipulation as a strategy to improve the biomass accumulation, metabolite concentration and overall metabolite yields of *Stevia rebaudiana* plants grown indoors under full artificial light, especially in locations that would otherwise be non-ideal. The focus of this study is on the use of standard red + blue horticulture lighting systems with small quantities of green light to maximize the plant productivity and energy use efficacies without the need for additional spectral content. Besides looking at the biomass and metabolite yields, this study also evaluated the effects of photoperiod manipulation on the photon conversion and energy use efficacies under a constant DLI and spectral composition. The photon conversion efficacy (PCE), expressed in mg mol^{-1}, represents the amount of Rebaudioside A (Reb A) + Stevioside (ST) that can be produced by 1 mol of photons, while the energy use efficacy (EUE), expressed in g kWh^{-1}, denotes the amount of electrical energy consumed to produce 1 g of Reb A and ST compounds.

3.3 Material and Methods

3.3.1 Plant Materials

Stevia rebaudiana seeds procured from Bakers Creek Heirloom Seeds, USA (https://www.rareseeds.com) (accessed on 10th January 2022) were washed under running tap water and dried on a filter paper prior to use. A 50- cell plug tray (54 cm × 28 cm × 5.7 cm) was filled with autoclaved potting soil (www.serbajadi.com.my) (accessed on 10th January 2022), then seeds were surface sowed with 1 seed per cell. The seeds were

purchased in batches for each experimental cycle. The soil and seed were sprayed with water and wrapped in clear plastic to prevent evaporation of moisture. The trays were placed in a climate controlled dark room for experiments with artificial light or in a greenhouse, both within the Plant Biotech Facility of University of Malaya, Kuala Lumpur. The temperature of the dark room and greenhouse were maintained at 25 °C ± 2 °C with a relative humidity of 70–80%. The dark room was housed in a building within the facility, with highly insulated walls, floors, and ceiling, and without any windows. The greenhouse had diffused colourless polycarbonate walls and roof, with 3 of the 4 walls not exposed to the external environment. The temperature of both rooms was controlled via air conditioning units that had an average cooling load of approximately 2500 BTU m^{-2} while the humidity in both rooms were controlled via a standalone humidifier with a built in sensor that was set to begin operations as the room humidity drops below 70%.

Five weeks after sowing, the seedlings were removed from the plug trays and transplanted into individual pots (12 cm × 12 cm × 10 cm) filled with autoclaved potting soil (www.serbajadi.com.my) (accessed on 10th January 2022). A total of 24 seedlings from all treatments were selected for transplanting. The seedlings were selected based on the mean height, discarding the outliers. The transplanted seedlings were watered sparingly, ensuring the topsoil remained moist while preventing water logging. The experiment was repeated 3 times from August 2018 to October 2019. Each cycle lasted for 175 days from the first sowing of the seeds. In order to ensure timelines are adhered to and to ensure a full 175 days per experimental cycle, the sowing and germination of the cycles has an overlap, with experimental cycle 2 starting before the end of experimental cycle 1, and cycle 3 starting before the end of experimental cycle 2. The plants under artificial light and under natural sunlight in the climate-controlled greenhouse (GH) had a planting density of 24 plants/m^2.

The Daily Light Integral (DLI) for the respective light treatments were computed as follows:

$$\text{DLI} = \frac{(\text{PPFD} \times 3600 \times \text{Photoperiod in Hours})}{1{,}000{,}000}$$

where, DLI = Daily Light Integral in mol $m^{-2}day^{-1}$, PPFD = Measured Photosynthetic Photon Flux Density in µmol m^{-2} s^{-1}.

3.3.2 Light Treatments

Four custom-built lighting systems were used for this study. Each system consisted of 144 high powered LEDs (Cree, USA and Osram, Germany) in a single channel (Figure 3.1A). The 144 LEDs were connected in series consisting of 96 Red LEDs with a peak of 630 nm, 24 Green LEDs with a peak of 550 nm and 24 Blue LEDs with a peak of 450 nm (Figure 3.2). The intensity of each unit was individually controlled by varying the supply current for each system, via the built-in potentiometer of the power supply units (Meanwell, Taiwan). Each system has a maximum wattage of 400 W, limited by the power supply units, to ensure the LEDs solder point temperature did not exceed the rated values provided by the manufacturers. Prior to installation at the facility, the photosynthetic photon flux (PPF) of the system, that denotes the total amount of light within the PAR range that is emitted by the luminaire, was measured in a 3-meter integrating sphere (GE Lighting, USA) at Novabrite Lighting Sdn Bhd, Malaysia (www.novabrite.com.my) (accessed on 10th January 2022) lighting laboratory. The lighting fixtures were set to its predetermined intensity based on the respective treatments before being measured in the integrating sphere. The measurements (photosynthetic photon flux, PPF and power, W) from the integrating sphere was recorded after 1 hour of operation. The photoperiods were controlled by the means of a standard timer (Hager EH711, Germany). The intensity of each artificial lighting treatment was adjusted to ensure all treatments had the same DLI of 7.2 ± 0.1 mol $m^{-2}day^{-1}$. The intensity,

photoperiod and light recipe for each treatment are as in Table 3.1. The plants exposed to the photoperiod named 16HI (where "I" indicates intermittent) were exposed to 5.3H Light/2.7H Dark on a continuous loop such that they received a total of 16 h of light intermittently over a period of 24 h (Table 3.1, Figure 3.2A). The photoperiods were selected to represent Stevia's critical photoperiod (12H), a 4-hour addition to its critical photoperiod (16H, 16HI), and 4-hours less than its critical photoperiod (8H). All systems were fitted with a digital energy meter (BAYITE-PZEM-061, China) to monitor and record the overall power and energy consumption throughout the duration of the experiments. The American Society of Agricultural and Biological Engineers (2017) standards were used to define the PAR and Plant Biologically Active Radiation (PBAR) range.

Table 3.1 : Light Treatments of Photoperiod Experiments

Parameters	Unit	8H	12H	16H	16HI	GH
UVA (380nm)	µmol $m^{-2}s^{-1}$	0	0	0	0	4.38 ± 0.9
Blue (450 nm)	µmol $m^{-2}s^{-1}$	50.00 ± 1.0	33 ± 0.7	25 ± 0.5	25 ± 0.5	78.55 ± 1.6
Green (550 nm)	µmol $m^{-2}s^{-1}$	12.26 ± 0.2	8.25 ± 0.2	6.25 ± 0.1	6.25 ± 0.1	119.10 ± 2.4
Red (630 nm)	µmol $m^{-2}s^{-1}$	186.74 ± 3.7	123.75 ± 2.5	93.75 ± 1.9	93.75 ± 1.9	135.83 ± 2.7
Far Red (730 nm)	µmol $m^{-2}s^{-1}$	0	0	0	0	91.05 ± 1.8
PPF 400-700 nm	µmol s^{-1}	414 ± 8.2	231 ± 4.62	175 ± 3.5	175 ± 3.5	N/A
PPFD [a] 400-700 nm	µmol $m^{-2}s^{-1}$	249 ± 5.7	165 ± 3.3	125 ± 2.5	125 ± 2.5	333.48 ± 6.7
PBAR [a] 280-800 nm	µmol $m^{-2}s^{-1}$	249 ± 5.7	165 ± 3.3	125 ± 2.5	125 ± 2.5	409.10 ± 8.2
DLI	mol $m^{-2}day^{-1}$	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	14.41 ± 0.3
Light Hours in a day	Hours (h)	8	12	16	5.3H × 3	12
Planting Density	Plants/m^2	24	24	24	24	24
Power	W	316	175	129	129	0

Note: [a] The Photosynthetic Photon Flux Density (PPFD),and the Plant Biological Active Radiation (PBAR) spectral wavelength ranges and definition were based on American Society of Agricultural and Biological Engineers (2017).

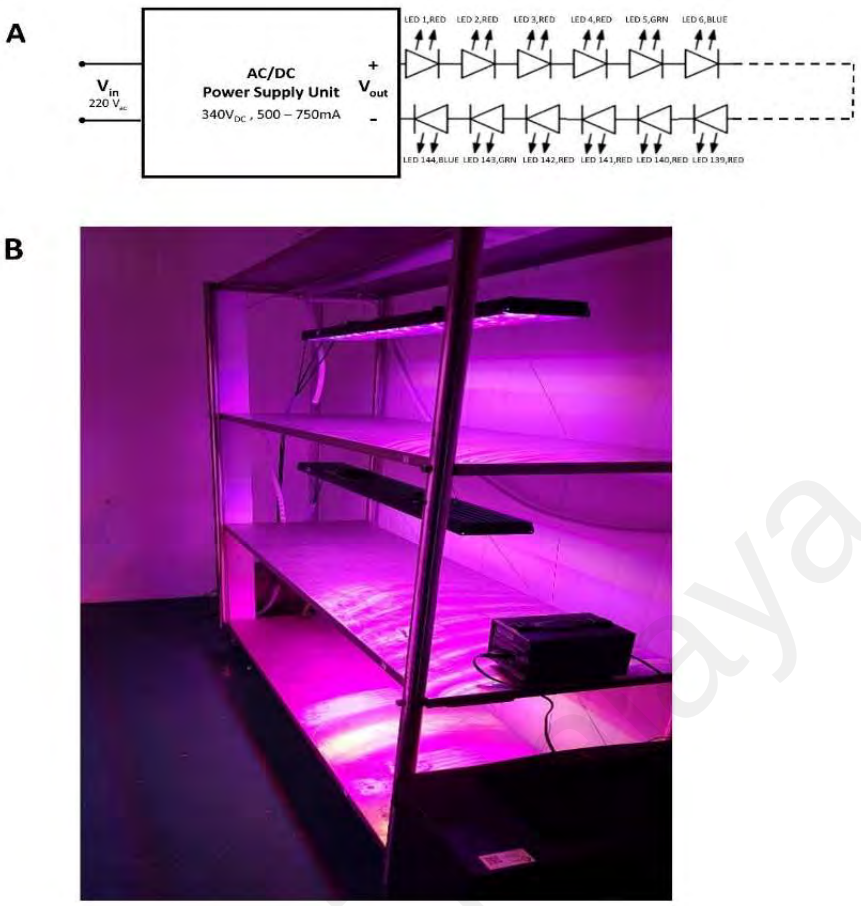

Figure 3.1 : Artificial lighting fixture. (A) Lighting system circuit. (B) Actual lighting fixture setup in growth room.

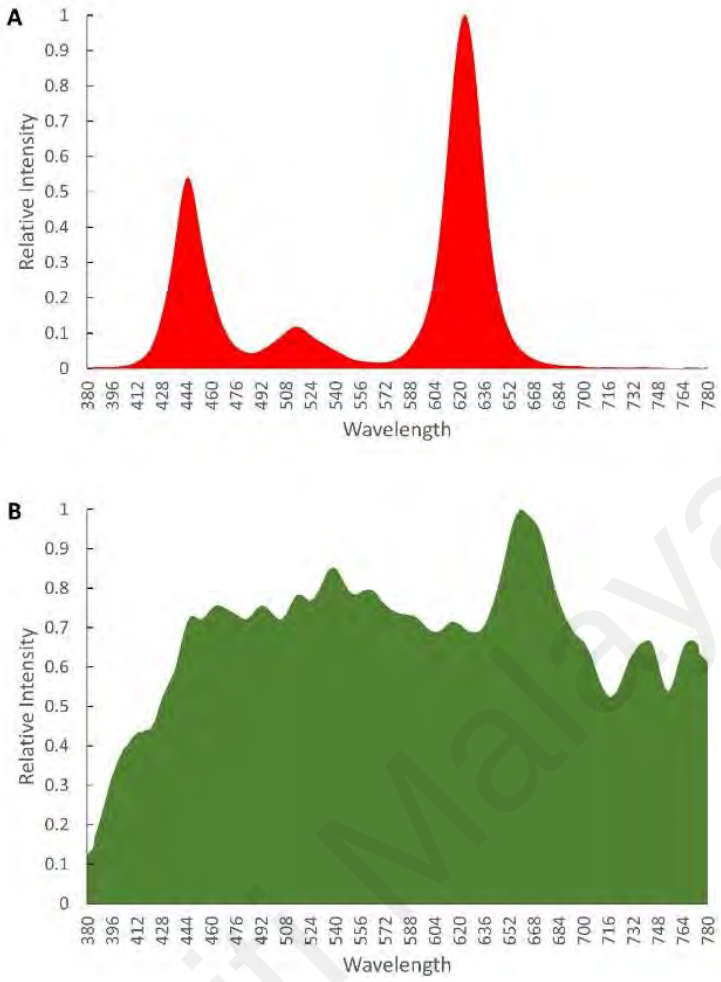

Figure 3.2 : Spectral distribution of Light Treatments used for Photoperiod experiments. (A) Artificial light treatment. All treatments used equal spectral distribution but with varying intensities and photoperiod. (B) Spectral distribution of natural daylight measured at the greenhouse.

The artificial lighting systems were installed on 4 separate racks. All 4 racks were located within the same climate controlled dark room. Each of the 4 racks had 3 growing levels installed with identical light treatments (Figure 3.1B). A black mesh material was used to shield the racks, preventing light trespass and interference between the different light treatments.

One experimental set of plants was grown in the climate-controlled greenhouse (GH) under natural light and photoperiod as a control for natural tropical sunlight and day-neutral photoperiod. The typical photoperiod in Kuala Lumpur, Malaysia (latitude 3°08′28.32″ N) is 12-h (Othman et al., 2018; Tan et al., 2008). As the intensity and

spectral distribution of the natural light captured in the greenhouse varied significantly throughout the day, light measurements were conducted at 5-min intervals, continuously over a 14-day period using a portable spectroradiometer (Asensetek, Taiwan) (Table 3.1, Figure 3.2B). The spectroradiometer was placed 30cm above the growing surface of the GH.

3.3.3 Plant Productivity Analysis

3.3.3.3 Biomass Yield

Plants were harvested at the end of each 175-day cycle, in November 2018, May 2019 and September 2019 by cutting all stems at 5 cm above the soil. Only samples that had not reached the flowering stage were harvested. The leaves and stems for each plant were separated and a digital scale (Shimadzu, Japan) was used to determine fresh weight. The leaves for all plants under the same photoperiod were collected and washed under running water. The leaves were drained in a mesh bowl before gently blotting with tissue paper. The leaves were dried in an oven (Binder, Germany) at 60 °C for 20 h, at which time a steady weight was achieved. The dried leaves and stems were cooled to room temperature before measurements of dry weight were made. After weighing, the leaves were packed with a desiccant (silica gel) in an airtight container. The samples were stored at -4 °C before LCMS analysis.

The percentage biomass partitioning towards the leaves were calculated using the formula:

$$\text{Leaf Biomass Partioning } = \frac{\text{Leaf DW}}{(\text{Leaf DW} + \text{Stem DW})}\%$$

3.3.3.4 Metabolite Yield

Although various components of SG can be separated via LCMS, this study focused only on ST and Reb A as these 2 components account for more than 90% of the total SG

present in Stevia leaves, and are compounds with the highest commercial demand at present (Ciriminna et al., 2019; Wojewoda et al., 2018). Analytical standards of ST (804.87 g mol^{-1}) and Reb A (967.01 g mol^{-1}) (purity > 98%) obtained from Sigma-Aldrich (Germany) were used as an external reference. The standard compounds were dissolved in 1 mL mixture of 70:30 *v*/*v* water and acetonitrile before injected into the LCMS apparatus to identify its peaks and to construct the standard curves for each compound using the methods outlined in FAO and WHO (2020).The dried leaves from all cycles were combined after the final harvest in September 2019 and were subjected to LCMS analysis to determine the percentage content of Reb A and ST. Ground dried leaves were extracted via the cold maceration method in which 0.5 g of ground leaves was mixed with 50 mL of 35:65 (*v*/*v*) ethanol and water. The sample was sonicated for 2 h and filtered. The resulting eluents were dried using a miVac centrifugal concentrator. Samples of 10 mg of the resulting extract were dissolved in 1 mL of 70:30 (*v*/*v*) mixture of water and acetonitrile. The sample was filtered using a PES membrane with 0.22µm pore size. LCMS analysis used an LC-MS QTOF apparatus (Agilent 1290 Infinity™) with a C18 column. A modified approach based on the assay methods outlined in FAO and WHO (2020) was used to quantify the concentration of ST and Reb A among the within the leaves of each treatment, expressed in percentage of mass of leaf dry matter (%*w*/*w*).

To obtain the quantity of the total ST and Reb A metabolite yields that can be realised, the mean combined Reb A and ST yields per plant, expressed in g $plant^{-1}$, was calculated as follows:

$$\text{Metabolite Yields} = \text{Mean Leaf DW} \times (\text{Reb A Concentration} + \text{ST Concentration})$$

where,

Mean Leaf DW = Mean leaf dry weight per plant in g;

Reb A & ST Concentration = Percentage composition per gram of leaf DW (Obtained from LCMS results).

3.3.4 Energy and Photon Efficacy

The lighting power density (LPD) representing the lighting electrical energy used per m^{-2} of growth area, directly related to the overall energy demand of a CEA, expressed as Wm^{-2}, was calculated as follows:

$$\text{LPD} = \frac{\text{Measured Lighting Power (W)}}{(\text{Growth area in m}^2)}$$

The photon conversion efficacy (PCE), the amount of Reb A + ST that can be produced with 1 mol of light, represented in mg mol^{-1} was calculated using the following equation:

$$\text{PCE} = \frac{[(\text{Reb A} + \text{ST Yield per plant (mg)}) \times \text{Planting Density}]}{(\text{DLI} \times \text{No. of Days})}$$

The photosynthetic photon efficacy (PPE) of the systems that describes the amount of photosynthetic radiation emitted by the system for every unit of electrical energy consumed, denoted in µmol J^{-1}, was calculated as follows:

$$\text{PPE} = \frac{\text{PPF (µmol s}^{-1})}{\text{Measured Lighting Power (W)}}$$

The total cooling power and energy measurements of the growth room and greenhouse were measured using a 3-phase power quality logger (Fluke 1735) that was connected to the input at the distribution panels at both locations. The greenhouse did not have any other electrical loads besides the air conditioning system. Although the growth room had the lighting and air conditioning loads, as each lighting system had its own logger, only the air conditioning loads were measured. The measurements were logged at 5-min intervals over a period of 10-days. To obtain the highest energy use, the measurements were done under full load conditions with growth areas filled to maximum density with

fully grown plants just before harvest. Past literature evaluating the power and energy consumption and loads within CEA systems typically normalised the energy and power consumptions to $1m^2$ of space (Graamans et al., 2018; Graamans et al., 2020). In this study, the results obtained from the data logging were used to calculate the cooling power density (CPD) expressed in W m^{-2}, normalised to 1 m^2 of growth space in both facilities.

$$\text{CPD} = \frac{\text{Measured Cooling Power (W)}}{\text{Total Growth Area } (\text{m}^2)}$$

The Cooling Power Density for the growth room was measured under light and dark conditions. As the greenhouse cooling system was influenced by the external ambient conditions, a 24-h average was used, resulting in the following values:

Using the values obtained in Table 3.2, the total energy density (kWh m^{-2}) for the growth room and greenhouse was calculated. These values present the total electrical energy consumed per m^2 of growth space over the 175 days growth cycle. The values were calculated using the following equations:

Greenhouse,

$$\text{Total Energy Density} = \frac{\text{CPD } (\text{Wm}^2) \times 24\text{hours} \times 175 \text{ days}}{1000}$$

Growth room,

$$\begin{aligned}\text{Total Energy Density} &= \text{Cooling Energy Density (Light)} \\ &+ \text{Cooling Energy Density (Dark)} + \text{Lighting Energy Density}\end{aligned}$$

where,

Cooling Energy Density (Light)

$$= \frac{\text{CPD (light)}(\text{Wm}^{-2}) \times \text{Light Hours} \times 175 \text{ days}}{1000}$$

Cooling Energy Density (Dark)

$$= \frac{\text{CPD (Dark)}(\text{Wm}^{-2}) \times \text{Dark Hours} \times 175 \text{ days}}{1000}$$

$$\text{Lighting Energy Density} = \frac{\text{LPD (Wm}^2) \times \text{Light Hours} \times 175 \text{ days}}{1000}$$

Table 3.2 : Cooling Power Density of Growth Room and Greenhouse

Growth Room		**Greenhouse**
Dark	Light	
32.78 ± 4 Wm^{-2}	110.75 ± 4.9 Wm^{-2}	139.34 ± 70 Wm^{-2}

As the artificial lighting and cooling systems were fixed for all experimental cycles, there were no difference in energy densities obtained between replicates of the same treatment.

The energy use efficacy (EUE) that described the realisable yield for every kWh of electrical energy consumed, expressed in mg kWh^{-1} was calculated for both the biomass and metabolite yields as follows:

$$\text{EUEbiomass} = \frac{\text{Mean Leaf DW (mg plant}^{-1}) \times \text{Planting Density}}{\text{Total Energy Density (kWh m}^{-2})}$$

$$\text{EUEmetabolite} = \frac{Metabolite\ yields\ (mg\ plant^{-1}) \times Planting\ Density}{Total\ Energy\ Density\ (kWh\ m^{-2})}$$

3.3.5 Statistical Analysis

The 1-way Analysis of Variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) post hoc test with $p < 0.05$ was used to identify the statistical significance and relationship between the results, while a 2-way ANOVA was used to

evaluate the interaction effects between the light treatments and experiment cycles. The IBM SPSS Statistics package (V25.0) was used for all statistical analysis.

3.4 Results

3.4.1 Interaction Effects

The results from the 2 -way ANOVA (Table 3.3) showed that there were no significant interactions between the replicates and light treatments (Replicate*Treatment), indicating that the effects of light treatment on the parameters were reproducible and were not caused or affected by the experiment replications. While there were statistically significant effects between the treatments was not unexpected, there were also statistically significant effects at $p < 0.05$ observed between the replicates within a treatment. However, further analysis of the estimated marginal means indicated that while the mean values for all treatments under replicate 2 varied compared to replicate 1, the overall pattern on the effectiveness of the different treatments remained the same through all replicates ensuring the validity and robustness of the data obtained. While the mean values for all parameters measured were higher under replicates 1 and 3 while having lower values in replicate 2, the Tukey's HSD for all 3 replicates were identical.

Table 3.3 : ANOVA Results on Interaction Effects

		Sum of Squares	df	Mean Square	F	Significance ($p < 0.05$)
Replicate	Leaf FW	11.163	2	5.581	4.284	0.015
	Stem FW	20.557	2	10.278	4.060	0.018
	Leaf DW	0.423	2	0.212	3.926	0.021
	Partitioning	0.004	2	0.002	3.407	0.034
	PCE	2.817	2	1.408	3.278	0.039
	Metabolite Yield	0.008	2	0.004	3.532	0.030
	EUE Biomass	282.013	2	141.007	3.846	0.022
	EUE Metabolite	5.376	2	2.688	3.415	0.034
Treatment	Leaf FW	4179.745	4	1044.936	801.957	0.000
	Stem FW	1522.918	4	380.729	150.375	0.000
	Leaf DW	87.250	4	21.813	404.547	0.000
	Partitioning	0.111	4	0.028	49.265	0.000
	PCE	1875.729	4	468.932	1091.497	0.000
	Metabolite Yield	4.610	4	1.153	1056.411	0.000
	EUE Biomass	112,535.142	4	28133.785	767.313	0.000

	EUE Metabolite	4861.022	4	1215.255	1543.934	0.000
Replicate * Treatment	Leaf FW	1.690	8	0.211	0.162	0.995
	Stem FW	4.418	8	0.552	0.218	0.988
	Leaf DW	0.114	8	0.014	0.264	0.977
	Partitioning	0.002	8	0.000	0.390	0.926
	PCE	1.439	8	0.180	0.419	0.910
	Metabolite Yield	0.005	8	0.001	0.538	0.828
	EUE Biomass	79.097	8	9.887	0.270	0.975
	EUE Metabolite	2.387	8	0.298	0.379	0.931
Error	Leaf FW	449.529	345	1.303		
	Stem FW	873.497	345	2.532		
	Leaf DW	18.602	345	0.054		
	Partitioning	0.194	345	0.001		
	PCE	148.220	345	0.430		
	Metabolite Yield	0.376	345	0.001		
	EUE Biomass	12,649.543	345	36.665		
	EUE Metabolite	271.555	345	0.787		

Note: 2 Way ANOVA with replication was used to analyse the interaction effects between the Light Treatments (Treatment) and Experiment cycles (Replicate). Values are mean (n = 24) per replicate at $p < 0.05$. As the lighting and cooling electrical energy were fixed, the energy density value did not vary by replicate.

3.4.2 Biomass Yield

The 16H treatment, that had the longest continuous photoperiod, resulted in the highest accumulation of fresh leaf and stem biomass, with 21% more fresh leaf per plant and 20% more fresh stem compared to the 8H treated plants that had the next greatest yield of fresh biomass (Figure 3.3A, B). GH treatment had the lowest fresh leaf and stem yield, 66% lower compared to the 16H treatment for both. The 16HI and 8H treated plants had the highest dry biomass partitioning towards it leaves at 34.77% and 33.73%, while the longer photoperiods of 16H, 12H and GH had similar portioning of 31% (Figure 3.3D). 16H treated plants had the highest dry leaf yield among all treatments. 16HI had significantly lower fresh leaf yields but plants in this group had dry leaf yields comparable to those from the 16H treatments, with 1.93 g dry leaf yield per plant. 8H and 12H had 1.30 g and 1.22 g respectively while the lowest dry leaf biomass was observed in plants grown under GH (Figure 3.3C).

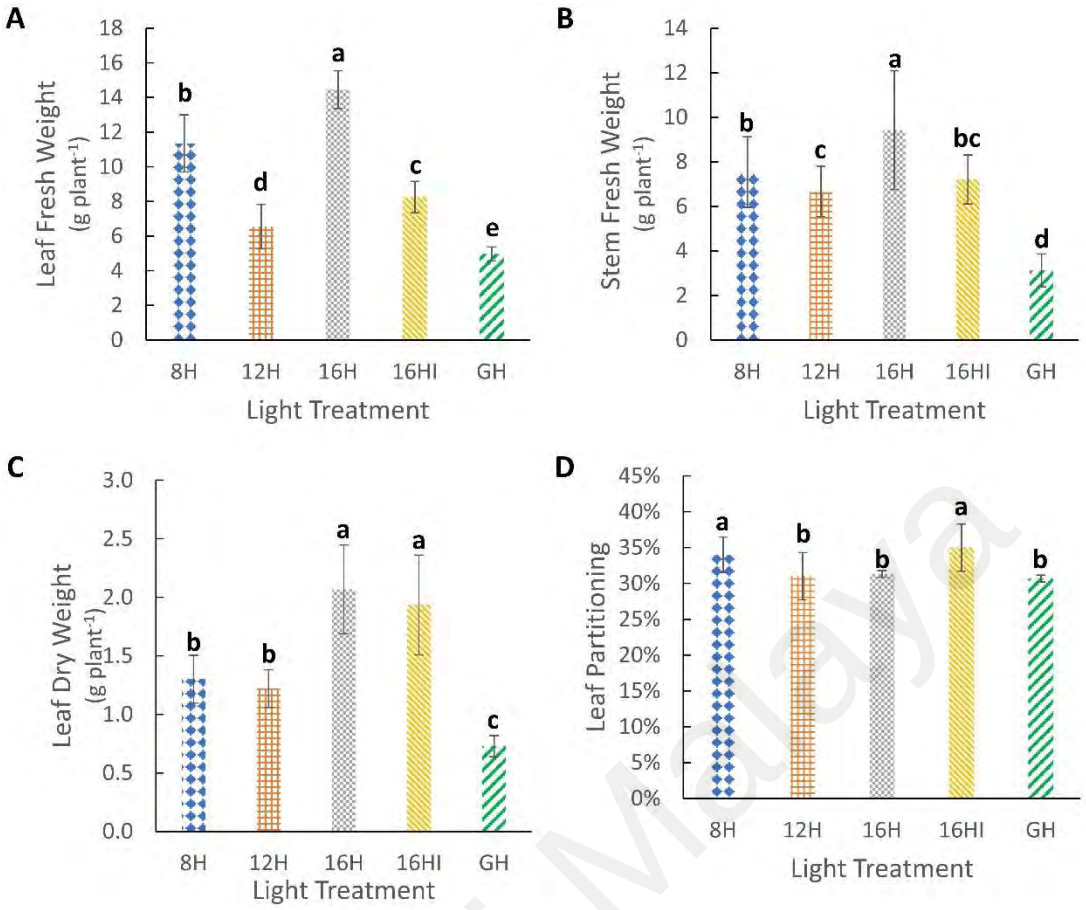

Figure 3.3 : Effect of photoperiod on fresh and dry biomass (A) Leaf fresh weight (g plant^{-1}). (B) Stem fresh weight (g plant^{-1}). (C) Leaf dry weight (g plant^{-1}). (D) Percentage dry biomass partitioning towards leaves (%). All measurements were obtained at 175 DAP. Values represents Mean (n = 72) ± Standard Deviation. Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at $p < 0.05$.

3.4.3 Metabolite Concentration and Yield

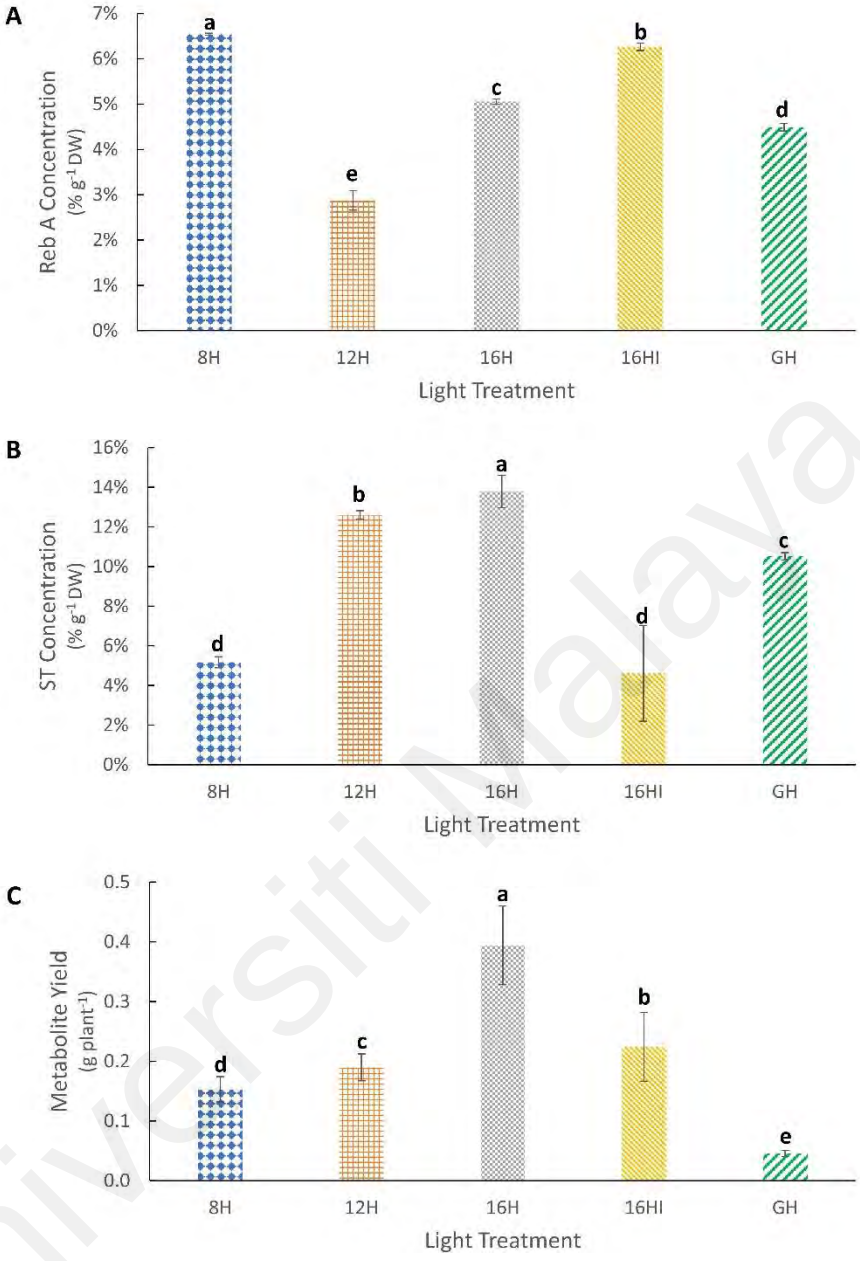

Figure 3.4 : Effect of photoperiod on metabolite accumulation in *Stevia rebaudiana* leaves. (A) Percentage concentration of Reb A in dry leaf weight (%). (B) Mean percentage concentration of ST in dry leaf weight (%). (C) Average metabolite (ST + Reb A) yield per plant (g plant−1). Values represents Mean (n = 72) ± Standard Deviation. Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at $p < 0.05$.

At 175 DAP, plants under the 8H photoperiod had the highest Reb A content at 6.54% w/w of dry leaf followed by 16HI (6.27% *w/w*), while 12H had the lowest Reb A content at 2.87% *w/w* (Figure 3.4A). Plants from the 8H and 16HI treatments, with the highest

Reb A content had the lowest ST accumulation in their leaves (Figure 3.4B). 8H (5.17% *w*/*w*) and 16HI (4.61% *w*/*w*) were the only treatments with ST content below 10% w/w, 56% and 31% lower than the 16H treatment that had the highest ST content of 13.79% *w*/*w*. The plants grown under natural sunlight and photoperiod (GH) had the lowest yield of 0.04 g per plant while the 16H treatment had the highest combined yield of ST and Reb A per plant at 0.39 g, 975% higher than that of GH, followed by 16HI (0.22 g), 12H (0.19 g) and 8H (0.15 g) light treatments (Figure 3.4C).

3.4.4 Energy and Yield Efficacy Analysis Results

The 8H and 12H treatments, both with higher PPFDs, had higher lighting power densities (LPD) at 316 W m^{-2} and 175.01 W m^{-2} respectively, compared to the 16H and 16HI treatments (Figure 3.5A). Among the artificial light treatments, the 16H treatment had the highest photon conversion efficacy, producing a combined 7.5 mg of ST and Reb A compound for every mol of light while the 8H treatment was the lowest, producing 2.92 mg of the same compound for every mol of light (Figure 3.5B). No significant changes in PPE were observed under the different photoperiods and intensities.

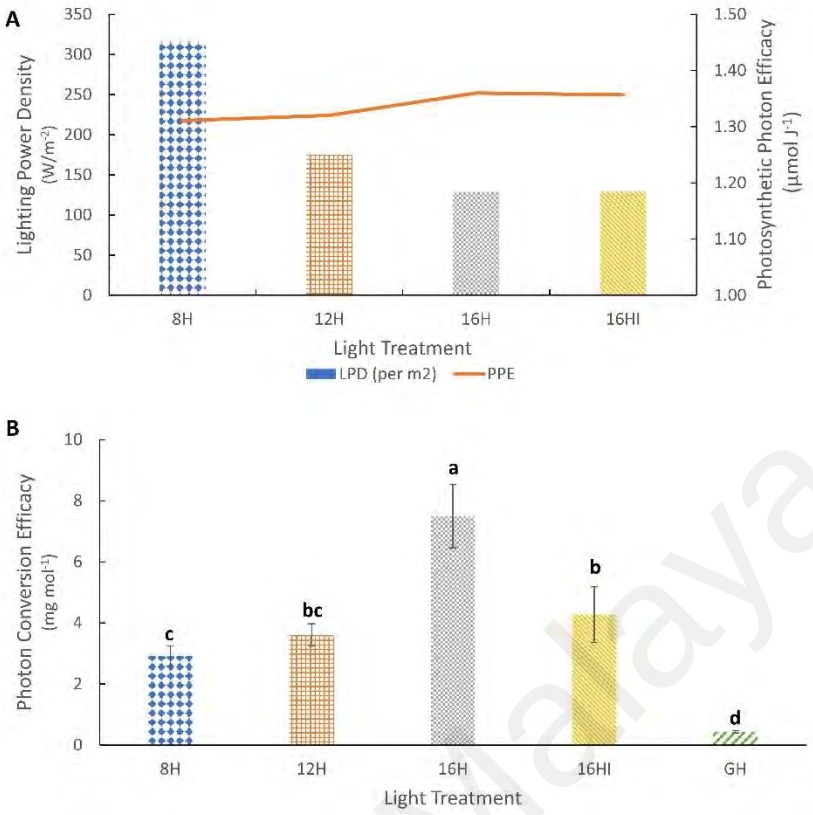

Figure 3.5 : Photosynthetic and Photon Conversion Efficacy (A) Lighting Power Density (Wm−2) and Photosynthetic Photon Efficacy (µmol J^{-1}) for different artificial light treatments. The bar charts indicate the Lighting Power Densities (LPD) for the different light treatments while the Line chart describes the Photosynthetic Photon Efficacies (PPE) for the same light treatments. (B) Photon Conversion Efficacy (PCE), the total amount of ST and Reb A produced for every mol of light (mg mol−1). Values represents Mean (n = 72) ± Standard Deviation. Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at p < 0.05.

The energy consumption of the growth room that used artificial lighting was significantly higher compared to the naturally lighted greenhouse, with the extended photoperiods of 16H and 16HI having the highest energy density of 763 kWh m^{-2} each (Figure 3.6A). The higher energy requirements of the artificial lighting systems were driven by the additional artificial lighting loads that were not present in the greenhouse that used natural sunlight with air conditioning for temperature regulation. Overall, the greenhouse had the lowest total energy consumption of 585 kWh m^{-2} (Figure 3.6B). Even though the 16H protocol had the highest energy consumption, it was the most efficient photoperiod, resulting in the highest EUE for both biomass (61.5 mg kWh^{-1}) and metabolite (12.4 mg kWh^{-1})

accumulation. Conversely, although the greenhouse had the lowest overall energy consumption among all experiments, it had the lowest EUE for biomass and metabolite accumulation at 29.89 mg kWh^{-1} and 1.87 mg kWh^{-1} respectively.

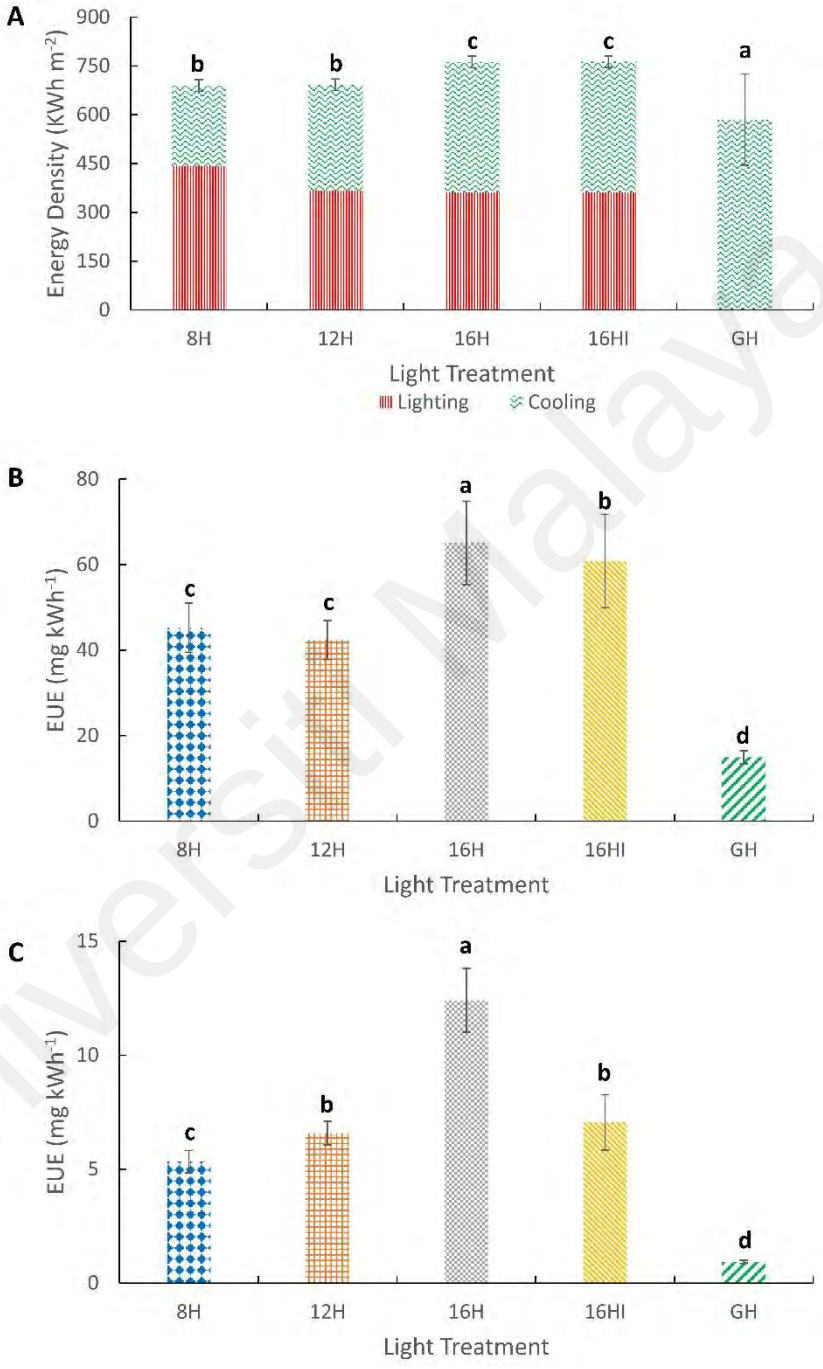

Figure 3.6 : Electrical Energy Consumption and Efficacies. (A) Total electrical energy consumption over 175 days growth cycle (kWh m^{-2}). (B) Energy Use Efficacy (EUE) of biomass production (mg kWh^{-1}). (C) Energy Use Efficacy of metabolite production (mg kWh^{-1}). All values normalised to 1 m^2 of growth space. Values represents Mean (n = 72) ± Standard Deviation. Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at $p < 0.05$.

3.5 Discussion

Indoor cultivation of plants in controlled environments under full artificial light has been shown to be not only economically viable for many plant species but also improved yields and quality (Hardanto & Sumarni, 2021; He et al., 2020b; Hwang et al., 2020). The productivity of such systems is however effected by the light quality, intensity, and photoperiod (An et al., 2021; de Andrade et al., 2021; Palmer & van Iersel, 2020). As artificial lighting systems in CEAs account for a significant portion of the energy usage and costs, (Graamans et al., 2018; Zhang & Kacira, 2020) it is important to ensure that qualitative and quantitative aspects are optimised for the plants being cultivated. At present, artificial lighting used in indoor cultivation are designed to provide photoperiodic extension, supplemental intensities, or both. These artificial lighting systems are often designed based on the ideal DLI requirements of the plants. DLI being a function of the photoperiod and intensity has a direct relationship on the energy consumption and cost within a CEA. A common approach towards achieving a high DLI is to have high light intensities and extended photoperiods. This does not only increase the overall lighting energy costs, but also indirectly increases the cost of cooling. While LEDs are more energy efficient compared to traditional fluorescent and discharge lamp technologies, it still converts up to 48% (Graamans et al., 2020) of the electrical energy into heat. Hence, a system having higher intensities, DLI or both, would contribute more internal heat build-up within a facility. Using DLI values equivalent that is typically based on natural daylight is also not efficient when using LEDs that can supply the specific wavelengths used by the plant to maximise the intended yields. Studies have reported the beneficial and detrimental effects of selected spectral content on the productivity of multiple plant species (Demotes-Mainard et al., 2016; Huché-Thélier et al., 2016). Therefore, the full spectral composition available in natural daylight may not be the most optimised spectra for the plant, and as such the DLI based on natural light alone is not a proper reference

towards maximising plant productivity as it does not consider the qualitative aspect of the light. This is apparent from studies showing that artificial lighting with different spectral composition but with identical PPFDs and DLI, having significantly different yields (Rengasamy et al., 2021; Zhen & Bugbee, 2020a).

Although past studies have shown that introduction of short night interruptions with red and far-red lights are able to extend the vegetative period of field grown Stevia plants (Ceunen et al., 2012a), the tropical climate in Malaysia, with a hot and humid climate with thunderstorms throughout the year limits large scale field cultivation (Abdulameer et al., 2018; Tan et al., 2008). Hence, this study is specifically focused on indoor cultivation in an environmentally controlled condition. Studies have shown that the SG and biomass accumulation in field grown Stevia plants vary significantly according to the seasons and other environmental factors such as humidity, temperature and water availability (de Andrade et al., 2021). Indoor cultivation would eliminate these uncertainties as all environmental conditions can be controlled and reproduced as desired, regardless of external environmental conditions, not requiring natural light, ensuring a stable and predictable yield. The photoperiod experimental system in the current study, was designed to provide an output within the PAR region with the intensities adjusted to achieve identical DLIs across all artificial lighting photoperiods. Past studies have shown that having a lower PPFD over a longer photoperiod to be more productive in lettuce and Mizuna, than having higher intensities with shorter photoperiods, with identical DLIs, as lower PPFDs promote more efficient photosynthetic activity in plants (Palmer & van Iersel, 2020). Besides improving plant productivity, lower light intensities would also require less lighting power, improving the lighting system efficacy due to reduced effects from the thermal and current droop within the LED package (Kusuma et al., 2020). The spectra distribution of the artificial lighting systems in this study were selected to match the peak sensitivity range of the photosynthetic pigments. Red and blue light were used

as the basal spectra as plant photosynthetic pigments chlorophyll a, chlorophyll b and carotenoid are most sensitive to these wavelength ranges (Leyla et al., 2018). Small amounts of green spectra were introduced to address the lower sensitivity areas of the chlorophyll absorption spectra between the green 500 nm to 600 nm range in order to provide the plants with a continuous spectrum that mimics the pattern of the chlorophyll absorption range and not just the peak wavelengths (Ouzounis et al., 2015; Zheng et al., 2019b). While the combination of red and blue spectra is known to be highly effective in stimulating photosynthesis, more recent studies have found that adding supplemental green light to match closer to the chlorophyll action spectra is beneficial in stimulating plant photosynthetic response as green light is able to penetrate deep through the canopy reaching leaves at lower levels, unlike red and blue light (Claypool & Lieth, 2020; Zhang et al., 2020b). The artificial lighting systems had a maximum wattage of 400 Ws and operating at a maximum of 80% of the maximum wattage, the maximum intensity was determined to be at 249 ± 5.7 μmol $m^{-2}s^{-1}$. This value was used for the shortest photoperiod of 8H and the DLI calculated was at 7.2 mol $m^{-2}day^{-1}$. This DLI value was then selected for all artificial lighting systems. Incidentally, it represented 50% of the average DLI obtained from natural sunlight in the greenhouse and was within the range reported by Evans et al. (2015) to be ideal for accumulation of ST and Reb A compounds. The treatments were set up to compare the effects of 8, 12 and 16 h of continuous light within a 24-h period. The 16HI treatment was designed to create a photo stressed environment by providing a shorter light and dark period of 5.3 h light and 2.7 h of darkness, with the cycle repeated 3 times daily resulting in a cumulative photoperiod of 16 h delivered intermittently over a 24-h period. Plants grown in a greenhouse (GH) with around 12 h of natural daylight were used as a reference for plant growth and metabolite analyses. To maximise the ST and Reb A yields in indoor cultivated Stevia plants, both, the dry leaf biomass and concentrations of ST and Reb A must be increased. There was a

small but statistically significant difference ($p < 0.05$) observed in the measurements between replicates 1 and 2 within each treatment. This observation is attributed to the genetic variation of the seeds. While the seeds were sourced from the same supplier and is of the same plant type, as it was procured in batches at different times over the course of 2 years, it was not possible to control or limit its genetic variability. Although there was no significant difference in germination rates observed for the different photoperiods, there were clear differences in the accumulation of biomass and metabolites that could influence the economics of plant productivity.

3.5.1 Plant Productivity

3.5.1.1 16 Hour Continuous and Intermittent Photoperiod Optimised Biomass Accumulation

The leaves are the most commercially important part of the Stevia plant as they have the highest concentrations of SG compounds. From the findings of this study, it was noted that the dry leaf weight was the best representation of the metabolite yields of the plants compared to the fresh leaf yields. While the results obtained for 16H, 8H and 12H were consistent with studies that noted the increase in dry leaf biomass under higher light intensities, photoperiod or DLIs (Ceunen & Geuns, 2013a; Ermakov & Kochetov, 1994; Yang et al., 2015; Yoneda et al., 2017b) the high yield from the 16HI was not expected given the significantly lower fresh leaf yield obtained. It was also observed that while both 8H and 12H treatments resulted in comparable dry leaf yields per plants, the 8H treatment resulted in significantly higher fresh leaf yields compared to 12H treated plants. This points to a higher moisture content of 89% in the leaves of 8H treated plants compared to 81% of those in plants treated under the 12H photoperiod. While the mean moisture contents of these treatments were in line with those from previous studies (Ceunen & Geuns, 2013c; Ceunen et al., 2012a; Rengasamy et al., 2021) that reported an average leaf moisture content of between 81% to 89%, the major difference between the

short 8H photoperiod and neutral 12H period suggests a higher water usage by Stevia plants under short photoperiod conditions. As the humidity within the growth room was maintained the same for all experimental photoperiods, the difference in leaf moisture content was a factor of the plant's photosynthetic activity instead of ambient conditions. The higher intensity of 8H light resulted in a higher rate of photosynthesis and subsequently higher leaf temperatures. This would result in the plants drawing more moisture from the soil. All artificial lighting treatments resulted in higher dry leaf biomass accumulation compared to the natural sunlight of GH. The significant difference observed in the fresh and dry leaf biomass yields among the different artificial lighting systems corroborates previous studies that found more efficient photosynthetic activity with lower light intensities under constant DLIs (Palmer & van Iersel, 2020) with the lowest intensities under 16H and 16HI having the highest dry leaf yields, a result of photosynthetic activity (Elkins & van Iersel, 2020a, 2020b; Palmer & van Iersel, 2020). Meanwhile, the GH treatment, with higher DLI, had lower dry biomass accumulation when compared to the 12H treated plants although both treatments had similar photoperiods. This finding also underlines the importance of the light quality over the intensity and DLI, providing an option to further optimise the biomass yields of indoor Stevia cultivation by manipulation of photoperiod and light quality. Although natural daylight had higher intensities, DLI, and had more spectral content, ranging from UV to beyond far-red, it had the lowest fresh and dry biomass accumulation, suggesting the inhibitive nature of certain spectral components within natural light. Yoneda et al. (2017a) reported shorter plants and lower biomass accumulation with the increase of blue spectral component in Stevia plants, comparable the findings in this study where the GH treatment under natural daylight, that had the highest blue spectral content, resulted in the lowest fresh and dry leaf biomass accumulation. Although studies have reported the supercharging effect of far-red spectral component (Demotes-Mainard et al., 2016;

Legendre & van Iersel, 2021; Zhen & Bugbee, 2020a, 2020c; Zhen et al., 2021) in increasing the productivity of photosynthetic activity in several plants, increasing its dry biomass yields, however this was not observed in this study. The higher far-red content of the natural sunlight under GH treatment did not result in any increase in biomass accumulation of Stevia plants, corroborating past studies that reported far-red spectral components to not having any positive effects on the yields of Stevia (Rengasamy et al., 2021).

The overall effect of the photoperiod and intensity over the DLI was observed throughout this study, where plants grown under the same spectral composition and DLI, but with shorter photoperiods had lower dry leaf yields compared to those under the 16H and 16HI treatments, although under higher intensities. It was observed that plants under the 12H treatment, with a photoperiod similar to the critical photoperiod of Stevia plants (Ceunen et al., 2012c; Zaidan et al., 1980), resulted in fresh and dry leaf yields more similar to the GH grown plants, compared to other artificial lighting treatments. While the similar traits between 12H and GH treated plants were not unexpected, the 8H and 16H treatments, with inverse intensity and photoperiods, had traits similar to each other, demonstrating the possible influence of high light intensities in reducing some of the effects of photoperiods shorter than the critical photoperiod of Stevia. Under all treatments, the dry stem biomass was significantly higher than the leaf biomass. Previous studies reported significantly higher leaf to stem biomass ratios, often with leaves accounting for more than 50% of the total biomass (Benhmimou et al., 2018; Ceunen & Geuns, 2013b). However, these findings in previous studies were based on fresh and not dry biomass and may have been influenced by the overall water content of the samples, not accurately representing the dry biomass partitioning. These findings highlight the importance and effects of the light quality and photoperiod on optimising biomass yields. Having higher intensities or DLIs may not always result in higher output if the way the light is delivered

(photoperiod) is not optimised. Even under conditions with identical spectral content, the biomass accumulation can be significantly improved by employing an optimised photoperiod strategy while maintaining the DLI. While having lower light intensities over extended photoperiods has proven to be the most productive approach, caution should be exercised to avoid using lighting levels that are too low. When lighting levels drop below an acceptable threshold, plants such as Stevia will begin to exhibit a light scavenging shade avoidance response, resulting in taller plants with fewer leaves, and would begin to transition to a reproductive stage where plants start to flower (Rengasamy et al., 2021). Stevia plants lose their commercial viability once they begin to flower as the content of desired metabolites deteriorates by up to 50% when there is no new vegetative growth (Rengasamy et al., 2021).

3.5.1.2 Continuous 16 Hour Photoperiod Increased Metabolite Concentration and Yields

Although ST and Reb A are two of the most abundant of all SG components, Reb A has significantly higher commercial value, with preference for use in the food and beverage industry due to a better taste profile that lacks the bitter aftertaste of ST (Ciriminna et al., 2019). The Reb A and ST concentrations obtained under GH treatment were consistent with field grown varieties, reported to be between 2% to 4% *w*/*w* for Reb A, and 5% to 10% for ST (Kurek & Krejpcio, 2019; Muthusamy & Munaim, 2019; Wojewoda et al., 2018). The short photoperiods of 8H and intermittent light from the 16HI treatment, had the highest percentage yields of Reb A, and the lowest ST content. Given the higher commercial value of Reb A, reaching USD70,000 per tonne, this is a preferred trait (Ciriminna et al., 2019). However, when considering the overall dry leaf biomass, the final realisable yield of these two compounds was significantly lower compared to that of the 16H treatment. The difference in concentration of Reb A and ST and the overall difference in ratio observed in this study corroborated the findings of previous studies

that reported higher percentage of ST and Reb A under long day conditions compared to short day conditions (Evans et al., 2015; Yoneda et al., 2017b; Zaidan et al., 1980). Evans et al. (2015) noted an increase in the concentration of ST and Reb A, as the DLI increased up to 10 mol $m^{-2}day^{-1}$, after which the concentration of ST reduced while Reb A and total SG percentage remained constant. The combined ST and Reb A metabolite yields per plant were highest under the 16H treatment (0.40 g $plant^{-1}$), 8.6 times the amount obtained in plants under the GH treatment (0.05 g $plant^{-1}$). All artificial lighting treatments, regardless of photoperiod and intensity, yielded higher than the GH grown plants with 16HI at 0.22 g (4.91×), 12H at 0.19 g (4.15×) and 8H at 0.15 g (3.35×) per plant. Studies have reported higher metabolite yields under lower DLIs (Evans et al., 2015) and long day conditions (Ceunen & Geuns, 2013b; Yoneda et al., 2017b). However, the increase in biomass accumulation caused by an extended vegetative period was attributed to this increase, rather than an increase of the metabolite concentration (Yoneda et al., 2017b). A similar observation was made in this study where the increase in metabolite yields was correlated with the increase in the dry biomass yields as opposed to the concentration of ST and Reb A. This result indicates the functional feasibility of implementing the strategy of optimising artificial lighting photoperiod to improve productivity as this does not compromise on the metabolite concentrations and yields. Often, the application of artificial lighting in commercial agriculture is focused on improving the biomass yields with no significant consideration for the metabolite contents. However, in plants with medicinal purposes such as Stevia, having both the biomass and metabolite yields is imperative towards maximising economic viability.

3.5.2 Extended Photoperiod Improved Efficacies

In CEAs, lighting energy costs often account for more than 50% of the total energy load and as such, careful consideration should be made to ensure the most efficient approach is taken (Graamans et al., 2018). The LPD and PPE of the artificial light treatment is a

representation of the equipment energy efficacy, and while it does not a reveal the effectiveness of the different photoperiods on biomass and metabolite accumulation, this is linked directly to the overall lighting energy requirements and costs. Increase in light intensities resulted in higher LPDs with the highest intensity of the 8H system consuming 316.6 W m^{-2}, or 2.45 times more than the LPD of the 16H system, that had the lowest intensity. The overall LPD does not increase linearly as the intensity is increased (Figure 3.6). While the intensity between 16H and 8H increased by 100%, the corresponding LPD increased by 145% from 128 Wm^{-2} (16H) to 316 Wm^{-2} (8H). This is due to the nature of LEDs that experience current droop, caused by an increase in current density of the chip surface leading to photon leakage among others, and thermal droop that causes a reduction in optical efficacy of the LEDs with an increase in junction temperature of the LEDs (Kusuma et al., 2020; Morgan Pattison et al., 2018). While the PPE values of the systems did not vary significantly across the different intensity and photoperiods it should be noted that these systems were designed and constructed as a prototype for experimental purposes using technology available in 2017 and have PPE values that are deemed to be low by current standards. Current technologies with significantly improved LED efficacies, and with superior commercial grade luminaire and control systems achieve photosynthetic photon efficacies (PPE), defined as the amount of lighting within the PAR range that can be produced per Joule (J) of electrical energy used, of between 2.7 to 3 µmol J^{-1} (Kusuma et al., 2020), up to 2.7 to 3 times more energy efficient compared to the LED components used for this study. This would result in possible further reduction of 16H system's LPD from 128.7 W m^{-2} to between 42.9 and 47.6 W m^{-2} (Graamans et al., 2018; Graamans et al., 2020; Kusuma et al., 2020) further reducing the overall electrical energy requirements and costs. As both LPD and PPE are indicators of the lighting system's electrical efficiencies, improvement in these parameters also translates to lower internal heat generation by the lighting systems. As the LED efficiencies

improve, the LPD reduces and the PPE increases, leading to a reduction in energy conversion to heat. This results in lower waste heat generated within the CEA, reducing the cooling requirements and costs (Graamans et al., 2018).

Overall, the artificial lighting systems had significantly higher photon conversion efficacies compared to those of the greenhouse. However, there were significant differences between the artificial lighting systems, with the highest efficacy observed under the 16H (7.50 mg mol^{-1}) treatment followed by the 16HI (4.23 mg mol^{-1}), 12H (3.62 mg mol^{-1}) and 8H (2.92m g mol^{-1}) treatments. This difference highlights the important role of the overall photoperiod and intensity of artificial light on the biomass and metabolite accumulation in Stevia, as all treatments had the same DLI and spectral component. The PCE decreased as the intensity increased and as the photoperiod reduced. An increase in intensity was not sufficient to counteract the effects of a shorter photoperiod. These findings are directly related to the lower efficiency of photosynthetic activity at higher light intensity in certain plants due to lower daily electron transport through photosystem II (Elkins & van Iersel, 2020a, 2020b; Palmer & van Iersel, 2020). The PCE is an important indicator of the economic viability of the lighting systems as artificial lighting requires additional energy input that translates to an increase in operating costs, hence it is essential that the yields are maximised for every mol of light delivered. The natural daylight of the GH treatment, although having higher DLI, intensity and a complete spectral content, had the lowest conversion efficacy of 0.44 mg mol^{-1}, 17 times that of the 16H systems. While the PCE is not relevant for natural light, as sunlight is free, it provides an insight into the photo sensitivity of Stevia plants. The significant variance between the PCE of natural and artificial light points to a lower photo saturation point of *Stevia rebaudiana* plants, not responding to higher intensities and DLIs, an observation that was also noted by Evans et al. (2015) and Yoneda et al. (2017b).

The lowest LPD and highest PCE of the 16H photoperiod makes this the most efficient among the artificial lighting treatments.

The energy density for the entire growing cycle was lowest under the naturally lighted GH compared to all artificial lighting setups. This finding was not unexpected and corroborates past studies that reported energy consumption in climate-controlled greenhouses to be lower than artificially lighted plant factories (Graamans et al., 2020; Weidner et al., 2021; Zhang & Kacira, 2020). However, unlike past studies that were based on northern latitudes with seasonal temperature variations, this study was based in the tropics, where the annual ambient temperature and photoperiod remains fairly constant throughout the year (Malaysian Meteorological Department, 2019) and cooling instead of heating was the primary energy load for the greenhouse. Comparing the artificial lighting setups and the greenhouse, it is apparent that while there is no lighting energy demand for the GH, it had significantly higher cooling energy consumption. In the growth room, the cooling load is directly related to the photoperiod. A longer photoperiod would require extended hours of additional cooling to mitigate the waste heat generated by the lighting systems. As the photoperiod extends to 16 h, the cooling energy requirements outweighed the lighting requirements. Hence, while the longer 16-h photoperiod was more productive, had lower LPD and the highest PPE, it also consumed the most electrical energy among all artificial lighting systems over the growth cycle. This finding may seem to be against the intention of optimising energy efficacy, however when comparing the energy use efficacies for all systems, the 16H photoperiod was the most efficient in both biomass and metabolite accumulation. While 16H consumed 11% more energy compared to 8H, it had 44% higher EUE in terms of biomass accumulation and 133% higher EUE in terms of metabolite yields. These findings indicate that while photoperiod manipulation does not affect the lighting equipment efficiency, it is extremely effective in improving the overall energy use efficacies, generating higher

yields per unit of electrical energy consumed. When coupled with the latest horticulture lighting systems that have higher PPEs (Kusuma et al., 2020), these efficacies can be further improved.

Ceunen et al. (2012a) recommended the use of night interruption via short photoperiods of red light during the dark period as a low-cost method to extend the vegetative stage of field grown Stevia plants. This was corroborated by Yoneda et al. (2017b) who reported that red and far-red night interruption results in an increase in biomass yields as compared to an 8 h photoperiod control. While the proposed method by Ceunen et al. (2012a) would be the preferred for field cultivation or cultivation under natural sunlight, the current study focused on the optimisation of the photoperiod under full artificial light, in an indoor setup, without any natural light in a tropical environment where outdoor cultivation would not be ideal. Although Yoneda et al. (2017b) reported improved yields under the night interruption approach, the overall highest biomass yield across all experiments was observed under treatments with higher intensities and longer photoperiods. As artificial lighting energy consumption accounts for more than 50% of the total energy cost within a CEA system, it is important to optimise the lighting setup, to maximise the yields, going beyond field grown benchmarks.

Besides drawing attention to the effect of light quality on plant productivity, the findings of this study also highlighted the influence of spectral composition on the effective DLI of Stevia plants. The plant productivity was higher under the selected wavelengths of the artificial light as compared to the full spectrum natural light. This allows for significantly lower DLIs within a CEA, lowering its energy requirements. As DLI is affected by both intensity and photoperiod, this study found that having lower intensities at longer photoperiods further optimised the energy requirements of artificial light, increasing plant productivity, reducing the overall lighting power load, and improving the overall system efficacy, compared to having higher intensities at shorter photoperiods. These results

validate photoperiod manipulation as a viable approach to improving productivity and increasing energy use efficacies for indoor cultivation of *Stevia rebaudiana*. Hence, in future design considerations for more efficient artificial lighting systems for indoor cultivation of Stevia, instead of trying to match the DLIs of field grown conditions, the light quality, intensity, and photoperiod should be prioritised.

3.6 Conclusion

The effect of photoperiod and intensities productivity and energy efficacy of indoor cultivation of *Stevia rebaudiana* was studied. The 16-h photoperiod delivered continuously or intermittently had the highest dry leaf yields. Although 16HI and 8H had higher concentrations of Reb A, the overall metabolite yields were highest under the 16H treatment driven by the overall higher dry biomass and ST yields. Although the GH had the lowest energy consumption, the highest energy use efficacies were obtained under 16H.

Based on this study, a 16-h photoperiod under red and blue artificial lighting systems supplemented with small amounts of green spectrum is recommended for indoor cultivation of *Stevia rebaudiana*. These conditions produced high biomass and metabolite yields, with a high energy use efficiency. The findings of this study also underlined the influence of light quality on determining the quantitative aspects of Stevia plants. Under the right spectral composition, the DLIs, intensities and photoperiods of artificial light can be further optimised to improve yields and energy efficacies.

In the next chapter, the effect of different spectral compositions was explored under a constant DLI, photoperiod and intensity.

CHAPTER 4 : EFFECT OF LIGHT QUALITY ON *STEVIA REBAUDIANA*

The use of different spectral compositions on the germination and growth of Stevia plants were evaluated in this chapter. The rate of germination and flowering, the biomass and metabolite yields were evaluated under constant DLI, photoperiod and intensity. This chapter has been published in 2022 under the title "Beyond *the PAR spectra: impact of light quality on the germination, flowering, and metabolite content of Stevia rebaudiana (Bertoni)"* in the "*Journal of the Science of Food and Agriculture"* with N Rengasamy, RY Othman, HS Che and JA Harikrishna as the authors.

4.1 Introduction

The quality, quantity, intensity, and duration of light are known influencers of plant growth and development, from dormancy and germination of seeds through to flowering and metabolite accumulation (Yadav et al., 2020). Besides facilitating the capture and conservation of energy through photosynthesis, light also plays a crucial role in seedling de-etiolation, stem elongation, phototropism, the movement of stomata and chloroplasts, shade avoidance response, development and maintenance of circadian rhythms, synthesis of metabolites and regulation of flowering time in plants (Liu et al., 2020).

4.2 Literature Review

Red and far-red (FR) irradiation are known to induce flowering in certain plants while blue light inhibits flowering (Huché-Thélier et al., 2016). However, these effects are species dependent and vary with spectral composition and intensity (Jones, 2018; Zheng et al., 2019b). Besides the main light harvesting photosynthetic pigments, chlorophyl a and b with peak absorption wavelengths of 430nm and 665nm respectively, most plants also have other photoreceptors with wavelength sensitivities going beyond the photosynthetic region (Ouzounis et al., 2015). Among these, the UVR8 (absorbing

wavelengths of 280-315nm), cryptochrome and phototropins (350-500nm), and phytochrome (600-800nm) are known to have roles in the regulation of germination, flowering, and metabolite accumulation (Zhang et al., 2020b; Zheng et al., 2019b).

The World Health Organisation (WHO) reported that 9% of the global population or 347 million people have been diagnosed with diabetes (Mukhtar et al., 2016). The global increase in the rates of obesity and its associated diseases including diabetes has given rise to demand for healthier alternatives to sugars (Mojto et al., 2019). The global market for low and non-calorific sugar substitutes was estimated to be approximately USD1.1 trillion in 2020 and is dominated by artificial or synthesised sweeteners lead by aspartame, followed by saccharin, acesulfame and sucralose (Li et al., 2020a). Although these artificial sweeteners are deemed to be reasonably safe for general consumption, multiple reports have raised concerns on the potential risks and effects these sweeteners have on consumer health, from the potential carcinogenic effects of aspartame, saccharin and acesulfame (Jiang et al., 2018; Rafati et al., 2018), to the gut microbe altering effects of sucralose (Schiffman & Rother, 2013). These artificial sweeteners are also not typically broken down in the human body, ending up as contaminants in groundwater and wastewater, leading to a growing environmental concern (Li et al., 2020a).

The demand for safer and healthier sweeteners has stimulated the development of natural sugar alternatives, including *Stevia rebaudiana,* a perennial plant from the Asteraceae family that is native to the highlands of Brazil and Paraguay (Geuns, 2010). Purified Stevia extract has been approved for use as a food additive and sweetener by the USA Food and Drug Administration (FDA), Foods Standards Australia New Zealand (FSANZ), and by the European Food Safety Authority (EFSA) ((FSANZ), 2008; Ciriminna et al., 2019; Saharudin et al., 2020b). The recognition by the FDA, EFSA and FSANZ has opened up the use of Stevia extracts in major markets, driving global demand

which is expected to grow from USD338m in 2014 to USD554M by the end of 2024 (Ciriminna et al., 2019).

In developed and developing countries in Asia, such as Malaysia, dried Stevia leaves and leaf extracts have been popularised not only as a natural low-calorie sweetener but also as an herbal supplement, based on reported anti-inflammatory, anti-hypertensive, and anti-hyperglycaemic properties (Abdulameer et al., 2018; Saharudin et al., 2020b). At present, unprocessed and processed Stevia leaves are imported from China, India and South America with little local cultivation due to the non-ideal photoperiodic conditions in this geographical region (Abdulameer et al., 2018). The total steviol glycoside (SG) content in field grown plants in their native environment ranges between 4% to 20% of the total dry leaf biomass, with Stevioside (ST) and Rebaudioside A (Reb A) being the main glycosides: ST at 5-10% of the total dry leaf biomass and Reb A at 2-5% (Ciriminna et al., 2019; Yoneda et al., 2017a). To ensure continuous supply throughout the seasons, to reduce over dependence on imported feedstock, and to cater to the ever-growing demand, there is a need to improve the yields of these high valued compounds by optimising the cultivation environment outside of its normal range of latitudes (Abdulameer et al., 2018; Abdullateef et al., 2015a).

Stevia rebaudiana being a short day (SD) plant with a critical photoperiod of between 12 to 13 hours (Ceunen et al., 2011), has a tendency for a shortened vegetative stage and early flowering when grown under a photoperiod of 12 hours or less. The amount of SG in the leaves reduces by up to 50% after flowering. When grown under day-neutral conditions, in tropical countries like Malaysia with an almost equal 12 hours of light and darkness, *Stevia rebaudiana* flowers as early as 7 weeks after planting, halting further vegetative development and resulting in low absolute SG yields (Abdulameer et al., 2018). The high market price of the compounds, especially Reb A that has estimated market value of USD73,000 per tonne (Ciriminna et al., 2019), makes *Stevia rebaudiana*

an ideal candidate for intensive cultivation in Controlled Environment Agriculture (CEA) or plant factories under full artificial light and in a vertical layout.

Previous studies on germination and flowering of Stevia plants did not fully consider the effects of different spectral compositions, focusing instead on the effects of monochromatic light sources. Studies that used white light from a fluorescent (FTL) light source, as a replacement for natural daylight or to extend the natural photoperiod, did not consider the spectral composition of these sources (Abdullateef & Osman, 2011; Simlat et al., 2016). The spectral distribution of white FTL and LED light sources can vary significantly between manufacturers and is highly dependent on the chip and phosphor materials used (Ahn et al., 2019). Past studies on yield improvements focused on manipulation of the photoperiod to extend the vegetative state. These studies used conventional incandescent lamps or red LEDs to extend the photoperiod from a short day to a long day condition (Ceunen et al., 2011; Zaidan et al., 1980), or used night interruption techniques to induce the plant to exhibit long day characteristics (Ceunen & Geuns, 2013a; Ceunen & Geuns, 2013c; Ceunen et al., 2012c; Yoneda et al., 2017b). However, these studies were based on field grown practises, under natural sunlight and not indoor cultivation under full artificial light. Growing small leafy plants such as Stevia in an indoor environment such as multitiered urbans farms (Wong et al., 2020) is becoming increasingly popular and commercially viable due to efficiency in use of space and reduction in the need for pesticide use (Pinstrup-Andersen, 2018).

With the recent developments in LED technology, the overall cost, performance, and capability of the available products has improved significantly, enabling the use very specific narrow bandwidth lights to undertake precision agriculture activities while still making good business sense, especially with regards to high valued crops such as *Stevia rebaudiana* (Virsile et al., 2020; Zhang et al., 2020b).

This study explored the use of supplemental lighting within and outside of the photosynthetically active radiation (PAR) region (UV-A, Far-red and green) together with a basal photosynthetic specific spectrum, to extend the vegetative period of the plant growth cycle and to increase the overall biomass and relative SG content, specifically the ST and Reb A yields, simulating a CEA vertical farm setup. Even though various steviol glycosides can be separated by High Performance Liquid Chromatography (HPLC), this study focuses on Stevioside and Reb A as these glycosides account for up to 90% of the total SG content of a Stevia plant (Wojewoda et al., 2018), and are also the two compounds with the highest commercial demand (Ciriminna et al., 2019). UV-A and Far-red spectra were used to evaluate their effectiveness in improving biomass and metabolite accumulation. The use of green light, a spectral range that is not popular at present in commercially available horticulture lighting systems, was also explored given its lower cost compared to FR and UVA LEDs. Past studies have reported the positive effects of supplemental UVA (Chen et al., 2019), FR (Legendre & van Iersel, 2021), and green (Claypool & Lieth, 2020) spectra on other plant species, resulting in significantly higher yields when used with red and blue base spectra, even at low supplemental intensities. These findings provide an opportunity for optimisation of indoor cultivation of Stevia by addition of low amounts of a third spectral component to maximise yield output. The current study also addressed the concerns of the low germination rate and precocious flowering of *Stevia rebaudiana*, when grown in day-neutral conditions. This is the first study to evaluate the response of *Stevia rebaudiana* plants under different supplemented lighting treatments in a controlled environment growth room, throughout its lifecycle, from germination to harvest.

4.3 Materials and Methods

4.3.1 Plant Materials

Stevia rebaudiana seeds purchased from Bakers Creek Heirloom Seeds, USA (https://www.rareseeds.com/) were washed under running tap water and dried on a filter paper prior to being surface sowed at a rate of 1 seed per cell in a 50-cell plug tray (54cm x 28cm x 5.7cm) that was filled with autoclaved potting soil (www.serbajadi.com.my). The trays were sprayed with water, wrapped in clear plastic and placed in a climate-controlled room under the respective light treatments within the Plant Biotech Facility (PBF) of University of Malaya, Kuala Lumpur. The temperature of the room was maintained at $25^{\circ}C \pm 2^{\circ}C$ with a relative humidity of 70-80%. The seedlings were watered sparingly throughout the period. The experiment was replicated 3 times from June 2018 till October 2019 with each cycle lasting 175 days from sowing (DAP).

4.3.2 Seed Germination study

The seeded trays were placed under each light treatment at a rate of 1 full tray of 50 seeds per light treatment. The rate of germination was observed on a weekly basis, every 7^{th} day, from week 0 (sowing) to Week 4. Data was collected at the same time of each week and the number of germinated seeds were recorded.

The weekly rate of germination was calculated as per the equation below:

$$\text{Rate of Germination} = (\text{Sum of germinated seeds} \div \text{Total Seeds Sowed})\%$$

Where,

Sum of Germinated Seeds = Total surviving germinated seeds at data collection time

4.3.3 Flowering study

Five weeks after sowing, seedlings were removed from the plug trays and transplanted into individual pots (12cm x 12cm x 10cm) filled with autoclaved potting soil (www.serbajadi.com.my). A total of 20 seedlings from each light treatment were selected for transplanting. The seedlings were selected based on the mean height, discarding the outliers. The samples were placed at a density of 20 plants per square meter, directly below the light source. The transplanted seedlings were watered sparingly, ensuring the topsoil remained moist while preventing water logging. The rate of flowering was recorded on a weekly basis (every 7^{th} day) from transplanting for a total of 140 days (175 DAP). The plant was deemed to have entered the flowering phase when the first flower bud fully bloomed.

The Weekly Rate of Flowering was calculated using the following formula:

$$\text{Weekly Rate of Flowering} = (\text{No. of samples in flowering stage} \div \text{Total samples})\%$$

Where,

No. of samples in flowering stage = total number of plants with at least 1 fully bloomed flower at time of data collection

4.3.4 Light Treatments

Table 4.1 : Spectral Composition of Light Quality Treatments

	Unit	RB	FR	UVA	BR	GR	FS
UV-A (380nm)	µmol $m^{-2}s^{-1}$	0%	0%	5%	0%	0%	2%
Blue (450nm)	µmol $m^{-2}s^{-1}$	30%	30%	30%	45%	25%	40%
Green (550nm)	µmol $m^{-2}s^{-1}$	0%	0%	0%	0%	10%	8%
Red (660nm)	µmol $m^{-2}s^{-1}$	70%	70%	70%	55%	65%	52%
Far Red (730nm)	µmol $m^{-2}s^{-1}$	1%	17%	1%	1%	1%	5%
Total PPFD	µmol $m^{-2}s^{-1}$	130± 2%	130± 2%	130± 2%	130± 2%	130± 2%	130± 2%
R:B		2.3	2.3	2.3	1.2	2.6	1.3
R:FR		92	4.3	90	100	84	10
Photoperiod in 24 Hours	Hours (h)	16	16	16	16	16	16

Note: UV-A and FR irradiation does not fall within the defined PAR region hence it is not considered when calculating the total PPFD. In the light recipes used for all experiments, the percentage addition of the supplemental non-PAR spectra in relation to the total PPFD is used to describe the dosage of non-PAR wavelengths.

Six custom built lighting systems, each consisting of 8 channels of high-powered LEDs were used (Osram Opto, Germany, Cree,USA, and Edison Opto,Chinese Taipei). Each channel (UV-A, Deep Blue, Blue, Green, Amber, Deep Red, Red and Far-Red spectra) was individually controlled from 0-100% intensity via a pulse width modulation (PWM) controller. Brief block diagram on the lighting system design and installation location is available in Chapter 6, Section 6.3.

A base photosynthetic spectrum of red+blue (RB) light was present in all artificial light treatments. Treatments were then dosed with supplemental spectra of UV-A (UVA), blue (BR), FR, Green (GR) and a combination of all 3 (UVA+FR+GR) (Figure 4.1, Table 4.1). The photosynthetic photon flux density (PPFD) of all systems was maintained at 130

µmol $m^{-2}s^{-1}$± 2 with the intensity measured at 30 cm from the base of the growing platform using a portable spectroradiometer (Assensetek, TW). The luminaires were installed 75 cm from the base of the growing platform and had a 16-hour light and 8-hour dark period.

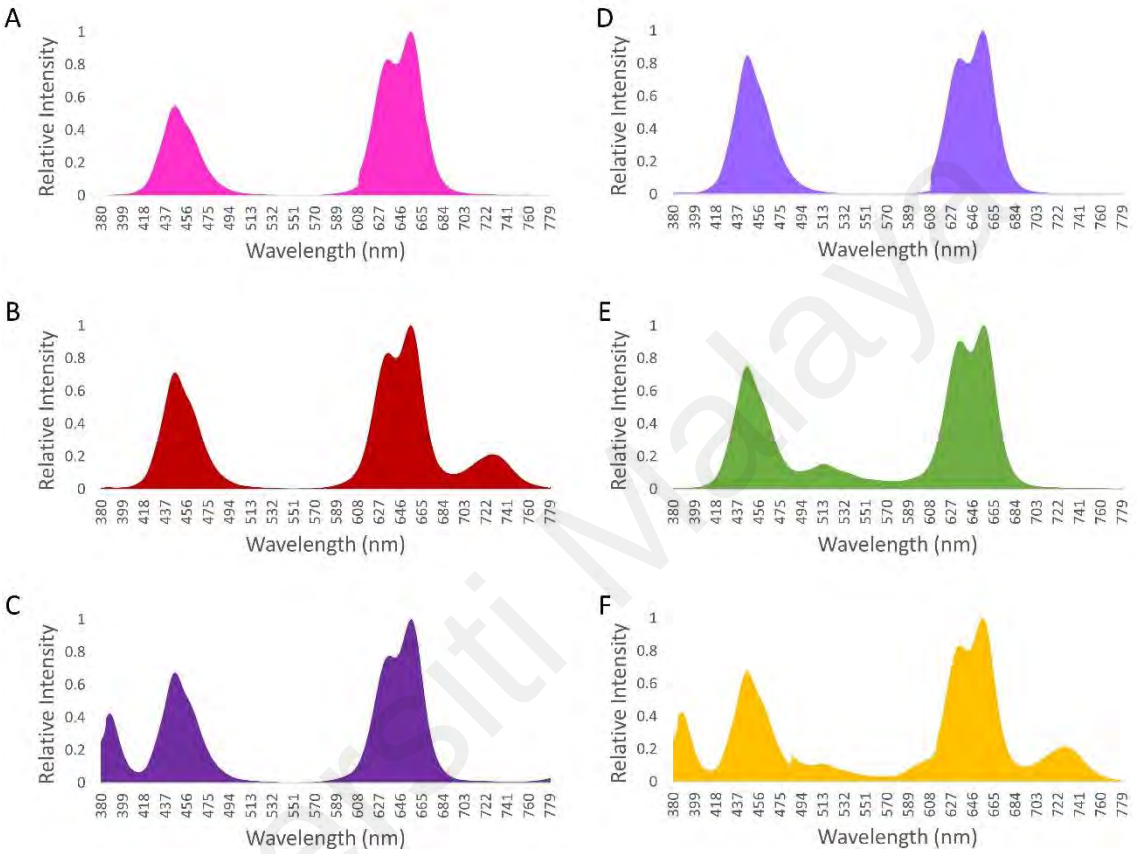

Figure 4.1 : Spectral distribution of Light treatments used in Light Quality studies. (A) RB: Base Red + Blue; (B) FR: Red+Blue+FR; (C) UVA: Red+blue+UV-A; (D) BR: Red+blue (Higher Blue content); (E) GR: Red+Blue+Green; (F) FS: Red+Blue+UV-A+FR+Green. All treatments had equal PPFD of 130 µmol $m^{-2}s^{-1}$.

4.3.5 Plant Growth

The height of seedlings was measured at 30 DAP (Week 4) using a standard metric ruler. The measurement was taken from the base of the plant to the topmost part of the plant. At 175 DAP, the final height of the plants was measured in the same method. A standard digital calliper was used to measure the stem and leaf thickness. The stem thickness was measured at 5 points along each stem, beginning from 5cm above the soil while the leaf thickness was measured across the centre of the leaf, at its widest point. 25 leaves,

sampled randomly beginning from 5cm from the base of the plant, was measured for each biological replicate. The measurements were repeated every cycle.

4.3.6 Biomass yield

The plants were harvested in November 2018, May 2019, and September 2019 by cutting the stems at 5cm above the soil after which the leaves and stems for each plant were separated and weighed using a digital scale (Shimadzu, Japan). The leaves of all plants under a specific light treatment were collected, washed under running water, and dried by letting the washed leaves sit in a mesh bowl for 1 hour before being blotted down gently with tissue papers. The leaves were then dried in an oven (Binder, Germany) at 60°C for 20 hours until it achieved a steady weight. Once cooled to room temperature, the samples were once again measured using the digital scale before being packed with silica gel desiccant in an airtight container. The dried samples were then stored at -4°C before being used for the LCMS analysis to determine its ST and Reb A composition. The stems of all plants were washed, dried and the weight determined using the same method as for the leaves. The percentage biomass partitioning towards the leaves were calculated using the formula:

$$\text{Leaf Biomass Partioning} = \frac{\text{Leaf DW}}{(\text{Leaf DW} + \text{Stem DW})}\%$$

Where,

Leaf DW = Leaf dry weight

Stem DW = Stem dry weight

The moisture content of the leaves was calculated based on the formula as follows:

$$\text{Moisture Content} = \frac{\text{LeafDW}}{\text{Leaf FW}}\%$$

Where,

Leaf DW = Leaf dry weight

4.3.7 LCMS Analysis

The extraction of the dried Stevia leaves was done using the maceration method. 0.5 g of ground up dried Stevia leaves was mixed with 50 mL of 35:65 (v/v) ethanol-water mixture. The sample was then sonicated for 2 hours and filtered to get the eluents, that were subsequently dried via a miVac centrifugal concentrator to remove the solvent. 10 mg of the resulting extract was then dissolved in a 1mL mixture of water and acetonitrile (7:3). The sample was then filtered using a PES membrane with pore size 0.22μm. The LC-MS QTOF apparatus (Agilent 1290 Infinity™) with a C18 column was used for the LCMS analysis. A modified approach based on the assay methods outlined in FAO and WHO (2020) was used to quantify the amount of ST and Reb A among the samples, expressed in percentage of mass of leaf dry matter (%w/w).

The Reb A to ST ratio was calculated using the simple formula of ratios:

$$\text{Reb A to ST Ratio } = \frac{\text{Reb A weight in mg}}{\text{ST weight in mg}}$$

4.3.8 Yield Per Plant

The Reb A and ST yields per plant under each light treatment was calculated as follows:

$$\text{Reb A} + \text{ST Yield Per Plant (g)}$$
$$= \text{Mean Leaf DW} \times (\text{Reb A Concentration} + \text{ST Concentration})$$

Where,

Mean Leaf DW = Mean Leaf dry weight per plant

Reb A & ST Concentration = Percentage composition per gram of leaf DW as obtained from LCMS Analysis

4.3.9 Statistical analysis

The data obtained was further analysed using the Analysis of Variance (ANOVA). Tukey's Honestly Significant Difference (HSD) post hoc test with a $P<0.05$ was then used to identify the statistical significance and relationship between the data. All statistical analysis was done using IBM SPSS Statistics 25 Software package.

4.4 Results

4.4.1 Effect of light quality on germination rate

Seeds under all 6 light treatments began germinating within 1 week after sowing. The UVA treated seeds had the highest rate of germination at 68%, significantly higher than the lowest rates observed under the BR (56%) and FS (57%) treatments. Seeds under the FR, RB, and GR treatments had a germination rate of 63%, 62% and 60% respectively (Figure 4.2). Except for the FS treatment that had an increment in germination by 1% from week 2 to week 4, percentage germination under all other treatments peaked at week 2. Seedling mortality was observed for the UVA, GR and RB treatments at 5%, 3% and 2%. The UVA treatment while improving the overall seed germination, had a negative effect on the survival of the seedlings when exposure was prolonged beyond 2 weeks. The 2 treatments with the lowest rate of germination, BR, and FS, were also the treatments with lower red to blue (R:B) ratios.

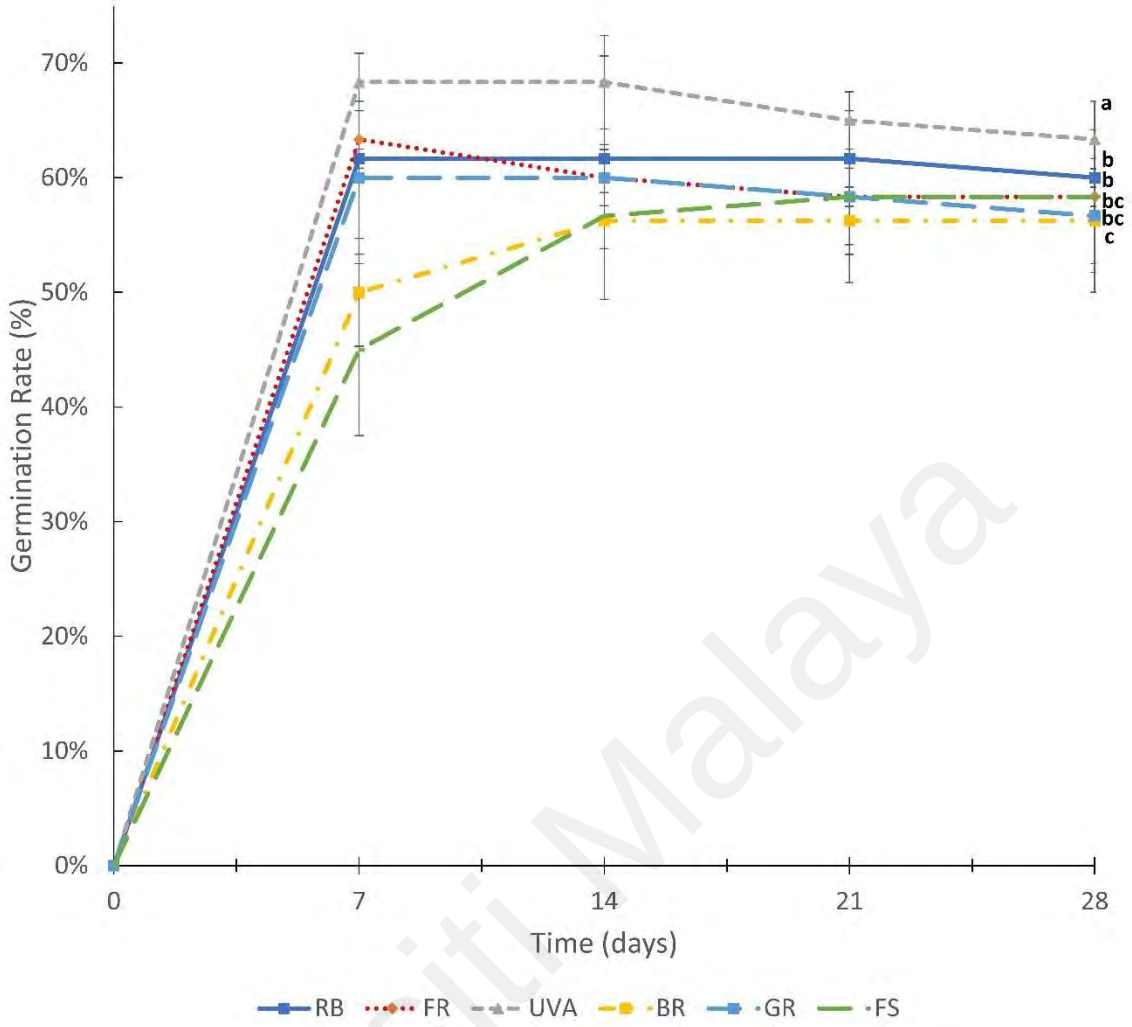

Figure 4.2 : The mean rate of germination of *Stevia rebaudiana* seeds under different light treatments observed on a weekly basis. A negative slope indicates mortality within the seedlings. Values represents Mean (n = 60) ± Standard Deviation. Different letters at the error bars indicate statistical significance determined by Tukey's HSD post hoc test at P<0.05.

4.4.2 Effect of light quality on seedling height

30 days after sowing, BR-treated seedlings had a mean height of 3.15cm, more than 2 times the mean height of seedlings grown under FR treatment (Figure 4.3). Treatments with lower red component in its spectral composition (BR, GR and FS) had taller seedlings while the three treatments with 70% red content (Table 4.1) had significantly shorter seedlings (Figure 4.3). Seedlings with supplemental green light had taller

seedlings compared to plants with a PAR range comprising of only red and blue wavelengths higher red composition.

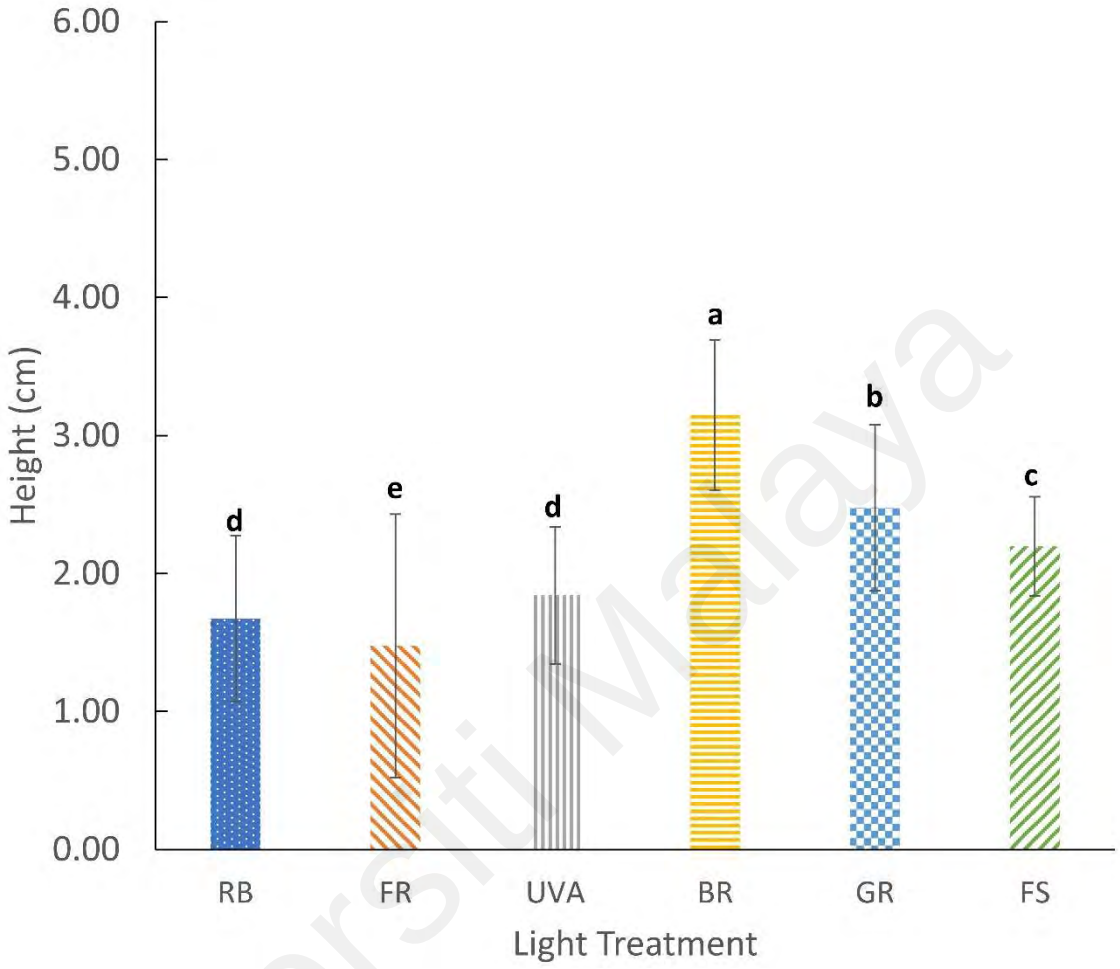

Figure 4.3 : Mean Seedling height under the different light treatments at 30 DAP. Values represent Mean (n =60) ± Standard Deviation. Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at P<0.05.

4.4.3 Effect of light quality on flowering time

The earliest flowering was observed at the 16th week after sowing, with the highest rate under the BR treatment followed by the FS and GR treatments at 34%, 17% and 7% of the total population, respectively (Figure 4.4). Plants under other treatments exhibited significant delays in flowering with flowering commencing at weeks 22 (FR), 24 (UVA) and 25 (RB) in all experimental cycles (Figure 4.4). BR treatment had a mean final percentage of plants with flowers of 44%, 7.3 times higher than that of RB and UVA that

had only 6% of its plants with flowers. GR treatment with a flowering percentage of 17%, while having similar R:B and F:R ratios as UVA and RB, has an overall lower absolute Red and Blue fluence rate as green light was supplemented to make up the PPFD. All samples that transitioned to the flowering phase continued to flower until the end of the experiments.

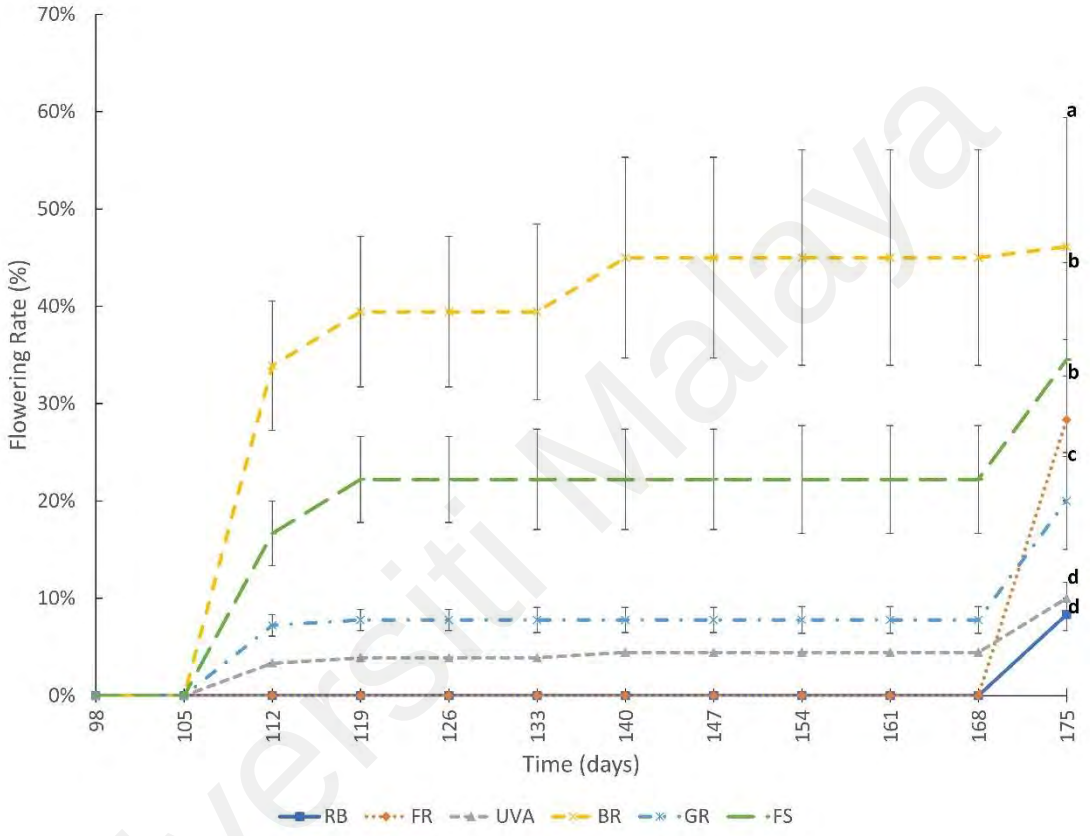

Figure 4.4 : Effect of Light Quality on Flowering. The cumulative weekly percentage of plants with flowers under different light treatments observed from the day of sowing of seeds. Values represents Mean (n = 60) ± Standard Deviation at 175 DAP (Week 25). Different letters at the error bars indicate statistical significance determined by Tukey's HSD post hoc test at P<0.05.

4.4.4 Effect of Light Quality on Plant Growth

The greatest height at 175 DAP was observed in plants under the multispectral FS treatment with a mean height of 55.65cm, more than 10% taller than samples grown under the BR treatment, and more than 42% taller than those under the RB treatment that had the shortest mean final plant height at 32.03cm (Figure 4.5A). Plants grown under the RB

treatment also had the thickest leaves, with a mean thickness of 0.51mm, 13% thicker than thinnest leaves obtained under the FS treatment (Figure 4.5B). Plants in treatments with lower red to blue ratio or with additional spectra had thinner leaves compared to the RB spectrum. The difference in spectral composition however did not have a major effect on the stem thickness, with only minor variations observed (Figure 4.5C). UV-A supplemented treatments (UVA and FS) had the greatest number of leaves with an average of 93.5 leaves per plant each (Figure 4.5D). Although both FS and BR treatments had similar red to blue ratios, the inclusion of an additional UV-A spectrum in FS had a positive effect on leaf number.

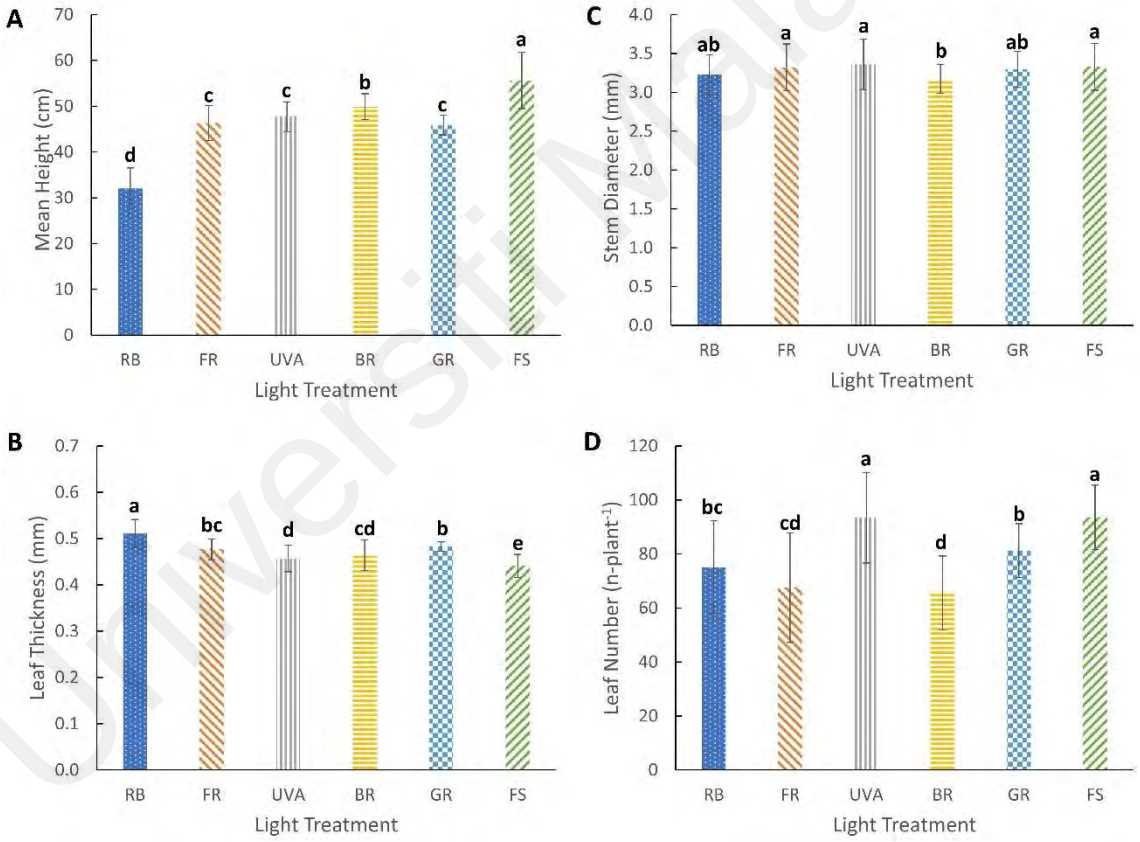

Figure 4.5 : Effect of Light Quality on Plant Growth. (A) The mean height of Stevia rebaudiana plants under different light treatments at 175 DAP. (B) The mean leaf thickness observed at 175 DAP. (C) The mean stem diameter measured at 175 DAP. (D) The mean number of leaves per plant, obtained at 175 DAP

4.4.5 Effect of Light Quality on Biomass and Metabolite Accumulation

The highest amount of fresh leaf biomass was obtained from plants under the green supplemented GR and FS treatments with a mean of 13.3g of fresh leaves per plant each, while the BR, FR and RB treatments yielded 8.8g, 9.65g and 9.78g, respectively (Figure 4.6A). The RB treated plants meanwhile had the lowest mean stem fresh weight, 55% and 53% less than those under the FS and FR treatments. Although the RB and FR treatments had an identical basal red and blue spectrum, the supplemented FR irradiation increased the stem fresh weight, also observed in the FS treatment, the only other light treatment with supplemental FR irradiation (Figure 4.6B). The average fresh weight per leaf was significantly higher under the GR treatment at 0.16g per leaf compared to 0.14g per leaf for plants under the FS treatment (Figure 4.6C). The lowest mean fresh weight per leaf was observed under the UVA treatment, averaging 0.12g per leaf.

Although plants grown under the RB treatment had the lowest leaf dry weight yield (Figure 4.6E) these plants had the highest dry matter partitioning towards leaves, with 35% of dry matter coming from leaves. The lowest percentage of dry leaf biomass partitioning was observed under FR, where the dry leaf yield was only 22% of total dry matter.

The GR and FS treated plants had higher mean dry leaf weight at 2.1g and 1.8g per plant respectively, while the RB spectrum the lowest yield of 0.98g per plant, 53.3% lower than GR treated plants (Figure 4.6E). Plants under the green supplemented GR treatments did not only have the highest leaf fresh and dry weight yields, but also had the leaves with the highest moisture content of 81.8% w/w (Figure 4.6F).

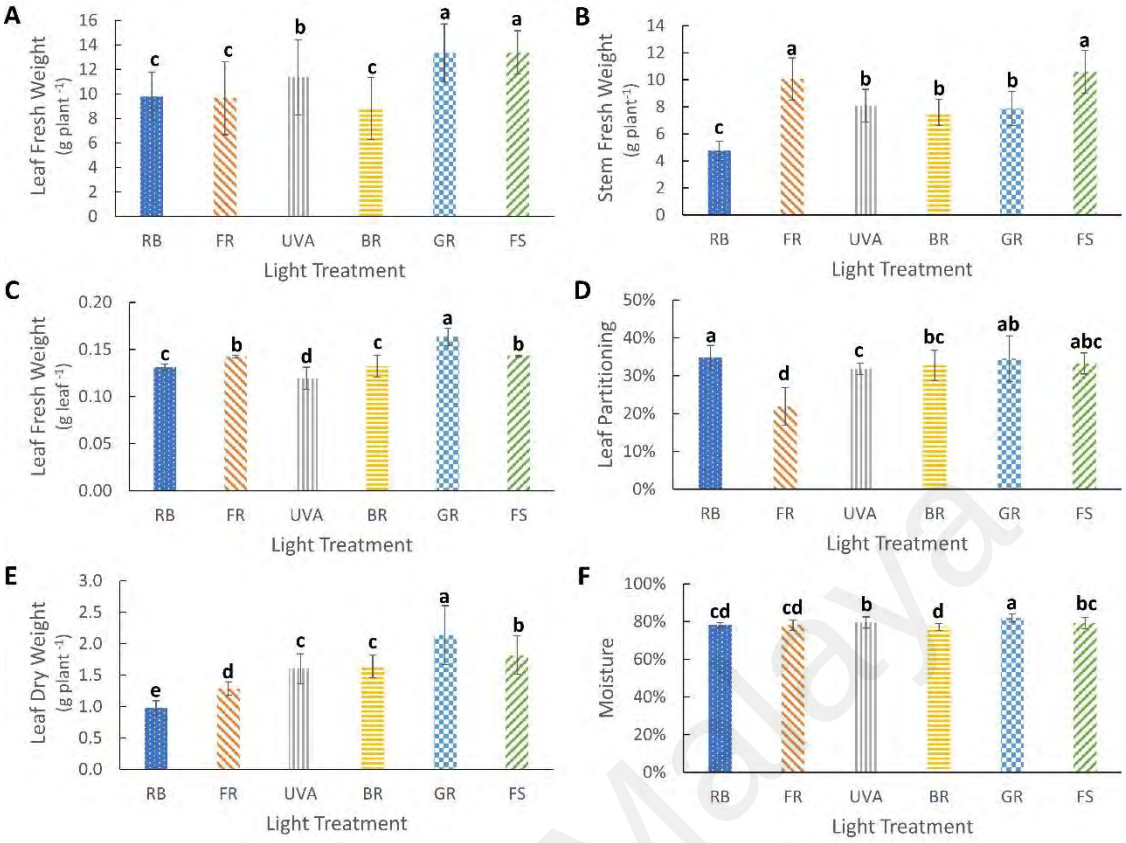

Figure 4.6 : Effect of Light Quality on Biomass. A) The mean leaf fresh weight per plant (g plant-1) of Stevia rebaudiana plants 175 DAP under different light treatments. (B) The mean stem fresh weight per plant observed at 175 DAP. (C) The mean fresh weight per leaf (g leaf-1) at 175 DAP. (D) The partitioning of dry biomass towards the leaves at 175 DAP under the different light treatments. (E) The mean dry weight yield of leaves per plant (g plant^{-1}). (F) The mean moisture content of leaves from plants grown under the different light treatments. Values represents Mean (n = 60) ± Standard Deviation at 175 DAP (Week 25). Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at P<0.05.

The FS treatment had the lowest Reb A content at 4.33% of its leaf dry weight (Figure 4.7A,) compared to the highest yield obtained in the GR treatment (7.70% w/w), that was followed by UVA (7.39% w/w) and BR treatments (7.25% w/w). The UVA treated plants had the highest ST yield at 20.05% w/w (Figure 4.7B). Unlike the yields of Reb A, ST accumulation was reduced under conditions with green supplemented light, with GR and FS treated plants having lower ST yields of 13.27% w/w and 13.45% w/w, respectively. Although the UVA treated plants had a lower Reb A to ST ratio, it had the highest combined Reb A and ST, and subsequently SG yield, totalling an average of 27.45% w/w

(Figure 4.7 C, D). The GR and UVA treated plants had the highest ST and Reb A yields of 0.45g and 0.44g per plants respectively while the lowest yield was observed in the base RB treatments, with a yield of 0.17g per plant (Figure 4.7D).

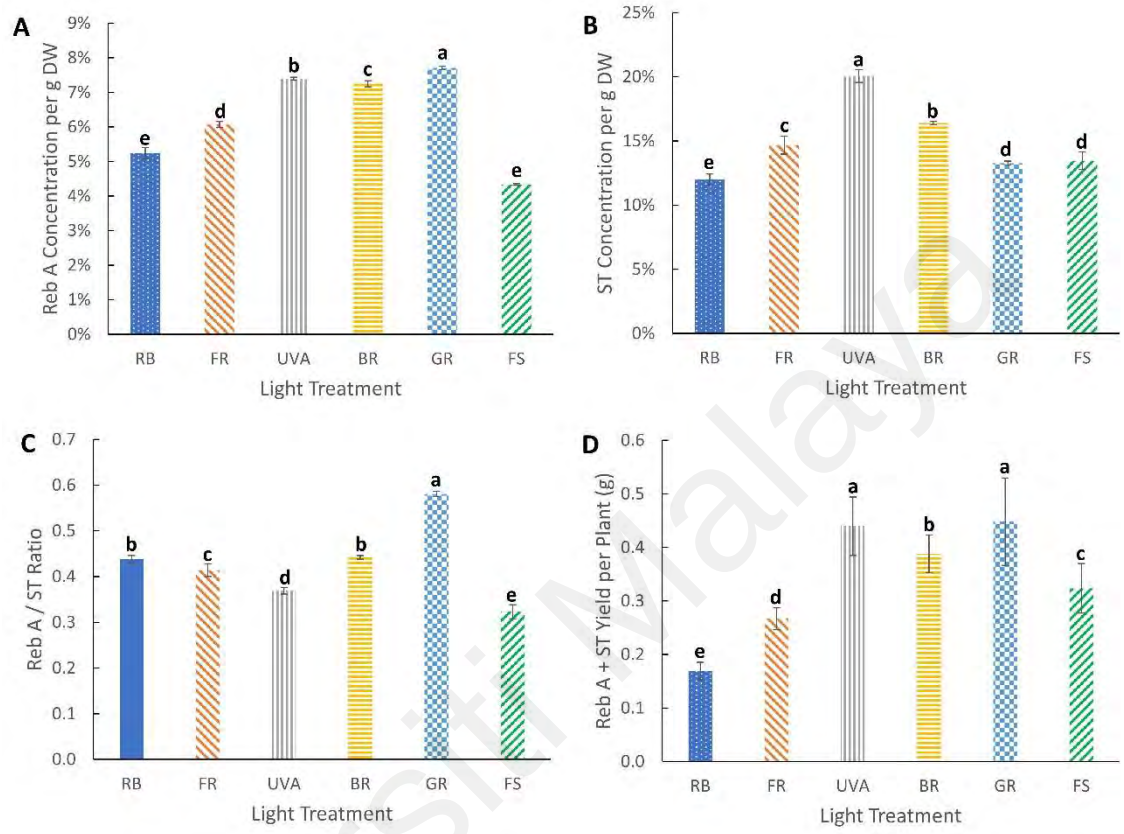

Figure 4.7 : Effect of Light Quality on Metabolite Accumulation. (A) Mean percentage composition of Reb A in dry leaf weight for *Stevia rebaudiana* plants under the different light treatments. (B) Mean percentage composition of ST in dry leaf weight under different light treatments. (C) The mean calculated Reb A to ST percentage ratios for Stevia rebaudiana leaves under different light treatments. (D) The mean yield of Reb A and ST per plant, expressed in grams, for all light treatments. Values represents Mean (n = 60) ± Standard Deviation at 175 DAP (Week 25).

4.5 Discussion

LEDs with narrow bandwidth, allow for the optimisation of light quality and quantity and can be used efficiently in a controlled growth environment. Driven by the increase in usage and popularity of LEDs in general lighting and automotive applications, the overall production costs and system efficiencies of LEDs have improved significantly (Kusuma

et al., 2020). The use of optimised lighting recipes within a controlled environment introduces an additional dimension where final yields such as that of valuable metabolites, can be improved beyond field grown varieties, further improving the economic feasibility for CEA cultivation of *Stevia rebaudiana* in countries where natural light conditions are not suitable for outdoor cultivation.

To study the effect of light quality, a treatment with basal PAR spectrum of red (660nm) and blue (450nm) (RB) (Figure 4.1,Table 4.1) was set-up to match the peak absorption spectrum of chlorophylls a and b while maintaining a red to blue ratio close to the daily weighted average observed under natural daylight conditions (Zheng et al., 2019b). The base spectrum peaks of 450nm and 660nm was also selected based on its popularity as the preferred spectral range for photosynthetic activity in commercial horticulture lighting systems(Wu et al., 2020a). Two treatments with spectral components outside the PAR spectrum (UVA and FR) having the same red: blue light ratio as RB were included to study the effects of non-PAR spectrum on *Stevia rebaudiana*. Two more light treatments with supplemental quantities within the PAR region (BR and GR) were developed. These light treatments had the overall base red and blue light composition reduced to accommodate the additional spectra without increasing the overall PPFD of each treatment. Lastly, the FS treatment incorporated the base red and blue spectra together with the UV-A, FR, GR, and BR components to create a broad-spectrum light source.

The ASABE (2017) standards were used to define the PAR and Plant Biologically Active Radiation (PBAR) range. A recent study by Zhen and Bugbee (2020c) put forward an argument for the PAR region to be redefined to include the FR spectra, however the efficacy of FR spectra in photosynthetic activity relies on the presence and wavelength range of other spectral components, with FR on its own only minimally increasing photosynthesis (Zhen & Bugbee, 2020c; Zhen & van Iersel, 2017). As this synergistic effect of FR on photosynthesis is associated with the Emerson effect as opposed to the

Stark-Einstein theory, to which wavelengths within the current PAR region comply, the present definition put forward by ASABE and the ASABE standards (ASABE, 2017) were used to define the PAR and Plant Biologically Active Radiation (PBAR) range for the current study.

4.5.1 UV-A improves germination in *Stevia rebaudiana*

Based on earlier reports for Stevia and several other plants, it was expected that the UVA supplemental light and treatments with higher red spectral composition would increase germination in *Stevia rebaudiana* (Demotes-Mainard et al., 2016; Huché-Thélier et al., 2016) and this was indeed the case (Figure 4.2). Phytochromes, primarily absorb in the red and far-red region of the spectrum from 600nm to 800nm, a known mediator of germination (Abdullateef & Osman, 2011; Zheng et al., 2019b). The highest final germination percentage of 68% observed under UVA in the current study was similar to the 67% observed under monochromatic red light in a specialised chamber by Abdullateef et al. (2015a) and significantly higher than Simlat et al. (2016) with 50% under blue monochromatic light and Abdullateef and Osman (2011) with 41% under monochromatic red light.

Blue light when dosed with a low fluence of UV-A irradiation, has been reported to trigger photoinduction of germination in seeds (Shinomura et al., 1996), an effect exhibited by the highest germination rate obtained in this study under the UVA light treatment (Figure 4.2B). These responses are however species and cultivar dependent with studies also reporting the inhibitory nature of red, blue and red+blue light with regards to germination (Simlat et al., 2016).

4.5.2 Blue light affects seedling height in Stevia

Ramírez-Mosqueda et al. (2016) reported Stevia seedlings grown under a red+blue (1:1) light were taller than those grown under monochromatic red, monochromatic blue, white

and fluorescent lights, in line with the findings in this paper where seedlings under the BR treatment, with a red to blue ratio closest to 1 (1.2) having the tallest seedlings (Figure 4.3). Seedlings in the current study were germinated from seeds and grown fully under the same light, unlike in other studies where germination was either under different lighting conditions before being transplanted into the light treatments (Esra et al., 2016b) or the seedlings were cultivated in vitro (Ramírez-Mosqueda et al., 2016).

4.5.3 Blue and far-red spectra promote early flowering in Stevia

Although red light triggers an inhibition reaction in the phytochromes (Kusuma & Bugbee, 2020; Wang & Folta, 2013), far-red light triggers initiation of flowering, resulting in a higher overall rate of flowering (Demotes-Mainard et al., 2016; Zheng et al., 2019b). This was observed in the current study where samples under the FR treatment and FS treatments with higher far-red content and lower R:FR ratio had a higher rate and an earlier start to flowering compared to treatments with higher R:FR ratios, except BR (Figure 4.4 A,B). Plants under the FR treatment, while having a slight delay in flowering compared to other treatments, had a high week-to-week flowering percentage towards the end of the experiment cycle resulting in a high overall final flowering percentage.

The BR and FS treatments with higher blue content flowered earlier and at a higher rate compared to the others (Figure 4.4) showing the rate of first flowering and the final percentage of flowering to correlate with the blue spectral content of the treatment. Blue light is known to trigger early flowering via the cryptochrome photoreceptors (Huché-Thélier et al., 2016; Zheng et al., 2019b). The earliest and the highest rate of flowering (Figure 4.4A,B) was obtained in conditions with a higher blue content and a lower red content (BR), indicating the strong influence of cryptochrome and the importance of the R:B ratio. Similar observations were noted in *A. thaliana*, where blue light accelerated flowering by 15 days compared to those grown under red light (Guo et al., 1998), and in roses where monochromatic blue light induced full floral development (Abidi et al.,

2013). In Chrysanthemum plants grown under white light conditions, the increase and decrease of blue content resulted in a delay in flowering, while red and blue light combination similar to the RB treatment in the current study has also been observed to delay flowering in *Fuchsia hybrida* cultivated in a controlled environment conditions (Huché-Thélier et al., 2016). Though species dependent, shorter wavelengths in the non-PAR ultraviolet region has been reported to not only delay flowering but also to decrease the rate of flowering in multiple species (Huché-Thélier et al., 2016) in line with the findings in this study where UV-A supplemented plants had the lowest rate of flowering together with the RB treated plants.

4.5.4 Non-PAR wavelengths encourage Stevia vegetative growth

Studies by Esra et al. (2016b), and Yoneda et al. (2017a) reported an increase in blue light composition leading to shorter plants in *Stevia rebaudiana,* contradicting the findings of this paper that had taller plants under treatments with low red to blue ratios compared to the rest (Figure 4.5A). It is worth noting that Yoneda et al. (2017a) reported taller plants under conditions with lower intensities of blue light compared to plants grown under white fluorescent lamps or monochromatic red light sources, where the plant height was greatest under conditions with a blue to red ratio of 0.12, while higher ratios resulted in shorter Stevia plants (Yoneda et al., 2017a). Similar observations of higher blue light leading to shorter plants were reported among other species, highlighting the inhibitive nature of blue light on stem elongation and plant height (Huché-Thélier et al., 2016) and the positive impact a higher composition of red light, and a lower R:FR ratio, has on promoting stem elongation (Demotes-Mainard et al., 2016). The positive effects of a higher R:FR ratio were observed in this study when comparing the RB and FR treatments, with plants under the FR treatments being taller than those under the RB treatment although both systems had identical basal red and blue spectra, indicating the promotion of stem elongation of plants under these treatments as a shade avoidance

response. Although species dependent, both red and blue light have been reported to mediate stem elongation (Demotes-Mainard et al., 2016; Huché-Thélier et al., 2016; Zhang et al., 2020b). The difference observed in this study compared to Yoneda et al. (2017a), and Esra et al. (2016b), can be attributed to the difference in the intensity of supplemental blue light and the absence of additional supplemental spectra (UVA, GR) in those studies.

Studies have also shown that the inhibitory and promotive nature of blue light on stem elongation is not only dependent on the absolute amount of blue light content within the spectra or the plant species, but also on the light intensity (Kong et al., 2018; Yoneda et al., 2017a). Blue light at intensities between 50 to 100 μmol $m^{-2}s^{-1}$ promote stem elongation but at higher intensities, this response reverses to inhibitory in nature, indicating the possibility that stem elongation responses at lower intensities are in fact a blue light mediated shade avoidance response (Johnson et al., 2020; Yoneda et al., 2017a). The effect of blue light intensity on plant growth was also observed in this study where the BR and FS treatments, the only systems with blue light intensities above 50 μmol $m^{-2}s^{-1}$ (Table 4.1), resulted in the tallest plants among the different treatments. The overall stem thickness among samples under all treatments had minor variations, with plants under the BR treatment having the thinnest stems (Figure 4.5 B), consistent with previous studies that found increased blue spectral content resulting in smaller stem diameter among Stevia plants (Yoneda et al., 2017a). Studies by Glowacka (2006) and Poudel et al. (2008) however reported supplemental blue light increased the stem thickness in tomatoes and grapes, indicating a species specific response.

Low light conditions, although having a low photosynthetic efficiency resulting in lower biomass accumulation, are known to result in smaller and thinner leaves, a response that is believed to increase the leaves ability to intercept light and aid in the reflection and scattering of light increasing the light absorption by the chloroplasts (Terashima et al.,

2011; Wu et al., 2017). Lower R:FR ratios are known to result in thinner leaves due to the shade avoidance response that triggers the plant to divert more biomass towards its stem (Demotes-Mainard et al., 2016). A similar observation was obtained in the current study where the FR treated plants had a mean lower leaf thickness compared to the basal RB treated plants that had the thickest leaves among all treatments (Figure 4.5 B). In this study, the thinnest leaves were obtained in plants under the FS treatment that not only had a higher R:FR ratio compared to other treatments (except FR) but also had a higher blue light content. Blue light has a species-specific response, increasing the leaf palisade and mesophyll thickness in pepper (Schuerger et al., 1997) and rapeseed leaves (Shengxin et al., 2016) but decreasing the overall leaf thickness in peach (Rapparini et al., 1999) and red leaf lettuce (Samuoliene et al., 2020a). While the UV-A spectrum is not within the blue spectra in the physics world, plants are known to perceive UV-A using the same blue sensitive cryptochrome photoreceptor (Chen et al., 2019; Zheng et al., 2019b). This explains the similar traits observed between UVA and BR treated plants. Green light, when supplemented with red and blue light results in thinner leaves of red leaf lettuce seedlings compared to a base red and blue light, and similar results were obtained in this study under the GR treatment (Figure 4.5 B) (Samuoliene et al., 2020a). UVA and FS treatments, the only light treatments with supplemental UV-A irradiation had the greatest number of leaves per plant with a mean of 93 leaves each, 30% more than plants under the BR treatment that had the lowest number of leaves (Figure 4.5 D). A similar observation was reported in indoor cultivated lettuce where supplemental UV-A irradiation resulted in an increase in biomass and number of leaves indicating a possible separate pathway for UV-A stimulated responses in plants (Chen et al., 2019).

4.5.5 Green light increases biomass yield in Stevia

Most research and development of modern LED based horticulture lighting systems has focused on red and blue light spectra based on earlier reports that suggested these as being

most efficient for photosynthesis (Bian et al., 2019; Claypool & Lieth, 2020; Zhang et al., 2020b). It has been reported that supplementation with red light did not have any significant effects in manipulating the relative percentage content of the SG with respect to its dry biomass, but was effective in extending the overall vegetative period of the plant, increasing the overall biomass and absolute SG yield (Ceunen & Geuns, 2013a; Ceunen & Geuns, 2013c; Yoneda et al., 2017b), consistent with this study where the RB treated plants had higher fresh biomass compared to the BR treated plants that were grown under lower red light spectra (Figure 4.6 A).

The contribution of the non-PAR UVA and FR spectra on photosynthetic activity was prevalent in this study with treatments consisting of these spectra resulting in higher dry biomass accumulation compared to the base RB treatment under the same PPFD. However, when considering the spectral range beyond the PAR bandwidth, FR, UVA, and FS treatments also had higher overall photon output within the PBAR range (Table 4.1) compared to other treatments and the integral of this additional non-PAR spectra is a possible contributor to the higher photosynthetic activity and subsequently an increase in dry biomass accumulation. There is increasing evidence on the effectiveness of alternative spectra such as far-red, green, and ultraviolet in regulating and promoting plant growth (Wang & Folta, 2013; Zhen & Bugbee, 2020b). Recent reports by Zhen and Bugbee (2020b) and Chen et al. (2019) highlighted the benefits of far-red and UV-A supplemental spectra on improving yields by enhancing photosynthetic activity. The findings of this study are consistent with these recent publications, with UVA, FR and FS treatments resulting in higher dry biomass accumulation compared to the basal RB spectrum (Figure 4.6 E). In this study, the photosynthetic efficacy of UVA range was higher in *Stevia rebaudiana* compared to the FR spectra, resulting in a higher dry biomass accumulation at a lower PFD (Table 4.1). The effects of UVA spectra in photosynthesis is however species dependent and it can have detrimental effects on certain plants (Chen

et al., 2019; Kusuma et al., 2020). Although FR spectra did result in higher total dry biomass accumulation, this was observed with a higher partitioning towards stems, and at a higher PFD compared to other treatments (Table 4.1). The observations of this study are consistent with known shade avoidance response of plants to higher R:FR (FR) ratios and increased blue spectral (BR) content where plants begin to prioritise strengthening their stems over leaves and increasing stem biomass accumulation (Johnson et al., 2020; Yoneda et al., 2017a). The Emerson effect, a typical characteristic FR spectra has on photosynthetic activity when used as supplemental lighting (Zhen & Bugbee, 2020c), was not observed in this study. Unlike other wavelengths within the PAR range, FR spectra does not have a linear relationship with regards the rate of photosynthesis and its photosynthetic effectiveness is dependent on its synergistic relationship with other spectral components, especially in the presence of shorter wavelengths (Kusuma & Bugbee, 2020; Kusuma et al., 2020; Zhen & Bugbee, 2020c). The lower amount of shorter wavelength spectral component in the FR treatment may have led to a muted reaction of the FR supplemental light on the overall photosynthetic activity (Zhen & Bugbee, 2020c).

Green light can penetrate deeper into individual leaves and the plant canopy than red and blue light, enhancing photosynthetic capacity within the leaf and plant, leading to higher biomass accumulation (Samuoliene et al., 2020a; Zhang et al., 2020b). Similar results were obtained in pepper (Claypool & Lieth, 2020), lettuce (Samuoliene et al., 2020a) and tomatoes (Bian et al., 2019) where supplemental green light resulted in higher net photosynthetic rates and in most cases, an increase in biomass yield compared to blue, red or blue+red lights. In this study, the samples were grown at a high density of 20 plants/m^2, almost 200% higher than field grown densities (Nakonechnaya et al., 2019), resulting in dense canopies. While these dense canopies may limit the access and absorption of red and blue wavelengths by lower leaves, the supplemental green spectra in GR and FS treatments were able to penetrate deep into the canopy, resulting in higher

photosynthetic activity and biomass accumulation (Zhang et al., 2020b). The leaves of the GR treated plants, while having the highest fresh and dry biomass, also had an 81.84% moisture content, the highest among all treatments (Figure 4.6 F). This was however comparable to previous studies by Ceunen and Geuns (2013c); Ceunen et al. (2011) and Ceunen et al. (2012b) hat reported a leaf moisture content of between 83% to 87% among Stevia leaves.

4.5.6 UV-A Improves accumulation of Reb A and ST in Stevia

The Reb A yields of 7.70%, 7.39% and 7.25% obtained under the GR, UVA and BR treatments (Figure 4.7 A) were higher than previously published field data which ranged from 1.8% to 7% w/w (Ceunen & Geuns, 2013a; Ceunen et al., 2011, 2012c; Wojewoda et al., 2018). Apart from RB (11.99%) treated plants, the yields from UVA (20.05%), BR(16.39%), FR(14.98%), FS(13.45% and GR (13.27) (Figure 4.7 B) were all higher compared to data for field grown plants that ranged from 5% to 13% w/w (Aghighi Shahverdi et al., 2019; Libik-Konieczny et al., 2018). Yoneda et al. (2017a) reported significant increment in Reb A and ST content of plants grown under blue and far-red doped red light compared to plants under monochromatic red, white FTL, and lighting conditions with higher far-red content. Blue and UV-A irradiation are known to stimulate synthesis and accumulation of secondary metabolites by upregulating the expression of genes for the synthesis of flavonoids, and increasing the accumulation of anthocyanin, carotenoid and chlorophyll in many plant species (Huché-Thélier et al., 2016; Zhang et al., 2020b; Zheng et al., 2019b). Simlat et al. (2016) reported that *Stevia rebaudiana* plantlets irradiated with blue light had a higher accumulation of carotenoids, phenolics and total soluble sugars, indicating the effect of blue light on the synthesis and accumulation of the biochemical compounds in Stevia plants. Green light has also been observed to promote accumulation of secondary metabolites in plants as a defence mechanism by initiating and upregulating the expression of specific genes under abiotic

and biotic stress conditions (Shafiq et al., 2020) besides enhancing antioxidant activity, and contents of antioxidant and aromatic compounds in leaves (Samuoliene et al., 2020a; Virsile et al., 2020). There are no known UV-A and green light specific photoreceptors, and it is believed that plant responses to these wavelengths are regulated via blue light sensitive cryptochromes and phototropins receptors (Virsile et al., 2020; Zhang et al., 2020b). It was observed in this study that a low dosage of UV-A had a greater effect on the overall glycoside accumulation, resulting in consistently higher Reb A and ST yields compared to plants grown under higher doses of blue light (Figure 4.7A,B). Green supplemental light, while resulting in significantly high Reb A yields, had relatively low ST yields (Figure 4.7A,B). In the SG biosynthesis pathway, the UDP-glycosyltransferase 76G1 (encoded by the gene UGT76G1) is known to convert ST to Reb A while UDP-glycosyltransferase 74G1 (encoded by the gene UGT74G1) converts steviolbioside to ST (Kim et al., 2019; Yoneda et al., 2017a). Yoneda et al. (2017a) reported the effects of light quality on the transcription of the UGT74G1 and UGT76G1 genes, with plants grown under monochromatic blue light and red light with high far-red (FR) content having the highest gene transcription levels. The findings of the current study suggest a possible effect of green light on enhancing the transcription of UGT76G1, increasing the conversion of ST to Reb A, resulting in a higher Reb A/ST ratio (Figure 4.7C), which could in the future be confirmed by enzyme or expression assays. This difference highlights the complexity of the photosensory network, suggesting a system, rather than a single specific photoreceptor, working in conjunction to control the plant response to light quality.

4.5.7 Commercial Application

Recent advances in big data, artificial intelligence (AI), internet of things (IOT) and cloud computing have facilitated the transformation of simple greenhouses into plant factories that employ precision agriculture techniques to optimise productivity and resource

utilisation, paving the way for modern, technologically advanced, energy efficient and sustainable agricultural practices (Graamans et al., 2020). Artificial lighting, having a broad effect in regulating growth, morphology, and metabolism in plants, is a key driver for this transition (Zhang et al., 2020b; Zheng et al., 2019b). Addressing the global stigma associated with genetic engineering and manipulation, Carvalho and Folta (2014) proposed the alternative term of 'Environmentally Modified Organisms' (EMO), where environmental conditions are controlled and modified. CEA typically used for commercial food production, is now increasingly being adapted globally for cultivation of high valued herbs, and *Stevia rebaudiana* is a strong potential candidate. Based on the results obtained in this study, it is apparent that cultivation of *Stevia rebaudiana* under full artificial light with optimised parameters in a CEA setup is not only possible but can also lead to significantly improved yields. While red and blue light has been proven to be efficient for normal plant growth, additional supplemental spectra such as UV-A and FR, not presently common in horticulture systems, can also lead to improvements in overall ST and Reb A yields, as exhibited by the findings in this study where plants under the FR and UVA treatments had 58% and 160% higher yields compared to those cultivated under the RB treatment at a supplemental dosages of 22.1 5 μmol $m^{-2}s^{-1}$ of far-red and 6.5 μmol $m^{-2}s^{-1}$ of UV-A spectra respectively (Figure 4.7 D). This study also highlighted the importance of green light in yield improvement of indoor cultivation of *Stevia rebaudiana* with the GR light treatment, where green light when used to replace part of the red and blue spectra, had a final ST and Reb A yield per plant of 0.45g, 165% higher than the base RB treatment, while maintaining identical PPFD and PFD values (Figure 4.7D and Table 4.1).

4.6 Conclusion

Considering the key parameters critical for indoor cultivation of *Stevia rebaudiana* plants i.e. the rate of germination, the rate of flowering, dry leaf yield, and the Reb A and ST

yields; red and blue light with an intensity of 130 µmol $m^{-2}s^{-1}$ when supplemented with 6.5 µmol $m^{-2}s^{-1}$ UV-A irradiation under a 16-hour photoperiod was the most beneficial lighting regime. This optimal lighting regime resulted in higher seed germination, lower rate of flowering, high dry leaf yield and superior ST and Reb A yields at 175 days after planting.

In the next chapter, higher fractions of UV-A and green spectral content together with a base red and blue light was used. The effect of pre-harvest treatments on Stevia biomass and metabolite accumulation was also evaluated.

CHAPTER 5 : EFFECT OF GREEN AND UV-A FRACTION, AND PRE-HARVEST TREATMENTS ON *STEVIA REBAUDIANA*

This chapter describes two different studies. In the first study, higher fractions of green (15 and 25 µmol $m^{-2}s^{-1}$) and UV-A light (11 and 15 µmol $m^{-2}s^{-1}$) compared to those used in the experiments described in Chapter 4 that had 13 µmol $m^{-2}s^{-1}$ of green and 6.5 µmol $m^{-2}s^{-1}$ of UV-A, were applied from germination to harvest. In the second experiment, monochromatic blue, UV-A and green light, as well as the multispectral red-blue, red-blue-UV-A and red-blue-green were used as 3-day and 10-day pre-harvest treatments. The effect of these treatment on the biomass and metabolite yields were evaluated.

5.1 Introduction

It is an established fact that light plays an important role in plant growth and development. Both, the quantity of light, determined by the intensity and photoperiod, and the quality of light that is dependent on the spectral composition, is critical not only for photosynthetic and photomorphogenesis but also for the accumulation of metabolites within higher level plants (Paradiso & Proietti, 2022; Zheng et al., 2019a). Light quality and quantity play a part in regulating plant growth and development throughout the developmental stages from germination through to flowering (Appolloni et al., 2022; Hernández et al., 2022).

5.2 Literature Review

In field grown conditions, the quality and quantity of the steviol glycosides (SG) that can be realised is highly dependent on several external factors such as the light quality, photoperiod, temperature, soil moisture, wind, and water availability (de Andrade et al., 2021; Rai & Han, 2022). Additionally, any pre- and post-harvest techniques applied during the cultivation and harvest may affect the SG content in Stevia leaves (de Andrade

et al., 2021). Previous studies on improving the productivity of Stevia plants either in field grown conditions or within a controlled environment agriculture (CEA) system have focused on either increasing the quantity (biomass yield) (Chowdhury et al., 2017; Idrees et al., 2018; Maniruzzaman et al., 2017; Melviana et al., 2021) or quality (metabolite concentrations) (Ai et al., 2022; Basharat et al., 2021; Hernández et al., 2022; Jarma-Orozco et al., 2020; Shulgina et al., 2021). As the compound of commercial interest is extracted from the dried leaves of the Stevia plants, it is imperative that both aspects, the biomass accumulation and metabolite concentration be improved to maximise the productivity of the plant. The main approaches towards improving SG biosynthesis and yields can be categorised into conventional and biotechnical methods. The conventional method includes physical (light quality, photoperiod, abiotic stress) and chemical (drought, salinity stress, nutrient) manipulation, while the biotechnical approach includes but is not limited to, micropropagation, induction of polyploidy, and genetic manipulation (Basharat et al., 2021; Libik-Konieczny et al., 2021; Rai & Han, 2022).

Past studies on the effects of artificial lighting on the growth and yield of indoor cultivated Stevia plants has focused on photoperiod manipulation (de Andrade et al., 2021; Rengasamy et al., 2022a; Yoneda et al., 2017b), the use of night interruption (Armizatul et al., 2010; Ceunen et al., 2012a), light intensity (Hernández et al., 2022; Nakonechnaya et al., 2019; Yoneda et al., 2017b), and on the use of different spectral composition (Shulgina et al., 2021; Yoneda et al., 2017a) . In the study described in this chapter, two different lighting strategies were evaluated with an aim to determine the approach that would result in the highest yield. The first strategy used varying fractions of green (550 nm peak), Ultraviolet A (UVA) (380 nm peak) supplemental spectrum with a base of red (660 nm peak) and blue (450 nm peak) light. This is an expansion from the work done in the preceding chapter where it was observed that green and UVA supplemented light produced the highest yields of ST and Reb A. In work described in the Chapter 4, only

single treatments incorporating UV-A and green were used. The effects of different levels of green and UV-A fraction were not evaluated. Studies have reported the positive effects of increasing green spectra intensity within a base red and blue light, on the biomass accumulation of multiple plant species especially under higher intensities (Langston et al., 2022; Paradiso & Proietti, 2022; Santin et al., 2021). Supplementary UVA radiation has been reported to increase biomass and secondary metabolite accumulation in some plants, although this is species dependent (Chen et al., 2019; Kong et al., 2019b; Lee et al., 2022; Nair et al., 2021; Samuoliene et al., 2020a).

In the second approach, plants cultivated either under a base red + blue light, or under natural sunlight were subjected to a pre-harvest lighting treatment under 3 trichromatic and 3 monochromatic light sources for 3 or 10 days. Recent studies have reported the beneficial properties of blue, green, red, and UVA pre-harvest lighting treatments on improving the accumulation of secondary metabolite in vegetables and other leafy greens (Deng et al., 2017; dos S. Nascimento et al., 2020; Dou et al., 2019; Hooks et al., 2021; Langston et al., 2022). Previous studies have reported the positive effects of short pre-harvest treatments of between 2 to 4 days for lettuce (Hooks et al., 2021; Hooks et al., 2022; Zhang et al., 2021) and longer treatments of 10-days and beyond in kale (Jiang et al., 2021). As there has been no publication on the effects of pre-harvest lighting on Stevia, both, the shorter 3-day and longer 10-day pre-harvest durations were used for this study. The use of pre-harvest lighting strategies would potentially allow crops to be cultivated either under natural sunlight or under a base red and blue artificial light during its vegetative stage, before being transferred under specialised lighting spectra to improve the plant's quality. This would allow for optimisation of electrical energy used for the lighting component in these systems. This is the first study to employ varying fractions of green, UVA and a combination of green and UVA on *Stevia rebaudiana* from

germination to harvest. This is also the first time the effects of pre-harvest trichromatic and monochromatic light on indoor grown Stevia plants were investigated.

5.3 Material and Methods

5.3.1 Plant Materials

Stevia rebaudiana seeds sourced from Baker Creek, USA (https://www.rareseeds.com/) was surface-sown in at a rate of one seed per cell of a 50-cell plug tray (54cm x 28cm x 5.7cm) that was filled with autoclaved potting soil, after being cleaned under running water and dried on a filter paper. The trays were sprayed with water, wrapped in transparent plastic, and set up in the Plant Biotech Facility (PBF) of University of Malaya, Kuala Lumpur, under the appropriate lighting conditions. The dark room (PF) where the artificial lighting experiments were conducted, and the greenhouse (GH) where experiments under natural sunlight were both housed within the same facility. The room's temperature was kept at 25±2 °C, while the relative humidity ranged from 70 to 80%. Throughout the time, the seedlings were watered only when needed. Each experimental cycle lasted for 175 days and was repeated 3 times. The experiments were run from October 2019 to September 2022. In the interest of time, the experimental cycles had an overlap where the germination stage of the subsequent cycles were started prior to the harvesting of the preceding experimental cycle. The planting density for all experiments were at 24 individual plants per m^2 of growth space, both in the PF and GH.

5.3.2 Light Treatments

In this study, 2 different experiments were employed. In the first experiment (Strategy A), the plants were grown from seed to harvest under the same light treatment (i.e. no separate pre-harvest light treatment was given). In the second experiment, plants were grown either under red + blue artificial light within the dark room (PF), or under natural sunlight within the greenhouse (GH) before being subjected to a lighting treatment for

the 3 or 10 days immediately prior to harvest. A total of seven custom built light emitting diode (LED) lighting systems, each consisting of 8 independently controlled channels of high-powered LEDs were used for all experiments (Osram Opto, Germany, Cree, USA, Edison Opto, Chinese Taipei). Each channel (UV-A, Deep Blue, Blue, Green, Deep Red, Red spectra) was individually controlled from 0-100% intensity via a pulse width modulation (PWM) controller to provide the desired spectral composition for each treatment as outlined in Table 5.1 and Table 5.2. Brief block diagram on the lighting system design and installation location is available in Chapter 6, Section 6.3. A portable spectroradiometer (Asenstek Lighting Passport, Taiwan) was used to measure the total light output of all treatments. The measurements were taken at the plant canopy level, 30cm from the base of the growing surface. Measurements were taken monthly throughout the experimental period to confirm that light output was constant. As the natural light in the GH varied significantly throughout the day, a portable spectroradiometer with data logging function (Nanolambda XL-500 BLE, South Korea) was used to log the spectral information at 5-minute intervals. A 14-day average was used to obtain the mean values for the natural sunlight. The RB and GH treatments were used as controls for artificial lighting and natural sunlight respectively.

5.3.2.1 Experiment 1: Green & UVA

In the first experiment, all light treatments had a base red and blue light and was then supplemented with varying degrees of green (GR1, GR2), UVA (UV1, UV2) light spectra. Two additional light treatments that combined both green and UVA were included as UVGR1 (UV1+GR1) and UVGR2 (UV2+GR2) (Table 5.1). All treatments had a photosynthetic photon flux density (PPFD) of approximately 130 μmol m^{-2} s^{-1}. The definition of PPFD were based on American Society of Agricultural and Biological Engineers (2017).

Table 5.1 : Spectral Composition of Artificial Lighting Systems and Natural Sunlight for Strategy A

	Unit	UV1	UV2	GR1	GR2	UVGR 1	UVGR 2	RB	GH
UV-A (380nm)	µmol $m^{-2}s^{-1}$	11.84	15.81	0.15	0.17	12.45	7.44	0	4.38
Blue (450 nm)	µmol $m^{-2}s^{-1}$	39.96	39.81	36.77	32.45	36.92	32.76	39	78.55
Green (550 nm)	µmol $m^{-2}s^{-1}$	1.77	2.31	15.02	25.1	11.41	24.03	0	119.1
Red (660 nm)	µmol $m^{-2}s^{-1}$	89.26	90.13	80.61	72.45	82.68	73.38	91	135.83
Far Red (740 nm)	µmol $m^{-2}s^{-1}$	3.37	2.63	1.18	1.36	2.93	2.61	1.3	91.05
Total PPFD[a] (PAR)	µmol $m^{-2}s^{-1}$	130.99	132.25	130.78	130	131.01	130.16	130	333.48
Total PFD[b] (PBAR)	µmol $m^{-2}s^{-1}$	146.2	150.68	132.11	131.52	146.39	140.21	131.3	428.91
Daily Light Integral (DLI)	mol m^{-2} day^{-1}	7.5	7.6	7.5	7.5	7.5	7.5	7.5	14.4
Photoperiod in 24 Hours	Hours (h)	16	16	16	16	16	16	16	12

[a] Photosynthetic Photon Flux Density (PPFD) range of 400 nm to 700nm was based on American Society of Agricultural and Biological Engineers (2017)

[b] Photosynthetic Biological Active Radiation (PBAR) range of 280 nm to 800nm was based on American Society of Agricultural and Biological Engineers (2017)

5.3.2.2 Experiment 2 : Pre- Harvest Treatments

The second experiment was further split into four different pre-harvest (PH) strategies. In PH1, the plants were cultivated for 165 days under the basal RB light treatment before being subjected to 10 days under the trichromatic UV2, GR2 (Table 5.1), and BR (Table 5.2), and the monochromatic green, blue and UVA irradiation. In PH2, plants were cultivated for 165 days in the greenhouse under natural sunlight (GH) before being subjected to the same treatments as in PH 1. Meanwhile, in PH3 and PH4, the plants were

cultivated under RB and GH respectively for a total of 172 days before being subjected to 3 days of pre-harvest lighting treatment as in PH 1 (Table 5.2, Figure 5.1).

Table 5.2 : Spectral Composition of Pre-harvest (PH) Light Treatment

		Monochromatic			Trichromatic		
		Blue	**Green**	**UV-A**	**UV2**	**GR2**	**BR**
UV-A (380nm)	µmol $m^{-2}s^{-1}$	0	0	20	15.81	0.17	0
Blue (450 nm)	µmol $m^{-2}s^{-1}$	55	0	0	39.81	32.45	58.5
Green (550 nm)	µmol $m^{-2}s^{-1}$	0	25	0	2.31	25.1	0
Red (660 nm)	µmol $m^{-2}s^{-1}$	0	0	0	90.13	72.45	71.5
Far Red (740 nm)	µmol $m^{-2}s^{-1}$	0	0	0	2.63	1.36	1.3
Daily Light Integral (DLI)	mol m^{-2} day^{-1}	3.2	1.4	1.2	7.5	7.5	7.5
Photoperiod in 24 Hours	Hours (h)	16	16	16	16	16	16

Strategy	Treatment	Days After Sowing of Seeds			
		0	165	172	175
PH1	XH	Red-Blue (RB)		UV2	
	XI	Red-Blue (RB)		GR2	
	XJ	Red-Blue (RB)		BR	
	XK	Red-Blue (RB)		Monochromatic UV-A	
	XL	Red-Blue (RB)		Monochromatic Green	
	XM	Red-Blue (RB)		Monochromatic Blue	
PH2	XN	Greenhouse (GH)		UV2	
	XO	Greenhouse (GH)		GR2	
	XP	Greenhouse (GH)		BR	
	XQ	Greenhouse (GH)		Monochromatic UV-A	
	XR	Greenhouse (GH)		Monochromatic Green	
	XS	Greenhouse (GH)		Monochromatic Blue	
PH3	3H	Red-Blue (RB)			UV2
	3I	Red-Blue (RB)			GR2
	3J	Red-Blue (RB)			BR
	3K	Red-Blue (RB)			Monochromatic UV-A
	3L	Red-Blue (RB)			Monochromatic Green
	3M	Red-Blue (RB)			Monochromatic Blue
PH4	3N	Greenhouse (GH)			UV2
	3O	Greenhouse (GH)			GR2
	3P	Greenhouse (GH)			BR
	3Q	Greenhouse (GH)			Monochromatic UV-A
	3R	Greenhouse (GH)			Monochromatic Green
	3S	Greenhouse (GH)			Monochromatic Blue

Figure 5.1 : Pre-Harvest Lighting Strategies. Plants grown under GH or RB before irradiated with different spectral compositions for last 3 or 10 days before harvest.

Note : All treatments that have '3' as the first digit represent a 3-day pre-harvest treatment while those that start with an 'X' had a 10-day pre-harvest treatment.

5.3.3 Seed Germination and Flowering

The seed germination and flowering study was only done for plants under lighting Strategy A. The seeded trays were placed under the respective light treatments (Table 5.1). 14-days after sowing (DAP), the number of seeds that sprouted were recorded. The rate of germination for each light treatment was then calculated using the following equation.

$$\text{Rate of Germination} = \frac{\text{Sum of Germinated Seeds}}{\text{Total Seeds Sowed}}\%$$

5-week-old seedlings were transplanted into individual pots (12cm x 12cm x 10cm) filled with autoclaved potting soil (www.serbajadi.com.my). A total of 20 biological replicates from each light treatment were selected for each experimental cycle. The number of plants that reach the flowering stage was recorded from the 6th week after sowing of seeds until the end of each experimental cycle. The rate of flowering for each week was calculated as follows.

$$\text{Weekly Rate of Flowering} = \frac{\text{No. of Samples in Flowering Stage}}{\text{Total No. of Samples}}\%$$

Where,

No. of Samples in Flowering Stage = Plants with at least 1 fully bloomed flower

5.3.4 Biomass Accumulation

The plants were harvested by cutting the stems 5cm above the soil after which the leaves and stems for each plant were separated and weighed using a digital scale (Shimadzu, Japan). The leaves of all samples under the same light treatment were accumulated, rinsed under running water, and dried in a mesh bowl for 1 hour before being blotted gently with

tissue papers. The leaves were then dried in an oven (Binder, Germany) at 60°C for 20 hours until it achieved a steady weight. Room temperatures samples were once again weighed using the digital scale prior to being packed with silica gel desiccant in an airtight container. The samples were stored at -4°C before being used for the LCMS analysis to determine its ST and Reb A composition.

5.3.5 Metabolite Accumulation

5.3.5.1 LCMS

Each sample of 0.5 g of ground up dried Stevia leaves was mixed with 50 mL of 35:65 (v/v) ethanol-water mixture and sonicated for 2 hours after which it was filtered to obtain the effluents that were subsequently dried using a miVac centrifugal concentrator to remove any residual solvent. 10 mg of the resulting extract was then dissolved in a 1mL mixture of water and acetonitrile (7:3). The sample was then filtered using a PES membrane with pore size 0.22µm. The Shimadzu LCMSMS 8050 with a C18 column was used for the LCMS analysis. A modified approach based on the assay methods outlined in FAO and WHO (2020) was used to quantify the amount of ST and Reb A among the samples, expressed in percentage of mass of leaf dry matter (%w/w). Analytical standards of ST and Reb A compounds with a purity >98%, obtained from Sigma-Aldrich (Germany) were used as a reference. Although there are more than 60 identified glycosides, in this study only the ST and Reb A compounds that are the most abundant were considered.

5.3.5.2 Metabolite Yields

The quantity of the total ST and Reb A metabolite that can be realised, the mean combined Reb A and ST yield per plant, expressed in g plant^{-1}, was calculated as follows:

$$\text{Metabolite Yields} = \text{Mean Leaf DW} \times (\text{Reb A Concentration} + \text{ST Concentration})$$

where,

Mean Leaf DW = Mean leaf dry weight per plant in g.

Reb A & ST Concentration = Percentage composition per gram of leaf DW (Obtained from LCMS results).

5.3.6 Statistical Analysis

Data obtained was analysed using the Analysis of Variance (ANOVA) to compare the means within and between the different light treatments. Tukey's Honestly Significant Difference (HSD) post hoc test with a significance of $P<0.05$ was used to further illustrate the statistical significance and relationship between the data. All analysis was done using IBM SPSS Statistics V25.0 software package

5.4 Results

5.4.1 Effect of Green and UVA Fractions on Germination and Flowering rates

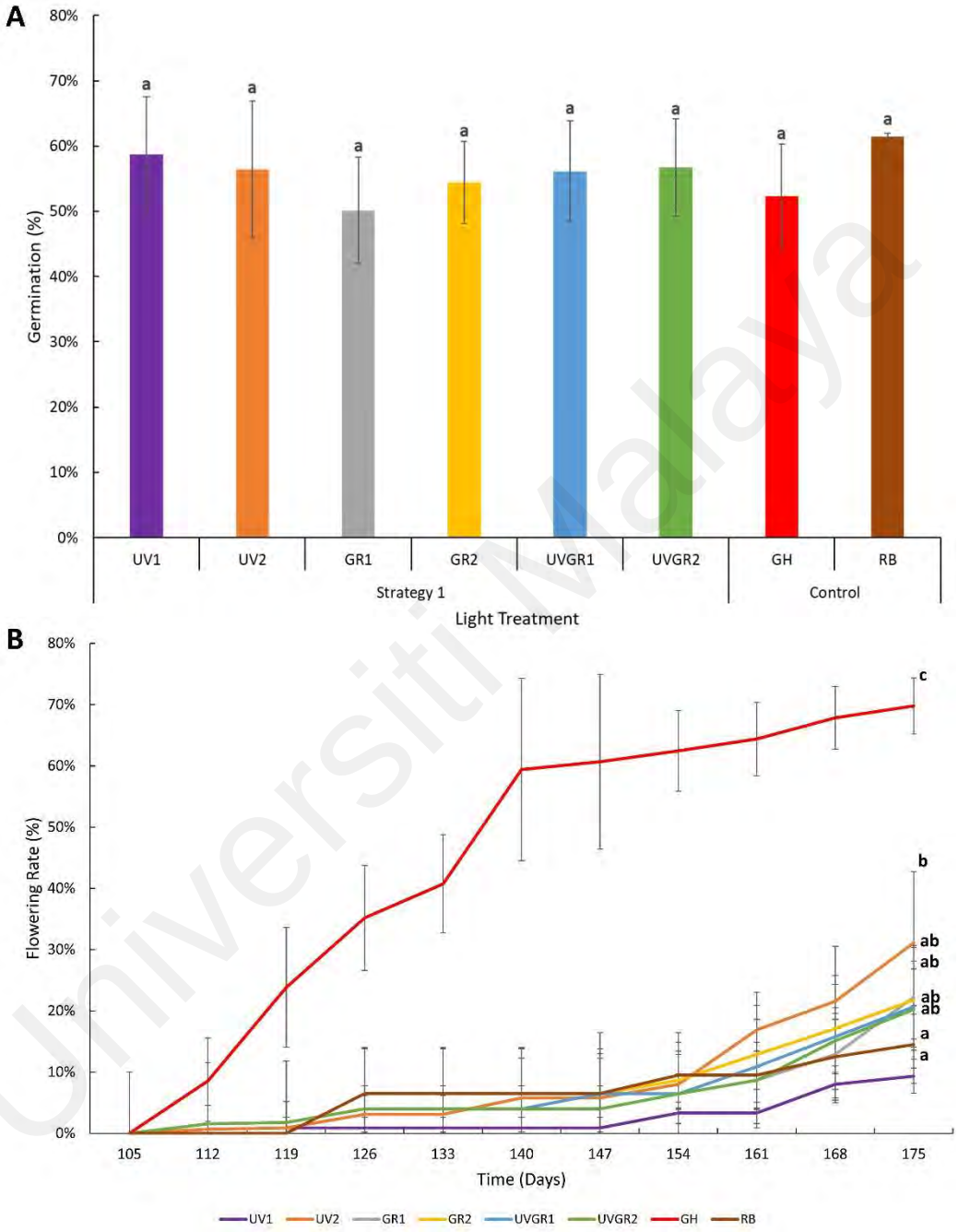

Figure 5.2 : Effect of Lighting Strategy on Germination and Flowering. (A) Mean rate of germination of *Stevia rebaudiana* seeds 14 days after sowing. (B) The cumulative weekly percentage of plants with flowers under different light treatments observed from the day of sowing of seeds. Values represents Mean (n = 60) ± Standard Deviation. Different letters at the error bars indicate statistical significance determined by Tukey's HSD post hoc test at P<0.05.

There was no statistically significant difference in the rate of germination between all light treatments in lighting Strategy A (Figure 5.2). The rate of germination varied between 50.15% (GH) and 61.50% (RB). The first signs of flowering were observed in week 16 (day 112) with GH registering 8.56% of its samples transitioning towards the flowering stage. Except for RB, all other artificial lighting treatments had between 0.67% (UV1, UV2) and 1.56% (GR1, GR2, UVGR1, UVGR2) of the total samples beginning to flower in Week 16. At the end of the experimental cycle of 175 days (Week 25), GH had the highest mean rate of flowering, with 69.78% of all samples entering the flowering stage, while the lowest rate of flowering was observed in UV1, with just 9.33%. It was observed that unlike UV1, the treatment with UV2 with a higher UV fraction, resulted in the second highest rate of flowering, with 31.11% of samples reaching the reproductive stage at the end of Week 25.

5.4.2 Effect of the Different Lighting Strategies on Biomass Accumulation

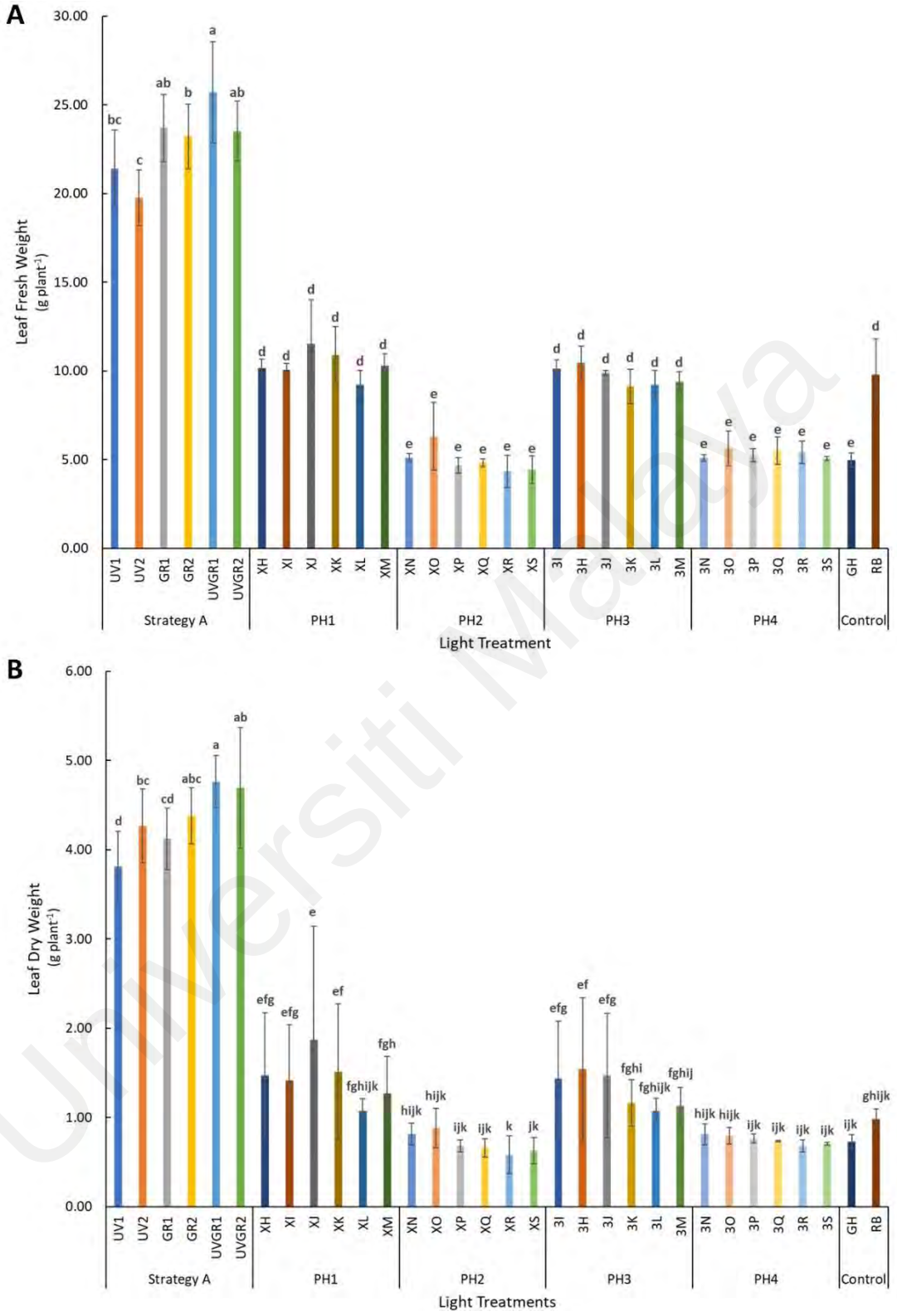

Figure 5.3 : Effect of Lighting Strategies on Fresh and Dry Biomass. (A) The mean leaf fresh weight per plant (g plant^{-1}) of Stevia rebaudiana plants 175 DAP under different light treatments. (B) The mean dry weight yield of leaves per plant (g plant^{-1}). Values represents Mean (n = 60) ± Standard Deviation at 175 DAP (Week 25). Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at P<0.05.

All lighting treatments under Strategy A recorded significantly higher fresh and dry biomass accumulation compared to all pre-harvest strategies and both GH and RB controls. UVGR1 had the highest fresh and dry leaf biomass accumulation of 25.70 g plant^{-1} and 4.76 g plant^{-1} respectively (Figure 5.2 A, B). While UV2 (19.75 g plant^{-1}) had the lowest fresh biomass yield among all treatments in Strategy A, UV1 (3.81 g plant^{-1}) had the lowest dry leaf yield within the same strategy (Figure 5.3A, B). Plants under Strategy A grown with supplemental green light fraction (GR1, GR2, UVGR1, UVGR2) had higher fresh biomass yield compared to treatments without any green spectral content (UV1, UV2). Treatments with both supplemental UVA and green were observed to result in higher dry biomass content compared to plants grown under red and blue light supplemented with either UVA or green.

There were no statistically significant differences in biomass between the pre-harvest strategies and its primary light treatments (Figure 5.3 A, B). Plants cultivated under RB produced 9.79 g plant^{-1} of fresh leaf biomass comparable to all pre-harvest treatments (PH1 and PH3) regardless of the duration of exposure (Figure 5.3 A). The highest fresh leaf yield from the pre-harvest strategy that had RB as its primary light treatment was observed under XJ that had 10-days of blue enhanced (BR) irradiation, followed by 3H that had 3-days of pre-harvest exposure under UV2 light treatment. Similarly, for plants grown under GH, the 3-day and 10-day pre-harvest treatment under artificial light did not result in significant changes in its fresh biomass accumulation (Figure 5.3 A). The GR2 pre-harvest treatments of 10-days (XO) resulted in the highest fresh biomass of 6.30 g plant^{-1} for plants with natural sunlight as its primary light source. All pre-harvest treatments that originated from RB resulted in an increase in dry biomass yield compared to the control RB (0.98 g plant^{-1}). The highest dry leaf yield was seen in the 10-day treatment under blue enhanced BR treatment with 1.87 g plant^{-1} (Figure 5.3 B). Plants grown under RB appeared to yield more dry biomass under dichromatic and trichromatic

lights treatments under both 10-day (XH, XI, XJ) and 3-day (3H, 3I, 3J) exposures compared to monochromatic light sources. Unlike plants treated under RB, no significant increase in dry biomass yield was observed in plants grown under the natural sunlight of GH (0.72 g plant^{-1}) (Figure 5.3B). Among all lighting strategies, XR resulted in the lowest fresh and dry biomass of 4.33 g plant^{-1} and 0.58 g plant^{-1} respectively.

5.4.3 Effect of the Different Lighting Strategies on Metabolite Accumulation and Yield

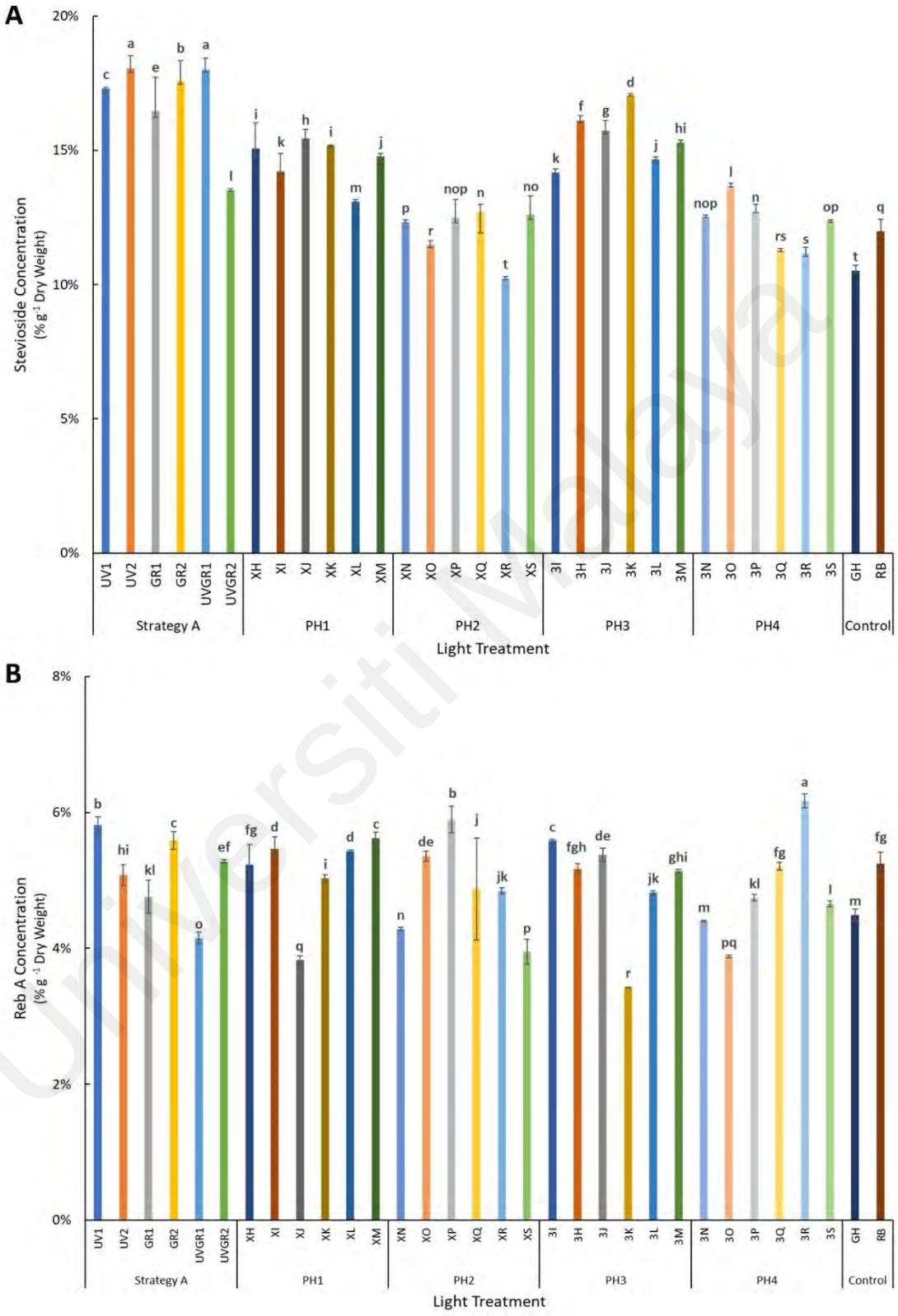

Figure 5.4 : Effect of Lighting Strategies on Metabolite Concentration. (A) Stevioside concentration per unit of dry leaves (% g^{-1} Dry Weight). (B) Reb A concentration per unit of dry leaves (% g^{-1} Dry Weight). Values represents Mean (n = 60) ± Standard Deviation. Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at $p < 0.05$.

Apart from GR1 and UVGR2, all other lighting treatments under Strategy A resulted in significantly higher Stevioside (ST) accumulation compared to all pre-harvest experiments and the controls. UV2 and UVGR1 had the highest ST concentration of 18% each followed by GR2 with 17.58% (Figure 5.4A). The lowest concentration of ST under Strategy A was observed in UVGR2 grown plants with 13.50%. Among the controls, the artificially lighted RB (12.00%) had a higher ST concentration compared to the natural sunlight grown GH (10.50%) plants (Figure 5.4A).

Among the pre-harvest treatment strategies, plants irradiated with RB as the primary light treatment had higher ST concentration compared to those that were grown under GH. This was observed in both 3-day and 10-day pre-harvest experiments (Figure 5.4A). All treatments under PH1 and PH3 (Figure 5.4A) had higher ST concentrations compared to the control treatment of RB. Higher ST accumulation between these two strategies were observed under the 3-day pre-harvest treatment (PH3) with the monochromatic UVA irradiation of 3K (17.02%) being the highest followed by 3H (16.11%) and 3J (15.73%) (Figure 5.3A). In PH2 and PH4 that had GH as the base treatment, it was observed that the monochromatic green light did not significantly affect the ST concentrations under both durations, with the 3-day 3R, and 10-day XR treatments resulting in 11% and 10.23% concentration respectively, comparable to the results of the GH control. The 3O treatment had the highest ST concentration among all treatments that originated from GH, with 12.66%.

Unlike the results for the ST concentration, the concentration of Reb A did not show a distinct superiority of Strategy A among the different light treatment and strategies (Figure 5.4B). In fact, 8 out of the 10 treatments that had the highest Reb A concentrations were obtained from the pre-harvest strategies. The highest concentration of 6.16% was obtained under the 3-day monochromatic green light pre-harvest treatment of 3R. XP, the 10-day pre-harvest treatment of blue enhanced BR spectra that like 3R, had the natural

sunlight of GH as its primary treatment. UV1 from Strategy A had the next highest concentration of 5.80% each. XM, GR2 and 3I had 5.60% of Reb A each, followed by XI, XL, 3J and XO completing the light treatment with the 10-highest concentrations of Reb A at 5.40% each. Interestingly the three treatments with lowest concentrations were also from the pre-harvest strategies with lowest concentration observed in 3K with 3.40% followed by XJ and 3O with 3.80% each (Figure 5.4B).

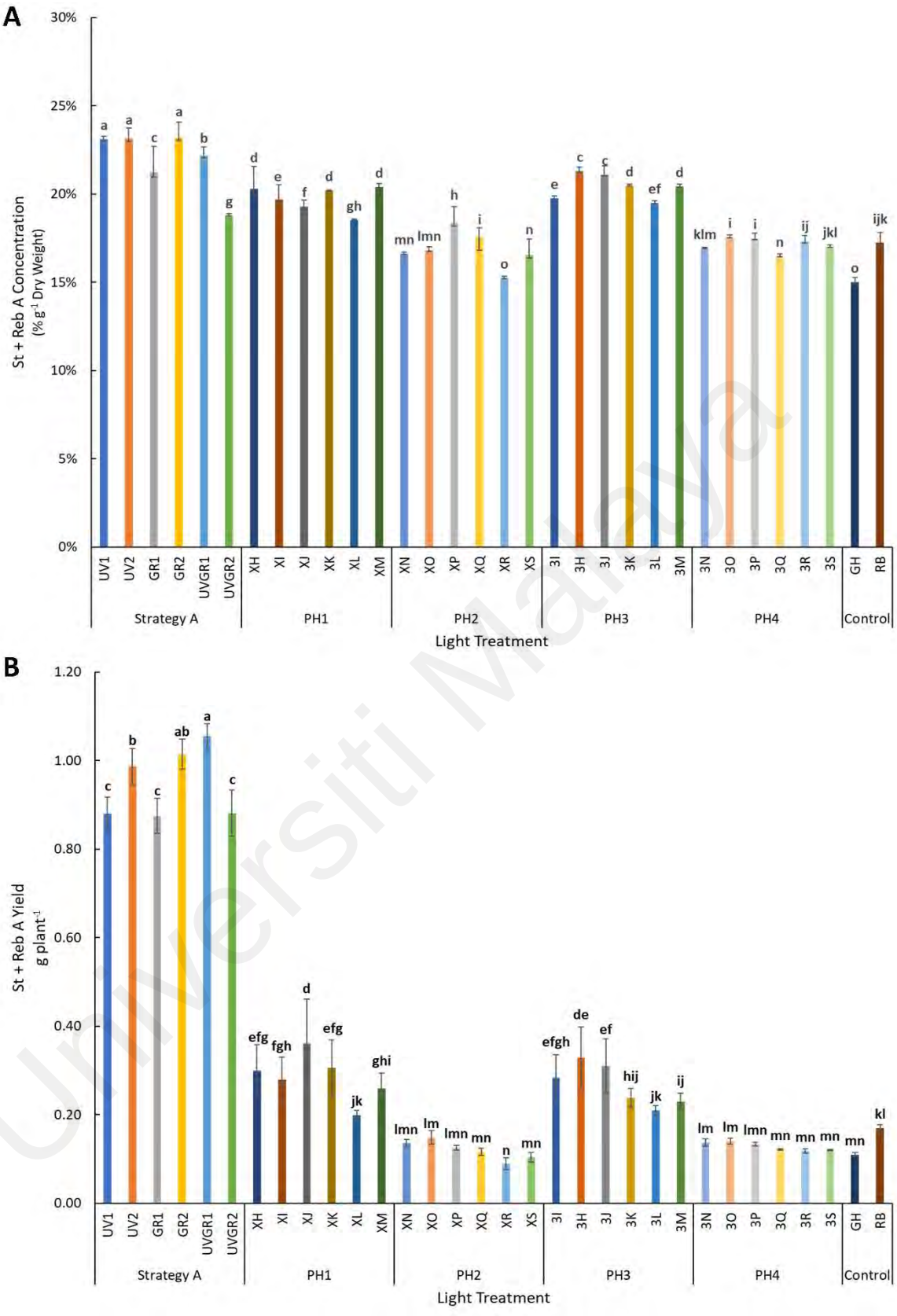

Figure 5.5 : Effect of Lighting Strategies on the Total ST + Reb A Concentration and Yields. (A) Mean Total ST + Reb A concentration per unit dry leaves (% g^{-1} dry weight). (B) Mean yield of ST + Reb A per plant (g $plant^{-1}$). Values represents Mean (n = 60) ± Standard Deviation. Different letters above the error bars indicate statistical significance determined by Tukey's HSD post hoc test at $p < 0.05$.

As ST and Reb A accounts for majority of the SGs within the leaves of Stevia plants (Raspe et al., 2022), for this study the combined concentrations and yields of ST + Reb A were used to exhibit the effect of the different light treatment and strategies on the secondary metabolite accumulation within *Stevia rebaudiana*. The GR2, UV2, and UV1 treatments under Strategy A had the highest overall metabolite concentrations of 23.10% each followed by UVGR1 (22.15%) (Figure 5.5A). Within the pre-harvest treatments, plants under PH1 and PH3, where RB was the primary light treatment, had higher secondary metabolite concentration that all pre-harvest strategies that had GH as its primary light source. The highest secondary metabolite concentration among the pre-harvest strategies were obtained in 3H and 3J with 21.10% each. Both treatments had RB as its primary treatment. It was observed that all pre-harvest strategies, regardless of if the primary source was from RB or GH, showed an improvement in secondary metabolite concentrations when compared to the controls. GH had the lowest combine concentration of 15.00% followed by XR (15.20%), and 3Q, XN, XS, and XO, all with GH as its primary irradiation, at 16.5% each (Figure 5.5A).

The overall yield of secondary metabolite that was obtained was influenced by both the dry biomass yield, and the overall concentrations of secondary metabolites (ST + Reb A). While the differences in secondary metabolite concentrations between the different light treatments varied between 15% to 23%, there was a significant difference at $P<.05$, observed in terms of the yields obtained as expressed in grams of ST and Reb A obtained per plant (Figure 5.5 B). Strategy A had significantly *($P<.05$)* higher yields among all strategies with UVGR1 yielding 1.05 g plant^{-1}, followed by GR2 and UV2 with 1.08 g plant^{-1} and 0.98 g plant^{-1} respectively. UV1, GR1 and UVGR1 had similar yields of 0.88 g plant^{-1} each. Between the four other groups of pre-harvest lighting strategies, PH1 and PH3 that had RB as the base radiation had a yield of between 0.17 g plant^{-1} (RB) and 0.36 g plant^{-1} (XJ). All treatments in these two strategies resulted in an improvement in terms

of yield compared to the RB control. Meanwhile, all treatments that had the GH base treatment (PH2 and PH4) resulted in lower yields compared to other treatments with yields of between 0.08 g plant^{-1} (XR) to 0.15 g plant^{-1} (XO) (Figure 5.5B). The GH control had a yield of 0.11 g plant^{-1} (Figure 5.5B). UVGR1 produced the highest yield and had 192% higher yield than the highest yields obtained from the pre-harvest strategy (XJ).

5.5 Discussion

The recent pandemic wreaked havoc on the global supply chain and stressed the healthcare systems across the globe, highlighting the importance of a stable and secure source of food, and the significance of maintaining a healthy lifestyle. The World Health Organisation (WHO) reported that more than 80% of the global population already use some form of herbal supplement and medication (Octavia et al., 2022). With the advent of the recent Covid-19 pandemic, there was a marked increase in the global consumption of herbal and dietary supplements as a precautionary measure (Radwan et al., 2022). Products derived from the leaves of the *Stevia rebaudiana* plants have been consumed historically around the world as a non-calorific sweetener. The acceptance and approval of Stevia extracts to be used as a food additive and sweetener by the USA Food and Drug Administration (FDA), Food Standards Australia New Zealand (FSANZ), and by the European Food Safety Authority (EFSA), has resulted in continuous increase in global demand for the product (Ilias et al., 2021; Rengasamy et al., 2022b; Wang et al., 2021). Besides being a natural non-calorific sweetener, Stevia also has a wide variety of therapeutic properties that can be beneficial to regulate diabetes, inflammation, hypertension, and obesity (Basharat et al., 2021; Ilias et al., 2021; Olas, 2022).

In Malaysia, products derived from Stevia are increasingly becoming popular as an herbal supplement (Saharudin et al., 2020a). At present, finished and semi-finished products derived from Stevia are imported from China, Japan, India, and South America, where

field cultivation take place during the warmer spring and summer seasons (Rai & Han, 2022). Stevia, being a day neutral plant, with a critical photoperiod of 12 to 13-hours, tends to flower as early as 7 weeks after germination. This has made commercial cultivation of Stevia plants under non-native environments such as Malaysia relatively challenging (Rengasamy et al., 2022b). Once transitioned to the flowering stage, Stevia plants stop further vegetative growth and the concentration of the ST and Reb A secondary metabolites in the leaves begins to reduce (de Andrade et al., 2021; Shulgina et al., 2021). This would result in an overall lower yield of ST and Reb A due to lower biomass and metabolite concentrations.

Controlled environment agriculture (CEA) systems that have duly regulated environmental conditions, often insulated from the external environment, are gaining traction as a popular choice for indoor agriculture (Shaari et al., 2021). A climate-controlled greenhouse, where except for the use of natural sunlight as its primary light, all other internal ambient conditions are fully controlled, is a common type of a CEA system (Graamans et al., 2020). These systems may use supplemental lighting to enhance the photoperiod or spectral components of the natural sunlight. The plant factory (PF) is another type of CEA system that is gaining popularity. Unlike the greenhouse, the PF is fully enclosed and fully uses artificial light. It can also be stacked into vertical farms where precision agricultural practices, that involve specific lighting, watering, cooling and nutrition schemes can be designed and delivered to specific plants across the various stages of it lifecycle (An et al., 2021; Olvera-Gonzalez et al., 2021b; Shaari et al., 2021). Lighting systems within a PF is the single biggest cost and energy consumer in the system. As the lighting energy alone can cost up to 70% of a CEA's operational expenditure, it is imperative that the most optimum lighting strategy is selected (Katzin et al., 2021; Kuijpers et al., 2021). Studies have shown that while a lighting product may be deemed to be extremely energy efficient, it may not be the most productive option to maximize

the yields and improve overall light use efficacies (Kuijpers et al., 2021; Kusuma et al., 2020; Pattison et al., 2018).

5.5.1 Effects of Varying Green and UVA Fractions on *Stevia rebaudiana*

Past studies have employed different lighting strategies to improve the biomass and metabolite yields of indoor and field grown Stevia plants. It has been reported that higher intensity (Hernández et al., 2022; Nakonechnaya et al., 2019; Rai & Han, 2022; Yoneda et al., 2017b) and a longer photoperiod (Ceunen & Geuns, 2013b; de Andrade et al., 2021; Yang et al., 2015; Yoneda et al., 2017b) result in an increase in biomass and secondary metabolite yields. However, these studies often did not take into account the daily light integral values of the different strategies, nor did it consider the spectral composition of the lighting systems. Ceunen et al. (2012a) and Rivera-Avilez et al. (2021) explored the use of night interruption to delay flowering, extending the vegetative stage of the plant and improving the overall secondary metabolite yields. The focus on these studies was to improve the quantitative aspect of Stevia cultivation by extending the vegetative stage and realized marginal improvement in metabolite concentrations. Meanwhile, past research on the effect of different spectral composition on Stevia mainly used red, blue, white, and far-red light (Melviana et al., 2021; Shulgina et al., 2021; Yoneda et al., 2017a). There have been limited studies on the effects of green and UVA light on the quality and quantity of Stevia plants. Chapter 4 reported the beneficial effects of supplemental green and UVA spectral components on the biomass and metabolite accumulation in Stevia plants. In the work reported in the current chapter, alternate fractions of green and UVA light were evaluated to identify the possibility of further enhancing the results reported in the preceding chapter and as reported in Rengasamy et al. (2022b).

In this study, no statistically significant difference in the rate of germination was observed among all light treatments of Strategy A. The rate of germination observed of between

50% to 61% was however within the range reported in previous studies (Aghighi Shahverdi et al., 2019; Kumar & Sharma, 2012; Macchia et al., 2007) but was lower than those reported by Abdullateef et al. (2015a) that obtained 67% germination under monochromatic red light, and Chapter 4 that reported a germination rate of 67% under UVA supplemented RB light. However, the study in the past chapter employed a lower fraction of UVA and radiation, at 6.5 μmol m^{-2} s^{-1}. The base RB light treatment that was identical between the present study and the study in the preceding chapter resulted in similar rate of germination of 62%.

Stevia being a day neutral plant is reported to transition early towards the flowering stage when cultivated under its critical photoperiod of 12 to 13 hours (de Andrade et al., 2021; Libik-Konieczny et al., 2021; Yoneda et al., 2017b). In Malaysia, where the annual daylight varies between 11.5 to 12.5 hours, Stevia plants have been reported to flower early, limiting its accumulation of biomass and reducing the overall metabolite yields (Abdulameer et al., 2018; Tan et al., 2008). In the current study, a distinct difference in the rate of flowering between plants grown under natural sunlight and photoperiod of GH, and those grown under a 16-hour photoperiod of artificial light was observed. GH, with higher DLI, intensity and a complete spectrum, resulted in a significantly higher rate of flowering compared to the rest, indicating a possible higher sensitivity of Stevia plants to photoperiods compared to intensity and DLI (de Andrade et al., 2021; Libik-Konieczny et al., 2021; Yoneda et al., 2017b). Far red and red light have been reported to influence flowering in certain plants including via the phytochrome photoreceptors, as does blue and UVA spectral composition via the cryptochrome photoreceptors (Ceunen & Geuns, 2013b; Santin et al., 2021). The effect of promoting or delaying flowering by these different spectral compositions are however species dependent (Paradiso & Proietti, 2022).

In this study the lowest rate of flowering was observed in UV1 and RB. Both treatments had similar red and blue fractions, while UV1 had a supplemental UVA irradiation. The finding of this study was similar to those of past studies that reported UVA supplemented light and RB base treatment to have lower rate of flowering (Rengasamy et al., 2022b). These two treatments had higher red spectral content, which has been reported to trigger a flowering inhibition reaction in the phytochromes, compared to others (Kusuma et al., 2020). However, the UV2 treatment, that had similar red content, resulted in the highest rate of flowering among the artificially lighted plants. While the UV2 treatment had similar red spectral content with UV1 and RB treatments, it had a higher UVA fraction. This increase in flowering may indicate a cryptochrome mediated response, as both UVA and blue light spectra are perceived by this photoreceptor. The UV2 treatment had the highest fraction of short (UVA and blue) wavelength spectral content among all treatments. Green light is perceived by both phytochromes and cryptochromes, hence it can inhibit or promote flowering, subject to the plant type and green light intensity (Meng & Runkle, 2019; Zheng et al., 2019b). Meng and Runkle (2019) reported that increasing intensities of green light from 0 to 25 μmol m^{-2} s^{-1} delayed flowering in short day plants. In this study, all treatments with supplemental green light had a similar rate of flowering, possibly indicating a stronger influence of the phytochrome mediated delay response.

The advantages and the positive effects of red and blue artificial light on the accumulation of biomass for indoor grown plants has been extensively researched. The red and blue light regions of the spectra are reported to be most efficient for photosynthetic activities given its proximity to the peak sensitivity of chlorophylls a and b (Paradiso & Proietti, 2022; Paucek et al., 2020a; Zheng et al., 2019b). Past studies on the effect of red and blue spectra on the rate of photosynthesis is often based on the McCree's action spectra, that is till this day used as an industry reference (Liu & Van Iersel, 2021; McCree, 1971; Zhen et al., 2021). However, there are known limitations to the action spectra put forward by

McCree (1971). The quantum yields were measured at very low PPFD values using narrow spectral wavebands (McCree, 1971; Zhen et al., 2021). Recent studies have highlighted an importance and influence of green spectral content on photosynthetic activity and biomass yields (Claypool & Lieth, 2020; Liu & Van Iersel, 2021; Meng & Runkle, 2019). As red and blue spectral components have higher absorption by the photosynthetic pigments, the light absorption of these wavelengths happens closer to the upper leaf surface, reducing the quantum yield of CO_2 assimilation in cells within the upper regions of the leaf and limiting light availability to the bottom part of a leaf (Liu & Van Iersel, 2021; Paradiso & Proietti, 2022). Meanwhile green light can penetrate deeper into the plant canopy, reaching lower levels and inducing a more balanced whole plant photosynthesis, encouraging biomass accumulation. The positive effects of green light on photosynthetic activity are however also dependent on the overall light intensity. Green light is more effective under higher overall light intensity (PPFD) as compared to situations with low light levels (Bian et al., 2018; Smith et al., 2017; Terashima et al., 2009). The effects of green supplemental light on the biomass accumulation of Stevia were visible in this study where all treatments with supplemental green spectra had higher fresh biomass compared to those with just UV or the base RB spectral composition. In this study, GR1 and GR2 that had green light fraction, had higher dry biomass compared to RB even though all treatments had identical PPFD. All three treatments had the same intensity and red to blue ratios but had very different productivity levels. The green light fraction that replaced part of the red and blue light in GR1 and GR2 enhanced the overall plant productivity.

Blue, green and UVA, perceived by the cryptochrome photoreceptor, have a common signaling pathway, while the Zeitlupe family of receptors perceive blue light (Samuoliene et al., 2020a). Past studies on the effects of UVA, either as a sole source or as supplemental light, yielded mixed results, exhibiting inhibitory traits in soybean, peppers,

and certain types of vegetables, while having positive effects on radish, lettuces, and other indoor grown plants (He et al., 2020b, 2021; Qian et al., 2020). The effects of UVA on biomass productions, photosynthetic activity, and metabolite accumulation are reported to be affected by the light intensity, photoperiod, spectral content of other light present and on the plant species (Brazaitytė et al., 2019; Samuoliene et al., 2020a; Samuoliene et al., 2020b). The production of plant biomass is, to a large extent, influenced by the DLI and light interception by the plant leaves (Chen et al., 2019). The higher DLI is associated with higher light intensity that falls onto the surface of the leaves of the plant and is assumed to increase photosynthetic activity and subsequently biomass accumulation. However, in this study, the PBAR intensities and DLI values for 280nm to 800nm were higher under the UV1 and UV2 light treatments that had a lower dry biomass yield compared to UVGR1 and UVGR2. This would suggest that the supplemental UVA spectra did not directly participate in the photosynthetic activity of Stevia plants. Most studies reported an increase in leaf area associated with exposure to UVA enriched light. A larger surface area would allow for more light to be intercepted, driving higher rates of photosynthesis (Chen et al., 2019; He et al., 2021). While the leaf area measurements were not done for this study, this would provide a possible explanation for the finding that all treatments with UVA supplementary light generated higher biomass compared to the base RB treatment. The addition of green light to UVA in UV1 and UV2 treatments appeared to provide a synergistic effect, increasing the dry biomass yield compared to all other treatments that only had either UVA or green, combining the positive effects of green and UVA.

In this study, across all strategies, the metabolite concentrations obtained were between 10.40% to 18.05% for ST, and between 3.40% to 6.10% for Reb A. These values reflect an improvement in the secondary metabolite concentrations compared to previous reported values of between 5 to 10% for ST and 2 to 4% for Reb A (Rai & Han, 2022).

The highest amount of ST and Reb A concentrations were obtained in plants under Strategy A, specifically those under UV1, UV2 and GR2 treatments. It was observed that plants grown under a higher UVA fraction in UV2 had higher ST concentrations compared to plants under UV1. UV2 however, had lower Reb A concentrations compared to UV1. The lower Reb A concentrations were in line with past studies that reported the shorter wavelengths of UVA and blue light leading to higher concentrations of ST in indoor grown Stevia plants (Yoneda et al., 2017a). Within the different green light fractions, it was observed that plants grown under the higher green fraction of GR2 had higher ST and Reb A concentrations compared to GR1. The higher ST and Reb A under higher green fraction was however contradictory to the findings reported in Chapter 4 that observed a decrease in ST concentrations while having a higher Reb A concentration in plants grown under the GR light treatment that had a green light fraction. The higher green light intensities in this study compared to the Chapter 4 (GR) may have been a factor in this variation. Higher photosynthetic activity has been described to be a factor affecting secondary metabolite accumulation in Stevia plants and, green light fraction is known to increase the rate of photosynthetic activity in multiple plant species (Hernández et al., 2022; Liu & Van Iersel, 2021; Paradiso & Proietti, 2022). While there are no green light specific photoreceptors, plants are believed to perceive green light via either the phytochromes or the cryptochromes. Previous studies have reported higher accumulation of SG under red light (Melviana et al., 2021; Yoneda et al., 2017a), or in the absence of blue light (Shulgina et al., 2021), highlighting a possible supporting role of phytochrome in accumulation of ST and Reb A in Stevia plants (Ceunen et al., 2012a; Hernández et al., 2022). While UV2 and GR2 both had high ST and Reb A concentrations, the UVGR2 treatment that had the UVA fraction combined with GR2, resulted in the lowest metabolite concentration among treatments in Strategy A. While UVGR2 treatment had higher Reb A concentrations compared to UVGR1, it was not sufficient to compensate

for the significantly lower ST concentrations obtained. The difference in the concentration of ST and Reb A observed in this study under the different light fractions indicate a possible effect of these different spectral compositions and the different fraction intensities on the expression levels of the various genes associated with the SG biosynthesis pathway.

In the SG biosynthesis pathway, the UDP-glycosyltransferase 85C2 (encoded by the gene UGT85C2) synthesizes steviolmonoside from steviol while UDP-glycosyltransferase 74G1 (encoded by the gene UGT74G1) converts steviolbioside to ST (Basharat et al., 2021). The UDP-glycosyltransferase 76G1 (encoded by the gene UGT76G1) is known to convert ST to Reb A (Basharat et al., 2021; Yoneda et al., 2017a). Past studies have shown that the levels of expression of the different genes within the pathway are influenced by light intensities, with higher light intensities resulting in higher levels of expression and subsequently higher metabolite accumulation (Hernández et al., 2022; Yoneda et al., 2017b). However, as the biosynthesis pathway is in a sequential form, the concentration of ST and Reb A produced at different light intensities does not depends exclusively on the greater expression of one gene with respect to the other (Hernández et al., 2022). A recent study by Hernández et al. (2022) reported a higher concentration of Reb A in plants irradiated with medium and high intensity light. While a higher level of expression was observed in the UGT74G1 gene, no changes were observed in UGT76G1. This finding outlined the effect of the preceding processes and its associated genes on the final accumulation of ST and Reb A. Without sufficient ST concentrations, it would not be possible to realize higher Reb A concentrations, even with a higher level of transcription in the UGT76G1 gene (Hernández et al., 2022). Meanwhile, Yoneda et al. (2017a) reported lighting treatments with red, blue and far-red spectral content increases the transcription levels of the UGT85C2 gene early on in the pathway, subsequently resulting in higher overall SG yields, while Melviana et al. (2021) reported an increase in

ST and Reb A concentrations, and the expression levels of UGT85C2, UGT74G1, and UGT76G1 genes in plants irradiated with far-red light. These past studies, similar to an early work by Ceunen et al. (2012a) observed an overall increase in SG contents but did not report any difference observed in the concentrations ratios of ST to Reb A (Libik-Konieczny et al., 2021; Rai & Han, 2022).

5.5.2 Effects of Pre-Harvest Lighting Strategies on *Stevia rebaudiana*

Pre-harvest lighting treatments, where plants are irradiated with a different spectrum from which they were predominantly cultivated for a short period prior to harvest, have been reported to be beneficial in improving the biomass and secondary metabolites in lettuce (Hooks et al., 2021; Hooks et al., 2022; Zhang et al., 2021), broccoli (Langston et al., 2022), kale (Deng et al., 2017; Jiang et al., 2021), basil (dos S. Nascimento et al., 2020; Dou et al., 2019), and in other herbs and leafy greens (Samuolienė et al., 2010). These studies used supplemental red, green, blue, far red and UVA light at varying intensities and photoperiods. Besides having a positive effect on the growth and metabolite accumulation in some plants, the use of pre-harvest lighting treatments is a potential cost-effective lighting strategy for indoor cultivation, as the use of higher powered lighting systems is limited to the short period prior to harvest (Hooks et al., 2021). At present there is no published literature exploring the use of pre-harvest lighting strategies to improve the quality and quantity of indoor grown Stevia. In this study, plants grown under RB and natural sunlight in the GH were subjected to 3 different pre-harvest light treatments that had the base RB (photosynthetic spectra) supplemented with green, UVA, and blue light spectra, and an additional 3 monochromatic light sources at low intensity to mimic a situation without photosynthetic activity (Figure 5.1).

The pre-harvest strategies, however, did not result in significant improvements of fresh and dry biomass over those obtained with Strategy A. A recent study by Hooks et al. (2021) reported that pre-harvest lighting treatment with blue or UVA irradiation resulted

in an increased fresh and dry biomass yield in lettuce, while a pre-harvest treatment with a combination of red and blue light did not. In this study, PH1 and PH3 had very similar dry biomass yields. No further significant gains in terms of dry biomass were obtained in the 10-day pre-harvest treatments XH, XH and XJ, compared to the 3-day treatment of 3H, 3I, and 3J under the same pre-harvest treatments. However, under monochromatic pre-harvest treatments, there was a significant increment observed in the 10-day UVA irradiated XK treatment that increased significantly in dry biomass compared to the 3-day 3K treatment. This difference observed between the different light treatments was in line with past literature that put forward the idea that the effectiveness of pre-harvest treatments not only depended on the light quality but also the period of exposure, as different spectral compositions would need different periods of exposure to be effective (Hooks et al., 2022). The monochromatic pre-harvest treatments resulted in lower dry biomass yield improvements compared to the multispectral pre-harvest treatments. This was not unexpected as previous studies have reported higher outputs under dichromatic red and blue light, or trichromatic red-blue-green pre-harvest lighting treatments, compared to monochromatic lights due to its ability to induce higher rate of photosynthetic activity (Izzo et al., 2020; Pattison et al., 2018).

Overall, the improvement in dry biomass yields among the pre-harvest strategies was only observed in plants that originated from the RB base spectrum and not from those under natural sunlight in the GH even though the GH had 100% higher DLI. Previous studies on lettuce reported that the effectiveness of supplemental pre-harvest treatment was affected by the DLI of the primary light source with plants under lower DLI responding more effectively, having higher biomass gains compared to those cultivated under higher DLIs (Hooks et al., 2021). Photoinhibition due to the high light intensity caused by supplementing the pre-harvest irradiation with natural sunlight was cited as a reason for this difference (Hooks et al., 2021; Hooks et al., 2022). However, in the present study,

the pre-harvest treatment was done in the dark room (PF) without any daylight, as such there is limited possibility a photoinhibition effect. It was more likely that plants cultivated under RB had more total leaf surface area (number of leaves and bigger surface areas) developed prior to the pre-harvest treatments, and subsequently was able to achieve a higher rate of photosynthesis than plants grown in the GH, as observed by the significantly higher fresh leaf biomass obtained under RB compared to GH. Although a significant increase in dry biomass was observed under PH1 and PH3, there was no improvement in fresh leaf biomass observed under PH2 and PH4. Cernusak (2020) suggested the observed improvement only in dry biomass yield as opposed to both dry and fresh biomass to be an effect off the compromise between the assimilate accumulation from photosynthesis and the water loss due to transpiration, as the stomata is a common gate for these two processes. Meanwhile, Lamalakshmi Devi et al. (2017) suggested that the observation of improvement only in dry biomass and not fresh biomass yield could instead be an effect of a faster rate of adaption of plant photosynthesis to the pre-harvest light treatments by the plant as compared to its leaf expansion growth and water balance.

Pre-harvest red light irradiation has been reported to increase lutein, beta-carotene and total phenol contents in basil, red pak choi and tatsoi, but not in beet or mustard (Langston et al., 2022). Short term pre-harvest blue light irradiation has been reported to have increase biomass accumulation and concentration of carotenoid and total phenolics in several plants (Azad et al., 2020; Loi et al., 2020; Zheng et al., 2019a). Although similar responses have been reported across multiple plants, an increasing number of studies have reported that the response of plants to light quality is also depended on plant species and cultivar (Alrifai et al., 2021).

Unlike the muted effects the various pre-harvest treatments had in terms of dry biomass accumulation, it was observed that the overall secondary metabolite accumulation improved, except for XR, significantly under all other treatments, regardless of its

originating light treatment, pre-harvest lighting treatment spectral composition, or the duration of treatment. The highest concentration of ST was obtained under 3K (17%), followed by 3H (16.5%). Both treatments had UVA spectrum delivered as a monochromatic source (3K) or combined with red and blue base lighting (3H). The lowest ST concentrations were observed in plants from GH that had monochromatic green light pre-harvest treatments of 3-days (3R) with 10.39%, and 10 days (XR) with 11.15%. Meanwhile, the highest Reb A accumulation was observed under the 3-day green monochrome light treatment of 3R (6.16%) while the lowest concentrations were obtained in 3K (3.43%) that 3-days of had monochromatic UVA pre-harvest irradiation. These findings were complementary to the findings from Strategy A and in line with past studies that reported the stimulatory effect of shorter blue and UV-A wavelengths on the accumulation of ST (Yoneda et al., 2017a), and the positive effect of green light on Reb A concentrations that was observed in Chapter 4. Overall, it was noticed that the 3-day pre-harvest treatments that contained blue and UVA spectral content, either as supplementary to RB (3H, 3K, XH) or as a monochromatic light source (XK, XM, 3L, 3M), had higher total ST and Reb A concentrations among all pre-harvest treatments. This was however only observed in plants that originated from RB and not from GH.

It was observed that there was a higher improvement in metabolite concentrations in the 3-day treatment of plants that were cultivated under either GH or RB, compared to the longer 10-day treatment. This was unlike the findings of biomass accumulation that saw the longer 10-day pre-harvest treatments having higher yields compared to the 3-day period. These results suggest that crop quality parameters are more sensitive than plant growth in response to short-term pre-harvest lighting, as plants typically adapt more quickly to changes in environmental factors including light amount, at the biochemical and cellular level than at the whole plant level (Lamalakshmi Devi et al., 2017). Under the pre-harvest treatments, the development of photosynthetic tissues and apparatus that

includes the mesophyl and chloroplasts may have been encouraged in plant leaves to adapt and capture more light (Kong et al., 2015), providing a foundation for improved biosynthesis of the secondary metabolites (Hooks et al., 2022). Overall, the metabolite yields (g plant^{-1}) obtained were significantly higher under Strategy A where no preharvest treatments were included. The higher biomass accumulation and metabolite concentration was a principal driver of this higher metabolite yields. The pre-harvest treatments for plants that originated from GH did not result in any significant improvement in metabolite yields while all pre-harvest treatments that originated from RB saw an improvement in yields compared to the RB control. However, unlike Strategy A, the improvements obtained in these pre-harvest strategies were mainly driven by an improvement in plant quality with little improvement in quantity.

5.6 Conclusion

In commercial application of CEAs, energy costs, especially artificial lighting energy costs are of a major concern. The selection of an appropriate lighting strategy would be able to influence the productivity and improve the energy use in these systems. In this study, it was observed that a full cycle treatment of UVGR1 and GR1 was the most productive in improving both the quality and quantity of indoor grown *Stevia rebaudiana* plants. It was also demonstrated that the use of pre-harvest lighting treatments, specifically for plants cultivated under a base red and blue photosynthetic spectrum, was a viable option to improve the overall quality and yields of Stevia.

In the next chapter, the environmental conditions and the overall electrical energy consumption profile of the greenhouse and growth room was evaluated.

CHAPTER 6 : ENERGY AND PHOTON CONVERSION EFFICACY ANALYSIS

In this chapter, the energy profile as well as the environmental condition of the greenhouse and growth room were analysed. The overall productivity, PCE and EUE of all strategies and treatments from Chapters 3 through 5 were compared and analysed to identify its overall effect in terms of improving plant quality and quantity, and to identify the most productive light treatment in terms of photon conversion and energy use.

6.1 Introduction

The increasing demand for food production driven by the rapid growth of the global population has accelerated the need for domestic cultivation of food crops across the globe. This has led to exploration and exploitation of large swatches of land, often with negative impact on the local biodiversity (Li et al., 2020b). Besides the impact on the environment, traditional open field farming is also susceptible to seasonal and weather changes, limiting the crop types and planting cycles, and needs extensive use of fertiliser and pesticides (Xu, 2019). These types of farms are often located far away from the general population, requiring additional transportation that can be across thousands of kilometres before reaching the consumer. Hence, it is not surprising that global food production is responsible for one third of the global greenhouse gas (GHG) emissions (Panchasara et al., 2021). Besides contributing to the emissions of GHG, the transportation also leads to wastage as up to 30% of the produce perishes during transit (Iddio et al., 2020).

6.2 Literature Review

Controlled environment agriculture (CEA) systems have been promoted as a possible solution to address some of the shortcomings of traditional agriculture (Van Gerrewey et al., 2022). Greenhouses, the most common type of CEA have been widely used in Europe

and in other parts of the world where seasonal variance in weather does not allow for year-round cultivation, and to cultivate non-native plants (Kuijpers et al., 2021; Nemali, 2022). Traditional greenhouses typically consist of a transparent or translucent structure that incorporates heating, irrigation, and ventilation. More modern structures include supplemental artificial lighting systems to extend the photoperiods during short days and may include carbon dioxide (CO_2) injectors to increase the CO_2 content within the facility (Katzin et al., 2021; Kozai et al., 2020). While these facilities enable year-round cultivation and improve resource use over traditional open field cultivation (Graamans et al., 2018), they normally require a similar footprint, and are mostly situated outside of the city limits. Plant factories or vertical farms are a more recent type of CEA that are made up of vertically stacked growing areas, equipped with heating, cooling, ventilation, irrigation and are fully artificially lighted (Vatistas et al., 2022; Xu, 2019). These structures are fully enclosed, often insulated against environmental factors such as temperature, humidity, and seasonal photoperiods. As plants cultivated in CEAs do not rely on external environmental factors, they can not only be stacked vertically, but the CEA facilities that house these plants can also be placed closer to the point of consumption, cutting down on transportation requirements and creating hyperlocal food production (Kozai et al., 2020). The use of plant factories for growing food crops has increased over the years, with multiple large scale start-ups (Plenty, Aerofarms, Infarm) coming on board and attracting major investments which have grown from USD 38.1m in 2016 to USD 406.54m in 2020 (i3, 2021b). Over a similar period, the commercialisation of highly energy efficient and cost-effective Light Emitting Diodes (LEDs) has led to the development and proliferation of precision horticulture lighting products that are more energy efficient than traditional technologies used in the older greenhouses (Katzin et al., 2021). The narrow band wavelengths of the LEDs provide an additional flexibility for customised solutions to maximise the productivity of the plants

under cultivation (Avgoustaki & Xydis, 2021; Kusuma et al., 2020; Paucek et al., 2020b). The legalisation of cannabis and hemp in North America, Europe and parts of Asia is also a major factor behind the increase in plant factory facilities in that region, as open field cultivation of these high valued crops is not permitted or is subjected to strict regulation (Hammond et al., 2020). Comparable to cultivation of food crops, cultivation of cannabis within a plant factory has been reported to result in significantly higher yields and improvement in product quality, often better than field grown or naturally lighted greenhouse grown materials (Magagnini et al., 2018; Namdar et al., 2019).

However, as a typical plant factory relies on supplied energy for lighting, heating, cooling, and other needs, this is a resource hungry solution. In unlighted greenhouses, the majority of electrical energy consumed is for heating and cooling, while in setups with supplemental artificial lighting, the lighting energy accounts for approximately 23% of total electrical energy demand (Katzin et al., 2021). It has been reported that artificially lighted greenhouses typically consume up to 100% more electrical energy than naturally lighted greenhouses but only result in an increase in yields of approximately 27% (Katzin et al., 2021). In plant factories, the biggest electrical energy consumer has been reported to be the artificial lighting system, accounting for 57% of the total electrical energy costs, followed by the heating and cooling costs at 37% (Avgoustaki & Xydis, 2021; Graamans et al., 2020). The high electrical energy requirements for CEAs, especially plant factories present a new challenge, where while addressing the long transit and associated sustainability issues, the increased energy need may negate the benefits (Weidner et al., 2021). Multiple studies have considered and modelled the electrical energy requirements of greenhouses and plant factories. As examples, Graamans et al. (2018); Harbick and Albright (2016) and Eaves and Eaves (2018) reported that plant factories have a higher energy use per unit growth area, but had an overall higher energy use efficiency, resulting in higher yields per unit energy consumed. Zhang and Kacira (2020) however reported a

higher energy use efficiency in greenhouses under warmer climatic conditions with plant factories being more efficient in cooler climates.

Most of these studies were based on environmental conditions in higher latitudes where seasonal weather variations are present. Limited studies have been done in tropical conditions such as Malaysia, where the weather remains relatively constant throughout the year with an average daily temperature of 27°C, a day and night temperature difference of between 7.6°C to 9°C (Malaysian Meteorological Department, 2019; National Renewable Energy Laboratory, 2021) and a natural day neutral photoperiod in the region of 12-hours (Abdulameer et al., 2018). Unlike greenhouses in higher latitudes where heating is required, in tropical environments, cooling load becomes the major energy requirement. Past studies on the energy consumption of CEAs mostly used lettuce as the candidate crop to model and evaluate resource use (Graamans et al., 2018; Graamans et al., 2020; Weidner et al., 2021; Zhang & Kacira, 2020) while high valued herbs and non-food crops such as *Stevia rebaudiana*, that can potentially improve the economics of CEAs were not considered. Previous studies on approaches to improve the efficiency of the plant factory and greenhouse CEAs have typically focused on the conversion to more efficient lighting technologies (Katzin et al., 2021; Kuijpers et al., 2021; Olvera-Gonzalez et al., 2021a), façade and overall design (Choab et al., 2020; Graamans et al., 2020; Yalçın & Ertürk, 2020), indoor climate control systems (Weidner et al., 2021; Yan et al., 2021), photoperiod and intensity control (Avgoustaki & Xydis, 2021; Nair et al., 2021), and on the use of photovoltaics (Bambara & Athienitis, 2019; La Notte et al., 2020). These studies focused on reducing the input energy as a means to improve the energy use efficiency. Li et al. (2020c), Chen et al. (2021), and Kong et al. (2019a) explored the use of red-blue ratios and the use of supplemental spectrum to improve the productivity and energy use efficiencies of lettuce. However, in these studies, the energy use efficacies were calculated purely for the electrical loads of the lighting

systems, unlike the current study that considers the total electrical energy load for the greenhouse and plant factories. This will provide a holistic view on the effects of spectrum optimisation on the energy use efficacies of the facility, including cooling loads. Studies have reported significantly higher efficacies when considering only the lighting systems and this value often reduces when the need for additional cooling or heating is taken into account (Katzin et al., 2021; Ouzounis et al., 2018).

In the current study, the total electrical energy demand for a climate-controlled greenhouse and growth room in a tropical environment was evaluated. The greenhouse and growth room were provided with identical environmental conditions with the light source being the only variable. The greenhouse was provided with only natural sunlight while the growth room was fully artificially lighted. *Stevia rebaudiana*, an herb popular in Malaysia as a naturally derived sugar substitute and as an herbal health supplement was used as the primary plant cultivated in both CEAs (Saharudin et al., 2020a). The Stevioside (ST) and Rebaudioside A (Reb A) compounds extracted from the leaves of Stevia plants are highly prized and have a market value of approximately USD70,000 per tonne (Ciriminna et al., 2019). The use of LEDs as the source of artificial lighting provided the opportunity for optimising the light spectrum used for the growth room. Apart from full spectrum, different light treatments, and their impact on the energy performance of the growth room were considered and compared with those of the climate-controlled greenhouse in this study. The optimisation of energy use via the application of the different lighting strategies and treatments was also evaluated from the perspective of the overall plant productivity, the lighting system energy efficacy, and the light use efficacies. The primary objectives of this study were to analyse the effects of tropical weather on the power consumption in the growth room and greenhouse, to appraise the artificial lighting system's effect on plant productivity and efficacies, to identify the most productive lighting strategy and treatment in terms of improving plant productivity and

quality, and to identify further opportunities for energy use optimisation. This was the first study of its kind to evaluate the total energy demand and energy use efficiency of indoor cultivation of Stevia in a tropical environment. All power, energy, yield, and efficacy values in this study were normalised to per unit growth area of $1m^2$.

6.3 Material and Methods

6.3.1 Layout and Location

The greenhouse (GH) and growth room (PF) were housed within the Plant Biotech Facility (PBF) of University of Malaya, Kuala Lumpur, Malaysia (3.1209° N, 101.6538° E). The GH and PF are separated by a preparation room (Figure 6.1). The growth room employed a stacked growing area to mimic a plant factory setup.

6.3.1.1 Greenhouse (GH)

The climate-controlled area of the GH had a footprint of $69.81m^2$ and a height of 2.90m, with 4 growing areas, each covering an area of $7.8m^2$ for a total of $31.2m^2$ of growing space (Figure 6.1 A). The growing areas are located 1m above the ground. The walls and roof of the structure are made of transparent polycarbonate material while the flooring is standard concrete. The GH does not have a supplemental lighting system. The GH had 2 air conditioning units that operate alternately on 4-hour cycles. The temperature within the GH was set to be maintained between 23°C and 25°C. The entrance to the GH is serviced by an enclosed walkway that is constructed of the same materials. An environment monitoring data logger (CM-0039, CO2meter.com, USA) was installed on the outer side of the wall of the enclosed walkway that is used to access the GH used as a reference point for the ambient temperature of the area surrounding the facility.

6.3.1.2 Growth Room (PF)

The PF is housed in a room within the PBF. The room was completely enclosed with no windows and with floors, walls and ceilings made of concrete. The internal wall of the

PF was further insulated with 75mm polyurethane panels. The PF had a footprint of 12.02m^2 with a height of 2.50m and is equipped with 4 racks. Each rack has 3 levels of growing space, each measuring 1.08m^2. Of the 12.96m^2 of available growing space, 10.8m^2 was used for this study. The PF climate-control system was serviced by 2 air conditioning units operating alternately at 4-hour intervals. The PF temperature was set to be within a range of 23°C to 25°C. The entrance to the PF is via the preparation room that had a similar construction to the PF, with concrete walls, floor, and ceiling.

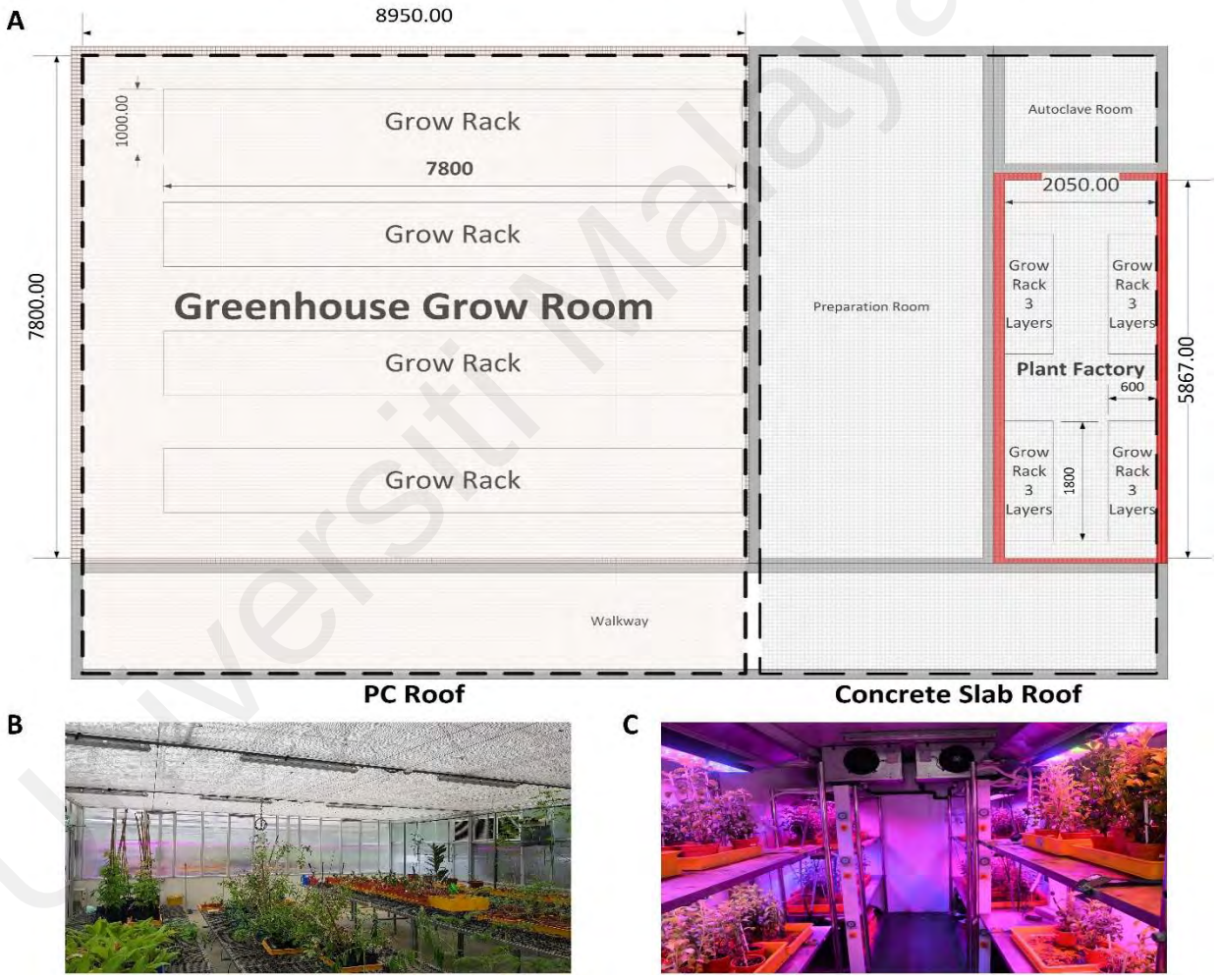

Figure 6.1 : Plant Biotech Facility with Greenhouse and Growth Room. (A) Overall layout of the Greenhouse growth room (GH) and Growth Room (PF). (B) Inside view of GH. (C) Inside view of PF.

6.3.2 Plant material

The plant materials were prepared as outlined in Chapters 3, 4 and 5 (Rengasamy et al., 2022a; Rengasamy et al., 2022b). *Stevia rebaudiana* seeds procured from Bakers Creek Heirloom Seeds, USA (www.rareseeds.com), were washed under running water and dried before being sowed in a 50 cell plug tray filled with autoclaved potting soil (www.serbajadi.com.my) at a rate of 1 seed per cell. The trays were then sprayed with water and placed under the different light treatments. 35 days after germination, the seedlings were transplanted into individual pots (12cmx12cmx10cm) filled with autoclaved potting soil (www.serbajadi.com.my). The seedlings were placed back under the respective light treatments and GH for another 140 days at a density of 24 plants m^{-2}. The total time from seed germination to first harvest was 175 days. At 175 days after planting (DAP) of the seeds, the plants were harvested by cutting of the stems 5cm above the surface of the soil. The experimental cycle was repeated 3 times for all artificial light treatments in the PF and under natural light in the GH.

6.3.3 Lighting Strategies and Treatments

The GH was not supplemented with artificial lighting. All lighting needs for plant photosynthetic activity were from natural sunlight with a photoperiod of 12-hours. The sunshade within the greenhouse was kept open to allow maximum light into the facility.

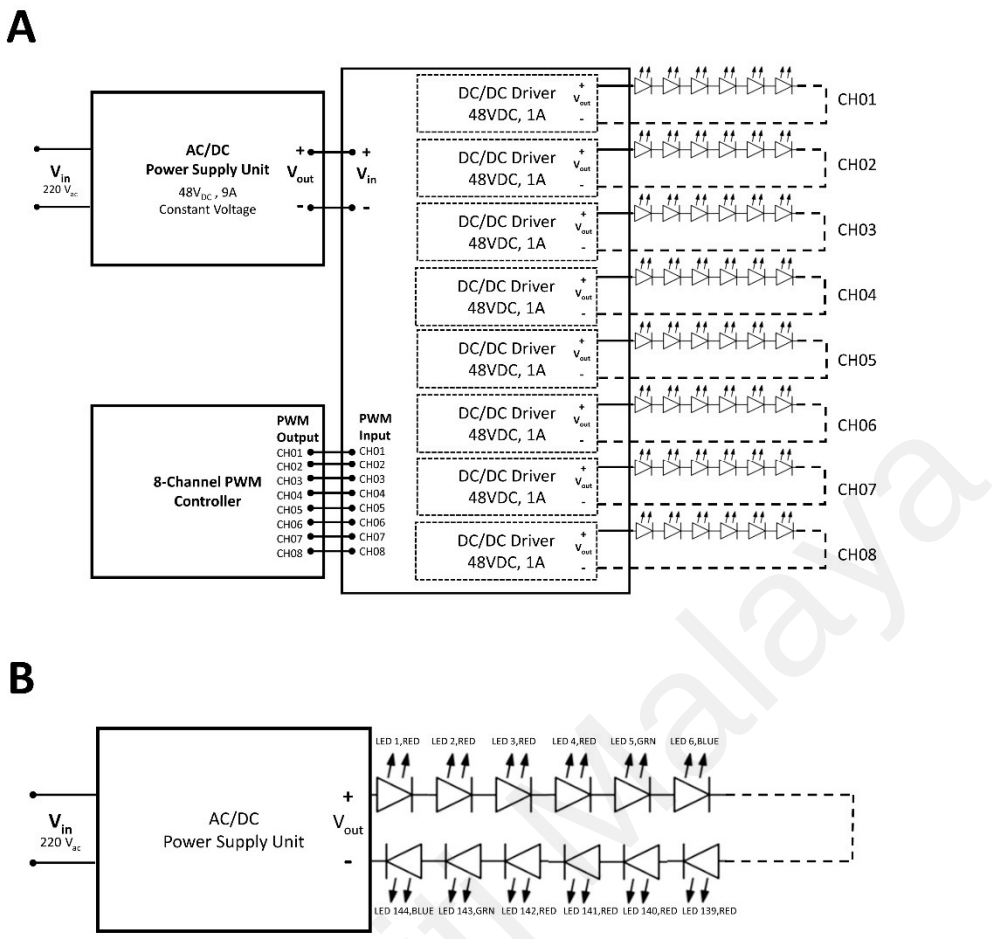

Figure 6.2 : Artificial Lighting System. (A) Lighting system circuit block diagram for Spectum and Pre-Harvest Strategies. (B) Lighting system circuit block diagram for Photoperiod Strategy.

The artificial lighting systems and strategies used were as described in Chapters 3, 4 and 5. The PF was installed with 10 custom built artificial lighting systems. Six systems consisted of 8 channels of high-powered LEDs (Osram Opto, Germany, Cree Inc, USA, and Edison Opto, Chinese Taipei) that were individually controlled via a pulse width modulator (PWM), capable of regulation from 0-100% output (Figure 6.2A). Another four systems used for the study on photoperiod had 144 high powered connected in series consisting of 96 Red LEDs (Osram Opto, Germany, Cree Inc, USA) with a peak of 630 nm, 24 Green LEDs with a peak of 550 nm and 24 Blue LEDs with a peak of 450 nm. The intensity of each unit was controlled by varying the supply current for each system, using the built-in potentiometer of the power supply units (Meanwell, Taiwan).

A total of 3 lighting strategies employing 39 different treatments were evaluated. The GH and a base red+blue (RB) artificial light treatment in the PF were used as controls. In the first strategy focused on the use of photoperiod manipulation (as in Chapter 3), while in in the second strategy, the primary focus was on the use of different spectral compositions (SS1) (as in Chapter 4) and different fractions of green and UVA (SS2) (as in Chapter 5). The third strategy employed the use of pre-harvest treatments of plants grown either in GH (PH2, PH4) or under RB (PH1, PH3) (as in Chapter 5).

Table 6.1 : Photoperiod Strategy (Chapter 3)

Parameters	Unit	8H	12H	16H	16HI
PPFD [a] 400-700 nm	µmol $m^{-2}s^{-1}$	249 ± 5.7	165 ± 3.3	125 ± 2.5	125 ± 2.5
PBAR [a] 280-800 nm	µmol $m^{-2}s^{-1}$	249 ± 5.7	165 ± 3.3	125 ± 2.5	125 ± 2.5
Photoperiod in 24 Hours	Hours (h)	8	12	16	5.3H × 3
Lighting Power	W	316	175	129	129

The plants exposed to the photoperiod named 16HI (where "I" indicates intermittent) were exposed to 5.3H Light/2.7H Dark on a continuous loop such that they received a total of 16 h of light intermittently over a period of 24 h.

Table 6.2 : SS1 (Chapter 4)

Parameters	Unit	FR	UV	BR	GR	FS
Total PPFD[a] (PAR)	µmol $m^{-2}s^{-1}$	130.0 ±2	130.0 ±2	130.0 ±2	130.0 ±2	130.0 ±2
Total PFD[b] (PBAR)	µmol $m^{-2}s^{-1}$	152.1±2	137.8±2	131.3±2	131.3±2	139.1±2
Photoperiod in 24 Hours	Hours (h)	16	16	16	16	16
Lighting Power	W	153.50	151.95	129.00	152.86	263.32

Table 6.3 : SS2 (Strategy A, Chapter 5)

	Unit	UV1	UV2	GR1	GR2	UVGR 1	UVGR 2
Total PPFD[a] (PAR)	µmol $m^{-2}s^{-1}$	130.99± 2	132.25± 2	130.78± 2	130±2	131.01± 2	130.16± 2
Total PFD[b] (PBAR)	µmol $m^{-2}s^{-1}$	146.2±2	150.68± 2	132.11± 2	131.52± 2	146.39± 2	140.21± 2
Photoperiod in 24 Hours	Hours (h)	16	16	16	16	16	16
Lighting Power	W	191	206.00	149.50	168.80	175.40	224.70

[a] Photosynthetic Photon Flux Density (PPFD) range of 400 nm to 700nm was based on American Society of Agricultural and Biological Engineers (2017)

[b] Photosynthetic Biological Active Radiation (PBAR) range of 280 nm to 800nm was based on American Society of Agricultural and Biological Engineers (2017)

All treatments in the spectrum strategies had a base of red and blue spectra that were then supplemented with other spectral components to create unique light recipes (Table 6.2, 6.3). The treatments were red+blue (RB), blue+red (BR) that had higher blue content, red+blue+far-red (FR), range of red+blue+green with increasing amounts of green spectral content (GR,GR1,GR2), range of red+blue+UV-A with increasing amounts of UV-A content (UVA,UV1,UV2), combination of GR1+UV1 (UVGR1), combination of GR2+UV2 (UVGR2) and a full spectrum wide band combination of red+blue+green+far-red+UVA (FS).

The pre-harvest strategy was split further into 4. PH1 involved cultivating the plants for 165 days under the basic RB light treatment before subjecting them to 10 days of trichromatic UV2, GR2, and BR irradiation as well as monochromatic green, blue, and UVA irradiation (Table 6.4). Before receiving the same treatments as PH1, plants in PH2 were grown for 165 days in a greenhouse with natural sunshine (GH). PH3 and PH4 involved growing the plants for 172 days under RB and GH, respectively, before putting them through the same 3 days of pre-harvest lighting as PH1. (Table 6.5).

Table 6.4 : Spectral Composition of Pre-harvest Light Treatment

		Monochromatic			Trichromatic		
		Blue	**Green**	**UV-A**	**UV2**	**GR2**	**BR**
Daily Light Integral (DLI)	μmol $m^{-2}day^{-1}$	3.2	1.4	1.2	7.5	7.5	7.5
Photoperiod in 24 Hours	Hours (h)	16	16	16	16	16	16
Lighting Power	W	20.00	30.00	50.00	206.00	168.80	129.00

Table 6.5 : Pre-Harvest Strategies

Strategy	**Treatment**	**Days After Sowing of Seeds**			
		0	**165**	**172**	**175**
PH1	**XH**	**Red-Blue (RB)**		**UV2**	
	XI	**Red-Blue (RB)**		**GR2**	
	XJ	**Red-Blue (RB)**		**BR**	
	XK	**Red-Blue (RB)**		**Monochromatic UV-A**	
	XL	**Red-Blue (RB)**		**Monochromatic Green**	
	XM	**Red-Blue (RB)**		**Monochromatic Blue**	
PH2	**XN**	**Greenhouse (GH)**		**UV2**	
	XO	**Greenhouse (GH)**		**GR2**	
	XP	**Greenhouse (GH)**		**BR**	
	XQ	**Greenhouse (GH)**		**Monochromatic UV-A**	
	XR	**Greenhouse (GH)**		**Monochromatic Green**	
	XS	**Greenhouse (GH)**		**Monochromatic Blue**	
PH3	**3H**	**Red-Blue (RB)**			**UV2**
	3I	**Red-Blue (RB)**			**GR2**
	3J	**Red-Blue (RB)**			**BR**
	3K	**Red-Blue (RB)**			**Monochromatic UV-A**
	3L	**Red-Blue (RB)**			**Monochromatic Green**

	3M	**Red-Blue (RB)**	**Monochromatic Blue**
PH4	**3N**	**Greenhouse (GH)**	**UV2**
	3O	**Greenhouse (GH)**	**GR2**
	3P	**Greenhouse (GH)**	**BR**
	3Q	**Greenhouse (GH)**	**Monochromatic UV-A**
	3R	**Greenhouse (GH)**	**Monochromatic Green**
	3S	**Greenhouse (GH)**	**Monochromatic Blue**

The control experiments were conducted under RB and GH with a spectral composition as in table below.

Table 6.6 : Control Experiments

	Unit	**RB**	**GH**
Total PPFD[a] (PAR)	µmol $m^{-2}s^{-1}$	130	333.48
PPF 400-700 nm	µmol s^{-1}		
Total PFD[b] (PBAR)	µmol $m^{-2}s^{-1}$	131.3	428.91
Daily Light Integral (DLI)	µmol m^{-2} day^{-1}	7.5	14.4
Photoperiod in 24 Hours	Hours (h)	16	12
Lighting Power	W	129.00	-

Notes:

[a] PAR and PBAR range used as per American Society of Agricultural and Biological Engineers (2017) definition.

[b] PPF values were based on spectral composition as the per light recipe and not based on maximum output values of the systems.

The photosynthetic photon flux density (PPFD), defined as the amount of PAR radiation in micromoles, that falls on a $1m^2$ surface each second, represented as µmol $m^{-2}s^{-1}$ is an important metric used to describe the light intensity of horticulture lighting (American

Society of Agricultural and Biological Engineers, 2017). All artificial lighting treatments used in this study had a similar PPFDs of 130 μmol $m^{-2}s^{-1}$ and a photoperiod of 16 hours per day from 7.00am to 11pm, resulting in a constant daily light integral (DLI) throughout the experimental period. Prior to installation, the fixtures were measured in an integrating sphere to obtain the photosynthetic photon flux (PPF) values for each light recipe, at the laboratory of Novabrite Lighting Sdn Bhd in Shah Alam, Malaysia (www.novabrite.com.my). Unlike the PPFD, the PPF represents the total PAR radiation that is emitted by a light source and is used to calculate the fixture efficacy. In photometric terms, the PPFD is like illuminance (lux), while the PPF is comparable to the total luminous flux (lumens) of a light source. Each growing area of $1m^2$ was installed with 1 artificial lighting system.

The experiments were conducted in stages over 4 years, from 2018 to 2022, to ensure that each light treatment had a 3 growth cycles, and a minimum of 60 biological replicates per cycle. An energy meter (BAYITE-PZEM-061) was installed in all artificial lighting systems to monitor and record the power and energy usage. The artificial lighting intensity and power was not changed throughout the experimental cycle.

6.3.4 Data Collection

6.3.4.3 Energy and Power

The total power and energy measurements for the GH and PF were measured and logged using a 3-phase power quality logger (Fluke 1735, FLUKE, USA) that was connected to the input at the distribution panels at both locations. The heating, ventilation, and air-conditioning (HVAC) system was the only load for the GH while the PF had the HVAC and lighting loads. In the PF, the logger was used to measure and log the cooling load only as the lighting loads had their own energy meters. The power and energy data were logged at an interval of 5 minutes over a period of 7 days for the PF and 10 days for the days. The data logging for the PF was conducted from the 29th of April to the 5th of May

2021 while the data collection for the GH was done from the 21st to the 30th of May 2021. The difference in measuring period between the PF and GH was due to the availability of the power quality logger. All ambient environmental data (ambient temperature, sunlight intensity) were measured during both sessions. All growth areas within the GH and PF were occupied with plants during the data logging period. To obtain a full load scenario during the data logging period, the artificial lighting load in the PF was set to maximum power of 250 Wm^{-2} with all 8 channels of the systems set to 100%. The power and energy data for each of the artificial lighting system in the PF was measured and logged during normal operations by means of individual energy meters (BAYITE-PZEM-061) that were installed at the direct current (DC) output of the power supply unit. As each of the lighting systems was installed to illuminate $1m^2$ of growth area, the lighting power density for the systems were equivalent to the system power as in Table 6.1.

The total power density for the GH, normalised to per unit growth area of $1m^2$, expressed in W m^{-2} was calculated as follows:

$$\text{Power Density GH} = \frac{\text{Total Measured 3} - \text{phase Power (W)}}{\text{Total Growth Area (m}^2\text{)}}$$

On the other hand, the power demand for each light treatment in the PF consist of two parts: the lighting power and the non-lighting power.

$$\text{Non Lighting Power Density for PF} = \frac{\text{Total Measured 3} - \text{phase Power (W)}}{\text{Total Growth Area (m}^2\text{)}}$$

$$\text{Lighting Power Density (LPD)for PF} = \frac{\text{Total Measured Lighting Power (W) for each system}}{\text{Growth Area (m}^2\text{) of each aritificial lighting system}}$$

The total power density for PF is the sum of both components :

$$\text{Total Power Density PF} = \text{Non Lighting Power Density PF} + \text{Lighting Power Density PF}$$

The total energy density for the experimental cycle of the GH, and the total lighting and non-lighting energy density per experimental cycle for all treatments of the PF, expressed in kWh m^{-2} were calculated using the following equations:

$$\text{Total Energy Density for GH} = \frac{\text{Mean Energy Measured (kWh)}}{\text{Total Growth Area } (m^2)} \times 24 \times \text{Experiment Cycle(Days)}$$

$$\text{Non Lighting Energy Density for PF} = \frac{\text{Mean Energy Measured (kWh)}}{\text{Total Growth Area } (m^2)} \times 24 \times \text{Experiment Cycle (Days)}$$

$$\text{Lighting Energy Density for PF} = \text{Mean Measured Lighting Energy} \times \text{Photoperiod} \times \text{Experiment Cycle (Days)}$$

$$\text{Total Energy Density for PF} = \text{Non Lighting Energy Density for PF} + \text{Lighting Energy Density for PF}$$

Where,

Mean Energy Measured = The hourly average data as measured by the 3-phase power quality logger

Mean Measured Lighting Energy = The hourly average lighting energy as measured by the energy meter at each system

Experiment cycle = 175 days

Photoperiod = Hours of artificial light as per Table 6.1

6.3.4.4 Biomass Yield

As stated in Chapters 3,4 and 5, at the end of each growth period, the plants were harvested by cutting the stems 5cm above soil surface. The leaves and stems were separated before the leaves were washed under running water and allowed to sit in a mesh bowl for 1 hour. The leaves were then gently blotted with tissue to remove remaining water before being dried in an oven (Binder, Germany) for 20 hours at 60°C. Once the dried leaves had cooled to room temperature, dry weight was measured using a digital scale (Shimadzu, Japan). The mean yield of dried leaf per unit growth area, expressed as g m^{-2} was calculated as follows:

$$\text{Dried Leaf Yield} = \frac{\text{Total Dried Leaf Weight (g)}}{\text{Total number of plant samples}} \times \text{Planting Density}$$

Where, Planting Density is 24 plants m^{-2}

6.3.5 LCMS Analysis

After being sonicated for two hours with 0.5 g of ground-up dry Stevia leaves and 50 mL of a 35:65 (v/v) ethanol-water mixture, the mixture was filtered to get the effluents, which were then dried with a miVac centrifugal concentrator to remove any remaining solvent. The resultant extract was then dissolved in 1 mL of water and acetonitrile at a concentration of 10 mg (7:3). Following that, the material was filtered using a PES membrane with 0.22 m pore size. The LCMS analysis was performed using a C18 column and either a Shimadzu LCMSMS 8050 (PH1,2,3,4, and SS 2) or LC-MS QTOF apparatus (Agilent 1290 Infinity™) (photoperiod strategy and SS1). The amount of ST and Reb A in the samples was quantified using a modified strategy based on the assay procedures described in WHO (2020), represented as a percentage of mass of leaf dry (% leaf DW^{-1}).

6.3.6 Metabolite Yields

To obtain the quantity of the total ST and Reb A metabolite yields that can be realised per m^2 of growth space, expressed in g m^{-2}, was calculated as follows:

$$\text{Metabolite Yields} = \text{Dried Leaf Yield} \times (\text{Reb A Concentration} + \text{ST Concentration}) \times \text{Planting Density}$$

where,

Reb A & ST Concentration = Percentage composition per gram of leaf DW (Obtained from LCMS results).

6.3.7 Efficiency and Efficacy Analysis

The energy use efficacy (EUE) that described the amount of metabolite yield obtained per kWh of electrical energy consumed, expressed in mg kWh^{-1} was calculated using the following formula :

$$\text{EUE} = \frac{\text{Metabolite Yield (g m}^{-2})}{\text{Total Energy Density (kWh m}^{-2})} \times 1000$$

The artificial lighting system's photosynthetic photon efficacy (PPE) that represents the efficacy of the lighting system to convert electrical energy to PAR radiation (American Society of Agricultural and Biological Engineers, 2017; Design Light Consortium, 2021), expressed as μmol J^{-1} were calculated as follows :

$$\text{PPE} = \frac{\text{PPF (}\mu\text{ mol s}^{-1})}{\text{Total Measured Lighting Power (W)}}$$

Where,

PPF value are as measured in the integrating sphere

The photon conversion efficacy (PCE) was used in this study to describe the effectiveness of the light recipes to convert moles of light to metabolite. The PCE calculation used in this study was modified from previous studies by Slattery and Ort (2015) and Kubota et al. (2016), to include the PBAR range, expressed in mg mol^{-1}, was calculated as follows:

$$\text{PCE} = \frac{\text{Metabolite Yield (mg m}^{-1})}{\text{PBAR}(\mu\text{mol m}^{-2}\text{ s}^{-1}) \times 3600 \times \text{Photoperiod(Hours)} \times \text{Experiment Cycle (Days)}}$$

Where,

PPF, Lighting Power, PBAR and Photoperiod as in Table 6.1

6.3.8 Indoor Air Quality

The air temperature, humidity, and CO_2 concentrations at a height of 1.5m above the ground in both the GH and PF were measured using an indoor air quality data logger (CO2meter.com CM00018,USA) at an interval of 5 minutes throughout the same period as the energy and power measurements. An additional measurement was made on the outside wall of the walkway that is shaded by the facility's roof overhang as a reference point of the ambient conditions.

6.3.9 Light

The PF lighting setup was designed to have a constant PPFD throughout the experimental cycles. The light output of each treatment was measured using a portable spectroradiometer (Asenstek Lighting Passport, Taiwan) at 30cm from the base of the growing platform. The light source was installed at 75cm from the growth area surface. The PPFD measurements were made monthly during the 3-year period to ensure a constant light output. Unlike the PF that had a constant intensity throughout, the natural light in the GH varied significantly throughout a day. Hence, a portable spectroradiometer

with data logging function (Nanolambda XL-500 BLE, South Korea) was used. The lighting measurements were logged in a 5-minute intervals from the 21st to the 30th of May 2021.

6.4 Results

6.4.1 Power Requirements of Greenhouse and Growth Room

The peak daily power consumption of the GH varied throughout the data collection period, having a maximum of 399.27 Wm^{-2} and minimum of 33.88 Wm^{-2}, with an average of 139.34 Wm^{-2} (Figure 6.3A). The daily values were closely associated with the ambient temperature and time, with lower temperatures during early mornings, late evenings, and nights resulting in lower power demand by the cooling system. The PF however, had a consistent trend in power consumption throughout the measurement period, unaffected by the variation in ambient temperature due to better insulation provided by the facility construction (Figure 6.3B). The power demand varied as per the start and stop times of the artificial lighting systems at 7.00am and 11pm respectively. Among the different artificial light treatments, the FS system had the highest average consumption of 259 Wm^{-2} followed by, UVGR2 (233.16 Wm^{-2}), UVA2 (220.69 Wm^{-2}) and UVA1 (210.75 Wm^{-2}). The lowest average power consumption recorded was under the FR (169.83 Wm^{-2}) system followed by the RB (175.43 Wm^{-2}) and GR (178.29 Wm^{-2}) systems.

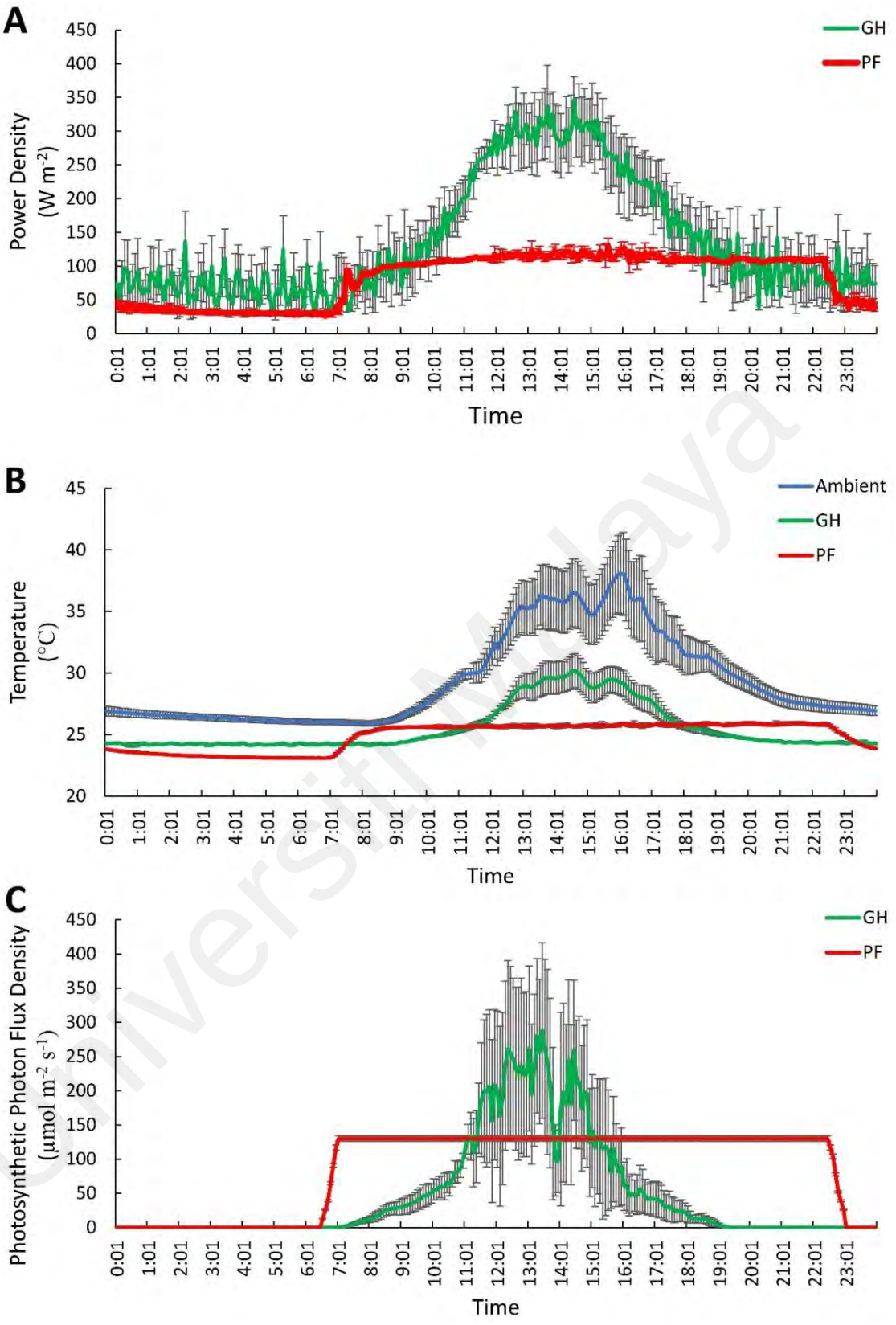

Figure 6.3 : Mean Power, Temperature and Photosynthetic Photon Flux Density for Greenhouse and Growth Room (A) GH and PF mean power demand (W m^{-2}) (B) Mean ambient, GH and PF temperature conditions. (C) Mean Photosynthetic photon flux density (PPFD) of GH and PF. PPFD values for PF does not include monochromatic pre-harvest lighting treatments.

Throughout the day, the peak power demand in the GH was during mid-day, from 11am to 4pm. This was also the period with the highest intensity of light, having PPFDs up to 400 µmol $m^{-2}s^{-1}$ (Figure 6.3 C), and highest ambient temperature. Meanwhile, the daily demand trend within the PF was related to the start and stop times of the artificial lighting. The power demand increases as the lights start the ramp up at 6.30am before reaching full power at 7.00am and the power consumption begins to reduce at 10.30pm as the lights begin the ramp down, turning off at 11pm (Figure 6.3 A,B,C). The non-lighting power, essentially the cooling requirement, ramps up as the internal heat generated by the lighting systems begins to increase and remains relatively constant throughout the operation time with an average of 110.75 Wm^{-2} during the artificial light operating hours, reaching a maximum of 128.25 Wm^{-2} and minimum of 88.60 Wm^{-2} (Figure 6.3 A). As the PPFD is fully controlled by the system, it remained constant at 130±2 µmol $m^{-2}s^{-1}$ throughout the operating period.

As natural light was the only source of photosynthetic radiation for the plants, the higher intensities, while preferred for photosynthetic activity, also resulted in higher ambient temperatures and lead to higher energy demand from the cooling system. As the PPFD increased throughout the day, so did the ambient temperatures, reaching a peak of 38.04°C within the facility (Figure 6.3 B). This caused the power demand for the cooling system to increase by an average 322% as the ambient temperatures increased from 27°C (98.76 Wm^{-2}) to 38°C (318.23 Wm^{-2}) . As the lighting power of the different lighting systems remained constant throughout the experiment, the cooling load was analysed separately (Figure 6.3 A) to evaluate any potential effects of variation in ambient temperature on the cooling power requirements. While the cooling demand within the PF appeared to be affected with the cooler temperature, it was noted that this cooler temperature was mostly observed during the night periods and early mornings, where the lighting systems are turned off or ramping up/down, hence the fluctuations observed. In

ambient temperature between 30°C to 36°C the average cooling power demand was 112.74 Wm^{-2}, with a maximum of 119.45 Wm^{-2} and minimum of 106.50 Wm^{-2}. Marginally higher cooling power consumption was observed when the ambient temperature exceeded 36°C, with an average of 117.13 Wm^{-2}, maximum of 125.25 Wm^{-2} and a minimum of 107.23 Wm^{-2}.

6.4.2 Total Energy Consumption of Greenhouse and Growth Room

The GH had the lowest mean electrical energy requirement over the experimental cycle of 175 days, at 584.43 kWh m^{-2} (Figure 6.4). It was also observed that all pre-harvest treatments that originated from GH has lower mean electrical energy consumptions of between 584.5 (3S) to 603.82 kWh m^{-2} (XN). Unlike the GH, the PF had both lighting and cooling requirements, and it was observed that under all treatments the overall lighting energy demand was higher than the cooling load. It should be noted that in this study, the cooling power density that was used to calculate the cooling energy density for all artificial lighting treatments, were based on the mean cooling power density obtained when the artificial lighting system was operating at a LPD of 250 Wm^{-2}. Consequently, all artificial light treatments had higher energy consumption than the GH with the full spectrum FS treatment being the most energy hungry system at 1084.09 kWh m^{-2}, followed by UVGR2 (975.95 kWh m^{-2}), UV2 (23.84 kWh m^{-2}) and UV1 (882.43 kWh m^{-2}). The BR and RB lighting treatments that had only red and blue spectrum, had the lowest energy consumption among all artificial lighting systems that had a 16-hour photoperiod at 707.90 kWh m^{-2} and 734.87 kWh m^{-2}, respectively (Figure 6.4). As expected, the shorter photoperiod of 8H and 12H resulted in a lower electrical energy consumption of 689.45 kWh m^{-2} and 691.86 kWh m^{-2} respectively. The overall electrical energy demand of the different treatments within the PF was primarily affected by the variation in lighting system power (Table 6.1). Multispectral treatments required higher electrical power and resulted in higher overall energy usage.

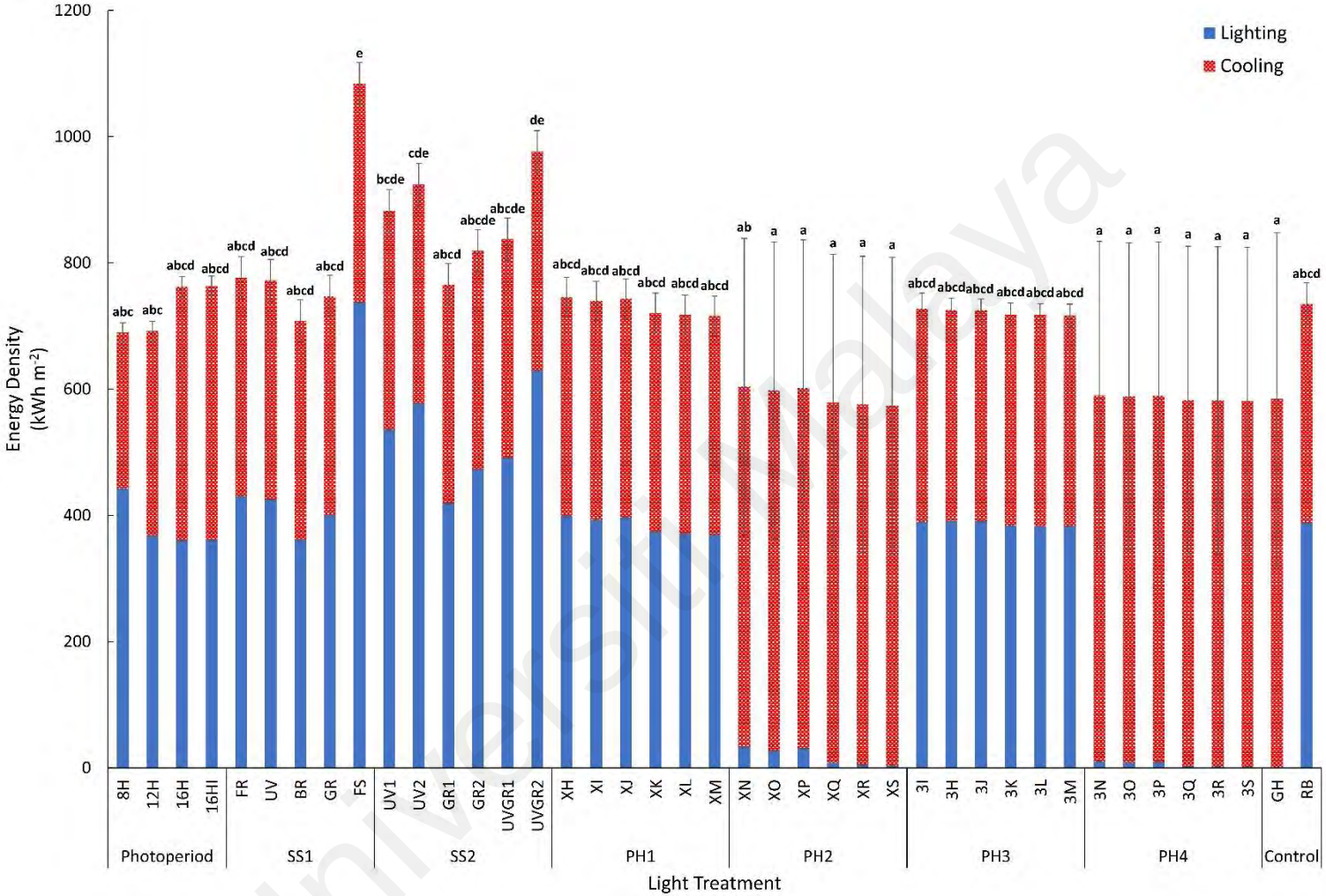

Figure 6.4 : Mean electrical energy density per experimental cycle of 175 days (kWh m^{-2})

6.4.3 Yield and Efficacy Analysis

Although GH had the lowest electrical energy consumption, it also resulted in one of the lowest average metabolite yields of 2.62 g m^{-2}, slightly higher than the two lowest yields obtained under pre harvest treatments of XR (2.12 g m^{-2}) and XS (2.48 g m^{-2}) that both originated from GH (Figure 6.5). All treatments in the PF had significantly higher yields, with the highest obtained under UVGR1 (25.30 g m^{-2}), followed by GR2 (24.34 g m^{-2}), and UV2 (23.67 g m^{-2}). The lowest yields in the PF were observed in plants from RB that were subjected to pre-harvest treatments of monochromatic green light for 10-days (XL) and 3-days (3L) with a yield of 4.77 g m^{-2} and 5.03 g m^{-2} respectively. All treatments under SS2 had significantly higher yields of between 21.12 g m^{-2} to 25.30 g m^{-2} compared to all other strategies. Comparatively, the next highest yields were obtained in treatment GR under SS1 with a yield of 10.75 g m^{-2}.

The lower metabolite yields from GH and the pre-harvest treatments that originated from GH, affected the EUE for these treatments. The lowest energy use efficacy (EUE) among all treatments at 4.45 mg kWh^{-1} (Figure 6.6) was obtained in XR, followed by 8H (5.33 mg kWh^{-1}), GH (5.51 mg kWh^{-1}) and RB (5.53 mg kWh^{-1}). The highest EUE was obtained under UVGR1 (30.24 mg kWh^{-1}), followed by GR2 (39.74 mg kWh^{-1}), GR1 (27.47 mg kWh^{-1}) and UV2 (25.65 mg kWh^{-1}).

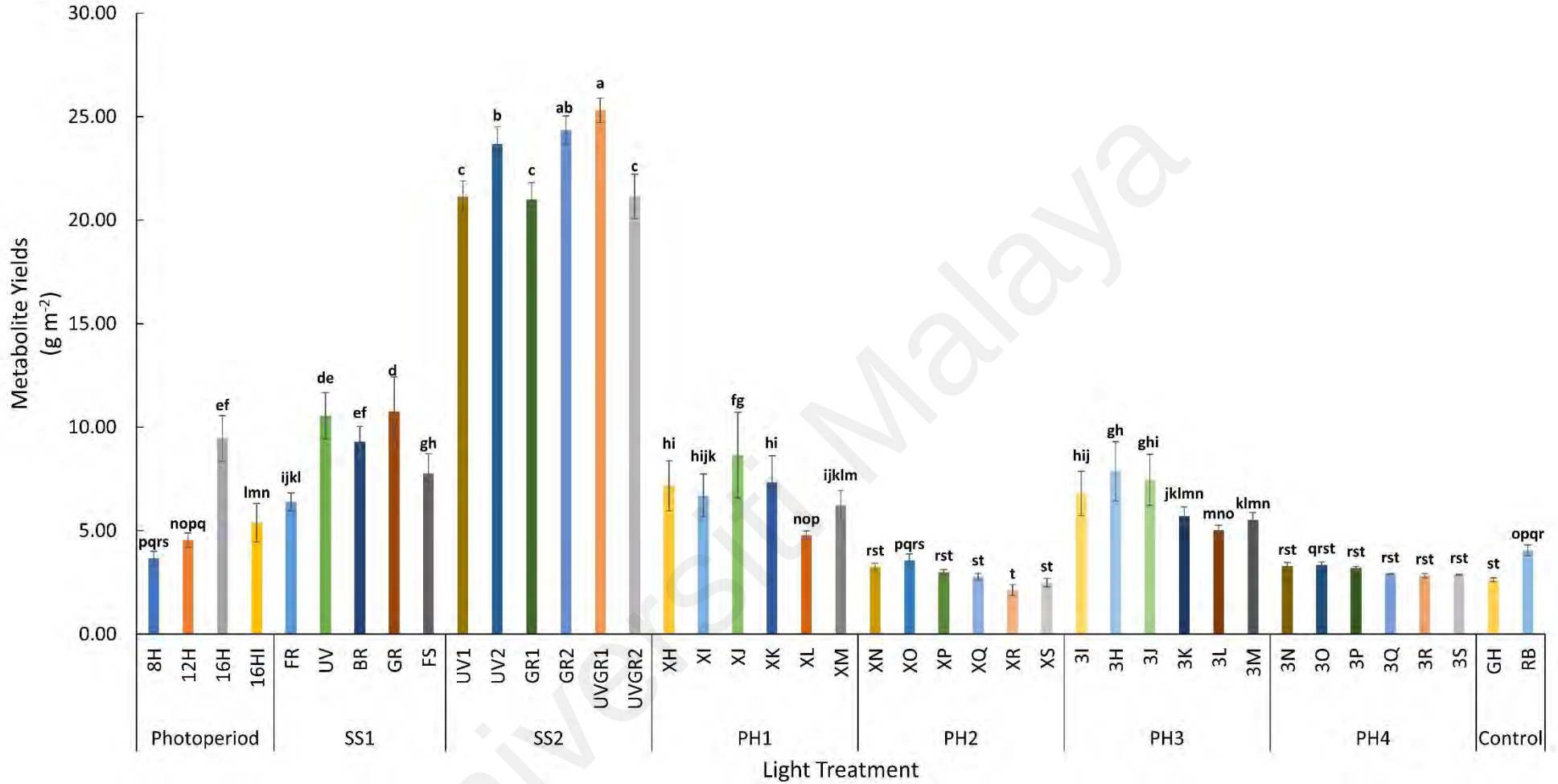

Figure 6.5 : Plant Productivity. The mean combined ST and Reb A metabolite yields that can be realised for every 1 m^2 of growth space occupied by each light treatment (g m^{-2}).

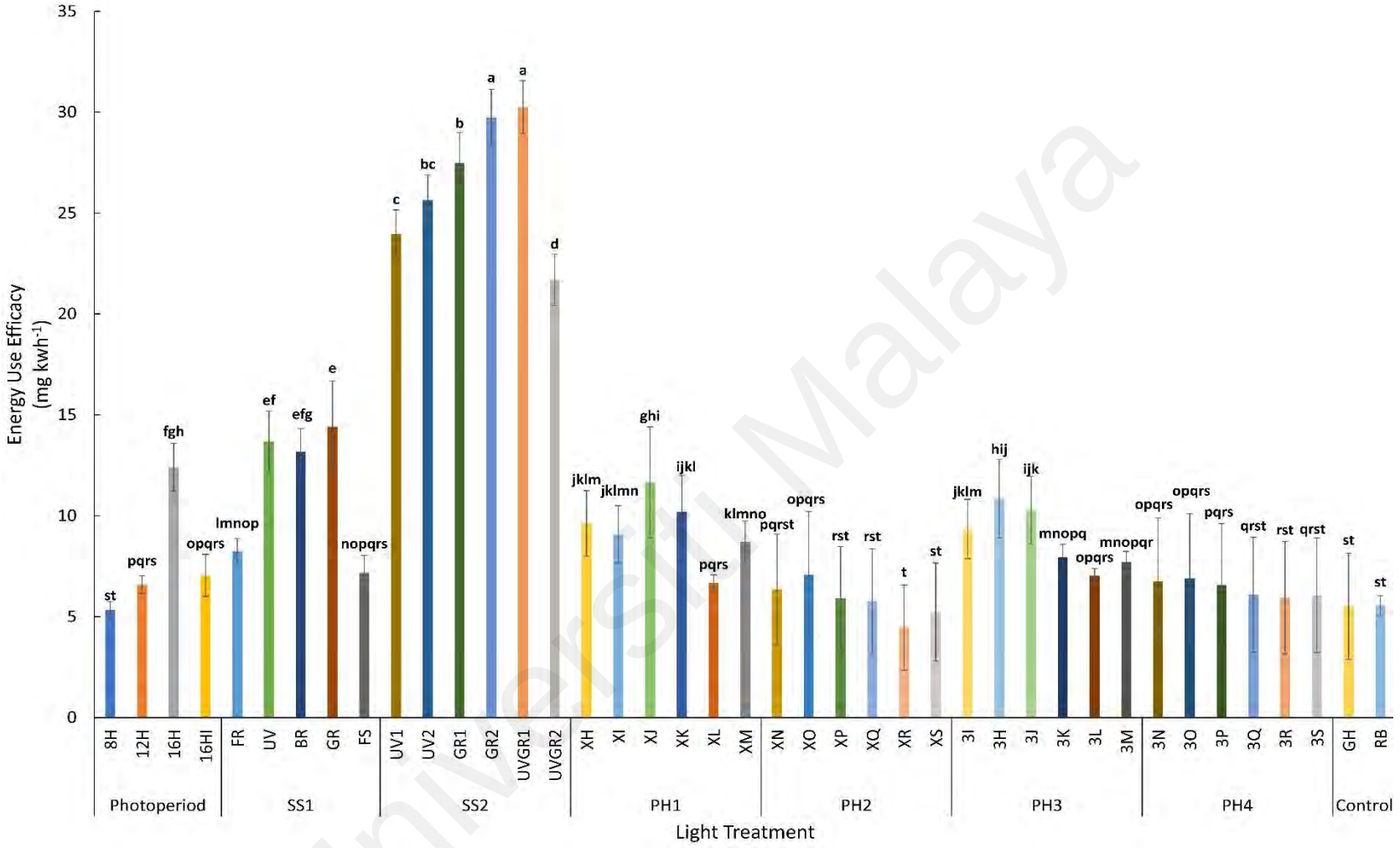

Figure 6.6 : Energy Use Efficacies. The mean Energy Use Efficacies for each light treatment representing the amount of ST and Reb A that can be realised for every unit of electrical energy consumed (mg kWh^{-1}).

The PPE of the artificial light reduced with the introduction of additional spectra, especially spectra beyond the PAR region of 400nm to 700nm such as far-red and UV-A. The BR treatment had the highest PPE of 1.41 µmol J^{-1} . The blue and red systems of RB and the photoperiod strategy systems (8H, 12H, 16H, 16HI) with only red and blue spectral the next highest PPE values of between 1.31 and 1.35 µmol J^{-1}. The lowest PPE were obtained in FS with 0.69 µmol J^{-1}, followed by UVGR2, UV2 and UV1 with 0.81 µmol J^{-1}, 0.88 µmol J^{-1} , and 0.95 µmol J^{-1}, respectively (Figure 6.7). Although the red and blue spectra of the 16H and 16HI treatment under Photoperiod Strategy were most efficient in terms of converting electrical energy to light within the PAR region, it had lower photon conversion efficacies (PCE) of 7.5 mg mol^{-1} and 4.2 mg mol^{-1} respectively. The multispectral light from the green supplemented GR2 (18.69 mg mol^{-1}) and GR1 (16.13 mg mol^{-1}), and UV-A supplemented UVGR1 (17.68 mg mol^{-1}), UVGR2 (14.47 mg mol^{-1}) and UV1 (14.86 mg mol^{-1}) had significantly higher PCE values (Figure 6.7). All treatments under SS 2 had significantly higher PCE values compared to all other treatments.

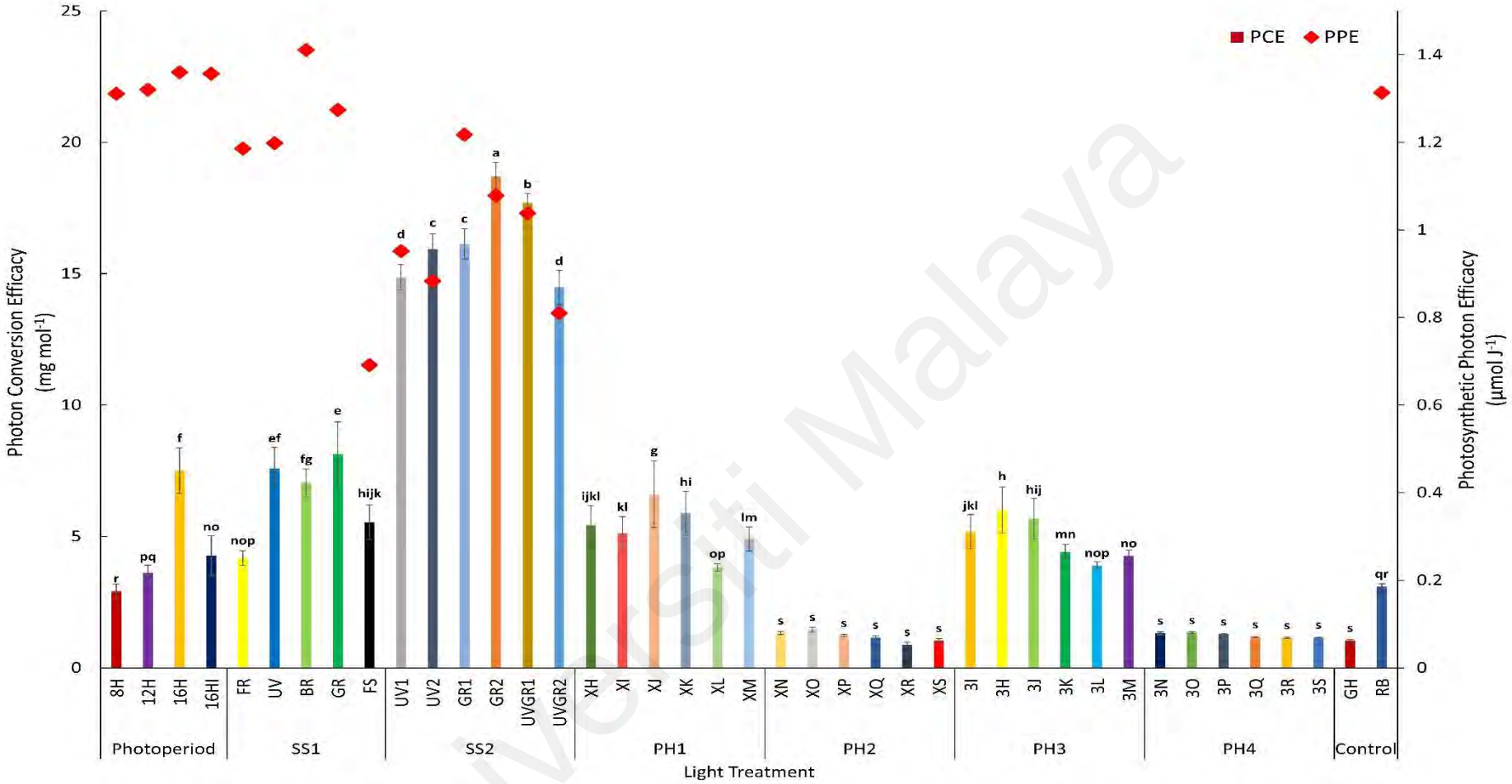

Figure 6.7 : Photon Conversion Efficacy and Photosynthetic Photon Efficacy. PCE represents the amount of ST and Reb A metabolite that can be produced by 1 mol of light delivered (mg mol^{-1}). PPE is the amount of light within the PAR spectrum that is produced by the lighting fixture for every unit of electrical energy consumed (µmol J^{-1})

6.5 Discussion

Cultivation of plants in climate-controlled greenhouses or plant factories allows for local production of non-native varieties that would otherwise have to be imported. It also provides an opportunity for hyperlocal production, uses less water than field cultivation, and allows reduced use of pesticides, making it more sustainable and the produce safer for general consumption (Hardanto & Sumarni, 2021). However, as the environmental conditions such as temperature, humidity and lighting within these systems are controlled, these incur additional energy and costs, not present in traditional farming practises (Shaari et al., 2021). Therefore, it is important to balance the benefits of CEA with the drawbacks, maximising productivity while optimising additional energy use. In tropical environments, cultivation of plants within CEAs are mostly limited to growing food and fruit crops such as strawberries, rock melons, tomatoes, and chilies. These type of crops are typically grown in greenhouses with natural ventilation, where the primary use of the greenhouse structure is to provide shade from the sun and rain. Like most tropical countries, Malaysia is blessed with a warm and humid climate, with an average annual maximum and minimum temperature of 32.67 °C and 24.24 °C respectively, and a neutral day length of 12-hours per day, throughout the year (Malaysian Meteorological Department, 2019). Malaysia also has an average annual rainfall of between 1800 mm and 3900 mm. The use of supplemental lighting in greenhouses in Malaysia is mostly limited to highland cultivation of ornamental plants, that mainly use standard white, or red and blue based light bulbs. From the findings in this study, two critical aspects were observed to influence the electrical energy consumption and overall efficacy of CEAs in tropical conditions. The aspects are, the artificial lighting recipe and system efficiencies, and the CEA type. These aspects not only affect the overall energy consumption of the respective systems but also provide an opportunity for optimisation of the overall energy usage in the CEAs by reducing energy demand and/or improving productivity.

6.5.1 Artificial Light Recipes and System Efficiency

Key factors influencing the economic feasibility of any form of business are its revenue and expenses. In CEAs, this translates to improving yields and reducing the energy costs. To optimise energy resource utilisation, and to improve economic feasibility, the emphasis would be on reducing the overall energy used (conservation), and to optimise the energy used, maximising output (efficiency). The lighting requirements in plant factories account for the largest purchased electrical energy load, up to 86% of total electrical energy used, regardless of geographic location and climatic conditions (Engler & Krarti, 2021; Weidner et al., 2021). In the current study, lighting accounted for between 51% and 68% of the total energy used within the PF. Previously and even today, CEAs used traditional lighting technologies as the primary artificial light source. High pressure sodium (HPS) and metal halide (MH) lamps are the preferred technologies for use as supplemental lighting in greenhouses, and as a primary light source in single layer plant factories (Elliott et al., 2020; Vatistas et al., 2022). While these traditional technologies are inexpensive and have PPEs of up to 2.1 μmol J^{-1} , they generate significant amounts of heat, making them unsuitable for vertical farm type CEAs (Elliott et al., 2020; Katzin et al., 2021). As these lamps are also not spectrally tuneable, growers are not able to optimise the light to suit the plant needs. The proliferation of LEDs in horticulture lighting addressed these short comings of past technologies, as LEDs do not only generate minimal heat compared to HPS, but also are available with a wide range of spectrums, facilitating highly customisable lighting solutions to meet a plant's specific needs (Avgoustaki & Xydis, 2021). It is widely known that LEDs are more energy efficient compared to traditional lighting technologies, both in general lighting and horticultural applications. Studies have shown that by switching to LEDs, total energy usage within a greenhouse can be reduced by up to 25% (Katzin et al., 2021).

However, as seen in the current study, by using an optimised light recipe, yields can be increased further, boosting overall plant productivity, and improving energy use efficacy. Light is an important resource for plants, influencing every aspect of growth from seed germination, vegetative growth through to reproduction via flowering and fruiting. Plants use light energy, harvested via photo pigments, chlorophyll a and b, to synthesise energy via photosynthesis (Appolloni et al., 2022; Demotes-Mainard et al., 2016; Paradiso & Proietti, 2022; Yadav et al., 2020). Besides using light for photosynthesis, light is also used for photosignaling to trigger a host of plant responses, including induction of flowering, elongation of plant stems, movement of chloroplasts, photomorphogenesis, circadian regulation and metabolite accumulation (Wu et al., 2020a). Most plants have a host of photoreceptors, with specific wavelength sensitivity for photosignaling purposes (Ouzounis et al., 2015; Zheng et al., 2019b). The phytochromes (PHY), absorb light in the red and far-red (FR) light spectrum from 600 to 800 nm, while the Cryptochromes (CRY) and phototropins (PHOT), with an absorption spectra in the 350nm to 500nm absorb ultraviolet A (UV-A) and blue light (Kuijpers et al., 2021; Sipos et al., 2020; Wang et al., 2014). The UVR8 photoreceptor with sensitivities in the range of 280nm to 315nm, is the primary photoreceptor for the detection of ultraviolet B (UV-B) radiation (Jenkins, 2017; Rai et al., 2021; Yadav et al., 2020). In this study, although all treatments had similar PPFDs, the productivity levels varied significantly. The higher green fractions of GR1 and GR2 resulted in 420% and 446% more dry leaf biomass respectively, compared to the dichromatic red and blue spectrum of RB. While red and blue light has been reported to be most ideal for photosynthesis (Claypool & Lieth, 2020), recent studies have reported a positive effect of supplementing green spectra with red and blue light to increase yields in certain crops (Appolloni et al., 2022; Bian et al., 2019; Zhang et al., 2020b). Unlike red and blue wavelengths, green and far-red spectra can penetrate deep through the leaves and plant canopy, reaching leaves at the lower levels, enhancing

overall plant photosynthetic rate, and increasing biomass accumulation (Zhang et al., 2020b; Zhen & Bugbee, 2020c). The positive influence of non-PAR spectra was also observed in this study with far-red and UV-A supplemented treatments resulting in higher yields compared to treatments that had only red and blue spectra. While FR had 19% more biomass compared to RB treated plants, plants grown under light with supplemental UV-A had yield improvements between 163% (UVA) and 485% (UVGR1). Besides improving biomass yields, studies have also reported improvement in metabolite accumulation in Stevia and in other plants when grown under UV-A supplementation (He et al., 2020b).

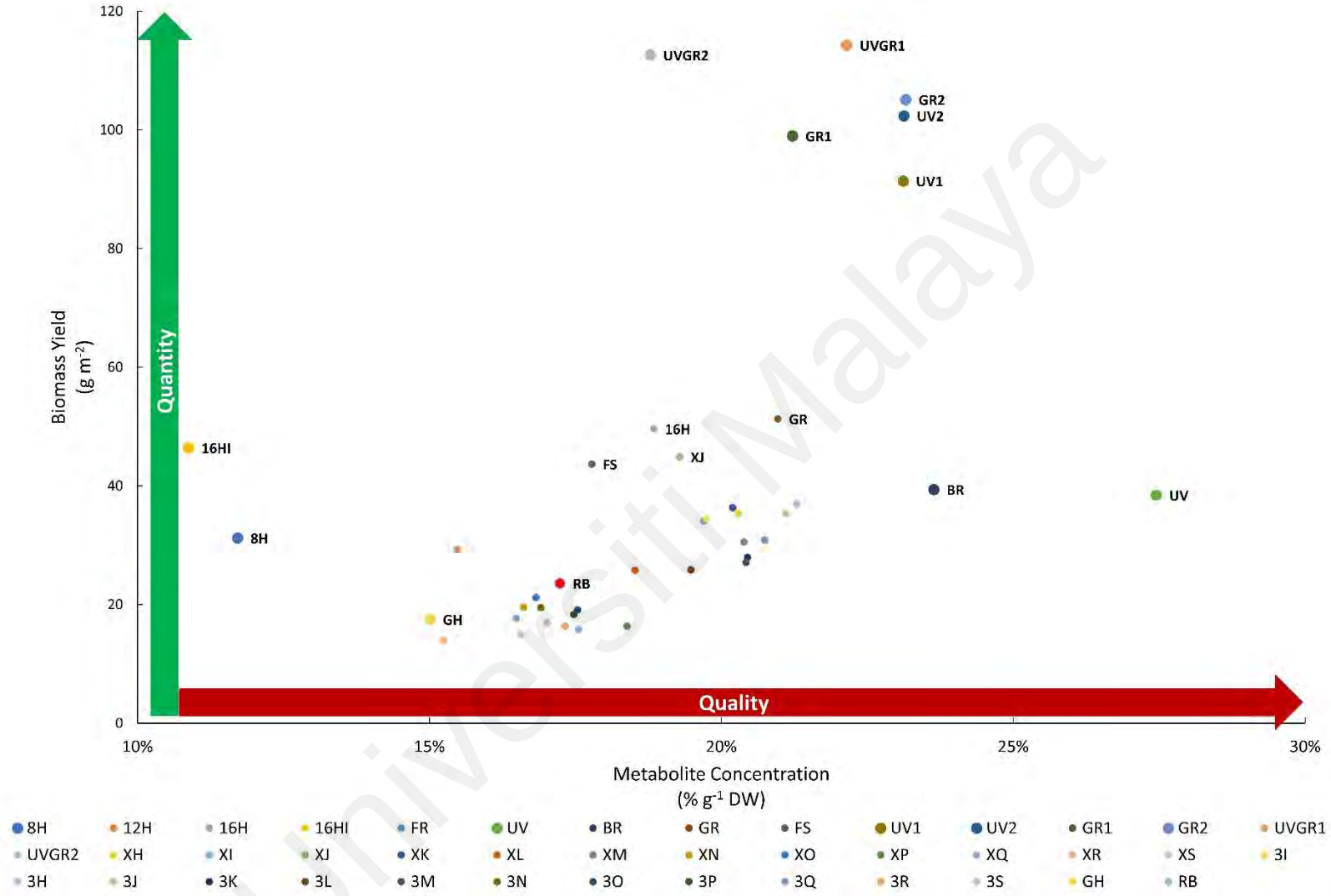

Figure 6.8 : Relationship between Biomass and Metabolite Yields

As observed in this study, the lighting treatments that had UVA and green supplemental spectra, especially under continuous 16-hour photoperiod throughout its growth cycle had considerably higher metabolite concentration compared to GH and the base RB. The shorter wavelengths of blue and UVA spectra have been reported to increase the production of secondary metabolites in several plant species including Stevia (Azad et al., 2020; Samuoliene et al., 2020a; Yoneda et al., 2017a). Comparing the different strategies, it was observed that the photoperiod strategy, was the least effective strategy to improve the secondary metabolite concentration in Stevia, while SS 1, specifically the UVA and BR treatment was the most effective, increasing the secondary metabolite concentration by 159% (UVA) and 137% (BR) respectively. Meanwhile, the pre-harvest treatments had varying degree of success in improving the plant quality. The most visible improvements were obtained in plants that originated from RB with an improvement of ST and Reb A metabolite concentration from 17.26 (RB) to between 18.52% (XL) and 21.29% (3I). An improvement in plant quality was also observed for plants within the pre-harvest treatment that originated from GH, with improving the overall secondary ST and Reb A metabolite concentration from 15.01% in the GH control to between 15.24% (XR) to 18.38% (XP). As plants typically respond faster to changes in environmental factors including light amount at the biochemical and cellular level than at whole plant levels, hence the short pre-harvest treatments were more effective in improving the overall metabolite concentration as compared to the overall biomass yield (Lamalakshmi Devi et al., 2017). In this study, the effectiveness of the different strategies with regards to improving plant quality and quantity was observed. The photoperiod strategies focused on improving the accumulation of biomass but did not result in significant improvement of combined ST and Reb A concentration (quality), while the pre-harvest treatments resulted in improvements of plant quality but not on quantity. The spectrum strategies, especially SS 2 that had higher fractions of UVA and green light were the most optimum,

improving both the biomass and metabolite accumulation significantly (Figure 6.8). In commercial application, this would be the preferred option, especially for indoor cultivation in PFs. While the pre-harvest treatments were not as effective in realising higher metabolite yields, its ability to improve the overall metabolite concentration within a short period of exposure makes it a viable option to improve plant quality.

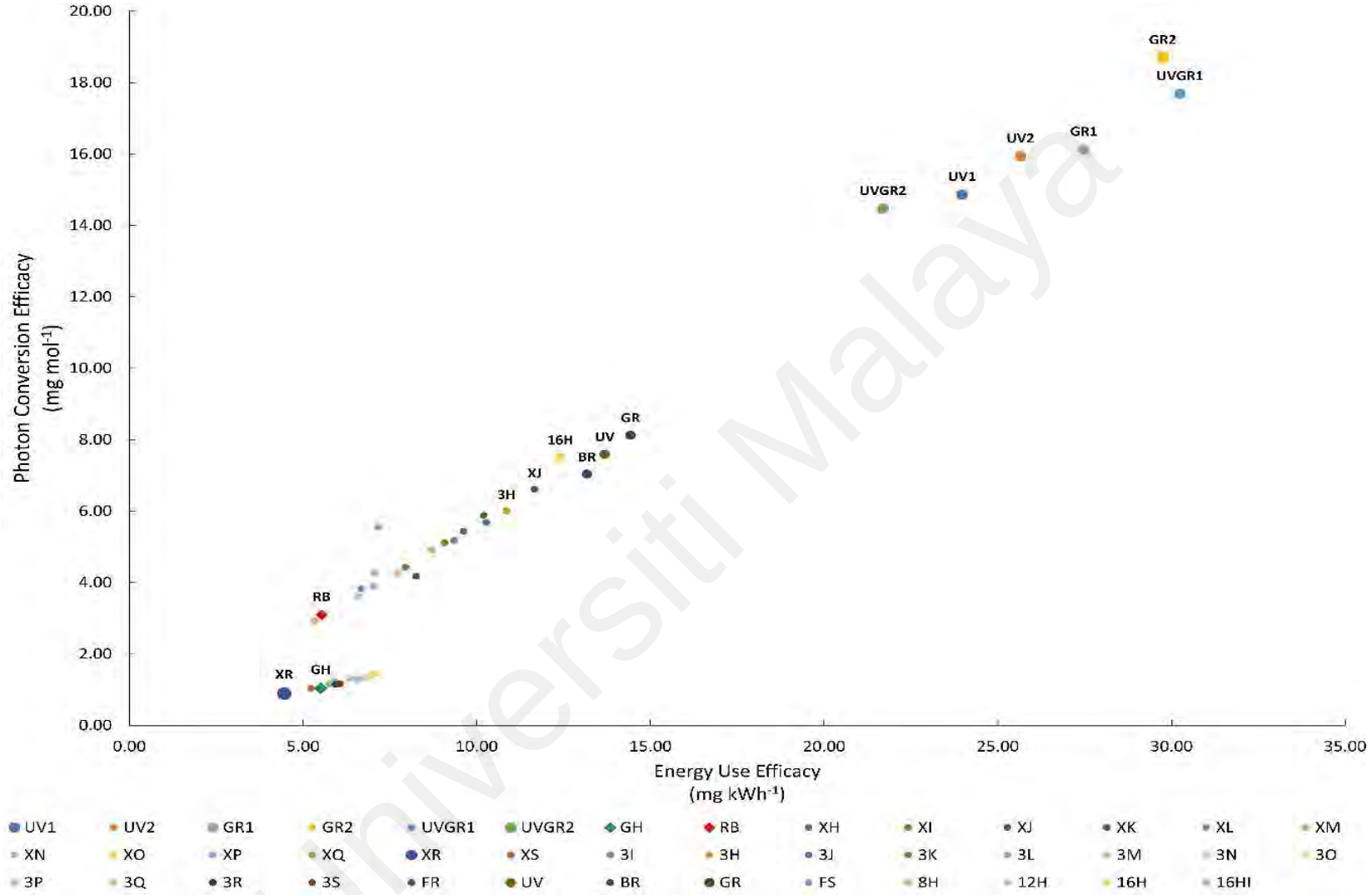

Figure 6.9 : Relationship between Photon Conversion Efficacy and Energy Use Efficacy of the different light treatments

Previous studies used light use efficiency (LUE) to describes how efficiently plants use light that it receives for growth. The LUE is calculated by dividing the total dry biomass obtained by the total incident light that the plant had received throughout the growth cycle (Legendre & van Iersel, 2021). While this provides a physiological measure of how efficiently plants use light, in contrast the PCE is based on the amount of light provided to the growing space, providing information on production efficiency, and subsequently affecting the overall system energy usage (Jayalath & van Iersel, 2021).

The photon conversion efficacies (PCE) that describe the amount of metabolite that can be obtained from 1mol of light delivered by these artificial lighting systems (Figure 6.7), were consistent with the overall plant productivity (Figure 6.5). In calculating the PCE, the full PBAR spectral range of the light from 280nm to 800nm was used, going beyond the PAR values of 400nm to 700nm. This is to take into consideration any contribution of non-PAR wavelengths in biomass accumulation. While the PAR and PBAR (Table 6.1) values for RB,BR,GR,GR1 and GR2 were the same, as the FR, FS, UVA, UV1, UV2, UVGR1 and UVGR2 light treatments had supplemental spectrum from beyond the PAR range, these treatments had PBAR values that were higher than the PAR values of 130 µmol $m^{-2}s^{-1}$. As DLI is defined as the amount of photosynthetically active photons within 400nm to 700nm that is delivered to $1m^2$ of area over a period of 24-hours, the spectral contents in the UV-A and far-red region were not considered when determining the values (Elkins & van Iersel, 2020a, 2020b). Hence, the DLI while accurately describing the total amount of photons within the PAR region that was delivered to the plant canopy, was not representative of the total visible light photons that were delivered to the plants in the systems that had supplemental UV-A and far-red light. Therefore, PCE was used to provide an accurate representation of the light recipe for plant productivity. The higher the PCE, the higher the yields that can be acquired from every mol of light received by the plant. As lighting in PF are provided artificially via LEDs, it is essential to use the

right light recipe with the highest PCE values to optimise the yields. In this study, the two treatments with the highest PCEs, GR1 and UVGR1 had 604% and 572% higher efficacies compared to the RB treatment. These findings illustrate how, by manipulating the spectral content of the lighting system, productivity and efficacies can be increased significantly, improving overall commercial feasibility. Although far-red radiation has been reported to supercharge photosynthesis in plants via the Emerson effect (Zhen & Bugbee, 2020c), this was not observed in the current study with the FR treatment resulting in only a 134% increase in PCE over RB, driven primarily by an increase in metabolite concentration rather than biomass accumulation . The UV-A spectrum had a significant effect in improving efficacies between 245% (UVA) and 515% (UV1) compared to RB.

The PCE and plant productivity information obtained, provide an insight into the spectral composition that is most effective in converting light into yields, focusing on efficiencies. To reduce the overall lighting energy consumed, the overall lighting system efficiencies need to be evaluated, concentrating on conservation of energy. A highly efficient system would be able to deliver the ideal spectral composition, with the highest PCE, while consuming lower energy, compared to a less efficient system. The PPE is a measure on the effectiveness of the lighting system, from a hardware perspective, in converting electrical energy to light within the PAR region. In this study, the highest PPEs were obtained in systems that had the mainly red and blue spectral contents, BR, and RB, including the photoperiod treatments, while the lowest PPEs were obtained in systems that had multiple spectrums that included non-PAR wavelengths. The FS, UVGR2, UV2, UV1 and UVGR2 systems had lower efficacies by 51%, 43%, 37%, 33% and 27% respectively, compared to BR. This finding was not unexpected as the addition of non-PAR wavelengths in these systems required additional energy input that drives up the power of each system (Table 6.1), and while the entire system power was considered, only the spectral content within the PAR range was used in determining the PPE. Green

light, while within the PAR region, did negatively influence the PPE of the systems, with higher intensities of green light fraction reduced the overall PPE of the systems. The GR, GR1 and GR2 systems with 13.0, 15.02 and 25.1 μmol $m^{-2}s^{-1}$ of green spectral content replacing the red and blue wavelengths (Table 6.1), had lower efficacies by 10%, 14% and 24% respectively, compared to BR. The global LED market has been focused on general lighting and automotive applications. Blue LEDs formed the basis for white general lighting LEDs, while red LEDs have been used for automotive and signalling applications. As such, more development has gone into these products in recent years and the overall electrical efficiencies of blue and red LEDs have increased to be in the region of 93% (Blue) and 81% (Red) (Kusuma et al., 2020). As green LEDs have not enjoyed the commercial success of red and blue LEDs, these have received less focus, resulting in green LEDs having efficiencies of approximately 42% (Kusuma et al., 2020). In this study, the light treatments under SS 2 had the most optimised balance between PCE and EUE, having the most productive effect on plant productivity and electrical energy use. In a commercial PF, this would be highly preferred as it can translate to higher revenue generated while maintaining an optimised cost in terms of energy use.

The findings of this study highlight the limitations of the current approach towards horticulture lighting systems. Lighting systems and light recipes that had the highest yields and were most efficient in converting light energy to biomass, had lower PPEs. While PPE has its limitations, it provides an overview of the overall system efficacy and areas for potential improvement as the spectral content within the PAR region represented a major portion of all lighting treatments in this study. The PAR region accounted for between 85% (FR) and 99% (RB, BR, GR, GR1, GR2), and any improvement in the efficiencies of the LEDs within this region will translate to a significant improvement in the overall system efficacies. It should be noted that while the photoperiod strategy lighting systems of 16H, 16HI, 12H and 8H system that had red, blue with some green

fraction, had the highest PPE value of between 1.31 to 1.36 μmol J^{-1} , these values were significantly lower than that of current available products in the market that have been reported to be in the range of between 3.0 to 3.2 μmol J^{-1} for a similar spectral composition (Elliott et al., 2020; Kusuma et al., 2020). The LEDs used in the current study were sourced and assembled in 2018. The potential improvement of up to 100% in terms PPE between the systems in this study and present technology can further improve the EUE and can lead to a reduction of the lighting power needs by up to 50%. This will reduce the overall energy usage of the PF, as any improvement in the artificial lighting systems within the PF will not only reduce its lighting energy load, but will also reduce its cooling load, as the lighting systems are the main source of heat within the PF (Graamans et al., 2018; Katzin et al., 2021; Kuijpers et al., 2021). The PCE is comparable to the power factor (pf) in an electrical system. A system with a lower pf would require more power to be transmitted to the load compared to a system with a pf close to unity. Likewise, in systems with a low PCE, more light would need to be delivered to the plants to achieve productivity similar to systems with higher PCE. The excess light provided, in terms of spectral content and intensity that is not used by the plants is not only wasted but may also cause detrimental effects. High light intensities have been known to cause photodamage in plants, while certain wavelengths have been reported to inhibit growth or induce undesirable traits in plants (Zheng et al., 2019b). Meanwhile, the PPE is analogous to the efficiency of a generator. The lighting fixture is responsible for converting electrical energy to light, and a more efficient system would result in lower waste heat generation. A lighting strategy with an optimised light recipe and a highly efficient fixture would operate similar to an electrical system that has a highly efficient generator feeding to a load with near unity power factor.

6.5.2 CEA Structure Type

The GH and PF had different sources of heat. The major heat build-up within the GH resulted from solar irradiation, with longer wavelengths beyond the visible spectrum heating the air within the GH, while in the PF, the internal heat was from the inefficiencies of the artificial lighting system. The overall energy demand within the GH was strongly influenced by the ambient conditions, increasing as the ambient temperature rises and reducing as the temperature dips (Figure 6.3 B). This influence of solar irradiation on the cooling requirements was not present within the PF that was insulated against natural light and ambient conditions. Past studies have reported the effect of ambient temperatures on the climate control energy requirements within greenhouses and plant factories (Weidner et al., 2021; Zhang & Kacira, 2020). However, as most of these studies focused on non-tropical conditions, heating loads were the primary consumer of energy in these systems, unlike in the present study where cooling rather than heating was required. Graamans et al. (2018) reported heating to be the biggest purchased energy load for greenhouses in Sweden, while cooling and dehumidification was the biggest electrical energy load in greenhouses situated in the United Arab Emirates (UAE). A similar observation was made by Zhang and Kacira (2020) who reported heating loads in the cooler climate of Duluth, Minnesota caused the overall electrical energy demand of greenhouses to increase between 50% to close to 100%, compared to greenhouses situated in warmer Phoenix, Arizona. Meanwhile, the lowest energy consumption was obtained in greenhouses situated in Riyadh, Saudi Arabia and Abu Dhabi, UAE (Zhang & Kacira, 2020). Weidner et al. (2021) reported a change in energy balance between greenhouses located in Stockholm, Sweden, and Singapore. While the supplemental lighting was the major energy load in Sweden, the cooling was the biggest load in Singapore, that has a tropical climate like Malaysia. Although these studies reported plant factories to have a higher electrical energy demand compared to greenhouses (Graamans et al., 2018;

Weidner et al., 2021; Zhang & Kacira, 2020), most of them found energy consumption in plant factories across locations to have similar energy consumption to one another, regardless of external climatic conditions (Graamans et al., 2018; Zhang & Kacira, 2020). Plant factories in Sweden, Netherlands, and the UAE (Graamans et al., 2018), and those in Saudi Arabia, UAE and the USA (Zhang & Kacira, 2020) did not have major differences in the overall energy requirements and energy balance, with higher lighting loads compared to cooling or heating, between locations. The findings from the current and past studies highlights the impact of the CEA structural type on the overall energy demand. While the use of transparent and opaque structures allows for the use of natural sunlight for plant growth, in tropical conditions, it also increases the internal temperatures of the CEAs, resulting in higher cooling requirements. In this study, the average daily cooling load in the GH was 139.21 W m^{-2} compared to 82.5 W m^{-2} for the PF.

Although the cooling load for the PF was significantly lower than that of the GH, it was observed that the overall electrical energy consumption of the PF was significantly higher, as the PF had cooling and lighting loads unlike the GH that only had a cooling load. As in past studies, the energy balance in the PF was dominated by the lighting requirements (Kozai et al., 2020; Zhang & Kacira, 2020). The total energy used per experimental cycle of 584.43 kWh m^{-2} in the GH was lower than that for 8H, the artificial lighting system in the PF that had the lowest total energy consumption of 689.45 kWh m^{-2}, for the same period (Figure 6.4). Past studies reported the annual lighting energy densities within PFs to be between 560 kWh m^{-2} $year^{-1}$ and 1374 kWh m^{-2} $year^{-1}$ (Vatistas et al., 2022). These studies however used lettuce and other leafy greens as its candidate plants unlike the current study that used Stevia. Comparable to past studies that had lighting loads that constituted between 50% to 86% of the total energy load (Graamans et al., 2020), the artificial lighting systems in the current study accounted for between 51% (BR) and 68% (FS) of the total electrical load in the PF. Contrasting with the PF that

required substantial amounts of additional energy input for lighting, the lighting requirement in the GH was fully provided by natural sunlight. While this may seem to be a bonus, as sunlight is free, this is associated with major drawbacks. While PF had a consistent PPFD throughout the photoperiod, the light intensity in GH varied throughout the day (Figure 6.3 C). Although the intensities within the GH do go beyond the values of the PF, this is only for a short period, typically between 11am and 3pm which reduces the functional photoperiod, where intensities are beyond 130 μmol $m^{-2}s^{-1}$, from 12 hours to a mere 4 hours a day. Plants that need higher intensities, and those that need longer photoperiods, will perceive this as a short-day condition and would begin to transition away from the vegetative stage (de Andrade et al., 2021). Where the vegetative plant parts are the desired product, this negatively impacts plant productivity, reducing yields, as observed in this study where the Stevia plants cultivated in the GH had metabolite yields of just 2.62 g m^{-2}, compared to the lowest yields obtained in the PF under whole cycle artificial light of 4.06 g m^{-2} under the RB treatment. It was also observed that as the light intensity in the GH reached a similar value as the PF at 130 μmol $m^{-2}s^{-1}$, the average ambient temperature increased to 36.08°C, while the average cooling load increased to 271.85 Wm^{-2}. The higher plant productivity in PF, albeit requiring higher electrical energy compared to GH, resulted in all artificial lighting treatments of spectrum Strategies 1 and 2 having significantly higher energy use efficacies over the GH and the base RB. FS, with the lowest EUE, was still 30% more efficient than the GH and RB, while GR2 that had the highest efficacy, was 540% more efficient. These findings are consisted with past studies that reported higher EUE and plant productivity in plant factories compared to greenhouses (Graamans et al., 2018; Kozai et al., 2020; Zhang & Kacira, 2020). The consistent environmental conditions, constant light intensity, and sufficient photoperiod in plant factories, were attributed to be the reasons behind this, as it permitted consistent yields and quality, regardless of seasons and other external factors. These findings outline

the importance of the CEA construction, especially in tropical environments. Past studies have highlighted the importance of greenhouse construction and selection of materials on the overall energy consumption (Engler & Krarti, 2021; Graamans et al., 2020). In this study it was noted that while a greenhouse type of construction permits the use of natural sunlight, it caused an increase in internal temperatures and required additional cooling energy compared to an enclosed plant factory type construction. Moreover, despite the relatively constant year-round photoperiod, the availability of natural light intensities in tropical conditions are not consistent: While there are no seasonal variations, there is frequent but intermittent cloud cover and rain which further reduces the effectiveness of the natural light in optimising plant productivity and EUE. Hence, for tropical conditions such as in Malaysia, an enclosed plant factory, insulated from the external conditions, under full artificial light would be the best option for consistent cultivation of non-native high valued herbs.

6.6 Conclusion

The findings of this study provided an insight into the energy requirements for greenhouse and plant factory type CEAs in a tropical condition. While GH consumed lower amounts of energy, the productivity and efficacies of the PF was far superior. Furthermore, it was observed that by selecting the optimised spectral content of artificial light, yields and efficacies can be further improved. It was also observed that the different lighting strategies have different benefits in terms of improving plant quality, quantity, or both. The selection of the most suitable light treatment should not only be based on its effect on plant productivity but also on its energy use efficacy. The PF under optimised light recipes would be the most productive and energy efficient approach towards cultivation of Stevia plants under tropical conditions.

CHAPTER 7 : CONCLUSION

This chapter provides the general conclusion of the thesis based on the results and discussion sections from chapters 3 to 6 and their relationship to the research objectives stated in Chapter 1. Here, the suggestions for future work as well as the novelty of this research are presented.

7.1 Conclusion

This study investigated the use of different solid state lighting strategies on the productivity and energy efficacies for indoor grown *Stevia rebaudiana* plants for use as a source of non-calorific sweetener food ingredient. Three different lighting strategies that used photoperiod, light quality (spectrum), and pre-harvest irradiation manipulation were evaluated. A total of 41 different light treatments were employed under these strategies. The research objectives were satisfied by these experiments and provide useful information that can be applied for indoor commercial cultivation of Stevia.

The work presented in Chapter 3, was an evaluation of the use of photoperiod manipulation as a lighting strategy to increase overall biomass and metabolite yields, as well as to improve the efficacy of the electrical energy used for indoor cultivation of *Stevia rebaudiana* in non-native environmental conditions. Stevia was grown under artificial lighting with red, green, and blue wavelengths with photoperiods of 8 h, 12 h, 16 h, and intermittent light amounting to 16/24 h, each with a constant Daily Light Integral (DLI). Yield was measured as leaf dry weight biomass in combination with Liquid chromatography–mass spectrometry (LCMS) analysis of Stevioside and Rebaudioside A content. Stevia plants under a continuous 16-h photoperiod (16H) had the highest productivity, resulting in the highest biomass accumulation and metabolite concentrations. The Stevioside and Rebaudioside A yields per plant were 975% higher than those obtained under natural daylight and day-neutral tropical photoperiod. Overall

energy use and photon conversion efficacies were also highest under 16H at 65.10 mg kWh^{-1} for biomass accumulation, 12.40 mg kWh^{-1} for metabolite yields and 7.5 mg mol^{-1} for photon conversion. These findings satisfied Research Objective 1 that was "*To identify the effects of artificial light intensity and photoperiod on the biomass and metabolite yields of indoor cultivated Stevia rebaudiana"* The findings in this chapter also demonstrated the potential optimization of energy used that can be realized by manipulating the intensity and photoperiod of artificial lighting within a PF. This approach can be realized with relative ease as it can be implemented simply by using a timer and dimming system. It is considered as a low hanging fruit for existing PFs that use the popular red+blue horticulture lighting systems. A simple switch from an 8-hour photoperiod to a 16-hour period resulted in 132% more metabolite yields for every kWh of electricity used.

Research Objective 2, "*To ascertain the effects of varying spectral compositions on the biomass and metabolite yields of indoor cultivated Stevia rebaudiana"* was achieved by the research presented in Chapter 4: Artificial lighting with LEDs was used to determine if different spectral compositions within and outside of the photosynthetically active radiation (PAR) range can be used to improve germination rates and yields for production of steviol glycosides in Stevia. Plants treated with red and blue light at an intensity of 130 µmol $m^{-2}s^{-1}$ supplemented with 5% of UV-A light under a 16-hour photoperiod produced the most desirable overall results with a high rate of germination, low percentage of early flowering, and high yields of dry leaf, Stevioside and Rebaudioside A, 175 days after planting. While red and blue light combinations are effective for plant growth, the use of supplemental non-PAR irradiation of UV-A wavelength significantly and desirably delayed flowering, enhanced germination, biomass, Rebaudioside A and Stevioside yields, while supplemental green light improved yield of biomass and Rebaudioside A, but not Stevioside. Overall, the combination of RB + UVA light resulted in the best

overall productivity for *Stevia rebaudiana*. The findings presented in this chapter showed how plant quality and quantity can be further improved by using alternative spectral contents that were previously neglected due to the low effect on a plant's photosynthetic rate. Using UV-A, green and far red, the metabolite yields were boosted significantly over photoperiod manipulation. These findings have potential application for indoor cultivation of Stevia and other medicinal plants at a commercial scale where both the biomass (quantity) and metabolite (quality) are of commercial importance.

Chapter 5 reported the findings of two experiments. In the first experiment, Stevia plants were germinated and grown under artificial light treatments that had different fractions of green, UV-A and a combination of green and UV-A spectral content in addition to a base red and blue light. All treatments had the same DLIs, intensities and photoperiods. It was observed that the while the different fractions did not influence the rate of germination compared to natural sunlight of the greenhouse, all treatments resulted in significant delays in flowering with the lowest rate of flowering in UV1 treatment that had 8.5% of UV-A spectral content with a base red-blue light. The highest dry biomass yields were obtained in treatments that had both green and UV-A spectral content of UVGR1 and UVGR2. Meanwhile, the highest concentrations of ST and Reb A metabolites were observed under treatments that had either UV-A or green, with UV1, UV2 and GR2 recording the highest concentrations. The highest realisable metabolite, represented by g plant^{-1} was under UVGR1 and GR2. In the pre-harvest experiments, plants cultivated under RB displayed a positive response to pre-harvest treatments, resulting in an increase of dry biomass accumulation under all treatments. Conversely, the dry biomass yields of plants originating from the greenhouse (GH) were not affected by the pre-harvest treatments. Plant quality saw an increase under pre-harvest treatments with plants that were grown under either the GH or RB resulting in higher metabolite concentrations under the different treatments. These improvements were however not

sufficient to improve the overall metabolite yields of the plants due to the poor biomass yield improvements. It was also observed that a 3-day pre-harvest treatment was sufficient to induce higher metabolite concentrations. Overall, from these experiments, it was seen that green and UV-A light fractions when used with a base red and blue light are able to further improve the quality and quantity of indoor grown Stevia. Meanwhile, the pre-harvest strategies, while not producing significantly improved biomass yield offer a viable option to improve the overall plant quality. These findings fulfil Research Objective 3 that was "*To investigate the effects of varying Green and Ultraviolet A (UVA) spectral fractions, and the use of Green and UVA pre-harvest treatments on the biomass and metabolite yields of indoor cultivated Stevia rebaudiana*". The findings of this chapter have a potential impact on indoor cultivation of Stevia. It was demonstrated that by using higher fractions of green and UV-A spectra, the overall realisable yields of the plant can be significantly increased. While the improvements in terms of plant quality was lower than what was observed in the work reported in Chapter 4, the biomass yields reported in chapter 5 were significantly higher. The positive effects of a short 3-day pre-harvest treatment on the metabolite concentrations in Stevia was also observed.

The energy and environmental conditions of both the GH and PF were evaluated in Chapter 6. The electrical energy consumption, temperature and light intensity profile was recorded over a 7 to 10-day period. Besides that, the overall electrical energy consumed during the entire growth cycle for the different light treatments used in the previous chapters were analysed. It was observed that ambient weather conditions had a profound effect on the energy demand of the GH, increasing with an increase in ambient temperatures, while the PF was immune to this fluctuation in external conditions. Overall, the full spectrum FS consumed the highest energy per growth cycle while GH had the lowest. It was also observed that the lighting systems consumed more than 50% of the electrical energy used in the various PF. While GH had the lowest energy consumption,

the overall energy use efficacy (EUE) of GH was also the lowest due to lower yields. The highest EUE was obtained in UVGR1 and GR2. All treatments under SS 2 that used green and UV-A fraction had significantly higher EUE values compared to all other strategies. This strategy also had the highest photon conversion efficacies (PCE) compared to all others even though the photoperiod systems had a higher photosynthetic photon efficacy (PPE). The findings of this part of the study illustrated the overall effect of the different strategies on the EUE and PCE, and also highlighted the limitations of using PPE alone as a measure of efficacy. This work also reinforces the potential use of different solid state lighting strategies to not only improve productivity but also energy use efficacies for indoor grown Stevia plants. Research Objective 4 "*To determine the lighting strategy that has the highest productivity and energy use efficacy for the indoor cultivation of Stevia rebaudiana*" was addressed in this chapter.

The interdisciplinary nature of agriculture engineering was observed throughout this study. It should be noted that for a successful indoor cultivation of *Stevia rebaudiana* or any other high valued plants, both the engineering and plant biological aspects must be optimized. Besides the effects of the different light parameters (intensity, photoperiod, spectrum) on the productivity and energy usage of the setup, the overall structural construction plays a major role too. Selecting the best design and materials can have a direct impact on the overall performance of the facility from the yield and cost perspective. Having a plant genotype that has been optimised for indoor cultivation can further boost the economics of an indoor agricultural setup. It is also imperative that the right type of crop, the most economically beneficial, be selected. This will add to the economic sustainability of indoor cultivation, making it a longer-term solution.

7.2 Novelty of Research

This study presented several new findings that were not previously reported in past literature. This is the first study to use green, UV-A and far-red spectral content together with red and blue light on Stevia plants. Unlike previous studies that limited the scope of research to either germination, flowering or growth, the present study presented a holistic approach, studying the effects of the different lighting strategies across all stages of the Stevia growth lifecycle, from germination to harvest. The current study is also the first study to use different fractions of green and UV-A, and to explore the use of pre-harvest treatments on Stevia. No other studies have reported the EUE, PCE and total energy used of indoor grown Stevia plants nor have there been any study conducted in the tropical climate of Malaysia.

7.3 Suggestions for Future Work

The use of LEDs as a sole source of light for indoor cultivation is an area of interest. Future work should consider the use of low and mid power LEDs as this will allow for a greater distribution of light while producing less heat. As low and mid-power LEDs do not require large heatsinks like the high-powered LEDs used for this experiments, their use can result in lower implementations costs. The use of alternative spectral ranges such as ultraviolet-B (UV-B), amber and lime should also be explored. Dynamic lighting is another approach to consider for future work, where the light intensity, photoperiod and spectral composition is varied across the different stages of plant growth. This would enable a more optimised light recipe, improving the EUE and PCE values. Future work may also involve the use of genotypes of Stevia that have been genetically improved to provide higher biomass and metabolite yields. The present study used commercially available seeds that were not subjected to selective breeding and selection. When used with an optimised lighting strategy, a superior genotype may be able to further improve yields, increasing commercial viability while enhancing energy efficacies. An in-depth

study on the effects of the different spectral composition, intensities and photoperiod on the expression levels of the various genes within the Stevia biosynthetic pathway has great potential for future research activities. This would enable further optimisation to improve the yields of higher value glycosides increasing the overall commercial viability of indoor cultivation of *Stevia rebaudiana* plants. As the use of artificial lighting and the general cultivation of *Stevia rebaudiana* indoors requires additional electrical energy input, more detailed studies should be conducted to evaluate the impact of these additional needs on the overall carbon footprint of these facilities and setups. A comparable study, evaluating the full cycle carbon footprint of indoor and outdoor cultivated Stevia plants would provide a greater understanding of the overall environmental impact of the different cultivation approaches.

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