Advancing Understanding of LED Light Impacts on Plant Phytonutrient Content for Application into Hydroponically Grown Leafy Greens.

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AUTHOR'S DECLARATION

"I hereby declare that this thesis is my original work, except where reference is made to other sources, and the work has not been submitted elsewhere, in whole or in part, to qualify for any other academic award of a higher degree elsewhere. All sections of this thesis with joint research/ collaborators have been appropriately acknowledged and referenced."

Candidate: Phoebe Sutton Date: 28th September 2022

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THESIS ABSTRACT

The majority of global population increases in the 21st Century will be concentrated in urban centres, where 'Hidden hunger', diets devoid of micronutrients, is common. Vertical farming is uniquely poised to address this shortfall due to its ability to produce food intensively under tailored LED lighting spectra. This thesis explores the contribution of light wavelengths and signalling pathways on the genetic expression of nutrient biosynthesis genes for carotenoid (pro-Vitamin A), tocopherol (Vitamin E) and ascorbic acid (Vitamin C) pathways, essential micronutrients that must be consumed via the diet, in *Arabidopsis* (Chapter 2). In addition, the impact of spectral quality on the growth and content of these nutrients was evaluated in Basil (Chapter 3) and Coriander (Chapter 4), two high value herbaceous crops commonly grown hydroponically in vertical farms.

Using publically available transcriptomic datasets, it was found that the transcriptional response of flux controlling genes to light quality in *Arabidopsis* was unique to each gene, but in general, MEP pathway genes are stimulated by red light (RL), whereas carotenoids are upregulated by both RL and blue light (BL). This study also found a novel increase in expression of tocopherol and ascorbic acid biosynthesis genes to BL. Crucially these responses were linked with photoreceptor input and associated light signalling components, including circadian input in all four pathways examined.

This thesis also examines the content of these phytonutrients and growth responses in two crop species, Basil and Coriander, grown under differing light regimes in controlled environments. The best overall commercially viable growth of Basil and Coriander was found in 25% BL 75% RL ($B_{25}R_{75}$) and $B_{50}R_{50}$, respectively. Whereas, ascorbic acid increases were associated with RL in Basil, and tocopherol and ascorbic acid increased with BL in Coriander.

This research has contributed new mechanistic understandings into the light regulation of phytonutrient pathways in *Arabidopsis*, and provided important insights into the differing effects of LED lighting regimes on phytonutrient content in valuable crop species. Such work constitutes a step towards the application of tailored spectra into Controlled Environment Agriculture and realising the potential of light quality to provide fresh and nutritious produce to urban populations.

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LIST OF ABBREVIATIONS

AA	Ascorbic Acid
ABA	Abscisic Acid
AsA	Ascorbate
A_{sat}	Saturated Light Assimilation Rate
B_{100}	100% blue light
$B_{75}R_{25}$	75% blue light 25% red light
$B_{50}R_{50}$	50% blue light 50% red light
$B_{25}R_{75}$	25% blue light 75% red light
BL	Blue light
βLCY	*LYCOPENE B-CYCLASE*
βOHase	*β-CAROTENE HYDROXYLASE*
CCA1	*CIRCADIAN CLOCK-ASSOCIATED 1*
CEA	Controlled Environment Agriculture
Chl	Chlorophyll
CMK	*4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase*
COP1	CONSTITUTIVELY PHOTOMORPHOGENIC 1
CRY	Cryptochrome
DHA	Dehydroascorbic Acid
DMAPP	Dimethylallyl Diphosphate
DXP	1-deoxy-D-xylulose 5-phosphate
DXR	*DXP REDUCTOISOMERASE*
DXS	*DXP-SYNTHASE*
εLCY	*LYCOPENE E-CYCLASE*
εOHase	*E-CAROTENE HYDROXYLASE*
FR	Far-Red
Fv/Fm	Photosynthetic Efficiency
GAP	D-glyceraldehyde 3-phosphate
GGDP	geranylgeranyl diphosphate
GGPP	Geranylgeranyl Diphosphate
GGP	*GDP-L-GALACTOSE PHOSPHORYLASE*
GH	Glasshouse
GL	Green Light
GME	*GDP-D-MANNOSE EPIMERASE*
GMP	*GDP-D-mannose pyrophosphorylase*
GSSG	Glutathione Di-sulphide
GSH	Glutathione
HDR	*HMBPP REDUCTASE*
HDS	*HMBPP SYNTHASE*
HGA	Homogentisic Acid
HIR	High Irradiance Response
HMPP	*4- hydroxy-3-methylbut-2-enyldiphosphate*
Hp	Hydroponics
HY5	*ELONGATED HYPOCOTYL 5*
IPP	Isopentenyl Diphosphate
LD	Long Day
LED	Light Emitting Diode
LFR	Low Fluence Response

MCT	*2-C-methyl-d- erythritol 4-phosphate cytidylyltransferase*
MDS	*2-C- methyl-D-erythritol 2,4-cyclodiphosphate synthase*
MEP	2-*C*-methyl-D-erythritol 4-phosphate
MPBQ	2,3-dimethyl-6-phytyl-1,4-benzoquinol
MVA	Mevalonic Acid
NPQ	Non-Photochemical Quenching
ORF	Open Reading Frame
PAR	Photosynthetically Active Radiation
PDP	Phytyl-diphosphate
PFD	Photon Flux Density
PFALs	Plant Factories with Artificial Lighting
ϕ	Quantum Yield
Phy	phytochrome
PIFs	Phytochrome Interacting Factors
PPFD	Photosynthetic Photon Flux Density
PRR	Pseudo-Response Regulator
PS	Photosystem
PSY	*PHYTOENE SYNTHASE*
qE	Energy Dependent Quenching
R_{100}	100% red light
RL	Red light
RLRC	Rapid Light Response Curve
ROS	Reactive Oxygen Species
RUPs	REPRESSOR OF UV-B PHOTOMORPHOGENESIS
SD	Short Day
Sn	Snijder Cabinet
tAA	Total Ascorbic Acid
TOC1	TIMING OF CAB EXPRESSION 1
UA	Urban Agriculture
UV	Ultraviolet
UVR8	UV RESISTANCE LOCUS 8
VAD	Vitamin A Deficiency
VDE	VIOLAXANTHIN DE-EPOXIDASE
VLFR	Very Low Fluence Response
VTC	VITAMIN C
VTE	VITAMIN E
ZEP	ZEAXANTHIN EPOXIDASE
ZT	Zeitgeber

CHAPTER 1

General Introduction

1.1. The Past, Present and Future of Agriculture

1.1.1. The Creation of Modern Agriculture

The world is on the precipice of a food production crisis, facing a triple threat of population growth, climate change and ecological breakdown (Ehrlich and Harte, 2015). When faced with a similar production crisis in the late 20th Century, the world undertook a Green Revolution (Wik et al., 2008). This introduced conventional intensified agricultural methods such as mechanisation, widespread use of chemical pesticides, herbicides, and fertilisers, methodical irrigation, and genetic modification (Pingali, 2012). However, the agroecosystems created have and continue to produce negative consequences with significant ecological implications for soil compaction, biodiversity loss (Defries and Uriarte, 2010), pollution of water bodies (Howarth et al., 1996), destruction of non-target species (Zaller and Brühl, 2019), and the rise of herbicide resistant weeds (Gilbert, 2013). Overall, since their creation, modern agroecosystems have caused a 60 % degradation in ecosystem services (Corvalan et al., 2005). This does not include the other fundamental inputs required into the current agriculture system, which include water, fossil fuels and land. FAO-UN (2017) reports that agriculture uses around 70 % of the fresh water in the world, increasing to 90 % in low rainfall areas, due to water exploitation from rivers and aquifers (FAO, 2017). Moreover, agriculture contributes 25 % of annual global greenhouse gases. Contributors to this include the manufacture of fertiliser (Matson et al., 1997) and the use of fossil fuels to power farming machinery and transportation of vegetable and fruit crops through global food chains, which together can account for as much as a third of the carbon footprint (M. Li et al., 2022). Finally, deforestation has accelerated since the green revolution (Defries and Uriarte, 2010), with the clearing of virgin land reducing the sequestration of carbon in these ecosystems (Krankina and Harmon, 2006).

As it functions currently, the global food production system is incompatible with ecological stability, sustainability, and current carbon targets (Matson et al., 1997; Pingali, 2012). A Second Green Revolution is required, one that recognises and mitigates the limitations of the first. Focus must be on “sustainable development” to tackle the effects of environmental

breakdown whilst increasing food production up to 70 % to feed a predicted global population of 9.8 billion by 2050 (FAO, 2009).

1.1.2. Urban Agriculture to Address the Nutritional Shortfall within Urban Populations

The future global population is predicted to be increasingly urban. By 2017, more than half the world, 4.1 billion people, had transitioned to live in urban areas. In the UK, 83 % of the population live in a city, and is expected to rise to 90 % by 2050 (United Nations Department of Economic and Soical Affairs, 2018). As populations shift from rural to urban, there is an accompanying employment shift from agriculture to industry or services. As a result, food production decreases, creating food insecurity within urban centres. In 2018, the FAO reported that 10 % of children (~ 3 million) and up to 16 % of adults with children suffer from food insecurity and food poverty in the UK (Corfe, 2018).

Areas devoid of access to fresh fruit and vegetables are called 'Food Deserts', and 76 % of UK Food Deserts are within cities (Wrigley, 2002). Urban Food Deserts correlate with a 'Hidden Hunger', where the overall calorie need is reached but there is an inadequate consumption of micronutrients, essential dietary compounds present in vegetables and fruits that are required for healthy bodily function (Ruel et al., 2017). At least one-third of the world's population suffers from malnutrition (Bailey et al., 2015), and over 2 billion of these are suffering from Hidden Hunger, which is associated with long-lasting effects on health, learning ability and economic loss (Thompson and Amoroso, 2004). As a result, the location of food deserts mirror incidence of mortality, from heart disease, to cancer, diabetes, and stroke, and adverse health effects including anaemia, depression of the immune system, and asthma (Smith, 2016; Wrigley, 2002). As urban populations continue to rise, so too will the incidence of food deserts, hidden hunger and the malaise associated.

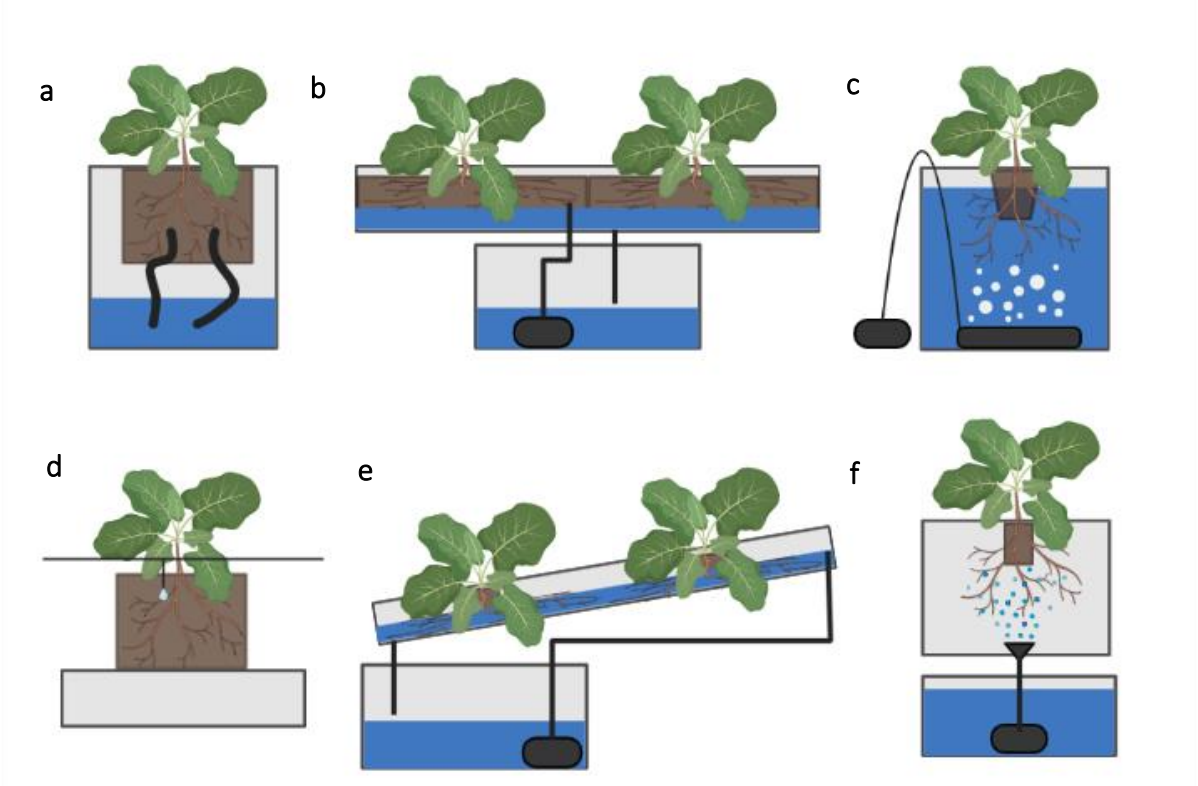
To deliver sustainable fresh food sources to address both the climate and health related aspects of the food supply, wherever possible food must be produced where populations reside (Li et al., 2022). Urban Agriculture (UA) is gaining traction worldwide to provide local, fresh food within cities by repurposing urban land for food production, processing and distribution (Armanda et al., 2019; Skar et al., 2020). This change to the food system can have

a multitude of social, economic and environmental benefits; including increased food and income security, local development and employment, greater fruit and vegetable consumption, improved physical and mental wellbeing, as well as increased knowledge and appreciation of food production within the population (Audate et al., 2019; Hoornweg and Munro-Faure, 2008). When implemented properly, these agriculture systems combat food insecurity in cities (Siegner et al., 2018), and will play a key role in remodelling and designing future cities (Gasperi et al., 2016; Skar et al., 2020) to fulfil the commitment in the UN Sustainable Development Goal 11, to making cities inclusive, safe, resilient, and sustainable. The movement is steadily growing with more than 800 million people involved in a form of UA in developed and developing countries (Edmondson et al., 2020; FAO, 2012).

1.1.3. Light Emitting Diode Driven Hydroponic Systems

The most technologically advanced form of UA within fully enclosed controlled environment agriculture (CEA) utilises two key technologies: hydroponics and LED lighting. Hydroponics of various forms have been used worldwide for over 40 years (Vermeulen et al., 2014). However, recent developments and reduced production cost of lighting emitting diode (LED) technology in conjuction with pressures on food production and carbon reduction have projected the potential of these technologies forward to create a commercially viable form of urban agriculture (Kozai and Niu, 2018).

Figure 1.1 Hydroponic methods of growing plants. The most commonly used forms of hydroponics include, (a) wick system – nutrient solution is drawn up a conductive material passively from a reservoir to the plant roots; (b) flood and drain – nutrient solution held in a reservoir is pumped up to flood a tray containing the plants which then drains back down; (c) deep-water culture – plants are suspended on top of the reservoir with the roots submerged into the aerated nutrient solution; (d) drip irrigation – nutrient solution is gradually added to rockwool substrate, commonly used in glasshouse production; (e) nutrient film technique – nutrient solution is continually pumped from the reservoir and passed over the roots of the plants located in a slanted tray or gulley; and (f) aeroponics – nutrient solution is delivered to plant roots by atomisation to create a fine mist. Created with BioRender.com



Hydroponics is a method of growing produce in a non-soil medium with plant nutritional needs supplied by a nutrient solution (Jones, 1982). Hydroponic systems can grow a wide variety of useful crops including, leafy greens, tomatoes, cucumbers, peppers, strawberries and microgreens (Swain et al., 2021). Hydroponic systems can supply the nutrient solution to plants in a variety of ways (Figure 1.1) that can be more suitable for particular crops. For example, many herb crops grow well in an ebb and flow system, where nutrient solution is supplied to a tray within which the crop is grown and then drains back to be used again. (Swain et al., 2021). It is increasingly common for hydroponic technologies to be paired with LED lighting. The rapid development of LED technology in the last decade holds many advantages over traditionally used horticultural lights such as fluorescent or High Pressure Sodium lamps (Bourget, 2008; Morrow, 2008; Wallace and Both, 2016). Firstly, LEDs are designed to generate selected wavelengths within small waveband range, which can be used to drive photosynthesis and to supply specific lighting needs of the crop (Izzo and Gómez, 2018; Massa et al., 2008). Secondly, LEDs are more efficient in converting energy to light, saving on 30 % electricity costs thereby also decreasing the carbon footprint (Kuijpers et al., 2021). Thirdly, their efficiency means they generate less heat giving them a cool emitting surface, allowing closer placement of the plants to the LED units without damage (Bourget, 2008; Izzo and Gómez, 2018). This

final feature makes LED lights particularly attractive for one of the most popular forms of urban agriculture, vertical farming.

1.1.4. Farming Vertically

Dickson Despommier was the first to popularise the idea of the vertical farm to the agriculture industry over a decade ago (Despommier, 2010), and today vertical farms can be found all over the world (Kozai et al., 2020). In the vertical farm, layers of crops are grown stacked on top of each other in a hydroponic system, with LED lights mounted on the underside of the shelving units (Despommier, 2013; Eigenbrod and Gruda, 2015; Thomaier et al., 2014). By supplying the lighting and nutritional needs of plants, vertical farms can be entirely self-contained units as small or large as required. Such systems can be installed in skycrapers (Despommier, 2010), building-integrated agriculture (Eigenbrod and Gruda, 2015), retrofitted into existing buildings (Specht et al., 2014), even growing in size to industrial, high scale intensive farm installations called plant factories with artificial lighting (PFALs), which output tonnes of produce a day (Kozai et al., 2020). By constructing farms in this way, vertical food production maximises land use efficiency (Barbosa et al., 2015), and produces more yield per unit area compared to horizontal hydroponic systems (Touliatos et al., 2016).

Some barriers into this latest agricultural innovation remain. The greatest criticism of CEA is the energy expense, mostly accounted to artificial lighting, particularly during a global energy crisis (Vaughan, 2022). However, this can be mitigated via sustainable energy generation combined with energy efficient design (Engler and Krarti, 2022), the implementation of lighting regimes optimised for energy use efficiency (Buyeye et al., 2022; de Carbonnel et al., 2022), or by growing the highest value crops. Other barriers include a lack of knowledge transfer within the industry, in addition to high capital investment costs for basic equipment requirements, such as pumps, tanks and lights (Warren et al., 2015). However, a PFAL and greenhouse system can be considered similar investments today due to the higher yield potential from vertical farming (Kozai et al., 2020). Moreover, the initial cost for a PFAL is expected to decrease over time (Kozai et al., 2020) especially as the number of companies specialising as systems providers providing ‘plug and play’ designs increase.

1.1.5. Opportunities in Vertical Farming

Urban vertical farming with LED lighting has grown in popularity due to its unique position to address the many shortfalls of modern agriculture, particularly in terms of increased yield and improved access to fresh produce for urban populations. Due to the fully controlled nature of the growth technique, these systems are attractive to academic researchers as they are highly suited to developing an intricate understanding of a cause-effect relationship between a single factor and plant growth, such as the light quality or nutrient solution composition. There is also great commercial interest in incorporating these advances into optimised growth 'recipes' that maximise growth potential of a chosen crop or target characteristic (de Carbonnel et al., 2022). Interest lies not only with elevating yield and reducing inputs but also on specialised crop production for plant-based compounds valuable for the cosmetic, aromatic, and pharmaceutical industries. The vast potential of vertical farming has driven over $1 bn of investments in 2021, with growth estimates up to $9.7 bn worldwide by 2026 (Namkung, 2022). In the near future, the range of plants grown in PFALs could satisfy local market demands for fresh salads, frozen and dried vegetables, sauces and pastes (Kozai et al., 2020).

As we move into this new era of indoor farming, it is vital to undertake extensive research into one of the most nuanced controllable inputs into vertical farms: the LED light control. The inherent features of LED lights allow for infinite variation of light spectral quality, intensity and duration, all of which have an impact on plant growth responses (Kozai, 2016). A growing body of evidence has demonstrated that plant nutritional content and the activity of antioxidant compounds including carotenoids, tocopherols and ascorbic acid (AA), is closely linked to lighting conditions due to photo-oxidative changes in plants (Samuoliene and Duchovskis, 2012; Taulavuori et al., 2017). Therefore, there is a great opportunity for vertical farms to supply a light environment tailored to consistently produce large quantities of fresh, locally produced and nutritionally enhanced crops to alleviate urban hidden hunger for antioxidant micronutrients and the maladies associated (SharathKumar et al., 2020).

1.2. Antioxidants are Essential for Living Organisms

1.2.1. Antioxidants Counteract Reactive Oxygen Species

Oxygen is infinitely useful in cellular processes due it its affinity for electrons. Therefore, oxygen derivatives are present in living systems as highly reactive oxidising molecules called reactive oxygen species (ROS) or free radicals. The most common are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$), ozone (O_3) and singlet oxygen ($^1O_2$). They are formed from a wide range of biochemical and physiological processes, and can therefore originate from endogenous sources, such as the mitochondria and chloroplast, or exogenous sources, for example ultraviolet light and environmental toxins (Krumova and Cosa, 2016).

The role of ROS in a living system is wide ranging and complex, and include use as an essential signalling network. In plants, ROS aid in the growth, development, metabolism and response to biotic and abiotic stresses (Mittler et al., 2022). Within humans, ROS function at the cellular level to regulate growth, programmed cell death, and signalling, whilst at the system level they can influence blood pressure, as well as cognitive and immune system function (Brieger et al., 2012). For this reason, there is a constant flow of production of ROS in healthy living systems (Sies and Jones, 2020). However, when these molecules are present in excess, this can result in uncontrolled oxidation leading to damage to nucleic acids, lipids, and proteins. This action has been linked to cell apoptosis and aging (Harman, 2002) and as a causal factors in several pathologies including cardio-vascular disease, diabetes, rheumatoid arthritis, cancer, and neurodegenerative disorders, including Alzheimer's and Parkinson's disease (Shenkin, 2006; Valko et al., 2007).

Antioxidants counteract oxidative damage to biomolecules caused by ROS. They may function as electron donors or acceptors, to stop the oxidising potential of ROS directly thereby preventing further chain oxidisation reactions, or by neutralising damaged cellular components (Monsen, 2000). Therefore, where the localisation of ROS production is implicated, antioxidant scavenging systems are also present to keep the system in equilibrium.

1.2.2. Phytonutrient Function in Plants and Humans

Many antioxidant micronutrients originate in plants, the most ubiquitous being glutathione and AA, including other isoprenoid protective pigments and compounds. Isoprenoids are the

largest and most diverse group of metabolites in all living organisms, with over 35,000 reported (Hunter, 2007). There are two sources of isoprenoids, the cytosolic mevalonic acid (MVA) pathway or the chloroplastic 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway (Hemmerlin et al., 2012; Lichtenthaler et al., 1997). Due to its chloroplastic location, the MEP pathway and its products are very sensitive to light (Ghassemian et al., 2006). The extensive nature of the isoprenoid family means that its products, in particular those from the MEP pathway, hold vital roles for plant survival including in photosynthesis (chlorophylls, plastoquinone) and respiration (ubiquinone), in hormonal regulation of growth and development (cytokinins, gibberellins, abscisic acid (ABA)) and as antioxidants, such as carotenoids and tocopherols (Figure 1.2) (Chappell, 2003; Croteau et al., 2000; Rodríguez-Concepción, 2006).

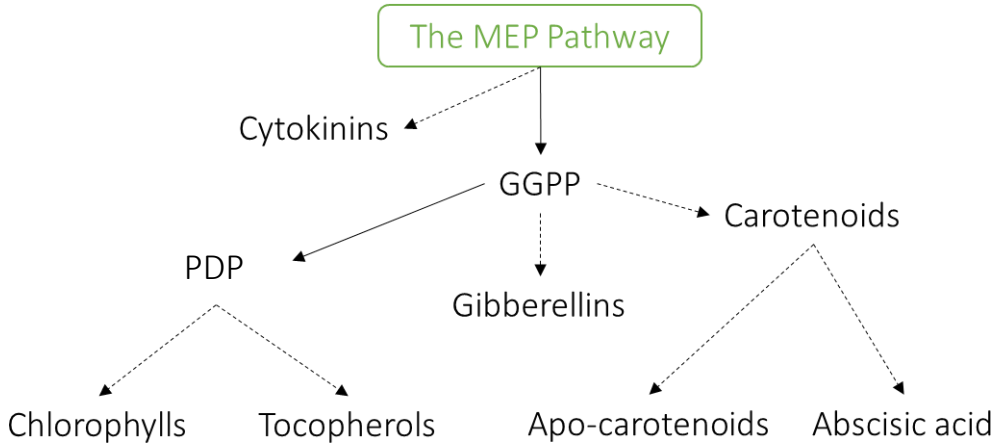


Figure 1.2 A variety of compounds are derived from the MEP pathway. The MEP Pathway is the source of isoprenoid compounds that have wide range of active roles in plant development. The resultant molecule geranylgeranyl diphosphate (GGDP) is the precursor of carotenoids, gibberellins, and the secondary molecule phytyl-diphosphate (PDP), which derives chlorophylls and tocopherols.

For humans, there are 30 essential micronutrients that can only be accessed via the diet with the exception of Vitamin D (Shergill-Bonner, 2017). Included in this list are plant-derived carotenoids and tocopherols, together with AA, which are antioxidants and micronutrients for humans. As such, they are essential elements for bodily function and long-term health and deficiencies will manifest as identifiable deficiency-associated disease. These micronutrients have wide ranging roles as co-factors in enzyme activity, metabolism and the production of

proteins, and protection from the oxidative damage of ROS (Shergill-Bonner, 2017). In addition, they exhibit anti-fungal, -bacterial, and –viral effects (Lattanzio et al., 2006; Seigler, 2001), and as such, have a high medicinal value. Of the 30 dietary micronutrients, some of the highest required are pro-Vitamin A (carotenoids), Vitamin E (tocopherols) and Vitamin C (ascorbic acid) (Geissler and Powers, 2005).

1.2.2.1. Carotenoids (Pro-Vitamin A)

Carotenoids are lipophilic C40 isoprenoids derived from the MEP pathway found within archaea, bacteria, fungi, algae and higher plants. The name carotenoids encompasses several types of molecules that are referred to as carotenes, until oxygenated when they become xanthophylls (DellaPenna and Pogson, 2006). After the combination of MEP pathway precursors, carotenoids are linear molecules until a branching point after the formation of lycopene (Figure 1.3). These branches are defined by molecules containing either a β-ionone and ε-ionone ring (β, ε), such as α-carotene that is oxygented to lutein (the most abundant xanthophyll in leaves), or two β-ionone rings (β, β) which contains β -carotene and zeaxanthin. Moreover, the xanthophylls of the β, β branch can derive other phytohormones such as the plant hormone abscisic acid (ABA) and strigolactones, important for plant growth and development (Cutriss and Pogson, 2006; Niyogi, 1999; Rock and Zeevaart, 1991).

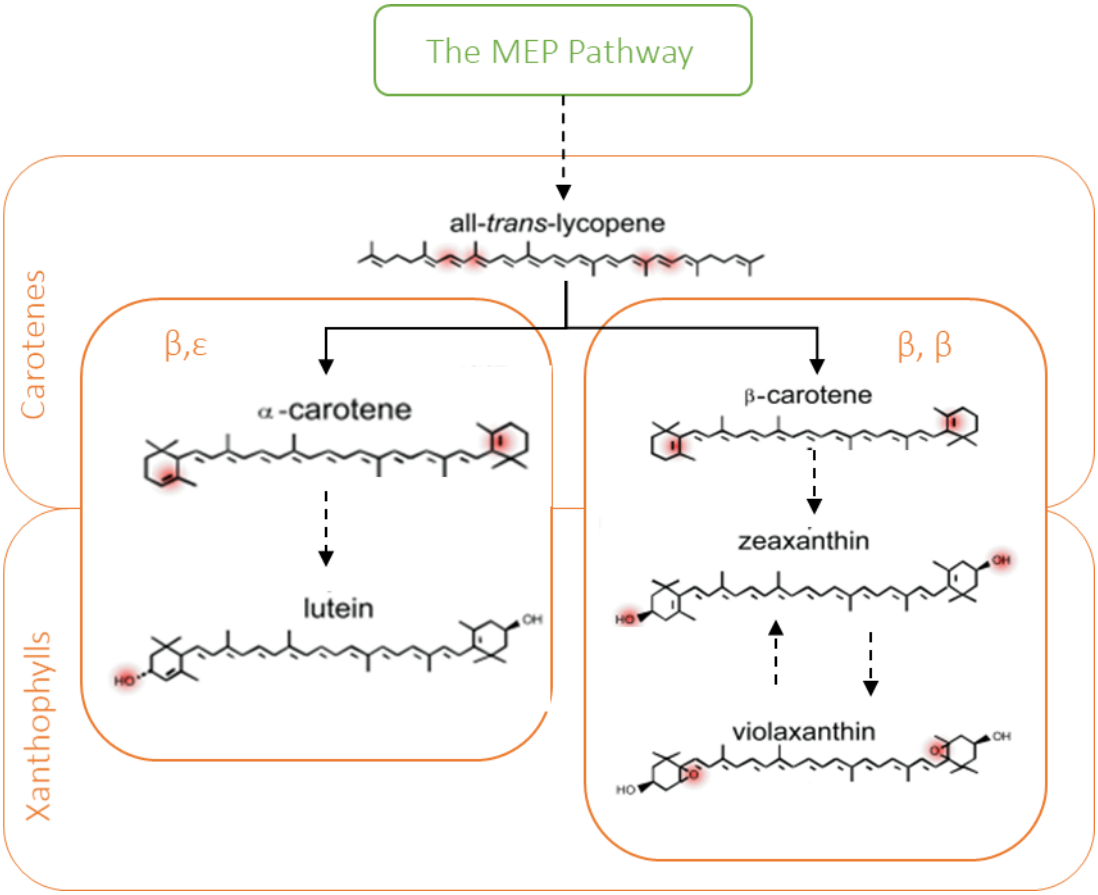


Figure 1.3 The chemical changes within the carotenoid pathway. Precursors from the MEP pathway generate linear carotenes culminating in lycopene, which branches into the β, ε branch and β, β branch, each defined by the addition of β-cyling or ε-cyling rings. The beginning of these branches a result in α-carotene and β-carotene respectively. These molecules are then oxygenated to the xanthophyll group, including lutein from the β, ε branch and zeaxanthin and violaxanthin from the β, β branch. Modifed from DellaPenna and Pogson (2006).

Within planta, carotenoid production is first stimulated by light upon emergence (Bouvier et al., 2005). Throughout the lifecycle carotenoids accumulate mainly in the leaves and fruits in chloroplasts and chromoplasts, respectively (Asensi-Fabado and Munne-Bosch, 2010). When sequestered in chromoplasts, carotenoids play an important role as colouring pigments and contribute to a plant's odour profile (Simkin et al., 2004; Sun et al., 2018). In chloroplasts, carotenoids play a vital role in photosystem assembly, light-harvesting complexes and photoprotection (Howitt and Pogson, 2006). Lutein, β-carotene and xanthophylls function as accessory pigments and structural elements of light-harvesting complexes absorbing light in

the blue and green spectra for photosynthesis, (Demmig-Adams and Adams, 1992; Holt et al., 2005; Liu et al., 2004; Pogson et al., 1996). When light harvesting causes the formation of ROS, carotenes and xanthophylls engage in non-quenching photoprotection and protect the light harvesting-complex from oxidative damage caused by excess excitation energy by dissipating it as heat (Holt et al., 2004). 'The Xanthophyll Cycle' between zeaxanthin and violaxanthin is the main mechanism by which carotenoids protect the photosynthetic machinery from bleaching, photoinhibition and UV-B exposure (Emiliani et al., 2018). It is theorised for xanthophylls to operate in similar functions across taxa in higher plants, as they are highly conserved (Niyogi et al., 2001). As their photoprotective function suggests, under high light, total xanthophyll content will increase up to 5-fold, a trend which reverses in low light (Falbel et al., 1994; Niyogi et al., 1998).

Because humans cannot synthesise vitamin A (retinol), carotenoids (pro-Vitamin A) are considered an essential dietary component for human health (Fraser and Bramley, 2004). Of these, β-carotene is the most common type of provitamin A in foods (Figure 1.3) (National Institutes of Health, 2021). However, the other carotenoids have a beneficial effect by providing antioxidant activity *in vivo* (Fiedor and Burda, 2014), in addition to anticancer and antifungal effects (Mayne, 1996; Palozza and Krinsky, 1992). Vitamin A has an essential role in numerous functions, as a chromophore (retinals) and a hormone (retinoic acids) for normal functioning of the visual system, cell growth, red blood cell production, immunity and reproduction (von Lintig et al., 2005). Vitamin A deficiency (VAD) during nutritionally demanding life stages in infants, young children and pregnant women can create Vitamin A deficiency disorders, the most common of which is xerophthalmia, the leading preventable cause of blindness in children in the world (Sommer and West, 1996). VAD also manifests as night blindness, often in pregnant women (Christian et al., 1998), anaemia (West et al., 2007), and can worsen infections (Scrimshaw et al., 1968). Significantly, Vitamin A supplementation has been shown to reduce mortality in children aged 6 – 59 months-old by 23 – 30 % (Fawzi et al., 1993), and in new mothers by 40 % (West et al., 1999). A recommended dietary intake of 3 – 6 mg β-carotene per day is therefore essential to provide Vitamin A precursors to avoid deficiency-associated disease (West and Darnton-Hill, 2008; Nutri-facts, 2020).

1.2.2.2. Tocopherols (Vitamin E)

Vitamin E consists of a collective group of lipid-soluble molecules constituting four tocopherols and four less prevalent tocotrienols. This group are amphipathic, containing a saturated tail (phytyl-PP) derived from the MEP Pathway, and a polar aromatic head group (homogentisic acid, HGA) from the Shikimate pathway (Mène-Saffrané, 2017). The four tocopherols, α-, β-, γ- and δ-tocopherol, are determined by the number and position of methyl substituents on the aromatic ring (DellaPenna and Pogson, 2006) (Figure 1.4). α-tocopherol has the highest Vitamin E activity of all tocopherols (Kamal-Eldin and Appelqvist, 1996; Saini and Keum, 2016). Similar to β-carotene, α-tocopherol is unanimously present in all parts of photosynthetic organisms (Asensi-Fabado and Munne-Bosch, 2010) but is mainly present in photosynthetic leaves, at 10 – 20 times greater content than in seeds (Asensi-Fabado and Munne-Bosch, 2010; DellaPenna and Pogson, 2006). As such, green leafy vegetables have a high percentage of α-tocopherol. Within plants, α-tocopherol functions directly as a singlet oxygen scavenger in photosynthesis, without which mutants suffer bleaching and lipid photo-destruction from increased un-quenched ROS generation when exposed to excess excitation energy (Kruk et al., 2005). Therefore, photoprotection is a key role of Vitamin E (Havaux et al., 2005) and high light, as an abiotic photoinhibiting stress, can actively increase tocopherol synthesis (Szymańska and Kruk, 2010).

In mammals, Vitamin E is an essential dietary nutrient that can only be acquired by the consumption of photosynthetic organisms. Originally discovered in 1922 as an important dietary factor for animal reproduction (Evans and Bishop, 1922), studies have since found the main role of Vitamin E is preventing the peroxidation of long-chain polyunsaturated fatty acids found in cell membranes by associating with them (Noguchi and Niki, 2019). Therefore, in mammals, including humans, Vitamin E is required for membrane stability and successful pregnancy (Traber, 2019). An inadequate dietary intake of Vitamin E is also known to cause cerebellar encephalomalacia in humans (Traber, 2019). Globally 13 % of blood serum is below the functional deficiency threshold concentration for α-tocopherol (Péter et al., 2015), which cause symptoms such as peripheral neuropathy, and increased incidence of myocardial infraction (Noguchi and Niki, 2019). Whereas, increased intake of Vitamin E is associated with potential health benefits, and classified as acting as a hypolipidemic, antihypertensive, neuroprotective and anti-inflammatory (Saini and Keum, 2016). Dietary supplementation with

Vitamin E can reduce the rate of miscarriage by 50 % (Shamim et al., 2015). A high intake of Vitamin E has been shown to reduce the risk of coronary heart disease by reducing oxidised cholesterols which are associated with cardiovascular disease (Bellizzi et al., 1994; Trpkovic et al., 2015). Moreover, α-tocopherol has been linked with immune system strength through its antioxidant function by activating t-cells and their proliferation (Molano and Meydani, 2012). With evidence for a wide variety of health benefits, the recommended daily dietary allowance of α-tocopherol is 15 mg for both men and women (Monsen, 2000).

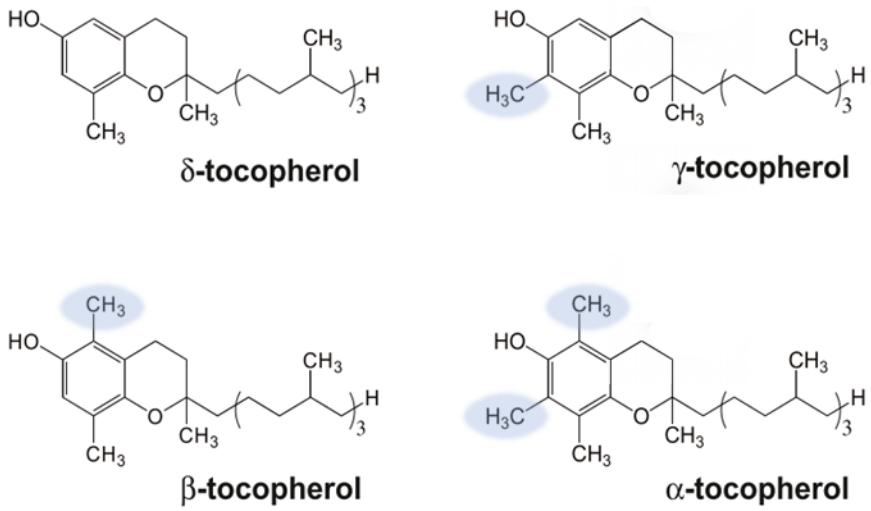


Figure 1.4 The chemical structure of the tocopherols. The tocopherol group δ-, γ-, β-, α-tocopherol are defined by the number and position of methyl groups, which impacts their antioxidant action. Reproduced from DellaPenna and Pogson (2006).

1.2.2.3. Ascorbic Acid (Vitamin C)

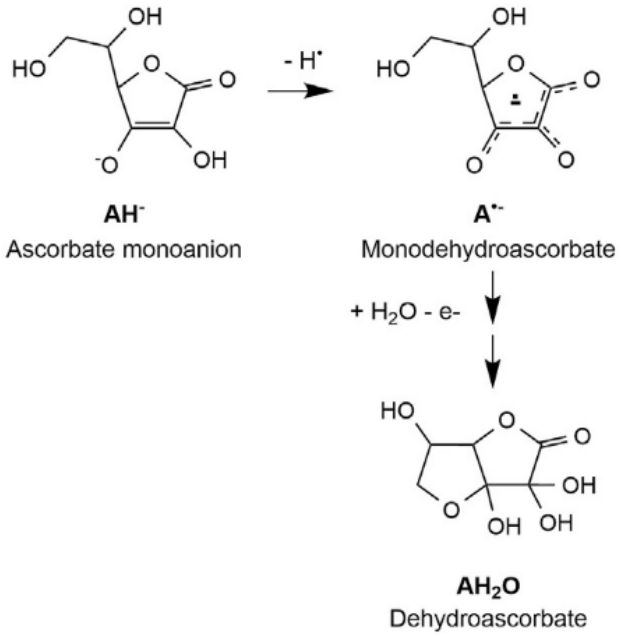
L-Ascorbic acid (AA, Vitamin C) is a soluble carboxylic acid with high redox potential found ubiquitously in eukaryotes. It is formed from hexose sugars and structurally consists of a five-carbon lactone ring with functional groups (Figure 1.5a) (Yoshimura and Ishikawa, 2018). AA can neutralise oxidative pressures in a variety of forms. As ascorbic acid and the deprotonated form ascorbate (AsA), it can act as a reducing agent by cycling between these forms and the radical anion, monodehydroascorbate ($A^{\bullet-}$). Monodehydroascorbate can then be oxidised further into dehydroascorbate (AH_2O) (Njus et al., 2020). Ascorbate can be recovered from AH_2O via the chloroplastic ascorbate-glutathione cycle (Figure 1.5b), utilising the redox potential of another stable antioxidant, glutathione, and the abundant NADP(H). The

mechanism of this cycle maintains concentrations of reduced glutathione (GSH), meaning that the presence of dehydroascorbate is minimised in optimal conditions (Foyer and Noctor, 2011).

AA is the major antioxidant of plants, found in all tissues but is mostly localised to the chloroplast (Smirnoff, 2000), where it holds a vital role in the preservation of photosynthetic function. AA accomplishes this by direct scavenging of ROS (Asada, 2002), and as an electron donor to recover photosynthetic machinery from photoinhibition after stress (Tóth et al., 2011). AA also assists in the function of the other photoprotective isoprenoid antioxidants by working as a co-factor in the xanthophyll cycle in the conversion of violaxanthin to zeaxanthin (Bouvier et al., 2005; Saga et al., 2010), and by regenerating oxidised tocopherol, thereby providing membrane protection (Szarka et al., 2012; Thomas et al., 1992). Interestingly, increasing Vitamin E levels appears to trigger compensatory changes in ascorbate and glutathione levels (Kanwischer, 2005; Li et al., 2010), demonstrating a feedback link with the isoprenoid pathway (Kobayashi and DellaPenna, 2008). Because of the pivotal role of AA in photosynthetic processes, AA shows light-dependent accumulation that can be modulated via light intensity and duration, as well as spectral quality (Paciolla et al., 2019). This can be utilised in CEA to increase AA content and delay the progression of leaf senescence (Barth et al., 2006).

Figure 1.5 The antioxidant action and cycling of Ascorbic acid. (a) The antioxidant action of ascorbic acid begins with the ascorbate anion (AH^-), and then oxidised further into monodehydroascorbate ($A^{\bullet -}$) and dehydroascorbate (AH_2O) (Adapted from Njus et al., 2020). (b) Ascorbate is recovered from dihydroascorbate via the action of glutathione (GSH) which is reduced into glutathione di-sulphide (GSSG) and itself is recovered by the redox potential of NADPH (Reproduced from Foyer and Noctor, 2011).

a



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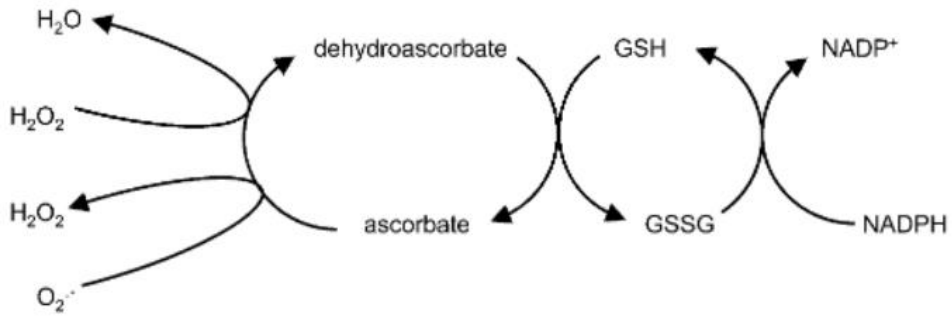


The antioxidant property of AsA is well known, however AA is also a vital player in the overall proper functioning of plants (Smirnoff, 1996; Smirnoff, 2000). AA holds a role in the synthesis of plant hormones, including ethelene, ABA, gibberellins and auxins (Arrigoni and de Tullio, 2002; Smirnoff, 2018), and in control of growth including cell division, elongation, differentiation and programmed cell death (Horemans et al., 2000; Liso et al., 1984). AA is also a co-factor for multiple enzymes (Ishikawa and Shigeoka, 2008), important for electron transport (Asada, 2002; Smirnoff, 1996) and in response to pathogen attacks (Conklin and Barth, 2004). Because of its broad interaction with hormones, ROS and light-induction, AA has a prominent role in global stress tolerance in plants (Hossain et al., 2017).

Humans cannot synthesize AA (Nishikimi et al., 1994), and so consumption of AA is required to reduce occurrence of chronic disease mostly due to its antioxidant properties (Carr and Frei,

1999). AA can also scavenge free radicals and prevent oxidative damage in humans, which makes it an effective molecule in anticancer treatment (Bjelakovic et al., 2004) and also AA is particularly required in the eyes for daily protection from solar radiation (Reim et al., 1978). AA is also involved in embryonic development, postnatal development, aging (Camarena and Wang, 2016) and is widely proclaimed for its beneficial effects on the human immune system. Therefore, AA is considered essential for human health (EFSA Panel on Dietetic Products, 2010), yet evidence suggests the deficiency prevalence is 5 %, even in industrialised countries (Granger and Eck, 2018). When the human body is Vitamin C deficient, it presents symptoms of weakness, gum disease, and bruising, also known as scurvy (NHS, 2020). Coronary heart disease and diabetes mellitus have also been related to AA-deficiency (Boekholdt et al., 2006; Paolisso et al., 1994). The recommended daily intake of Vitamin C is 75 – 90 mg/ day (Monsen, 2000). Plants are considered the most important source of AA, especially since the bioavailability of AA from plants is higher than that from artificial supplements (Carr and Vissers, 2013), likely due to the co-consumption of other micronutrients such as Vitamin E (Tanaka et al., 1997).

1.2.3. Biofortification

The importance of carotenoids, tocopherols and ascorbic acid to the human diet cannot be underestimated. Moreover, the carotenoid, tocopherol and AA antioxidants in plants are tightly interlinked with multiple interactions (Jiang et al., 2021). Primary examples of this are the sharing of MEP pathway precursor metabolites in carotenoid and tocopherol synthesis, and the recovery of redox potential of both of these molecules by AA. This makes them particularly interesting candidates for enhancement in plants. As such, there have been efforts to enhance nutritional content of plants through cultivar selection (Grusak and Dellapenna, 1999; Mou, 2005), and transgenic modulation of biosynthetic genes in the nutritional pathways (Dellapenna, 1999). However, there are limitations to these approaches for the antioxidants of interest in this study as discussed below.

Vitamin C has long been a target for biofortification, and research has shown some success in improving AA content in plants (Paciolla et al., 2019). However, the gains have not been enough to benefit the relevant stakeholders and the challenge to increase Vitamin C levels remains difficult due to the myriad of systems integrated with AA, which influence the

biosynthetic, recycling, and catabolic pathways (Cruz-Rus et al., 2012). Molecular studies have also shown genetic alteration of the tocopherol pathway can enhance α-tocopherol concentrations (Cho et al., 2005), yet, none of these discoveries have been commercially incorporated and distributed as a solution to vitamin deficiencies (Fitzpatrick et al., 2012).

Vitamin A deficiency is prevalent in preschool-age children in up to 122 countries worldwide, and especially in lower income countries, and estimated to blind 250,000 – 500,000 children every year in the period 1995 – 2005, half of them dying within 12 months of losing their sight (World Health Organization, 2009). Therefore, carotenoid biosynthesis has been the target of successful metabolic bioengineering in several crop plants (Giuliano, 2017; Römer and Fraser, 2005; Sandmann, 2001). A well-known example of this is 'Golden Rice', where carotenoid synthesis genes were inserted into the rice genome to relieve Vitamin A deficiency in developing countries (Beyer et al., 2002; Paine et al., 2005). This Genetically Modified cultivar was predicted to save up to 40,000 lives per year or 13,82,000 healthy life years annually in Disability Adjusted Life Years (Tang et al., 2009). However, genetic alterations can have a major negative effect on the physiology of plants, such as retarded early growth and development (Azari et al., 2010). In addition, there is public distrust towards genetically modified products, and new developments can be restricted in their implementation and dispersion, which, in the case of Golden Rice, took over 10 years to be released to the public, and is still under debate (Potrykus, 2012). Therefore, even when major gains are made using genetic engineering, it is not guaranteed to alleviate deficiency.

Major public health benefits could be achieved if the content of these vitamins within vegetables, fruits and leafy greens were elevated using a mechanism that did not rely on genetic modification and was more available to purchase (Poiroux-Gonord et al., 2010). Selective breeding can utilise the genetic diversity that exists within a species to generate new cultivars. This is especially poinient for CEA production as the vast majority of plant varieties available are specifically for outdoor growth and so have been selectively bred for attributes such as seasonality or disease resistance, often at the expense of yield and quality (Russel, 2013). However, these characterstics are not required within CEA facilities, and therefore potential improvements in yield, quality and plant nutritional composition can be made with development of vertical farm breeding lines.

Due to their role in stress responses, the production of carotenoids, tocopherols and ascorbic acid is reactive to environmental inputs. Research has shown that light is one of the most significant environmental parameters for phytonutrient production (Bian et al., 2015; Paradiso and Proietti, 2021), and has a primary role in the production of carotenoids (Welsch et al., 2000), tocopherols (Szymańska and Kruk, 2010), and ascorbic acid (Paciolla et al., 2019), likely due to their prominent roles in photoprotection and localisation to the chloroplast. The potential to improve crop yield and quality according to light quality in a controlled-environment agricultural facility was first proposed by Hanyu and Shoji (2000). In the field, environmental factors such as light, temperature, humidity and CO_2 concentration have impacts on crop quality but cannot be controlled (Gruda, 2005). In a glasshouse environment, temperature and CO_2 concentration are moderated given the prevailing sunlight to maintain optimum conditions. However, in a vertical farm with LED luminaires growers can now select the precise intensity and proportion of wavelengths of light to deliver to their crops which is tailored towards enhancement of specific plant attributes without the requirement of genetic modification (Massa et al., 2008). The combination of industrial urban vertical farming and photobiology research presents a unique opportunity to remedy urban hidden hunger, by targeting the light regulation of these three vital antioxidants, thereby making available the production of local, cheap, highly nutritious food.

1.3. Light as a Powerful Manipulator of Plant Growth and Development

The sun delivers electromagnetic energy to the surface of the earth, from gamma rays (high energy) to radio waves (low energy). The visible light range lies in the middle of this spectrum between ultraviolet (UV) and far-red light (FR) (400 – 700 nm) (Kozai, 2016). This range of wavelengths is fundamental to all life on earth due to the evolution of plants that capture and utilise this energy in photosynthesis (Horton et al., 1996). In addition to providing a fundamental energy resource for growth, light provides vital information about the surrounding environment, and acts as a source of developmental cues (Fankhauser and Chory, 1997). Therefore light quality, consisting of intensity (number of photons), spectra (the wavelength composition), and photoperiod (duration of light), is one of the principle environmental signals for plants (Kozai, 2016). Due to their nature as sessile organisms, plant survival is dependent on adaptation to the local environment, which is accomplished by light

detection leading to changes in gene expression (Chen et al., 2004). Therefore, in order to successfully grow plants in CEA under artificial lighting, a depth of understanding is required for how wavelengths of light regulate signalling cascades to modify plant growth and secondary metabolite production.

1.3.1. Photosynthesis

Within CEA, the most commonly discussed wavelengths for horticultural lighting are 400 – 700 nm, classified as photosynthetically active radiation (PAR) so called because wavelengths across this range facilitate photosynthesis (McCree, 1971). Photosynthesis is a process in which light energy is used to convert CO_2 and water into usable sugars.

Photosystem I (PSI) and Photosystem II (PSII) perform the initial light harvesting steps in the chloroplast. These photosystems consist of pigments, chlorophylls a and b (Chl a and Chl b) in addition to carotenoids, contained within a core complex and peripheral antenna system (Cao et al., 2018). Chl a is sequestered in the core complexes of the photosynthetic reaction centres, in addition to β-carotene, a primary carotenoid that absorbs light in the blue region. Additional pigments, Chl a and Chl b as well as carotenoids, reside in the peripheral antenna to increase absorption across the visible light spectra due to their differing absorption spectra. Chl a absorbs predominantly in the red light (RL) wavelengths (660 – 700 nm), whereas Chl b can absorb red (660 nm) in addition to wavelengths in the blue region (475 nm) (Caffarri, 2014; Milne 2015). The two photosystems differ in absorption spectra due to their constitution of light absorption pigments. PSI contains chlorophylls that absorb light above 700 nm and is therefore FR light sensitive. Whereas PSII contains Chl a that absorbs light at 660 nm, as well as Chl b and carotenoids, and can therefore absorb blue and red wavelengths (Blankenship, 2021). PSII is the primary photosystem in photosynthesis. After absorption, the energy is passed down to PSI via a tightly regulated sequence of chemical reactions in the electron transport chain to be captured as usable energy by fixing carbon into sugars.

Due to the maximum absorption peaks of the chlorophylls at red (~660 nm) and blue (~450 nm) (Caffarri, 2014; Milne 2015), the first horticultural lighting units focused only on delivering red and blue at these wavelengths (Runkle, 2016). These LED lighting units resulted in good yields of many plant species, including lettuce, in controlled environments (Dougher

and Bugbee, 2001; Hanyu and Shoji, 2000; Kim et al., 2004; Yorio et al., 2001). As lighting developed, green (white) light was also included into manufactured horticultural LED units primarily for improved visual observation of the crop and the assessment of plant health. Under red and blue light only, plants appear discoloured, therefore additional green or white light aids in the identification of disease, malnutrition and pests. It was generally believed that the vast majority of green light (GL) was reflected and not useful to the plant. However, despite a weak absorption by chlorophyll, a substantial amount (70 – 80 %) of green radiation is absorbed by the leaf (Sun et al., 1998). As the upper canopy absorbs the vast majority of the red and blue wavelengths, GL can penetrate deeper in the leaf profile (Sun et al., 1998). There it scatters between cellular components within the leaf, and drives photosynthesis through abundant lower chloroplasts (Sun et al., 1998; Terashima et al., 2009). Research has uncovered evidence that the addition of GL can aid the biomass accumulation of some species, including lettuce and basil (Johkan et al., 2012; Schenkels et al., 2020). FR wavelengths (> 700 nm) in combination with RL wavelengths (660 – 680 nm) can also increase the rate of photosynthesis to result in biomass accumulation (Li and Kubota, 2009). By driving the RL inclined PSII and the FR inclined PSI, energy is passed through the photosystems more efficiently via the Emerson Effect (Lysenko et al., 2014; Zhen and van Iersel, 2017).

Greater light intensity has also been used to increase photosynthesis and elevate yields (Liu and van Iersel, 2021). However, when light energy reaches the plant in excess of that which can be used in photosynthesis, excess oxidative radicals are produced which can damage plant organ and function, leading to photoinhibition. This overwhelms the existing scavengers, such as α-tocopherol which neutralises singlet oxygen from PSII, so plants have developed photoprotective systems to function as sinks for excess energy. There are several forms of fast, and slow acting mechanisms, together known as non-photochemical quenching (NPQ) (Dall'Osto et al., 2007; Long et al., 2022). The major form and fastest-activating NPQ is energy dependent quenching (*qE*), which includes 'The Xanthophyll Cycle. The carotenoid xanthophylls reside in the light harvesting antenna proteins of PSII, and reduce ROS formation through thermal dissipation of energy. The violaxanthin cycle is responsible for 80 % of *qE* in *Arabidopsis*, where a change in lumen pH triggers enzymes to convert violaxanthin to zeaxanthin via antheraxanthin, thereby activating quenching (Niyogi et al., 1998). Some species also have a lutein epoxide cycle which assist in the *qE* mechanism of NPQ, although

luteinis more often associated with assisting in light harvesting (Dall'Osto et al., 2007; Jahns and Holzwarth, 2012). The threshold of activation of *qE* is negatively affected by biotic stress, including drought, temperature extremes and nutrient deficiency. Therefore the presence of *qE* is directly linked to plant fitness, with enhancement associated with increased biomass production (Hubbart et al., 2018).

1.3.2. Photoreceptors Control Light Coordinated Photomorphogenesis and Metabolic Changes

The spectral components of incident light vary according to the environment, the time of day and the presence of other plants, and therefore conveys information about the surroundings. In order to detect spectral quality, plants absorb the wavelength composition of the light by specific photoreceptors (Briggs, 2001). The main photoreceptors responsible for photomorphogenic responses are phototropins and cryptochromes (BL and UV-A), phytochromes (RL and FR), and UV RESISTANCE LOCUS 8 (UVR8; UV-B) (Figure 1.6). Upon activation by their associated wavelengths, photoreceptors translocate to the nucleus where have been associated with triggering genetic changes to affect growth and phytochemical production and accumulation (Lefsrud et al., 2008), thereby adapting plant development within different light environments (Walters et al., 1999).

1.3.2.1. Blue Light

Blue light controls the direction of growth and the flattening of leaves in response to light via by phototropin and ZTL/ADO families of photoreceptors (Briggs and Christie, 2002; Legris et al., 2021). However, the cryptochromes (CRY) control the main growth and developmental responses to blue light variations in the wavelength, intensity, and diurnal variation of the irradiation (Yu et al., 2010). Cryptochrome photoreceptors, CRY1 and CRY2, absorb light from the UV-A to blue range (380 – 440 nm) via the attached flavin adenine dinucleotide chromophore (Cashmore et al., 1999). When exposed to blue light CRY1 and CRY2 proteins are translocated to the nucleus, where they form nuclear photobodies (Yu et al., 2009). The timescale is dependent on the fluence rate of the blue light, but can occur within minutes (Yu et al., 2007).

In Arabidopsis, CRY1 and CRY2 have a partially overlapping function in mediating blue light growth responses, including the inhibition of hypocotyl elongation (Lin et al., 1998), circadian clock entrainment (Somers et al., 1998), guard cell development and stomatal opening (Kang et al., 2009; Mao et al., 2005), and cotyledon opening (Ohgishi et al., 2004). Blue light also impacts upon pigment synthesis including anthocyanin accumulation (Ohgishi et al., 2004) and chlorophyll synthesis in de-etiolation (Xu and Ma, 2009). Mochizuki et al. (2004) found that as little as 5 $\mu mol.m^{-2}.s^{-1}$ of blue light (470 nm) acts as an activation threshold for photosynthesis genes in *Arabidopsis*, and PSII core proteins are proposed to be maintained via a blue/ UV-A pathway (Christopher and Mullet, 1994). The primary effect of blue light on chlorophyll biosynthesis has been reported in previous studies using lettuce, cucumber, and spinach (Hogewoning et al., 2010; Lee et al., 2010; Matsuda et al., 2007). Blue light has also been shown to contribute to the production of nutraceutical compounds, such as antioxidants and total phenols that can be beneficial to human health (Nájera et al., 2022). Specifically blue light effects the rate of carotenogenesis (Giliberto et al., 2005), the accumulation of tocopherols (Samuoliene et al., 2012; Samuolienė et al., 2017) and AA (Zha et al., 2020).

1.3.2.2. Red and Far-Red Light

Light ranging from 600 – 780 nm is detected by phytochromes. *Arabidopsis thaliana* has five members of the phytochrome protein family, phytochrome (phy) A-E, which are conserved in all angiosperms (Li et al., 2015; Mathews et al., 1995). Phytochrome exists as a soluble dimer with each monomer containing the light absorbing chromophore, phytochromobilin (Lagarias and Rapoport, 1980). The use of phytochromobilin allows phytochrome to exist in two photoreversible states. Phytochrome is first synthesised in the cytosol and accumulates in the dark as a biologically 'inactive' R-absorbing (Pr) form with a RL peak absorption at 660 nm. Upon exposure to RL, Pr phytochrome undergoes a conformational change to photoconvert into the 'active' FR absorbing (Pfr) form, which has a FR absorption maxima at 730 nm. Pfr then reverts back to Pr upon absorption of FR light or exposure to the dark (Quail, 2002). Upon conversion to the active Pfr form, the phytochrome molecule is rapidly translocated to the nucleus to accumulate in nuclear speckles (Nagatani, 2004; van Buskirk et al., 2012). This process is rapid and can be detected within 5 minutes of RL absorption (Quail, 2002), but is overturned if FR light is supplied early enough to revert phytochrome to the inactive Pr form

(Al-Sady et al., 2006). This characteristic of the phytochrome photoreceptor allows it to create a dynamic photoequilibrium dependent on the red : far-red light ratio (R:FR) or day length which is highly functional for regulating developmental responses (Rockwell, 2006).

phyB and phyA are the most studied of the family and function separately in the most crucial roles for plant development. Of these, phyB is the predominant phytochrome for the vast majority of RL responses (Wang, 2015). phyB coordinates responses via the R/FR photoreversible nuclear localisation (Trupkin et al., 2014), as a low fluence response (LFR) (Shinomura et al., 1996), which initiates signalling cascades resulting in induction or repression of target nuclear genes (Huq et al., 2003). The photoreversible characteristic of phyB enables signalling processes dependent on the balance of active or inactive phytochrome. Therefore, R:FR can be used as a proxy for day length and detection of shading events both of which alter the quantity of FR light (Kircher et al., 2002). Changes in photoperiod are the primary cause for flowering (Chory et al., 1996; Halliday et al., 1994), whilst a high level of shading, or low R:FR, can inhibit germination (Shinomura et al., 1996) and trigger shade avoidance responses, resulting in stem and leaf elongation via auxin regulation (Morelli and Ruberti, 2000), reduced branching, early flowering and negative effects on chlorophyll synthesis and other metabolites (Casal, 2013; Franklin, 2008). Plants can be shade-tolerant or shade-sensitive, and this is dependent on species responsiveness to R:FR. In general, plants adapted to open environments display stronger shade avoidance responses (Dudley and Schmitt, 1995; Power et al., 2021).

On the other hand, phyA has a unique response mechanism. While RL phyB-E responses are photoreversibile by FR (R/FR), phyA responses are not. Instead, phyA is the only known photoreceptor to absorb and become activated by FR, coordinating very low fluence responses (VLFR) for RL or FR and high irradiance responses (HIR) by accumulating for up to 2 h under continuous FR (Shinomura et al., 1996). These conditions control differing responses that are mostly phyA mediated. VLFR initiates germination and de-etiolation processes (Botto et al., 1996; Shinomura et al., 1996), whilst FR-HIR can inhibit hypocotyl growth and unfold cotyledons (Casal et al., 2000; Yanovsky et al., 1995). Moreover, phyA is light liable and rapidly degrades under light exposure, therefore decreases in abundance and influence after dawn, after accumulating during the night. This places phyA as a likely sensor of dawn, triggering flavonoid biosynthesis and other protective antioxidants and hormones for the prevention of

biotic and abiotic stress (Seaton et al., 2018). phyA accomplishes these responses by directly targeting genes across a wide range of pathways (Chen et al., 2014). Between phyA and phyB, phytochrome is involved with physiological responses including germination, de-etiolation, hypocotyl inhibition (Franklin and Quail, 2010), and stomatal opening (Wang et al., 2010). Phytochrome also has a role in metabolic responses, including initiation of the MEP pathway (Rodríguez-Concepción et al., 2004), carotenoid pathway (Ernesto Bianchetti et al., 2018), tocopherol (Alves et al., 2020) and AA accumulation (Thomsen et al., 1992).

1.3.2.3. UV-B Light

The UV radiation output by the sun is divided into three wavebands: UV-C (100 – 280 nm), UV-B (280 – 315 nm) and UV-A (315 – 400 nm). Due to UV absorption of the atmospheric ozone layer, UV-B radiation is the highest energy wavelength that can reach the surface of the earth (Björn, 2007). UV-A radiation is of slightly lower energy and not filtered by ozone, and contributes up to 95 % of total UV exposure (Moan, 2001). Both UV-B and UV-A are strong oxidising forces for living things, causing damage to DNA, proteins, and membrane lipids, which in excess can inhibit proper processes and functioning (Casati and Walbot, 2004; Jansen et al., 1998; Rozema et al., 1997).

UVR8 is the most recently discovered photoreceptor and absorbs UV-B light (Rizzini et al., 2011). Under white light, UVR8 can be found in both the cytoplasm and the nucleus as a dimer. When UV-B is absorbed by the 14 associated tryptophan chromophore (Christie et al., 2012), it immediately monomerises and accumulates in the nucleus in as little as 5 minutes (Hofmann, 2012; Kaiserli and Jenkins, 2007). The re-dimerization process is orchestrated by UV-B induced REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUPs) and WD40-repeat proteins (Heijde and Ulm, 2013). Therefore, UVR8 is self-regulating and under diurnal photoperiods, establishes a dimer/monomer photo-equilibrium (Findlay and Jenkins, 2016).

UV-B detection and signalling coordinates photomorphogenic and widespread metabolic alterations that have developmental and metabolic protective functions (Yadav et al., 2020)). However, responses to UV-B radiation are dependent on the wavelength, fluence rate and exposure. Brief exposure to longer UV-B wavelengths at low fluence rates stimulates modifications of plant architecture, such as inhibiting stem growth, reducing leaf expansion and promoting branching (Kim et al., 1998; Suesslin and Frohnmeyer, 2003), and producing

UV-protective flavonoids and other protective antioxidants, including α-tocopherol (Brown and Jenkins, 2008; Frohnmeyer et al., 1999; Szymańska et al., 2015). In contrast, high fluence rates of short UV-B wavelengths activate genes involved in in defence, wound, or general stress responses, including jasmonic acid, ethylene and salicyclic acid (Izaguirre et al., 2007; Stratmann, 2003; Ulm and Nagy, 2005). The induction of these defensive genes is inhibited by the presence of antioxidants (Green and Fluhr, 1995; Surplus et al., 1998). Other factors can also alter the responsiveness to UV-B including the extent of acclimation, developmental stage of the plant, the interaction of other environmental factors (Hectors et al., 2007), and the genetic variation in UV tolerance and responsiveness within and between species, including *Arabidopsis* (Kalbina and Strid, 2006), maize (Correia et al., 1999), and rice (Sato et al., 2003).

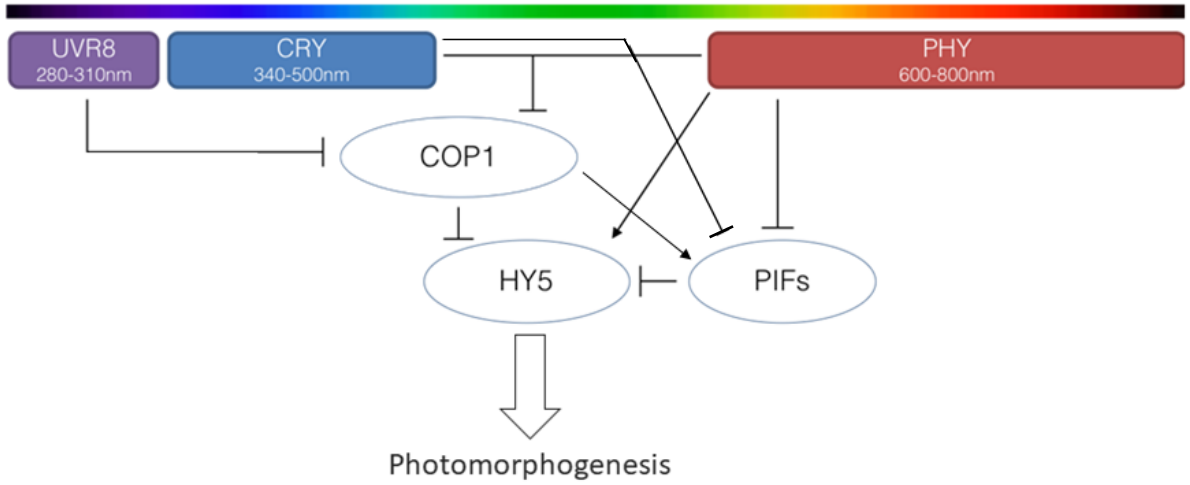


Figure 1.6 Overview of the light signalling pathways. Ranges of the electromagnetic spectrum are absorbed by photoreceptors UV RESISTANCE LOCUS 8 (UVR8), cryptochromes (CRY; Blue light and UV-A), phytochromes (PHY; Red light and Far-red). After detection of wavelengths within their range, photoreceptors interact with downstream signalling partners including CONSTITUTIVELY PHOTOMORPHOGENIC 1 *(*COP1), the transcription factors PHYTOCHROME INTERACTING FACTORS (PIFs) and ELONGATED HYPOCOTYL 5 (HY5) that enact transcriptional gene changes to promote photomorphogenesis. An inhibiting interaction is represented with a barred line, whilst a positive action is represented by an arrow.

1.3.3. The Light Signalling Network: COP1, HY5 and PIFs

Light detected by photoreceptors transcriptionally modulates almost 20 % of the genes in *Arabidopsis* via a shared signalling network (Yadav et al., 2020). To enact specific aspects of plant growth and development, photoreceptors direct genetic expression within the nucleus by interaction with transcription factors and coregulatory molecules to trigger signalling cascades (Chen et al., 2004). The primary signalling components of the light regulation pathways are CONSTITUTIVELY PHOTOMORPHOGENIC 1 *(*COP1), and the transcription factors ELONGATED HYPOCOTYL 5 (HY5) and PHYTOCHROME INTERACTING FACTORS (PIFs) that generally promote and repress photomorphogenesis, respectively (Figure 1.6).

1.3.3.1. COP1

COP1 is a E3 ubiquitin ligase that has several functional domains to interact with numerous target proteins (Schwechheimer and Deng, 2000). Involved in multiple light signalling pathways, COP1 represses positive photomorphogenic regulators in darkness and targets them for destruction by interacting with SPA1 (SUPPRESSOR OF PHYA) (Hoecker, 2017). Additionally, phyA and phyB, and CRY2 are also known to undergo degradation through direct interaction with COP1 (Podolec and Ulm, 2018). Under exposure to light, COP1 is inactivated by dissociation of SPA from COP1 via direct interaction by both phyA and phyB, and CRY1 and CRY2 (Liu et al., 2011a; Sheerin et al., 2015), and excluded from the nucleus (Subramanian et al., 2004). This stabilises the presence of positive photomorphogenic transcription factors, such as HY5, by reducing their degradation by COP1 (Hardtke et al., 2000; Pacín et al., 2014). In contrast COP1 is a positive regulator of UV-B responses, physically interacting with UVR8 (Oravecz et al., 2006) and promoting protein stabilisation in UV-B, most likely as a UVR8-COP1-SPA1 complex (Favory et al., 2009b; Huang et al., 2013).

1.3.3.2. HY5

The basic leucine zipper (bZIP) transcription factor HY5 functions as a master signal integrator of light and temperature impacts on growth (Koornneef et al., 1980) and metabolite content, such as pigments (Hoai Nguyen, 2020), in addition to hormone, nutrient, abiotic stress and ROS signalling pathways (Gangappa and Botto, 2016). When the repression of the COP1-SPA

complex is released by the photoreceptors, HY5 accumulates in the nucleus and binds to complementary motifs the promoter of light responsive genes (Chattopadhyay et al., 1998; Lee et al., 2007a). HY5 implements transcriptional and post-transcriptional effects by affecting the expression of over 1,100 genes (Zhang et al., 2011a). Both cryptochromes and phytochrome signalling cascades coalesce in HY5 via COP1, and therefore HY5 holds a central role as a positive regulator of photophormogenesis genes responding to both red and blue light (Griffin et al., 2020). HY5 also has a dominant function in UV-B signalling (Brown et al., 2009), and is associated with approximately half of UVR8-regulated genes (Brown and Jenkins, 2008).

1.3.3.3. PIFs

The Phytochrome Interacting Factors are basic-helix-loop-helix (bHLH) transcription factors (Toledo-Ortiz et al., 2003), of which *Arabidopsis* contains eight (PIF1 -8) (Lee and Choi, 2017; Monte et al., 2007), that lie at the centre of a variety of light-mediated responses in growth and metabolism (Castillon et al., 2007). Generally, PIFs function as negative regulators of phytochrome photomorphogenic development, and therefore maintain the skotomorphogenic state in darkness, as confirmed by the *pifQ* mutant photomorphogenic phenotype (Leivar, Monte, Oka, et al., 2008). Individually, each PIF has shared and specific roles and functions, and they work as a signalling hub to regulate a wide range of photomorphogenic responses (Leivar and Quail, 2011), including germination (Oh et al., 2004), chlorophyll synthesis (Leivar, Monte, Oka, et al., 2008; Shin et al., 2009), circadian regulation (Shor et al., 2017), hormone signalling and stress responses (Paik et al., 2017), and leaf senescence (Song et al., 2014). PIFs transcriptional regulation is accomplished by binding directly to the promoter of target genes (Leivar et al., 2012; Toledo-Ortiz et al., 2010), which repress or in some cases positively modulate genes to optimise growth under photoperiodic conditions (Soy et al., 2014). PIFs accumulate in the dark, when phytochrome is in its biologically inactive form, with their abundance regulated and stabilised by the COP1-SPA complex and DET1, to synergistically enhance repression of photomorphogenesis (Xu et al., 2014). The accumulation of PIFs in darkness can also work to indirectly down-regulate chlorophyll and carotenoid synthesis (Leivar et al., 2009; Moon et al., 2008).

When under light conditions, activated phytochomes are translocated to the nucleus whereupon they form nuclear bodies in association with PIFs (Wang, 2015). The Pfr form of phyA and B, in coordination with DELLA proteins (negative modulators of PIFs) and COP1 (Li et al., 2016), bind to PIFs and promote their degradation in the nucleus (Al-Sady et al., 2006; Shen et al., 2008), thereby releasing the PIF repression of photomorphogenic development (Leivar and Quail, 2011). Cryptochromes repress PIF4 to regulate shoot branching (Zhai et al., 2020) and can also interact with PIF4 and PIF5 to promote stem growth under limiting blue light (Pedmale et al., 2016). Similarly, UVR8 inhibits hypocotyl elongation by degradation of PIF4 and PIF5 (Tavridou et al., 2020), this is accomplished byUVR8 binding to COP1, thereby disrupting the stabilisation that COP1 provides to PIF5 (Sharma et al., 2019).

1.3.4. Crosstalk within Light Pathways

In addition to the linear pathways described above, research is uncovering ever more interactions between photoreceptors and light signalling components demonstrating a complex interconnectivity within the light signalling system. The light signalling pathways naturally cross over due to their common transcription factors and target responses for growth and metabolism. In this fashion, PIFs and HY5 can work within a regulatory module by binding to the same G-box target motifs in the promoters of common target genes (Leivar et al., 2009). They have been shown to act in cooperation, with HY5 and PIF3 binding to specific separate motifs, for anthocyanin biosynthesis (Shin et al., 2007), or in an antagonistic manner, where they bind to the same motif, for ROS-responsive genes (Chen et al., 2013), ethylene signalling (Jing and Lin, 2020), and carotenoid biosynthesis genes (Toledo-Ortiz et al., 2014). Meanwhile, PIFs may regulate phyB abundance creating feedback loops within the RL signalling pathway (Leivar, Monte, Al-Sady, et al., 2008). Alternatively, cross-regulation can occur between BL and RL photoreceptors, where cryptochrome photoreceptors have been identified as potential targets of phytochrome kinase activity (Chen et al., 2004), indicating direct crosstalk between light signalling pathways. Moreover, many physiological growth responses require synergistic co-action of both photoreceptors and their downstream signalling partners. For example, phyB and cryptochromes are interdependently involved in the inhibition of hypocotyl growth and cotyledon unfolding of seedlings (Casal and Boccalandro, 1995). UV-B can also act synergistically with RL to mediate cotyledon opening via phyB (Boccalandro et al., 2001).

Spectral absorption overlap can also lead to crosstalk, as in the case of UVR8 and cryptochromes. BL can induce expression of the UV-B signalling repressor RUP2, which is typically expressed by UV-B signalling in a negative feedback loop (Gruber et al., 2010). Moreover, GL has been shown to partially inactivate cryptochrome signalling (Bouly et al., 2007), which may provide a mechanism for green wavelengths to regulate photomorphogenic responses (Liu et al., 2011b; Sellaro et al., 2010). In other cases, light cues from multiple sources can be integrated to finely regulate a response, such as shade avoidance. Typically, perception of low R:FR by phytochrome induces shade avoidance responses by accumulation of PIFs. However, low BL in combination with low R:FR enhances shade avoidance responses such as petiole elongation by acting on the same PIF transcription factors (de Wit et al., 2016). Green light can also be associated with triggering shade avoidance type growth (Zhang et al., 2011b), likely associated with the low BL response due to the inactivation of cryptochrome by green light (Bouly et al., 2007). Competing vegetation absorbs red and blue wavelengths and transmits FR and green light, therefore detection and reaction to low ratios of R:FR and blue to green light (B:G) is evolutionarily advantageous. Finally, UV-B and RL found at high levels in unfiltered sunlight work to inhibit shade avoidance responses (Fraser et al., 2016). This is a prime example of the convergence of multiple photoreceptors upon a shared signalling network to coordinate a response, and highlights the broad scope that must be used when undertaking assessment of light signalling responses, both physiological and metabolic.

1.3.5. Photoperiod and the Circadian Clock

Plants experience daily environmental changes in light (photocycles) and temperature (thermocycles) that vary by season and latitude. Consequently, they have evolved an endogenous circadian clock with a period of approximately 24 hours, which ensures that internal biological processes are appropriately synchronized with the daily changes in the environment (Dodd et al., 2005; Michael and McClung, 2003). Precise timing creates a balance between supply and demand of metabolites, enzymes, and products at the correct time of day via a complex interplay of sensing, signalling and output that aid in the health and daily function of plants (Dodd et al., 2005; Michael et al., 2003; Woelfle et al., 2004). In the model plant *Arabidopsis thaliana*, around 30 % of genes show circadian regulation. These circadian-regulated genes feature in hormone and stress response pathways, metabolism, and other

important processes such as photosynthesis, and the synthesis of pigments and antioxidants (Covington et al., 2008; Michael and McClung, 2003).

The *Arabidopsis* circadian clock and its components is a very active area of research, and its core has been described (Hsu and Harmer, 2014; Webb et al., 2019). Briefly, the central circadian oscillator consists of gene encoded proteins: *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*), and *TIMING OF CAB EXPRESSION 1* (*TOC1*), a member of the pseudo-response regulator (PRR) protein family among others (PRR5, PRR7 and PRR9) (Figure 1.7) (Gendron et al., 2012). These genes form a negative transcriptional–translational feedback loop beginning with the 'morning loop' consisting of morning expressed *CCA1* and *LHY* genes, whose proteins promote *PRR7* and *PRR9* whilst repressing the expression of 'evening loop' genes, *TOC1* and *PRR5* (Alabadí et al., 2001; Lu et al., 2009). Due to the repression of PRR7 and PRR9, *CCA1* and *LHY* levels decrease at dusk, allowing the expression of the 'evening loop' genes, which in turn begin upregulating 'morning loop' gene transcription in preparation for dawn expression (Figure 1.7) (McClung 2006).

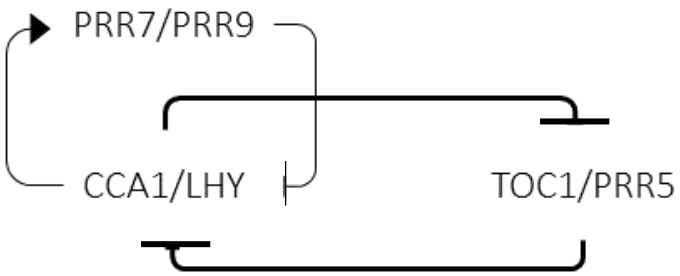


Figure 1.7 The main components of the circadian clock in Arabidopsis. A negative transcriptional-translational feedback loop is created with CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY), TIMING OF CAB EXPRESSION 1 (TOC1), and various pseudo-response regulator (PRR) genes. Adapted from McClung (2006) and Gendron et al. (2012).

To synchronise the clock signalling phases with diurnal fluctuations of the environment, the clock receives input from environmental time cues. Light is one of the primary methods for clock entrainment into set cycles upon emergence (Somers et al., 1998). Phytochromes

strongly influence clock output (Castillon et al., 2007; Hu et al., 2009). In fact, the resetting of the circadian oscillator is a very low fluence response (Nagy et al., 1993) enacted via phyA in direct or indirect associations (Seaton et al., 2018; Wang, 2015). Cryptochromes also contribute to circadian entrainment by acting within the clock mechanism to methylate and degrade CCA1 RNA (Wang et al., 2021). In addition, phyA directly interacts with CRY1 and CRY2 (Ahmad et al., 1998) and therefore, cryptochromes likely act as signal transduction components downstream of phyA (Devlin and Kay, 2000). This input into circadian control is redundant between CRY1 and CRY2 but as circadian rhythmicity is maintained in the *phyAphyBcry1cry2* mutant, additional components may also be involved (Yanovsky et al., 2000).

The interaction of cryptochromes and phytochromes ensures that the circadian clock is set at dawn and functioning appropriately to coordinate essential functions such as de-etiolation, flowering time, the establishment of the photosynthetic machinery and the generation of protective compounds (Covington et al., 2008). Many of these compounds originate from the MEP pathway and other downstream pathways such as chlorophyll, carotenoids, tocopherols and AA biosynthesis. Therefore, the genes encoding enzymes in these pathways demonstrate diurnal fluctuations in their expression (Dudareva et al., 2005; Hsieh and Goodman, 2005; Vranová et al., 2013). CCA1 is an important circadian component of HY5 regulation, which affects a vast swathe of photomorphogenic responses (Kay et al., 2015), and PRR9/PRR7/PRR5 are negative regulators of the chlorophyll, carotenoid, AA, and tocopherol biosynthetic pathways. This strongly suggests that circadian influences are important and interlink with light signals for the biosynthesis of these protective compounds (Fukushima et al., 2009).

1.4. Light-Targeted Production of Crops within Controlled Environment Agriculture

Both physiological and phytochemical activities are strictly correlated with the light environment (Bian et al., 2015; Paradiso and Proietti, 2021). Growth and phytonutrient content are intrinsic to plant well-being and are held in an optimal balance depending on the light environment. Therefore, optimisation of the light input for enhancement of growth may negatively affect metabolites or vice versa, in part because they are both dependent on MEP

pathway products, be that hormones or antioxidants (Chappell, 2003). In a commercial environment where growth of the crop cannot be compromised, study into the enhancement of nutritional content must also be paired with observation of potential growth effects to ensure a balance between biomass accumulation and nutritional content is found. However, the response to light quality for biomass, morphogenesis and the accumulation of bioactive compounds is species, even variety, dependent (Matsuda et al., 2004; Naznin et al., 2019; Ohashi-Kaneko et al., 2007; Taulavuori et al., 2013). This was found to be true for tocopherol antioxidant activity, for which light quality showed varying effects dependent on the crop tested (Samuoliene and Duchovskis, 2012). Thus, the light control opportunity presented by CEA has attracted bountiful research to untangle the effects of light on growth and secondary metabolites, with focus on specific high value crops to determine the optimal lighting conditions for maximising commercially desirable traits. Often this results in studies observing the effect of monochromatic or dichromatic lighting qualities on physiology or overall secondary metabolite content in order to establish a general effect that could be beneficial.

Current literature already indicates similar effects between crop varieties that could be taken forward towards an optimised crop specific light recipe. Lettuce is one of the most studied crops due to its fast growth and sensitivity to different light qualities (Dougher and Bugbee, 2001; Inada and Yabumoto, 1989). In general, higher proportions of RL will increase Lettuce biomass growth (Chen et al., 2016; Son and Oh, 2013). There is also evidence that UV and FR supplementation can also increase biomass and leaf area, relatively, however these wavelengths should be applied conservatively as a limited treatment (Chen et al., 2014; Chen et al., 2019; Eok Kim et al., 2013; Kobayashi et al., 2013; Li and Kubota, 2009; Ohashi-Kaneko et al., 2007; Pinho et al., 2017; Stutte et al., 2009). Inclusion of GL has seen beneficial effects in biomass production due to the penetration of this light through the canopy, and would be best applied where quality of the crop must be assessed by eye or where crop production is grown particularly dense (Johkan et al., 2012). High proportions of BL favour the accumulation of chlorophyll, carotenoids and anthocyanins (Goto, 2012; Shoji et al., 2010; Stutte et al., 2009), and it is important to note that RL in Lettuce was correlated with decreased chlorophyll, carotenoids, anthocyanins, and vitamin C (Chen et al., 2014; Johkan et al., 2010; Stutte et al., 2009; Zhang et al., 2018b). However, no consistent induction of Vitamin C by any light quality has been observed.

Other examples of studies on the effect of light quality in crops include Rocket, which shows a particularly strong response to FR light, increasing stem length and decreasing phytonutrient content (Nicole et al., 2019). Whereas for Spinach, RL increases biomass (Goins and Yorio, 2000; Li et al., 2011; Naznin et al., 2019), whilst BL doesn't contribute to dry weight but does affect the development of the leaves (Li et al., 2011; Park et al., 2019) and the accumulation of β-carotene, lutein (Li et al., 2011) and other carotenoids and chlorophylls (Ohashi-Kaneko et al., 2007). Studies with Spinach also support increased RL (Nicole et al., 2019; Ohashi-Kaneko et al., 2007) and BL (Gao et al., 2020; Park et al., 2019) proportion for improved AA production.

These studies highlight the variability between and within species and the differential photomorphogenic effects of wavelengths on growth and secondary metabolites. In the case of plant vitamin production, there is also a lack of mechanistic association with the light signalling components to the responses seen at present. A deeper photobiological understanding of light regulation on the biosynthesis pathways of carotenoids, tocopherols and ascorbic acid will unlock possible manipulation via light in crop plants to elevate the content of these phytonutrients and the health benefits associated with their consumption. Such an understanding could be applied to improve the performance and quality of crops grown within a commercial CEA facility.

1.5. Thesis Aim and Objectives

The aim of this thesis is to advance understanding of photobiological inputs to essential dietary antioxidants, in order to attain the goal of improving the nutritional quality of crops grown in CEA via LED lighting manipulations. This will be achieved by investigating the mechanism of light regulation in the model plant *Arabidopsis thaliana*, and to associate those findings with the ability of LED lighting to influence the phytonutrient content of herbaceous crops grown in a flood-and-drain hydroponic system, whilst monitoring commercially desired growth traits. Specifically, the objectives of this project are:

1. Investigate the BL, RL, FR and UV-B light regulation on key genes within the biosynthetic MEP pathway, in addition to the carotenoid, tocopherol, and ascorbic acid pathways in *Arabidopsis* (Chapter 2).
2. Explore using bioinformatics the light regulation of key genes within the phytonutrient pathways via common light signalling components HY5 and PIFs, in addition to photoperiodic or circadian inputs in *Arabidopsis* (Chapter 2).
3. Measure the effect of blue and red wavelengths in monochromatic and dichromatic mixes on the growth and nutritional content of Basil and Coriander (Chapter 3 and 4).
4. Explore the potential to include UV-B and FR wavelengths with commercial spectrum in supplementary light regimes and their effects on growth and phytonutrient content of Basil and Coriander (Chapter 3 and 4).
5. Quantify the growth and phytonutrient accumulation of Basil and Coriander grown under four different commercial spectra in a hydroponic system designed for commercial production at May Barn Consultancy Ltd (Chapter 3 and 4).

CHAPTER 2

Exploration of Light Regulation Within the Biosynthesis Pathways of Three Vitamins Crucial to Human Health

2.1 INTRODUCTION

Plant responses to light quality constitute physiological but also metabolic changes (Bian et al., 2015; Paradiso and Proietti, 2021). Therefore, recent studies have focused on increasing knowledge of plant genetic plasticity to light quality in order to understand how to use this environmental stimulus to drive a desired response. Historically, genetic modification has been the primary tool in manipulating crop plant production (Jiang et al., 2021). However, the advent of CEA opens the possibility of using light manipulations to target and control desired crop traits, such as particular growth patterns and the production of desired compounds or metabolites (SharathKumar et al., 2020). Secondary metabolites of particular interest are antioxidants and vitamins such as the carotenoids (pro-Vitamin A), tocopherols (Vitamin E) and ascorbic acid (Vitamin C) (see Section 1.2). The value of these metabolites to human consumption and industry provides a high commercial interest in understanding the mechanistic cause and effect of spectra and the timing of wavelength delivery to increase a crop's nutritional value within CEA. Applications of this knowledge in increasingly popular local vertical farm systems could help to tackle micronutrient deficiencies found in urban environments (Ruel et al., 2017), resulting in holistic health benefits within society (Specht et al., 2014).

Current evidence indicates that for many antioxidants, transcriptional regulation of their biosynthetic genes is the first point of control for their accumulation in response to environmental cues (Dietz, 2014; von Lintig et al., 1997). Light has been recognised as an important environmental input to modulate the production of antioxidants (Bian et al., 2015). However, to advance understanding of light-based regulation on their biosynthesis, multiple aspects of light quality influences such as specific photoreceptors and signalling components must be examined, particularly upon flux-controlling biosynthetic genes. These include studies to clarify the role of light in the regulation of dietary nutrient production in plants focusing on the dissection of the effect of monochromatic fluence, the role of wavelengths supplementary to PAR, and also identification of the role of specific photoreceptors and signalling components and the temporal influences of photoperiod and circadian rhythm.

2.1.1 Rate Limiting Genes Control the Flux of Antioxidant Biosynthetic Pathways and Production of Their Precursors

Carotenoids, tocopherols and ascorbic acid are synthesised in the plant tissues via a string of metabolite interconversions facilitated by functional enzymes within individual biosynthetic pathways (Figure 2.1). Metabolic flux is characterised as the flow of molecules through such metabolic networks as compound biosynthesis pathways (Ratcliffe and Shachar-Hill, 2006). The rate of metabolic flux can be limited by the availability of precursor metabolites and enzymes within the pathway to undertake the required metabolic steps. Molecular investigation and identification of such bottlenecks facilitates the understanding of the factors that contribute to limiting metabolic flux. This promotes flux-controlling biosynthesis genes as prime targets for manipulation to deliver maximum impact into the performance of the metabolic pathway. Therefore, to assess the role of light in the metabolic flux of dietary antioxidants, the response of rate-limiting enzymic genes within the biosynthetic pathways to different light wavelengths must be defined.

2.1.1.1 MEP Pathway

In plants, the MEP pathway provides most isoprenoid precursors for essential compounds involved in photosynthesis, including the biosynthesis of carotenoids, chlorophylls and tocopherols, as well as growth regulators (gibberellins, cytokinins, abscisic acid (ABA), strigolactones) and monoterpenes for interaction with the environment (isoprene, monoterpenes, diterpenes) (Bouvier et al., 2005; Umehara et al., 2008; Vranová et al., 2013). Due to their role as photoprotectors, the production of carotenoids and tocopherols is tightly interlinked via their MEP pathway origin (Jiang et al., 2021). Therefore, the MEP pathway is key for the analysis of regulatory mechanisms of carotenoid and tocopherol biosynthesis.

The precursors for all isoprenoids are the molecules isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). In plants, they originate from either the cytolsolic MVA pathway or the chloroplastic MEP pathway (Hemmerlin et al., 2012; Lichtenthaler et al., 1997; Rodríguez-Concepción and Boronat, 2002; Shanker Dubey et

al., 2003). While both pathways produce IPP and DMAPP, the current evidence only supports a very limited exchange of this precursor between the cytoplasm and the chloroplasts (Rodríguez-Concepción, 2010), therefore focus will be on the MEP pathway. The chloroplastic MEP pathway is a relatively recent discovery (1990s), and is only conserved within bacteria, algae and higher plants (Zhao et al., 2013). All enzymes involved in the function of the MEP pathway are nuclear encoded and imported into the plastids (Bouvier et al., 2005; Cordoba et al., 2009; Rodríguez-Concepción and Boronat, 2002; Vranová et al., 2013).

A simplification of the MEP pathway is as follows: D-glyceraldehyde 3-phosphate (GAP) and pyruvate are condensed initially into 1-deoxy-D-xylulose 5-phosphate (DXP) by *DXP-synthase* (*DXS*), which is then isomerized to MEP by *DXP reductoisomerase* (*DXR*). A further four consecutive steps are catalysed sequentially by *2-C-methyl-d- erythritol 4-phosphate cytidylyltransferase* (*MCT*), *4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase* (*CMK*), *2-C- methyl-D-erythritol 2,4-cyclodiphosphate synthase* (*MDS*), and *4-hydroxy-3-methylbut-2-enyldiphosphate* (HMBPP) *synthase* (*HDS*). The final enzymic step is enacted upon HMBPP by *HMBPP reductase* (*HDR*) to create IPP and DMAPP (Figure 2.1a) (Pu et al., 2021; Zhao et al., 2013). The addition of three IPP units to the MEP product DMAPP produces GGDP, a common precursor for carotenoid synthesis, chlorophyll synthesis and PDP used in tocopherol synthesis (Rodríguez-Concepción and Boronat, 2002; Vranová et al., 2013).

Biochemical study of the enzymic genes has identified *DXS* (At4g15560) to be the main rate-determining step of the MEP Pathway in *Arabidopsis* (Figure 2.1a) (Banerjee et al., 2013; Enfissi et al., 2005; Estevez et al., 2000; Wright et al., 2014). Transcriptional control is known to be the first regulatory step for DXS, its activity is also allosterically regulated through inhibition by IPP/DMAPP (Banerjee et al., 2013; Ghirardo et al., 2014), whilst DXS protein abundance is regulated by an as yet unidentified MEP pathway products (Guevara-García et al., 2005; Han et al., 2013). Other flux-controlling enzymes of the MEP pathway are *DXR* (At5g62790) (Mahmoud and Croteau, 2001) for which overexpression shows enhanced flux through the MEP pathway (Carretero-Paulet et al., 2006; Estevez et al., 2000), and *HDR* (At4g34350) (Botella-Pavía et al., 2004; Kim et al.,

2009). The rate-limiting capacity of *DXR* and *HDR* are variable dependent on plant species and conditions (Cordoba et al., 2009).

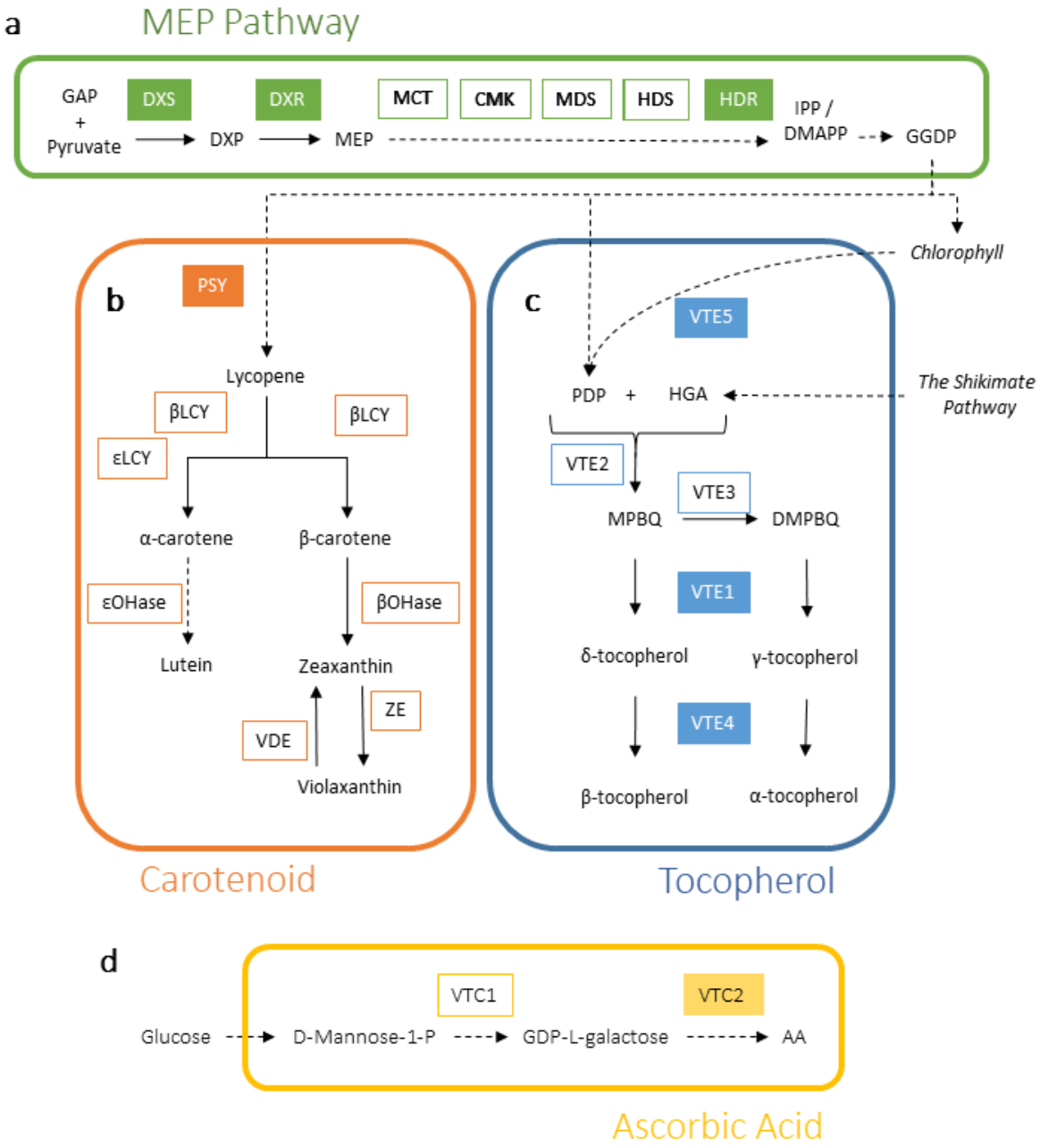


Figure 2.1 Simplified diagram of the isoprenoid biosynthesis pathways including (a) the MEP, (b) Carotenoid, and (c) tocopherol pathways, and (d) the ascorbic acid biosynthesis pathway. Direct steps along the pathways are connected with a solid black line and contracted steps are indicated with a dashed line. The boxes surround genes encoding the enzyme associated with the metabolic step of the adjacent arrow, with the rate-limiting enzymic genes studied in this chapter in coloured boxes. Abbreviations can be found within the main text.

2.1.1.2 Carotenoid Biosynthesis Pathway

Carotenoid synthesis is strongly linked to the MEP pathway (Rodríguez-Concepción, 2010). Dependence on the availability and flux of the MEP pathway was elegantly demonstrated by providing the substrate of DXS in excess and observing the acceleration of carotenoid synthesis in tomato fruits (Lois et al., 2000). At the beginning of carotenoid synthesis, *PHYTOENE SYNTHASE* (*PSY*) condenses two GGDP from the MEP pathway (Cazzonelli and Pogson, 2010) as the first committed step for the biosynthesis of carotenoids. The resulting compound 15-*cis*-phytoene undergoes several desaturation and isomerization steps to convert to all-*trans*-lycopene. After the formation of lycopene, carotenoid synthesis divides into two branches distinguished by different cyclic end-groups (Figure 1.3).

In the β, β-branch, *LYCOPENE B-CYCLASE* (*βLCY*) adds two β-inone rings to generate β-carotene. *B-CAROTENE HYDROXYLASE* (*βOHase*) oxidises β-carotene to derive the compounds used in the photo-protective xanthophyll cycle (Fiore et al., 2006). The core components of the xanthophyll cycle are zeaxanthin and violaxanthin, which are interconverted by ZEAXANTHIN EPOXIDASE (ZEP) and VIOLAXANTHIN DE-EPOXIDASE (VDE), respectively (Figure 2.1b). These compounds form part of the NPQ mechanism to protect plants from photodamage (Jahns and Holzwarth, 2012). The terminal products of the xanthophylls, violaxanthin and neoxanthin, converted by neoxanthin synthase (NXS) (Neuman et al., 2014), can be further modified to produce apocarotenoids such as phytohormones, signalling molecules and volatiles, including the drought sensitive hormone ABA (Hou et al., 2016; Nambara and Marion-Poll, 2005; Wasilewska et al., 2008). Along the alternative β, ε-branch, lycopene can be cyclized by both *βLCY* and *LYCOPENE E-CYCLASE* (*εLCY*) to generate α-carotene, with one β- and one ε-inone ring. Additions of functional groups by *E-CAROTENE HYDROXYLASE* (*εOHase*) to the respective rings converts α-carotene into lutein, another xanthophyll (Figure 2.1b) (Cunningham and Gantt, 1998; Moise et al., 2014; B. Pogson et al., 1996).

Much of the regulation for carotenoid synthesis centres on PSY (At5g17230) as the first and rate limiting step of the pathway (P. D. Fraser et al., 1994; Rodríguez-Villalón et al., 2009b; Ruiz-Sola and Rodríguez-Concepción, 2012). Transcription has been recognized

as the first regulatory step for PSY (von Lintig et al., 1997). PSY is encoded by multi-gene families in most plant species, except for *Arabidopsis* where PSY is encoded by a single gene (Hannoufa and Hossain, 2012). Molecular up-regulation of *PSY* expression has repeatedly demonstrated to increase carotenoid production in various plant species (Botella-Pavía and Rodríguez-Concepción, 2006; Fraser and Bramley, 2004; Rodríguez-Villalón et al., 2009b). Additionally, up-regulation of *PSY* activity in deetiolating seedlings has been shown to initiate a feedback mechanism that eventually results in the post-transcriptional accumulation of higher DXS protein levels (Guevara-García et al., 2005; Rodríguez-Villalón et al., 2009b). A coordinated induction of *DXS* and *PSY* has also been observed when carotenoid production is boosted during fruit ripening (Botella-Pavía et al., 2004; Fanciullino et al., 2008; Lois et al., 2000). Therefore, *PSY* is a most common target for genetic manipulations of carotenoid production in staple crops such as rice and tomato (Fraser et al., 2002; Pogson and Rissler, 2000; Römer et al., 2000; Rosati et al., 2000; Shewmaker et al., 1999).

2.1.1.3 Tocopherol Pathway

The tocopherols consist of a hydrophobic HGA 'head', supplied from the shikimate pathway, and a hydrophilic PDP 'tail' (Figure 2.1c) (DellaPenna and Pogson, 2006; Mène-Saffrané, 2017). PDP is derived from two sources, GGDP generated in the MEP Pathway or the phosphorylation of free phytol from chlorophyll degradation to PDP, catalysed by VITAMIN E 5 (*VTE5;* At5g04490; Phytol Kinase). The enzyme *VTE2* (At2g18950; Homogentisic Acid Phytyl Transferase) combines HGA and PDP as 2-methyl-6-phytylplastoquinol (MPBQ), the pathway then splits into two. MPBQ can be directly cyclised into δ-tocopherol and then methylated to β-tocopherol by *VTE1* (At4g32770; Tocopherol Cyclase) and *VTE4* (At1g64970; γ-Tocopherol Methyltransferase), respectively. Alternatively, MPBQ is methylated to 2,3-dimethyl-6-phytyl-1,4-benzoquinol (DMPBQ) by *VTE3* (Methyltransferase), which generates γ-tocopherol then α-tocopherol, again via *VTE1* and *VTE4*, respectively (Figure 2.1c) (Mène-Saffrané, 2017).

Several of the tocopherol biosynthesis genes encode enzymes with rate-limiting activity in the tocopherol biosynthesis pathway (Figure 2.1c) (Fritsche et al., 2017). *Arabidopsis*

mutants of *VTE1* lack all tocopherols and showed accumulation of its substrate DMPQ (Kanwischer, 2005; Porfirova et al., 2002). On the other hand overexpression of *VTE1* increases the tocopherol levels in *Arabidopsis* (Kanwischer, 2005; Szymańska and Kruk, 2010), tobacco (Yabuta et al., 2013) and lettuce (Lee et al., 2007b). Manipulations of *VTE4* alter the composition of tocopherols in plants. Limitations of this enzyme can cause accumulation of γ-tocopherol (Kanwischer, 2005), whereas overexpression of *VTE4* can increase the α-tocopherol composition in *Arabidopsis* and potentially total Vitamin E content also (Li et al., 2010c; Shintani, 2002). *VTE2* deficiency in *Arabidopsis* causes a lack of all tocopherol derivatives and pathway precursors (Sattler et al., 2004), whilst overexpression of *VTE2* increases total tocopherol concentration in both seeds and leaves (Collakova, 2003; Savidge et al., 2002), indicating a rate limiting gene. Transient expression of *AtVTE2* individually and in combination with At*VTE1* in *Nicotina benthamiana*, elevated the α-tocopherol content in leaves (Sathish et al., 2018). Further evidence shows that the combined overexpression of *VTE2* and *VTE4* have the greatest effect in increasing Vitamin E activity, showing a 12-fold increase in *Arabidopsis* (Collakova, 2003) and 19-fold increase in lettuce (Li et al., 2010c). Chlorophyll and α-tocopherol co-occur in green tissues (Booth, 1963) and are linked via the activity of *VTE5* (Kim et al., 2014). As a phytol kinase, the activity of this enzyme increases the supply of chlorophyll degradation-derived phytol PDP, which has been highlighted as a limiting factor for tocopherol synthesis in tomato (Ischebeck et al., 2006; Valentin et al., 2006). Tocopherol synthesis is highly dependent on PDP (Almeida et al., 2016; Ischebeck et al., 2006; Zhang et al., 2015a), therefore, boosting the source of PDP has been highlighted as a focal point for tocopherol enhancement (Quadrana et al., 2013). In summary, current evidence supports a role of VTE1, VTE2, VTE4 and VTE5 as a flux controlling enzymes in the tocopherol biosynthetic pathway (Figure 2.1c).

2.1.1.4 Ascorbic Acid Biosynthesis

Derived from AA, the AsA molecule is the most abundant soluble antioxidant and essential to the function of the carotenoids and tocopherols, acting to salvage and recycle the nutrient radicals for further use (Szarka et al., 2013). However, as AsA synthesis occurs in the cytosol, it is metabolically and spatially separated from the MEP, carotenoid

and tocopherol pathways (Fenech et al., 2021). The major route of AA biosynthesis in plants occurs through The Smirnoff–Wheeler pathway, where d-mannose and l-galactose are considered the primary compounds (d-mannose/l-galactose pathway) (Paciolla et al., 2019; Wheeler et al., 1998). Firstly, D-Glucose 6-P is sequentially transformed into D-mannose 1-P. *GDP-D-MANNOSE PYROPHOSPHORYLASE* (*VTC1/ GMP*) with *GDP-D-MANNOSE EPIMERASE* (*GME*) then converts D-mannose into GDP-L-galactose. Next, *GDP-L-GALACTOSE PHOSPHORYLASE* (*GGP*), produces L-galactose 1-P, which via a futher 2-step process generates L-ascorbic acid (Figure 2.1d) (Fenech et al., 2020). The concentration of AsA can vary when under severe oxidative stress (Fenech et al., 2020) due to hydration to DHA and subsequent reduction back to ascorbate via the Halliwell-Foyer-Asada cycle (Asada, 2002). Therefore, the control of the pathway was difficult to determine, but eventually it was found that the flux of the pathway is mainly controlled by GGP (Fenech et al., 2020), or in *Arabidopsis* by the two paralogs *VITAMIN C 2* and *5* (*VTC2* and *VTC5*) (Figure 2.1d) (Bulley et al., 2009; Yoshimura et al., 2014).

VTC2 (At4g26850) is expressed primarily in green tissues, and is upregulated more than other biosynthetic genes (Figure 2.1d) (Müller-Moulé, 2008; Yabuta et al., 2007), especially by high light which increases the AsA pool size, as well as abundance of *VTC2* in *Arabidopsis* (Dowdle et al., 2007) and tomato (Zhang et al., 2021). *VTC2* gene expression levels strongly linked to the resultant AsA levels in plants (Dowdle et al., 2007; Laing et al., 2004; Tamaoki et al., 2003; Yoshimura et al., 2014), with overexpression of *VTC2* potentially increasing the AA levels several-fold (Zhou et al., 2012). Therefore, *VTC2* (GGP) is identified as the rate-limiting gene in the Smirnoff-Wheeler Pathway in a wide variety of species including tobacco (Bulley et al., 2009), tomato (Bulley et al., 2012; Mellidou et al., 2012; Wang et al., 2013), kiwifruit (M. Li, Ma, Liang, et al., 2010), citrus (Alós et al., 2013), blueberry (Liu et al., 2015), strawberry and potato tubers (Bulley et al., 2012). VTC2 is localized in two compartments, the cytosol and the nucleus, suggesting enzymatic and regulatory functions for this protein (Müller-Moulé, 2008). This is supported by evidence of feedback control by an upstream open reading frame (ORF) in the *VTC2* gene which facilitates translational repression by ascorbate found in *Nicotiana benthaminana* (Fenech et al., 2020; Laing et al., 2015; Li et al., 2018; Zhang et al., 2018a).

This secures the role of VTC2 as a rate limiting gene (Fenech et al., 2020) and makes it the focus for engineering increases in AA concentrations (Broad et al., 2020).

2.1.2 Evidence of Light Control Over Phytonutrient Availability

Studies have identified evidence of light modulation upon phytonutrient biosynthesis, through the production of their metabolic products (Paradiso and Proietti, 2021) and direct measurement of biosynthesis gene transcription and regulation, including the MEP pathway, carotenoid, tocopherol, and AA biosynthesis. Light responses involve specific wavelength absorption and subsequent signalling via photoreceptors and regulatory partners such as HY5 and PIFs to coordinate of genetic regulation (see Section 1.3). In addition, the majority of the genes in the aforementioned pathways have circadian influence, often showing peaks at dawn or within the morning phase (Covington et al., 2008). Many of these responses have been associated with the rate-limiting genes identified.

2.1.2.1 Light Regulation of the MEP-Pathways of Isoprenoid Biosynthesis

MEP biosynthesis gene transcript accumulation is triggered by light exposure at key developmental stages such as de-etiolation in *Arabidopsis* (Botella-Pavía et al., 2004; Ghassemian et al., 2006; Hsieh and Goodman, 2005) and fruit ripening in tomato (Lois et al., 2000). Abiotic high light stress conditions also increase MEP pathway activity across the plant kingdom, from unicellular microalgae, *Haematococcus pluvialis*, to Spinach (Hu et al., 2019; Rivasseau et al., 2009). Red light in particular stimulates upregulation of all of the flux-controlling MEP pathway genes, *DXS*, *DXR* and *HDR* (Chenge-Espinosa et al., 2018). Mutant studies have shown that the red light photoreceptors, phyB and phyA are instrumental in orchestrating the changes in isoprenoid pathway activities by red and far-red light, respectively (Meier et al., 2011; Rodríguez-Concepción et al., 2004). Moreover, the phytochome interacting factors PIF1, PIF3 and PIF5 are implicated as general regulators of *DXS, DXR* and *HDR* (Chenge-Espinosa et al., 2018; Mannen et al., 2014). PIFs act as negative transcriptional regulators within the phytochrome signalling pathway,

therefore their involvement supports the role of red light in activating transcription within the MEP biosynthesis pathway.

UV-B may also contribute to MEP pathway regulation as a variant of *DXS* in rice was induced to nine-fold of normal expression after UV-irradiation (Kim et al., 2005). Further evidence of UV-B-mediated signalling in the accumulation of downstream MEP pathway products can be observed in the production of flavonoids or ABA in various species exposed to UV treatment (Gil et al., 2012; Park et al., 2013a; Shamala et al., 2020). Interestingly, transcription of MEP pathway genes also reflects light-dark oscillatory patterns, influenced by the circadian clock (Cordoba et al., 2009). A gene co-expression network showed significant correlation with circadian clock genes, in general these were the 'morning loop' genes (*PRR9*, *CCA1*, *LHY*) (Vranová et al., 2013). Analysis of *DXS*, *HDR* and *PSY* gene sequences showed the presence of multiple LHY/CCA1-binding elements in their promoter regions (Pokhilko et al 2015) and circadian oscillation in gene transcripts has also been associated in the accumulation of the downstream products of the MEP pathway, such as chlorophyll, monoterpenes, ABA, carotenoids and tocopherols (Dodd et al., 2005; Dudareva et al., 2005; Fukushima et al., 2009).

2.1.2.2 Light Modulation of the Carotenoid Biosynthesis Pathway

Evidence of light activation exists throughout the carotenoid biosynthesis pathway, especially during de-etiolation when the expression of many of the carotenoid synthesis genes rapidly increases (Bouvier et al., 2005; Stange and Flores, 2012). As their photoprotective function suggests, under high light, total xanthophyll content will increase in *Arabidopsis*, with up to 3-fold increases in wheat and barely; a trend which then reverses in low light (Falbel et al., 1994; K. K. Niyogi et al., 1998).

Light regulation of carotenoid accumulation in carrot roots has long been observed (Fuentes et al., 2012; Rodriguez-Concepcion and Stange, 2013; Stange et al., 2008). The modulation of carotenoids in tomato fruits by red, blue, far-red, green and white light have also been observed on carotenoid production and accumulation in tomato (Ntagkas et al., 2020). The concentration of lycopene has been raised in tomatoes by overexpressing cryptochrome (Giliberto et al., 2005) or by illuminating fruits with red

light (Llorente et al., 2016). Therefore both cryptochromes (Liu et al., 2018) and phytochromes (Ernesto Bianchetti et al., 2018), in particular phyA (Alba et al., 2000) have been linked to carotenoid production in tomato fruit ripening. Furthermore, red-light induced carotenoid accumulation in tomato fruits is subject to reversal by FR exposure, highlighting the role of phytochromes in the modulation of tomato carotenogenesis (Alba et al., 2000; Schofield and Paliyath, 2005; Thomas and Jen, 1975).

The influence of light on carotenoid synthesis and gene expression has also been reported in green tissues, such as the deetiolation of mustard and tobacco seedlings (von Lintig et al., 1997). Blue light was observed to increase a range of isoprenoid products including α- and β-carotenes and lutein in microgreens (Samuolienė et al., 2017), whereas carotenoid products accumulate differentially under red and blue light in leafy greens (Lefsrud et al., 2008). The xanthophyll biosynthetic genes are up-regulated upon exposure to red, blue and white light in tobacco (Woitsch and Römer, 2003), with VDE transcript levels in tobacco increasing under high light exposure (Bugos et al., 1999).

The light control of carotenoid synthesis lies disproportionately on the rate-limiting gene PSY. In *Arabidopsis PSY* expression is rapidly increased upon de-etiolation and is highly co-expressed with MEP Pathway genes under light-induction (Botella-Pavía et al., 2004; Meier et al., 2011; von Lintig et al., 1997). Expression of *PSY* is responsive to white, blue, red and far-red light (Li et al., 2020; Meier et al., 2011; Stange and Flores, 2012), which is followed by an increase in PSY protein and enzymatic activity (Welsch et al., 2000). Cryptochromes have been suggested to coordinate the blue light regulation of *PSY* (von Lintig et al., 1997), whilst phyA and phyB mediate the up-regulation of *PSY* transcripts and protein, under red and far-red light (Li et al., 2008a; von Lintig et al., 1997; Welsch et al., 2000, 2003).

The light signals from cryptochromes and phytochromes act upon *PSY* expression via HY5 and PIFs (Meier et al., 2011; Xie et al., 2019). Phytochrome signalling coordinates the binding of both transcription factors to light-responsive elements in the promoter of PSY (Toledo-Ortiz et al., 2010; von Lintig et al., 1997). Specifically, PIF1 binds to the G-box motif in the promoter of *PSY*, and in collaboration with PIF3, downregulates *PSY* expression, thereby negatively regulating carotenoid biosynthesis. Exposure to red light

releases PIF repression after just 1 h, allowing for rapid production of carotenoids upon de-etiolation (Toledo-Ortiz et al., 2010).

PSY expression follows a diurnal fluctuation of repression in the dark that released upon exposure to light. This diurnal pattern is attributed to PIF1, which represses *PSY* gene expression in the dark, and HY5 which upregulates the expression in the light. Together they act antagonistically upon the same G-box sequence in the *PSY* promoter, acting as a module to balance *PSY* expression (Toledo-Ortiz et al., 2010, 2014). Circadian influences are also present (Covington et al., 2008; Fukushima et al., 2009) and mutant studies revealed that the morning components of the circadian clock, LHY and CCA1 assist in the coordination of the reliable dawn peak of *PSY* by binding to the morning LHY-CCA1 complex in the promoter region of *PSY* (Pokhilko et al., 2015). These mechanisms of regulation ensure that *PSY* peaks simultaneously with *DXS* and *HDR* in the MEP pathway to set up the photosynthetic apparatus and photoprotection for the day ahead.

2.1.2.3 Modulation of Tocopherol Biosynthesis by Light

Tocopherols mainly function as antioxidants, protecting lipids and the photosynthetic machinery from damage (Havaux et al., 2005; Maeda et al., 2005; Munné-Bosch and Alegre, 2002). Considering that tocopherol synthesis is located in the plastids, it is expected that light will have an effect on the accumulation of tocopherols. Current research in *Arabidopsis* shows that in coordination with their photoprotective role, high light conditions increase tocopherol synthesis (Havaux et al., 2005; Krieger-Liszkay and Trebst, 2006; Szymańska and Kruk, 2010). Similarly, higher tocopherol content was found in high-altitude plants exposed to greater light intensities and UV-B spectral content, which can cause oxidative damage to tissues and photosynthetic machinery (Szymańska et al., 2015). More specifically, UV-B irradiation of *Arabidopsis* caused increases in γ-tocopherpol (Emiliani et al., 2018).

Further spectral effects of light on tocopherol accumulation has been observed, with elevated levels in microgreens under blue light, and green light in baby leaf lettuce and microalgae (Ljubic et al., 2021; Samuoliene et al., 2012; Samuolienė et al., 2017). In baby leaf lettuce, differential accumulation of tocopherols by light shows that for γ-tocopherol

green light has a stronger effect than blue light, whereas in red light, α- and β- tocopherol were preferentially accumulated (Samuoliene and Duchovskis, 2012). Park et al. (2013) demonstrated that ginseng roots under sole blue LED treatments (465 nm) contained significantly higher amounts of α-tocopherol in comparison to fluorescent lamps. In tomato, modulation of overall tocopherol content was observed when fruits ripened under different wavelengths of light (red, blue, far-red, green and white), indicating involvement of several photoreceptors (Ntagkas et al., 2020).

Few studies exist that link light signalling components with regulation of tocopherol biosynthesis genes. High light conditions have been found to increase the expression of *VTE5* in tomato, in addition to *VTE2* (Gramegna et al., 2019) and *VTE1* (Kobayashi and DellaPenna, 2008) and a *VTE1* homologue was linked to blue light signalling in tea plants (Zheng et al., 2019a). A genetic screen in *Arabidopsis* found a chloroplast-based gene *(At2g41040)*, with similar methyltransferase activity to VTE4, that was misregulated in a *cry1 hy5* mutant (Kleine et al., 2007). Therefore, evidence indicates that CRY1 has a role in the high light response of tocopherol accumulation and regulates the genetic response through HY5. Also, in tomato, cryptochromes have been associated with the accumulation of tocopherols (Fantini et al., 2019), together with phytochromes. Specifically, overexpression of phyB elevates levels of α-tocopherol (Alves et al., 2020). Searches of the promoters of the tocopherol biosynthetic pathway genes in tomato has discovered common cis-regulatory elements, which suggests control by transcription factors (Quadrana et al., 2013) such as PIF3, which has been shown to have a repressive role on the conversion of GGDP into the tocopherol precursor PDP in tomato (Gramegna et al., 2019).

Finally, α-tocopherol content follows a diurnal rhythm in leaves (Munné-Bosch and Alegre, 2002). In *Arabidopsis*, the circadian components CCA1 and LHY have been linked with direct control of *VTE1* expression (Abuelsoud et al., 2020), and PRR9/7/5 may also play a role in regulating the diurnal cycle (Fukushima et al., 2009), showing that photoperiodic and circadian regulation are also factors in the control of tocopherol production. Yet, α-tocopherol diurnal patterns varies across Mediterranean plant species, therefore such responses may be adaptive and require further investigation (Oliván and Munné-Bosch, 2010).

2.1.2.4 Light Modulation of Vitamin C Production

Due to its prolific role in plant function, the regulation of AA in plant cells comes from various levels, including photosynthetic signals (Bartoli et al., 2016; Karpinski et al., 1997; Yabuta et al., 2007; Zhang et al., 2021), carbohydrate availability (Ntagkas, Woltering, Bouras, et al., 2019; Tamaoki et al., 2003), and pathogen defence (Kiddle et al., 2004). AA levels in plants change drastically responding to environmental status, such as temperature, water (reviewed in Ishikawa et al., 2018), and similarly to the isoprenoid pathways, light is an important environmental cue for AA biosynthesis.

There is a well documented positive correlation between ascorbate levels and increasing light intensity, seen in leaves and leafy crops (Bartoli et al., 2006; Dowdle et al., 2007; Fukunaga et al., 2010; Grace and Logan, 1996; Massot et al., 2012) including lettuce (Gómez and Jiménez, 2020; Min et al., 2021; Zha, Liu, et al., 2019; Zhou et al., 2021), spinach (Eskling and Åkerlund, 1998), and cabbage (Lei et al., 2009); and in several fruit species including tomato (Ntagkas, Woltering, Nicole, et al., 2019), kiwi (Li, Ma, Liu, et al., 2010), apple (Li et al., 2009) and grapefruit (Cakmak et al., 1995). In addition to intensity, photoperiod and daily light integral also contribute to this effect (Nicole et al., 2019). However, caution should be used with high light treatments as it can induce excessive oxidative stress that in turn lowers yield (Gómez and Jiménez, 2020; Zha, Liu, et al., 2019), and its accumulation may be higher in continuous low level irradiation (Riga et al., 2019) under red and blue light (Shen et al., 2014; Zha, Zhang, et al., 2019).

Phytochromes have been proposed to regulate some of the AA pathway (Thomsen et al., 1992), and may be involved in R:FR mediated changes in AA levels as seen in *Phaseolus vulgaris* (Bartoli et al., 2009) and tomato fruits (Labrie and Verkerke, 2014). However, phytochrome driven response is not be ubiquitous in all plant species, as ascorbic acid content in red leaf lettuce was not affected by supplemental red light (Li and Kubota, 2009; Ohashi-Kaneko et al., 2007). In addition, there is an indication that monochromatic blue light has a positive influence on concentrations of AA (Lester and Lester, 2015) in leafy greens such as cabbage (80 $\mu mol.m^{-2}.s^{-1}$) (Li et al., 2012; Paradiso and Proietti, 2021), lettuce and komatsuna (300 $\mu mol.m^{-2}.s^{-1}$) (Ohashi-Kaneko et al., 2007). AA accumulation has also been noted in tomato fruit ripening under supplementary blue

light (250 µmol.m^{-2}.s^{-1}) (Ntagkas, Woltering, Nicole, et al., 2019), whereas linear increases of AA with BL intensity have been observed in citrus fruits (50 µmol.m^{-2}.s^{-1} and 100 µmol.m^{-2}.s^{-1}) (Zhang et al., 2015a), and in lettuce AA content was highest in a 75 % blue to 25 % red light treatment (200 µmol.m^{-2}.s^{-1}) (Zha et al., 2020). The enhancement of AA by blue light is attributed to regulation of biosynthesis genes at the transcription level (Zhang et al., 2015a), therefore it is likely that cryptochromes are involved in the AA biosynthesis pathway in a similar manner to CRY1 involvement in high irradiance responses (Kleine et al., 2007).

Experiments with AA-deficient plants suggest a protective role of AA against the damaging effects of UV-B in *Arabidopsis* (Conklin et al., 1996; Gao and Zhang, 2008). Similarly, AA improvements have been recorded in UV-B for soybean sprouts (Xu et al., 2005) and cucumber, where also an associated role of HY5 in the response was reported (Liu et al., 2019). AA expression is linked with other transcription factors beyond HY5, including some associated with ethelene or ABA (reviewed Mellidou and Kanellis, 2017). Experiments in rice show that light signals are likely to act on the light-responsive cis-elements found in the promoters of AA biosynthetic genes (Gao et al., 2011). In Kiwi, a G-box motif has been identified in the *VTC2* homologue and is responsible for the light responses (Li et al., 2013).

AA levels also increase and decrease in periods of illumination and darkness respectively, which correlates with transcripts of *VTC2* expression patterns (Dowdle et al., 2007; Gao et al., 2011; Maruta et al., 2008; Yabuta et al., 2007). In this respect, there is evidence to support that *VTC2* expression shows diurnal and circadian regulation resulting in fluctuations in ascorbate biosynthesis (Dutilleul et al., 2003; Gao et al., 2011; Tamaoki et al., 2003; Yoshimura et al., 2014). Expression of *VTC2* shows nightly increases in transcript levels (Abuelsoud et al., 2020) to produce a peak of mRNA transcripts in the early morning (Dowdle et al., 2007) and a corresponding accumulation of leaf AA content 8 hours later (Massot et al., 2012). Morning expression peak of *VTC2* supports the biosynthesis of AA for maximum light intensity later in the day (Dowdle et al., 2007). Data in *Arabidopsis* indicates that the diurnal expression of *VTC2* is controlled through the morning loop of the circadian clock as demonstrated by the conservation of *VTC2*

expression pattern under constant light (Dowdle et al., 2007) and its misregulation in a *cca1 lhy* mutant (Abuelsoud et al., 2020).

2.1.3 Chapter Aims and Objectives

Due to the vast amount of publicly available genomic experiments, *Arabidopsis thaliana* is a good model to investigate transcriptional responses. In *Arabidopsis*, researchers are able to explore a wide range of experimental conditions in WT and mutant genotypes, extracting their genes of interest from the full-genome published to evaluate as yet unstudied effects upon the transcriptional regulation of those genes. Whilst it is important to recognise that further regulatory mechanisms may occur after transcription, affecting protein abundance and activity, as the primary regulatory response, changes in gene transcription may be taken as an indication for the requirement of that protein under the experimental conditions. Therefore, using published genomic datasets and experimental measurements of gene transcription on the model plant *Arabidopsis thaliana*, this chapter aims to determine the role of light as a regulatory influence upon the biosynthesis of isoprenoid precursors, carotenoids, tocopherols, and ascorbic acid. Focus is on the rate-limiting genes previously described, which are as follows: *DXS*, *DXR* and *HDR* for the MEP pathway, *PSY* in the carotenoid pathway, *VTE1*, *VTE4* and *VTE5* for the tocopherol pathway, and *VTC2* for the ascorbic acid biosynthesis pathway (Figure 1).

The objectives of this Chapter are to investigate:

1. The effect of blue and red wavelengths on the expression of these genes, including the role of cryptochromes and phytochromes, respectively;
2. The role of light signalling partners *HY5* and *PIFs* on the expression of rate-limiting genes;
3. The promoter regions of the selected target genes, using bioinformatic approaches to identify potential regulatory motifs linked to light, photoperiodic and circadian modulation and evaluate the potential light-circadian networks.
4. The effect of far-red and UV-B supplementation as mechanisms of additional spectral influence.

2.2 MATERIALS AND METHODS

2.2.1 Genomic Expression Analyses

Throughout this chapter, *Arabidopsis* transcript abundance data was searched from the following publicly available genome-wide transcriptomic datasets using BL, RL, FR, UV-B, and WL. Data was averaged and fold change was used to more easily determine the effect of the light treatment by dividing the value for light treatment over the dark control, and the impact of the photoreceptors under the light treatment by diving the average in the photoreceptor mutant by the WT. The Log2 value of the resulting fold change was used to present the results on the same scale.

4.5 days BL RNA-seq dataset GSE58552 (He et al., 2015) extracted samples from 4.5 day old dark-grown WT, in addition to WT and *cry1cry2* seedlings grown in continuous BL (15 $\mu mol.m^{-2}.s^{-1}$). Data is displayed in Figure 2.2a and 2.8a.

4 days RL Microarray dataset GSE31587 (Hu et al., 2013) was used for Figures 2.3a, and 2.7b. Data was generated using 4-day old LER WT and *phyABCDE* seedlings grown in darkness or continuous red light (50 $\mu mol.m^{-2}.s^{-1}$).

Far-red Dataset GSE28297 (Leivar et al., 2012) measured WT and *pifQ* mutant grown in WL (19 $\mu mol.m^{-2}.s^{-1}$; R:FR ratio = 6.48) for 2 days at 21°C. Seedlings were then maintained in the same fluence rate of WL supplemented with far-red light (R:FR = 0.006) for 1, 3 or 24 h before harvesting. Control seedlings were maintained in parallel in the same fluence rate of WL for 24 hr before harvesting. Microarray data was used for Figures 2.4a and 2.8a.

UV-B For Figures 2.4b and 2.8b, microarray data with accession number E-MEXP-1957 (Favory et al., 2009a) contains data of Col-0 WT and *uvr8-6* photoreceptor mutant grown under continuous white light (3.6 $\mu mol.m^{-2}.s^{-1}$) supplemented with UV-B tubes (1.5 $\mu mol.m^{-2}.s^{-1}$) with either 345-nm cutoff filter (- UV-B) or 305-nm cutoff filter (+ UV-B). In addition to a 4 day treatment, filters were exchanged to provide a UVB treatment at 1 and 6 h before harvesting.

hy5 Microarray data for Figure 2.5a was provided by dataset GSE62119, generated with 3-day old WT and *hy5* mutant seeds grown under continuous white light (no intensity data provided) (Kawashima *et al*., unpublished data).

pifQ Figure 2.5b and 2.9 displays microarray data from the GSE17159 dataset using 2-day old Col-0 WT and *pifQ* mutants (non-functioning *PIF1*, *PIF3*, *PIF4* and *PIF5*) grown in darkness, constant red light (6.7 µmol.m^{-2}.s^{-1}) or 1 h red light before harvest (7.5 µmol.m^{-2}.s^{-1}) (Leivar et al., 2009).

Photoperiod and Circadian Photoperiodic expression data used for Figure 2.6 and 2.10, was taken from the DIURNAL database published by (Mockler et al., 2007). Data was collected on LER WT seedling, grown on agar with 3% (w/v) sucrose for 8 days under long day (16/8 hr L/D, 100 µmol.m^{-2}.s^{-1}) or short day (8/16 hr L/D, 100 µmol.m^{-2}.s^{-1}) light conditions at 22°C. Data was accessed via the DIURNAL online portal.

2.2.2 Experimental Plant Material

Col-0 WT, and light component mutants *phyAB* and *cry1cry2*, were sterilised with 10% (v/v) bleach solution for 8 minutes, washed with RO water and then sown in 0.5 basal MS media with vitamins and without sucrose. The seeds were vernalised at 4°C for 72 h and triggered to germinate with a WL treatment for 3 h, followed by a dark treatment for 21 h. Plants were exposed to a short term treatment (darkness followed by 2 h light) and long term treatment (continuous light) of RL (50 µmol.m^{-2}.s^{-1}) or BL (20 µmol.m^{-2}.s^{-1}) before harvesting after 4 days. Harvesting was performed under green light with liquid nitrogen. The material was stored at -80°C before processing for qPCR.

2.2.3 RNA Extraction, cDNA Synthesis and qPCR Analysis

RNA Extraction RNA extraction was performed using a SpectrumTM Plant Total RNA Kit (Sigma Aldrich) and cleaned with an RNase Free DNase Set (Qiagen). RNA was quantified with 2 µg of RNA on a Nanodrop 2000c Spectrophotometer (Thermo Scientific). cDNA was synthesised with a ScientificTM RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher) according to manufacturer's instructions using oligo dTs.

qPCR Analysis RT-qPCR analysis was performed using 5 µl PrimerDesign PrecisionPLUS qPCR Master Mix premixed with SYBR Green, 1 µl of 3 µM forward and reverse primer, 1 µl of sample cDNA, and 2 µl of sterilised water in a 10 µl reaction volume. The reaction was performed using a Stratagene Mx qPCR Machine (Agilent Technologies) with the following thermal cycling program: 95°C for 10 min, followed by 40x cycles of 95°C for 10 sec, 60°C for 10 sec, and 72°C for 15 sec followed by melting curve from 65°C to 95°C to ensure primer targeting specificity. Results were analysed using Stratagene Mx PRO software and Microsoft Excel. Results were normalised to the light-stable *PP2A* reference gene (Klie and Debener, 2011) and relative gene expression was calculated as described in Pfaffl's methods for analysing LightCycler PCR data (Pfaffl, 2001). A full list of primers is presented in Appendix Table 6.1.1.

2.2.4 Promoter Motifs

The promoter region 2 Kbp upstream of the start codon of the genes of interest in the biosynthesis pathways was searched for protein motif binding elements indicating an interaction or transcriptional control by circadian and light control elements using the Patterns Locator online bioinformatics tool (Mŕazek and Xie, 2006).

The circadian clock regulatory elements in Arabidopsis are grouped into three major 'modules' (Staiger et al., 2013). The G-box (CACGTG), the Morning Element (ME, CCACAC) and the HUD, also known as a PBE-box, (CACATG) motifs represent a 'Morning Module' that is associated in genes expressed at dawn; while the Evening Element (EE, AAAATATCT) and the GATA motif (GGATA) consist of an 'Evening Module' that is linked with evening-expressed transcripts. The third 'Midnight Module' includes the Starch Box (SBX: AAGCCC), the Telo-Box (TBX: AAACCCT) and the Protein Box (PBX: ATGGGCC), which is enriched in genes that have midnight-specific expression (Staiger et al., 2013). Searches also included the CCA1 binding site (AAAATCT or ACAATCT), and T1ME (TGTG) (Huang et al., 2012).

Motifs that are demonstrated to bind PIF and HY5 transcription factors were also searched. These include E-box variants (CANNTG), including G-box (CACGTG) and PBE-box (CACATG/ CATGTG) or G-box coupling elements (GCEs) containing the 'ACGT' motif

core which bind to PIFs (Kim et al., 2016a; Pu et al., 2021; Toledo-Ortiz et al., 2003; Zhang et al., 2011a; Zhang et al., 2013).

In addition to CGE-related sequences, including the Z-box (ATACTGTGT), CA (GACGTA) and CG hybrids (GACGTG), as well as G-box (CACGTG), C-box (GACGTC) and A-box (TACGTA) which interact with HY5 (Lee et al., 2007a; Zhang et al., 2011a). Motifs and their associated bindings are listed in the Appendix Figure 6.1.5.

2.2.5 ATTRACTOR Search

The *Arabidopsis Thaliana* Transcriptional Circadian Network (ATTRACTOR, https://greennetwork.us.es/ATTRACTOR/) was used to explore the influences of circadian clock and light signalling on the genes of interest within the biosynthesis pathways. The service integrates transcriptomic data with cistronic data, generated using ChIP-seq on central transcriptional factors and regulators, including CCA1, LHY, TOC1, PRR5, PRR7, PRR9, LUX, ELF3, ELF4, PHYA, PHYB, CRY2, PIF5, PIF4 and PIF3.

2.2.6 Statistical Analysis

Genomic Datasets Expression profiles of published RNA-seq and microarray datasets were analysed using Microsoft Excel, where significant differences in gene expression ratios were analysed with the Mann-Whitney-Wilcoxon test for all genes and were adjusted using the Benjamini–Hochberg false discovery rate (FDR) at the significance level of 0.05, where triplicate results existed. Otherwise, the F statistic was generated to verify equal variance between groups before performing a paired Student's *t*-test. The statistical analyses used are listed in the figure legend.

Light Exposure Experiment qPCR expression data was generated in biological and technical triplicate for all light conditions tested. Differences between treatments was statistically validated with a Student's *t*-test using Microsoft Excel.

2.3 RESULTS

As previously mentioned, examination of the literature identified several rate limiting biosynthesis genes: *DXS*, *DXR* and *HDR* of the MEP pathway, *PSY* in the carotenoid pathway, *VTE1*, *VTE4* and *VTE5* of the tocopherol pathway, and *VTC2* of the ascorbic acid biosynthesis pathway (Figure 2.1). To examine the impact of different wavelengths of light within the biosynthesis pathways, bioinformatics and qPCR were employed as exploratory bioinformatics and molecular biology methods to analyse transcriptional control via light quality and major photoreceptors including cryptochromes, phytochromes and UVR8, upon the expression of the target genes. Further published genomic datasets were analysed to verify photoperiodicity of these genes. Due to their close association as isoprenoids, the MEP, carotenoid and tocopherol pathways are presented together first, with the data for AA biosynthesis following.

2.3.1 Blue Light Impacts Isoprenoid Gene Transcription Through Cryptochromes

The effect of long-term (4.5 days continuous) exposure to BL on the expression of the enzymic genes was examined in *Arabidopsis* WT and *cry1cry2* mutant using the published RNA-sequencing dataset GSE58552 (He et al., 2015) (Figure 2.2a). qPCR data was gathered via a light exposure experiment, whereby *Arabidopsis* WT and *cry1cry2* mutants were grown under 4 days continuous BL and expression measured by qPCR (Figure 2.2b). Results per pathway are summarised below.

MEP Pathway The RNA-seq datasets and qPCR experiment both showed BL supressed *DXS* expression and induced *DXR*. However, the RNA-seq data showed *HDR* expression was significantly repressed under BL, confirmed by FDR and *t*-test analysis (Figure 2.2a), whereas in the qPCR experiment (Figure 2.2b), *HDR* expression was induced by BL. In the published dataset, expression was misregulated in the *cry1cry2* mutant, indicating the expression pattern is driven by cryptochrome. This effect was not significant in the qPCR data (Figure 2.2b).

Carotenoid Pathway A significant increase in *PSY* expression under BL can be seen in both the RNA-seq and qPCR data (Figure 2.2). However, whilst the involvement

of cryptochromes is clear, the way they effect PSY expression is not, as the double mutant shows increase in expression compared to WT in RNA-seq data, but decrease in the experimental data, with both results statistically significant by *t*-test (Figure 2.2).

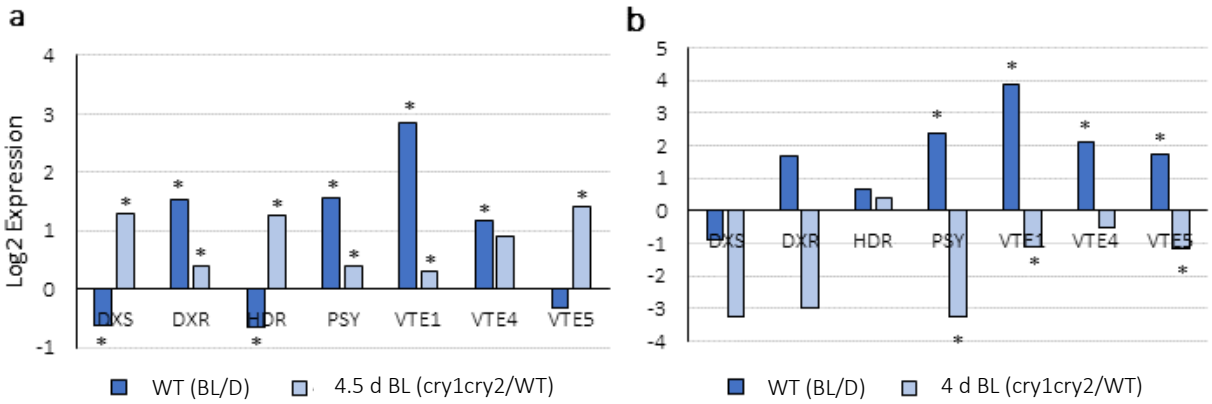


Figure 2.2 Influences of long exposure of blue light on isoprenoid biosynthesis gene expression. (a) RNA-seq data (GSE58552) of *Arabidopsis* WT and *cry1cry2* mutants as log2 expression of genes under 4.5 days continual blue light (15 µmol.m^{-2}.s^{-1}, n = 2), with (b) qPCR gene expression of plants grown under 4 days continual blue light (20 µmol.m^{-2}.s^{-1}) or dark (D) control (n = 3). Data shows treatment relative to D control (dark blue) and cryptochrome double mutant (*cry1cry2*) relative to the treatment (light blue). Bars indicate the average expression of biological replicates, with asterisks indicating a significant difference calculated by *t*-test ($p < 0.05$) between the treatments indicated in the figure legends.

Tocopherol Pathway The gene *VTE1* showed the greatest increase in expression under BL for both the RNA-seq and pPCR data (Figure 2.2). This elevated expression was significant, with cryptochrome playing a significant role. Whereas, expression of *VTE4* and *VTE5* differed between the genomic data and qPCR experiment. A significant increase in *VTE4* expression was seen in the published data, but not for *VTE5*. In comparison, both *VTE4* and *VTE5* expression was significantly higher in response to BL compared to darkness in the qPCR data (Figure 2.2).

Overall, despite some differences within the MEP and tocopherol pathways between the two data sources, these data show a consistent positive influence for BL compared to darkness in at least one rate-limiting gene of the three isoprenoid biosynthesis pathways

examined. Interestingly, in the genomic dataset the expression in the *cry1cry2* mutant increases relative to the WT BL expression in all genes. This suggests that cryptochromes could have a blanket suppressant effect on the biosynthesis genes. However, this was not replicated in the qPCR experiment where all genes with the exception of *HDR* decreased in the *cry1cry2* mutant, demonstrating the opposite principle, that cryptochromes have a positive impact on gene expression throughout the isoprenoid pathways. At present, the current data cannot clarify the effect of cryptochrome on these genes, but hint at a role of blue light in their modulation. While similar, there are slight differences between the genomic datasets and the qPCR, including the age of the seedlings, and the duration (4 days vs 4.5 days) and intensity of the illumination, which may point to the need for finer characterisation of BL effects.

2.3.2 Red Light Increases Isoprenoid Gene Transcription via Phytochromes

Expression data from a genomic dataset (GSE31587) (Hu et al., 2013) of LER WT and *phyABCDE* mutants grown under 4 days continuous RL (Figure 2.3a), were used to investigate the contribution of red light and phytochromes to the expression of the isoprenoid rate limiting genes. Once again, qPCR gene expression extractions of *Arabidopsis* Col-0 WT and *phyAB*, the main two phytochromes, were grown in similar conditions of 4 days continuous RL (Figure 2.3b).

MEP Pathway Across both data sets, MEP Pathway genes in general show a positive response to RL, and show an attenuated expression in the phytochrome mutants (Figure 2.3). Whilst this trend was significant in the genomic data, this response was muted within the qPCR data. One of the reasons for the observed variations may be due to the stronger phytochrome mutant (*phyABCDE*) used within the genomic data, compared to a weaker *phyAB* mutant used for the experimental data.

Carotenoid Pathway The data collected from the genomic datasets and qPCR experiment were closely correlated. Both found that RL increased *PSY* expression above expression levels in the dark, which was statistically significant by *t*-tests (Figure 2.3). This expression was also severely reduced in the phytochrome mutants, indicating this is a phytochrome-driven response.

Tocopherol Pathway RL increased the expression of all tocopherol biosynthesis genes examined to varying degrees, in both the genomic datasets and experimental data (Figure 2.3). *VTE1* and *VTE5* in particular displayed significant increased expression in RL. In the phytochrome mutants, *VTE5* expression was decreased compared to WT, suggesting a positive role for phytochrome. Opposingly, both datasets showed an increase in phytochrome mutant expression for *VTE4*, significantly so in the qPCR data, indicating a suppressive effect of phytochrome for this gene (Figure 2.3b). Finally, the role of phytochrome was less clear for *VTE1* expression in the phytochrome mutant, with small but opposing effects for the genomic and qPCR data, respectively (Figure 2.3).

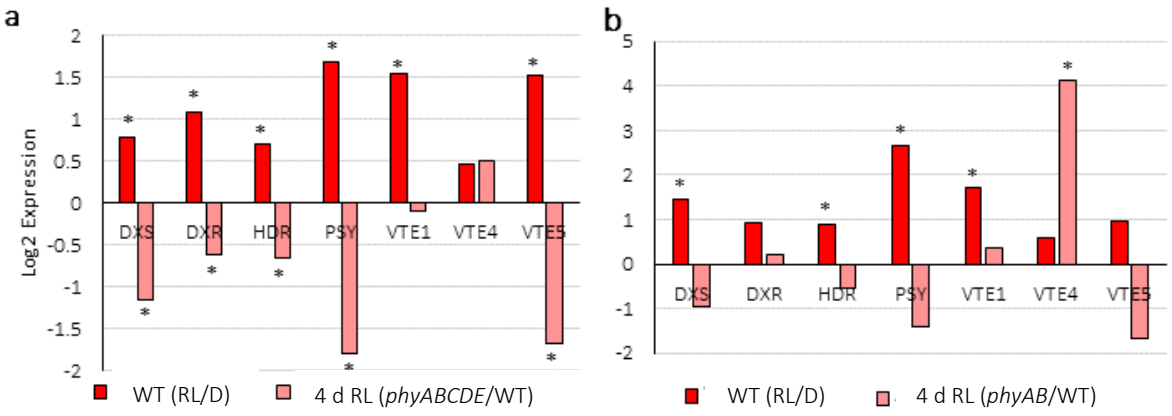


Figure 2.3 Impact of red light via phytochrome on expression of key isoprenoid pathway genes. (a) Microarray data (GSE31587) of the transcription abundance of *Arabidopsis* isoprenoid genes in WT plants grown under 4 days red light (RL) (50 µmol.m^{-2}.s^{-1}, n = 2), compared to mutant *phyABCDE* expression. (b) qPCR expression of WT and *phyAB* mutants under 4 days continuous RL (50 µmol.m^{-2}.s^{-}1, n = 3). Data shows treatment relative to D control (dark red) and phytochrome mutants (*phyABCDE* or *phyAB*) relative to the treatment (light red). Bars indicate the average expression of biological replicates, with asterisks indicating a significant difference ($p < 0.05$) between the treatments indicated in the figure legends, as calculated by *t*-test.

The genomic datasets indicate that red light is overall a positive influence on the biosynthesis pathways of MEP products and carotenoids, which matches previous published results (Chenge-Espinosa et al., 2018; Meier et al., 2011), and demonstrates a novel role of phytochrome and RL in the biosynthesis of tocopherols. The lack of

correlation between the published datasets and experimental data is likely due to the difference in phytochrome mutants and ecotype used, and therefore indicates a redundancy between phytochrome members outside of phyA and phyB in the coordination of genetic responses within the pathways. In addition, the age of seed used to collect the experimental data may also have impacted on the greening responses that are associated with these pathways.

To determine the contribution of specific phytochrome photoreceptors to the RL responses seen, the two most influential, phyA and phyB, were examined in the GSE3811 microarray (Tepperman, Hwang and Quail, 2006) using WT *Arabidopsis* and phytochrome mutants phy*A*, phy*B* and phy*AB* under 1 h RL (Appendix Figure 6.1.3). Overall, the decrease in transcripts of all genes in the phy*AB* mutant illustrate a complementary redundancy and therefore highlights the importance of phytochrome in the correct expression of these genes at least in short term illumination, but there are clear preferential contributions of the individual phytochromes as well. Within the MEP pathway, *DXS* is primarily influenced by phyA, in *DXR* both phyA and phyB play an inhibitory role, whilst *HDR* has an equal contribution of both phyA and phyB. For *PSY* in the carotenoid pathway, phyA is the dominant phytochrome photoreceptor under 1 h RL, however phyB also plays a role as there is a greater response seen in the double mutant (Appendix Figure 6.1.3). For the tocopherol genes, *VTE1* is not strongly expressed in WT but an increase in expression is seen in the *phyA* mutant, and an equal decrease in expression between the *phyB* and *phyAB* mutant. Again, in *VTE4* there is little expression in WT but a decrease in expression for *phyB* and *phyAB* mutant indicating phy involvement. Finally, *VTE5* expression is strongly repressed in *phyB* indicating a stronger contribution to the regulation of this gene by phyB (Appendix Figure 6.1.3).

2.3.3 Wavelengths Outside of PAR Influence Biosynthesis of Isoprenoids via Gene Expression

Visible wavelengths are not the only light energy able to influence plant growth and development. FR (700 – 780nm) and UV-B light (280 – 315 nm) are included in the extended range of wavelengths outside of PAR and have proven to have photomorphogenic effects including the shade avoidance response and inhibition of

stem growth, respectively (see Section 1.3.2) (Kim et al., 1998). Evidence has also shown that FR light may negatively impact on the availability of photosynthetic pigments, including carotenoids (Bou-Torrent et al., 2015). Furthermore, UV-B wavelengths may positively affect the accumulation of photoprotective isoprenoid metabolites, including anthocyanins, carotenoids and flavonoids (Huché-Thélier et al., 2016). Therefore, published genomic datasets were searched to observe any effect of FR light and UV-B on the transcription of rate limiting genes in the MEP, carotenoid and tocopherol biosynthesis pathways.

Far-red Data taken from GSE28297 (Leivar et al., 2012) (Figure 2.4a) shows that all durations of FR light supplementation (0.006 R:FR), either short (1 h), medium (3 h) and long-term (24 h), negatively influenced the expression of all genes examined. However, the extent of the effect was dependent on the length of exposure. Only *DXR* and *VTE5* show a significantly reduced expression after 1 h FR by FDR analysis. *DXS*, *PSY* and *VTE5* show the greatest expression reduction after 3 h FR, whereas the other genes show incrementally stronger reduction with additional FR exposure resulting in the strongest transcription suppression at 24 h. The largest repression is seen in *VTE4* after 24 h FR supplementation, whilst *VTE1* shows the smallest response across all durations.

UV-B Short (1h), medium (6 h) and long (4 day) term exposure to UV-B (305 nm) was examined in the published genomic data set E-MEXP-1957 (Figure 2.4b) (Favory et al., 2009a). MEP pathway gene expression was down regulated across all exposures, with the exception of *DXR* and *HDR*, which became induced after 4 days of UV-B treatment. Conversely, *PSY* is positively affected consistently by UV-B, with the highest increased expression after 1 h UV-B, and induction remaining at 6 h and 4 days UV-B (Figure 2.4b). Of the tocopherol genes, *VTE4* shows differential expression across exposure: at 1 h and 4 days expression was enhanced, but at 6 h UV-B expression was repressed. *VTE5* shows a consistent repression across all treatment periods, the greatest at 6 h UV-B (Figure 2.4b). It is notable that the expression changes of these aforementioned genes were only marginal. However, *VTE1* shows a significant increase in expression at all levels of exposure, with the highest increase being after 6 hr UV-B (Figure 2.4b). UVR8 is the only photoreceptor reported to detect UV-B light, so as expected, all genes showed altered responses in the *uvr8* mutant (data not shown). However, a statistically significant

decrease in expression of the *uvr8* mutant compared to WT was only detected in *HDR* and *VTE1* by FDR analysis (data not shown), revealing a significant regulatory role of UV-B via UVR8 for these genes only.

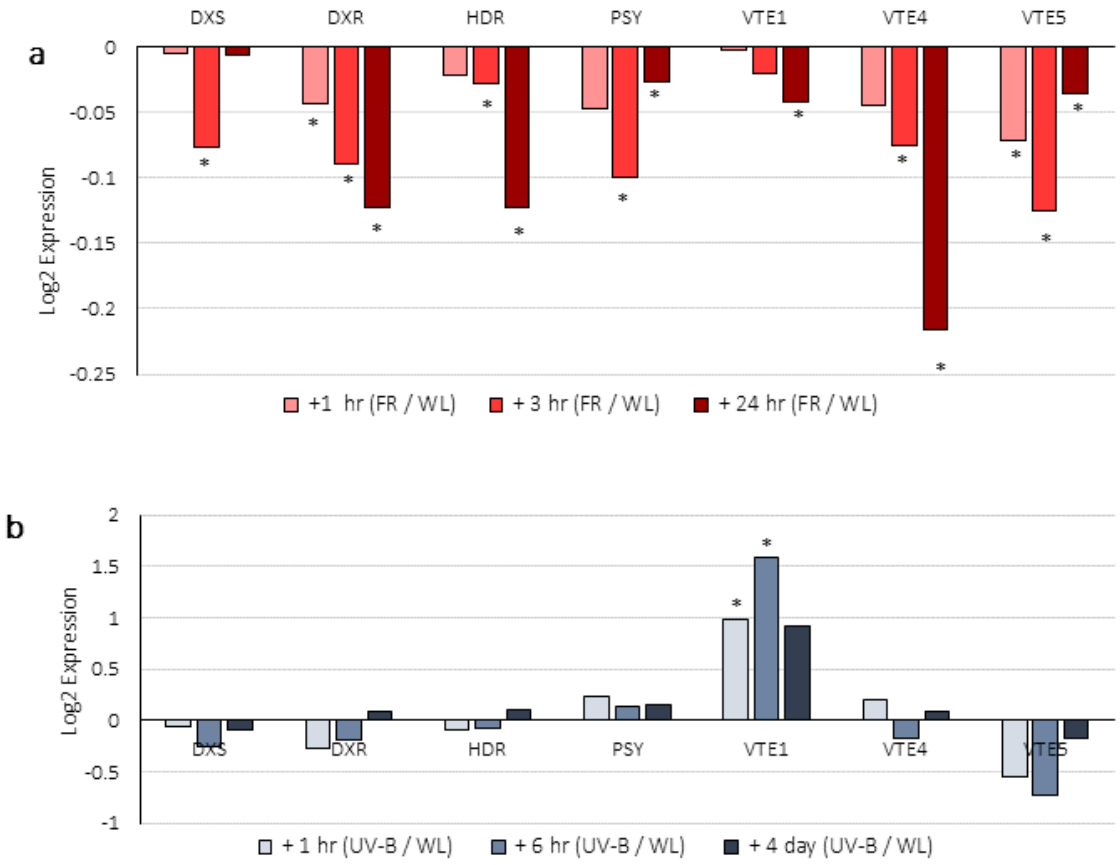


Figure 2.4 Isoprenoid genes show reduction under FR light and selective induction under UV-B. Microarray data of gene expression as measured in *Arabidopsis* WT grown under (a) white light (WL) control (19 µmol.m^{-2}.s^{-1}), and WL supplemented with 1, 3, and 24 h FR light (R:FR = 6.48, GSE28297, n = 3) and (b) WL control (3.6 µmol.m^{-2}.s^{-1}) compared to supplemented WL with 1, 6 h and 4 day UV-B illumination (1.5 µmol.m^{-2}.s^{-1}, E-MEXP-1957, n = 3). Bars indicate the average abundance of biological replicates, with asterisks indicating a significant difference calculated by *t*-test ($p < 0.05$) between the treatments indicated in the figure legends.

Together these data show that wavelengths outside of PAR do have an effect on isoprenoid synthesis by altering the expression of key genes within the MEP, carotenoid

and tocopherol pathways. In general, FR suppresses all three biosynthesis pathways. Whilst UV-B has a repressive effect on the MEP pathway, the carotenoid gene PSY showed a positive response to supplementation, yet the variations in the gene expression within the tocopherol pathway showed the strongest elevation and the strongest suppression seen of all the genes (Figure 2.4b). The strength of these effects seem to be dependent on the length of exposure, which could be used to activate or supress specific areas of the biosynthesis pathways and manipulate the biosynthesis of their products.

2.3.4 The Role of Light Signalling Components in Photoreceptor-Mediated Transcriptional Regulation of the Isoprenoid Pathways

Photoreceptor signalling cascades coalesce on HY5 and PIFs, which act to promote and antagonise photomorphogenesis respectively (Toledo-Ortiz et al., 2014; Yadav et al., 2020). The previous data analyses uncovered photoreceptor influences on the expression of isoprenoid pathway rate limiting genes by wavelengths within PAR and the wider spectral range. Therefore it is reasonable to predict that both HY5 and PIFs also play a role in transcriptional regulation of the MEP pathway genes, for which a RL effect (40 $\mu mol.m^{-2}.s^{-1}$) is already reported in young *Arabidopsis* seedlings (3 days old) (Chenge-Espinosa et al., 2018). To explore the action of light signalling components within the isoprenoid and related antioxidant pathways, genomic datasets of *hy5* (GSE62119; Kawashima *et al*., unpublished data; Figure 2.5a) and *pifQ* mutants (GSE17159; Leivar et al., 2009; Figure 2.5b) were examined.

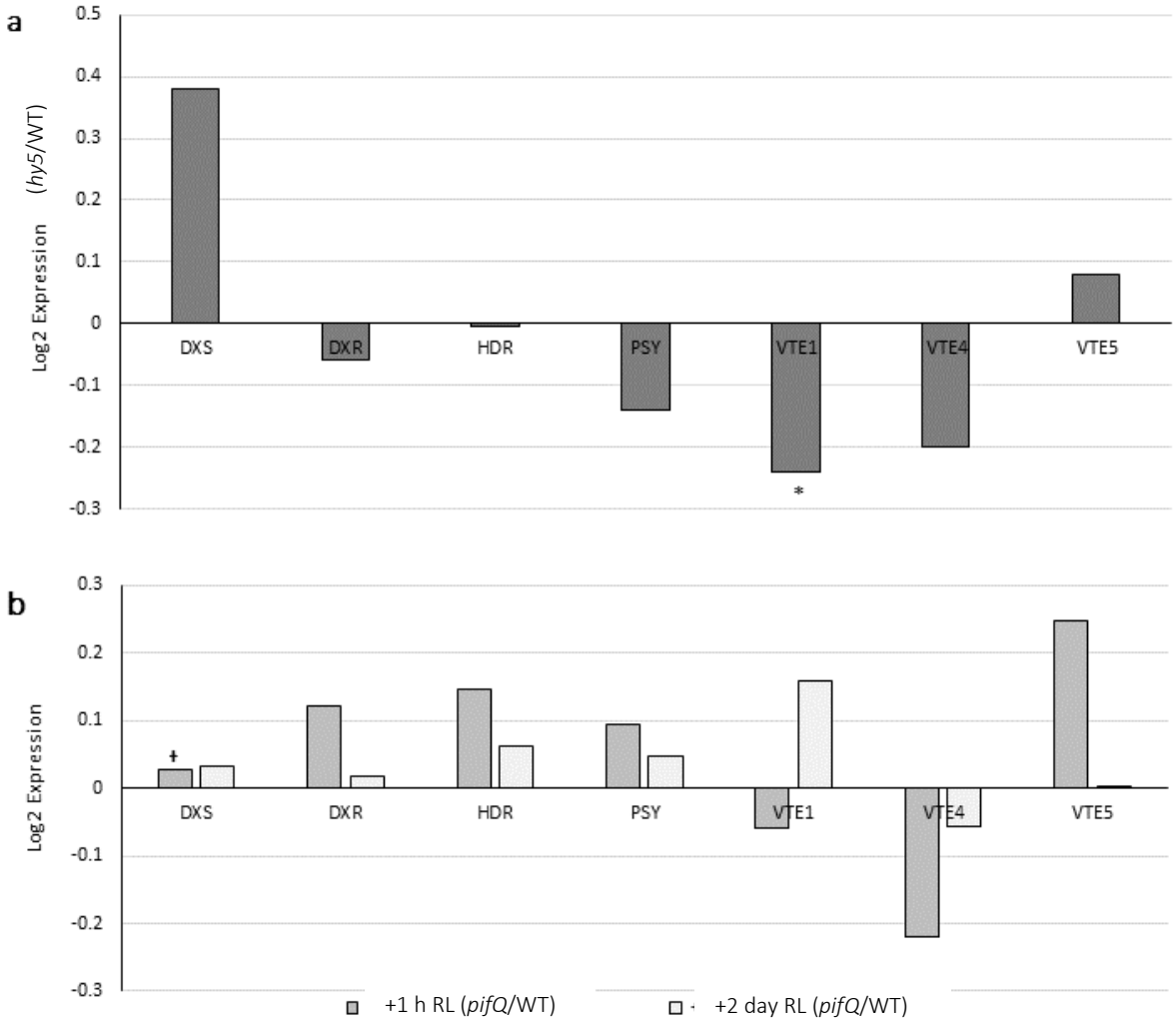


Figure 2.5 Exclusion of light signalling partners PIFs and HY5 alters the expression of isoprenoid genes. Microarray data of (a) log2 expression of the fold change between WT and *hy5* mutants grown under 3 days white light (no intensity provided, GSE62119, n = 3), and (b) WT and *pifQ* mutants compared under 1 h RL (7.5 µmol.m^{-2}.s^{-1}) and 2 days RL (6.7 µmol.m^{-2}.s^{-1}, GSE17159, n = 3). Statistical significance was detected with FDR analysis as indicated by the cross, or *t*-test when denoted by an asterisk ($p < 0.05$).

The genomic dataset GSE62119 (Kawashima *et al*., unpublished data) evidenced a role of HY5 signalling in all genes except *HDR*. As would be the case for HY5 regulated genes, reductions in expression were seen in the *hy5* mutant for *DXR*, *PSY*, *VTE1* and *VTE4* (Figure 2.5a). The reduction of *VTE1* transcription was significant by *t*-test analysis ($p < 0.05$), indicating that *HY5* plays an important role in the upregulation of *VTE1*. Interestingly, increases in expression were found in *hy5* mutant for *DXS* and *VTE5*,

suggesting a suppressive influence of HY5 on their expression (Figure 2.5a). This is unusual as *HY5* is more commonly associated with the promotion of photomorphogenesis, as seen in the case of the other genes analysed.

As the action of PIFs is to repress transcriptional regulation of genes by phytochrome, it is expected that a positive log2 expression will be recorded when comparing *pifQ* expression to the WT treated with RL. This was generally true, although the effect of the *pifQ* mutant varied across the genes and a significant increase in expression in the *pifQ* mutant was only found for *DXS* (Figure 2.5b). However, *pifQ* demonstrated a transient repressive effect on the regulation of *VTE1* and *VTE4*, which places them as PIF-activated genes.

These genomic data analyses support the hypothesis that light signals detected by the cryptochromes and phytochromes are passed onto the light signalling partners HY5 and PIFs, which could interact with the binding motifs found in the promoters of the isoprenoid biosynthesis pathways (Appendix Figure 6.1.5), resulting in changes in expression.

2.3.5 The Photoperiodic Cycling of Isoprenoid Biosynthesis Genes Could be Circadian Regulated

For many light regulated genes, gene expression across a 24 h day is not constant and instead fluctuates according to demand for the resulting protein. This is often an adaptive response for optimal function of processes creating diurnal cycles of expression. Light can play a major role in the regulation of such fluctuations as it enables timing of responses to environmental events such as dawn (Dodd et al., 2005). Blue and red wavelengths also entrain the circadian clock, a 24 h oscillating mechanism that can preserve the timing of genetic regulation regardless of the changes in the light environment (Somers et al., 1998). Knowing the timing of peak expression of the flux controlling biosynthesis genes and the mechanism for regulation can enable optimal application of stimulatory lighting treatments to alleviate bottlenecks, i.e. when expression is lowest.

The expression profile of the isoprenoid genes was examined for photoperiodic transcript accumulation peaks using long-day (LD; 16/8 hr Light/Dark) and short day (8/16 L/D) datasets published by the DIURNAL project (Mockler et al., 2007). By comparing the expression profiles of two day lengths, consistencies between them can be identified which indicates potential circadian regulation.

MEP Pathway Expression levels for MEP pathway genes *DXS* and *HDR* presented a phase peak at zeitgeber (ZT) 4 and lowest expression at ZT12 in both long day (LD) and short day (SD) datasets (Figure 2.6a, b). Whilst *DXR* showed near-constant expression throughout the day with a variable peak expression at respective dusk; ZT16 in LD (Figure 2.6a) and ZT8 in SD (Figure 2.6b).

Carotenoid Pathway For carotenoid synthesis, the DIURNAL LD and SD datasets showed that *PSY* expression peaked consistently at dawn (ZT0) after steadily accumulating over the previous 16 hours (Figure 2.6c, d).

Tocopherol Pathway In LD and SD, both *VTE1* and *VTE4* showed a primary peak at ZT4, but height of expression was greater for *VTE1* in SD (Figure 2.6e, f). In the LD data, these genes also showed a secondary peak of lower amplitude at dusk (ZT16). Whereas, expression for *VTE5* showed a gradual increase to peak at ZT8 under LD or ZT4 under SD and then a gradual decrease after. In both conditions, *VTE5* expression was lowest 4 hours after dusk (Figure 2.6e, f).

All the genes examined display diurnal fluctuations in expression, with *DXR* and *VTE5* in particular displaying photoperiodic changes in expression (Figure 2.6). However, the vast majority of genes preserved the expression patterns between the different photoperiods examined, indicating the potential for circadian influence. To discover circadian regulation of gene expression, further datasets of plants grown under continuous light and under entrainment conditions from the DIURNAL project (Mockler *et al.*, 2007) were examined (Appendix Figure 6.1.4). These data reveal that *DXS*, *HDR, PSY, VTE1, VTE4* and *VTE5* maintain a cycling rhythmicity in entrainment conditions, implicating circadian regulation for these genes.

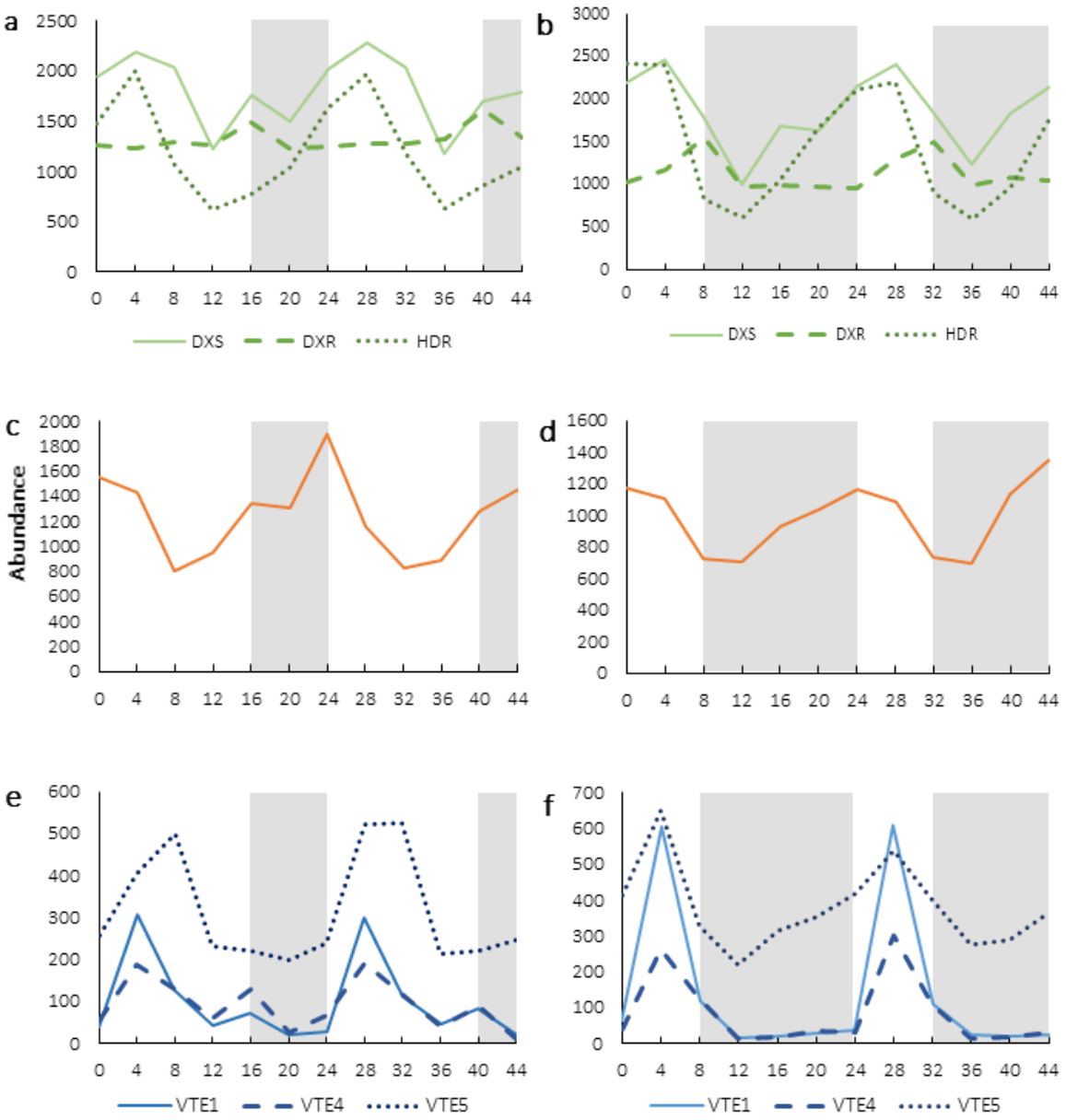


Figure 2.6 Isoprenoid genes show photoperiodic fluctuation with circadian input. Fluctuations in expression of biosynthesis genes within the MEP (a, b), carotenoid (c, d) and tocopherol (e, f) pathways, as found under a long-day (16/8 h L/D) diurnal cycle and (a, c, and e) short-day cycles (8/16 h L/D) (b, d, f). Data collected from the Mockler (2007) DIURNAL database.

Moreover, circadian factors binding motifs were found within the promoters of all the genes (Appendix Figure 6.1.5), including factors involved in the morning and evening loops, such as CCA1, LHY, TOC1 and PRRs. Also searched was ATTRACTOR, a database of transcriptomic data with cistronic data that displays gene expression profiles indicating stimulatory or repressive influences of associated circadian signalling molecules at particular time points. The gene entries for the isoprenoid biosynthetic genes in ATTRACTOR list CCA1, LHY and PRRs as regulatory components for the rate limiting biosynthesis genes (Appendix Figure 6.1.6), which is further supported by the presence of binding sites found within the promoters (Appendix Figure 6.1.5). The notable exception within these data is *DXR*, which did not have accessible entries within the continuous light or entrainment datasets form the DIURNAL project, nor from the ATTRACTOR online database. This is indicative of a lack of circadian regulation for *DXR*, which is also the only gene to show a photoperiodic alteration in expression in response to dark to light transitions, with the peak at dusk dependent on the light-dark transition.

2.3.6 Blue and Red Light Influence the Genetic Control of Ascorbic Acid Production

Blue light Using the genomic dataset (GSE58552) (He et al., 2015) the long-term effect of BL on *VTC2* gene expression was examined (Figure 2.7a). A significant increase in expression was found compared to dark conditions, by *t*-test in the genomic data (Figure 2.7a) and qPCR data (Appendix Figure 6.1.1). However, the *cry1cry2* mutant response was significantly elevated compared to WT blue light responses, which indicates that cryptochromes play a repressive role in *VTC2* expression (Figure 2.7a).

Red light Expression of *VTC2* increased under long-term (4 days; GSE31587) (Hu et al., 2013) RL exposure (Figure 2.7b). Phytochrome plays a significant role in the expression of *VTC2* under these conditions as expression significantly declined in comparison to the WT in the *phyABCDE* mutant (Figure 2.7b) and under similar conditions in the *phyAB* mutant (Appendix Figure 6.1.2).

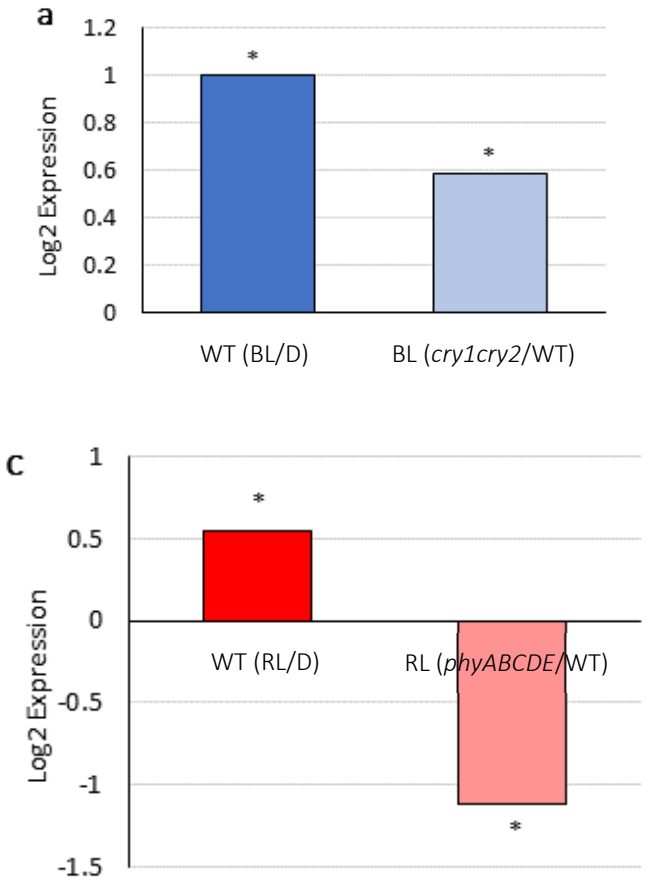


Figure 2.7 *VTC2* expression is controlled by cryptochromes and phytochromes. Microarray data of *VTC2* transcription abundance under (a) blue light (GSE58552) over 4.5 days (15 µmol.m^{-2}.s^{-1}, n = 2) as measured in WT and *cry1cry2* mutants, and (b) red light (GSE31587) delivered for 4 days (50 µmol.m^{-2}.s^{-1}, n = 2) continuously in WT and *phyABCDE* mutants. Bars indicate the average expression of biological replicates, with significant difference ($p < 0.05$) between the treatments indicated in the figure legends marked with asterisks as calculated by *t*-test.

2.3.7 The Duration of Supplementary Wavelengths Impacts Genetic Control of Ascorbic Acid Production

Published genomic data was examined for the effect on *VTC2* expression of supplementation with FR and UV-B light over a short-, medium- and long-term basis. The genomic dataset GSE28297 (Leivar et al., 2012) compared *Arabidopsis* WT under control WL conditions and WL supplemented with FR light (R:FR = 0.006) for 1, 3, or 24 h. *VTC2*

expression decreased with increasing exposure to supplementary FR light (Figure 2.8a) with significantly decreased levels of expression after 3 and 24 h FR supplementation.

To test the potential involvement of UVR8 in the response, the genomic dataset E-MEXP-1957 (Favory et al., 2009a) was examined for expression data of WT against *uvr8* mutants grown under control conditions or 1 h, 6 h or 4 days of UV-B supplementation. Contrary to FR light, UV-B supplementation produced a strong increase in transcript levels after 1 h, which remained positive but gradually reduced in strength of response over application time (Figure 2.8b). Expression is reversed in the *uvr8* mutant at all time points, demonstrating a positive role of UVR8 receptor signalling in the effect of UV-B supplementation on *VTC2* expression.

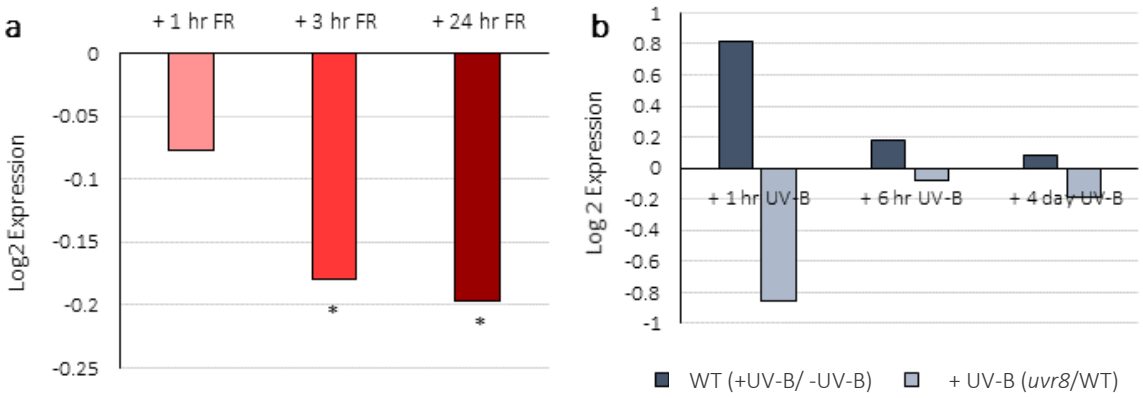


Figure 2.8 The influence of wavelengths outside of PAR on *VTC2* expression can be seen in genomic data of (a) WT plants under WL control (19 µmol.m^{-2}.s^{-1}) and 1, 3, and 24 h FR light supplementary to WL control (R:FR = 6.48, GSE28297, n = 3), and (b) WT and *uvr8* mutants under WL (3.6 µmol.m^{-2}.s^{-1}) and WL supplemented with 1, 6 h and 4 days UV-B light (1.5 µmol.m^{-2}.s^{-1}, E-MEXP-1957, n = 3). Bars indicate the average expression of biological replicates, with asterisks indicating a significant difference ($p < 0.05$) between the treatments indicated in the figure legends, as calculated by *t*-test.

2.3.8 Transcriptional Signalling Partners in the Light-Mediated Genetic Control of Ascorbic Acid Production

As light wavelengths were shown to impact on the expression of *VTC2*, the potential downstream signalling mechanism was explored. The GSE62119 genomic dataset (Kawashima et al., unpublished data) was used to compare the expression of *hy5* mutants against WT (*hy5*/WT) which gave a positive log 2 expression ratio (0.36) that was not significant by *t*-test (data not shown). This indicates that HY5 is may not be a factor involved in the light-regulation of *VTC2*. Next, the potential contribution of PIF1, 3, 4 and 5 was examined using the *pifQ* mutant under RL (dataset GSE17159; Leivar et al., 2009). Evidence of potential PIF influence was seen as a significant reduction of *VTC2* expression was present in the *pifQ* mutant (Figure 2.9). This result would indicate that PIFs may act as activators of the expression of *VTC2*.

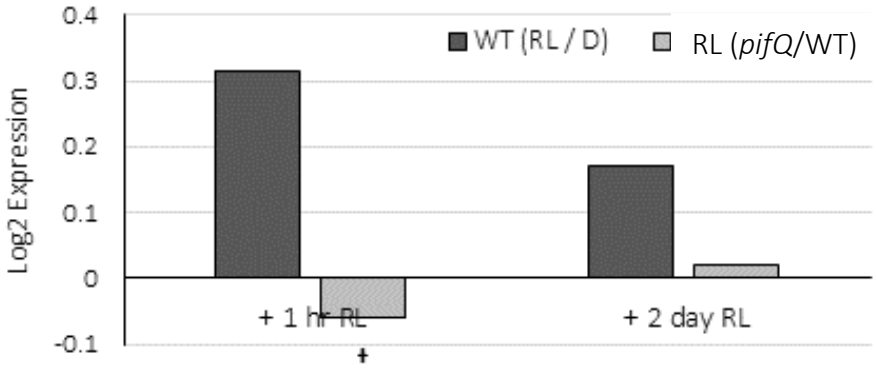


Figure 2.9 Genomic data reveals role of PIFs in *VTC2* expression. Expression of *VTC2* in WT and *pifQ* mutants was compared under 1 h red light (RL, 6.7 µmol.m^{-2}.s^{-1}) and 2 days RL (7.5 µmol.m^{-2}.s^{-1}, GSE17159, n = 3). Bars indicate the average expression of biological replicates, with a cross denoting a significant difference ($p < 0.05$) between the treatments indicated in the figure legends, as calculated by FDR.

2.3.9 Genetic Control of Ascorbic Acid Production Shows Photoperiodicity

Further to light signalling partners, photoperiodicity and circadian regulation was explored for *VTC2* using the DIURNAL database datasets (Mockler et al., 2007). Under long-days, *VTC2* shows 12 h oscillation periods, with approximately equal peak amplitude phases at ZT4 and ZT16 (Figure 2.10a), but under short days, only the morning peak was

conserved (Figure 2.10b). Examination of expression under circadian entrainment and continuous light found a single peak of *VTC2* expression at relative 'dawn' and lowest expression between ZT8 and ZT12 (Appendix Figure 6.1.4). This suggests that there could be a circadian component modulating the timing of expression of *VTC2* to peak expression at dawn, but photoperiod is also involved in establishing the presence of a secondary peak under long days. Analyses of the *VTC2* promoter region discovered circadian motifs to bind TOC1, PRRs, and LHY, in addition to a Morning Element and one starch box from the midnight module (Appendix Figure 6.1.5). Interestingly, a binding motif from each of the Morning, Evening and Midnight circadian regulatory element modules was found (Staiger et al., 2013), indicating that the expression of *VTC2* is regulated throughout the day. The ATTRACTOR entry for *VTC2* confirmed inputs for LHY and PRRs on two separate points of expression (Appendix Figure 6.1.6).

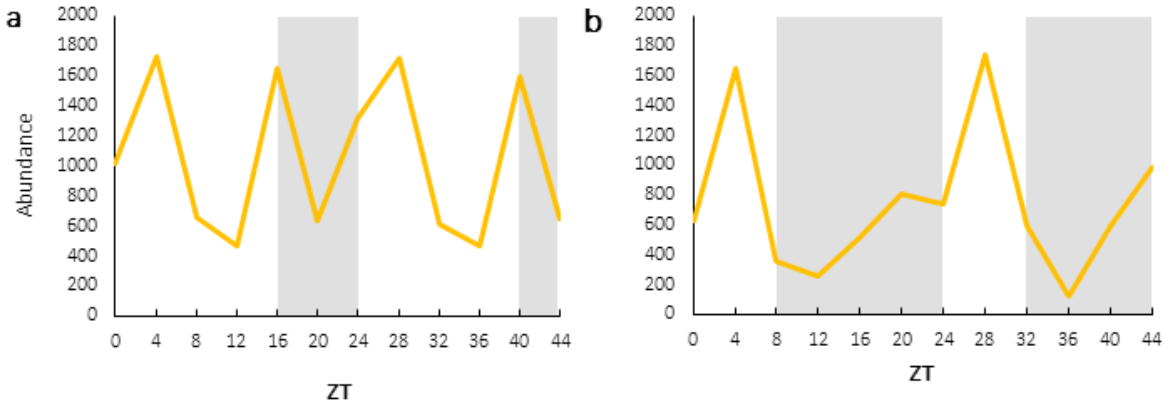
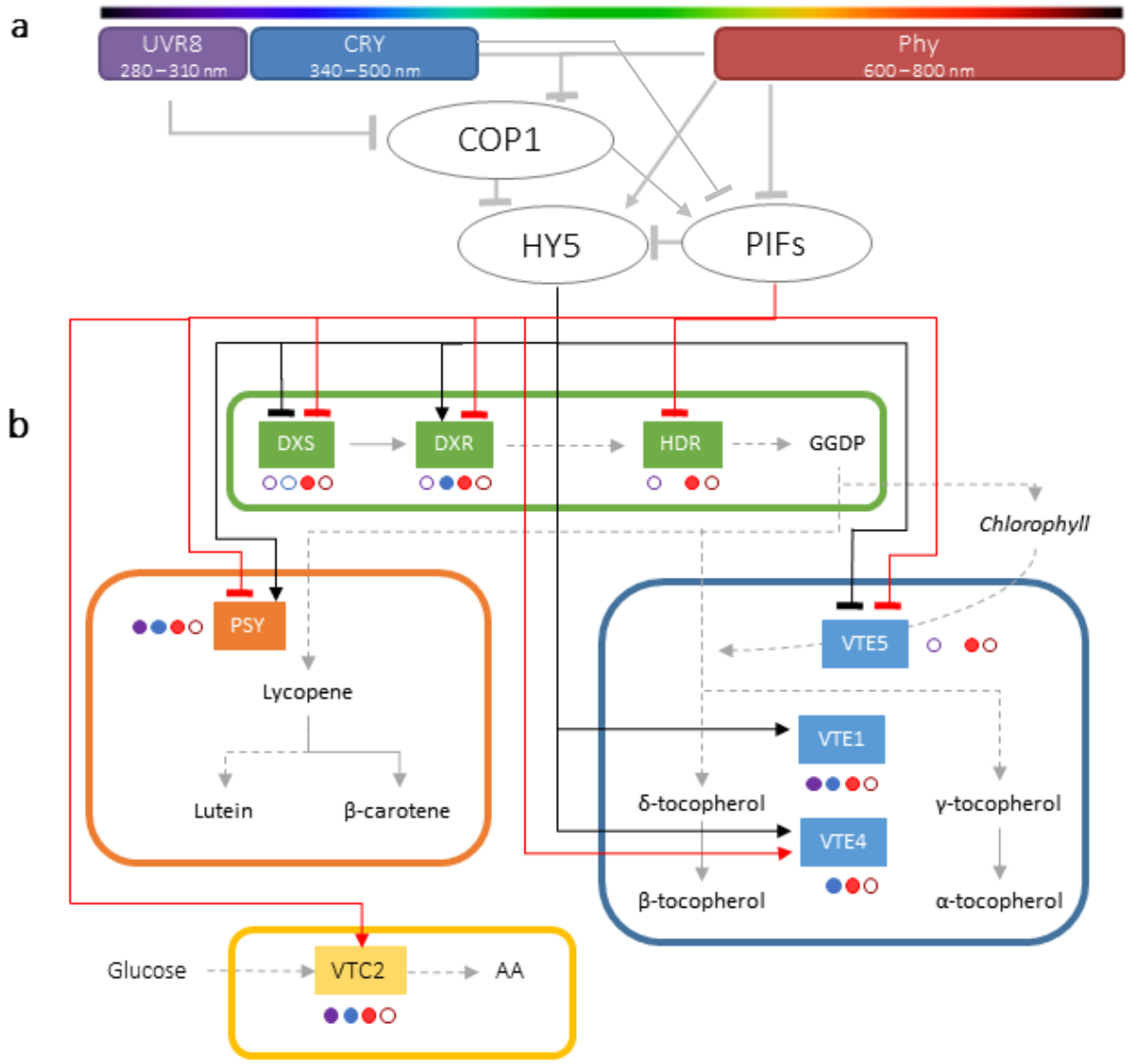


Figure 2.10 VTC2 diurnal expression is circadian regulated. (a) Normal diurnal fluctuations of VTC2 expression under a long-day cycle (16/8 h L/D) as compared to (b) expression in plants entrained on a short day diurnal cycle (8/16 h L/D). Data collected from the Mockler (2007) DIURNAL database.

2.4 DISCUSSION

The advent of controlled environment agriculture opens the door for high level manipulations of the light environment to target desired crop traits (Bian et al., 2015). Light is most known for its photomorphogenic effects on plant architecture (Paradiso and Proietti, 2021), however light can also significantly effect secondary metabolite production, including important dietary vitamins and their precursors, such as carotenoids (pro-Vitamin A), tocopherols (Vitamin E) and AA (Vitamin C) (see Section 2.1.2). In order to begin developing a light regime to target the production of these beneficial compounds, the effect of specific wavelengths on the expression of biosynthesis genes must be understood. This chapter addressed that first step by systematically exploring the input of light quality, photoreceptors, light signalling pathways and temporal influences of photoperiod and circadian rhythm on the expression and regulation of rate limiting genes within the phytonutrient biosynthesis pathways. The findings are summarised in Figure 2.11 and discussed for each pathway below.

Figure 2.11 Light control of rate-limiting genes within phytonutrient biosynthesis pathways. Simplified diagram of (a) the light signalling pathways and (b) the isoprenoid biosynthesis pathways, including the MEP, carotenoid, tocopherol pathways, and the ascorbic acid biosynthesis pathway featuring the flux controlling enzymic genes only (coloured boxes), and the effect of light wavelengths and transcription factors HY5 and PIFs on their transcription. Light wavelength input into the regulation of the biosynthetic genes are denoted by coloured circles for UV-B (purple), blue (blue), red (red) and far-red (dark red) wavelengths, where filled circles indicate a positive effect on transcription and outlined circles represent a negative effect. Where no circles are present the effect was not certain. The effect of transcription factors HY5 (black lines) and PIFs (red lines) is shown as positive (arrow) or negative (barred line). Direct biosynthetic steps along the pathways are connected with a solid grey line and contracted steps are indicated with a grey dashed line. Abbreviations can be found in Section 1.3.



2.4.1 The MEP Pathway Gene Expression is Primarily Upregulated by Red Light and Repressed by Blue, UV-B and FR Light via Light Signalling Pathways

The MEP Pathway is a major source of isoprenoid precursors for the creation of chlorophylls, growth regulating hormones and phytonutrients, specifically carotenoids and tocopherols (Bouvier et al., 2005; Vranová et al., 2013). The chloroplastic location and photosynthetic role of a number of isoprenoid products (chlorophylls, carotenoids, phylloquinone, plastoquinone, tocopherols) targets the pathway for light regulation (see Section 2.1.2.1). Previous study of the MEP pathway has shown a light-mediated

upregulation of gene transcripts (Cordoba et al., 2009). Here, genomic analysis of both published and experimental data revealed that the expression of rate limiting genes *DXR*, *DXS* and *HDR* show differential responsiveness to specific wavelengths.

Cryptochrome 1 has been linked to regulation of gene transcription within the cytosolic isoprenoid MVA-pathway (Rodríguez-Concepción et al., 2004); however, there has been very little exploration into blue light control of the chloroplastic MEP Pathway. Novel evidence presented here show *DXS* expression decreased with long-term exposure to BL (Figure 2.2), with relative increases in *cry1cry2* mutant expression suggesting that cryptochrome suppresses expression of the MEP pathway gene. On the contrary, exploration into acute genetic responses found that *DXS* expression increased under short-term (2 h) blue light (Appendix Figure 6.1.1), indicating that the transcriptional response of *DXS* is sensitive to the duration of BL exposure. This dose response could be of adaptive benefit to increase isoprenoid production under acute blue light exposure, which is most abundant at dawn and midday (Abdel-Rahman et al., 2017). For *DXR*, transcripts increased under long-term blue light, which is also likely linked to the cryptochrome photoreceptor (Figure 2.2). However, conflicting effects of BL were seen on *HDR* expression, which decreased in the genomic data but increased in the qPCR data, therefore the effect of blue light could not be clarified. Importantly, the differences seen between the two datasets could be due to the age of the seedlings used in the long term exposure study, which differed by 12 h and therefore transcript abundance is likely to have been effected by diurnal fluxes in gene expression (Cordoba et al., 2009).

Peaks of MEP gene expression are commonly co-expressed with core circadian genes from the morning loop (PRR9, CCA1, LHY) (Cordoba et al., 2009; Pokhilko et al., 2015). Early morning temporal regulation ensures the biosynthesis of plastidial isoprenoid products during the height of the day, when the photoprotective effects of the resultant antioxidants are most required (Walter et al., 2009). For *DXS* and *HDR,* morning abundance was conserved in short-day and long-day photoperiods (Figure 2.6a, b), under continuous light and entrainment conditions (Appendix Figure 6.1.4), indicating circadian influence. A recent mathematical modelling of the diurnal patterns for *DXS* and *HDR* included circadian influences of morning loop elements LHY and CCA1. The same study

also discovered the presence of LHY/CCA1 binding elements in the promoter regions of *DXS* and *HDR* (Pokhilko et al., 2015). Thorough investigation of the promoter regions also found HUD/PBE-box and GATA boxes in addition to the binding motif T1ME, which binds TOC1 and other Pseudo-Response Regulators (PRRs) throughout the promoters of *DXS, DXR* and *HDR* (Appendix Figure 6.1.5). It should be noted that *DXR* did not show any circadian data within ATTRACTOR, nor the DIURNAL datasets, and showed only photoperiodic modulation to the expression peak (Figure 2.6a, b). This suggests that DXR is the most resilient or non-responsive of the MEP pathway genes tested to light quality changes. Interestingly, the ATTRACTOR database listed repression by PRR5 at ZT10 in *HDR* expression (Appendix Figure 6.1.6), which is possibly why the genomic data, harvested at ZT12, would have shown HDR in repression in comparison to the qPCR data which was harvested at dawn. Studies on short term irradiation (Appendix Figure 6.1.1) also indicate that to increase MEP gene expression, exposure to short term blue light could be beneficial to activate both *DXS* and *DXR* expression. Whilst these results show the promising impact of BL on abundance of MEP pathway genes, BL duration and intensity needs to be further characterised. Such experiments should explore the effect of BL on the 24 h expression of MEP pathway genes in addition to circadian effects using photoperiodic and continuous light regimes.

The observed impact of BL via cryptochromes could also be linked with downstream signalling components. HY5, the positive photomorphogenic transcription factor, in particular plays a major role in blue light signalling (Yadav et al., 2020), and has been linked to the CRY2 regulation of MVA-pathway genes (Rodríguez-Concepción et al., 2004). HY5 regulates the MEP pathway in *Arabidopsis* young seedlings with differential contribution to the expression of *DXS, DXR and HDR* during deetiolation (Chenge-Espinosa et al., 2018). To further explore the role of HY5 and light, a published *hy5* mutant genomic dataset was searched (Kawashima *et al*., unpublished data). Interestingly, a positive effect of HY5 on *DXR* was suggested by Chenge-Espinosa et al. (2018), and by the genomic dataset analyses (Figure 2.5), presenting a positive signalling pathway for the activation of *DXR* transcription. On the other hand, no effect of HY5 on HDR was observed in the mutant dataset nor the published study (Chenge-Espinosa et al., 2018) (Figure 2.5), thereby supporting the view that HDR is an enzyme whose

regulation by BL may be milder and whose light effects are less dependent on transcription (Chenge-Espinosa et al., 2018). Analysis of the genomic *hy5* dataset found that *DXS* expression increased in the *hy5* mutant, indicating a potential repressive role for HY5 and a possible mechanism for downregulation of *DXS* under long exposure to BL. However, this contradicts results from Chenge-Espinosa et al (2018) who found that *DXS* transcription was reduced in a *hy5* mutant, demonstrating a positive role for the photomorphogenic signalling component. However, these experimental conditions differ significantly in the wavelength and length of illumination used, which was continuous WL in the *hy5* dataset and 6 h of RL in Chenge-Espinosa et al. (2018), both using 3 day-old seedlings. Because both red and blue light signalling pathways converge on HY5 within a larger regulatory framework (Griffin et al., 2020; Zhang et al., 2017) the impact on DXS within a *hy5* mutant could have been differentially effected by the wavelengths applied. Alternatively, the intensity used in the genomic dataset was not listed, and could have varied substantially to the published study. Moreover, the difference in illumination period between the two studies is likely to have influenced expression levels, especially as *DXS* transcripts show diurnal fluctuations and circadian inputs as explained above (Figure 2.6a, b). Therefore, whilst these results show that *DXR* is positively regulated by cryptochromes and HY5, and that *HDR* is unlikely to be regulated by blue light nor HY5, the true impact of hy5 on the expression of MEP pathway genes cannot be concluded. How photoreceptor signals are conducted via the light signalling components to the MEP pathway genes is a rich source of further study. Such studies could use fluence response curves to explore the effect of light intensity and spectra on HY5 as a downstream component of gene transcription to establish the maximal response intensity of HY5 regulation.

On the other hand, the expression of the rate limiting MEP genes under long-term red light showed a clear positive response in the genomic and experimental data (Figure 2.3). Whilst there was a potential reduction in response due to seed age in the experimental results, both of these data sets are in agreement with Chenge-Espinosa et al. (2018) who revealed a role of monochromatic red light in stimulating the upregulation of *DXS*, *DXR* and *HDR* in young *Arabidopsis* seedlings. Moreover, a potential dose duration effect was seen in further genomic data sets where the expression of genes was higher after 4 days

RL than with 1 h (Appendix Figure 6.1.2). Alternatively, this could be an artifact of an intensity-based effect between the short-term RL (8 $\mu mol.m^{-2}.s^{-1}$) and long-term RL (50 $\mu mol.m^{-2}.s^{-1}$) as higher intensity light can be part of the mechanisms to increase gene transcription and MEP pathway products (Lefsrud et al., 2006; Rossel et al., 2002). This possibility is supported by evidence from the qPCR data, where similar *DXS* and *HDR* expression levels were recorded between short- and long-term red light exposures of the same intensity (50 $\mu mol.m^{-2}.s^{-1}$) (Figure 2.3b and Appendix Figure 6.1.2). Therefore, current observations indicate that RL could be supplied to elevate MEP pathway gene expression, with induction proportional to intensity, yet further experimentation including fluence response experiments would be needed to clarify the links between RL intensity and transcriptional activation of the MEP-pathway.

Importantly, these results also show that responses were attenuated in phytochrome mutants, demonstrating the requirement of phytochromes for the RL expression of MEP genes. Previously, down regulation of downstream MEP pathway products was observed in a *phyB* mutant, which indirectly implicated phyB in the regulation of MEP pathway genes (Rodríguez-Concepción et al., 2004). The phytochrome photoreceptors serve to coordinate different responses; phyB is associated with the major red light driven responses, whilst phyA is more commonly associated with low fluence responses or far red light and rapid responses to red-light (Shinomura et al., 1996; Wang, 2015). Genomic data showed that in short-term RL exposure both phyB and phyA contribute to the RL expression of *DXR* and *HDR*, whilst *DXS* is primarily influenced by phyA (Appendix Figure 6.1.3). Despite the differing contributions of phyA and phyB to the modulation of individual genes, expression of all MEP pathway genes examined was reduced under FR light supplementation (Figure 2.4a). However, *DXS* did show a different expression pattern, where significant suppression was only seen at 3 h FR supplementation. It is unknown whether the preferential contributions of the phytochromes resulted in the differential FR expression patterns seen under FR. However, phyA has been shown to impact the FR light induction of plastid isoprenoid pathway genes (Meier et al., 2011), therefore this could be a contributing factor. Another possibility is that other signalling components could also modulate expression under red and FR light. For instance, PIFs play a major role in phytochrome signalling pathways as antagonists to RL responses

(Leivar and Quail, 2011). PIFs generally have a repressive effect on the transcription of MEP genes (Chenge-Espinosa et al., 2018). *DXS* was reported to show a robust response to RL (Leivar et al., 2009), and the *pifQ* mutant accumulates high levels of *DXS*, *DXR* and *HDR* transcripts in the dark and under red light (Chenge-Espinosa et al., 2018). Also increments in expression of all three genes were observed in the *pifQ* genomic experiments analysed (GSE17159; Figure 2.5b). Therefore, current evidence indicates that PIFs are negative modulators of the rate limiting steps of the MEP pathway and could contribute to expression of MEP genes in RL and FR light.

PIFs and HY5 can work antagonistically by both binding to G-boxes in promoter regions of target genes (Kim et al., 2016b; Toledo-Ortiz et al., 2003). Several G box related sequences were found in the *DXR* promoter, and demonstrated to be directly upregulated by HY5 in ChIP experiments, and showed a potential non-preferential interaction with PIF1 (Chenge-Espinosa et al., 2018). Despite a lack of G-boxes present in the promoter regions of *DXS*, ChIP experiments demonstrated direct binding of PIFs and HY5 to the promoter region (Chenge-Espinosa et al., 2018), and it was previously suggested that PIF and HY5 regulation og *DXS* is accomplished via PBE-boxes and G-box Coupling Element, respectively (Pu et al., 2021). As these cis-acting elements are located very close to each other, it is postulated that the binding of one or the other regulator interferes with the binding of the other, thereby generating an antagonistic mechanism as described for *PSY* (Toledo-Ortiz et al., 2010, 2014). *PIF1*, *PIF3* and *PIF5* in particular are implicated in *DXS* transcript accumulation in *Arabidopsis*, with *PIF1*, and to a lesser extent *PIF3*, involved in *HDR* transcript levels regulation (Chenge-Espinosa et al., 2018). In the *HDR* promoter, a PBE-box and GCE/ACE motif were found (Appendix Figure 6.1.5), however HY5 did not bind in ChIP, but a significant binding of PIF1 was observed in the dark, most likely at the PBE-box (Chenge-Espinosa et al., 2018). Therefore, light modulation of *HDR* likely occurs by a de-repression of PIF1/PIF3, rather than a transcriptional activation due to HY5. Alternatively, ione study proposed that PIF5-could play a positive role in the accumulation of chlorophylls and carotenoids, however this interpretation may not be fully accurate as PIF-OX used in this study can create dominant negative effects of other PIFs known to be negative modulators of the expression of the MEP pathway genes (Mannen et al., 2014). In addition, my analysis of the ATTRACTOR

entries show timed repression at ZT4 by PIF5 and PIF4 for *HDR* and *DXS* respectively (Appendix Figure 6.1.6). At present, evidence suggest a role for both HY5 and PIFs in light responsive regulation of the MEP pathway that is unique to the individual rate limiting genes *DXS*, *DXR* and *HDR*. PBE boxes bind PIFs (Pu et al., 2021), but are also known as HUD and involved in the morning module of the circadian clock (Huang et al., 2012). Interestingly, both *DXS* and *HDR* contain the HUD/ PBE box, as well as various C- and E-boxes that can also bind PIFs and HY5 (Appendix Figure 6.1.5). Therefore the ZT4 morning expression peak found in the photoperiodic expression profiles is likely the result of both circadian and light signalling converging in specific promoter elements. Further studies on the interaction of PIFs and the circadian clock components should clarify if together they control the peaking time of the rate limiting steps of the MEP-pathway, maximizing the activity of the production of isoprenoids to moments of the day where the precursors are needed for multiple functions (Vranová et al., 2013).

UV-B radiation levels fluctuate according to season and weather, therefore plants must establish protective mechanisms from the oxidative damage these wavelengths can cause (Jansen et al., 1998). Only recently have studies suggested that isoprenoids could participate in UV protection in plants (García et al., 2016). Even more recently, mutants deficient in *DXS* and *HDR*, were shown to have a decreased tolerance to UV-B stress due to reduction in antioxidant MEP pathway products, including carotenoids and tocopherols (Emiliani et al., 2018). Therefore, it is reasonable to hypothesise an effect of UV-B light on the regulation of MEP pathway genes. UV was previously reported to increase expression of a *DXS* gene in *Cuminum cyminum L*. (Ghasemi et al., 2019), and in rice (referred to as *DXS3*) (Kim et al., 2005), with the latter study finding no significant trend for *Arabidopsis*. Whereas, the bioinformatic analysis performed in this chapter showed short-term (1 and 6 h) exposure to UV-B slightly down-regulated *DXS*, *DXR* and *HDR* expression, with marginal increments for *DXR* and *HDR* after 4 days continual exposure (Figure 2.4b). Although the variation in expression was small, and not in the positive direction as initially hypothesised, the photoreceptor UVR8 did show a significant impact on *HDR* expression. Therefore, whilst little response is seen by *HDR* to blue light, UV-B via UVR8 may have a stronger influence on *HDR* expression. However, for the other rate limiting steps of the MEP-pathway, UV-B may reduce their expression in *Arabidopsis*.

This may indicate links between UV-B and damage in the plastids that lead to changes in flux through key chloroplastic pathways (Santos et al., 2004). This response could be dose related, as MEP pathway genes were not listed in a low-fluence genetic screen in *Arabidopsis* (Brown and Jenkins, 2008). Further experiments with different doses between 1 – 6 h and intensities of UV-B would be necessary to clarify if, as initially hypothesized, long-wave UV-B can activate the production of photoprotective antioxidants (Brown et al., 2005), at what point UV-B photoprotective mechanisms are stimulated (Brown and Jenkins, 2008), and whether, under excessive UV-B exposure, a chloroplast under stress will shut down such pathways.

These results show that wavelength and duration of the illumination inputs contribute to the expression of MEP pathway genes. Therefore, light presents a valid method to attempt the manipulation of the flux of the MEP pathway by influencing the transcription of the rate limiting genes. However, the presented data also show that holistic effects of light such as the timing of peak expression must be considered. As phytochromes and cryptochromes are part of the entrainment inputs of the circadian clock (Devlin and Kay, 2000; Wang, 2015), future experiments should explore whether the circadian entrainment and photoperiodic cycling these MEP pathway enxymes change depending on the spectral composition.

2.4.2 Carotenoid Pathway – Holistic Light Control Through PSY

The carotenoid biosynthesis pathway begins with precursors derived purely from the MEP pathway (Rodríguez-Concepción, 2010). Therefore it is no surprise that intrinsic links have been found between the rate limiting gene of the carotenoid pathway *PSY* (Ruiz-Sola and Rodríguez-Concepción, 2012) and the MEP pathway gene *DXS* in particular (Botella-Pavía et al., 2004; Meier et al., 2011; Rodríguez-Concepción, 2010). Transcription of *PSY* and other carotenoid genes is triggered rapidly upon seedling emergence to light and is crucial to the establishment of the photosynthetic machinery due to its role in light harvesting, and photoprotection (Dall'Osto et al., 2007; Toledo-Ortiz et al., 2010). The induction of *PSY* transcription has been seen in a range of

monochromatic wavelengths including blue, red, and far-red (von Lintig et al., 1997; Welsch et al., 2003), each of which were explored in this chapter.

The results presented here show an induction of *PSY* transcripts under BL, with an involvement of cryptochromes in the expression, although the effect is yet to be fully clarified (Figure 2.2). Cryptochromes coordinate several blue light responses (Yang et al., 2017) and have been implicated in the expression of carotenoids in tomato via overexpression genotypes (Giliberto et al., 2005; Giuliano et al., 2008). However whilst the involvement of cryptochromes in blue light regulation has been suggested (von Lintig et al., 1997) this has not previously been demonstrated in *Arabidopsis*. As carotenoids have a photoprotective role it would not be unusual for the rate limiting gene of the biosynthesis pathway to be blue light regulated, especially as blue light is heavily implicated in stress responses (Hideg et al., 2018) in a similar manner to UV-B (Yadav et al., 2020). UV-B has shown to be indirectly involved with increases in carotenoid and antioxidant content (Emiliani et al., 2018; Ghasemi et al., 2019). Indeed, under differing exposures of UV-B, *PSY* expression increased with highest expression found after just 1 h of exposure (Figure 2.4b), similar to that found in UV-B regulated genes in *Cuminum cyminum* L. (Ghasemi et al., 2019). However, *PSY* expression in the *uvr8* mutant whilst reduced, was not statistically significant (data not shown). These results show that blue and UV-B wavelengths have an effect on carotenoid biosynthesis, yet further exploration by genetic studies using *cry1*, *cry2* and *cry* double mutants, in addition to *uvr8*, at several light intensities and duration is required for greater mechanistic insight.

Previous studies have experimentally linked *PSY* expression with red and far red light (Li et al., 2008a; von Lintig et al., 1997; Welsch et al., 2000, 2003). Clear red light induction of *PSY* expression was also found in these analyses after long-term (Figure 2.3) and short term red light exposure (Appendix Figure 6.1.2), with a dependence on phytochrome in both cases. Under short dose (1 h) of low fluence RL (8 μmol.m^{-2}.s^{-1}), the phytochrome control of *PSY* at transcript and protein level is dominated by phyA with moderate contribution of PhyB (Appendix Figure 6.1.3) (Tepperman et al., 2006). phyA has a prominent role in short-term red light responses, as it is light liable. Therefore this dominant role of phyA under short red light doses may be related to the high activity of PSY at dawn (Seaton et al., 2018). phyA is also the sole phytochrome contributor to

modulation of *PSY* transcripts under long term FR (von Lintig et al., 1997). Long-term FR-HIR is associated with inhibition of stem elongation under continuous FR, where transient high activation of phyA is induced as well as a rapid turnover of the protein and end of the acute responsiveness (Parks and Quail, 1993). Such a response could be linked with the reduction in *PSY* expression under long term FR in the genomic data sets which applied a strong dose of FR (R:FR = 0.006) for up to 24 hrs (Figure 2.4a). Despite the detailed work on red light responses, there is scope for further studies on the effects of FR on gene transcription within the carotenoid biosynthesis pathway, including FR enrichment (low R:FR ratios) and FR-HIR responses, that only temporarily activate phyA. Results may find that the ratio of red: far-red in leaves could have a more nuanced effect on carotenoid synthesis and trigger changes in accumulation of carotenoids as seen in tomato fruits (Alba et al., 2000; Schofield and Paliyath, 2005).

Both HY5 and PIFs transcription factors have been observed to interact with *PSY* and its homologues in *Arabidopsis* and other plants to modulate the expression of carotenoids (Chenge-Espinosa et al., 2018; Toledo-Ortiz et al., 2010). In tomato plants, blue light and red light act via HY5 and PIFs upon *PSY* to increase lycopene content (Xie et al., 2019). *Arabidopsis* genomic dataset analyses for *PSY* confirmed the role of HY5 as a positive regulator, and PIFs as a negative regulator of *PSY* expression (Figure 2.5). These two regulator molecules act antagonistically upon *PSY* expression, by binding to motifs within the promoter of PSY (Toledo-Ortiz et al., 2010), including C- and E-box motifs found throughout the promoter of *PSY* (Appendix Figure 6.1.5), and two G-boxes (Chenge-Espinosa et al., 2018), which is disproportionately found in phyA-regulated genes (Hudson and Quail, 2003). Due to the multiple regulatory components binding to the same motifs in the *PSY* promoter, it could present an interesting model of antagonistic and synergistic modulation between wavelengths, depending on which component is prevalent due to the light environment. Therefore lighting spectra is an option to further explore the modulation of *PSY* accumulation and activation of the production of carotenoids.

In addition to light quality, *PSY* transcript levels also showed diurnal fluctuation, characterised as a dawn peak in both short- and long-day cycles (Figure 2.6c, d), and

oscillations are present in circadian entrainment (Appendix Figure 6.1.4), extending to a circadian control of *PSY* peaking times. The regulatory mechanism of *PSY* expression throughout the day could be linked to the circadian associated motifs found within 2Kbp of the promoter region and includes binding sites for CCA1, and LHY, which relate to morning expression patterns (Appendix Figure 6.1.5). In addition PSY also has a PRR binding site and T1ME, that can bind TOC1 to complete the reciprocal circadian regulation (Alabadí et al., 2001; Huang et al., 2012). Interestingly, ATTRACTOR lists PRR5 as having a regulatory input into the circadian cycle of *PSY* in the evening time (Appendix Figure 6.1.6), and PPRs have been previously linked to the control of the carotenoid pathway (Fukushima et al., 2009). Overall, the bioinformatics studies on *PSY* show that beyond a very active light quality regulation, circadian inputs are very likely involved in tuning the diurnal expression pattern resulting in a dawn peak in transcription (Figure 2.6c, d). Throughout the plant lifecycle, from de-etiolation to flowering, cryptochromes and phytochromes interact to ensure the circadian clock is set at dawn and functioning appropriately (Somers et al., 1998). It is likely that *PSY* expression, so closely associated with de-etiolation and light responses (Rodríguez-Concepción, 2010; Rodríguez-Villalón et al., 2009a), is also a target of cryptochrome and phytochrome interaction, setting it as a key gene for light control of carotenoid biosynthesis.

The positive input of both red and blue light, in addition to the reciprocal binding of HY5 and PIFs to the *PSY* promoter and the strong evidence of circadian regulation complete a complex picture of multifactorial light regulation for carotenoid biosynthesis. Therefore, further experimentation should focus on understanding the diurnal *PSY* expression profile under spectral compositions found in natural light environments, with geotemporal variation based on global position, time of day and weather conditions. Combined with additional molecular characterization of the light and circadian components identified as potential regulatory components, these studies would clarify how these regulatory forces interact to create an adaptive method of transcriptional control through the prevalence and timing of light wavelengths.

2.4.3 Tocopherol Pathway

The family of tocopherols have essential functions as antioxidants to protect against lipid peroxidation but also as radical scavengers in photoprotection (Munné-Bosch, 2007). Measurements of light effects on tocopherol content show increases in high light conditions (Krieger-Liszkay and Trebst, 2006; Szymańska and Kruk, 2010), under elevated blue light in microgreens (Samuolienė et al., 2017), or in green light in microalgae and in baby leaf lettuce (Ljubic et al., 2021; Samuoliene et al., 2012). But despite the close relation to the MEP pathway and chlorophyll metabolism (see Section 2.1.1.3), and evidence of different wavelengths influencing the production and accumulation of tocopherols (see Section 2.1.2.3), there is little information on photoreceptor and genetic regulation of tocopherol biosynthesis. As such, the evidence presented in this chapter represents the first methodical analysis of light inputs upon rate-limiting enzymic genes, *VTE1*, *VTE4* and *VTE5* of the tocopherol biosynthesis pathway in the model plant *Arabidopsis*.

2.4.3.1 *VTE1* and *VTE4* Show Similar but Distinct Responses to Light Quality

Of all four tocopherols, α-tocopherol has the most potent antioxidant activity thereby the most desirable tocopherol to target for enhancement in food crops (Kamal-Eldin and Appelqvist, 1996). α-tocopherol is the terminal product of tocopherol biosynthesis, requiring first *VTE1* and then *VTE4* for generation (Fritsche et al., 2017). The sequential nature of these enzymic genes appears to generate common responses to light quality. For instance, *VTE1* and *VTE4* both show identical expression peaks over long- and short-day photoperiods (Figure 2.6e, f). However, the level of expression of *VTE1* was predominantly higher than that of *VTE4*. Moreover, from the evidence collated in this chapter, *VTE1* expression is primed to dominate *VTE4* expression under all the light conditions tested, particularly under monochromatic blue and red light (Figure 2.2 and 2.3, respectively). In addition, *VTE4* repression under FR light supplementation was greater than *VTE1* at all exposures examined (Figure 2.4a), and under UV-B supplementation, *VTE1* transcription was significantly induced, whereas *VTE4* expression

did not show a clear response (Figure 2.4b). Observing the pathway overall, and assuming transcript levels reflect on protein content and enzymatic action for the two resultant enzymes, this would create conditions more favourable to γ-tocopherol accumulation rather than α-tocopherol (Figure 2.12). However, in green tissues, α-tocopherol is by far the most abundant (Asensi-Fabado and Munne-Bosch, 2010), which can be linked to the expression of *VTE4* in *Arabidopsis* (Li et al., 2010c; Shintani, 2002). However, current studies show that isolated increases of *VTE4* alone cannot be achieved through a targeted light recipe, but light qualities could be used to increase tocopherol levels in general by targeting multiple rate limiting steps (see Section 2.1.2.3). Interestingly, differences in tocopherol composition using light was achieved in baby leaf lettuce, where blue and green light supplementation promoted γ- and α-tocopherol accumulation, and red light supplementation had significantly positive effect on α- and β-tocopherol over the other tocopherols (Samuoliene et al., 2012), showing that light quality has a good potential to modulate the accumulation of desired tocopherols.

Expression analyses of *VTE1* and *VTE4* show transcriptional upregulation under monochromatic blue and red light, with evidence of the involvement of cryptochromes and phytochrome photoreceptors (Figure 2.2 and 2.3, respectively). Overexpression of *PHYB* in tomatoes resulted in higher levels of α-tocopherol at all sampling stages (Alves et al., 2020). Meanwhile, blue light effects have been reported on tocopherol content and the role of cryptochromes in the accumulation of metabolites including tocopherols has been previously documented for tomatoes (Fantini et al., 2019). More specifically, *VTE1* has previously been linked to blue light signalling in tea plants (Zheng et al., 2019a), therefore, the induction of blue and red light within the tocopherol biosynthesis pathway in *Arabidopsis* corroborates light effects observed in other species (Kleine et al., 2007). The mechanism is likely HY5 as *hy5* mutants show a significant role of this transcriptional regulator in activation of *VTE1* and *VTE4* expression in *Arabidopsis* (Figure 2.5a). Mechanistically such regulation could be enacted upon the repeated C-box and E-box motifs upstream of *VTE1* and *VTE4*, which are binding sites for regulation by light signalling components HY5 and PIFs (Appendix Figure 6.1.5). Expression analysis in *pifQ* mutants suggests that PIFs also serve as inducers of transcription for *VTE1* and *VTE4*

particularly in the case of acute red light responses (Figure 2.5b). Whilst PIF activity is generally associated with transcription repression, PIFs have a dual capacity to function also as transcriptional activators (Leivar and Quail, 2011). As the family of PIFs is large, different members could have individual regulatory roles, for example PIF3 in tomato represses light-mediated tocopherol synthesis (Gramegna et al., 2019), whereas PIF4 does not have a role (Rosado et al., 2019). Further analysis of the role of individual PIFs and other light signalling components is required to give further clarification and discover the transcriptional control by light signalling components within tocopherol synthesis.

In addition to PAR wavelengths, FR and UV-B light responses were also examined in genomic experimental datasets. Under supplementary FR light, repressive effects on transcription for both *VTE1* and *VTE4* were observed, but with a disproportionately larger effect on *VTE4* (Figure 2.4a). This is especially interesting as ATTRACTOR listed phyA as a positive expression input for *VTE4* (Appendix Figure 6.1.6), but a stronger contribution of phyB was seen in a microarray examining different phytochrome contributions (Appendix Figure 6.1.3). As far-red responses are attributed to phyA (Mateos et al., 2006), these data may point at different modes of phyA action under RL and FR light, including FR-HIR and low R:FR ratio conditions (Parks and Quail, 1993). In support of this option, FR light reduced the concentration of α-tocopherol in *Raphanus* seedlings in comparison to WL (Lichtenthaler and Becker, 1975), therefore the original observation of FR reduction of tocopherol genes may be linked at perception of low R:FR ratios by phyA. This could be an adaptive mechanism, as when the plant is shaded photosynthesis can increase in efficiency due to balanced energy flux through PSI and PSII (Zhen and van Iersel, 2017), potentially leading to a reduced requirement of photoprotective molecules. In contrast, UV-B supplementation induced a large increase in *VTE1* transcripts, which correlates with a previous study that found higher levels of γ-tocopherol in *Arabidopsis* illuminated with UV-B (Emiliani et al., 2018). UV-B also promotes *VTE4* in short doses (1 h), which could lead to increased α-tocopherol content as is seen in several species under UV-B radiation (Munné-Bosch and Alegre, 2002) (Figure 2.4b). Therefore the FR and UV-B wavelengths could be applied as supplementary lighting treatments to respectively suppress or enhance the transcription of tocopherol biosynthesis. However, further understanding

is required as to the dose and duration effects of FR and UV-B on these genes in order to gain precision in the accumulation of the most valuable end product, α-tocopherol.

In addition to light quality inputs, diurnal expression of tocopherol genes was observed in bioinformatic studies of long-day and short-days and in the ATTRACTOR dataset, with a conserved peak expression for *VTE1* and *VTE4* at ZT4 (Figure 2.6e, f and Appendix Figure 6.1.6). As the cycling of *VTE1* and *VTE4* can still be observed in continuous light and in entrained conditions, it is likely that this expression pattern is circadian (Appendix Figure 6.1.4). In this respect, the circadian regulators PRRs (Fukushima et al., 2009) have been identified as inputs into the diurnal regulation of tocopherol genes, and mutants of *CCA1* and *LHY* have misregulated expression of *VTE1* (Abuelsoud et al., 2020). Interestingly, ATTRACTOR also lists LHY and PRR5 as inputs into *VTE1* and *VTE4* expression, respectively (Appendix Figure 6.1.6). The presence of CCA1 and LHY binding motifs in the *VTE1* promoter and LHY and PRR motifs within the *VTE4* promoter present further evidence of a potential direct mechanism of regulation by circadian components, that awaits exploration in the future (Appendix Figure 6.1.5). The content of α-tocopherol also follows a diurnal rhythm in leaves, but this response can vary according to the species (Munné-Bosch and Alegre, 2002; Oliván and Munné-Bosch, 2010) which may hint at a species-specific combination between light quality and photoperiodic responses. Thorough molecular research is required to disentangle the regulation of tocopherol biosynthesis by light and its links to photoperiodic and circadian control, to enable the application of these learnings in the enhancement α-tocopherol enhanced in crops under commercial CEA growing settings.

2.4.3.2 Light Influences on VTE5 are Distinct from Other Tocopherol Synthesis Genes

It was known since the 1960's that tocopherol and chlorophyll are closely linked in green tissues (Booth, 1963). The relatively recent discovery of the complete biosynthesis pathways of these two molecules has established that chlorophyll and tocopherols are not only associated via the supply of GGPP from the MEP pathway (Bouvier et al., 2005), but in a more intimate way through chlorophyll metabolism. The enzymic gene *VTE5* converts the chlorophyll-derived free phytol in phytyl-phosphate that is then

phosphorylated into PDP, one of two starter molecules of the tocopherol pathway (Kim et al., 2014; Szymańska et al., 2015). *VTE5* function is therefore rate-limiting and provides an additional dynamic to address when observing the effect of light on tocopherol synthesis.

Significant effects on *VTE5* transcription under BL and RL was found in this study. However a lack of consensus between the genomic and qPCR datasets under BL mean that intensity and duration effects are present, so the exact role of BL signals in the *VTE5* transcriptional control remain unclear (Figure 2.2). BL has been associated with chlorophyll synthesis in algae (Matters and Beale, 1995); simultaneous BL regulation of *VTE5* with chlorophyll production could be a conceivable advantage to coordinate turnover of chlorophyll to ensure proper function and concurrently to facilitate tocopherol production. In addition, *VTE5* was clearly red light induced in a phy dependent manner in all datasets analysed-mediated (Figure 2.3), with a likely prominent role of phyB on the modulation of its transcriptional control (Appendix Figure 6.1.3). This observation is in agreement with the overwhelming effect of phytochrome and RL on chlorophyll synthesis (Masoner and Kasemir, 1975) as studies of phyB in rice indicate (Inagaki et al., 2015). Analysis of mutants of HY5 and PIFs, suggest that contrary to expectation, HY5 may play an inhibitory role, similar to what was seen for *DXS*, in addition to inhibition by PIFs by red light signals (Figure 2.5). PIFs and HY5 have the potential to directly interact with *VTE5* promoter, via the C- and E-box promoter motifs discovered in this study (Appendix Figure 6.1.5); this possibility and their involvement requires further study.

Interestingly, a significant reduction of *VTE5* expression is seen under FR light after only 1 h of exposure (Figure 2.4a). Chlorophyll synthesis is stimulated by exposure to red light, a response that is reversible by far-red light (Price and Klein, William, 1961), therefore, the drop in *VTE5* expression could be associated with the decrease in chlorophyll synthesis under FR light. In addition, *VTE5* was downregulated under all UV-B exposures, similar again to *DXS*, however *VTE5* was the most highly downregulated of all the isoprenoid genes tested (Figure 2.4b). This is unexpected considering the reported role of VTE5 in protection from high light, whereupon damaged chlorophyll is degraded and the phytol salvaged to produce protective tocopherol (Almeida et al., 2016; Patil and

Senthil-Kumar, 2020). To support this possibility, studies using *vte5* RNAi lines in tomato showed lower accumulation of tocopherol and a decreased tolerance to high light stress (Spicher et al., 2017). UV-B stress triggers damage in a similar manner to high light (Jansen et al., 1998), thereby it would be expected that *VTE5* expression could increase to provide precursor for a highly effective antioxidant. However, under a low level of UV-B induced stress, before damage, accumulation of chlorophyll has been documented in UV-B treated plants (Favory et al., 2009a), which could be associated with an initial decrease in tocopherol production in a temporary flow of intermediaries to chlorophyll metabolism, before damage and the need of antioxidants is perceived by the plant.

In addition to spectral quality inputs, *VTE5* expression showed differential 4 h peak shifts between diurnal cycling in long- and short-day conditions (Figure 2.6e, f) which was also seen under continuous light and entrained conditions (Appendix Figure 6.1.4). This would suggest a strong photoperiodic control of its expression. However, binding motifs for PPRs and LHY were identified in the *VTE5* promoter (Appendix Figure 6.1.5), and the action of these circadian modules was also listed in the ATTRACTOR database (Appendix Figure 6.1.6), hinting at a circadian contribution to *VTE5* expression that is likely linked to its photoperiodic regulation and, due to its close association, circadian or photoreceptor regulation of chlorophyll degredation.

The results presented here show that within the tocopherol pathway, *VTE5* has a clear light regulation, with impact of spectral light quality within and surrounding PAR, in addition to photoperiodic and circadian control. For these reasons, *VTE5* is a very promising candidate for further characterisation as an integral point in the light-mediated regulation of tocopherols. Up-regulation of this gene by proper light environments could provide increases in tocopherol precursors, that alleviate identified bottlenecks within the tocopherol pathway (Quadrana et al., 2013).

2.4.4 Ascorbic Acid Pathway – Novel Discovery of Spectral Regulation for VTC2

As a derivative to AA, AsA is the most abundant lipophilic nutrient present in leaves (Smirnoff and Wheeler, 2000), and has a unique role as an antioxidant that acts to regenerate reduced xanthophylls and tocopherols via the ascorbate–glutathione cycle.

Therefore, to maintain a proper function of both carotenoids and tocopherols pathways, AA is necessary (Kobayashi and DellaPenna, 2008; Saga et al., 2010). AA levels are highly responsive to environmental signals including light, temperature and water (reviewed by Ishikawa et al., 2018). Of these the light input is best studied, but the vast majority of research is based on intensity driven responses (Page et al., 2012; Tamaoki et al., 2003). Light intensity upregulates genes and enzymes in the AA pathway, determining the availability of AA (Zha, Liu, et al., 2019). High-light induced transcription is related to photosynthesis (reviewed by Bartoli et al., 2016) and has been applied as end-of-production treatments for the improvement of nutritional quality and increments in shelf-life in lettuce (Gómez and Jiménez, 2020; Min et al., 2021) and tomatoes (Ntagkas, Woltering, Nicole, et al., 2019). However, if over applied, these treatments can lead to an imbalance in ROS production and thereby reduce yield (Gómez and Jiménez, 2020; Zha, Liu, et al., 2019). Due to its intricate relationship to the chloroplastic-based isoprenoid pathways it was hypothesised that the expression of the rate limiting gene of the ascorbic acid biosynthesis pathway would also be effected by wavelengths in a similar manner to the isoprenoid pathways, and could therefore be used to deliver a non stress-inducing light treatment for elevated AA content.

In addition to light intensity, published data indicate that BL or combination of RL and BL increases levels of AA in lettuce and komatsuna (Ohashi-Kaneko et al., 2007, 2013). One study went further, evaluating the performance of Lettuce under continuous ratios of 25% BL and 75% RL ($B_{25}R_{75}$), $B_{50}R_{50}$ and $B_{25}R_{75}$. The results showed that plants grown under the highest proportion of BL ($B_{75}R_{25}$) had increased AA pool size and elevated *VTC2* expression (as well as other AA biosynthesis genes) (Zha et al., 2020). Expression analyses have linked high-light responsive genes to CRY1 (Kleine et al., 2007) and *VTC2* is a high light responsive gene (Linster and Clarke, 2008). *VTC2* transcript abundance is impacted in the cryptochrome mutant, and based on the *Arabidopsis* genomic data, transcription is shown to be elevated in long-term BL (Figure 2.7a and Appendix Figure 6.1.7). Moreover, *VTC2* is also significantly induced by red light in a phytochrome-dependent manner (Figure 2.7b), but the quantity of expression could be dependent on duration of the illumination. Under high light treatment, *VTC2* expression was up-regulated after 2 h but then decreased (Zhou et al., 2021). Similarly, the expression of *VTC2* under short-

term RL exposures is higher in long-term (Appendix Figure 6.1.7). Whilst these results need to be corroborated by further qPCR testing, this evidence supports the identification of *VTC2* as a phy-dependent early response gene (Tepperman et al., 2006). phyA dominates expression of RL induced early response genes (Tepperman et al., 2006), and when the contributions of phytochrome to *VTC2* were examined, it was found to be strongly phyA regulated (Appendix Figure 6.1.3). Evidence of phyA regulation in a FR-HIR response can also be seen in the suppression of *VTC2* transcripts under FR supplementation (Figure 2.8a). This FR-effect has been previously documented in leaves of the common bean (*Phaseolus vulgaris L.*) that were grown under a low R:FR ratio, where ascorbate rapidly reduced over a single photoperiod (Bartoli et al., 2009). Moreover, the effect of shading for seven days in tomato downregulated *VTC2* in both leaves and fruits, resulting in a measured 50% reduction in total ascorbate in leaves and 10% reduction in fruits (Massot et al., 2012). This analysis shows that *VTC2* transcripts respond positively to long term BL and most strongly to short-term RL via phyA, in addition to decreased expression under long term FR. These responses should be considered when designing LED lighting treatments that target AA production.

Experiments with AA-deficient plants also suggest a protective role of AA against the damaging effects of UV-B (Conklin et al., 1996; Gao and Zhang, 2008). My studies showed that a short 1 h dose of supplementary UV-B can increase *VTC2* expression (Figure 2.8b). Similarly, AA improvements have been recorded in UV-B for soybean sprouts (Xu et al., 2005) and cucumber, with HY5 involvement in the response (Liu et al., 2019). However, the bioinformatic studies conducted on light signalling components presented here found a significant contribution of PIFs to the expression of *VTC2* (Figure 2.9) but not of HY5 (data not shown). Light responsive promoter regions have been previously located for *VTC2* between -70 and -40 bp of the start codon (Gao et al., 2011) and a G-box in the promoter of *VTC2* (GGP) in kiwi fruit leaves has been identified as a motif involved in the light-regulated expression of this gene (Li et al., 2013). However, a promoter analysis of *VTC2* in *Arabidopsis* did not locate any G-boxes, but instead found several other light-responsive motifs to which both PIFs and HY5 can bind (Appendix Figure 6.1.5). Transcription factors have been proposed to govern AA levels when exposed to oxidative stress or at particular stages of growth, some of which are associated with ethylene or

ABA, but none so far have been linked with light responsiveness (Mellidou and Kanellis, 2017). While, not yet fully conclusive, data gathered supports the involvement of PIFs in the light regulation on *VTC2*, and this is an area that requires further study to clearly establish if PIFs could be modulators of *VTC2* expression and its relation to phyA involvement as established earlier.

In addition to light-regulated motifs, circadian clock motifs that bind PRRs and LHY were also found in the *VTC2* promoter (Appendix Figure 6.1.5), giving further support to the PRR input listed on ATTRACTOR (Appendix Figure 6.1.6), and misregulated expression of *VTC2* found in a *cca1 lhy* mutant (Abuelsoud et al., 2020). Interestingly, a dawn expression peak was found in continuous light and in entrained data sets (Appendix Figure 6.1.4), which was also observed in long-and short-day expression data (Figure 2.10), hinting at circadian regulation, possibly via CCA1 and LHY. However, the expression of a secondary peak around dusk differed between the day lengths, indicating VTC2 also has photoperiodic oscillatory behaviour that could be malleable. Diurnal rhythms of AsA have been recorded previously, with diurnal variation in transcript levels of *VTC2* correlating to leaf AsA content measured 8 h later (Dutilleul et al., 2003; Massot et al., 2012). Therefore, whilst VTC2 is subject to circadian morning expression, light duration could be used to control the timing of *VTC2* expression peak and thus leaf AA and AsA content a few hours later.

In summary, the data presented here represents evidence of circadian and spectral quality- regulation of the AA biosynthesis flux-limiting gene *VTC2* in *Arabidopsis.* However, these influences may not be universal in crop plants, as BL and RL modulation of AA content was observed in lettuce but not seen in spinach (Ohashi-Kaneko et al., 2013), nor in another type of red leaf lettuce (Li and Kubota, 2009). Therefore, further studies of the effect of light on AA in multiple species, particularly of high value crop plants, would be required before confirming a modified light environment for the manipulation of the AA levels.

2.5 CONCLUSIONS

Overall, data gathered in this chapter support the hypothesis that light signals can be utilised to impact on the expression of rate-limiting vitamin biosynthesis genes. MEP pathway gene expression is primarily driven by red wavelengths, whereas carotenoid, tocopherol and ascorbic acid biosynthesis genes are positively influenced by blue, red and UV-B wavelengths (Figure 2.11). Application of these findings could 'level out' expression peaks and alleviate flux bottlenecks in the biosynthesis pathways resulting in greater dietary vitamin content. For example, a potential dynamic lighting regime to increase the expression of these genes would consist of an increase in red light in the afternoon/dusk period to boost expression of MEP pathway genes when they are at their lowest diurnal expression before night time. This treatment paired with a short increase of blue and UV-B wavelengths in the morning after dawn, will target enhancement of carotenoid, tocopherol and ascorbic acid biosynthesis genes. In general, FR light is not likely to increase vitamin content as FR supplementation reduced expression in all genes analysed, potentially linked to the role of these molecules in photoprotection.

However, the vitamin pathways are highly integrated with light influences enacted uniquely upon each gene though the interaction of light signalling components including photoreceptors, HY5, PIFs and circadian elements. Therefore, more holistic experiments on light quality, duration and timing of application with effects observed across pathways would be required. To build upon this work, further molecular genetic studies on the light signalling components that could deliver light quality, quantity and circadian inputs should be conducted for the case of phytonutrients accumulation, in particular for tocopherols and ascorbic acid. Potential feedback between pathways, such as between DXS and *PSY,* must also be better explored, together with effects of ecotype and additional environmental cues that can happen together with light in nature, such as temperature changes. As evidence gathered here indicate, any applied lighting regime must monitor for knock-on effects to circadian regulation and plant function but also measure the end-product to ensure that light influences on genes expression result in a measurable increase in the target compound.

CHAPTER 3

Light Effects on the Growth and Vitamin Content of High Value Herb Basil (*Ocimum basilicum*)

3.1. INTRODUCTION

The Basil genus, *Ocimum*, comprises of more than 30 herbaceous plants and shrubs native to the tropical and subtropical regions of Asia and Africa, and subsequently introduced to Europe and America (Sullivan, 2009). Prized for its attractive aroma, there are over 40 domesticated cultivars specialised for culinary and ornamental markets that vary in size, colour and fragrance, including lemon, liquorice and fruity (Simon and Morales, 1999). The most popular commercial cultivars are from the *Ocimum Basilicum* species, the sweet Basil group; commonly associated with dark green leaves and small white flowers, it is classified as a tall slender type (Darrah, 1974) growing to 30 -60 cm in height with large leaves around 5 cm in length (Sullivan, 2009). *O. Basilicum* is characterized by a large diversity among its genotypes from various aroma types, leaf size and shape, leaf and stem colour, inflorescence colour and structure, grow habit and seed morphology (Dudai, et al., 2018). In addition to the great range of morphological traits within this group, there is also variation in chemical composition including phenolic compounds (Marotti et al., 1996; Simon and Morales, 1999).

3.1.1. Secondary Metabolites of Basil and Their Properties

All Basil varieties synthesise phenylpropenes to give the characteristic flavour, in addition to essential oils and nutritional compounds including carotenoids, tocopherols and ascorbic acid (Sullivan, 2009). It is these compounds which makes Basil one of the most important domesticated herbs globally. Widely used in cuisine from Italy to Thailand, Basil compliments vegetables, meat, fish, stews, sauces, dressings, and tomatoes most commonly (Makri and Kintzios, 2008). The aromatic essential oils from the leaves and flowers also hold value to the perfume and beauty industries. In addition, Basil secondary metabolites hold several pharmacological properties (Singh and Chaudhuri, 2018).

Throughout history, Basil was a traditional remedy for ailments including stress, respirational issues, gastrointestinal problems, cardiovascular difficulties, sore throat and toothaches (Prakash and Gupta, 2005). In the modern-day, scientific studies have revealed anti-viral, anti-microbial, antioxidant, and anti-cancer properties attributed to both essential oils and phytonutrients (Ahmed and Masoud, 2014; Singletary, 2018). Rosmarinic acid (RA) is the major phenolic compound in Basil, followed by Caffeic Acid (Jayasinghe et al., 2003; Lee and Scagel,

2009). Both compounds are potent antioxidants (Chen and Ho, 1997; Jayasinghe et al., 2003), with antimicrobial activity (Bais et al., 2002; Widmer and Laurent, 2006) and RA in particular having anti-inflammatory activity (Sanbongi et al., 2004). In addition, Basil phytonutrients, including vitamins and minerals, have similar reported health benefits, and can have beneficial synergistic effects with the essential oil RA (Filip, 2017; Jayasinghe et al., 2003; Singh and Chaudhuri, 2018). This includes the nutrients of focus in this chapter, carotenoids (β-carotene and lutein) (Daly et al., 2010), tocopherols (Gómez-Coronado et al., 2004) and AA (Dumbravă and Moldovan, 2012; Murárikóvá and Neugebauerová, 2018) (Table 3.1). Unlike the essential oils, these compounds are essential to the human bodily function (see Section 1.2.2) and highlights Basil as a vehicle for delivering these nutrients to a typical diet.

Lutein (+ zeaxanthin)	β-carotene	α-tocopherol	Ascorbic Acid	Source
		4.05		Gómez-Coronado et al. (2004)
			27.1	Dumbravă and Moldovan (2012)
6.6 ± 0.4	18.4 ± 1.9			Daly et al. (2010)
			3.43 – 22.2	Murárikóvá and Neugebauerová (2018)
			10.05 – 15.25	Majkowska-Gadomska, Kulczycka and Dobrowolski (2017)
			15.03 – 26.31	Burdina and Priss (2016)

Table 3.1 Phytonutrients of interest in mature Basil originating from local markets or grown in university greenhouses, taken from the literature (mg/ 100g).

3.1.2. Growing Basil in Hydroponics with Artificial Light

The variety of compounds with culinary, nutritional and medicinal value has made Basil a popular herb and high value crop for indoor agriculture. With its short stature and quick cropping time, the herbaceous crop is highly suited to hydroponic vertical farming (Larsen et al., 2020). Using this farming method in itself can benefit the nutritional content of Basil, compared to conventional soil-based systems, under the same environmental conditions, hydroponic cultivation has been shown to improve the antioxidant capacity of the crop, increasing Vitamin C and Vitamin E, as well as total phenolic content and RA (Sgherri et al., 2010).

Basil prefers to grow in full sun conditions, and grows more vigorously with decreased competition for water and light (Sullivan, 2009). Indoor hydroponic cultivation within a vertical farm increases the availability of water and nutrients, and can fully control the light input to the crop thereby providing prime conditions for growth. The optimal light recipe for Basil is highly sought by indoor vertical farm growers. Different ratios of wavelengths have been tested in Basil and optimisation has proven to be complex (Tarakanov et al., 2012), with differing effects on growth, and nutrient accumulation observed in the numerous studies conducted (Larsen et al., 2020; Paradiso and Proietti, 2021).

Published studies summarized in Table 3.2 have shown that across cultivars at least 10% BL is required to give a good growth response in Basil (Bantis et al., 2016; Naznin et al., 2019; Pennisi, Blasioli, et al., 2019; Yelton et al., 2017) with some findings suggesting blue should be supplied at a higher proportion than red light (R:B = 0.7) (Piovene et al., 2015). Other studies have revealed a potential role of GL in promoting Basil fresh weight gain and height (Amaki et al., 2011; Bantis et al., 2016; Dou et al., 2019b; Yelton et al., 2017), an effect that has also been seen in lettuce (Kim et al., 2004; Nguyen et al., 2021). The effect of supplementary FR and UV-B lighting on Basil is little explored. In general, plants display classical symptoms to these wavelengths, including shade avoidance responses (SAR) and UV responses (see Section 1.3.2). There is some evidence to suggest a sensitivity of Basil to FR, resulting in SAR including elongated stems and reduced leaf area, but this varies by cultivar (Bantis et al., 2016; Larsen et al., 2020). Similarly for UV-B, there is evidence indicating sensitivity such as reduced stem lengths (Chang et al., 2009; Dou et al., 2019b).

3.1.3. Secondary Metabolites in Basil Vary Due to Spectral Composition

Photosynthesis, growth and phytonutrient content are intrinsically linked. Photosynthesis is required to provide sugars for growth, and antioxidant phytonutrients are required by the photosynthetic machinery to both assist in the harvesting of light, but also aid in protection from oxidative damage from free radicals. The three phytonutrients studied in this thesis, carotenoids, tocopherols and ascorbic acid, have a direct role in the function and maintenance of photosynthesis to enable growth of the plant (see Section 1.2.2), and published literature suggests their accumulation is impacted by light quality in mature Basil (Table 3.3). Red light exposure may increase α-tocopherol content, but decrease AA and lutein as well as total carotenoid pigments (Samuoliene et al., 2016). Alternatively, under 17 % BL fraction total carotenoid content was maximised in Basil (Naznin et al., 2019) and in Sweet Basil grown with 32% blue light, total phenolic content was increased and was also found to have the best flavour, highest spice and richest aroma (Yelton et al., 2017). UV-B induces essential oil production and is required for the proper development of volatile oil glands in Sweet Basil (Ioannidis et al., 2002). Indeed, UV-B effects volatile content, including major volatiles linalool and eugenol (Chang et al., 2009), in a dose dependent manner (Kakani et al., 2003). Comparatively, total flavonoid content, total phenolic content, as well as anthocyanins and ascorbic acid increased with UV-B (Ghasemzadeh et al., 2016; Sakalauskaitė et al., 2012). However, UV-B has no observed effect on the photosynthetic pigments, chlorophylls and carotenoids (Sakalauskaitė et al., 2012).

In the spectral mixes tested to date, general trends reveal that Basil phytonutrient content is most sensitive to BL, with total phenolics, chlorophylls and carotenoids contents at their highest value under the largest proportion of BL (Table 3.3). Examination of these studies together suggest that BL at 20% or more of the total PPFD is required to target increased secondary metabolite production in Basil.

Cultivar	Light Condition	Parameter	DLI ($mol.s^{-1}.day^{-1}$)	Plant Age	Source
Aton	B_{100}		6.9	8 weeks	
	$B_{75}R_{25}$				
	$B_{50}R_{50}$				
	$B_{25}R_{75}$				
	R_{100}	H FW			Schwend et al. (2016)
	GH	H FW	6.9	8 weeks	
	$B_{41}R_{59}$ (R:FR=10.6)				
	$B_{10}R_{90}$ (R:FR=2.6)	H			
	$B_{19}R_{81}$ (R:FR=8.3)	FW (out of LEDs)			
Dolly	$B_9G_{19}R_{70}FR_1$	FW	9.7	35 days	
	$B_{33}G_{14}R_{51}FR_1$	FW	17.3		Larsen et al. (2020)
	$B_{65}G_7R_{26}$	FW	17.3		
	B_{100}		13		

Emily	$B_9G_{19}R_{70}FR_1$	LA	9.7	35 days	
	$B_9G_{17}R_{60}FR_{14}$		17.3		
	$B_8G_{15}R_{53}FR_{25}$	H DW	17.3		
G Lemon Basil	$B_{17}R_{83}$		11.5	5 weeks	
	B_9R_{91}	FW DW L#			Naznin et al. (2019)
	B_5R_{95}				
	R_{100}	H			
Genovese	$B_{66}R_{33}$		12.4	39 days	
	$B_{50}R_{50}$				
	$B_{33}R_{66}$				Pennisi et al., 2019)
	$B_{25}R_{75}$	FW			
	$B_{20}R_{80}$				
Improved Genovese Compact	$B_{12}G_{44}R_{44}$	H	12.9	3 weeks	
	$B_{24}G_{41}R_{35}$				Dou et al. (2020)

	$B_{12}R_{88}$ $B_{24}R_{76}$	LA FW DW			
Lettuce Leaf	$B_{35}G_{24}R_{37}$ (R:FR=5.7) $B_{12}G_{19}R_{61}$ (R:FR=5.5) $B_{8}G_{2}R_{65}$ (R:FR=2.5) $B_{14}G_{16}R_{53}$ (R:FR=2.8) $B_{20}G_{39}R_{35}$ (R:FR=8.2)	H DW	10.1	4 weeks	Bantis, Ouzounis and Radoglou (2016) [+]
N/D	$B_{38}G_{37}R_{25}$ (R:B = 0.7) $B_{22}G_{54}R_{24}$ (R:B = 1.1) $B_{20}G_{50}R_{30}$ (R:B = 1.5) $B_{7}G_{53}R_{40}$ (R:B = 5.5) $B_{14}G_{79}R_{7}$	FW	11.5	46 days	Piovene et al. (2015)

Red Rubin	$B_{12}G_{44}R_{44}$ $B_{24}G_{41}R_{35}$ $B_{12}R_{88}$ $B_{24}R_{76}$	 FW DW LA FW DW H	12.9	3 weeks	Dou et al. (2020)
Red Rubin x Mountain Athos	$B_{35}G_{24}R_{37}$ (R:FR=5.7) $B_{12}G_{19}R_{61}$ (R:FR=5.5) $B_8G_2R_{65}$ (R:FR=2.5) $B_{14}G_{16}R_{53}$ (R:FR=2.8) $B_{20}G_{39}R_{35}$ (R:FR=8.2)	LA H DW	10.1	4 weeks	Bantis, Ouzounis and Radoglou (2016) [+]
Sweet Basil	Blue Blue-Green Green Red W ($B_{17}G_{17}R_{17}W_{49}$)	 FW	2.9	10 weeks	Amaki et al. (2011)

	$B_0G_8R_{92}$		12.6	9 weeks	
	$B_8G_8R_{84}$				
	$B_{16}G_8R_{76}$	FW			Yelton, Byrtus and Chan (2017)
	$B_{24}G_8R_{68}$				
	$B_{32}G_8R_{60}$				
Sweet Genovese	GH	H	25.9	3 – 4 leaf pair growth + 2 weeks	Chang, Alderson and Wright (2009)
	GH + UV-B	DW	+ N/D		
Thai Basil	GH		17.3	3 – 4 leaf pair growth + 7 days	Sakalauskaitė, Viskelis and Duchovskis (2012)
	GH + UV-B		+ 2 kJ m^{-2} day^{-1}		
	GH + UV-B	H LA FW DW	+ 4 kJ m^{-2} day^{-1}		
Green and Purple Basil	Control	H LA	9.2 – 12.9	38 - 44 days	Dou, Niu and Gu (2019b)
	+ 1 hr 2 days UV-B		+ 18.7 kJ m^{-2} day^{-1}		
	+ 2 hr 2 days UV-B		+ 37.4 kJ m^{-2} day^{-1}		
	+ 1 hr 5 days UV-B		+ 18.7 kJ m^{-2} day^{-1}		

+ 2 hr 5 days UV-B	+ 37.4 kJ m^{-2} day^{-1}

Table 3.2 LED light conditions found to maximise growth measurements of various Basil cultivars in published studies. Light condition notation is divided into blue (B), green (G), red (R) or white (W) light proportion with the percentage value in subscript (i.e. 38% blue, 26% green and 36% red light, $B_{38}G_{26}R_{36}$), and is accompanied by the daily light integral (DLI). The parameter abbreviation is noted against the light quality in which the highest value was recorded in the published study. Plants were grown hydroponically unless indicated that they were grown in soil ($^{+}$). WFL, White fluorescent light; W, white light; GH, ambient light in glasshouse; FW, Shoot fresh weight; DW, Shoot Dry weight; LA, Leaf Area; H, Stem length; L#, Leaf number; N/D, Not Disclosed.

Cultivar	Light Condition	Parameter	DLI ($mol.s^{-1}.day^{-1}$)	Plant Age	Source
Genoverser	HPS + Blue / + Blue-violet	Phytonutrients RA	25.9	79 days	Taulavuori et al. (2018) [+]
Genovese	$B_{66}R_{33}$		12.4	39 days	Pennisi et al. (2019)
	$B_{50}R_{50}$				
	$B_{33}R_{66}$				
	$B_{25}R_{75}$	AO Chl TFC			
	$B_{20}R_{80}$				
G Lemon Basil	$B_{17}R_{83}$	Car	11.5	5 weeks	Naznin et al. (2019)
	$B_{9}R_{91}$	AO Chl			
	$B_{5}R_{95}$				
	R_{100}				
Improved Genovese Compact	$B_{12}G_{44}R_{44}$		12.9	3 weeks	Dou et al. (2020)
	$B_{24}G_{41}R_{35}$	Anth			
	$B_{12}R_{88}$				
	$B_{24}R_{76}$	AO TFC TPC			

	$B_{49}R_{51}$				
Lettuce Leaf	$B_{35}G_{24}R_{37}$ (R:FR=5.7) $B_{12}G_{19}R_{61}$ (R:FR=5.5) $B_8G_2R_{65}$ (R:FR=2.5) $B_{14}G_{16}R_{53}$ (R:FR=2.8) $B_{20}G_{39}R_{35}$ (R:FR=8.2)	TPC	10.1	4 weeks	Bantis, Ouzounis and Radoglou (2016) [+]
N/D	$B_{38}G_{37}R_{25}$ (R:B = 0.7) $B_{22}G_{54}R_{24}$ (R:B = 1.1) $B_{20}G_{50}R_{30}$ (R:B = 1.5) $B_7G_{53}R_{40}$ (R:B = 5.5) $B_{14}G_{79}R_7$	TPC AO TFC	11.5	46 days	Piovene et al. (2015)

Red Rubin	$B_{12}G_{44}R_{44}$ $B_{24}G_{41}R_{35}$ $B_{12}R_{88}$ $B_{24}R_{76}$ $B_{49}R_{51}$	Anth Chl TFC Anth AO Chl TFC TPC	12.9	4 weeks	Dou et al. (2020)
Red Rubin x Mountain Athos	$B_{35}G_{24}R_{37}$ (R:FR=5.7) $B_{12}G_{19}R_{61}$ (R:FR=5.5) $B_8G_2R_{65}$ (R:FR=2.5) $B_{14}G_{16}R_{53}$ (R:FR=2.8) $B_{20}G_{39}R_{35}$ (R:FR=8.2)	TPC	10.1	4 weeks	Bantis, Ouzounis and Radoglou (2016) [+]
Sweet Basil	Blue Blue-Green Green Red WL (B1:G1:R1:W3)	EO (linalool)	2.9	10 weeks	Amaki et al. (2011)

Sweet Genovese	Elevated R @ 638 nm Elevated R @ 665nm Sole R @ 638 nm Sole R @ 665nm	α-T AA Lutein β-carotene	17.3	19 days	Samuoliene et al. (2016) [+]
	GH GH + UV-B	Volatiles	25.9	3 – 4 leaf pair growth + 2 weeks	Chang, Alderson and Wright (2009)
Thai Basil	GH GH + UV-B GH + UV-B	Anth AA AO TPC	17.3 + 2 kJ m^{-2} day^{-1} + 4 kJ m^{-2} day^{-1}	3 – 4 leaf pair growth + 7 days	Sakalauskaitė, Viskelis and Duchovskis (2012)

Table 3.3 LED light conditions found to maximise the antioxidant, phenolic, and flavonoid content of various Basil cultivars in published studies. Light condition notation is divided into blue (B), green (G), red (R) or white (W) light proportion with the percentage value in subscript (i.e. 38% blue, 26% green and 36% red light, $B_{38}G_{26}R_{36}$), and is accompanied by the daily light integral (DLI). The parameter abbreviation is noted against the light quality in which the highest value was recorded. Plants were grown hydroponically unless indicated that they were grown in soil ([+]). AA, Ascorbic Acid; AO, Antioxidant Capacity; Anth, Anthocyanin content; α-T, α-Tocopherol; Car, Total Carotenoids; Chl, Total Chlorophyll; EO, Essential Oils; GH, Ambient light in Glasshouse; TFC, Total Flavonoid Content; TPC, Total Phenolic Content; RA, Rosmarinic Acid.

3.1.4. Aims and Objectives

The aim in this chapter is to compare the growth response and phytonutrient content (carotenoid, tocopherol and ascorbic acid) of common CEA-grown, high value herb Basil cv. *Sweet Genovese* grown in different controlled environments and under several light qualities varying in blue, red, far red and UV-B content. This will inform understanding of tailored lighting regime for *O. Basillicum* required to strike an optimum balance between maximising growth and nutrient content. The objectives within the chapter are:

1. To compare growth and phytonutrient content of Basil grown in different controlled environment (CE) agricultural systems.
2. To verify an optimal blue and red ratio for Basil growth as seedlings and mature plants, in addition to phytonutrient content at harvest.
3. To explore the effect of supplementary lighting with UV-B and FR wavelengths located outside of PAR on Basil growth and nutritional content.
4. To quantify the effect of spectral mixes delivered by four commercial luminaries on Basil development and quality in a commercial setting.

3.2. METHODS

3.2.1. Experiment Comparing Controlled Environments

Glasshouse. In April and May 2021, Basil cv. *Sweet Genovese* (CN seed, Lot no. 50113) were sown into 9 cm plant pots in M3 soil (Levington) and placed in a glasshouse at Lancaster University (GH) with 16 h supplementary lighting provided by white Senmatic FL300 Sunlight LED units to give an average light intensity of 119 µmol.m^{-2}.s^{-1}; intensity and quality varied according to the weather conditions (Appendix Table 6.1.2). The hourly temperature ranged between 16 and 37 °C, and relative humidity (RH) from 12 to 53 %, (average temperature 24 ± 4 °C, RH 30 ± 9 %) as recorded by an Ektron II C sensor (HortiMaX B.V., Pijnacker, The Netherlands) suspended in the middle of the glasshouse. After 2 weeks, Basil plants were thinned to one plant per pot, total of 28 pots, and left to grow for a total of 4 weeks. Plants were rotated throughout the experiment to account for positional effects. Physiological measurements were recorded (as per Section 3.2.6), and samples harvested with liquid nitrogen for freeze-drying. After freeze-drying, samples were stored at -80 °C until further analysis (Section 3.2.7 – 10). The treatment was repeated twice.

Controlled Environment Cabinet. Basil plants were grown Snijder growth cabinets (Scientific BV Microclima 1750, Snijder Labs, The Netherlands), from this point forward addressed as 'Sn', set to 20 ± 1 °C, RH 55 %. Light intensity of the cabinets was filtered (LEE filters #209) to 150 µmol.m^{-2}.s^{-1} ± 10 %, photoperiod was set to 16 h. Basil plants were harvested as per the Glasshouse. The experiment grew 28 plants and was repeated twice.

Hydroponics. The vertical hydroponic growth facilities (Hp) were self-assembled in a CE Room at Lancaster University, as described in Appendix 6.2.1. Temperature was measured at the level of the hydroponic trays using an EasyLog temperature probe (Lascar Electronics, UK) and found to be 22/20 °C L/D, RH was 50 ± 5 %. On the basis of preliminary studies (Appendix 6.2.1.3), Basil seed was sown into 2 cm x 2 cm rockwool plugs that had been previously soaked in a specialised nutrient solution tailored to the water composition at Lancaster University generated by May Barn Consultancy Ltd. (for recipe see Appendix 6.2.1.2). The 28 plugs were placed in irrigation trays with a germination hood to maintain humidity, and put into the hydroponic system under the light treatment. Plants were irrigated for 30 minutes twice a day

with nutrient solution. After two weeks seedlings were thinned to just one seedling per plug and transplanted into individual 9 cm pots with soaked clay pebbles. Plants were grown for a total of 4 weeks under five GE Arize Lynk (GEHL48HPKB1) luminaires spaced to deliver 150 µmol.m^{-2}.s^{-1} for 16 h per day, then harvested as per the glasshouse plants. A spectral breakdown for all treatments is available in Appendix Table 6.1.2.

3.2.2. Experiment on Monochromatic and Dichromatic Light Quality in Seedlings

Basil seeds were sterilised with 10% (v/v) bleach treatment for 8 min before being washed a minimum of 5 times with RO water. Seeds were sown directly onto 1 M MS + vitamins agar media (6 % w/v), and no sucrose, within 9 cm plates. Ten seeds were sown per plate with equidistant spacing. Plates were sealed with Micropore tape and placed directly into Clima Red/Blue Light Cabinets, model EB2-N-PB (Snijder Labs, The Netherlands) in a 16/8 h L/D cycle at 22/20 °C day/night temperature. Plants were grown for 2 weeks under monochromatic blue (B_{100}) and red (R_{100}), or dichromatic blue and red treatments at 75% blue and 25% red ($B_{75}R_{25}$), $B_{50}R_{50}$, $B_{25}R_{75}$, in either High intensity (unfiltered), Medium (1 stop neutral filer, LEE filter #209) or Low intensity (2 stop neutral filter, LEE filter #210). Light intensities for each regime are described in Table 3.4. Three plates were sown per treatment and rotated throughout the experiment. Measurements of the seedlings included seedling establishment, fresh weight of the shoot using a microbalance, length of the hypocotyl measured from the base of the stem to the tip using ImageJ, and assessment of the growth stage via a numeric key (Table 3.5). The experiment was repeated three times and results were collated.

	B_{100}	$B_{75}R_{25}$	$B_{50}R_{50}$	$B_{25}R_{75}$	R_{100}
High	125	160	200	200	200
Medium	60	80	100	100	125
Low	20	40	50	50	40

Table 3.4 Light intensities (µmol.m^{-2}.s^{-1}) delivered to seedlings under monochromatic and dichromatic conditions in light cabinets.

#	Key
1	Leaves closed
2	Cotyledons opened
3	True leaves sprouting
4	True leaves mature
5	Second leaf pair sprouting

Table 3.5 The number code used to assess the development stage of Basil seedlings grown for two weeks in plates.

3.2.3. Experiment with Monochromatic and Dichromatic Light Quality on Mature Plants

Basil plants were grown for 4 weeks in the self-built hydroponic system as described in Appendix 6.2.1, under monochromatic blue (B_{100}) and red (R_{100}), or dichromatic blue and red treatments at 75 % blue and 25 % red ($B_{75}R_{25}$), $B_{50}R_{50}$, $B_{25}R_{75}$. Light was delivered by LED strip lighting at wavelengths of 450 nm (B) and 660 nm (R) (RS Components, Appendix 6.2.1.4). Light intensity for each treatment was set to 150 µmol.m^{-2}.s^{-1} +/- 10 % (spectral breakdown in Appendix Table 6.1.2), with a photoperiod of 16 h (DLI 8.64 mol.m^{-2}.day^{-1}). Twenty eight pots were rotated regularly within the tray to account for any lighting discrepancies and the experiment was repeated twice. Upon harvest, experimental measurements were gathered according to Sections 3.2.6 – 10.

3.2.4. Experiments with Supplementary Light Treatments

UV-B The control and treatments were performed simultaneously within a controlled environment room at Lancaster University with an impermeable light barrier between them (Appendix Figure 6.2.8). Five GE Arize lamps were suspended above each hydroponic flood and drain tray to provide an intensity of ~150 ± 5 µmol.m^{-2}.s^{-1} (16/8 h L/D, 22/20 °C day/night, RH 50 ± 5 %). Two UV-B bulbs (~313 nm, Q-Panel Lab Products) were also suspended above the treatment tray to provide UV-B irradiance at 0.45 +/- 0.03 W.m^{-2} for 4 h in the middle of the photoperiod to give a total UV-B dose of 6.48 kJ.m^{-2}.day^{-1} beginning 6 days before harvest (22 DAS). UV-B is a high energy wavelength which is harmful to living tissue (see Section

1.3.2.3). Therefore, the UV-B dose was selected based on previous published data on UV-B supplementation with Basil and Coriander. In these studies UV-B irradiation strength ranged from 0.2 – 3.5 $W.m^{-2}$, with treatment periods over 1-8 h over one day to 6 days (Chang et al., 2009; Dou et al., 2019a; Fraser et al., 2017; Mosadegh et al., 2018, 2019; Nascimento et al., 2020). Using this range, a 'low' irradiation (0.45 $W.m^{-2}$) was selected combined with a reasonable dose period similar to natural exposure (4 h mid-photoperiod for 6 days) with the aim to create a strong enough treatment to elicit UV-B responses without creating a stressful environment. No filter was applied to the UV-B lamps as it was determined that the potential additional PAR wavelengths from the UV-B bulbs was not detectable within error as measured by a PAR meter (PG100N, UPRtek). Plants were sown, irrigated and harvested as described in Section 3.2.3. Upon harvest, experimental measurements were gathered according to Sections 3.2.6 – 10. The experiment was repeated, however the secondary replica was excluded from analysis as it was affected by drought.

Far RedFive GE Arize Lynk lamps were mounted above a flood and drain tray within the vertical hydroponic system located a controlled environment room (22/20 °C L/D, RH 50 ± 5 %). To achieve the lowest R:FR ratio possible (~1) with the equipment available, the GE luminaires were filtered with a SC50 filter (LEE Filters). Light intensity was 150 +/- 10 $\mu mol.m^{-2}.s^{-1}$, with a 16 h photoperiod. Two tracks of FR LED strip lighting (RS components) were mounted in between the GE luminaires (see Appendix 6.2.1.4). The FR treatment used (R:FR ~1) is comparable to natural sunlight, and is not considered a strong dose, therefore a long FR treatment period was used, beginning on the 10^{th} day before harvest for 16 h per day (PFD 235 $\mu mol.m^{-2}.s^{-1}$). A fan was used to draw air across the tray to cool the lights and maintain temperature as close to the rest of the room as possible. Plants were sown and irrigated as described in Section 3.2.3. Upon harvest, experimental measurements were gathered according to Sections 3.2.6 – 10. The effects of FR were compared to those observed under control conditions without the FR LED supplement. Only one experiment was performed due to time constraints.

3.2.5. Experiment on Commercial Spectra at May Barn Consultancy Ltd.

Plants were harvested at 4 weeks old from May Barn Consultancy Ltd. in September 2020 on site in Nantwich. Basil cv. *Sweet Genovese* was grown in Rockwool sheets within a flood and

drain vertical farm system, and irrigated with specialised nutrient solution. Environmental conditions were controlled at 26 ± 1 °C and 55 ± 3 % RH. Plants were grown over 4 levels within one rack, each with a different horticultural LED spectra (from top to bottom): Heliospectra Siera Propagation High Blue (HSP-HB), Heliospectra Siera Indoor Production (HSIP), Current by GE Arize Lynk Vegetative High Blue GEHL48HPPV (GE-HB) and Valoya L28 AP673L (AP673L). The light intensity and spectra composition within each level is recorded in Appendix Table 6.1.2. Physiological measurements were recorded as per Section 3.2.6 on 28 plants sampled randomly from a single rockwool sheet. Tissue samples were transported from the site in dry ice and stored in -80 °C at Lancaster University until pigment analysis or freeze-dried for nutritional analysis by HPLC and UV-Spectrophotometer as described in Section 3.2.10.

3.2.6. Physiological Measurements

Several physiological measurements were taken upon each mature plant at harvest. Firstly, shoot fresh weight (FW) was immediately measured using a balance. Total leaf number was counted with leaves above 2 mm included. Leaf thickness was carefully measured upon a fully emerged leaf using callipers. Stem length was recorded with a photo and processed with Image J, measuring from the base of the shoot to the base of the upper petiole. Leaf area was the final destructive process: all leaves were stripped from the plant and passed through a leaf area machine (LI-3100, LICOR). Random plants were selected for sampling for pigment analysis, UV-spectrophotometry, or HPLC analysis. Tissue samples were collected by harvesting mature leaves of six plants together, thereby each treatment was sampled as a whole and averaged within the sample. All other plants were processed for dry weight. For dry weight (DW), the whole shoot was put into an envelope and placed in a drying oven at 60 °C for a minimum of 72 h until dry, whereupon it was weighed. Percentage DW was calculated by dividing DW by FW and multiplying by 100.

3.2.7. Photosynthetic Measurements

Rapid Light Response Curves Measurements were taken on a mature leaf in receipt of full illumination whilst growing with an infrared gas analyser (LI-6400XT, LICOR) and RBG light source (LICOR) according to the LICOR manual. The RGB light source was set to the same B, R, G ratio of the light treatment that the plants had been grown under, at a high intensity

(1500 µmol.m^{-2}.s^{-1}), for a minimum of 30 minutes before starting the rapid light response curve (RLRC) by sequentially lowering the intensity. Intensities measured were 1500, 1250, 1000, 800, 600, 400, 200, 100, 50, 25 µmol.m^{-2}.s^{-1}. Readings were allowed to stabilise for a minimum of 2 minutes between intensities before logging. C_i increased with decreasing intensity, from ~200 µmol.mol to ~400 µmol.mol. Temperature was set to ambient, and CO_2 was maintained at 400 ppm. The light response curve was fitted to averaged data with a non-rectangular hyperbola model (Marshall and Biscoe, 1980) using custom code with R Language and environment function nls() (R Studio, version 4.2.1) provided by Dr Samuel Taylor. After fitting of the line the values of light saturated rate of CO_2 uptake (A_{sat}) and maximum quantum yield of CO_2 (ϕ) were returned.

Chlorophyll Florescence Measurements were recorded with an infrared gas analyser (LI-6400XT, LICOR) and chlorophyll florescence head (LICOR). Plants were dark adapted for a minimum of 30 minutes before commencing chlorophyll florescence measurements upon a mature fully expanded leaf. F_0 and F_m were recorded with the test light off and antic light on. Illumination was then provided at 200 µmol.m^{-2}.s^{-1} with the same R:G:B composition as the growth conditions of the tested plants. F'_m was recorded once light-acclimated. Methods were adapted from (Murchie and Lawson, 2013). The parameters of F_v/F_m and NPQ were calculated within the device by the same methods outlined in (Maxwell and Johnson, 2000).

MultispeQ Measurements of Photosystem II efficiency (F_v/F_m) and non-photochemical quenching without dark adaption (NPQt) (Tietz et al., 2017) were preformed using the open-source MultispeQ v1, a hand-held device made by PhotosynQ, and the associated platform for collaborative research (www.photosynq.org) (Kuhlgert et al., 2016). The measurements were performed by clamping the device on a fully expanded leaf located at the top of the canopy, in receipt of full illumination, and selecting the protocol Photosynthesis RIDES.

3.2.8. Pigment Analysis

Chlorophyll and carotenoid quantification was performed using frozen plant tissues ground using pestles and mortars and mixed with volumes of ice-cold 80 % (v/v) acetone and centrifuged at 10,000 rpm for 15 min until pellets were white. Samples were then measured in a 96-well microplate reader (SpectroStarNano, BMG Labtec, Aylesbury, UK) and chlorophyll and carotenoid concentration calculated as described in Lichtenthaler and Buschmann (2001).

3.2.9. UV-Spectrophotometer Analyses of Carotenoid and Tocopherol Content

Standards of β-carotene (SIGMA) and α-tocopherol (SIGMA) were created by dissolving a small amount in hexane and ethanol, respectively. Stock standard solutions were diluted until within range of the UV-Spectrophotometer, whereupon an absorbance scan was performed using a Spectrasil cuvette (TSL Spectrasil, Torr Scientific Ltd.). Maximum absorption was found for each standard, 450 nm for β-carotene and 292 nm for α-tocopherol, and used for further analysis. Serial dilutions of each standard was made and absorption measured. Concentration of the standards at each dilution was calculated using the Beer-Lambert Law (Formula 1).

Formula 1 $$\boldsymbol{A} = \varepsilon \mathrm{m}\, \mathrm{l}\, \mathrm{C}$$

Where: *A* = *Absorbance*

εm = *Molar Extinction Coefficient*

l = *Length of the Light Path*

C = *Concentration*

The εm value used for β-carotene in 100 % hexane at 450 nm was 2500 (SIGMA) and for α-tocopherol in 100 % ethanol at 292 nm was 3260 (SIGMA). Linear regression analysis of the standard dilution absorbance values was used to create calibration curves to calculate concentration in the samples (Table 3.6).

Sample extraction was performed on freeze-dried tissue subsequently ground under liquid nitrogen. 50 mg was weighed in a glass vial and 3 ml of ethanol (+ 0.1% (w/v) butylated hydroxytoluene [BHT]), 1 ml of HPLC water and 1.5 ml of hexane (+ 0.1% (w/v) BHT) was added. The mix was vortexed for 1 min and centrifuged at 2500 rpm for 5 minutes at 4 °C. The organic hexane layer was removed to a separate glass amber vial on ice and extraction was repeated with hexane twice for exhaustive extraction. The hexane layer was diluted three fold in the cuvette to bring the absorbance within range of the UV-Spectrophotometer.

Standard	Max Absorbance (nm)	Equation	R^2 value

ß -carotene	450	y = 4x	1
α -tocopherol	292	y = 3.0675x	1

Table 3.6 Equations derived from linear regression analysis of nutritional standards, used to quantify concentrations in plant material by UV-Spectrophotometry.

3.2.10. High Performance Liquid Chromatography Quantification of Lutein, β-Carotene, α-Tocopherol and Ascorbic Acid

Lutein, β-carotene and α-tocopherol content were quantified, by High Performance Liquid Chromatography (HPLC) at the School of Food Science and Nutrition of Leeds University. Under the supervision of Dr. Ng'andwe Kalungwana and Dr. Christine Bosch a suitable method was developed for the measurement of carotenoids and tocopherols in green leaf material (Appendix 6.2.2).

For each treatment, fully expanded leaves of 6 adult plants were randomly chosen, collated together in a tube and placed in liquid nitrogen. Samples were then freeze-dried and stored in -80 °C until analysis. Freeze-dried samples were packed into insulated boxes with dry ice and transported in darkness to Leeds University, where upon they were placed immediately in -80 °C. Samples were ground in liquid nitrogen and 50 mg transferred to a 15 ml glass test tube; four independent extractions were undertaken per treatment. Extraction was performed as per Section 3.2.9 with the addition of 100 µl of echinenone internal standard before the first extraction (absorbance = 0.810). The extracted hexane was concentrated by evaporation in a Techne Sample Concentrator under a nitrogen gas current, then re-suspended in 200 µl of the mobile phase acetonitrile: methanol: dichloromethane [43:43:14] (v/v/v). Extract was filtered with a PFTE filter disc with 0.22 µm membrane into an amber HPLC vial, before injection into the HPLC at 10 µl (Shimadzu System LC-20AD SP, autosampler LC 20AC, diode detector SPD-M20A, and fluorescence detector RF-10A). An isocratic method was used with mobile phase of acetonitrile: methanol: dichloromethane [43:43:14] (v/v/v) on the Acclaim™ column C30, 4.6 x 250 mm, with 5 µm particle size (Thermo-Fisher Scientific). Flux was fixed at 1 ml/min at room temperature, with 30-minute analysis time. Lutein and ß-

carotene were read at 450 nm using the SPD detector and α-tocopherol was read at 290 nm with the fluorescence detector.

Vitamin C, defined as total ascorbic acid (tAA), comprises of AA and dehydroascorbic acid (DHA). 50 mg of ground tissue was placed in a 50 ml falcon tube and 2 ml of 4.5 % (w/v) metaphosphoric acid (MPA) solution was added. The mix was vortexed for 1 min before centrifuging for 2 minutes at 4000rpm. To quantify tAA, 1 ml of supernatant was removed to a 5 ml amber glass vial and mixed with 200 µl of dithiothreitol (20 mg/ml) before being left to stand in the dark at room temperature for 2 h. Samples were filtered with a PFTE filter disc with 0.22 µm membrane into an amber HPLC vial, before injection of 20 µl into the HPLC (Dionex ICS-3000). An isocratic method was used with mobile phase of water: acetonitrile: formic acid [99:0.9:0.1] (v/v/v) at Lancaster University on the Agilent InfinityLab Poroshell 120 EC-C18, 3 x 100 mm, with 4 µm particle size. Flux was fixed at 0.5 ml/min at 20 °C, with 15-minute analysis time. Ascorbic acid was read at 245 nm.

For the calibration curve, SIGMA standards of lutein, β-carotene, all-RAC α-tocopherol and L-ascorbic acid were used. Different concentrations of the standards were injected: 6.25, 12.5, 25, 50, 100, 200 µg/ml for lutein and β-carotene; 125, 250, 500, 1000 ng/ml for α-tocopherol; and 50, 100, 150, 200, 250 µg/ml for L-ascorbic acid, and the peak area of the standard was measured. A linear regression analysis (Table 3.7) and Formula 2 was used to calculate concentrations of secondary metabolites.

Standard	Equation	R^2 value
Lutein	y = 0.0000429x + 47.7401338	0.98
ß-carotene	y = 0.0000106x + 1.8463967	0.99
α-tocopherol	y = 0.0079495x + 22.5195323	0.99
L-ascorbic acid	y = 0.8862x - 6.1834	0.99

Table 3.7 Equations derived from linear regression analysis upon standard calibration curves, used to quantify concentrations in plant material (ng/ml for α-tocopherol and µg/ml for all others).

Formula 2 $$\boldsymbol{Concentration}\ (\mu \boldsymbol{g/mg}) = (\boldsymbol{m} * \boldsymbol{x} + \boldsymbol{K}) * (\boldsymbol{DF})/\boldsymbol{w} * \mathbf{1000}$$

Where: *m* = *Value of Slope*

x = *Area Calculated from the Chromatogram*

K = *b Value of the Linear Regression*

DF = *Dilution Factor*

W = *Weight in Grams*

3.2.11. Statistical Analysis

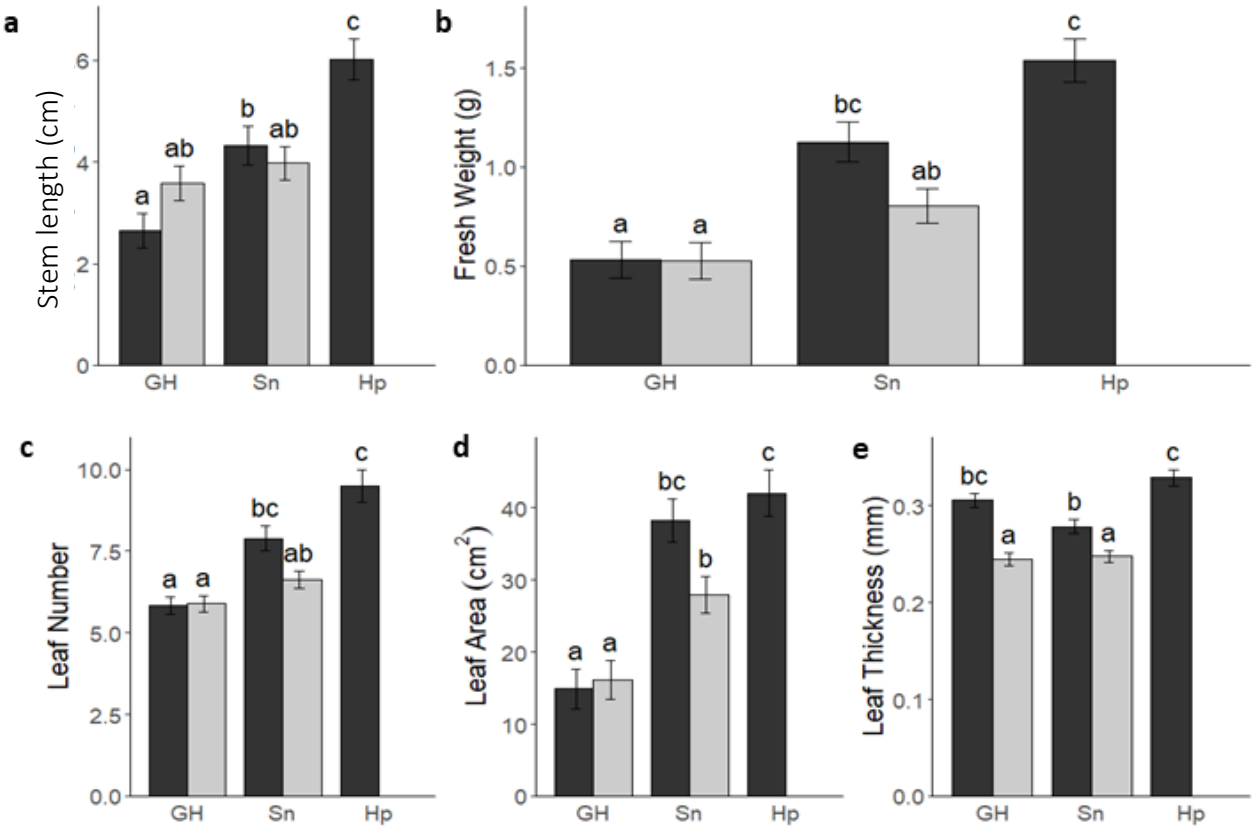
A two-way ANOVA was used to determine significance of measurements between lighting treatments and replica number, one-way ANOVAs were used when no repeated experimental data were available. The residuals of the models were checked for normality, and data was $\log_e$ transformed where appropriate to satisfy the normality assumption of the model. Estimated marginal means were calculated on original or back-transformed data and plotted data was on the original scale. A sidak post hoc test was used to assess the differences between groups. Statistical tests were performed using R (RStudio, version 4.1.2).

3.3. RESULTS

3.3.1. Growth in Controlled Conditions within Compost and Hydroponics

To evaluate the growth of Basil in controlled environments, three different methods where examined: GH and Sn which used compost, and Hp which used rockwool cubes surrounded by clay pebbles. (Figure 3.1). Hp plants were grown under commercial LED horticultural lighting (Current powered by GE Arize Lynk), and the fluorescent lighting in Sn was filtered to match the intensity of these lights (~150 $\mu mol.m^{-2}.s^{-1}$). Spectral qualities were measured and are recorded in Appendix Table 6.1.2. Temperature of Hp and Sn was also matched. The light quality and temperature in GH varied according to the weather between April and May 2020 (average temperature 22 ± 5 °C, RH 30 ± 15 %, total light energy intensity 22 $W.m^{-2}$ (Apr) and 34 $W.m^{-2}$ (May), with a minimum of ~10 $W.m^{-2}$ maximum of 157 and 184 $W.m^{-2}$, respectively). Repeated experiments were performed for GH and Sn treatments but not for Hp due to time constraints. The experimental repeats showed similar trends, although the effects on leaf thickness and dry weight were statistically significant between repeats ($p<0.001$). Nevertheless, the growth conditions had a significant effect on all measurements: height, fresh weight, dry weight, leaf number, leaf area, and leaf thickness ($p < 0.001$ for all parameters).

Figure 3.1 The growth of basil in different controlled environments. Basil grown in compost within a glasshouse between April and May under supplementary white light LEDs (GH) or Snijder cabinet with fluorescent lights (150 $\mu mol.m^{-2}.s^{-1}$) (Sn), was compared against hydroponically (Hp) grown Basil grown under a commercial LED fixture (150 $\mu mol.m^{-2}.s^{-1}$). All treatments used a long day (16 hr) photoperiod. Physiological measurements include, (a) stem length (cm), (b) fresh weight (g), (c) total leaf number, (d) leaf area (cm^2), and (e) leaf thickness (mm). Dark grey bars display estimated marginal means from the first experimental replica, and light grey bars for the second replica (n = 28 per replica). Error bars represent standard error. Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p< 0.05$). Data that share a letter are not statistically significant.



The growth data showed GH plants were shorter, had less fresh weight, a reduced number of leaves and subsequently less leaf area than plants grown in Sn and Hp (Figure 3.1). These results indicate that environments with greater control provide better conditions for growth. Interestingly, the Hp plants grew significantly taller than the Sn plants. Hp plants were also heavier with more dry weight percentage mass (Appendix Table 6.1.3), likely due to an increased number of leaves that were thicker with more leaf area (Figure 3.1). Overall, the plants in Hp were more commercially valuable, asserting that production systems can have an impact on yield and that hydroponic vertical farming is a viable solution for growing high quality Basil.

3.3.2. Effect of Light Quality on Basil Growth Physiology Across Development Stages

To assess the impact of light on early development Basil plants were grown in plates to seedling stage (2 weeks) within a light cabinet under monochromatic blue and red light (B_{100} and R_{100}, respectively), and three dichromatic mixes ($B_{75}R_{25}$, $B_{50}R_{50}$ and $B_{25}R_{75}$).

Establishment. A seedling was defined as established if actively growing at the time of measurement, and overall rates were good (80 % +), with the highest in the medium intensity treatment (Table 3.8). Between B_{100} and R_{100}, establishment was higher under RL only, but establishment was lower with increasing RL in the dichromatic treatments averaged across the intensities.

Intensity	B_{100}	$B_{75}R_{25}$	$B_{50}R_{50}$	$B_{25}R_{75}$	R_{100}
Low	93%	88%	85%	83%	92%
Medium	98%	92%	90%	90%	98%
High	98%	95%	83%	92%	97%

Table 3.8 Establishment success of Basil seedling grown under different intensities of light qualities. Seedlings were grown for two weeks in plates within a controlled environment cabinet (16/8 h L/D cycle at 22/20 °C day/night temperature). For intensities see Table 3.4.

Hypocotyl length. A two-way ANOVA found that light quality significantly affected hypocotyl lengths ($p < 0.001$). As shown in Figure 3.2a, both R_{100} and B_{100} grew longer seedlings, but only the monochromatic RL condition significantly increased in height with intensity (Appendix Figure 6.1.9). B_{100} and the dichromatic treatments were similar in heights and the response to intensity but overall the effect of intensity was marginally significant ($p < 0.05$). It is expected that BL will shorten hypocotyls (Folta and Spalding, 2001), and this response was observed, as B_{100} is shorter than R_{100}, and $B_{75}R_{25}$ is shorter than $B_{50}R_{50}$ and $B_{25}R_{75}$. Despite this, seedlings under B_{100} and R_{100} were significantly taller than seedlings under dichromatic light qualities, which is an undesirable trait resembling etiolation and therefore indicates a suboptimal light environment. Alternatively, this could be accounted for by seed age as the monochromatic experiments were performed a significant period earlier than the dichromatic experiments, and a trend can be seen whereby the monochromatic seedlings outperform the dichromatic treated seedlings in all the parameters measured.

Fresh weight. Figure 3.2b shows a similar effect of light quality on fresh weight ($p < 0.001$). The greatest FW was found in R_{100}, followed by B_{100}. The fresh weight significantly increased with intensity in the monochromatic conditions ($p < 0.05$), and also in the dichromatic conditions, albeit marginally. Although R_{100} resulted in the highest FW across all

intensity treatments, seedlings under B_{100} showed the greatest gains in FW with increasing intensity. Intensity was a significant factor across all light qualities tested ($p < 0.001$).

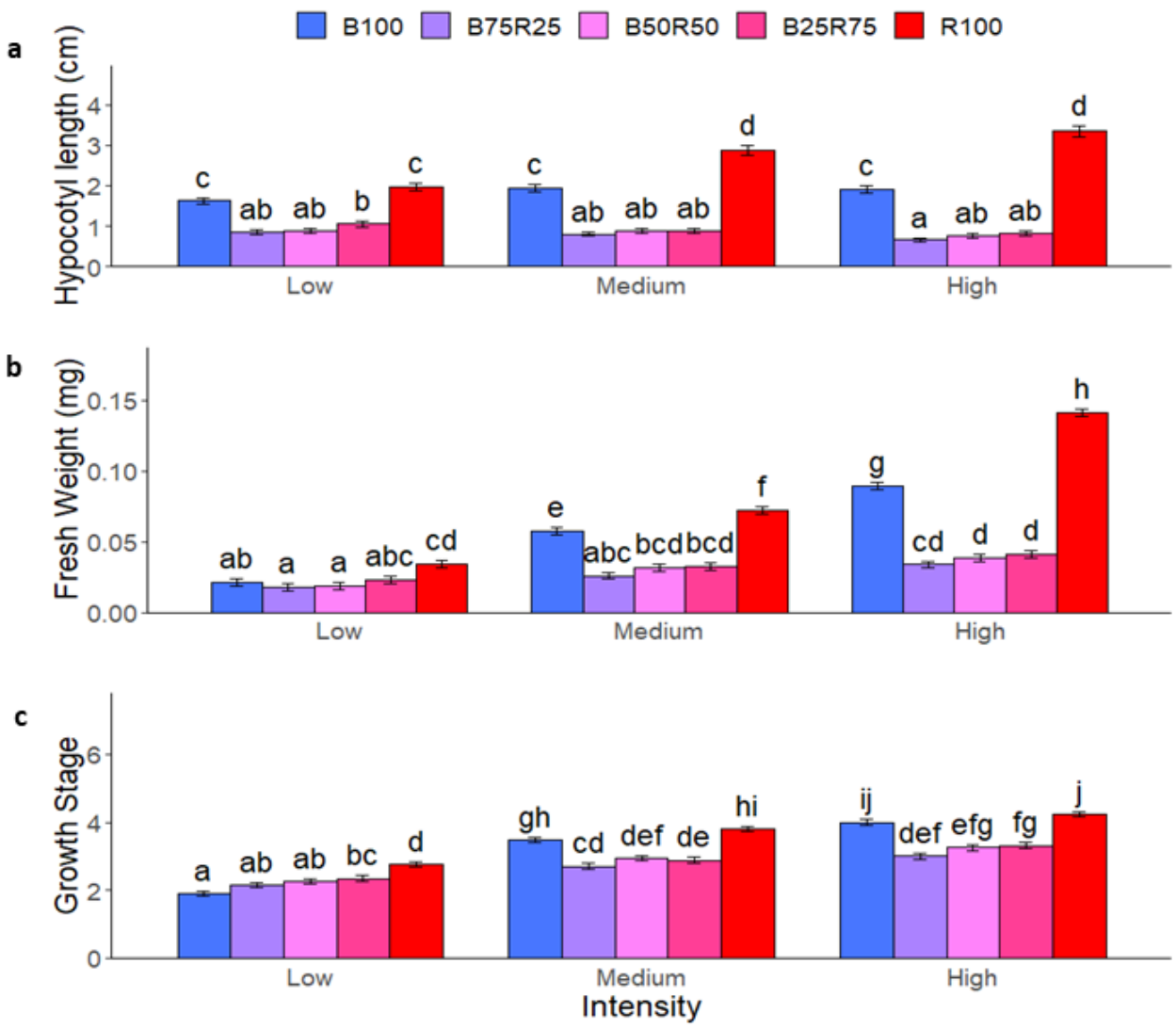


Figure 3.2 Growth of seedling basil plants under blue and red light mixes. Measurements of (a) hypocotyl length (cm), (b) fresh weight of the shoot (g), and (c) the growth stage of basil seedlings grown for 14 days under (i) 100% blue light (B_{100}), (ii) 75% blue and 25% red ($B_{75}R_{25}$), (iii) $B_{50}R_{50}$, (iv) $B_{25}R_{75}$, and (v) R_{100}, under low, medium and high intensities (from left to right). The intensities used (Table 3.4) and growth stage key (Table 3.5) can be found in the methods. Error bars represent standard error (n = 60). Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

Development. Seedling development was measured by assigning a numerical value to defined growth stages as outlined in Table 3.5. As shown in Figure 3.2c, seedlings were more developed with increasing light intensity ($p < 0.001$). Red light produced the most developed seedlings across the light conditions tested. However, although B_{100} produced the least developed seedlings under low light intensity, it was not significantly different from R_{100} at the highest light intensity. Therefore, results obtained show that light quality was a significant factor in the development of Basil seedlings ($p < 0.001$). In the medium and high intensities, both B_{100} and R_{100} seedlings were more developed than the dichromatic conditions, which could again be attributed to the loss of vigour with seed age in the dichromatic tests as described for seedling height. However, the trends within the dichromatic conditions across the intensities also showed greater seedling development with higher RL content indicating that a greater quantity of RL aids in the growth and development of Basil seedlings. However this could be confounded by the additional photons supplied in the RL treatment compared to the BL treatment.

Overall, these data show that both light quality and intensity affect seedling growth and development (interaction for all parameters, $p < 0.001$). R_{100} is best for producing the "heaviest" seedlings. However, they are less developed than seedlings grown under blue light as assessed by leaf emergence (Table 3.5). Therefore, the extra FW gains are likely from the increased hypocotyl extension under R_{100} and so do not necessarily create desirable seedling growth (Figure 3.2). Between the dichromatic treatments, only slight differences were found, however, treatments with greater RL performed better than those with more BL, growing seedlings with similar height but greater fresh weight and more advanced growth stages.

To explore whether light quality could differentially affect growth of Basil across development stages, Basil plants were grown to maturity (4 weeks) in a hydroponic flood and drain system under the same monochromatic and dichromatic light conditions (B_{100}, $B_{75}R_{25}$, $B_{50}R_{50}$, $B_{25}R_{75}$, R_{100}). Experimental repeats with the mature plants were plotted separately (Figure 3.3). Looking at the data overall, the two-way ANOVA found a significant effect of the experimental repeats on fresh weight ($p < 0.05$), leaf number ($p < 0.001$), leaf area ($p < 0.001$), and leaf thickness ($p < 0.001$). There was some variation seen between repeats especially with $B_{50}R_{50}$, which displays a significant elevation of growth across most of the observed physiological parameters in the first experimental repeat. The plants in this repeat were treated following

the same protocol, however it is possible that the environmental parameters such as temperature could have varied as this was not measured at the level of the plants constantly. Therefore, the statistical significance between repeats is likely due to the inconsistent results in the $B_{50}R_{50}$ experiments, especially as in most cases the inter-experimental variation within the other light treatments was not significant by a Sidak post-hoc test.

Upon harvest, physiological measurements were gathered on mature plants according to Section 3.2.6. The undesirable stem elongation under R_{100} in seedlings continued into adult plants, as can be seen in Figure 3.3a, where the $B_{50}R_{50}$ and R_{100} light qualities recorded significantly longer stem lengths ($p < 0.01$). Similarly, FW and DW shows significant discrepancies between light qualities ($p < 0.001$; Figure 3.3b, Appendix Table 6.1.3). The effect of light treatment on FW is difficult to determine due to the high variation between experimental repeats. For example, results show the highest accumulation of biomass in the first repeat of $B_{50}R_{50}$, and the lowest in the second repeat. Most interestingly the lowest FW also had a disproportionately high DW percentage, indicating that the plant cell in this trial were not turgid. $B_{75}R_{25}$ is another light quality with significant variations between independent experimental repeats; the first is similar to B_{100} and the second to $B_{25}R_{75}$, again making it difficult to ascertain the effect of the light condition. Whilst statistically significant differences were not found, results point at plants in $B_{75}R_{25}$ having the capacity to accumulate biomass equally as well as in $B_{25}R_{75}$, which also grew consistently heavier than B_{100} and R_{100}

In the mature plants there was no observable difference in leaf number, with exception of the aforementioned outlier results of $B_{50}R_{50}$, which created a significant effect of light treatment ($p < 0.001$; Figure 3.3c). However, differences between light qualities in leaf area ($p < 0.001$; Figure 3.3d) were observed, with the greatest areas found under $B_{50}R_{50}$ and $B_{25}R_{75}$. When combined, these results show that the reduced leaf areas seen in the other treatments are a result of smaller leaves. For example, plants in the second repeat of $B_{50}R_{50}$ in Figure 3.3d had the same leaf number as plants in other light treatments, however the leaf area measurement was the lowest of all treatments, indicating that the leaves were less expanded and smaller overall. Other examples of this effect of light quality on leaf area include B_{100} and $B_{75}R_{25}$. The trends seen between individual experimental repeats in leaf area strongly reflects those seen in fresh weight and stem length, suggesting a correlation between these factors. This is expected as FW is a product of the stem and leaves, both size and number. However, the dry

weight fraction does not follow the same trend with the highest DW% found in repeat 2 of $B_{50}R_{50}$, and the lowest DW% found in $B_{25}R_{75}$ (Appendix Table 6.1.3).

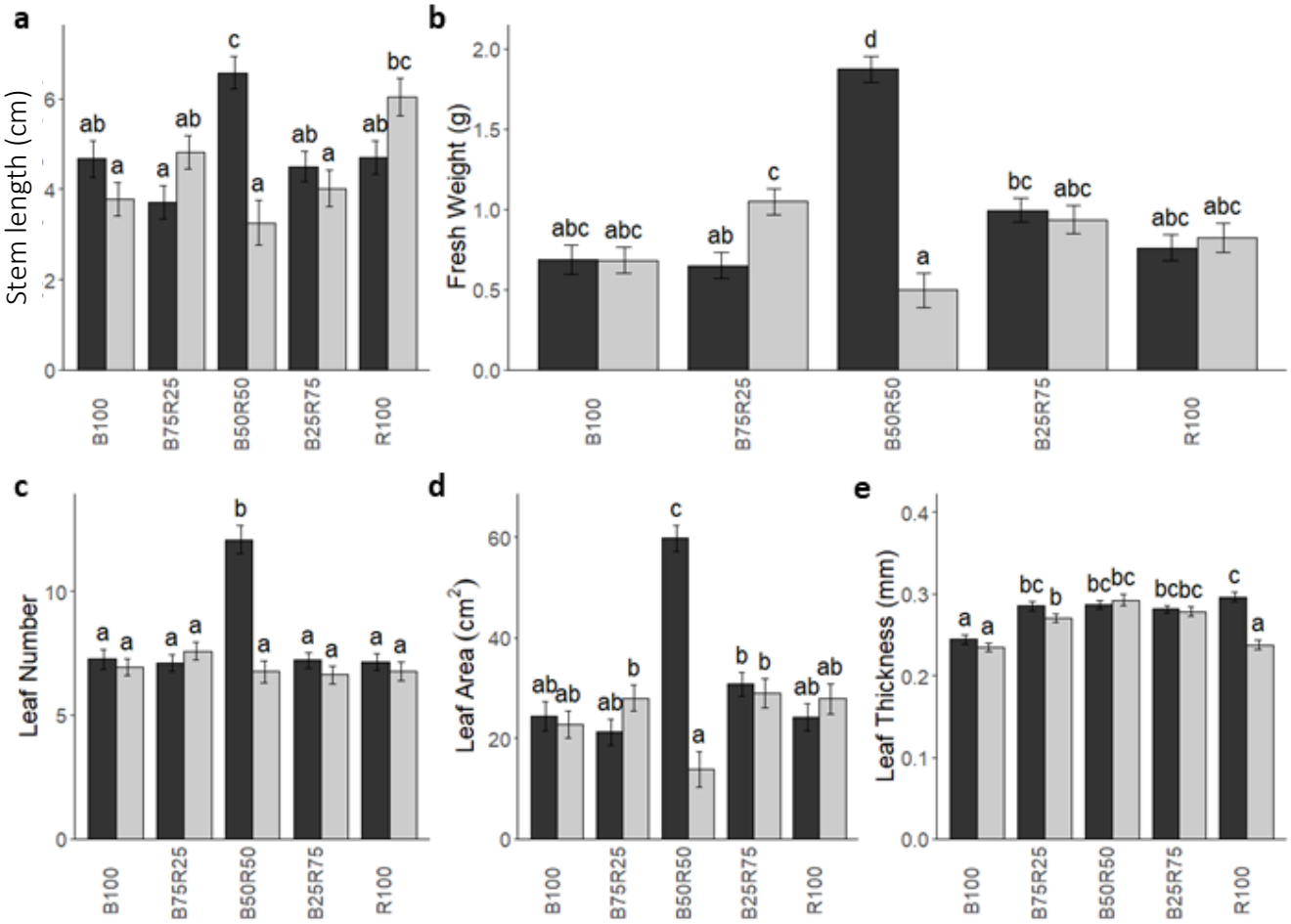


Figure 3.3 Mono- and dichromatic light treatments on the growth of adult basil plants. Physiological measurements including (a) stem length (cm), (b) fresh weight (g), (c) total leaf no, (d) leaf area (cm^2), and (e) leaf thickness (mm) of mature basil plants grown for 4 weeks under 100% blue light (B_{100}), 75% blue and 25% red light ($B_{75}R_{25}$), $B_{50}R_{50}$, $B_{25}R_{75}$ and R_{100} light conditions (150 $\mu mol.m^{-2}.s^{-1}$; 16/8 L/D). Dark grey bars display estimated marginal means from the first experimental repeat, and light grey bars for the second. Error bars represent standard error. Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p< 0.05$). Data that share a letter are not statistically significant.

Leaf thickness is an important quality marker for growers as thicker leaves are less likely to become damaged upon packaging and therefore last better on shop shelves. Light quality has

shown to have an effect on leaf thickness, with blue light promoting thicker leaves (Zheng and van Labeke, 2017). However, within the presented experiment, results varied from the published data and the thinnest leaves were found in plants grown under B_{100} (Figure 3.3e), with little variation between the dichromatic treatments, and variable thickness seen in R_{100} ($p < 0.001$). Therefore, for the Basil variety and plant age tested, monochromatic blue light is not suitable for generating thicker leaf growth. However, adding a relatively small amount of red light (25%) raises the thickness to that comparable to other light qualities.

The results on adult Basil plants show that light quality can significantly impact important growth parameters. Due to a significant interaction between light quality and replica number for all parameters tested ($p < 0.01$) these results also show the variability of biological responses with environmental inputs, demonstrate the importance of constantly monitoring all environmental parameters that can impact growth. This is highlighted by the outlier results obtained in experimental repeat 1 for $B_{50}R_{50,}$ on which we cannot be certain whether the result obtained is attributed to the lighting condition or another environmental factor that we did not monitor, such as the local temperature. When these results are not taken into account, the best performing light quality for the growth of Basil is $B_{25}R_{75}$. This shows that while RL may be the dominant wavelength, BL is necessary to optimize growth parameters in Basil, resulting in good height, biomass accumulation and leaf number of the plants.

3.3.3. Supplementation with UV-B and FR Wavelengths and Their Effects on Basil Growth

Whilst the PAR wavelength range supplies the photosynthetic energy required for growth, UV-B and FR wavelengths found either side of the PAR range are more renowned for their photomorphogenic effects. UV-B wavelengths trigger metabolic changes to stunt growth (Favory et al., 2009a), whereas FR activates shade avoidance responses to promote elongation (Franklin and Whitelam, 2005). These effects were explored on mature Basil grown hydroponically under horticultural LED lighting (GE Arize) supplied at 150 $\mu mol.m^{-2}.s^{-1}$. The supplementary UV-B treatment was delivered by fluorescent bulbs (6.48 $kJ.m^{-2}.day^{-1}$ for 6 days pre-harvest). To achieve the FR treatment (R:FR ~ 1.0) the horticultural lights were filtered in addition to supplementation with FR LEDs in the final 10 days of growth. Spectral breakdowns can be found in Appendix Table 6.1.2. Note that due to a malfunction, the repeat experiment

of –UV-B and +UV-B treatments was affected by drought and were therefore not included in this analysis. Only one repeat of the FR treatment was performed due to time constraints. Despite data being limited to a single experimental replica, a one-way ANOVA found significant effect of the treatments on height, fresh weight, dry weight, leaf area, leaf thickness ($p < 0.001$), and leaf number ($p < 0.01$) (Figure 3.4).

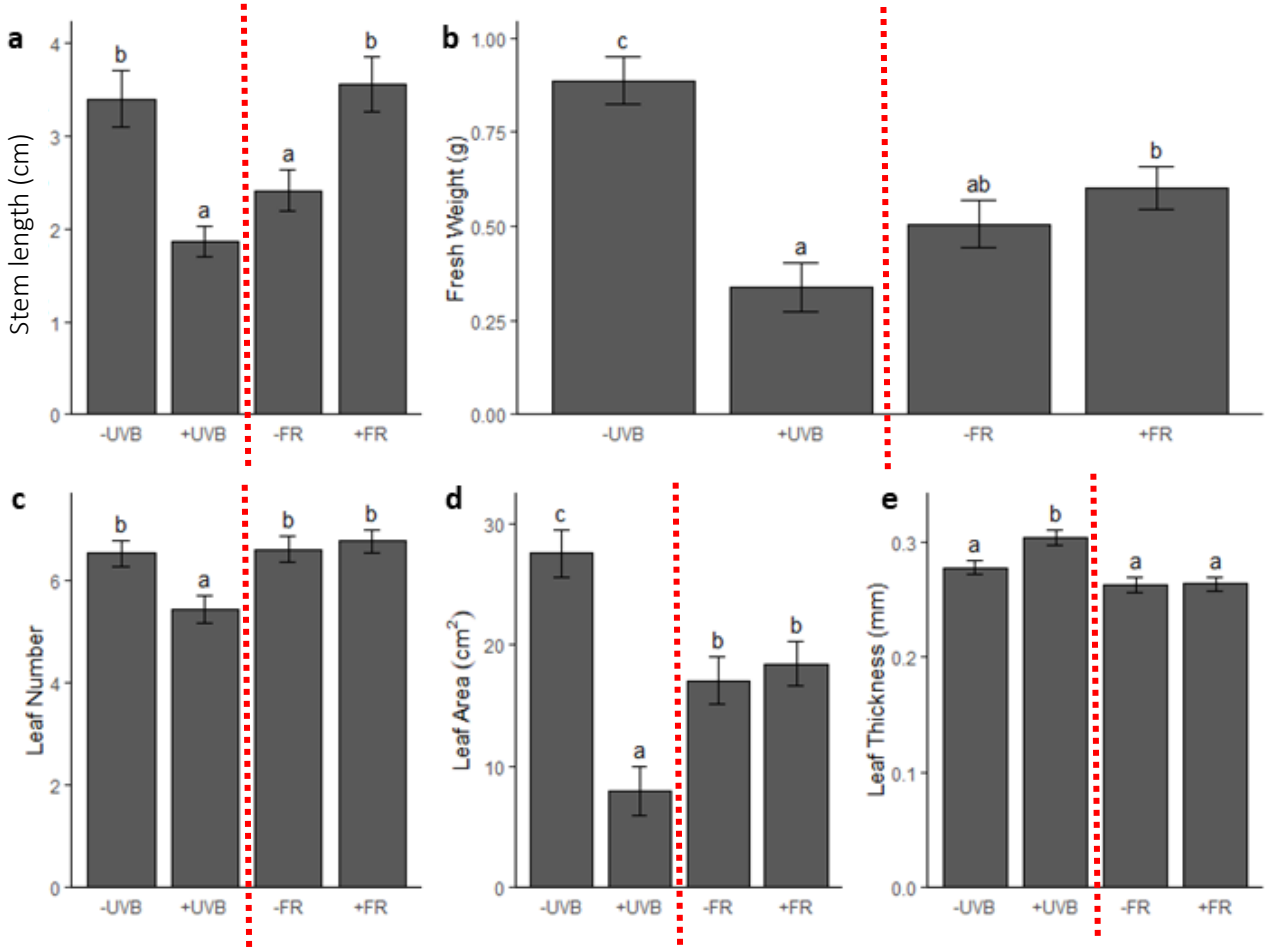


Figure 3.4 Basil growth under supplemental UV-B and FR. Mature basil under GE lighting (150 µmol.m^{-2}.s^{-1}) supplemented with UV-B fluorescent tubes (6.48 kJ m^{-2} day^{-1} delivered as 4 h treatment at midday) for 7 days or filtered (SC50, LEE Filters) GE lighting (150 µmol.m^{-2}.s^{-1}) supplemented with FR LEDs (R: FR =1) for 10 days before harvest. Data shows physiological measurements of (a) stem length (cm), (b) fresh weight (g), (c) total leaf no, (d) leaf area (cm^2), and (e) leaf thickness (mm). Error bars represent standard error. Statistical analysis was performed with a one-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p< 0.05$). Data that share a letter are not statistically significant.

The UV-B light results show evidence of the classical stem length UV responses (Jenkins, 2009; Ulm and Jenkins, 2015) (Figure 3.4); plants with UV-B were significantly shorter and lighter than control plants. However, increased branching and effects on leaf development, another reported effect of UV-B lighting (Teramura and Sullivan, 1994), was seen and in fact leaf count was lower under UV-B supplementation. Despite a small difference between leaf numbers, a large difference was seen in leaf areas, indicating that leaves were smaller with UV-B treatment. Upon harvesting it was observed that the UV-B treated leaves were thicker (Figure 3.4e). Observations also included an upward curl of leaves treated with UV-B, compared to a normal downwards cup of the leaf (Appendix Figure 6.1.9).

FR supplementation in Basil also demonstrates some classical responses (Franklin and Whitelam, 2005), primarily significant elongation of the stem (Figure 3.4a) and a marginal increase in FW and DW % (Appendix Table 6.1.3). In the mature plants little difference was found in leaf number, leaf area and leaf thickness under FR supplementation, whereas typical FR responses would result in a reduction in these parameters. However, these responses were reported in significantly lower R:FR ratios (< 0.1) (Franklin and Whitelam, 2005), whereas my FR treatment was comparatively mild (R:FR ~1).

These results indicate that supplementation with wavelengths outside of the PAR range has impacts across plant growth and development. In my experimental set up, UV-B specifically shortened stems, reduced leaf area and thickened leaves, and FR promoted elongation of stems.

3.3.4. The Impact of Spectral Design on Basil Growth in a Commercial Hydroponic System

Due to the commercial applications of this research, it is suitable to document the effects of current commercially available horticultural spectra upon the growth of Basil grown in a hydroponic system comparable to that found in the latest vertical farms. For the comparison of commercial LEDs with different horticultural spectral qualities, May Barn Consultancy Ltd. grew Basil plants in their hi-spec flood-and-drain hydroponic system under four different LED fixtures: HSP-HB ($B_{30}G_{33}R_{37}$), HSIP ($B_{15}G_{16}R_{69}$) and GE-HB ($B_{40}G_{1}R_{59}$), and AP673L ($B_{11}G_{21}R_{68}$). The spectral quality of these fixtures were unique to each other containing different R:B, B:G

and R:FR ratios. Full spectral breakdowns are available in Appendix Table 6.1.2. It is important to note that the AP673L LEDs were delivering ~100 µmol m^{-2} s^{-1} – a lower PPFD than the other luminaires.

Results obtained (Figure 3.5a) show that the height of the plants was effected by the lighting treatments ($p < 0.001$); all groups were found to be significantly different by Sidak post-hoc. The tallest plants were grown in under HSIP, followed by HSP-HB then GE-HB. The decrease in height in HSP-HB and GE-HB is likely accounted for by the spectra, as both contain higher proportions of blue light, which is widely demonstrated to inhibit stem growth (Lin et al., 1998). Meanwhile, the shortest plants were grown under the lowest intensity spectra, AP673L, which is likely attributed to the lack of light energy for growth.

As shown in Figure 3.5b, the fresh weight and dry weight of the plants was also dependent on the lighting environment ($p < 0.001$). The HSP-HB performed best for fresh weight, with similar results for HSIP and GE-HB, and a significant drop in weight under the AP673L. This is likely due to the difference in lighting intensity, which was lowest in AP673L. However, the distribution of the measurements suggests that the Heliospectra lighting tended towards producing heavier plants at the higher end of the observed range (Figure 3.5b). This is interesting as the only spectral similarities between them are the proportion of BL and GL (Appendix Table 6.1.2), which has been associated with increases in biomass for Basil (Dou et al., 2019b).

The trend in leaf number reflected that of fresh weight, and was again significantly affected by the lighting treatment ($p < 0.001$) (Figure 3.5c). Interestingly the plants with the largest number of leaves was found under GE-HB, however the spectra that produced the highest number of leaves most consistently was HSP-HB. It is possible that the high blue aspects of these spectra could have stimulated increased leaf production; however, a similar leaf number to GE-HB was seen in HSIP. Again the spectra with the worst performance was the AP673L however this was the spectra with the lowest intensity (Figure 3.5c), which supports a relevant role of light intensity to match with light quality effects.

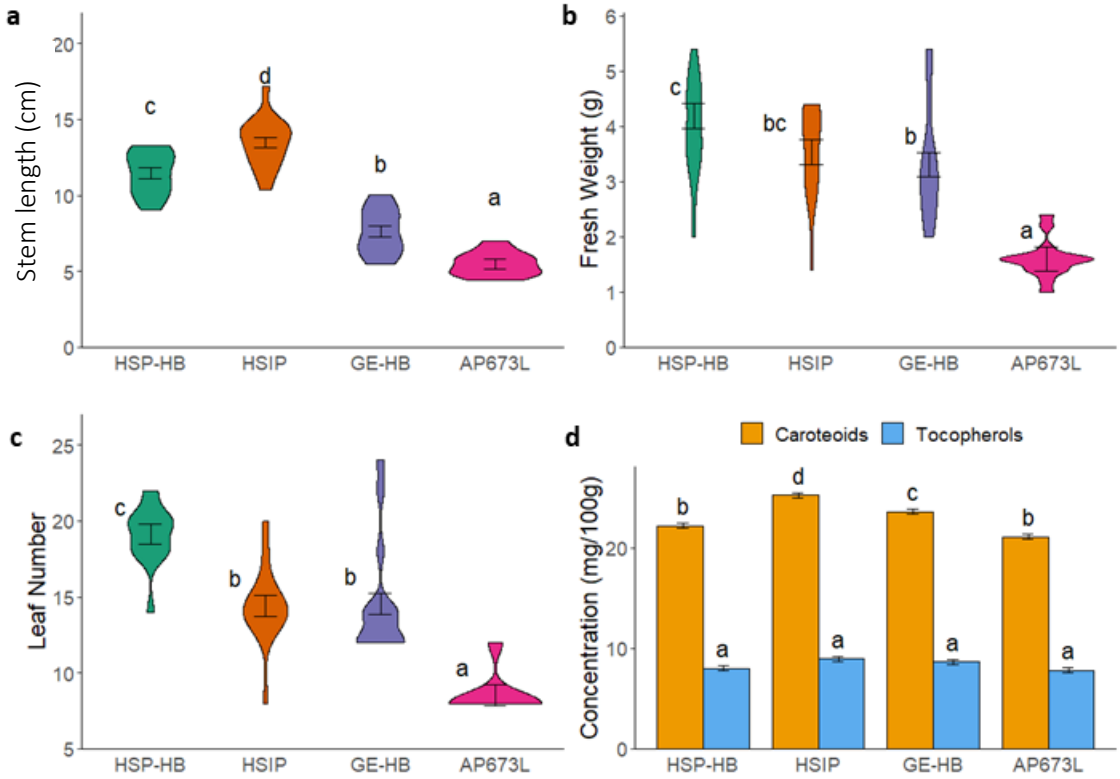


Figure 3.5 Growth and quality of Basil grown in a hydroponic system at May Barn Consultancy Ltd. Mature basil under four commercial lighting spectra: Heliospectra Siera Propagation High Blue (HSP-HB), Heliospectra Siera Indoor Production (HSIP), Current by GE Arize Lynk Vegetative High Blue (GE-HB) and Valoya L28 AP673L (AP673L). Intensity and spectral breakdowns can be found in Appendix Table 6.1.2. Data shows physiological measurements of (a) stem length (cm); (b) fresh weight (g); (c) total leaf no; and (d) UV-spectrophotometric measurements of carotenoid and tocopherol content (mg/100g DW). Error bars represent standard error. Statistical analysis was performed with a one-way ANOVA (a-c) and a two-way ANOVA (d). Statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

3.3.5. The Effect of Spectral Wavelengths on Phytonutrient MEP Pathway Derivatives: Carotenoids and Tocopherols

To properly assess the impact of spectra on Basil nutritional quality, quantification of phytonutrient content of antioxidants of MEP-isoprenoid origin, carotenoids and tocopherols, was determined by UV-spectrophotometry and HPLC analysis. Sample extractions for UV-

spectrophotometer analysis and HPLC analysis were performed following the same protocol. However, spectrophotometry provides quantification of the total content whereas HPLC is a more accurate method to separate individual molecules. Selected metabolites for analysis included Lutein and β-carotene representing the two branches of the carotenoid biosynthesis pathway (Figure 1.3), and of the four tocopherols produced in plants, α-tocopherol was selected as it is the most powerful and abundant tocopherol in green tissues (Kamal-Eldin and Appelqvist, 1996).

Figure 3.5d presents UV-spectrophotometer quantification analysis of carotenoid and tocopherol content in Basil plants grown under four commercial LED lighting fixtures. No difference was found between treatments for tocopherols by Sidak post-hoc test. However, carotenoid content was greatest under HSIP, followed by GE-HB, with HSP-HB and AP673L showing no statistical difference. This demonstrates the ability of spectra to significantly influence the carotenoid content of the resultant crop ($p < 0.001$). These effects can be analysed based on the proportions of wavelengths present in the lighting spectra (see Appendix Table 6.1.2). However, this is complicated by the presence of multiple wavebands (R, B, G, and FR) and deeper understanding cannot be achieved without isolating the spectral inputs.

To assign causal relationship to the input of red and blue wavelengths on the content of carotenoids and tocopherols, UV-spectophotometer and HPLC measurements were undertaken on plants grown under monochromatic and dichromatic light qualities. The measurements performed with a UV-spectrophotometer showed an interaction between phytonutrient content and the light quality ($p < 0.001$) (Figure 3.6a). Tocopherol content was observably elevated in $B_{75}R_{25}$ treatment, although not found to be significantly different by Sidak post-hoc test. Carotenoid content was also elevated in $B_{75}R_{25}$ and $B_{50}R_{50}$. The lowest average content was found in B_{100} (Figure 3.6a). The UV-spectrophotometer results point at a BL dominant spectra for driving carotenoid and tocopherol accumulation.

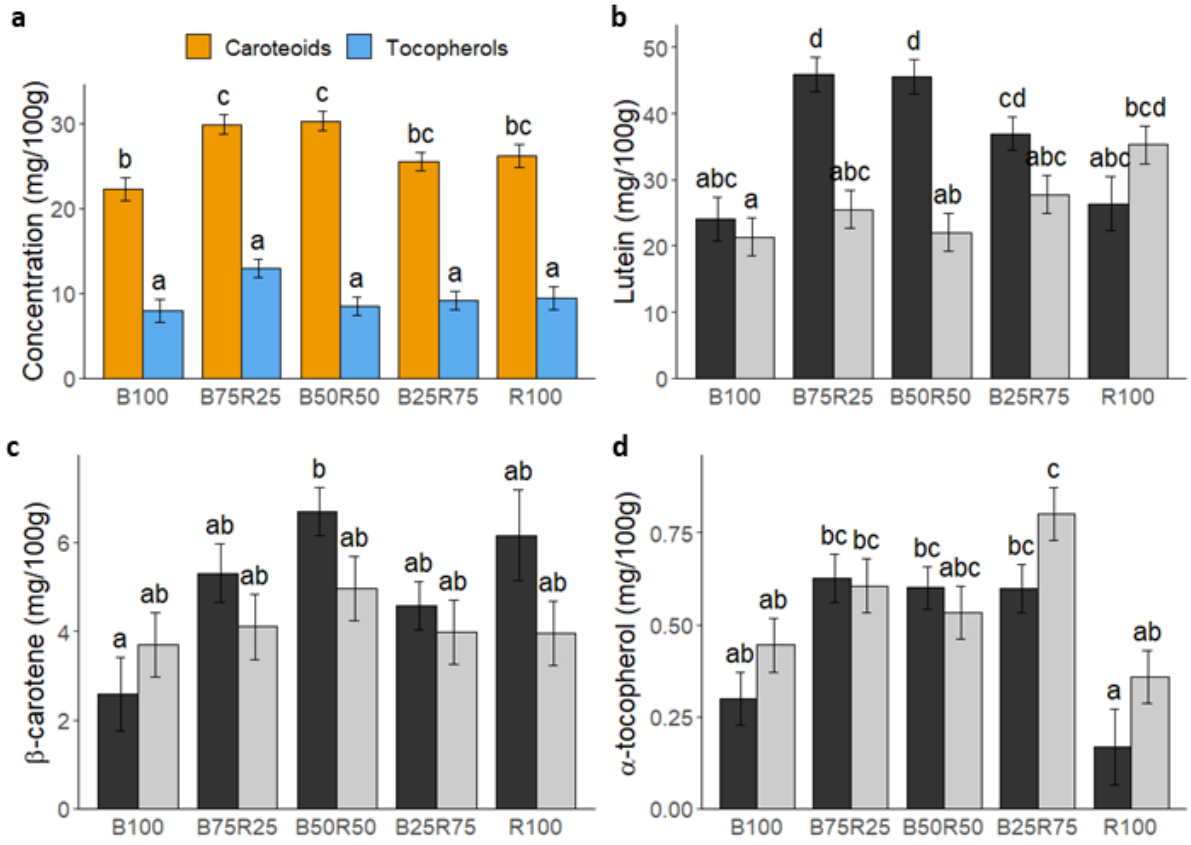


Figure 3.6 Phytonutrient quantification in Basil grown under different ratios of red and blue light. Mature hydroponically-grown Basil leaves were measured by (a) UV-spectrophometer for total carotenoids and tocopherols; and HPLC for quantification of (b) lutein, (c) β-carotene, and (d) α-tocopherol (mg/100g DW). Light regimes are the same as described in Figure 3.3. Dark grey bars display estimated marginal means from the first experi mental replica, and light grey bars for the second replica (b – d). Error bars represent standard error (n = 4). Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a Sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

The HPLC measurements were able to evaluate the light effects on individual compounds. The lighting treatments significantly influenced the content of lutein ($p < 0.001$; Figure 3.6b). Overall, the first experimental repeat showed similar trends to the UV-spec, with the highest phytonutrient content in treatments $B_{75}R_{25}$ and $B_{50}R_{50}$. B_{100} again had the lowest phytonutrient content, which was consistent across repeats. However, in the secondary repeat R_{100} showed comparable lutein content to $B_{25}R_{75}$, and was higher than the levels recorded for $B_{75}R_{25}$ and

$B_{50}R_{50}$. This indicates that lutein is responsive to RL. The combination of blue and red wavelengths is also a significant factor for β-carotene content in Basil ($p < 0.01$; Figure 3.6c). On average, B_{100} and $B_{50}R_{50}$ were the least and most effective treatment for β-carotene, respectively. R_{100} also performed comparatively well to $B_{50}R_{50}$ in the first experimental repeat. However, significant variation between the individual repeats was seen for Lutein ($p < 0.001$) and β-carotene ($p < 0.05$), which was similar to the variations observed in the growth measurements and could therefore be related to plant growth differences. Alternatively, the carotenoids are light liable which, in addition to their role as antioxidants, makes them prone to rapid degradation. Despite, following best practice protocols of protection from light contamination and storage in -80 °C, the differences between replicates could have occurred during storage or transportation to Leeds University for analysis.

On the other hand, α-tocopherol content remained consistent across independent replicate experiments ($p < 0.1$). The monochromatic treatments B_{100} and R_{100} resulted in the lowest concentrations of α-tocopherol, and whilst not statistically significantly different, the means for B_{100} were slightly higher than R_{100} (Figure 3.6d). Across the dichromatic mixes, α-tocopherol levels were relatively equal, with a small spike in the second replica of $B_{25}R_{75}$. Nonetheless, the light environment can significantly impact on the α-tocopherol content of Basil ($p < 0.001$). These results support a role of both BL and RL in the production of tocopherols, more so RL in the $B_{25}R_{75}$ condition, and importantly the unsuitability of monochromatic light.

UV-B reportedly acts to increase nutritional content of plants (Nájera et al., 2022), whilst FR can reduce phytonutrients (Bou-Torrent et al., 2015). Therefore, to evaluate if this trend applies to Basil, the nutritional content of carotenoids and tocopherols was measured with and without moderate supplemental UV-B and FR light. There was a significant effect of these treatments on the resulting nutritional content when measured by UV-spectrophotometry ($p < 0.001$) (Figure 3.7a). However, for UV-B the result was opposite the expected trend with carotenoid content decreasing with additional UV-B (Figure 3.7a). When measured by HPLC, UV-B supplementation showed a decrease in lutein content with a small concurrent increase in β-carotene, indicating a shift from the β, ε branch of the carotenoid biosynthesis pathway to the β, β branch (see Section 1.2.2.1.; Figure 3.7b). However, these shifts in lutein and β-carotene concentrations between UV-B treatments was not found to be significant by sidak post hoc test. For tocopherol content, UV-spectrophotometry indicated a decrease in overall

tocopherol content (Figure 3.7c). However, the more specific measurement of α-tocopherol content by HPLC showed a small increase with additional UV-B (Figure 3.7c). Therefore, whilst none of these differences registered as significant, it does imply, in a similar way to the carotenoids, that UV-B treatment decreases overall tocopherol content but triggers a shift in tocopherol production to α-tocopherol, the most powerful antioxidant. On the other hand, additional FR was associated with a significant decrease in total carotenoids (Figure 3.7a). Similarly, total tocopherol content also decreased with supplementary FR. Due to time constraints, HPLC analysis was not performed on these samples.

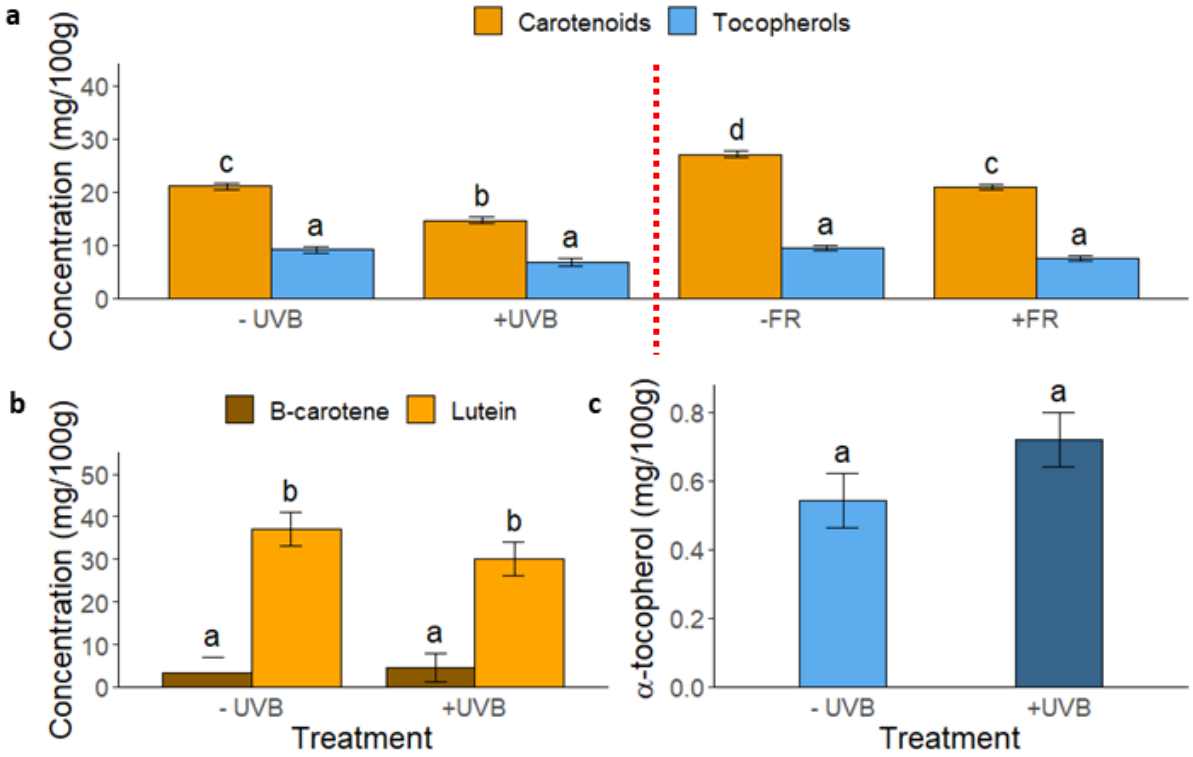


Figure 3.7 Quantification of carotenoids and tocopherols in Basil grown under supplementary wavelengths of UV-B and FR. Four week old hydroponically-grown Basil leaves (n = 4) were measured by (a) UV-spectrophometer for total carotenoids and tocopherols; and HPLC for quantification of (b) lutein and b-carotene, and (c) a-tocopherol (mg/100g DW). Light regimes were the same as described in Figure 3.4. Error bars represent standard error. Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

Similar to light quality, environmental fluctuation can also effect phytonutrient content (Csambalik et al., 2019). When comparing plants grown in compost conditions in GH and more closely controlled Sn, there was no detectable statistical difference between these conditions and the nutritional content analysed with both UV-spectrophotometry and HPLC (Figure 3.8). However, the mean value of carotenoid content was greater in the controlled conditions of Sn. HPLC analysis revealed lutein specifically was increased under Sn conditions (Figure 3.8b). Interestingly, α-tocopherol content was elevated in GH plants in comparison to Sn plants when analysed by HPLC (Figure 3.8c), whereas no difference was detected with the UV-

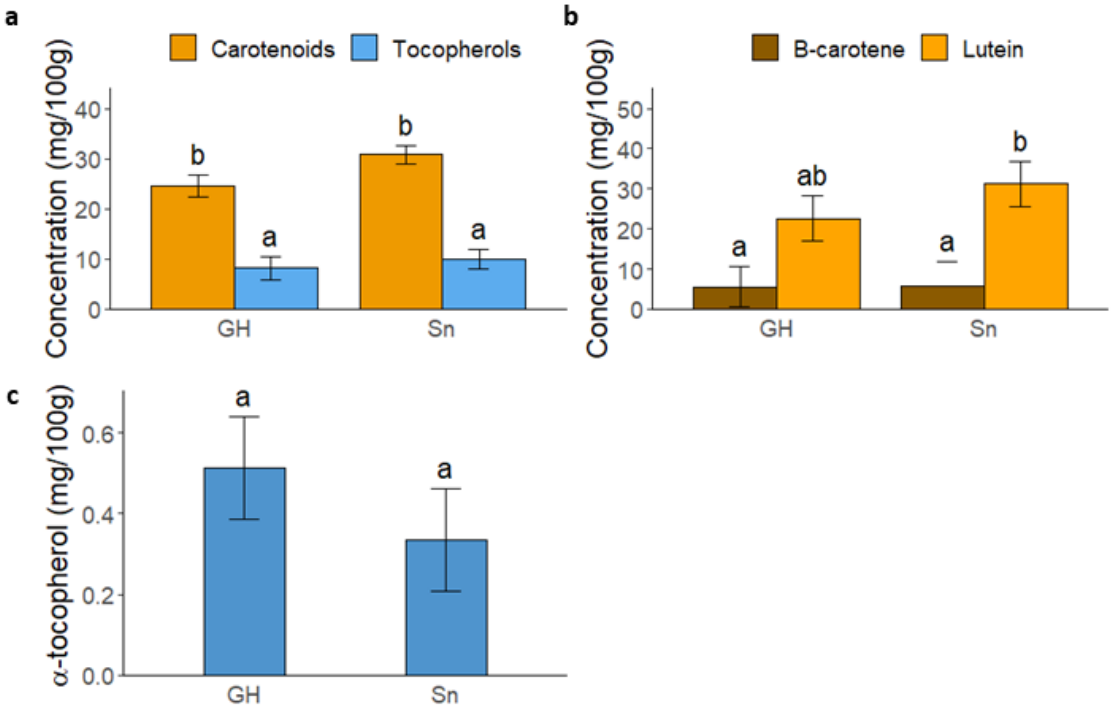


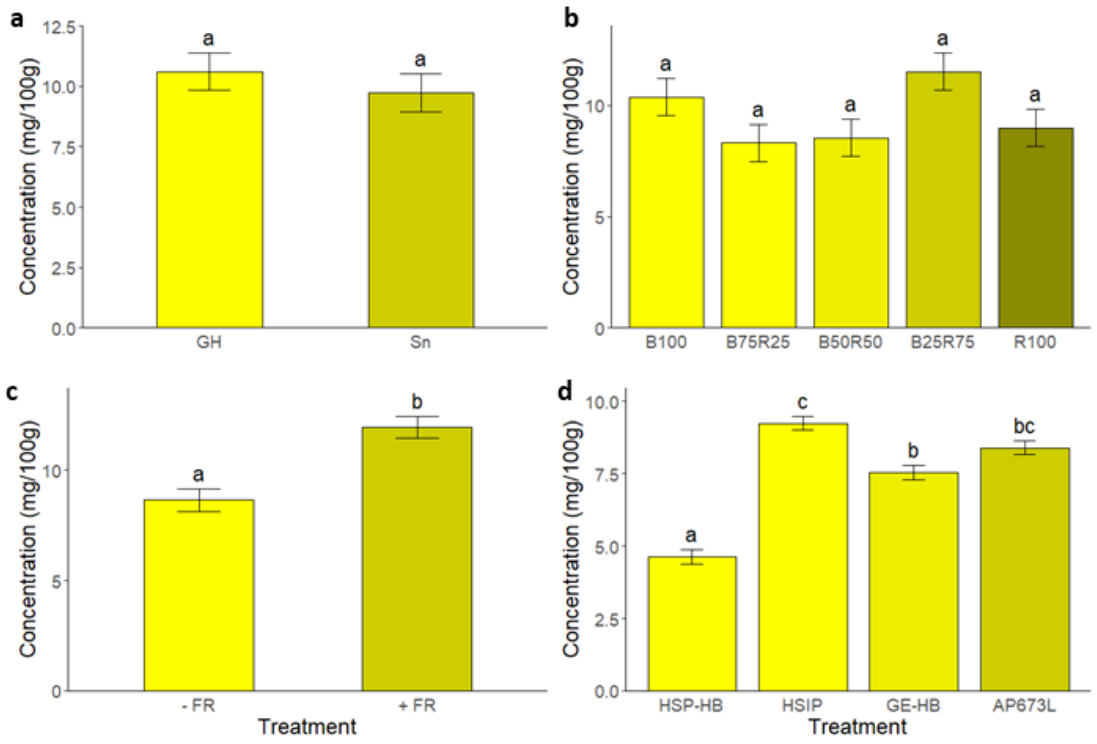
Figure 3.8 Nutritional content of Basil grown in conventional controlled environment conditions. Basil was grown in a glasshouse (GH) or Snijder cabinet (Sn) to maturity and sampled for (a) UV-spectrophotometer analysis of total carotenoids and tocopherol content, and HPLC quantification of (b) carotenoids β-carotene and lutein, and (c) α-tocopherol (mg/100g DW). Light regimes were the same as described in Figure 3.1. Error bars represent standard error. Statistical analysis was performed with a two-way ANOVA (a, b) and one-way ANOVA (c) (n= 4). Statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p< 0.05$). Data that share a letter are not statistically significant.

spectrophotometer. These results indicate that carotenoids and tocopherols react differently to environmental stability, with carotenoids accumulating with increased stability and tocopherols with decreased stability.

3.3.6. Ascorbic Acid Content

AA in the reduced form ascorbate has an overarching role to recycle the oxidised xanthophylls and tocopherols thereby restoring their function as antioxidants (Section 1.2.2.3) (Akram et al., 2017). As such, tAA is a major antioxidant group in leafy vegetables including Basil (Table 3.1) and is a target for quality improvement (Min et al., 2021). tAA of mature Basil was measured by HPLC under different growth conditions and light qualities and the results analysed by one-way ANOVA and sidak post-hoc. No difference in tAA content was found between GH plants compared to Sn (Figure 3.9a). Similarly, when comparing the mono-and dichromatic light qualities, no statistically significant difference was found (Figure 3.9b). However, the observed trend reveals that $B_{25}R_{75}$ may induce elevated levels of tAA. Of the supplementary treatments tested, only FR could be measured for tAA content. The analysis reveals a significant increase in tAA under FR supplementation ($p < 0.001$) (Figure 3.9c), an unexpected result as tAA content reduces under lower R:FR in beans (Bartoli et al., 2009). Finally, there was also significant variation found under the four commercial spectra ($p < 0.01$) (Figure 3.9d). HSIP contained the highest levels of tAA, followed by AP673L. The lowest content of tAA was found in HSP-HB. Interestingly, spectra with higher red content in general accumulated more tAA, which may indicate a predominance of phytochrome in the regulatory production of ascorbic acid in Basil.

Figure 3.9 Total ascorbic acid content of Basil grown under various spectral qualities. HPLC measurements of total ascorbic acid (mg/100g DW) in 4 week-old Basil leaves grown (a) in glasshouse (GH) or Snijder cabinet (Sn), (b) hydroponically under different ratios of blue and red light qualities, (c) supplemented with or without FR light, and (d) under four commercial LED lighting spectra. Light regimes are the same as described in Figure 3.1, 3.3, 3.4, and 3.5, respectively. Error bars represent standard error (n = 3). Statistical analysis was performed with a one-way ANOVA. Statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p< 0.05$). Data that share a letter are not statistically significant.



3.3.7. Photosynthetic Pigments are Measurably Impacted by Light Quality

A pigment assay was performed to measure the content of both forms of chlorophyll and the total carotenoid content of the samples collected under several light qualities. Light energy is harvested for photosynthesis via chlorophylls (Chl *a* and Chl *b*) and carotenoids held in the core complexes and peripheral antennae of PSI and PSII, which vary in pigment composition and therefore absorption (see Section 1.3.1). Furthermore, the number of PSI and PSII complexes can be altered dependent on the surrounding light quality to optimise light absorption and photosynthetic efficiency, or over shorter time scales the association of main light harvesting complex LHCII can be adjusted (Wientjes et al., 2017). These shifts that can be measured as the ratio of Chl *a* to Chl *b* (or PSI/PSII chlorophyll ratio). Generally, the trends in chlorophyll content and carotenoid content were correlated, demonstrating the close relationship between the two molecule families (Figure 3.10).

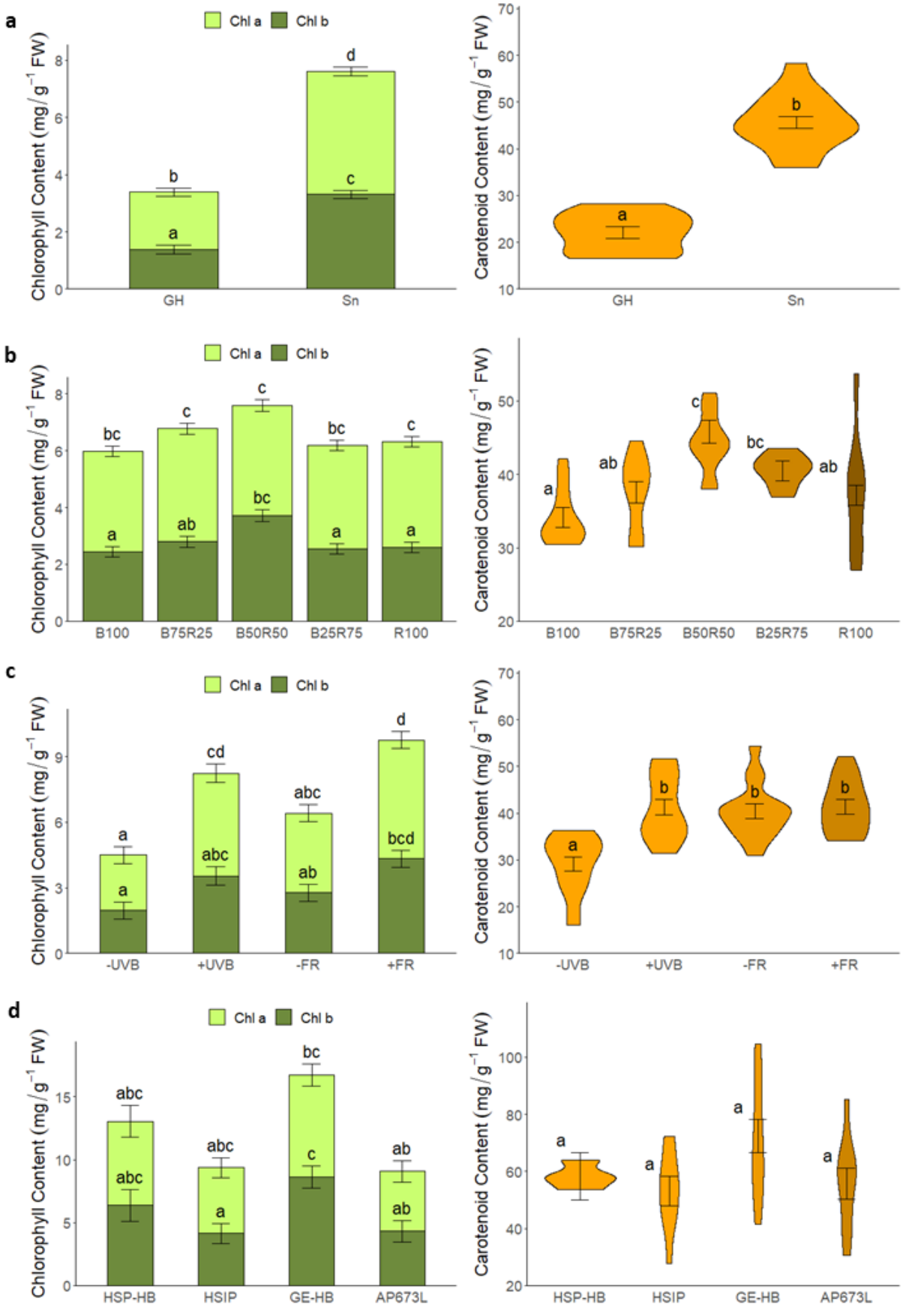

a
Chl a
Chl b
Chlorophyll Content (mg/g⁻¹ FW)
GH
Sn
Carotenoid Content (mg/g⁻¹ FW)
b
B100
B75R25
B50R50
B25R75
R100
c
-UVB
+UVB
-FR
+FR
d
HSP-HB
HSIP
GE-HB
AP673L

Figure 3.10 Photosynthetic pigment content in Basil grown under various light qualities. Total chlorophyll and carotenoid content (mg/g FW) of mature basil, for (a) controlled environment growth conditions, (b) different ratios of blue and red light, (c) supplemental UV-B and FR lighting, and (d) samples from the facility at May Barn Consultancy Ltd. Light conditions are as described in Figure 3.1, 3.3, 3.4, 3.5, respectively. Chlorophyll a and Chlorophyll b content was calculated from FW measurements using formulae published in Lichtenthaler and Buschmann (2001). Data represent the average of a minimum of three biological replicates and error bars show the standard error. Statistical significance of FW and chlorophyll abundance between each light treatment was calculated using two-way ANOVA (chlorophyll) and one-way ANOVA (carotenoid) with sidak post hoc testing ($p < 0.05$). Data that do not share a letter are statistically significant.

Light treatment was a significant factor for total chlorophyll and carotenoid content ($p < 0.001$). Among the light regimes tested, $B_{50}R_{50}$ was the most favourable R:B ratio to overall chlorophyll and carotenoid content (Figure 3.10b). In this treatment, Chl *b* was significantly increased which decreased the Chl *a*:*b* ratio to roughly 1, the lowest of all the treatments (Table 3.9). Chl *b* is contained within PSII and enables absorption into the blue region. The carotenoid accessory pigments also aid in blue-green light absorption (Holt et al., 2005), therefore changes can be associated with spectra

Supplementation with both UV-B and FR light caused significant changes in chlorophyll and carotenoid content ($p < 0.001$) (Figure 3.10c). In the UV-B treatment is was noted that the plants appeared visibly darker green in appearance, and the data shows that chlorophyll content roughly doubled compared to the control, but the appearance could also be attributed to increased anthocyanin production (Krizek, 2004; Ubi et al., 2006). The plants under the additional FR treatment contained more chlorophyll. This was unexpected as FR supplementation is associated with suppression of chlorophyll synthesis (Bou-Torrent et al., 2015), but instead, this could be reflective of a decreased turnover of pigments. Notably, the UV-B treatment increased the Chl *a:b* ratio slightly, whereas a decrease was recorded for the FR treatment (Table 3.9). Interestingly, carotenoid content significantly increased after UV-B supplementation, but no significant difference was found between the FR treatment and control (Figure 3.10b).

While the Sn treatment was conducive to increased chlorophyll and carotenoid content compared to GH ($p < 0.001$) (Figure 3.10a), the pigment concentrations measured in the controlled environments experiment were substantially lower than those measured in the hydroponic system of the experiment with commercial lighting at May Barn Consultancy Ltd. (Figure 3.10d). The lowest Chl *a:b* ratios were found in GE-HB (0.94) and HSP-HB (1.05), which also had the highest blue spectral content of the fixtures tested (Table 3.10; Appendix Table 6.1.2). In addition, the spectra with the lowest total chlorophyll contents also had the lowest R:FR ratio, which unlike the results of the FR supplementation experiment, follows the known literature on FR light negatively affecting chlorophyll synthesis (Bou-Torrent et al., 2015). However, these spectra HSIP and AP673L were also similar in R:B ratio and B:G ratio, which could also have impacted on the total chlorophyll content which was significantly affected by lighting treatment ($p < 0.001$). Meanwhile, no effect of treatment was seen on carotenoid pigments.

Overall, chlorophyll concentration in Basil can be maximised with an R:B ratio close to 1, and UV-B supplementation. However, it is likely that addition of FR to the spectrum risks reduction of total chlorophyll although this remains to be firmly established. Moreover, highly controlled environments appear to be favourable to the accumulation of chlorophyll, possibly due to reduced oxidative stress.

3.3.8. Spectral Impacts on the Function of Photosynthesis

The presence of carotenoids in the photosynthetic system also aids in the dissipation of heat via the xanthophyll cycle, thereby protecting the photosynthetic machinery from photoinhibition (Emiliani et al., 2018). The heat sink capacity of the carotenes is denoted as NPQ, and this was measured using chlorophyll fluorescence on dark-adapted plants. The NPQ values (listed in Table 3.9), reveal that there is little observable difference between all the treatments tested with a few notable exceptions. Treatment with differing B and R light mixes significantly influenced NPQ ($p < 0.001$). $B_{75}R_{25}$ and $B_{50}R_{50}$ give the highest NPQ values, however there was also significant variation between the biological replicate experiments ($p < 0.001$), for example with R_{100} and B_{100}, which could be related to temperature changes between the experiments as NPQ is responsive to temperature in other species such as wheat and pine trees (I. A. Hassan, 2006; Porcar-Castell, 2011). NPQ ($p < 0.05$) measurements for the

plants grown under supplemental UV-B and FR treatment were found to be significantly different compared to control by one-way ANOVA. GH and Sn plants are also similar in NPQ capacity; however, treatment was significant ($p < 0.01$) and the NPQ value for Hp was greater demonstrating a higher capacity for managing photosynthetic stress in these plants (Table 3.9).

Treatment	Repeat	Chl a:b	Fv/Fm	NPQ	A_{sat}	Θ
GH	1	1.46	0.79 ± 0.004 ab	1.20 ± 0.175 ab	18.7	0.026
	2	-	0.80 ± 0.004 a	1.38 ± 0.143 a	19.0	0.044
Sn	1	1.30	0.80 ± 0.004 ab	0.94 ± 0.062 b	-	-
	2	-	0.79 ± 0.004 b	1.38 ± 0.173 a	16.4	0.029
Hp	-	-	0.79 ± 0.004 ab	1.51 ± 0.119 a	17.1	0.030
B100	1	1.44	0.80 ± 0.008 ab	0.07 ± 0.010 b	10.1	0.019
	2	-	0.81 ± 0.006 a	1.29 ± 0.143 a	16.3	0.034
B75R25	1	1.42	0.80 ± 0.002 ab	1.57 ± 0.339 a	16.0	0.030
	2	-	0.80 ± 0.012 ab	1.26 ± 0.339 a	19.7	0.031
B50R50	1	1.05	0.80 ± 0.004 ab	1.40 ± 0.070 a	20.0	0.045
	2	-	0.80 ± 0.005 ab	0.46 ± 0.265 b	18.9	0.031
B25R75	1	1.43	0.78 ± 0.009 ab	1.13 ± 0.364 a	16.8	0.031
	2	-	0.79 ± 0.010 ab	1.31 ± 0.222 a	19.7	0.039
R100	1	1.44	0.78 ± 0.015 bc	0.15 ± 0.088 b	15.7	0.031
	2	-	0.75 ± 0.023 c	1.25 ± 0.134 a	13.1	0.031
- UVB	-	1.29	0.76 ± 0.006 b	1.11 ± 0.119 b	13.7	0.027
+ UVB	-	1.32	0.77 ± 0.006 b	1.30 ± 0.158 ab	16.7	0.029
- FR	-	1.31	0.78 ± 0.006 ab	1.21 ± 0.150 ab	9.5	0.010
+ FR	-	1.25	0.79 ± 0.005 a	1.36 ± 0.146 a	16.0	0.023

Table 3.9 Photosynthetic measurements of basil plants from each of the experimental conditions tested. Measurements were collected by extraction or LICOR on a fully expanded exposed leaf of a mature plant (n = 4) and include chlorophyll a:b ratio, photosystem II efficiency (Fv/Fm), non-photochemical quenching (NPQ), light saturated rate of CO_2 uptake (A_{sat}, µmol (CO_2).m^{-2}.s^{-1}), and quantum yield (ϕ, µmol (CO_2).$µmol^{-1}$ (photons)). Letters indicate statistical significance as calculated by Sidak posthoc test, with groups of analyses separated by a thicker line.

Chlorophyll fluorescence also provides the value for maximum quantum efficiency of PSII (Fv/Fm). A healthy range of Fv/Fm for most plant species is 0.79-0.84 (Maxwell and Johnson, 2000), and in Basil the range is around 0.77 – 0.81 (Stetsenko et al., 2020; Taulavuori et al., 2018). Basil plants grown for this chapter were mostly within this range (Table 3.9). However, relative to the other treatments, Fv/Fm was lowest in both the UV-B supplementary and control treatments ($p < 0.01$), suggesting that the environment in the controlled room was not optimal. Light quality was a significant factor ($p < 0.001$) under mixed BL and RL treatment. Fv/Fm was low for Basil plants grown in R_{100}, whereas the highest reading of Fv/Fm was found in B_{100}. This indicates that while both light qualities can drive photosynthesis, blue light may be more effective at stimulating PSII function.

RLRCs were used to further explore the photosynthesis rate (A_{sat}) response to Basil plants grown under the different spectral ratios and environments (Appendix Figure 6.1.2). The plants grown in Hp showed higher A_{sat} at the lower intensities between 0 – 800 µmol.m^{-2}.s^{-1} than the other GH and Sn controlled environments (Appendix Figure 6.1.10). These figures also show increased photosynthetic rates under higher light intensity for the dichromatic mixes, which is to be expected as chlorophyll utilises both wavelength ranges to drive photosynthesis (McCree, 1971). However, the trends between the monochromatic treatments reveal that both B_{100} and R_{100} are comparable across intensities to the dichromatic mixes in repeat 1 and 2, respectively (Appendix Figure 6.1.10). Therefore results support that photosynthesis in Basil can be driven by monochromatic light alone. Comparing A_{sat} of B_{100} experiment 1 and R_{100} experiment 2, B_{100} (16.3 µmol.m^{-2}.s^{-1}) performed marginally better than R_{100} (15.7 µmol.m^{-2}.s^{-1}) (Table 3.9), but when accounting for the Fv/Fm values, overall BL in Basil may be better at driving photosynthesis than RL, possibly due to BL driven changes in stomatal opeing (Inoue and Kinoshita, 2017). Data showed that stomatal conductance under RL (0.09 – 0.16 mmol.m^{-2}.s^{-1})

was roughly half of that under BL (0.21 – 0.32 mmol.m^{-2}.s^{-1}). However, the highest A_{sat} values were found for the dichromatic mixes, $B_{50}R_{50}$ and $B_{75}R_{25}$ in particular, highlighting the importance of both wavelengths for optimal photosynthetic function (Table 3.9).

From the RLRC non-rectangular hyperbola model (Marshall and Biscoe, 1980), it was found that the Quantum Yield (ϕ) varied across the treatments (Table 3.9). Similar to Fv/Fm, lower ϕ was found in the GH and Sn plants on average, than Hp plants. In addition, whilst high ϕ was seen across the monochromatic and dichromatic spectra, with values approaching or surpassing 0.04 µmol.µmol^{-1} in the $B_{25}R_{75}$ and $B_{50}R_{50}$ treatments, the highest ϕ value overall was found in $B_{50}R_{50}$ (0.045 µmol.µmol^{-1}) (Table 3.9). Overall, these data demonstrate, a superior photosynthetic response of Basil in Hp against plants grown in GH and Sn. Between the monochromatic light conditions, B_{100} surpassed R_{100} in measurements of Fv/Fm and A_{sat} providing evidence that BL is more efficient at driving photosynthesis than RL alone. However, the dichromatic light qualities showed similar and more consistent responses, highlighting the need of both wavelengths.

Spectra	Chl a:b	Fv/Fm	NPQt
Heliospectra Propagation High Blue	1.05	0.74 ± 0.021 a	0.70 ± 0.187 a
Heliospectra Indoor Production	1.26	0.73 ± 0.006 a	0.80 ± 0.054 a
GE Arize High Blue	0.94	0.73 ± 0.007 a	0.84 ± 0.060 a
Valoya L28 AP673L	1.10	0.74 ± 0.006 a	0.71 ± 0.052 a

Table 3.10 Photosynthesis measurements taken at May Barn Consultancy Ltd. on Basil grown under commercial spectra. Measurements on mature basil include Chlorophyll *a:b* ratio, and photosystem II efficiency (Fv/Fm), and non-photochemical quenching without dark adaptation (NPQt) measured using a MultispeQ.

In the experiments conducted with plants harvested from May Barn Consultancy Ltd to evaluate impact of commercially available LEDs with differential spectral quality, measurements were conducted with the portable MultispeQ (Table 3.10). Compared to measurements with LICOR, the MultispeQ readings for Fv/Fm and NPQt (a non-dark adapted value of NPQ) are lower, and so comparisons cannot be made between the MultispeQ data and the LICOR. Results obtained within these samples show no significant variation between the Fv/Fm values, indicating that all the plants were under similar stresses and PSII was

performing similarly. The highest NPQt value was recorded in plants grown under the GE-HB spectra, which correlates with the highest total carotenoid content found in the chlorophyll assay in addition to a low R:B and high B:G spectral ratio. However, differences in the NPQt values for the other treatments were not statistically significant (Table 3.10). HSIP-HB showed the lowest NPQt and also the greatest variation, which could be due to instrumental error. Whilst the MultispeQ instrument was useful the results did not reflect the wide range of physiological differences between plants grown in the different spectra, therefore, more sensitive equipment is required to be able to discern photosynthetic differences between spectral treatments of the same species.

3.4. DISCUSSION

3.4.1. Hydroponics Generated a Higher Yield Than in Other Compost-Based Controlled Agriculture

The case for expanding hydroponic vertical faming is supported by evidence that, compared to traditional glasshouse indoor cultivation, vertical hydroponic production can reduce water inputs (Barbosa et al., 2015), increase yield per unit area (Touliatos et al., 2016), and even boost nutritional content (Treftz and Omaye, 2016). For example, Basil grown in hydroponics in a glasshouse, accumulated 50% more vitamin C over plants grown in soil (Sgherri et al., 2010). However, the benefit of growing plants under artificial lighting is contested. Some studies have found glasshouse conditions with natural light produce Basil with greater fresh weight, leaf area and plant height over various LED conditions (Carvalho et al., 2016; Schwend et al., 2016). However, these experiments were conducted in the summer months, where plant growth greatly benefits from the elevated light intensities that cannot be feasibly replicated in an indoor growth facility. These favourable growth conditions in glasshouses are not consistent, unlike light environments that can be created with fluorescent or LED lighting fixtures.

Compared to some LED regimes, Basil grown under white fluorescent lights generated higher dry weight (Frąszczak et al., 2015), greater fresh weight, plant height, leaf area (Bantis et al., 2016; Frszczak et al., 2014) and essential oil content (Frąszczak et al., 2015). In contrast, the data presented here showed that over the same area, Hp techniques under LED lighting grew Basil greater in fresh weight yield, height, leaf number, area and thickness, as compared to plants grown in compost in GH with supplementary LED lighting and in Sn with fluorescent lighting (Figure 3.1). The production of biomass was likely driven by a superior photosynthetic performance (Table 3.9), as seen across light intensities in the light response curve (Appendix Figure 6.1.10), which reached the same maximum light saturation levels of 1400 $\mu mol.m^{-2}.s^{-1}$ as reported previously for glasshouse-grown Basil (Chang et al., 2008). Moreover, in hydroponic Basil, the high NPQ value (Table 3.9) indicates a relatively high accumulation of xanthophylls, including photoprotective zeaxanthin and lutein, which act to thermally dissipate excess excitation energy (Jahns and Holzwarth, 2012; Ruban, 2009; Ruban and Murchie, 2012). As powerful antioxidants, their presence raises the nutritional profile of the hydroponically-grown crop.

Growth, chlorophyll and carotenoid content of Basil was lowest in GH crops (Figure 3.8 and 3.10). Over the experiment, the temperature of the Snijder cabinet and hydroponic growth room was maintained at 22/20 °C, whereas in the glasshouse, plants were exposed to temperature and humidity ranges of 20 °C and 40 %, respectively. Antioxidant essential oils rosmanic acid and caffeic acid in Basil were depleted when grown in a glasshouse due to exposure to such abiotic stresses (Ruban et al., 2007; Schwend et al., 2016). Therefore, it is likely that the nutritional improvements in the Sn plants are due to the stable environment created in the growth cabinet (temperature, humidity and light intensity), allowing more directed energy towards growth and development, without additional stress, which can deplete antioxidant reserves. However, consumption of radical scavenging capacity by oxidative pressures can simultaneously trigger a constant production of more powerful antioxidants such as α-tocopherol (Munné-Bosch, 2005) and ascorbic acid, which has a function in recycling both carotenoids and tocopherols (Noctor and Foyer, 1998). Indeed, levels of α-tocopherol and tAA were elevated in GH Basil (Figure 3.8, 9). Hydroponically-grown Basil plants had superior AA content over soil-grown plants within a glasshouse environment (Sgherri et al., 2010). Hydroponics has the capacity for boosting nutritional yield by providing optimised conditions for their synthesis and accumulation. Therefore, the challenging environmental fluctuations of a glasshouse envrionment paired with favourable growth conditions supplied by hydroponics is likely to have boosted AA production but also conserved the antioxidant potential. However, the response of AA to environmental stresses must be further characterised to enable knowledgeable interventions.

Alternatively, elevations of tocopherols and tAA in GH Basil could be attributed to the different spectral environments, for which GH Basil received significantly greater amounts of BL as well as UV irradiance, than Sn plants (Appendix Table 6.1.2). Blue and UV wavelengths have been previously associated with the increase in tocopherols and AA (Emiliani et al., 2018; Park et al., 2013b; Xu et al., 2005; Zha et al., 2020). However, the inconsistency of the spectral environment in glasshouses makes it difficult to explore the specific contribution of spectral quality. Therefore this study went on to examine the spectral effects on growth and nutrition utilising the wavelength targeting specificity capacity of LED-driven hydroponic production in a tightly contolled environment, with the aim to be able to trigger phytonutrient production using light without subjecting plants to environmental stress and thereby consuming the antioxidant capacity and compromising growth.

3.4.2. Blue and Red Light Consistently Effected Growth Across Development Stages

Previous studies have shown that the contribution of red and blue light has an effect on Basil architecture and quality as microgreens and mature plants, and suggests that for growth and metabolite content Basil prefers a lighting environment with a higher percentage of blue (20 %+) than usually found in horticultural dichromatic mixed LED lighting (10 %) (Table 3.2, 3.3). Therefore, this study explored the effect of specific red and blue ratios at two key developmental stages, seedlings (2 weeks) and mature plants (4 weeks).

Plants coordinate growth and developmental responses to blue and red light via the cryptochrome and phytochrome photoreceptors, respectively (see Section 1.3.2.), including control of stem length. In *Arabidopsis*, phytochrome B is required to prevent an elongated stem phenotype under white and red light (Reed et al., 1993), whilst blue light is known to inhibit stem elongation through cryptochrome (Ahmad et al., 2002), with both light signalling responses coordinated via common light signalling partners and the hormones auxin and giberellin (Folta et al., 2003). However, whilst the mechanisms of photoreceptor light detection and light signal transduction are highly ubiquitous within higher plants, the strength and modulation of deetiolation and growth responses that are triggered can vary across species (Chen et al., 2004; Johnson et al., 2020). This variation is a result of adaptive responses to a species' native environment and is required to be understood in order to effectively exploit light-controlled growth in CEA. In Basil under monochromatic conditions, elongated stems were observed in seedlings under BL and for both seedling and mature plants under RL (Figure 3.2a and 3.3a). This correlates with published studies where elongation responses have been seen in spectra with 90 – 100 % BL accompanied by a shift in dry matter content from leaves to stems (Larsen et al., 2020), and in spectra with greater contribution of red light (Table 3.2) (Bantis et al., 2016; Naznin et al., 2019; Schwend et al., 2016). These results suggest that stem elongation under monochromatic light is a common response across Basil cultivars. As Basil is grown and harvested for the leaves, these results show that monochromatic light conditions are not conducive to commercially desired plant architecture, as biomass accumulation is altered to favour stem elongation. Moreover, epinasty (curling or rolling of leaves) under 100% red light was also seen in this study (Appendix Figure 6.1.9). Such 'Red Light Syndrome' responses have also been recorded in other species such as cucumber (Davis and Burns, 2016)

and generate an unfavourable leaf morphology for light interception and therefore reduced biomass accumulation, as well as reducing customer appeal (Amaki et al., 2011). Basil growth responses under high proportions of both red and blue could result from a weaker responsiveness of phytochromes, cryptochromes, or their downstream signalling partners to modulate stem growth (Karnachuk et al., 2001; Takase et al., 2003). In the case of BL, the elongated shade-avoidance-response phenotype has been attributed to a decrease in phytochrome activity (Kong et al., 2018). Alternatively, different species could hold affinities for specific wavelengths to trigger desired growth responses. A recent investigation into the absorption of Basil extractions found peak absorption of blue light at 435 nm, rather than the 450 nm specified by the McCree curve for photosynthetic output and therefore most commonly used in horticultural lighting design (McCree, 1971). However, when the 435 nm wavelength was delivered in a 1.5:1 ratio with red light, Basil increased in yield (Riha et al., 2020). These results demonstrate the viability of studies like mine to explore and verify the growth responses to blue and red light in crops grown in artificial lighting environments. Moreover, they present further mechanistic study into light responses observed and further scope in the field for photobiological study into specific wavelength energies to be delivered for optimised growth.

In previous studies, mature (46 days) (Piovene et al., 2015) and microgreen (17 days old) Basil (Lobiuc et al., 2017) showed greater fresh weight yield under greater proportions of blue light over red. On the other hand, the effect of supplemental blue (450 nm) and blue-violet (420 and 440 nm) on glasshouse 'Genoverser' grown under high pressure sodium lamp showed no effect on shoot elongation, and instead biomass and number of leaves was highest in the control (HPS only) and lowest in blue-violet light (Taulavuori et al., 2018). My results showed that for Basil seedlings (14 days-old) the spectra for most ideal growth was $B_{25}R_{75}$ (Figure 3.2). This spectral preference carried over into the mature (4 week-old) Basil, which demonstrated the most consistent performance across the growth parameters, including higher stem length, fresh weight and dry weight under $B_{25}R_{75}$ (Figure 3.3, Appendix Table 6.1.3), and shows the necessity of both blue and red wavelengths for control of growth and leaf elongation. Moreover, this is consistent with the conclusions of (Pennisi, Blasioli, et al., 2019) that an R:B ratio of 3 was the most optimal for Basil growth.

Interestingly, despite a good photosynthetic yield (ϕ) in $B_{25}R_{75}$ and the other dichromatic mixes, Basil had the highest photosynthetic capacity and greatest PSII efficiency under monochromatic blue (Table 3.9; Appendix Figure 6.1.10), suggesting blue light drives photosynthesis better in Basil. Whilst, no effect on photosynthesis was seen on glasshouse 'Genoverser' grown under HPS with supplemental blue (450 nm) and blue-violet (420 and 440 nm) (Taulavuori et al., 2018), other studies have found higher photosynthetic rates and greater fresh weight yield under greater proportions of blue light (Piovene et al., 2015). In an alternative study, net photosynthesis rates in both green and purple variety Basil were highest at $B_{49}R_{51}$ (Dou *et al.*, 2020). In my experiments, the photosynthetic performance under $B_{50}R_{50}$ was also good, which may have contributed to the exceptionally high growth results for the first experimental repeat of $B_{50}R_{50}$ treatment. It is not clear whether this result can be genuinely attributed to lighting treatment alone, as the second $B_{50}R_{50}$ experimental repeat showed the lowest growth results. However, the disproportionally high DW percentage in the secondary repeat indicates a lack of leaf expansion, which suggests an issue occurred with irrigation or other environmental condition such as temperature, preventing the plants in this repeat from achieving the high growth rates of the first. If that is the case, then it is possible that an equal balance of blue and red light is optimal for Basil growth and requires further investigation. What can be concluded is that light quality is not the only parameter impacting growth in a hydroponic system, and that the yield potential of Basil, seen in the first repeat of $B_{50}R_{50}$, is particularly high.

3.4.3. Phytonutrient Content can be Influenced by Blue and Red Wavelengths

Basil is highly valued for its production of aromatic and flavour compounds, and previous research has found their content to be light dependent (Table 3.3) (Amaki et al., 2011; Ichimura et al., 2008; Nicole et al., 2019; Yelton et al., 2017). The major phenolic RA significantly increased with supplementary BL LEDs (Taulavuori et al., 2013), and also under RL (Shiga et al., 2009) which was confirmed to be regulated by phytochrome (Schwend et al., 2016). Overall studies indicate that a majority contribution of RL with a significant proportion of BL is required for essential oil and volatile production in Basil (Table 3.3). However little study has been conducted on the light regulation of the nutritional compounds most beneficial for human health. Carotenoids and tocopherols are important isoprenoid phytonutrient derivatives from the MEP pathway. In addition to ascorbic acid, they are essential components of the human

diet, but also play a key role in proper plant functioning acting as antioxidants and photoprotectors (see Section 1.2). It is expected that their production and accumulation is associated with light inputs in Basil (Table 3.3). Therefore, this study explored the effect of blue and red lighting ratios on the content of these phytonutrients in Basil plants.

The two main carotenoids, Lutein and β-carotene, showed similar responses to R:B ratios, but did appear to vary slightly. Overall, R_{100} accumulated carotenoids well, but significant enhancements of carotenoids was seen in $B_{75}R_{25}$ and $B_{50}R_{50}$ (Figure 3.6). $B_{50}R_{50}$ also stimulated concurrent increases in the chlorophyll and carotenoid photosynthetic pigments (Figure 3.10), in addition to the highest NPQ vales (Table 3.9), suggesting that a significant proportion of BL and RL is beneficial to the accumulation of these compounds. In fact, red wavelengths have been shown to have negative effects on carotenoid pigments in 19-days-old Basil (Samuoliene et al., 2016). Increased proportions of RL as well as sole illumination by RL, reduced lutein significantly by up to 35.8%. Similarly, β-carotene content decreased under increasing RL (-43.6%) and sole RL (-34.5%) (Samuoliene et al., 2016). These results likely indicate that CRYs in addition to phys are needed for optimal activation of the carotenoid pathway, potentially acting upon the functional copy of *PSY*, the main rate determining biosynthesis enzyme, which is found in Basil (Torre et al., 2016). Basil *PSY* expression could be responsive to red and blue light in a similar manner to *Arabidopsis PSY* (Stange and Flores, 2012; von Lintig et al., 1997), or tomato *PSY1*, which subsequently elevates lycopene, a precursor to lutein and β-carotene (Xie et al., 2019). In addition, light quality could also be differentially affecting the activity of the downstream enzymes in the carotenoid pathway, thereby impacting on the accumulation of specific carotenoids. The different trends of lutein and β-carotene content seen across the light qualities tested here may provide support for this and therefore suggest an interesting area for future exploration.

Tocopherol biosynthesis genes found in *Arabidopsis* are conserved in other species, yet their regulation by light is just beginning to be explored (Li et al., 2008b). In tomato, light quality has been shown to impact tocopherol content (Ntagkas, Woltering, Nicole, et al., 2019) but more detailed work on the effect of light quality on α-tocopherol biosynthesis has not been published. The biosynthesis gene *VTE1*, one of the rate limiting steps of the biosynthesis pathway, was found to be correlated to BL in tea plants (Li et al., 2008b) and the same gene was found to be upregulated by BL in *Arabidopsis* in Chapter 2. In addition, RL positively

impacted on all the genes described as rate limiting steps of the tocopherol biosynthesis pathway, and in agreement, elevated α-tocopherol has been observed under supplemental RL in young Basil plants (Samuoliene et al., 2016). However, in this study of Basil, α-tocopherol accumulation required both BL and RL and showed little preference for ratio, except for one high result in one experimental repeat under $B_{25}R_{75}$ (Figure 3.6d). This indicates that both BL and RL activate the production of tocopherols in Basil, however the influence of cryptochromes or phytochromes on the individual genes within the pathway may vary.

Similar to α-tocopherol, tAA content was not effected by blue and red ratios except a small peak was recorded under $B_{25}R_{75}$ (Figure 3.9). Samuoliene et al. (2016) also observed in Basil that AA content was increased in elevated RL regardless of the wavelength of RL used. tAA has also been shown to be modulated with RL in Mustard (Thomsen et al., 1992) and tomato fruits (Labrie and Verkerke, 2014). Broccoli placed under a post-harvest RL treatment saw a delayed reduction in AA content and therefore delayed senescence. It was found that RL upregulated AA metabolism genes in Broccoli, including biosynthesis genes (*VTC2*) and regeneration genes (Ma et al., 2014). In contrast, AA pool size correlated with increasing blue light content in lettuce. But this was likely due to regeneration rather than biosynthesis as blue light stimulated expression of regeneration genes more than biosynthesis genes (Zha et al 2020). However, in Chapter 2, *VTC2* expression was found to be impacted by RL and BL in *Arabidopsis*. Therefore, for Basil and *Arabidopsis* evidence indicates an association of AA modulatory genes with phytochrome and cryptochrome signalling, that could be conserved amoung species.

Overall, this evidence indicates that light has a functional role in the accumulation of these beneficial compounds in Basil, likely via similar light signalling forces upon the biosynthesis genes as seen within *Arabidopsis*, albeit with differential spectral responses relating back to varying activation of enzymes (Lewinsohn and Gijzen, 2009). The species specificity of light regulation could be implicated with evolutionary pressures associated with the native lighting environment of a species, and therefore a fitness adaptation. To explore the mechanism of light regulation in Basil further, experiments addressing the transcript accumulation of Basil homologues of the MEP, carotenoid, tocopherol and AA biosynthesis genes in response to blue and red light would clarify the transcriptional activation of the pathways by specific wavelengths, which is known to be the first level of control in their biosynthesis and accumulation. It would also be of interest to determine whether the observed fluctuations

under spectral qualities are related to enzymatic activities within the pathway. Furthermore, evaluation of the degradation of these compounds should be explored, as light may also play a role in metabolite turnover.

3.4.4. UV-B Produced Negative Responses in Growth and Carotenoid Content but Positive Effects on Tocopherol

UV-B radiation is a high energy wavelength found in natural sunlight, which triggers photomorphogenic and metabolic adaptive responses (Jenkins, 2009). In particular, leaf expansion and other developmental aspects including cell division, stomatal differentiation and senescence, are under control of UV-B signalling pathways (Wargent et al., 2009). UV effects on yield can vary between crop species, for example, solar UV-B inhibits growth in many crops including soybean and pepper, whilst in corn and potato growth can be stimulated (Teramura and Sullivan, 1994). The evidence of UV-B supplementation on Basil is also variable. One study found supplementary UV-B fluorescent tubes caused no significant differences in height and leaf number, but leaf area was reduced (dose not measured) (Johnson et al., 1999). Whereas another study did show reduced plant height, increased dry weight and thickness of the leaves after 2 weeks of UV-B treatment (2.1 kJ m^{-2} day^{-1}) (Chang et al., 2008). However, a more recent study found that in Thai Basil cultivar a dose of UV-B (4 kJ m^{-2} day^{-1}) increased Basil height, leaf area, fresh and dry weight (Sakalauskaitė et al., 2012). My Basil plants presented typical responses to the UV-B dose supplied (6.48 kJ m^{-2} day^{-1}) including inhibited stem elongation, thicker leaves, and reduced leaf area (Figure 3.4). These effects were also correlated with a total reduction in biomass reflected as decreases in fresh and dry weight (Appendix Table 6.1.3). Interestingly Chang, Alderson and Wright (2008) also use the '*Sweet Genovese'* cultivar and their growth results most closely match the results presented here and thereby offer support to the findings. Future studies should explore the differences among cultivars, as one light regime could affect the morphology of Basil drastically differently as seen between the aforementioned studies and the effect on height (Chang, Alderson and Wright, 2008; Sakalauskaitė, Viskelis and Duchovskis, 2012).

Differences between these studies can be attributed to dose as well as cultivar specific responses (Sakalauskaitė et al., 2012). My results showed further effects on Basil cv Sweet Genovese such as upwards curling of the leaves and a 'glossy' shine, likely due to increased thickness of waxy cuticles (Strid et al., 1994)(Figure 3.4), as well as a deeper colour and

measurable increase in chlorophyll concentration (Figure 3.10). Other similar observational effects on Basil leaves was reported with a similar dose but with no effect on chlorophyll content (Mosadegh et al., 2019). Instead, higher doses of UV-B decreases Basil chlorophyll content (Dou et al., 2019a; Mosadegh et al., 2019), which is also seen in spinach (DeLong and Steffen, 2011). This decrease was attributed to degradation and inhibition of the photosynthetic machinery at higher UV-B doses, which was compounded by the low PPFD levels used in the controlled environment study (Dou et al., 2019a). Some carotenoids, including β-carotene, are associated with chlorophylls to function as antioxidants and protect the photosynthetic machinery from oxidative damage from environmental stresses, such as UV-B radiation. This study found a decrease in carotenoid content under UV-B supplementation (Figure 3.7a and b), which could also be attributed to an overload of high energy wavelengths consuming the antioxidant capacity held within carotenoids (Neugart et al., 2020). The elevation of chlorophylls combined with the depletion of carotenoids suggests that the carotenoids are fulfilling their role to protect the photosystems from photooxidative destruction due to UV-B radiation, especially as this did not coincide with a decrease in Fv/Fm or NPQ under UV-B (Table 3.9).

Increases in secondary metabolite production are a documented UV adaptive response in Basil (Table 3.2). A two week treatment of UV-B for 1.5 h day^{-1} increased volatiles four-fold in Basil, and the effect could easily be detected by the enhanced aroma (Johnson et al., 1999). With a 2-week treatment of daily UV-B for 3 h (2.1 kJ m^{-2} day^{-1}), the total content of volatiles significantly increased (Chang et al., 2009). The effects are possibly dependent on UV dose, genotype, temperature and the time of year as a short 1 h day^{-1} UV-B treatment had no effect on the qualitative or quantitative composition of volatiles (Kakani et al., 2003). Comparatively, total flavonoid content, total phenolic content, as well as anthocyanins and ascorbic acid increased with UV-B (Ghasemzadeh et al., 2016; Sakalauskaitė et al., 2012). Due to sampling limitations, effects of UV-B on ascorbic acid content could not be measured in this study. However, a non-significant increase of α-tocopherol was detected by HPLC (Figure 3.7c). UV-B associated increases in α-tocopherol have been recorded previously in spinach (DeLong and Steffen, 2011). Due to the role of α-tocopherol as a protective molecule preventing lipid peroxidation and oxidative damage, it is feasible that elevations of α-tocopherol production are an adaptive response to alleviate damage. Mechanistically, Basil possesses copies of the MEP pathway genes, in addition to tocopherol biosynthesis genes *VTE1, VTE3* and ascorbic acid

genes *VTC2* and *VTC4* (Dudai, Carp, et al., 2018; Rastogi et al., 2014). Therefore, accumulation responses could be enacted by UV-B induced transcriptional changes in the biosynthetic pathways as was seen in *Arabidopsis*, where strong induction of *VTE1* and *VTC2* was seen under UV-B exposure (Chapter 2). Overall, whilst UV-B can result in phytonutrient changes, further evidence is required as to the mechanism and optimal dose to produce a controlled response for induction of production with minimised UV induced turnover of the protective products.

3.4.5. Far-Red Supplementation Stimulated Elongation and Decreased Carotenoid and Tocopherol Content but Increased Ascorbic Acid

In contrast to UV-B responses, FR light is anticipated to elongate stem and leaves, due to shade avoidance responses (Franklin and Whitelam, 2005). FR light induced elongation of leaves and stems is linked with changes in auxin biosynthesis and sensitivity, with PIFs in coordination with phyA playing a crucial role in the orchestration of the response (Hersch et al., 2014; Tao et al., 2008). Elongation responses have been seen in a previous study in Basil (R:FR = 5 or 3) (Meng and Runkle, 2019). The R:FR ratio generated in this study by FR supplementation (~ 1.0) was relatively low and comparable to normal sunlight in vegetative shade, which delivers ratios between 1.2 to 0.05 (Franklin and Whitelam, 2005). Nevertheless, the FR supplementation resulted in Basil plants with increased height and fresh weight as growth resources were allocated to stem growth, confirming a photomorphogenic sensitivity to FR light (Figure 3.4). Biomass accumulation could also be enabled by FR light enhancement of photosynthetic energy and efficiency by stimulation of PSI. This drives electrons through the light cycle of PSI, thereby removing electron pressure from PSII which increases NPQ relaxation of the *qE*-quenching and also results in higher photosynthetic efficiency of PSII (Meng and Runkle, 2019; Zhen and van Iersel, 2017). Increases in NPQ due to FR has been seen in marcoalgae (Zheng et al., 2019b), and also *Arabidopsis* and *Nicotiana* under low light (30 $\mu mol.m^{-2}.s^{-1}$) conditions (Kono et al., 2020). Whilst the light intensity in this experiment was moderate (~150 $\mu mol\ m^{-2}\ s^{-1}$) marginal increases in NPQ and Fv/Fm was observed in the present study (Table 3.9), possibly due to these effects of FR on the photosystems (Long et al., 2022; Zhen and van Iersel, 2017).

In addition to stimulating changes in plant growth hormones, FR radiation is also linked to decreases in chlorophyll pigmentation and plant nutrition due to the inactivation of phytochrome and the release of negative transcriptional regulators PIFs, which down regulate

chlorophyll and carotenoid biosynthesis (Bou-Torrent et al., 2015; Franklin and Whitelam, 2005; Huq et al., 2004; X. Liu et al., 2017). To balance the activity of the photosystems as an adaptive response to light quality, the association of accessory pigments in the antennae (containing Chl b) to the core reaction complex (containing Chl a) can change, resulting in shifts of the Chlorophyll *a:b* ratio. As FR light or shaded conditions predominantly activates PSI, a common response is to decrease Chl *a:b*, up to three fold, thereby increasing light absorption in the higher energy wavelengths (400 – 500 nm) (Dale and Causton, 1992; Wientjes et al., 2017). Despite the same decrease of Chl *a:b* seen in lettuce (Zou et al., 2019), no change was seen previously in Basil seedlings (Meng and Runkle, 2019). In this study, the photosynthesis pigment assay did register a shift from Chl a to Chl b, lowering the Chl *a:b* ratio, however there was also an unexpected increase in total chlorophylls (Figure 3.10c; Table 3.9). Both blue and red light are effective for induction of chlorophyll synthesis, triggering a signalling cascade via positive photomorphogenesis transcription factor HY5 (Lee et al., 2007a; Okamoto et al., 2020), however, supplementary FR can decrease Chl content (Bou-Torrent et al., 2015). It is possible that chlorophyll synthesis still takes place via blue or red light signalling, with low levels of supplemental FR, as would occur in natural sunlight levels of FR. However, the observed increase is more difficult to explain and may be attributed to an effect of small temperature changes between the treatments, which was not continuously monitored. Whilst temperature was controlled as far as possible, the addition of FR LEDs did add heat into the grow area. While extreme heat (40°C) will negatively effect chlorophyll synthesis (Mohanty et al., 2006), in my experiments the differences remained in close range of the set point of 22°C ± 2°C. As Basil is of Mediterranean origin, it is possible that chlorophyll accumulation is positively correlated with temperature as observed in maize and *Arabidopsis* (Toledo-Ortiz et al., 2014) and that an increase of one or two degrees in the average temperature could result in the greater chlorophyll accumulation observed under the FR LEDs.

Carotenoid production is similarly linked to chlorophyll via the MEP Pathway (Vranová et al., 2013) and is also predicted to decrease under supplemental FR, as seen in lettuce (Zou et al., 2019), due to the activation of PIFs by shade resulting in downregulation of carotenoid biosynthesis genes (Bou-Torrent et al., 2015). Spectrophotometric quantification revealed that supplementary FR did significantly decrease carotenoid content, as predicted, in addition to a slight decrease in tocopherols (Figure 3.7). Concurrent decreases in chlorophyll, carotenoids and tocopherols could indicate an effect of FR light on the MEP pathway, which provides

precursors for all of these essential molecules. Indeed, FR was found to have repressive effects on the expression of MEP biosynthesis genes in *Arabidopsis* (see Chapter 2), which could impact production of all downstream products.

Increasingly, AA content is inversely correlated to the R:FR ratio, showing increased production under a lower R:FR ratio with minimal impact of blue and red light proportions as seen earlier (Figure 3.9). This finding is of particular note as additional red light has also been seen to increase AA in Basil (Samuoliene et al., 2016). The documented positive response of AA to both red and FR wavelengths is of particular interest for future studies. Overall, these results support the notion that ascorbic acid production and accumulation in Basil is predominantly regulated by phytochrome. Therefore RL manipulation in conjunction with FR supplementation could be a point of control for AA content, either by activating the biosynthesis pathway or potentially decreasing AA turnover, respectively. This mechanism should be used cautiously in a commercial setting however as additional FR light may simultaneously lower the nutritional value of the crop via a decrease in carotenoids and tocopherols.

3.4.6. Spectral Design has a Significant Impact on the Growth of Basil in a Commercial Hydroponic System

There is a vast selection of spectral compositions in the commercial horticultural LED market, containing the full spectral range including all PAR wavelengths in addition to UV and FR. This study explored the quality of Basil grown under four of the many commercial offerings from three of the major manufacturers, including two from Heliospectra (HSP-HB and HSIP), one from GE (GE-HB), and a final luminaire made by Valoya (AP673L). These spectra differed in the ratio of red and blue light, as well as the proportion of B:G and additional FR light (Appendix 6.1.2). Intensity of the fixtures also varied from ~ 250 $\mu mol.m^{-2}.s^{-1}$ (HSP-HB and GE-HB) to ~ 210 $\mu mol.m^{-2}.s^{-1}$ (HSIP), and down to 150 $\mu mol.m^{-2}.s^{-1}$ (AP673L).

Overall, HSP-HB ($B_{30}G_{33}R_{37}$) produced the Basil crop with the most valuable growth habits under relatively equal proportions of blue, green and red wavelengths, and a small contribution of far-red light (R:FR = 11.2). The crop produced under this spectrum was mid-range in height, but competed with the highest fresh weight production and high leaf count (Figure 3.5). Interestingly, the height measurements of the crop across the four spectra roughly followed

the spectral value of R:FR as a function of intensity (Appendix Table 6.1.2). I previously found that Basil produces a stem sensitive FR response (Figure 3.4), and therefore the difference in height could be attributed to the spectral input of FR promoting stem elongation. For example, HSIP produced the tallest crop under a high intensity with one of the lowest R:FR ratios. AP673L ($B_{11}G_{21}R_{68}$) had a similar low R:FR ratio but also low intensity overall; low light availability for photosynthesis and growth explains why this Basil was the shortest despite the lengthening effects of a low R:FR ratio. Whilst the effect of FR light has not been explicitly investigated in Basil, the effect of R:FR ratio in commercial lighting that includes FR wavelengths can be explored in the published literature. '*Atom*' cv. demonstrated elongation under the commercial spectra with the lowest R:FR ratio (Schwend et al., 2016), as did '*Lettuce Leaf*' cv. in another study (Bantis et al., 2016). However the second cultivar in that same study ('*Red Rubin x Mountain Athos*' cv.) did not show the same physiological response to FR light (Bantis et al., 2016). This suggests that Basil has a cultivar-dependent growth response to FR. Alternatively, the effect of FR has been demonstrated to be dependent on R:B ratios (Meng and Runkle, 2019), which also fluctuated between the studies (Table 3.2). This illustrates the interconnected nature of photoreceptors and growth responses, with both phytochromes and cryptochromes enacting developmental signalling of red: far-red and blue light (Griffin et al., 2020; Pedmale et al., 2016a).

While biomass accumulation has also been correlated with low R:FR ratios, frequently this resource is allocated the stem creating undesirable growth (Bantis et al., 2016; Schwend et al., 2016). Instead, the results obtained here support a positive role of light intensity with fresh weight gain, as well as greater leaf number in HSP-HB (Figure 3.5). Higher light intensities drive greater photosynthetic output and therefore more biomass accumulation up to the point of photoinhibition. Temperature could also be a factor, as higher light intensities will result in greater heat production by the LED fixtures. There was a differential distribution of temperature across the grow areas; however it was not related to intensity. Instead, temperature increased down the rack, where the highest rack (HSP-HB) had a temperature of 25 °C and the lowest rack fitted with AP673L had a temperature of 27 °C. In terms of spectral content, the earlier experiment on blue and red proportions found higher fresh weight in light regimes with greater proportions of red light than blue light (Table 3.2, Figure 3.3b). However, HSP-HB has a roughly equal proportion of blue to red light (R:B ratio = 1.3) but also includes the highest GL content among the commercial LEDs tested (Appendix table 6.1.2). Blue and

red wavelengths are absorbed by the upper leaves of a crop, allowing GL to penetrate further into the canopy to be absorbed by accessory pigments in the lower leaves (Meng et al., 2019; Terashima et al., 2009; Wang and Folta, 2013). This can increase light interception and photosynthetic rate (P_N) (Terashima et al., 2009) as seen in lettuce (J. Liu and van Iersel, 2021), resulting in increased shoot mass and leaf area in lettuce (Kim et al., 2004), and increased shoot mass, height and leaf area in tomato (Wollaeger and Runkle, 2014). Moreover, due to the lack of absorption of GL in the upper canopy, a green-enriched light environment signals shade conditions similar to enriched FR light. As such, GL has been observed to trigger shade-avoidance-like responses independent of cryptochrome and phytochrome signalling in wild-type *Arabidopsis* including leaf elongation and positional hyponasty (Meng et al., 2019; Zheng et al., 2013). In Basil cultivars, increasing GL content between 12 – 45 % has been documented to increase stem and leaf elongation, increase yield and leaf number, and decrease secondary metabolites including anthocyanin, phenolics and flavonoids, similar to shade avoidance responses (Dou et al., 2019b; Schenkels et al., 2020). These responses are likely attributed to GL absorption by cryptochrome, an action that can partially deactivate cryptochromes (Bouly et al., 2007) which counters cryptochrome suppression of shade avoidance responses, and reverses blue light mediated growth including stomatal opening, hypocotyl elongation inhibition and anthocyanin accumulation (Fraser et al., 2016; Zhang and Folta, 2012). In addition, decreased UV-A and blue light content can also mediate shade avoidance responses via CRYs accumulating and physically interacting with PIFs (Pedmale et al., 2016a). Therefore, the deactivation of cryptochromes by GL exacerbates the shade avoidance type response (Fraser et al., 2016) possibly contributing to the results under HSP-HB. It would be of interest to address in future experiments the effects of GL on Basil growth and development, and the inclusion of GL into commercial spectra. Particularly as green wavelengths can also aid the grower in inspection of the crop, making the identification of disease and malnutrition easier.

As for antioxidant content, carotenoid, tocopherol and tAA content was highest in HSIP ($B_{15}G_{16}R_{69}$), which has one of the lowest R:FR ratios (Figure 3.5d and 3.9d). Despite the strong associations of AA content with high intensity light (Bartoli et al., 2006; Massot et al., 2012), in my experiment, R:FR ratio was the most prominent factor for predicting AA content among the commercial spectra. This is evidenced particularly by AP673L, which generated the second highest tAA concentration of the four spectra, despite delivering the lowest intensity light but,

similar to HSIP, consisted of a low R:FR ratio. This is further supporting evidence of FR acting as a positive control for ascorbic acid content in Basil. Unlike tAA, the elevated levels of carotenoids under HSIP (Figure 3.5d) does not match with decreased carotenoid content found in the FR supplementation experiments performed at Lancaster University (Figure 3.7a). However, the R:FR ratio of HSIP was 8.5, not as low as the 1.0 ratio used in the FR supplementation experiment and therefore the effect on secondary metabolite production was likely mitigated. Moreover, evidence suggests that inclusion of FR can increase photosynthetic efficiency and decrease the energy load on PSII, therefore reducing inhibition and the requirement of non-photochemical quenching by carotenoids and scavenging of radicals by tocopherols (Zhen and van Iersel, 2017). In this way, mild FR supplementation can reduce turnover of photoprotective antioxidants and allow their accumulation.

Alternative factors remain temperature and light intensity, both of which can elevate antioxidant content (Wang and Zheng, 2001), but also the proportion of red and blue wavelengths can play a role in the type of antioxidants accumulated, for which red light can increase β-carotene content as seen in the light quality experiments conducted (Figure 3.6) and in agreement with the close relationship of phytochrome to carotenoid synthesis (Toledo-Ortiz et al., 2010; von Lintig et al., 1997). These environmental and spectral factors could also explain the similar carotenoid contents of HSP-HB and AP673L, which was unexpected due to the significantly higher light intensity of HSP-HB (257 $\mu mol.m^{-2}.s^{-1}$ and 154 $\mu mol.m^{-2}.s^{-1}$, respectively). For instance, the two degree increase in temperature for the AP673L grow area compared to HSP-HB, in addition to the greater R:B ratio (6.1 in AP673L verses 1.3 for HSP-HB), could have aided the accumulation of carotenoids to levels comparable to that of a higher intensity environment (Appendix Table 6.1.2). Whereas, HSIP also contains a higher proportion of RL to BL (R:B = 4.7) under a high intensity and therefore results in the maximal carotenoid content.

A final factor effecting carotenoid accumulation could be the GL content as seen by the measurements of NPQ a response associated with photoprotective carotenoids. The highest NPQt value was recorded in plants grown under the GE-HB spectra ($B_{40}G_{1}R_{59}$) (Table 3.10), in correlation with the highest total carotenoid content (Figure 3.10d). Whereas, HSP-HB ($B_{30}G_{33}R_{37}$) showed the lowest NPQt. Both spectra had very similar intensity, R:B values, and quantity of blue photons which is also associated with carotenoid biosynthesis (von Lintig et

al., 1997) (Appendix Table 6.1.2). However, the spectra HSP-HB and GE-HB differed drastically in GL content: GE-HB contained very little GL, in comparison to HSP-HB which contained nearly equal amounts of BL and GL. As stated previously GL has been shown to negatively effect secondary metabolite production in Basil (Dou, Niu and Gu, 2019a), and therefore this discrepancy between the two spectra could also have impacted on carotenoid accumulation. However this has not been studied and requires further scientific investigation.

Overall, my results show complexities of predicting how phytonutrients will respond in Basil plants when in a mixed spectral setting, and suggest that more research is required to untangle the interactive effects of spectra, intensity and temperature. Experimentation with the descriptive spectral metrics of R:B, B:G and R:FR will likely help in the interpretation of spectral effects.

3.5. CONCLUSIONS

This chapter achieved the aim of comparing the growth a phytonutrient content of Basil under several different lighting qualities. Importantly, it was discovered that monochromatic red light was detrimental for growth across developmental stages, resulting in elongated stem and poor leaf growth. It was found that, similar to the published literature, Basil showed consistent fresh weight gain and leaf area under predominantly RL mixes, with a contributing proportion of BL in the range of 20 %, which in the case of these experiments was under $B_{25}R_{75}$. In addition, α-tocopherol and AA content was elevated under $B_{25}R_{75}$, similar to the 20% BL requirement found within literature to elevate phenolic, flavonoid and antioxidant content. Significantly, evidence indicated a strong influence of phytochrome with AA content and R:FR ratio that is of interest for further study. Comparatively, carotenoid content was most strongly accumulated under $B_{50}R_{50}$, demonstrating a requirement of both phytochromes and cryptochromes. My results also hinted at an important role of GL on yield gains and secondary metabolite production in Basil, which should be explored further. Greater resolution into these responses could be gained by measuring other light impacted metabolites, such as essential oils, or by undertaking molecular measurement of the genetic response of phytonutrient biosynthesis genes in Basil. Moreover, whilst these results apply to Basil cv. 'Sweet Genovese', further research with multiple cultivars and species will clarify responses that are cultivar specific or variable depending on species.

CHAPTER 4

Light Effects on The Growth and Vitamin Content of High Value Herb Coriander (*Coriandum sativum*)

4.1. INTRODUCTION

The cultivated herbal plant Coriander (*Coriandum sativum* L.) is an annual umbellifer plant within the *Apiceate* family (Bhat et al., 2014). The whole of the plant can be consumed from root to stem, including leaves and fruits (seeds), each with a characteristic aroma (Sharma and Sharma, 2012). Native to Italy, the plant is grown commercially in numerous countries in North Europe, North Africa and Asia due to its high value within cosmetics and as a flavouring agent (Sahib et al., 2013). Culinary uses for Coriander have been found in 5th Century Chinese cookbooks (Diederichsen, 1996), and today Coriander is used in dishes worldwide as a garnish or ingredient ranging from salads to curries and chutneys (Bhat et al., 2014; Diederichsen, 1996). Whilst widely consumed for its unique taste, Coriander also has the potential for promoting well-being through its high content of bioactive compounds, including essential oils, tocopherols, carotenoids and vitamins (Macleod and Islam, 1976; Sahib et al., 2013).

4.1.1. Uses for Secondary Metabolite Production in Coriander

Coriander has been used medicinally since ancient times with references found in Egyptian papyrus and classical Greek and Latin literature (Diederichsen, 1996). The herb is still used today in traditional medicines to treat mild illnesses from coughs and abdominal discomforts, to more serious uses as an antiseptic and to treat dysentery (Gantait et al., 2022; Sahib et al., 2013). Modern science has confirmed that Coriander extracts have antimicrobial, anti-anthelmintic, antioxidant, anti-diabetic, diuretic, anti-inflammatory, and anti-anxiety effects (Bhat et al., 2014; Freires et al., 2014; Sahib et al., 2013; Singletary, 2016).

These beneficial effects on human health are attributed to the high contents of polyphenols, essential oils and vitamins in Coriander (Nadeem et al., 2013). A rich mixture of phenols and aldehydes has been detected by HPLC coupled to mass spectrometric detection (Kaiser et al., 2013; Sahib et al., 2013). The main essential oil of Coriander is linalool (60 – 80 %), but content can vary depending on the region of origin, cultivar and plant maturity (Sahib et al., 2013; Singletary, 2016). Of particular interest is the content of phytonutrients in the leaf such as pro-vitamin A (β-carotene) at comparative levels to tomato (Kandlakunta et al., 2008), in addition to significant Vitamin C (tAA) content and sources of α-tocopherol (Table 4.1) (Bhat et al., 2014; Singletary, 2016). Due to the antioxidant properties of each of these compounds (see Section

1.2.2), they are associated with some of the beneficial health effects of Coriander (Sahib et al., 2013). Because of this, interest and scientific study into Coriander is focused on methods of increasing productivity and quality through agri-biotechnological intervention (Gantait et al., 2022).

Lutein (+ zeaxanthin)	β-carotene	α-tocopherol	Ascorbic Acid	Source
4.7 ± 0.4	9.5 ± 1.1			Daly et al., 2010
	98.7 ± 17.6	22.6 ± 1.0	15.9 ± 2.2	Dias et al., 2011
		2.50		Saini and Keum, 2016
	6.1 ± 0.1		98.1 ± 0.3	Singh et al., 2001
	0.16 ± 0.0			Kandlakunta et al., 2008

Table 4.1 Contents of phytonutrients of interest (mg/100g) in mature Coriander taken from the literature.

4.1.2. The Effect of Light on Coriander Growth and Phytonutrient Content

Overseas production of Coriander is marked by an undesireable lack of leaf and abundance of stem for marketable packs. Importantly, hydroponic production under LEDs presents an opportunity to increase the leaf quality of Coriander produced locally. Previous studies on Coriander have examined the aromatic differences between seeds and leaves (Shahwar et al., 2012), or wild and cultivated varieties (Eyres et al., 2005), and a small number have looked at the effects of the environmental growth conditions on both physiology and the composition of secondary metabolites (Table 4.2 and 4.3). Overall, Coriander shows stronger growth responses to greater proportions of RL including increased stem length and biomass gains (de Clercq and van Labeke, 2022; McAusland et al., 2020; Naznin et al., 2016; Nguyen et al., 2020; Ohashi-Kaneko et al., 2013). However, evidence shows that BL may be more stimulatory for secondary metabolites, including chlorophyll content and flavonol index (de Clercq and van Labeke, 2022; Matysiak and Kowalski, 2021), as well as antioxidant capacity (Naznin et al., 2016; Nguyen et al., 2020). It is interesting to note that Coriander may be particularly sensitive to the addition of FR and UV light in height and secondary metabolite regulation, respectively (Fraser et al., 2017; Matysiak and Kowalski, 2021).

Cultivar	Light Condition	Parameter	DLI (mol.m^{-2}.day^{-1})	Plant Age	Source
Cruiser	WL WL + UV-B WL + FR WL + FR + UV-B	H	3.0	4 weeks	Fraser et al., 2017 $^{+}$
N/D	R_{100} B_{100} G_{100} $B_{13}R_{87}$ $B_{12}R_{82}Fr_{6}$	H FW DW LA	11.5	4 weeks	Nguyen et al., 2020
	Fluorescent R_{100} B_{100} BR	FW DW H L# L#	11.5	N/D	Ohashi-Kaneko et al., 2013
	$B_{17}R_{83}$ $B_{9}R_{91}$ $B_{5}R_{95}$	L# FW DW FW DW	6.9	4 weeks	Naznin et al., 2016

	R_{100}	H			
	W_{100}	LA	4.6	12 weeks	Matysiak and Kowalski, 2021 +
	$B_{50}R_{40}W_{10}$				
	$B_{20}R_{70}W_{10}$	LA			
	$B_{20}R_{70}W_{10}$ + FR	FW H LA			
	$B_{66}R_{33}$		14.1	6 weeks	de Clercq and van Labeke, 2022 +
	$B_{50}R_{50}$				
	$B_{33}R_{66}$				
	$B_{20}R_{80}$	H			
Green Aroma	R_{100}	H LA FW DW L#	8.6	3 weeks	McAusland et al., 2020 +
	B_{100}				
	BR	LA			
	$B_{38}G_{26}R_{36}$	LA			
Slow Bolt	W	H	3.0	4 weeks	Fraser et al., 2017 +
	W + UV-B				
	W + FR				
	W + FR + UV-B				

Table 4.2 LED light conditions and maximised growth measurements of various Coriander cultivars. Light condition notation is divided into blue (B), green (G), red (R) or white (W) light proportion with the percentage value in subscript (i.e. 38% blue, 26% green and 36% red light, $B_{38}G_{26}R_{36}$), and is accompanied by the daily light integral (DLI). The parameter abbreviation is noted against the light quality in which the highest value was recorded. Plants were grown hydroponically unless indicated that they were grown in soil ($^{+}$). B, Blue light; BR, blue red light; DLI, Daily light integral DW, Shoot Dry Weight; FW, Shoot Fresh Weight; FR, Far red light; G, Green light; H, Stem Length; LA, Leaf Area; L#, Leaf Number; N/D, Not Disclosed; R, Red light; W, White light; UV-B, Ultraviolet-B.

Cultivar	Light Condition	Parameter	DLI ($mol.m^{-2}.day^{-1}$)	Plant Age	Source
N/D	R_{100} B_{100} G_{100} $R_{87}B_{13}$ $R_{82}B_{12}Fr_{6}$	AO TPC AA Chl AA Chl	11.5	4 weeks	Nguyen et al., 2020
	$R_{83}B_{17}$ $R_{91}B_{9}$ $R_{95}B_{5}$ R_{100}	AO	6.9	4 weeks	Naznin et al., 2016
	W_{100} $R_{40}B_{50}W_{10}$	Chl Chl FI	4.6	12 weeks	Matysiak and Kowalski, 2021

	$R_{70}B_{20}W_{10}$ $R_{70}B_{20}W_{10}$ + FR				
	$R_{33}B_{66}$ $R_{50}B_{50}$ $R_{66}B_{33}$ $R_{80}B_{20}$	Chl FI TPC TPC Chl	14.1	6 weeks	de Clercq and van Labeke, 2022
Slow Bolt	W W + UV-B W + FR W + FR + UV-B	AO	3.0	4 weeks	Fraser et al., 2017 [+]
Green Aroma	R_{100} B_{100} RB $R_{36}G_{26}B_{38}$	 Aromatics	8.6	3 weeks	McAusland et al., 2020 [+]

Table 4.3 LED light conditions that maximise the antioxidant, phenolic, and flavonoid content of various Coriander cultivars. Light condition notation is divided into blue (B), green (G), red (R) or white (W) light proportion with the percentage value in subscript (i.e. 38% blue, 26% green and 36% red light, $B_{38}G_{26}R_{36}$), and is accompanied by the daily light integral (DLI). The parameter abbreviation is noted against the light quality in which the highest value was recorded. Plants were grown hydroponically unless indicated that they were grown in soil ([+]). AA, Ascorbic Acid; AO, Antioxidant Capacity; Chl, Total Chlorophyll; TPC, Total Phenolic Content; FI, Flavonol Index.

4.1.3. Aims and Objectives

In this chapter, the growth and production of carotenoids, tocopherols and AA in Coriander cv. '*Cruiser'* is compared when grown in different controlled environments and under monochromatic, dichromatic and mixed spectral light qualities. The content of these phytonutrients was measured in relation to the light spectrum to advance knowledge of *C. sativum* light responses with the aim of improving commercial production using a lighting recipe. Therefore, the objectives of this chapter are:

1. To compare growth and phytonutrient content of Coriander grown in different controlled environment agricultural systems.
2. To determine an optimal blue and red ratio for Coriander growth as seedlings and mature plants, in addition to phytonutrient content at harvest.
3. To explore the effect of supplementary lighting with UV-B and FR wavelengths located outside of PAR on Coriander growth and nutritional content.
4. To quantify the effect of spectral mixes delivered by four commercial luminaries on Coriander development in a commercial setting.

4.2. METHODS

4.2.1. Experiment Comparing Coriander Grown in Controlled Environments

Twenty-eight Coriander cv. '*Cruiser*' (CN seed, Lot no. 45156) plants each were sown and grown in GH. Sn and Hp environmental conditions as described in Section 3.2.1. The only difference was that after 2 weeks, Coriander plants were thinned to one pair of plants originating from the same seed per pot. After 4 weeks plants were harvested for analysis as described in Section 4.2.6. A spectral breakdown for all treatments is available in Appendix Table 6.1.2.

4.2.2. Experiment on Seedlings in Monochromatic and Dichromatic Light

Coriander seeds were sterilised with 10% (v/v) bleach treatment and 2% (v/v) tween20 for 8 minutes before being washed a minimum of 5 times with RO water. Seeds were sown directly onto germination media (Conn et al., 2013) within 9cm plates. Five seeds were sown per plate with equidistant spacing. As each seed contained two mericarps, this equated to sowing 10 potential seedlings per plate. Plates were sealed with Micropore tape and light treatments were applied as per Section 3.2.2. Measurements of the seedlings included percentage seedling survival at the point of measurement, fresh weight of each shoot using a microbalance, length of the hypocotyl measured using ImageJ, and assessment of the growth stage via a numeric key (Table 4.4). During the experiment, the plates were rotated three times a week to accommodate for lighting discrepancies. The experiment was repeated three times.

#	Key
1	Cotyledons unemerged from seed
2	Cotyledons fully emerged
3	First True leaf
4	Two True leaves

Table 4.4 Key for number code used to assess the development stage of Coriander seedlings grown for two weeks in plates.

4.2.3. Experiment on Mature Coriander Under Monochromatic and Dichromatic Light

Coriander plants were grown for 4 weeks in the self-built hydroponic system as described in Section 3.2.1, under monochromatic blue (B_{100}) and red (R_{100}), or dichromatic blue and red treatments at 75 % blue and 25 % red ($B_{75}R_{25}$), $B_{50}R_{50}$, $B_{25}R_{75}$. Experimental parameters were identical to Section 3.2.3, for a spectral breakdown see Appendix Table 6.1.2. The position of the pots was changed three times a week to account for positional effects. Measurements were collected according to 4.2.6. The experiment was repeated.

4.2.4. Experiment on Coriander with Supplementary Light Treatments

Far Red and UV-B treatments were applied as described in Section 3.2.4. Plants were sown and irrigated as described for the hydroponic system in Section 3.2.1. The position of the pots was changed three times a week to account for positional effects. Measurements were collected according to 4.2.6. The experiment was repeated.

4.2.5. Experiment on Commercial Spectra at May Barn Consultancy Ltd.

Samples were grown and harvested from May Barn Consultancy Ltd. in September 2020 on site in Nantwich as per Section 3.2.5. Coriander cv. *Cruiser* was grown under 4 horticultural LED spectra: Heliospectra Siera Propagation High Blue (HSP-HB), Heliospectra Siera Indoor Production (HSIP), Current by GE Arize Lynk Vegetative High Blue GEHL48HPPV (GE-HB) and Valoya L28 AP673L (AP673L). The light intensity and spectra composition within each level is recorded in Appendix Table 6.1.2.

4.2.6. Measurements Taken

Physiological Several physiological measurements were taken upon harvest. Each Coriander seed contains two mericarps that can produce a plant; in the event both mericarps germinated, the tallest of those was selected for measurement. Unless otherwise stated, data was collected on a total of 28 plants per experimental repeat as described in Section 3.2.6.

Photosynthetic Rapid light response curves, chlorophyll florescence and measurements with the MultispeQ device were all performed as recorded in Section 3.2.7.

Pigments Chlorophyll and carotenoid quantification was performed as described in Section 3.1.8.

UV-Spectrophotometry Samples were extracted and measured according to Section 3.1.9.

HPLC tAA was measured according to Section 3.1.10.

4.2.7. Statistical Analysis

A two-way ANOVA was used to determine significance of measurements between lighting treatments and experimental repeat, one-way ANOVAs were used when no repeated experimental data were available. The residuals of the models were checked for normality, and data was $\log_e$ transformed where appropriate to satisfy the normality assumption of the model. Estimated marginal means were calculated on original or back-transformed data and plotted data was on the original scale. A sidak post hoc test was used to assess the differences between groups. Statistical tests were performed using R (RStudio, version 4.2.1).

4.3. RESULTS

4.3.1. Comparing Coriander Growth in Different Controlled Environments

Coriander was grown using three different controlled environment cultivation methods: GH, Sn and Hp (Figure 4.1). The light intensities of Hp and Sn conditions were matched at ~150 $\mu mol.m^{-2}.s^{-1}$, whereas the GH treatment was under daylight conditions which reached a maxima of 157 $W.m^{-2}$. All light qualities were broad spectra containing blue, red and green wavelengths, however the diversity of wavelengths increased across the conditions, with Hp containing the narrowest spectral bands and GH the widest (spectral qualities in Appendix Table 6.1.2). Plants were significantly taller when grown in the greenhouse and Snijder cabinet ($p < 0.001$). When observing fresh weight, Snijder-grown plants were heaviest, whilst the weight of the hydroponic-grown plants was comparable to the greenhouse ($p < 0.001$). Interestingly, despite treatment significantly affecting dry weights ($p < 0.001$), the percentage DW (calculated as shoot DW (g) divided by shoot fresh weight (g) multiplied by 100) was comparable between all the conditions (Appendix Table 6.1.4). This shows that similar biomass grew between treatments, and that differences in fresh weight are likely due to differential water retention within the plant shoots. Leaf number ($p < 0.001$), leaf area ($p < 0.001$) and leaf thickness ($p < 0.001$) was impacted significantly by the growth conditions (Figure 4.1c-e). Sn plants grew the highest number of leaves, which equated to the highest leaf areas. Whilst Hp plants grew fewer leaves than plants grown in GH and Sn, leaf areas were comparable across all growth conditions. This indicates that Coriander grown in hydroponics grew larger leaves, which were also thicker (Figure 4.1e), possibly due to the acute delivery of photosynthetically active blue and red wavelengths.

There was variation between the repeat experiments for all growth parameters measured ($p < 0.001$). Differences between repeat experiments was expected and found in GH-grown plants due to fluctuations in the environmental conditions. However, significant differences were also detected in plants grown in Sn, which were maintained a more controlled environment. Unfortunately, a secondary experiment in Hp conditions was not performed due to time constraints, so the question of which controlled environment produced more consistent growth cannot be fully explored. Overall, these initial results shows that the growth of Coriander can be variable and may be sensitive to multiple environmental inputs, including

light, nutrient availability and temperature. Nevertheless, the experiment demonstrates that hydroponics are a viable method of growing Coriander, generating fresh weight and leaf area yields comparable to a greenhouse-grown crop.

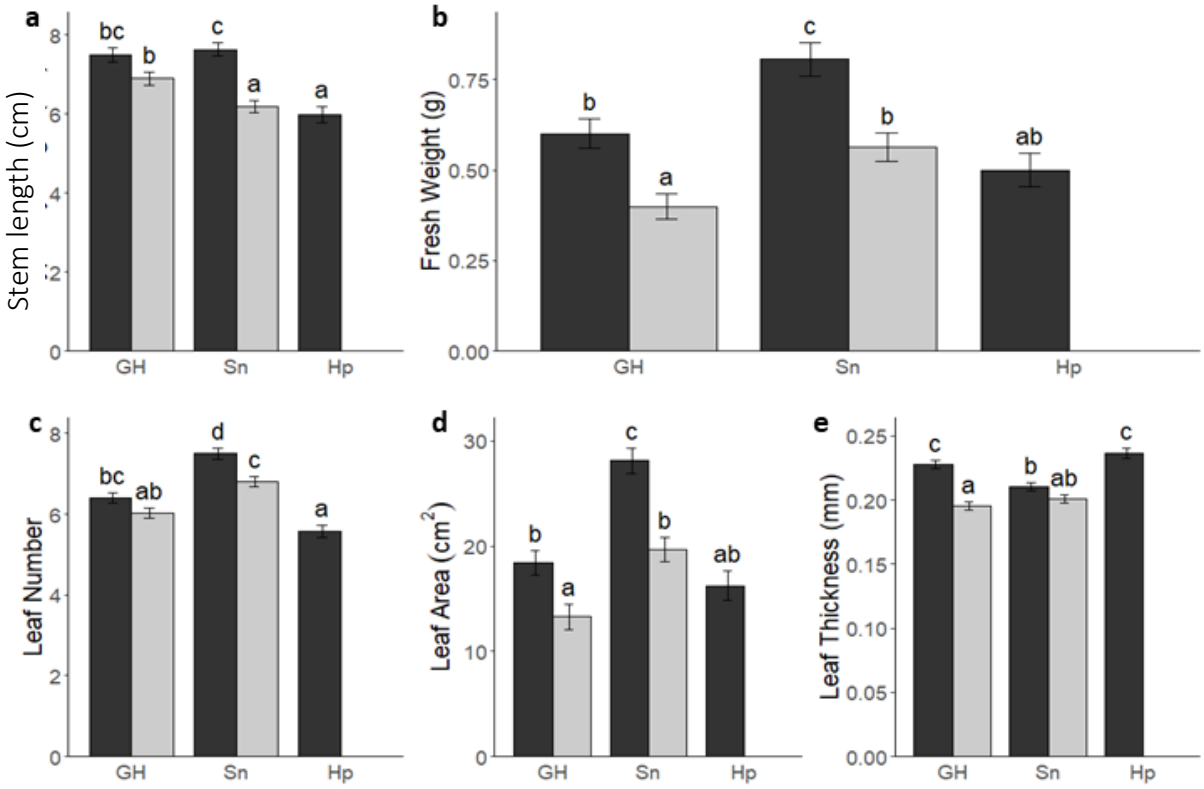


Figure 4.1 The controlled environment growth method on the growth of coriander. Coriander grown in soil within a greenhouse between April and May under supplementary white light LEDs (GH) or Snijder cabinet with fluorescent lights (150 µmol.m^{-2}.s^{-1}) (Sn), was compared against hydroponically (Hp) grown coriander grown under a commercial LED fixture (150 µmol.m^{-2}.s^{-1}). All treatments used a long day (16 hr) photoperiod, and lasted 4 weeks. Physiological measurements include, (a) stem length (cm), (b) fresh weight (g), (c) total leaf number, (d) leaf area (cm^2), and (e) leaf thickness (mm). Dark grey bars display estimated marginal means from the first experimental repeat, and light grey bars for the second (n = 28 per repeat). Error bars represent standard error. Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

4.3.2. Observing Responses to Light Quality in Seedling Physiology

Blue and red wavelengths contain energy for photosynthesis and have important photomorphogenic effects. To ascertain their impact on Coriander at different life stages, blue and red wavelengths were delivered as monochromatic and dichromatic treatments. The seedling stage of Coriander was assessed by germinating and growing seeds for 2 weeks under five different ratios of blue and red light, with three levels of intensity: low, medium and high (detailed in Table 3.4). The effect on seedling establishment and growth in terms of hypocotyl elongation, fresh weight gain and developmental progress was assessed. Results were analysed with a two-way ANOVA and sidak post hoc test.

Seedling Establishment. Seedlings were counted as established if they survived to the point of measurement, which varied drastically from 23 to 91 %. The lowest percentage rates were found in $B_{75}R_{25}$ and $B_{50}R_{50}$. Both B_{100} and R_{100} were relatively equal, but the highest success rate occurred in $B_{25}R_{75}$, suggesting that Coriander seedling growth is more successful in light qualities with higher red light content but that blue light is also required. There was little difference between the intensity within the light qualities tested for the successful establishment of Coriander (Table 4.5).

Intensity	B_{100}	$B_{75}R_{25}$	$B_{50}R_{50}$	$B_{25}R_{75}$	R_{100}
Low	60%	30%	35%	86%	62%
Medium	68%	23%	38%	79%	68%
High	67%	42%	53%	91%	47%

Table 4.5 Percentage of Coriander seedlings established after two weeks of growth in a controlled-environment cabinet under different light qualities and intensities (See Table 3.4). Seeds were sown on germination media within plates.

Hypocotyl Elongation. Light intensity had an impact on seedling height ($p < 0.001$; Figure 4.2a). Increases in intensity caused height decreases in the light qualities of R_{100}, $B_{25}R_{75}$ and $B_{50}R_{50}$. Intensity did not change seedling height for B_{100} and little change was also seen in $B_{75}R_{25}$. Comparing the dichromatic treatments only, hypocotyls were shortest in the highest intensity blue content, $B_{75}R_{25}$. However, seedlings were taller under monochromatic conditions over dichromatic conditions, with B_{100} comparable to R_{100}. Overall, spectra had a

significant impact on hypocotyl length ($p < 0.001$), and the longest plants were grown in monochromatic conditions (Appendix Figure 6.1.11).

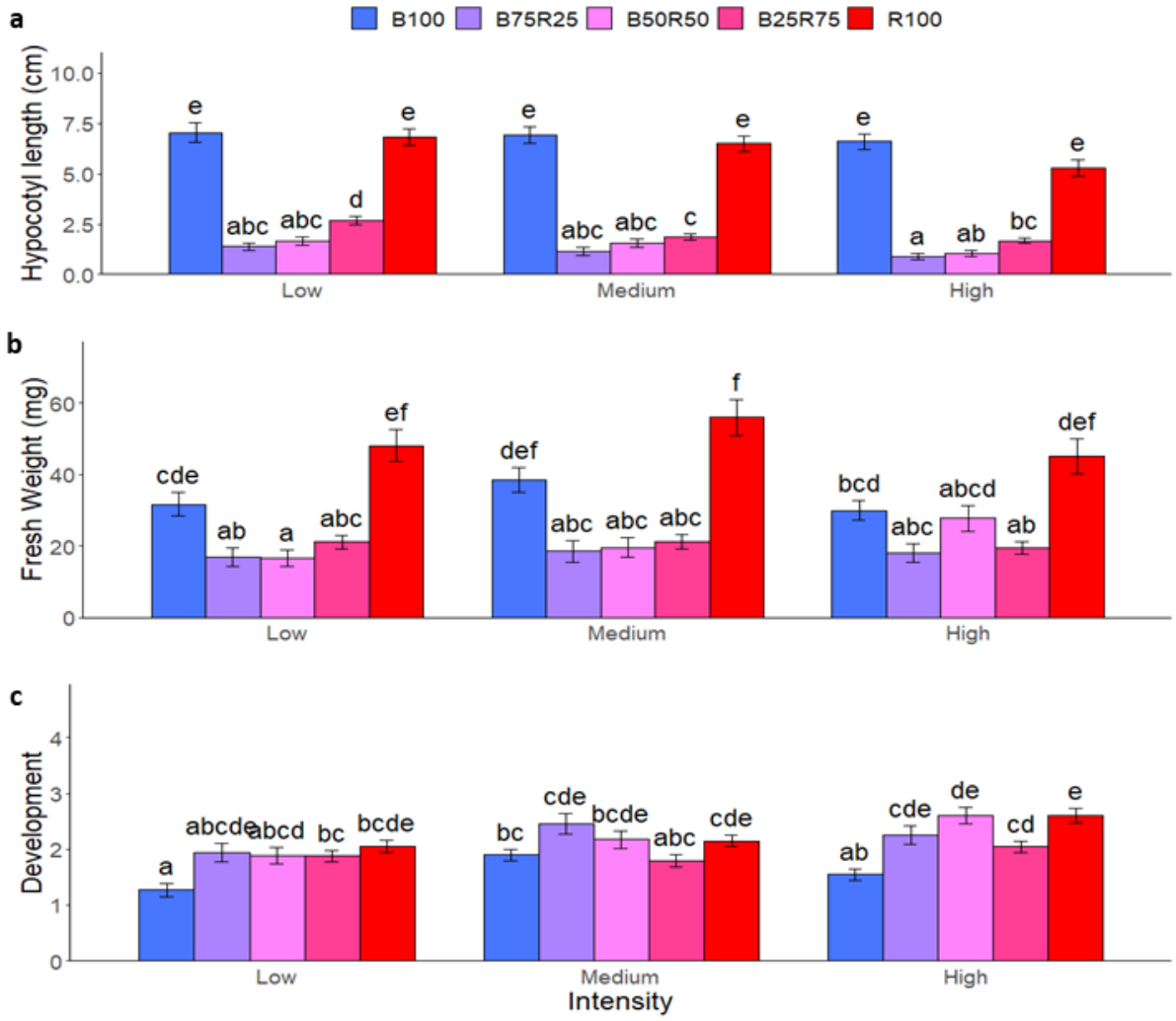


Figure 4.2 Blue and red light mixes required for growth of coriander seedlings. Measurements of (a) hypocotyl length (cm), (b) fresh weight of the shoot (g), and (c) development of coriander seedlings grown for 14 days under (i) 100% blue light (B_{100}), (ii) 75% blue and 25% red ($B_{75}R_{25}$), (iii) $B_{50}R_{50}$, (iv) $B_{25}R_{75}$, and (v) R_{100}, under low, medium and high intensities (from left to right). The intensities used (Table 3.4) and growth stage key (Table 4.4) can be found in the methods. Error bars represent standard error (n = 60). Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p< 0.05$). Data that share a letter are not statistically significant.

Biomass accumulation. Seedling fresh weight did not significantly alter with light intensity across all spectra tested. However, the ratio of blue and red wavelengths significantly affected seedling fresh weight ($p < 0.001$), with heavier seedlings found under the monochromatic blue

and red conditions. Within the dichromatic treatments, $B_{25}R_{75}$ tended to grow heavier seedlings, with the exception of the highest intensity for which $B_{50}R_{50}$ had the heaviest seedlings (Figure 4.2b). In addition, whilst not statistically significant, both monochromatic conditions gained slightly more weight under the medium intensity relative to the low and high.

Development. Development assessment was based on leaf emergence and growth as described in Table 4.4. The seedlings became increasingly developed under greater light intensity ($p < 0.001$, Figure 4.2c) and this varied between light quality ($p < 0.001$). An interaction was seen between the two factors ($p < 0.01$) meaning that the difference in means between the intensity levels is different depending on the lighting spectra, and there were few significant differences found between the treatment groups by Sidak posthoc test (Figure 4.2c). In low intensity light levels, B_{100} seedlings were least developed and there were little differences between the other spectra. At medium intensity, seedlings were most developed in $B_{75}R_{25}$, and interestingly, at the higher intensity, the most advanced development was seen in R_{100} and $B_{50}R_{50}$. Both of these light qualities showed a positive correlation between developmental response and intensity however, this trend was not statistically significant.

Overall, these results show a requirement of both wavelengths for proper deetiolation the absence of which results in elongated growth causing heavier seedling fresh weights. Monochromatic blue light was the most detrimental to seedling growth, but under high light intensity red light can generate comparable growth to dichromatic conditions, showing that red light is important to Coriander seedling growth and development.

4.3.3. Light Quality Influences Growth Parameters in Mature Coriander

Based on observations of effects of light quality on Coriander seedling growth and development (see Section 4.3.2), Coriander was grown to maturity (4 weeks) under the same ratios of blue and red at a light intensity of 150 $\mu mol.m^{-2}.s^{-1}$, and measurements on growth were recorded, including stem elongation, biomass accumulation and leaf anatomy. The experiment was repeated, and the trend was consistent trend between repeats, particularly in the $B_{25}R_{75}$ and R_{100} treatments (Figure 4.3).

Stem elongation. The length of Coriander stems varied by light quality ($p < 0.001$). Similar to the seedlings, there was low variation between stem lengths in the dichromatic mixes and longer lengths were seen in the monochromatic red and blue wavelengths (Figure 4.3a). Of the three dichromatic mixes tested, $B_{50}R_{50}$ showed the longest stems when averaging between the experimental repeats. There was variation between the repeats ($p < 0.001$), and significant variation within repeats for R_{100} and B_{100} according to a Sidak post hoc test.

Biomass accumulation. The variation within experimental repeats was observed in fresh weight ($p < 0.001$), and was most pronounced in B_{100}, $B_{75}R_{25}$ and $B_{50}R_{50}$ (Figure 4.3b). Across these light conditions, fresh weight increased with increasing R light fraction, to reach the heaviest weight for Coriander plants in the second repeat of $B_{50}R_{50}$ ($p < 0.01$). Despite variation between repeats in stem length, $B_{25}R_{75}$ and R_{100} showed consistency in fresh weight between repeats, producing fresh weights to match the average of $B_{75}R_{25}$. However, DW varied by treatment (<0.05) and repeat ($p < 0.001$) with the highest DW % in $B_{50}R_{50}$ and $B_{25}R_{75}$ around 3 times greater than the lowest percentage in the B_{100} and R_{100} conditions (Appendix Table 6.1.4). This is further evidence to show the importance of both blue and red light in the growth of Coriander.

Leaf anatomy. In general, blue light increases branching and leaf expansion (Ohgishi et al., 2004), and in this experiment, the ratio of blue light influenced leaf number in Coriander ($p < 0.001$). In general, leaf number was highest in $B_{75}R_{25}$ and $B_{50}R_{50}$, but lowest under B_{100} (Figure 4.3c). The effect of light quality was also reflected in leaf area ($p < 0.001$, Figure 4.3d), but the results were not consistent and an effect of the experiment repeat was seen ($p < 0.001$). $B_{50}R_{50}$ registered the greatest leaf area, and lowest leaf area was in B_{100}. There was very little variation in the thickness of leaves, however both light quality and experiment repeat was statistically significant ($p < 0.001$ and $p < 0.05$, respectively). Interestingly, a significant drop in leaf thickness was recorded in the second experimental repeat of B_{100} and R_{100}, the same repeat that had elongated stems (Figure 4.3e) showing the plants in these repeats had both elongated stems and leaves.

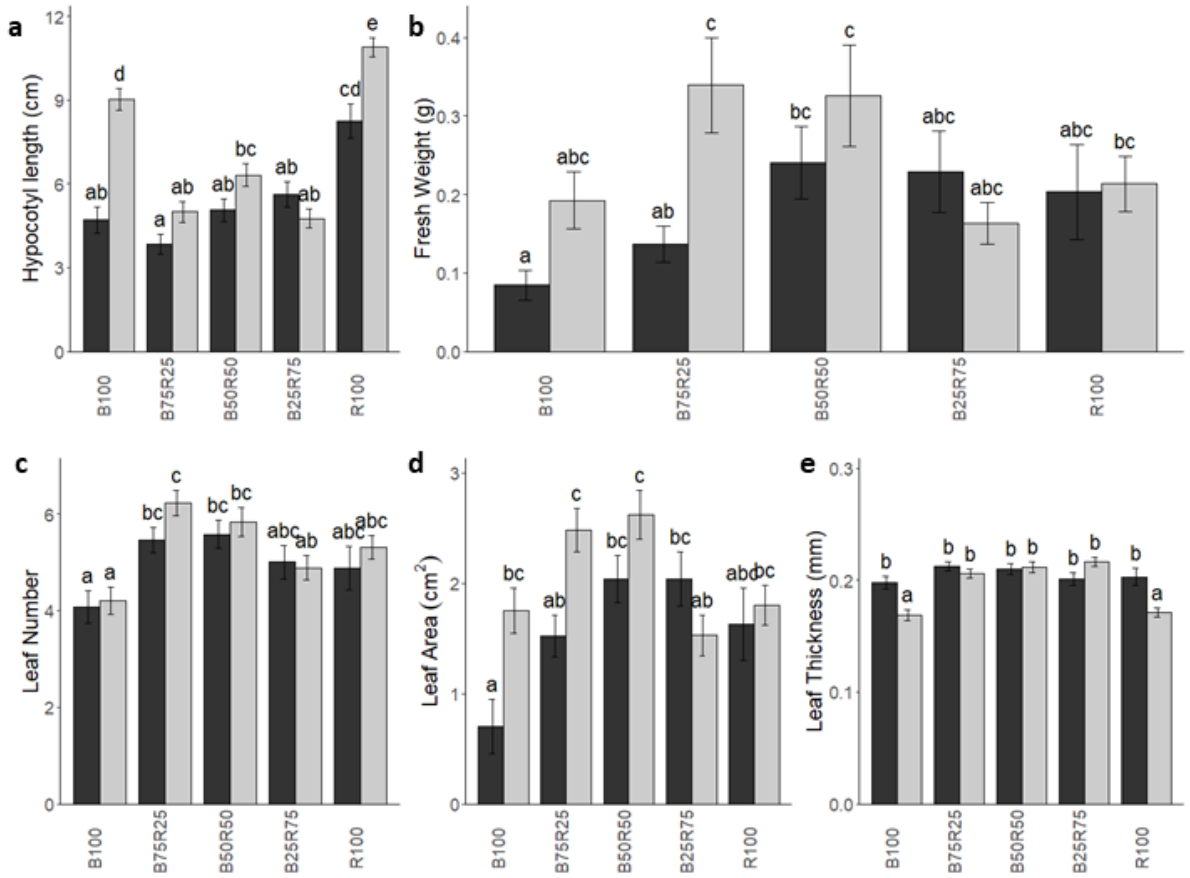


Figure 4.3 The ratio of blue and red wavelengths affects the growth of mature Coriander plants. Physiological measurements including (a) stem length (cm), (b) fresh weight (g), (c) total leaf no, (d) leaf area (cm^2), and (e) leaf thickness (mm) of mature coriander grown for 4 weeks under 100% blue light (B_{100}), 75% blue and 25% red light ($B_{75}R_{25}$), $B_{50}R_{50}$, $B_{25}R_{75}$ and R_{100} light conditions (150 μmol.m^{-2}.s^{-1}; 16/8 L/D). Dark grey bars display estimated marginal means from the first experimental repeat, and light grey bars for the second (n = 28 per repeat). Error bars represent standard error. Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

4.3.4. Investigating the Growth Response of Coriander to Supplementary Lighting Outside of PAR

In order to address whether supplementary light regimes with UV-B and FR light could have effects on mature Coriander, an experiment was set up where Coriander plants were grown under GE commercial LED lighting supplemented with either UV-B fluorescent lighting or FR LED lighting in the final days of the grow cycle. To investigate the propensity of these wavelengths to alter plant morphology, the effect of 6.48 $kJ.m^{-2}.day^{-1}$ UV-B radiation and R:FR ratio of ~1 was determined on stem extension, leaf physiology and biomass (Figure 4.4, Appendix Table 6.1.4). With the exception of height ($p<0.05$), no significant effect of light on the measured physiological attributes was found by two-way ANOVA. No effect of experimental repeat was observed, demonstrating that the results were comparable between experiments.

Observation of the trends shows that stem lengths were longer with the addition of both UV-B and FR in comparison to their controls, with a corresponding increase in fresh weight for UV-B but not for FR (Figure 4.4a and b). Leaf numbers were essentially identical between the treatments; however, there was a slight tendency towards higher leaf areas with additional UV-B, and lower leaf area with supplementary FR (Figure 4.4c and d). No observable differences could be seen in leaf thickness (Figure 4.4e). Overall, these results demonstrate that supplementary lighting can impact Coriander growth, however the effect is not significant which could either be due to an effect of the applied dose or responsiveness of Coriander to these wavelengths.

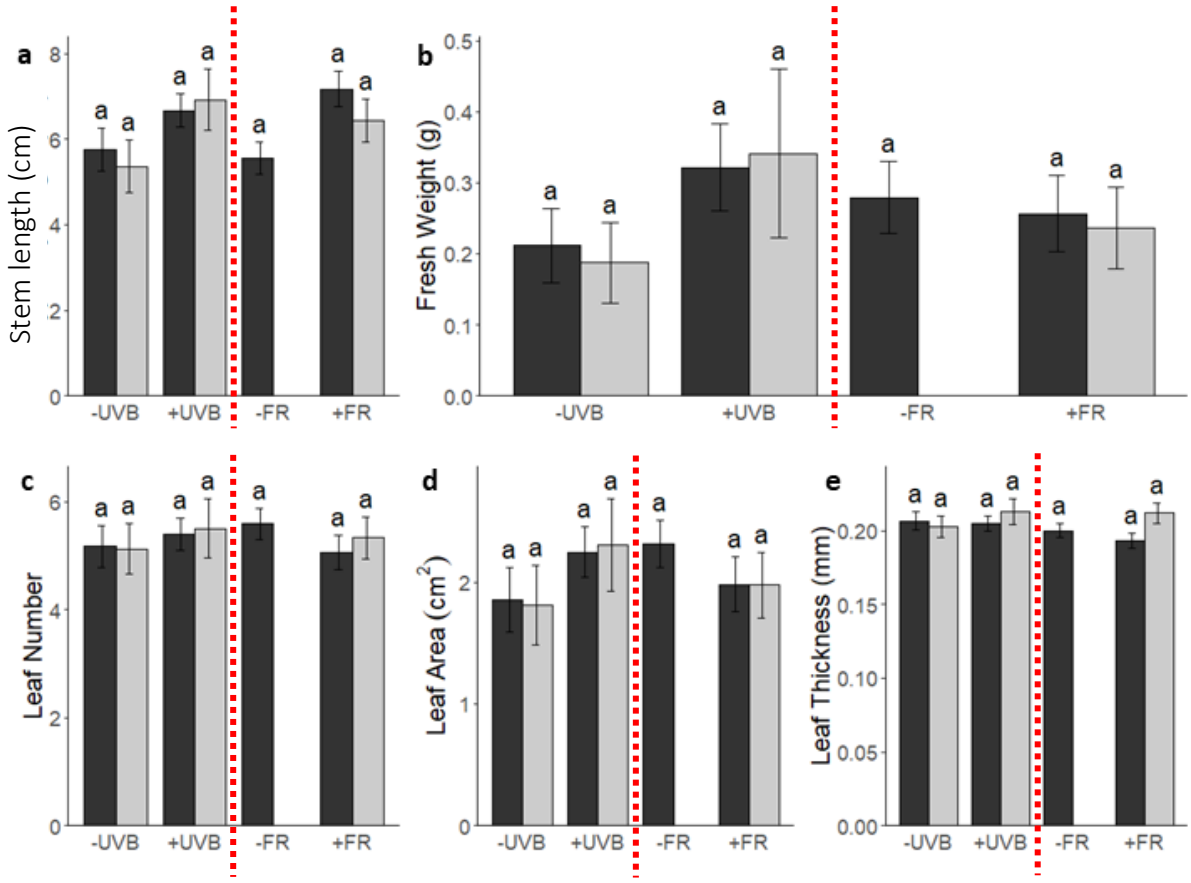


Figure 4.4 Coriander growth was unchanged under supplemental UV-B and FR. Mature coriander under GE lighting (150 µmol.m^{-2}.s^{-1}) supplemented with UV-B fluorescent tubes (6.48 kJ.m^{-2}.day^{-1}, delivered as 4 h treatment at midday) for 7 days or filtered (SC50, LEE Filters) GE lighting (150 µmol.m^{-2}.s^{-1}) supplemented with FR LEDs (R: FR =1) for 10 days before harvest. Data shows physiological measurements of (a) stem length (cm), (b) fresh weight (g), (c) total leaf no, (d) leaf area (cm^2), and (e) leaf thickness (mm). Error bars represent standard error. Statistical analysis was performed with a two-way ANOVA, and statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

4.3.5. Choice of Commercial Spectra Influences Coriander Growth

The previous experiments with blue and red ratio (Section 4.3.2 and 4.3.3) showed an effect on Coriander growth, therefore it was of interest to test commercial LEDs with different light composition to establish their effect on Coriander production. To do this, Coriander cv '*Crusier*' were grown at May Barn Consultancy Ltd. within a multilevel vertical racking system under four commercial LED horticultural fixtures: two Heliospectra luminaries (HSP-HB and HSIP), one GE luminaire (GE-HB), and finally a luminaire from Valoya (AP673L). The composition of these spectra were unique, varying in R:B ratio and the proprtion of GL or FR light. The lights were set to similar intensity (~250 µmol.m^{-2}.s^{-1}) with the exception of AP673L which was 100 µmol m^{-2} s^{-1} lower (spectral details listed in Appendix Table 6.1.2). Figure 4.5 and Appendix Table 6.1.4 show the physiological measurements of Coriander grown under these spectra. The differences in spectral quality had a significant effect on height ($p < 0.001$), fresh weight ($p < 0.001$), dry weight ($p < 0.01$), and leaf number ($p < 0.001$). The longest stems and highest fresh weights were found under the Heliospectra luminaries (HSP-HB and HSIP). HSP-HB also yielded the greatest leaf number but showed the largest spread in the data. This variation could be due to differences in light quality and intensity, which dropped off towards the edges, and was not controlled for as these plants were grown on a rockwool matt and were not moved throughout the experiment. Alternatively, the leaf growth could be effected by the spectrum of the incident light which can vary according to the thickness of the canopy, as red and blue wavelengths are absorbed with the remaining light consisting disproportionately of FR and green wavelengths. Interestingly, the GE-HB lights, although they were the highest intensity (267 µmol.m^{-2}.s^{-1}), did not grow stems or leaf numbers significantly different to AP673L at the lowest intensity (154 µmol.m^{-2}.s^{-1}), suggesting an effect of spectra. However, fresh weight of the GE-HB was significantly greater then AP673L according to Sidak post hoc test. Despite this, the AP637L has the greatest DW % of all spectra (Appendix Table 6.1.4). Whilst unable to be confirmed by leaf thickness measurements on the Coriander under these growth conditions, such a result could be due to increased expansion and turgidity of leaves in the higher intensity lighting conditions.

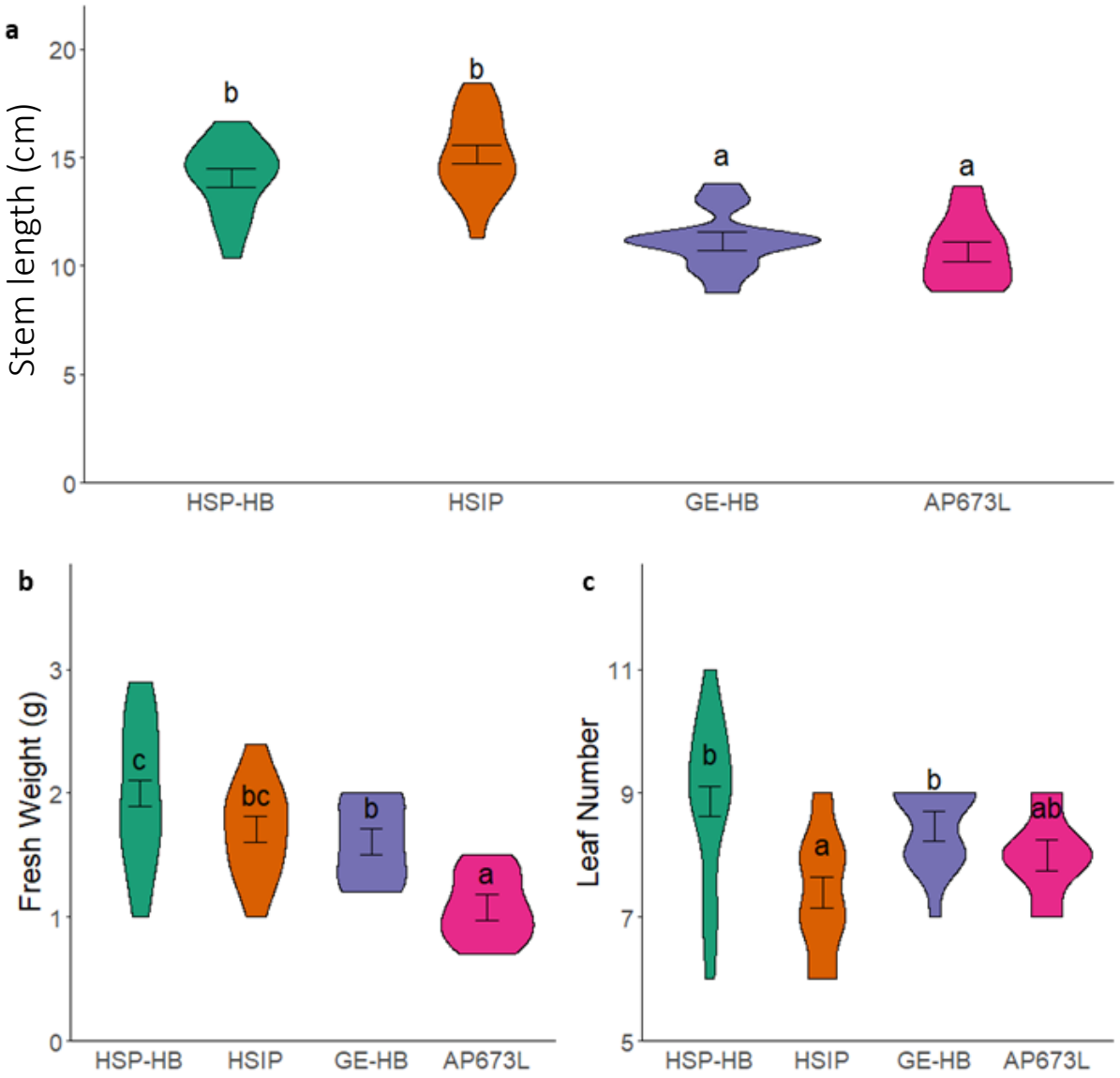


Figure 4.5 Commercial spectra altered the growth of coriander in a hydroponic system at May Barn Consultancy Ltd. Mature coriander under four commercial lighting spectra: Heliospectra Siera Propagation High Blue (HSP-HB), Heliospectra Siera Indoor Production (HSIP), Current by GE Arize Lynk Vegetative High Blue (GE-HB) and Valoya L28 AP673L (AP673L). Intensity and spectral breakdowns can be found in Appendix 6.1.2. Data shows physiological measurements of (a) stem length (cm); (b) fresh weight (g); and (c) total leaf no. Error bars represent standard error (n = 28). Statistical analysis was performed with a one-way ANOVA. Statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

4.3.6. Phytonutrient Content of Coriander Leaves is Influenced by Light Quality

Light intensity and spectral composition is linked to the production of chlorophylls and photoprotective antioxidants, in particular β-carotene, α-tocopherol and ascorbic acid, which are known to accumulate in Coriander (Table 4.1). Therefore, the nutritional content of Coriander was measured to explore the effect of light quality delivered by controlled environment cultivation, blue and red ratio, supplemental lighting and commercial luminaires (described in Section 4.2.1, 4.2.3, 4.2.4, and 4.2.5, respectively). The carotenoid and tocopherol content of mature Coriander leaves was quantified using UV-Spectrophotometry (Figure 4.6), tAA content was measured with HPLC (Figure 4.7), and chlorophyll and carotenoid content via microplate assay (Figure 4.8).

Carotenoids and Tocopherols When observing the effect of blue and red ratios, the total content of both carotenoids and tocopherols decreased as the percentage of red light increased ($p < 0.001$) (Figure 4.6b). This trend can clearly be seen in the monochromatic and dichromatic light conditions, where the highest concentration of both carotenoids and tocopherols was found in B_{100}, the lowest in R_{100}. Only $B_{50}R_{50}$ diverges from the trend, with a slight increase compared to the other dichromatic ratios (Figure 4.6b). These results demonstrate a positive response of MEP pathway derived vitamins to increasing blue light, but also suggest that equal proportions of blue a red have a cumulative effect.

Analysis of Hp Coriander was not performed due to time constraints. However, Sn plants had significantly higher total carotenoid content than GH plants, but did not differ in total tocopherol content ($p < 0.001$; Figure 4.6a). The light quality of the fluorescent bulbs in the Sn treatment was more consistent in addition to the environmental parameters, suggesting that carotenoid content could be more sensitive to these differences. Tocopherol content was also not responsive to the supplementary UV-B and FR lighting treatments, which did not differ compared to the control. However, carotenoids did decline with the addition of both supplementary wavelengths ($p < 0.001$; Figure 4.6c), suggesting that carotenoids are quite sensitive to environment and light quality.

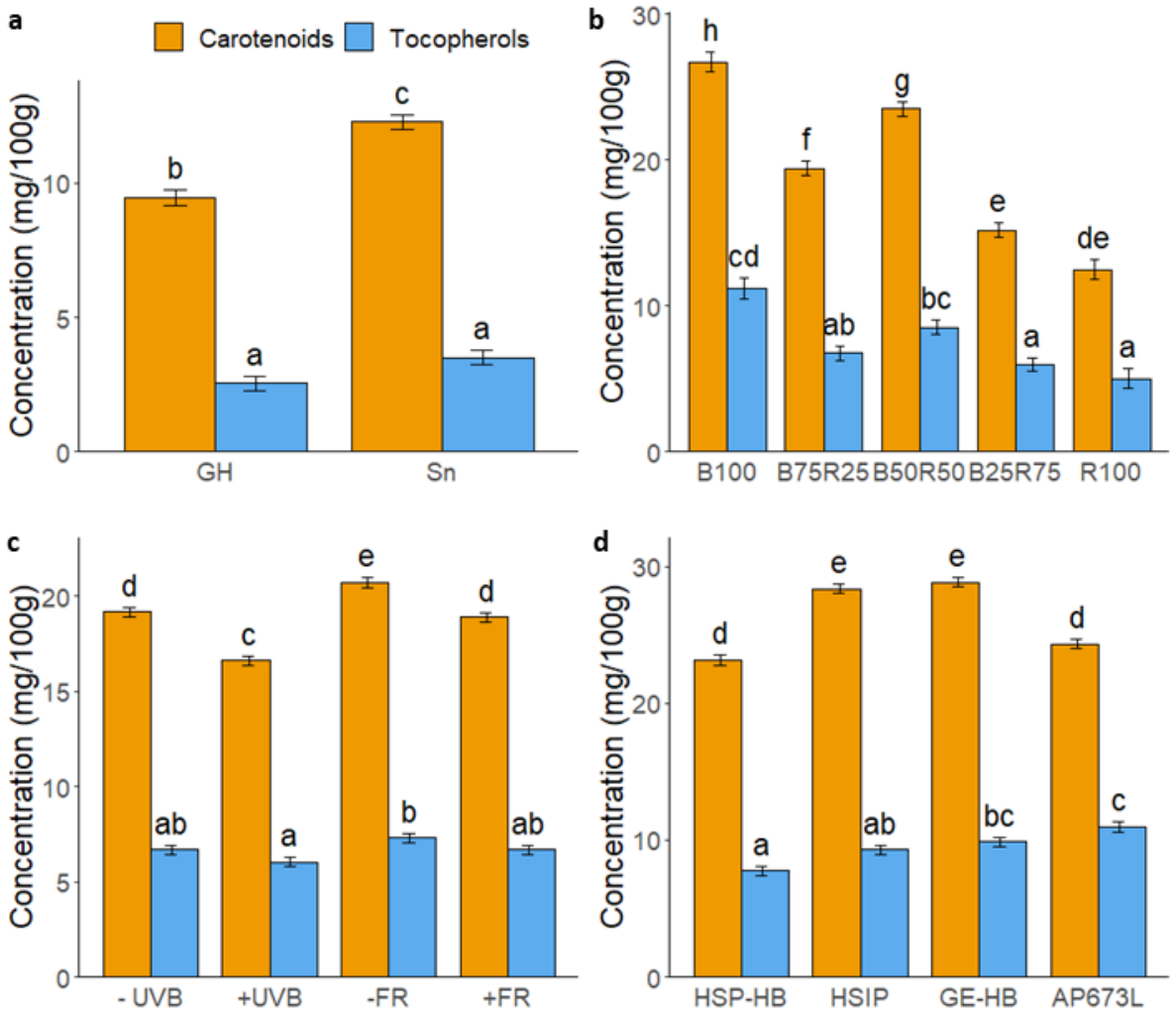


Figure 4.6 Concentration of total carotenoids and tocopherols in Coriander under experimental light conditions. Mature hydroponically-grown Coriander leaves were measured by UV-spectrophometer for total carotenoid and tocopherol content (mg/ 100 g DW) under different light treatments. Light regimes and experimental conditions are the same as described in (a) Figure 4.3, (b) Figure 4.1, (c) Figure 4.4, and (d) Figure 4.5. Bars display estimated marginal means (n = 4) and error bars represent standard error. Statistical analysis was performed with a two-way ANOVA, and statistical comparisons was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

Significant differences in both carotenoid and tocopherol content were seen in the samples grown under different LED spectra at May Barn Consultancy Ltd ($p < 0.001$; Figure 4.6d). The highest tocopherol content was seen in AP673L, and the lowest in HSP-HB. These spectra differed in intensity and red light proportion, for which AP673L and HSP-HB were lowest,

respectively. Both HSIP and GE-HB had similar concentrations of tocopherols and the greatest amounts of carotenoids. Interestingly, these two spectra differed greatly in R:B ratio, B:G ratio and R:FR ratio. Overall, these initial studies show that tocopherols are less sensitive to environmental and light inputs than carotenoids, which was effected by both. Tocopherols appeared to accumulate in lower light intensities and predominant blue light spectral contribution, similar to carotenoids.

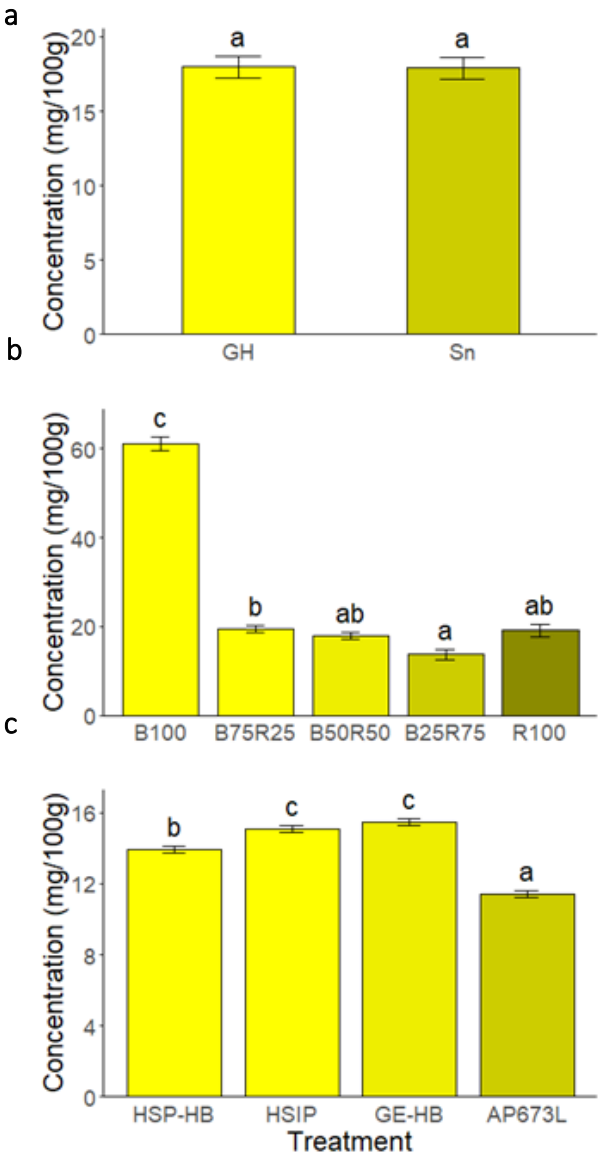


Figure 4.7 Total ascorbic acid content of Coriander varied under spectral qualities. HPLC measurements of total ascorbic acid (mg/100g DW) in mature Coriander leaves grown (a) in greenhouse (GH) or Snijder cabinet (Sn), (b) hydroponically under different ratios of blue and red light qualities, and (c) under four commercial LED lighting spectra. Light regimes are the same as described in Figure 4.1, 4.3, and 4.5, respectively. Error bars represent standard error (n = 3). Statistical analysis was performed with a one-way ANOVA. Statistical comparisons between lighting conditions was done using a sidak post-hoc test ($p < 0.05$). Data that share a letter are not statistically significant.

Ascorbic Acid. No effect on tAA was found between GH and Sn conditions (Figure 4.7a). However, a significant effect of treatment on tAA was found for monochromatic and dichromatic light qualities and at May Barn Consultancy ($p < 0.001$). tAA was three times higher under B_{100} than under other dichromatic and monochromatic light qualities (Figure 4.7b). Moreover, GE-HB (high blue light content and high intensity) contained the highest levels of tAA, whilst lower quantities were detected in HSP-HB and AP367L (Figure 4.7c). These results

suggest that light intensity and quality, the proportion of blue light in particular, have a strong impact on the accumulation of ascorbic acid in Coriander.

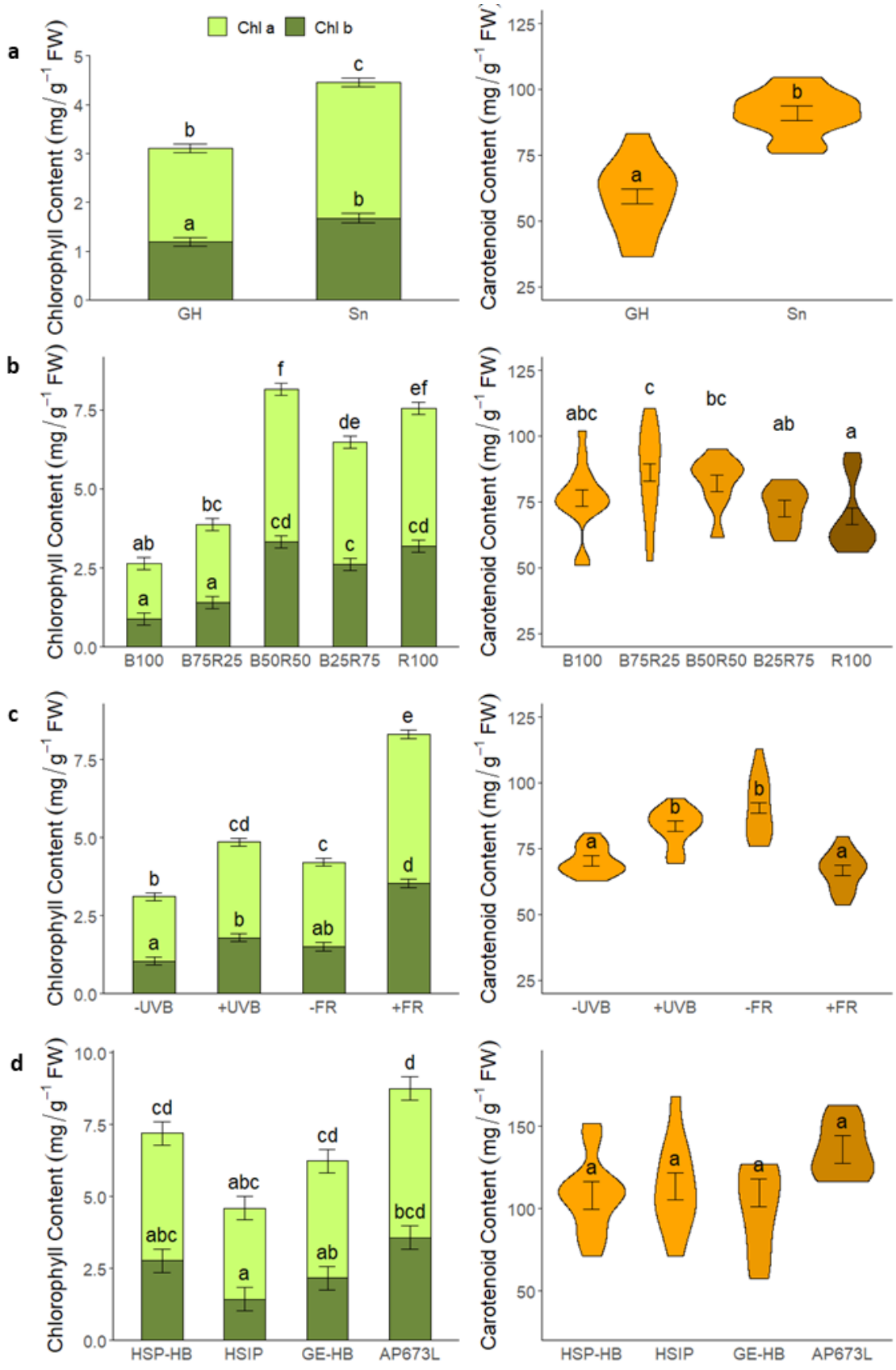


Figure 4.8 Fluctuations of chlorophyll and carotenoid content in Coriander is dependent on spectra. Total chlorophyll and carotenoid content (mg/g FW) of mature coriander, for (a) controlled environment growth conditions, (b) different ratios of blue and red light, (c) supplemental UV and FR lighting, and (d) samples from the facility at May Barn Consultancy Ltd. . Light conditions are as described in Figure 4.1, 4.3, 4.4, 4.5, respectively. Chlorophyll a and Chlorophyll b content was calculated from FW measurements using formulae published in Lichtenthaler and Buschmann (2001). Error bars show the standard error (n = 3). Statistical significance of FW and chlorophyll abundance between each light treatment was calculated using two-way ANOVA (chlorophyll) and one-way ANOVA (carotenoid) with sidak post hoc testing ($p < 0.05$). Data that do not share a letter are statistically significant.

Chlorophyll and Carotenoids. The accumulation of photopigments chlorophyll ($p < 0.001$) and associated carotenoids ($p < 0.01$) was significantly different for Coriander plants grown in GH and Sn conditions (Figure 4.8a). Total chlorophyll and carotenoids both increased in Sn. Chl a and Chl b increased at relatively similar rates due to the comparable Chl *a:b* ratio (Table 4.6), which suggests the increase is due to an environmental input rather than spectral.

When measured under different ratios of blue and red wavelengths, chlorophyll and carotenoid content varied significantly ($p < 0.001$) (Figure 4.8b). The lowest total chlorophyll content was found in B_{100}, and second lowest in $B_{25}R_{75}$, and the content was significantly higher in all the other ratios by Sidak post hoc test. Unfortunately, variation between experiments obscure any trends in Chl *a:b* with spectral composition (Table 4.6). As for carotenoid content the highest average was seen in $B_{25}R_{75}$, but this treatment also showed the greatest variance among technical replicas within experiments. The highest value of total carotenoids with minimal variance is $B_{50}R_{50}$, therefore chlorophylls and carotenoids coalesce on $B_{50}R_{50}$ as an optimal ratio (Figure 4.8b).

The effects of supplemental wavelengths were significant for chlorophylls and carotenoids ($p < 0.001$). Supplementation with UV-B increased carotenoids and total chlorophyll, with a simultaneous decrease in Chl a:b ratio indicating a greater increase in Chl b, which absorbs in the blue spectral range (Blankenship, 2021), over Chl a (Figure 4.8c). Similarly, an increase in total chlorophyll is seen with supplementary FR, and an even greater decrease in Chl *a:b*.

However, this is accompanied by a significant decrease in carotenoid content, showing spectral effects on accumulation (Figure 4.8c).

Finally, chlorophyll content also varied significantly under the commercial spectra tested at May Barn Consultancy Ltd ($p < 0.001$), with HSIP significantly lower and AP673L significantly higher (Figure 4.8d). The opposite trend was seen in the Chl *a:b* ratio which was highest and lowest for HSIP and AP673L, respectively (Table 4.7). The HSIP and AP673L lights were similar in light quality but were applied with a high and low intensity, respectively. Whereas, both HSP-HB and GE-HB, which are composed of similar intensity and R:B ratios, were similar in chlorophyll content. In addition, no significant differences were seen in the total carotenoid content between the spectra, however AP673L was higher on average, which may indicate an effect of intensity (Figure 4.8d).

Taken together, whilst these results do not allow definitive conclusions to be drawn, they have demonstrated that some properties of light spectra, intensity and growth method have marked effects on photosynthetic pigments in Coriander, which require further investigation.

4.3.7. Photosynthetic Efficiency Measurements under Different Lighting Conditions

Photosynthesis is an essential process for plant growth and is closely linked to the phytonutrients of interest in this study. Therefore photosynthetic performance of mature Coriander under the experimental light conditions was measured by chlorophyll fluorescence (Table 4.6), and RLRC (Appendix Figure 6.1.12). The parameters collected include photosynthetic efficiency (Fv/Fm), NPQ, and from the model: maximum gross assimilation rate (A_{sat}), and quantum yield (ϕ).

Controlled Environments. The photosynthetic parameters of Hp plants revealed lower photosynthetic efficiency. Hp plants also had a noticeably low ϕ and the lowest Fv/Fm ($p < 0.001$) compared to GH and Sn treatments. This likely explains why the RLRC of Hp plants did not compare well to the other controlled environments (Appendix Figure 6.1.12). As the experimental repeat could not be performed due to time constraints, it is unclear whether these results were linked to the experimental design or a real effect of the treatment. However, the fitted equation also showed that hydroponic plants had comparatively high A_{sat},

demonstrating that the photosynthetic function of the plants was comparable in light saturating conditions. Sn plants were more consistent in photosynthesis measurements between the repeats compared to GH. The heat sink capacity (NPQ) varied across all treatments, and no significant trend could be seen (Table 4.6).

Light Quality. Of the various ratios of blue and red light used, the $B_{75}R_{25}$ treatment recorded the highest A_{sat} across the experimental repeats (Table 4.6; Appendix Figure 6.1.12). All Fv/Fm values were ~ 0.80-0.82 ($p < 0.05$), indicating the plants were not stressed and functioning well. The highest ϕ was found in $B_{25}R_{75}$ at 0.035 µmol.µmol^{-1}, compared to the lowest in B_{100} at 0.022 µmo.µmol^{-1}, indicating an improved photosynthetic performance under red light. NPQ values for lighting treatment and experimental repeat were significant ($p < 0.001$). The dichromatic mixes $B_{75}R_{25}$, $B_{50}R_{50}$ and $B_{25}R_{75}$ had the highest NPQ values at 1.97, 1.72 and 2.27, respectively. The mix of performances for each parameter across the lighting treatments indicates that particular photosynthetic activity may be stimulated by specific lighting conditions, however overall photosynthesis is more resilient to stress within the dichromatic mixes.

In contrast, there is no significant difference in photosynthetic efficiency, in either Fv/Fm or NPQ at different light intensities for Coriander grown with and without supplementary UV-B and FR. However, the LRC response for the FR control condition is not normal, and as the experiment was only performed once, it is not possible to confirm whether this is due to the treatment or an anomaly (Appendix Figure 6.1.12). Nevertheless, on average NPQ is lower with FR, which is in line with expectations, due to the increased stimulation of PSI by FR light (Zhen and van Iersel, 2017)

Table 4.6 Photosynthetic measurements of mature coriander plants from each of the experimental conditions tested. Measurements were collected by extraction or LICOR on a fully expanded exposed leaf (n = 4) and include chlorophyll a:b ratio, photosystem II efficiency (Fv/Fm), non-photochemical quenching (NPQ), light saturated rate of CO_2 uptake (A_{sat}, µmol (CO_2).m^{-2}.s^{-1}) and quantum yield (ϕ, µmol (CO_2).umol^{-1} (photons)). Letters indicate statistical significance as calculated by Sidak posthoc test, with groups of analyses separated by a thicker line.

Treatment	Repeat	Chl a:b	Fv/Fm	NPQ	A_{sat}	Θ
GH	1	1.61	0.82 ± 0.006 a	1.72 ± 0.092 a	23.3	0.030
	2	-	0.82 ± 0.002 a	1.48 ± 0.118 a	19.0	0.044
Snijder	1	-	0.81 ± 0.006 ab	1.41 ± 0.226 a	21.1	0.028
	2	1.66	0.83 ± 0.004 a	1.37 ± 0.170 a	23.7	0.032
Hydro	-	-	0.80 ± 0.011 b	1.54 ± 0.150 a	23.6	0.019
B100	1	1.41	0.82 ± 0.002 a	1.44 ± 0.167 cde	14.0	0.022
	2	1.95	0.81 ± 0.005 a	1.69 ± 0.108 bcd	22.4	0.032
B75R25	1	-	0.82 ± 0.004 a	1.15 ± 0.111 e	28.0	0.032
	2	1.77	0.82 ± 0.004 a	1.97 ± 0.038 ab	17.5	0.031
B50R50	1	1.45	0.81 ± 0.007 a	1.72 ± 0.130 bc	-	-
	2	1.87	0.82 ± 0.004 a	1.72 ± 0.111 bc	26.1	0.033
B25R75	1	1.47	0.80 ± 0.028 a	1.39 ± 0.076 de	22.7	0.033
	2	1.89	0.81 ± 0.007 a	2.27 ± 0.080 a	26.2	0.035
R100	1	1.37	0.81 ± 0.004 a	1.45 ± 0.187 cde	17.7	0.027
	2	1.83	0.80 ± 0.004 a	1.68 ± 0.171 bcd	-	-
- UVB	1	1.98	0.79 ± 0.031 a	1.34 ± 0.493 a	22.7	0.032
	2	-	0.81 ± 0.004 a	1.52 ± 0.339 a	-	-
+ UVB	1	1.71	0.81 ± 0.004 a	1.53 ± 0.161 a	23.4	0.033
	2	-	0.82 ± 0.004 a	1.63 ± 0.454 a	-	-
- FR	-	1.80	0.82 ± 0.006 a	1.44 ± 0.113 a	17.3	0.009
+ FR	1	-	0.82 ± 0.003 a	1.31 ± 0.181 a	19.1	0.027
	2	1.35	0.82 ± 0.004 a	1.18 ± 0.596 a	19.7	0.026

Commercial Lighting. For the plants grown at May Barn Consultancy, photosynthetic measurements were recorded with a MultispeQ and therefore, only Fv/Fm and non-dark adapted NPQ (NPQt) were measured (Table 4.7). Of these, no significant differences were found by one-way ANOVA in Fv/Fm, as the plants were grown in similar conditions. However, for NPQt, GE-HB had the highest value but was similar to HSP-HB, both high blue content spectra, and was significantly higher than AP673L, which had the lowest intensity, indicating a potential influence of both spectral quality and intensity on NPQt under these growth conditions.

Spectra		Chl a:b	Fv/Fm	NPQt
Heliospectra Propagation High Blue	(HSP-HB)	1.60	0.77 ± 0.010 a	0.47 ± 0.084 ab
Heliospectra Indoor Production	(HSIP)	2.20	0.77 ± 0.008 a	0.42 ± 0.066 ab
GE Arize High Blue	(GE-HB)	1.88	0.77 ± 0.004 a	0.49 ± 0.032 a
Valoya L28 AP673L	(AP673L)	1.46	0.77 ± 0.033 a	0.36 ± 0.065 b

Table 4.7 Photosynthesis measurements taken at May Barn Consultancy Ltd. on mature coriander grown under commercial spectra. Measurements include Chlorophyll a:b ratio, and using a MultispeQ to measure photosystem II efficiency (Fv/Fm), and non-photochemical quenching without dark adaptation (NPQt) on a fully expanded exposed leaf (n=4).

4.4. DISCUSSION

4.4.1. Coriander Yield and Quality Increased in Controlled Conditions

Coriander is cultivated for its wide use in culinary dishes due to its distinct flavour, but Coriander is also a high value crop due to the high contents of phytonutrients beneficial to human health (Nadeem et al., 2013). Traditionally Coriander has been grown in greenhouses, but hydroponic vertical farming cultivation methods with greater climate control are emerging as new technologies for CEA in which to grow Coriander. To test the validity of these methods, Coriander was grown in compost within GH, Sn and Hp conditions, and the growth response and nutritional content of the Coriander was measured. Coriander growth was best overall in Sn, within a tightly controlled environment, compost substrate and under broad spectrum light. Coriander plants grown in Sn were the heaviest, with the most leaves and highest leaf area (Figure 4.1). In addition, the Sn plants contained elevated levels of carotenoids, confirmed by both UV-spectrophotometery and microplate assay (Figure 4.6b and 4.8a). These measurement techniques determine total carotenoids, therefore we cannot be certain if the changes are in photosynthetic carotenoids as accessory pigments, or in photoprotective carotenoids involved in NPQ both of which are effected by light conditions. Chlorophyll and carotenoid content were both elevated in Sn plants compared to GH (Figure 4.8a), which would suggest a greater quantity of photosynthetic carotenoids. However, NPQ was elevated in GH conditions (1.72) compared to Sn conditions (1.41) (Table 4.6), which indicates a shift in production of protective carotenoids. This is possibly due to increased stress from fluctuations in light intensity, humidity and temperature, in the glasshouse that could have triggered oxidative damage (Xie et al., 2019). For example, a period of high light intensity can lead to photoinhibition and the production of reactive oxygen species, which are quenched by carotenoids as antioxidants (see Section 1.2.2.1). In addition, the greater control of environmental conditions in the Snijder cabinet (i.e. fixed light intensity, temperature and humidity) could have decreased abiotic stress and reduced the requirement for the antioxidant function of carotenoids, thereby lowering their consumption and allowing their accumulation (Howitt and Pogson, 2006). Future experimentation by measurement of specific photoprotective xanthophyll carotenoids and dietary carotenoids (β-carotene) or of genes related to oxidative stress would further dissect these forces and confirm whether the

protective controlled environment is conducive to improving the nutritional value of Coriander.

It is interesting to note that first repeat in the Snijder cabinet appeared to outperform the rest of the experimental repeats (Figure 4.1); however, these plants also resulted in the lowest DW percentage (Appendix Table 6.1.4). This indicates that whilst still generating a significantly higher biomass, some of the observed gains were due to additional turgidity of the plant cells. A variation in growth between experimental repeats was seen in the greenhouse and even within those in the Snijder cabinet despite a high consistency in photosynthesis yield between those experiments (Table 4.6). Temperature has been shown to impact plant growth, termed thermomorphogenesis, resulting in transcriptional and epigenetic regulation and morphological traits, including increased growth rates of stems and leaves (Casal and Balasubramanian, 2019; Ding et al., 2020). Climate control is less precise in a greenhouse, with temperature ranging from 16 and 37 °C and therefore variation is expected between experimental repeats. However, it is surprising that one of the Snijder repeats outperformed the other, considering that all variables were identical. It is possible that the plants were exposed to microclimates within the cabinet. To control for this in future, additional monitoring of temperature could be performed by thermal imaging or by placing temperature probes at the level of the plants, meanwhile increased rotation throughout the experiment will control for potential microenvironments within the cabinet, minimise variation and ensure reproducibility. Alternatively, as the methods, environmental settings and seed stock were identical, the cause of the Snijder variation could be natural variation within the plants. It would be interesting to determine by future experimentation the extent of biological variation within seed stock, and whether particular cultivars are more consistent in their growth responses.

4.4.2. The Presence of Blue and Red Light is Optimal for Coriander Growth

Blue and red wavelengths are primary drivers of photosynthesis and photomorphogenesis, which effect growth rates, architecture and secondary metabolite production, all valid contributors to a high value commercial crop (see Section 1.3). However, different plant species, even cultivars within species, demonstrate different reactions to blue and red light (Paradiso and Proietti, 2021), which could be associated to an adaptive response to light

quality. Therefore, it is important to assess the impact of these fundamental wavelengths on Coriander growth and nutrition, which was performed by applying a spectrum of blue and red ratios to Coriander seedlings.

The germination of Coriander is irregular (Gantait et al., 2022), therefore this study presents a valuable result by demonstrating that seedling establishment is maximised under $B_{25}R_{75}$ (Table 4.5). Both blue and red wavelengths support seedling development via their respective photoreceptors: cryptochromes and phytochromes (Neff and Chory, 1998). Phytochrome initiates germination as a very low fluence response (<1 µmol.m^{-2} red light) via phyA and phyB (Casal and Sánchez, 1998; Shinomura et al., 1996). Consistent with the sensitivity of this response, the germination was conserved at all tested intensities; even in the B_{100} treatment due to phyB absorption of blue light (Chen et al., 2004). However, both wavelengths are required for development and establishment of the seedling after germination by triggering further responses including the establishment of photosynthetic machinery, leaf development, and entrainment of the circadian clock and the genetic cascades associated (von Arnim and Deng, 1996). In the case of Coriander, $B_{50}R_{50}$ emerged as the best light quality overall, maintaining a compact seedling, with advanced development and high fresh weight gain (Figure 4.2).

Furthermore, both monochromatic blue and red wavelengths grew Coriander seedlings with long stems (Figure 4.2a). As mentioned previously, blue and red have synergistic roles in the development of a seedling, and both cryptochromes and phytochromes contribute to the inhibition of stem elongation (von Arnim and Deng, 1996). Therefore, the elongation seen may be due to improper de-etiolation, and that lack of synergistic effects that cannot be initiated by the monochromatic wavelengths independently. Moreover, classical skotomorphic features are also present, including hooked hypocotyl and pale yellow cotyledons (Josse and Halliday, 2008) (Appendix Figure 6.1.11). Interestingly, in R_{100} deetiolation improved with increasing intensity, with effects such as shorter hypocotyls and expansion of the cotyledons, indicating that for Coriander in monochromatic red light, deetiolation is a HIR. Growth under monochromatic blue light however, did not change with intensity. There could be several reasons for this: firstly, this could indicate that red light is essential to proper deetiolation of Coriander as in *Arabidopsis* (Tepperman et al., 2006). Secondly, this may have been due to the lower intensities applied in the B_{100} treatment due to equipment constraints, despite that the

intensities tested are likely to be saturating for cryptochrome responses (Pooam et al., 2021). However, to ensure that this is not a confounding factor, future studies should use the same light intensities in addition to providing precise monitoring and control of the growth environment to ensure parity between treatments.

To examine whether these effects were conserved with further development, Coriander was grown to mature stage under the same blue and red ratios at the same intensity. Parallels in growth trends were found between seedlings and mature plants. Like the seedlings, mature Coriander were tallest in the R_{100} treatment (Figure 4.3a). This is consistent with the results of previous studies where, between monochromatic red and blue, R_{100} gave the tallest stem measurements, whilst B_{100} gave the shortest shoot length (Naznin et al., 2016; Nguyen et al., 2020). A poor growth response to B_{100} was seen as low germination rate and low leaf number in McAusland et al. (2020), and is also seen in the present study which found the lowest fresh weight, leaf number and leaf area under B_{100} (Figure 4.3). This demonstrates that extension of the growing stage cannot compensate for the poor growth established under monochromatic conditions in seedlings. However, results from Ohashi-Kaneko et al. (2013) showed that R_{100} gave the best growth results against monochromatic blue and $R_{50}B_{50}$, which grew plants with the lowest number of leaves and the shortest plants, respectively (Ohashi-Kaneko et al., 2013). Moreover, in comparison to different ratios of red and blue light, Coriander was tallest in the highest proportion of red light (de Clercq and van Labeke, 2022). Differences between studies could be due to a number of factors, including environmental discrepancies, lighting quality and intensity, but also developmental stage at measurement and cultivar which differed in the published studies (Table 4.1). Therefore, future studies into these effects should consider the effects of blue and red ratio on several cultivars of Coriander, to capture any effect of light on morphology and function that is conserved across development and across cultivars, which intriguingly might uncover a distinct genetic adaption in Coriander light responses.

In this study, $B_{50}R_{50}$ grew superior Coriander cv. '*Cruiser'* to maturity with stems that were the longest of the dichromatic conditions, in addition to the highest biomass (fresh and dry weight) and leaf area of all spectral conditions (Figure 4.3). In a similar study, Coriander weight also increased with blue light proportion but only up to 17 % (Naznin et al., 2016). However further publications support greater biomass (Nguyen et al., 2020) and better overall growth for Coriander under mixed blue and red lighting (McAusland et al., 2020). Such improvements are

likely with the presence of both wavelengths due to their synergistic effects on leaf morphology and photosynthesis (Zheng and van Labeke, 2017). Changes in the ratio of red to blue can influence photosystem stoichiometry (PS I/ PS II ratio) (Landi et al., 2020). The advanced growth responses in $B_{50}R_{50}$ was correlated with a good light response curve performance (Appendix Figure 6.1.12). Therefore equal presence of both wavelengths can help optimise photosynthesis driving the harvest of photosynthetic energy by both photosystems and other accessory pigments including carotenoids, thereby limiting photoinhibition (Zhen and van Iersel, 2017). These results together with the literature shows that an R:B ratio of 1 or above is most suitable for Coriander growth.

4.4.3. Antioxidant Content in Coriander is Stimulated by Blue Light

Research into the influence of light on secondary metabolite production in Coriander has shown greater production of total phenolics in mixed light with the highest red light content (de Clercq and van Labeke, 2022). However it is likely that the higher concentrations of aromatics would be unpleasant in red light (soapy, sweet and musty aromatic nuances) (McAusland et al., 2020). On the contrary, Naznin et al. (2016) found Coriander grown under the lowest R:B ratios gave the highest antioxidant activity and R_{100} gave significantly lower activity, whilst Nuguyen et al. (2019) found the highest antioxidant capacity under B_{100} light. This study focused on the contents of antioxidant phytonutrients in Coriander, where it was found that total carotenoid and tocopherol content was highest in B_{100} and decreased with increasing red light fraction (Figure 4.6). Intriguningly, *VTE1,* a rate limiting gene of the tocopherol biosynthesis pathway in A*rabidopsis* (Kanwischer, 2005; Porfirova et al., 2002), has previously been linked to blue light signalling in tea plants (Zheng et al., 2019a), and a similar genetic mechanism may be occurring in Coriander via the VTE1 gene present in the genome (Song et al., 2020). In addition, a far higher tAA content was found under B_{100} (Figure 4.7), and due to its role in recycling and stabilisation of carotenoids and tocopherols could have participated in their accumulation under the same lighting treatment (Bouvier et al., 2005; Saga et al., 2010; Thomas et al., 1992). Blue light has been associated with secondary metabolite production, particularly photoprotective compounds, in other plant species (Landi et al., 2020). Overall, these results suggest a strong component of cryptochrome signalling in Coriander antioxidant production, possibly due to a high irradiance response driven by the

high-energy blue wavelengths. Therefore, B_{100} appears to be the best spectra to grow Coriander purely to maximise phytonutrient content. However, although the phytonutrient content was higher in B_{100} than under $B_{50}R_{50}$, the latter was superior to the other dichromatic mixes tested (Figure 4.6), and therefore represents a good choice for a Coriander crop with a favourable phenotype and high phytonutrient content.

4.4.4. Coriander Physiological Response to Supplementary Lighting is Limited, but Phytonutrient Content was Impacted

Supplementary wavelengths outside of PAR (400 – 700 nm) have been documented to induce changes in growth and metabolic responses in Coriander. With supplementary FR (R:FR ratio unknown), Coriander showed increased fresh weight and height (Matysiak and Kowalski, 2021). Classical shade avoidance responses were documented by Fraser et al (2017) in the cultivar '*Slow Bolt'*. A treatment of very low R:FR (= 0.05) reduced total leaf area and number of petioles. The petioles that remained were significantly elongated, as well as the stems resulting in tall spindly plants. However, UV-B light (1.5 $\mu molm^{-2}s^{-1}$ or 0.6 W m^{-2}) inhibited this response in both *'Slow Bolt'* and '*Cruiser*' cultivars, creating a more compact plant (Fraser et al., 2017). Further, when combining FR and UV-B treatments the authors established that a 4 h dose of UV-B was sufficient to antagonise shade avoidance responses and inhibit stem elongation, with a 12 h dose resulting in the shortest plants. Inhibition effects of UV-B were also seen when applied in a glasshouse environment (Fraser et al., 2017). Meanwhile, (Hassan et al., 2020) indicated that treatment with UV-A could increase total phenolics, total flavonoids, and antioxidant activity. However, the effect of UV-B and FR treatments on Coriander growth and phytonutrient content has not been widely explored and therefore, preliminary experiments, modelled on Fraser et al. (2017), were performed to examine these effects.

In this study no significant differences in growth were found either with supplementary light treatments of UV-B (0.45 +/- 0.03 $W.m^{-2}$ for 4 hours = 8.6 $J.s^{-1}$) or FR (R:FR ~ 1) (Figure 4.4). Trends showed a leaf area increase under UV-B and decrease in FR, similar to Fraser et al., (2017). However, stems tended to increase with both UV-B and FR, contrary to stem reduction reported under UV-B (Fraser et al., 2017). Finally, fresh weight tended to increase under UV-B but decrease in FR, which contrasts to the documented increases in fresh weight under FR (Matysiak and Kowalski, 2021). However, it is important to note that the effects of UV-B and

FR are dependent on the duration, and strength (irradiance and intensity, respectively) of the treatments. Although the applied treatments in these experiments were inspired by Fraser et al. (2017), the FR treatment could not be recreated due to equipment constraints, and instead a much higher R:FR ratio was applied more akin to natural sunlight than a shaded condition. For the UV-B treatment irradiance was also slightly lower that that published in Fraser et al. (2017), therefore it is likely that the resulting treatments were too weak to elicit the same significant growth responses. Moreover, the authors noted that there could be an influence on the timing of the dose, with the final third of the day being most effective (Fraser et al., 2017), whereas this in the present study treatment was applied at midday, in a similar manner to natural sunlight when UV exposure is greatest between 11 am and 3 pm. Similar to the priming of immune responses by CCA1 at dawn to 'anticipate' dawn sporulation (Wang et al., 2011), it is possible that photoprotective mechanisms are coordinated via circadian regulation to be most active at midday as an adaptive response to potential oxidative damage from UV-B radiation. Therefore, if this is the case the applications of UV-B at this time had least effect on morphology explaining the differing effects observed in this and previous studies.

UV-B treatment increased chlorophyll and carotenoids measured in a microplate assay (Figure 4.8) but total carotenoids decreased with UV-B when measured using UV-spectrophotometry (Figure 4.6). Due to the techniques used, the type of carotenoids was not specifically measured, therefore it is unclear whether photosynthetic or photoprotective carotenoids were effected differently. However, no difference could be seen between control and UV-B treatment in the RLRC (Appendix Figure 6.1.12) or PSII efficiency (Table 4.6), indicating that the UV-B treatment did not compromise the photosynthetic machinery likely due to an increase in photosynthetic carotenoids seen in the microplate assay. Moreover, NPQ under UV-B increased on average (Table 4.6) which has previously been shown to facilitate dissipation of excessive energy as a photoprotective mechanism (Allorent et al., 2016). The decreased carotenoids in the UV-Spectrophotometer (Figure 4.6c) can be associated with consumption of these carotenoids to maintain photosynthetic yield under the damaging high energy irradiation of UV-B (Howitt and Pogson, 2006). On the other hand, total carotenoid content decreased with additional FR (Figure 4.6c) and a significant decline in carotenoid content was recorded in the microplate assay (Figure 4.8). Attenuation of carotenoid content under FR lighting has been seen in sweet peppers (Kim and Son, 2022), and maize (Cohen and Goodwin,

1962). It is likely that these results are due to a decrease in carotenoid production under FR, as repression on carotenoid biosynthesis genes was observed in *Arabidopsis* under FR (see Chapter 2). On the other hand, a significant increase in chlorophyll content was recorded for both UV-B and FR, opposite to the trend found in Fraser et al. (2017). However, a higher chlorophyll index was found under supplementary FR in Nguyen et al (2019). In the present study, elevated chlorophyll could be explained by a slight increase in temperature due to the additional LED and fluorescent lighting for FR and UV-B, respectively. A similar issue was observed in studies of Basil, and addressed in 3.4.4.

No change in tocopherol content was seen for UV-B or FR treatments (Figure 4.6c), which suggests that the carotenoid pathway in Coriander is more responsive to supplementary light than the tocopherol pathway. Alternatively, alterations in temperature impact the composition of phytonutrients, and carotenoids could be more sensitive to temperature than tocopherols. Temperature does effect carotenoid accumulation but the direction of the effect can vary between species. For example, increasing temperature caused a linear increase in lutein and β-carotene in Kale, but a linear decrease in Spinach (Lefsrud et al., 2005). Similarly, an experiment with stress inducing temperatures in Soybean found significant increases in α-tocopherol and decreases in δ- and γ-tocopherol under higher temperatures (Chennupati et al., 2011). Such an effect could be occurring in Coriander to alter tocopherol composition but give no overall change in tocopherol content, albeit on a smaller scale due to the smaller differences in temperature. Moreover, it is possible that an increase in temperature could have contributed to the decline in carotenoids under FR light, and is a valid topic for further experimentation into how temperature might effect phytonutrient production in Coriander.

4.4.5. Choice of Commercial Spectra Influences Coriander Growth

There is large spectral variation within commercial LED fixtures, containing differing proportions of the wavelength ranges within PAR and into FR. This study examined Coriander growth under four unique spectral compositions from 4 commercial luminaries, three of which, HSP-HB ($B_{30}G_{33}R_{37}$), HSIP ($B_{15}G_{16}R_{69}$) and GE-HB ($B_{40}G_{1}R_{59}$) were of comparable intensity (> 210 µmol m^{-2} s^{-1}), and the fourth, AP673L ($B_{11}G_{21}R_{68}$) was significantly lower intensity (~154 µmol m^{-2} s^{-1}) (full spectral breakdowns can be found in Appendix Table 6.1.2.). The effect

of these spectra and intensity were analysed on Coriander physiology and nutrient content to determine commercial value.

Biomass and architecture. Maximum fresh weight was seen in HSP-HB (Figure 4.5b). Examination of the spectral content reveals the R:B ratio to be relatively even at 1.3 (Appendix Table 6.1.2), similar to the optimal ratio ($B_{50}R_{50}$) found for fresh weight production in the dichromatic experiment (Figure 4.3). It can be noted that GE-HB also contains an low R:B ratio of 1.5 but grew Coriander significantly lighter than HSP-HB. However, despite a similar R:B ratio, these two luminaires differ dramatically in their contribution of green wavelengths, with HSP-HB consisting of an equal proportion of blue, red and GL whilst GE-HB contains almost no green wavelengths (Appendix Table 6.1.2). The addition of GL to create RGB spectra has been cited as a cause for increased photosynthesis rate (CO_2 assimilation rate, A_{sat}) which is highest under RGB lighting in Coriander, and likely attributes to biomass accumulation especially as light intensity increases (McAusland et al., 2020). In addition, it is suggested that the addition of GL may benefit plants at the later stages of growth due to the increased penetration of green wavelengths through the dense canopy and the ability to drive photosynthesis in the understory (Meng et al., 2019).

As HSIP plants had the lowest leaf number, the fresh weight gains seen are likely due to long stems. The HSIP spectra contains an explicit far-red peak to create a R:FR ratio of ~ 8.5 (Appendix Table 6.1.2). The interaction of red and far-red wavelengths present in the tested spectra could directly trigger photomorphogenic effects to result in stem elongation (Morelli and Ruberti, 2000). The elongation of stems is a shade avoidance response, believed to enhance fitness and survival of plants within a canopy (Franklin and Whitelam, 2005). Elevated FR irradiation causes photoconversion of phytochrome into an inactive form (see Section 1.3.2.2), which allows the antagonistic regulation of auxin via phytochrome signalling partners, phytochrome interacting factors (PIFs), specifically PIF4, PIF5 and PIF7 (Ma and Li, 2019). Intriguingly, no significant difference in stem length was found in Coriander grown under supplementary FR (R:FR = 1.4) in earlier experiments with supplementary lighting (Figure 4.4a). However, the FR treatment was applied 10 days before harvest, whereas the light quality in HSIP was consistent throughout the growth stages. FR can deactivate phytochrome driven responses, and it was previously determined that both B and R wavelengths are necessary for proper de-etiolation and development of Coriander stems. The extended exposure to FR could

therefore have a stronger effect on development. Taken together, these data suggest that the interaction of intensity and strength of FR treatment on Coriander growth would be an interesting line of enquiry for future experimentation.

In addition, these wavelengths are not delivered in isolation, and are combined with blue and green wavelengths. GL has been observed to induce shade avoidance responses in lettuce and kale similar to FR (Meng et al., 2019). In Basil, stem elongation has been observed with increasing proportions of GL at 32 and 45 % (Dou et al., 2019b), and additional green wavelengths also triggers increased biomass, leaf number, and area which has been associated with a shade avoidance responses (Schenkels et al., 2020). In Coriander, Ohashi-Kaneko et al. (2013) found a greater leaf number under fluorescent light, which contains green wavelengths, over RL, BL and RLBL only LED treatments. Both HSP-HB and HSIP have high proportions of GL, which could be generating shade avoidance phenotypes in Coriander thereby contributing to the extended stems, and the increased leaf number in HSP-HB (Figure 4.5). Moreover, the reduced leaf number in HSIP and AP673L, could be due to the contribution of FR in the spectra, which reduces leaf number in Coriander (Fraser et al 2017).

When comparing spectra delivered at similar intensity (HSP-HB, HSIP and GE-HB), Coriander under GE-HB are significantly shorter (Figure 4.5a). This is unsurprising, as the GE-HB spectra contains a high proportion of blue light that is associated with the inhibition stem length (Lin et al., 1998), which was also seen in earlier experiments (Figure 4.3). It is interesting to note, that GE-HB ($B_{40}G_1R_{59}$) and AP673L ($B_{11}G_{21}R_{68}$) did not differ in stem length, when AP673L contains green and FR wavelengths and therefore has associations with stem elongation. In fact, AP673L is similar proportionally to HSIP ($B_{15}G_{16}R_{69}$), which grew the heaviest and some of the tallest Coriander. However, AP673L was delivered at ~100 $\mu mol.m^{-2}.s^{-1}$ less than HSIP and GE-HB. Photosynthetic activity increases with incident light intensity (Bukhov et al., 1995), therefore plants under AP673L has less available energy for growth despite the lengthening pressures typically found with green and FR wavelengths as explained above (Morelli and Ruberti, 2000; Schenkels et al., 2020). This suggests that the spectral and intensity based influences on stem length between GE-HB and AP673L likely cancelled out, resulting in equally tall Coriander in both treatments. This indicates that both spectral effects and intensity play a role in the final height of the crop, and that one factor can be used to balance out stimulatory or inhibitory effects of the other.

The fresh weight results between AP673L and GE-HB also demonstrate another potential impact of intensity. The GE-HB plants were significantly heavier than those under AP673L despite the similar length and leaf number between the two treatments (Figure 4.5). In addition, AP673L generated the highest percentage dry weight and had a greater overall biomass (Appendix Table 6.1.4). Therefore, the fresh weight difference could be accounted towards fresh mass gained through increased turgidity in plants under GE-HB (Barrs and Weatherleyt, 1962), which could be attributed to the higher intensity of the lights driving photosynthesis, transpiration and therefore increased water uptake. Alternatively, blue light controls stomatal aperture and conductance via phototropins and cryptochromes respectively (Boccalandro et al., 2012), therefore it possible that the greater BL quantity of GE-HB affected water uptake and retention in the tissues. It would be interesting to experiment further on the change in biomass and physiology that may occur when the intensity of the four commercial spectra is varied, and therefore explore which factor holds the greatest influence on Coriander growth: spectra or intensity.

Chlorophyll. Chlorophyll is an essential plant chemical that is effected by phytochrome and light intensity. Lower light intensity and high red light proportions result in elevated chlorophyll contents (Bukhov et al., 1992; da Silva Ferreira and Sant'Anna, 2017; Rezai et al., 2018) whilst FR wavelengths contribute to reduced chlorophyll biosynthesis (Casal et al., 1987). In Figure 4.8d, the lowest intensity light (AP673L) contained the highest chlorophyll content. Meanwhile, the HSIP spectra, which contains FR wavelengths and delivered at a high intensity, contained the lowest chlorophyll content. It is also suggested that green wavelengths reduced pigmentation in two lettuce species similar to FR (Meng et al., 2019), however correlations between GL and chlorophyll content were not seen here. Coriander plants with low chlorophyll contents are less visually appealing to customers, and potential biomass accumulation is negatively impacted by reduced photosynthetic capacitiy. High Fv/Fm (a measure of PSII efficiency) was found to correspond to the highest chlorophyll contents in Coriander (Matysiak and Kowalski, 2021). In this experiment, no change was seen in Fv/Fm, but differences can be seen in the Chl a:b ratios. Light quality impacts Chl a and b ratios by changing the association of antenna complexes thereby altering the energy distribution between Photosystem II and I, and optimising light absorption for photosynthesis (Landi et al., 2020; Zheng and van Labeke, 2017). Interestingly, HSP-HB and GE-HB which have similar R:B ratios and intensity also have

similar Chl a:b ratios (Appendix Table 6.1.2 and Table 4.7). HSIP and AP673L have the highest and lowest Chl a:b ratios, respectively, yet both have high R:B ratios and share other spectral composition similarities in terms of green and FR light proportions. It is possible that instead the intensity differences between HSIP (257 µmol m^{-2} s^{-1}) and AP673L (150 µmol m^{-2} s^{-1}) are affecting the production of chlorophyll. Chl a plays central role in the reaction centres of both photosystems and the light reaction of photosynthesis altogether, whereas Chl b is present at higher concentrations in the peripheral antenna (Cao et al., 2018). The photosynthesis is optimised under prevailing light quality conditions by linking the activity of the PS to the balance of Chl a:b (Dale and Causton, 1992). Therefore, to accommodate for the increased light energy under higher intensities, Coriander shifted the production of Chlorophyll b to greater quantities of Chlorophyll a, as seen previously in barely seedlings (Raul De la Torre and Oliver Burkey, 1990) and tomato (Davies et al., 1986) that were exposed to high light intensity. Overall, these results demonstrate reactivity of chlorophyll to spectra and light intensity that could be used to increase chlorophyll content in Coriander grown under artificial lighting, thereby increase the visual appeal of plants and potentially boosting yield, however further work is required before this link can be firmly established.

Antioxidant Accumulation. Modifications of secondary metabolites relating to aroma in Coriander, were found to be due to variances in spectra and not PPFD (McAusland et al., 2020). Indeed, similar levels of carotenoids and tocopherols were found in the lowest intensity spectra (AP673L) as in the highest (HSP-HB) (Appendix Table 6.1.2). However, the HSP-HB spectra that resulted in the greatest growth response, in terms of fresh weight and leaf production, also resulted in the lowest carotenoid content, whilst HSIP and GE-HB had the highest contents (Figure 4.6d). This might suggest a trade-off between plant biomass and phytonutrient production in a similar fashion to that proposed for resource allocation to biotic defence (Zuest and Agrawal, 2017), particularly as the MEP pathway originates the production of terpenoids released by plants in response to herbivore attack (Sharma et al., 2017), in addition to the carotenoids and tocopherols. Alternatively, the rate of carotenoid production was the same in these three spectra, but that the greater size of the plants under HSP-HB meant that the concentration of carotenoids in mg/100g was lower. On the other hand, spectral impacts could be involved. For example, the elevated content in GE-HB can be explained by the significantly higher blue light content, which was seen in the earlier

experiments with blue and red ratios to be associated with increased levels of carotenoids and tocopherols (Figure 4.6a). However, a spectral cause for how HSIP has produced matching quantities is difficult to evaluate with the same principle because the two spectra differed greatly in quantities of BL and GL, but contained similar absolute quantities of RL (Appendix Table 6.1.2). It is possible that rather than the addition of blue light, it is the quantity of red light that is the predictive variable for carotenoid and tocopherol content in Coriander. This is supported by the results of HSP-HB and AP673L, which delivered approximately equal intensities of red light wavelengths and did not differ in carotenoid content (Figure 4.6d and Appendix Table 6.1.2). Therefore, the activation of phytochrome could be implicated in the production and accumulation of MEP pathway phytonutrient products in Coriander although further research would be require to determine if this is the case.

Interestingly, the spectral trend in carotenoids was replicated in tAA content (Figure 4.7c). However this appears to be more closely related to an intensity effect, as the tAA content was significantly lower in the lowest intensity treatment, AP673L, than all the other spectra (Figure 4.7c; Appendix Table 6.1.2). High light intensity has been shown to elevate AA content in *Arabidopsis* leaves (Yabuta et al., 2007), lettuce (Chengbo et al., 2021; Min et al., 2021), and tomato fruits (Zushi et al., 2020). However, there also seems to be a spectral factor, as GE-HB and HSP-HB are similar in intensity yet the tAA content was lower in HSP-HB. The disproportionate elevation of tAA under B_{100} as previously demonstrated shows a strong induction effect of blue light (Figure 4.7a). AA content is elevated with blue light irradiation in single cell algae (Shigeoka et al., 1979), tomato fruits (Zushi et al., 2020), citrus fruits (Zhang et al., 2015b), as well as cabbage seedlings and other edible vegetable sprouts (Kang et al., 2020). The effect of blue light has been linked to cryptochrome and changes in gene expression within the AA biosynthesis and recycling pathways (Kang et al., 2020; Zushi et al., 2020). Therefore, similar effects could be occurring in both GE-HB and HSP-HB with their high blue spectral content. However, the latter spectra also contains a significant (~1/3) proportion of GL, which may act as an antagonist to blue light responses (Meng et al., 2019), explaining the lower content of AA in HSP-HB compared to GE-HB (Figure 4.7). Regardless, the ability to improve AA content in Coriander via additional blue light is a commercially valuable prospect, and presents opportunity for further discovery into the optimal spectral quality for high growth and AA content.

4.5 Conclusions

The experiments reported in this chapter provide a tantalising glimpse of the potential for manipulations of spectral quality of the plant growth environment for optimising the growth and phytonutrient content of Coriander. Increasing the level of control within the growth environment can have a positive effect on leaf area and number, offering a route to increase the leaf quality of market Coriander. However, measurements of specific antioxidant phytonutrients, such as β-carotene and xanthophylls or α-tocopherol and other tocopherol family members would further enrich understanding of whether Coriander with a raised nutritional composition is a commercial possibility. This would allow a deeper characterisation of different antioxidants with differential roles in the plant, but all relevant for human nutrition. Examination of their accumulation under different light quality and intensities would provide greater insight into plant protective mechanisms, particularly under supplementary UV-B and FR light. Moreover, greater control over temperature and positional effects during studies, would deepen understanding of these responses. This could be achieved by the placement of multiple probes within a bed or the use of thermal imaging cameras to highlight thermal anomalies. This will eliminate a variable that that could have had an unaccounted effect in these experiments and is known to impact on growth, chlorophyll production and phytonutrient content, and likely decrease the variation between repeats and add further weight to the conclusions of this chapter.

Nevertheless, several important conclusions can be presented which advance our understanding of Coriander light responses. It was shown that both blue and red wavelengths were required for proper de-etiolation and plant growth throughout the lifecycle. Moreover, within these experiments, a 50:50 proportion of blue and red wavelengths resulted in the best overall growth of commercially viable Coriander that is still carotenoid dense. Intriguingly, the accumulation of carotenoids and tocopherols were observed to increase with blue light content. However, results obtained may indicate that instead red light quantity is the controlling spectral factor over antioxidant concentration, with a role for phytochrome in their biosynthesis and accumulation. In contrast, AA was most closely associated with blue light and cryptochrome signalling which is consistent with the results of previous studies in a variety of other species. Future experimentation could focus on investigating the effect of varying intensity of selected spectra on the growth and phytonutrient content of Coriander.

CHAPTER 5

General Discussion

5.1. Realising the Potential of LED Light Regimes in CEA

The phenomena of vertical farming is becoming increasingly popular worldwide (Kozai et al., 2020). In addition to providing increased yield over field conditions (Barbosa et al., 2015), vertical farming has the potential to have a positive knock-on effect on wider environmental problems by the circular use of resources such as water and fertilisers. In doing so, this will prevent the exploitation and pollution of the environment (Cowan et al., 2022) and free up farmland to grow more field-based crops or to restore natural ecosystems (Stein, 2021). Moreover, there is vast potential for creating optimised LED lighting regimes for leafy green crops in vertical farms to improve light-regulated traits, including plant architecture and secondary metabolite production along with antioxidants beneficial to human health (Bian et al., 2015). Such tailored lighting regimes have the potential to increase the profitability and commercial viability of vertical farm systems, which will advance their installation into urban sites, increasing access to fresh, nutrient-rich foods for urbanised populations that are disproportionately likely to suffer from a hidden hunger for micronutrients (Wrigley, 2002). Therefore, understanding the impact of light quality on plant growth and nutritional content holds great commercial and social value (Specht et al., 2014). To that end, this thesis explored the contribution of light wavelengths and signalling pathways on the genetic expression of nutrient biosynthesis genes for carotenoids, tocopherols and AA in *Arabidopsis* (Chapter 2) and evaluated the impact of spectral quality on the growth and content of these nutrients in Basil (Chapter 3) and Coriander (Chapter 4), two high value herbaceous crops.

5.1.1. The Commonalities of Light Control on Growth and Phytonutrient Content

Commonalities appeared in the patterns of growth between the crop species examined. Elongation of seedlings in monochromatic BL and RL was observed in both Basil and Coriander (Figure 3.2 and 4.2), which suggested the requirement of both wavelengths for correct seedling development, in particular for the inhibition of hypocotyl elongation. Both phytochromes and cryptochromes are implicated in the de-etiolation of seedlings in *Arabidopsis* and work together either redundantly or additively to modulate responses (Casal and Boccalandro, 1995; Neff and Chory, 1998). In the transition from skotomorphogenesis (Josse and Halliday, 2008) into light adapted growth, BL and RL photoreceptors trigger vital processes including cotyledon

opening (Casal et al., 2000; Ohgishi et al., 2004; Yanovsky et al., 1995), the inhibition of hypocotyl elongation (Casal et al., 2000; Lin et al., 1998; Yanovsky et al., 1995), and greening of the cotyledons (Botto et al., 1996; Xu and Ma, 2009), where the photosynthetic machinery is established, including the isoprenoid products chlorophyll and accessory carotenoid pigments (Cao et al., 2018). The requirement of both wavelengths continued throughout development of Basil and Coriander, which is evidenced by the greatest mean fresh weight for both crops occurring under the $B_{50}R_{50}$ dichromatic light quality (Figure 3.3b and 4.3b). After de-etiolation, both BL and RL have specific effects on photosynthesis and photomorphogenesis. RL is the main driver of photosynthetic chlorophyll in the core of PSI and PSII (Blankenship, 2021; McCree, 1971), which provides energy for growth, whereas BL aids in the optimisation of photosynthesis by regulating stomatal opening (Kang et al., 2009; Mao et al., 2005) and promoting photomorphogenic leaf expansion (Vandenbussche et al., 2007). These responses may be subject to crosstalk effects similar to de-etiolation (Neff and Chory, 1998), but overall, both wavelengths contribute to generating fresh weight biomass.

Similarly, when growth was examined under spectra delivered by commercial lighting, HSP—HB ($B_{30}G_{33}R_{37}$) produced the heaviest plants with the highest average leaf number in both species (Figure 3.5 and 4.5). Intriguingly, this spectral composition also contains a roughly equal proportion of BL and RL, with a relatively equal R:B ratio of 1.3 (Appendix Table 6.1.2). This links the results of the two experiments, and demonstrates that the effects of light quality on growth are conserved in both species. The favoured equal proportion of BL and RL could be associated with the origin of Basil and Coriander, which is Africa and Italy, respectively (Sahib et al., 2013; Sullivan, 2009). Due to the angle of the sun, the surface solar spectrum contains greater quantities high-energy wavelengths around the equator such as UV (Cicarma et al., 2009); therefore, both of these species may be adapted to greater spectral proportions of BL, resulting in a favoured R:B of ~1. It has been suggested that plant light absorbance spectra could be used as a guide for designing species-specific lighting spectra (Riha et al., 2020). For instance, both Basil and Coriander show an absorption peak of 435 nm, and when BL is supplied at this wavelength for Basil there is a significant improvement in both yield and physiological parameters (Riha et al., 2020). Intriguingly, the absorbance of both Basil and Coriander at 450 nm is equal to the absorbance of RL at 665 nm, which could also explain the preference of $B_{50}R_{50}$ light quality in both species. Future research would benefit from

consideration of a species' origin, natural habitat and light absorption spectra to develop understanding of macro influences into plant responses to light quality.

The impact of light wavelengths on the rate limiting biosynthesis genes for the studied nutrients in the model plant *Arabidopsis* was explored using bioinformatics and measurements of gene expression by qPCR (Chapter 2). The activity of the MEP pathway enzymes, DXS, DXR and HDR, regulate the production of the carotenoid and tocopherol precursor (Botella-Pavía and Rodríguez-Concepción, 2006; Estevez et al., 2000; Mahmoud and Croteau, 2001). RL has been shown to upregulate MEP pathway genes previously in *Arabidopsis* (Chenge-Espinosa et al., 2018), which was also observed in this study, in addition to a repressive role of BL in MEP pathway regulation (Figure 2.2 and 2.3). Moreover, the carotenoid and tocopherol rate limiting genes (*PSY, VTE1* and *VTE4*) were targets for RL and BL regulation (Figure 2.11). Whilst *PSY* has previously been reported to be responsive to BL and RL (Li et al., 2020; Stange and Flores, 2012), this novel discovery for tocopherol biosynthesis genes provides a mechanism for the modulation of tocopherol composition seen in Baby Leaf Lettuce under BL and RL (Samuoliene et al., 2012). The MEP pathway products are wide ranging and central to the function of higher plants, therefore copies of their biosynthesis enzymes can also be found in Basil (Rastogi et al., 2015; Torre et al., 2016) and Coriander (Song et al., 2020), in addition to *PSY*, *VTE1* and *VTE4*. Interestingly, in Basil the $B_{50}R_{50}$ light quality accumulated the highest carotenoid content (Figure 3.6), which correlates with evidence of RL MEP pathway stimulation and BL driven synthesis of the downstream carotenoid pathway. Similar patterns of total carotenoid content were seen across the commercial LED spectra in both high value crops (Figure 3.5d and 4.6d). A preferential accumulation of carotenoids under BL has also been observed in Lettuce varieties (Goto, 2012; Stutte et al., 2009) and in Spinach (Li et al., 2011; Ohashi-Kaneko et al., 2007). Confirmation of the light influences on the genetics including the measurement of protein and enzyme activity in *Arabidopsis*, Basil and Coriander would expand the insight from these experiments, and aid in the expansion of research into other species.

Plants that are shade-sensitive upon exposure to low R:FR will commonly elongate the stem and leaves via auxin regulation (Morelli and Ruberti, 2000) in order to seek light when shaded by neighbours (Franklin, 2008). Both Basil (Bantis et al., 2016; Larsen et al., 2020) and Coriander (Fraser et al., 2017) were recorded to display such responses under shading. This study also found the longest stems of Basil and Coriander under HSIP ($B_{15}G_{16}R_{69}$) (Figure 3.5a

and 4.5a), which contained a relatively low R:FR ratio (Appendix Table 6.1.2), indicating that both species were responsive to shade-induced elongation. As the response to shade is adaptively linked to the natural environment of a species (Dudley and Schmitt, 1995; Power et al., 2021), it is possible that similar FR light responses were seen in both species again due to their shared Equatorial origin (Sahib et al., 2013; Sullivan, 2009). Furthermore, the effect of FR light on the production of nutrients was also explored in all three species. In general, FR supplementation reduces carotenoid content in an adaptive readjustment of the photosynthetic machinery (Bou-Torrent et al., 2015), and has been noted to reduce phytonutrient content in crops such as Rocket (Nicole et al., 2019). Similarly, it was found in Basil and Coriander that supplementation with FR light decreased carotenoid content and, in a novel finding, tocopherol content (Figure 3.7a and 4.6c), which is in agreement with reduced genetic transcription activity of all biosynthesis genes in all pathways of the nutrients studied in *Arabidopsis* (Figure 2.4a and 2.8a) (Leivar et al., 2012). Tocopherols are an integral part of the photoprotective machinery and, similar to carotenoids, scavenge ROS to prevent photoinhibition (Kruk et al., 2005), therefore are likely to be under similar regulation to carotenoids under FR light. However, FR was found to increase chlorophyll content in Basil and Coriander (Figure 3.10c and 4.8c). This result was unexpected for FR supplementation, which is more likely to decrease chlorophyll synthesis (Franklin and Whitelam, 2005; Huq et al., 2004), potentially by decreasing MEP pathway biosynthesis (Figure 2.4a), which provides precursors for chlorophyll (Chappell, 2003; Croteau et al., 2000). That an increase of chlorophyll was seen in both crop species suggests that an external factor in the experimental set up used to grow both sets of plants could have been involved. Indeed, whilst a fan was used to try to regulate temperature in the hydroponic shelving unit, the heat from the supplementary LEDs could have impacted on the biosynthesis of chlorophyll, in a similar fashion to *Arabidopsis*, where chlorophyll accumulation is positively correlated with temperatures ranging from 17 – 27 °C (Toledo-Ortiz et al., 2014). This mechanism utilises the HY5-PIF regulatory module to adjust the expression of genes required for photosynthesis and photoprotection in response to light and temperature changes, in order to optimise photosynthetic performance and growth in *Arabidopsis* (Toledo-Ortiz et al., 2014). Alternatively, the constant turnover of chlorophyll could have been impacted by FR via a decrease in VTE5 transcript abundance to cause slower degradation, which is little explored in the literature (Lin et al., 2014; Perkins and Roberts, 2011). As this was an effect seen in both

species, this is a point of further exploration to untangle the potential effect of temperature on chlorophyll synthesis and of FR light on chlorophyll turnover.

On the other hand, UV-B specifically increased α-tocopherol content in Basil as measured by HPLC (Figure 3.7c). Interestingly, the strongest induction of biosynthesis genes in *Arabidopsis* by UV-B was seen for VTE1 (Figure 2.4b). The VTE1 enzyme is responsible for the creation of y-tocopherol which leads to α-tocopherol in the tocopherol biosynthesis pathway (DellaPenna and Pogson, 2006) (Figure 2.1). Therefore, the results in *Arabidopsis* could suggest that a similar of effect of UV-B is occurring on the *VTE1* gene in Basil to result in elevated concentrations of α-tocopherol in leaf tissues. Individual antioxidant compounds act in combination with other antioxidants producing synergistic or antagonistic effect that can affect total antioxidant capacity (Niki and Noguchi, 2000). The combination of antioxidant properties among lycopene, β-carotene, tocopherols and AA produce synergistic antioxidant effects (Liu et al., 2008). The production of these compounds is also linked, for example increasing tocopherol levels appears to trigger compensatory changes in ascorbate and glutathione levels (Kanwischer, 2005; Li et al., 2010c), demonstrating a feedback link with the isoprenoid pathway (Kobayashi and DellaPenna, 2008). In general, the content of tocopherol is inversely correlated with the content of ascorbate and glutathione, due to the salvaging of tocopherols from radicals by ascorbate-glutathione-mediated reduction (Szarka et al., 2012). Therefore, α–tocopherol deficiency leads to an increase in ascorbate and glutathione (Kanwischer, 2005). However, in some cases, such as sunflower cell lines, high tocopherol can correlate with higher content levels of ascorbate (Caretto et al., 2002). This was found in Basil where the highest recorded AA and α-tocopherol levels coincided, particularly for the HSIP and $B_{25}R_{75}$ light conditions (Figure 3.5d, 3.6d and 3.9). Similarly, in Coriander, the highest levels of AA and total tocopherols were seen in the B_{100} light treatment (Figure 4.6b and 4.7b). This could be associated with the expression of both tocopherol and AA biosynthesis genes in *Arabidopsis*, which are stimulated by BL and RL via cryptochromes and phytochromes, respectively (Figure 2.2, 2.3 and 2.7). Therefore whilst the consumption of ascorbate by the recycling of tocopherols is a factor affecting the accumulation of these antioxidants in the plant tissues, so too is the regulatory light input into the biosynthesis of these compounds. This presents a wide avenue for further exploration into overcoming the consumption limitation of antioxidant

availability by using light wavelengths to increase the concentration of competing antioxidants simultaneously across multiple plant species.

5.1.2. Light Effects can be Species-Specific

Whilst the effects of light on growth were largely similar between the two crop species examined, the response of antioxidant accumulation to light quality was not as comparable. Coriander showed a stronger induction of carotenoids and tocopherols under BL than Basil for which both BL and RL demonstrated a role in the accumulation of antioxidants (Figure 4.6b and 3.6). Examination of the published literature showed that for Basil a BL proportion of 20 % was optimal for targeting secondary metabolite production (Bantis et al., 2016; Dou et al., 2020; Naznin et al., 2019; Pennisi, Sany, et al., 2019; Piovene et al., 2015) (Table 3.3). Whereas for Coriander, secondary metabolite production peaked under spectra with 30 % content of BL (de Clercq and van Labeke, 2022), rising to 50 % (Matysiak and Kowalski, 2021) or even 100 % in some studies (Nguyen et al., 2020). These results show that there is a fundamental difference in the light response of isoprenoid secondary metabolite production between Basil and Coriander. In a similar fashion to growth responses, plant light absorbance spectra could also provide insights into the differential accumulation of secondary metabolites between species. Examination of the published absorption spectra for Basil and Coriander shows that the 435 nm absorbance for Coriander was higher than Basil (Riha et al., 2020), however, further study into the relationship between light absorbance and secondary metabolite content is required.

Discrepancies between AA content in the commercial light treatments was also seen between the two species, in particular, the quantity of AA in HSP – HB ($B_{30}G_{33}R_{37}$) was relatively low in Basil and higher in Coriander (Figure 3.9d and 4.7c). As the HSP – HB spectra has a low R:B ratio due to the higher content of BL, this could also be associated with the stronger response of AA to BL in Coriander (Figure 4.7b). Published studies also show an association of Coriander secondary metabolite production with UV light (Matysiak and Kowalski, 2021) therefore, Coriander secondary metabolite production may be sensitive to higher energy wavelengths. A BL sensitivity of AA content has also been observed in other leafy greens, such as lettuce and komatsuna (Ohashi-Kaneko et al., 2007; Zha et al., 2020) but also in tomato (Ntagkas, Woltering, Nicole, et al., 2019) and citrus fruits (Zhang et al., 2015b). Such enhancement of AA under BL can be attributed to transcriptional regulation of the AA biosynthesis pathway (Zhang

et al., 2015b), as was seen for the rate limiting gene *VTC2* in *Arabidopsis* by cryptochrome (Figure 2.7a). On the other hand, *Arabidopsis VTC2* was also upregulated by RL (Figure 2.7b), and AA content in Basil was more closely associated with RL, the highest content in the $B_{25}R_{75}$ dichromatic light treatment (Figure 3.9b). Moreover AA significantly increased under FR light (Figure 3.9c), further indicating phytochrome control over AA production in Basil. This is contrary to a reduction in AA associated with FR supplementation in *Phaseolus vulgaris* (Bartoli et al., 2009), and to the reduced transcription of the AA biosynthesis gene *VTC2* in *Arabidopsis* (Figure 2.8a). However, the FR treatment in Bartoli et al. (2009) and the genomic dataset (Leivar et al., 2012), were a lot stronger (R:FR <0.2) than the FR supplementation applied in this study (R:FR ~1), therefore previous data only associates decreased AA content with strong FR. Instead, the increased AA content observed in this study could be due to the reduced requirement of AA in recovering photoprotective antioxidants (Saga et al., 2010; Szarka et al., 2012) under sunlight levels of FR (Franklin and Whitelam, 2005), as photosystems function more efficiently with supplementary FR and less likely to suffer from photoinhibition requiring NPQ (Lysenko et al., 2014; Zhen and van Iersel, 2017).

Preliminary growth results under supplementary FR also showed greater elongation of Basil stems than Coriander (Figure 3.4a and 4.4a). The extent of plant light responses can be related to evolutionary adaptation(Han et al., 2019; Théry, 2001) in particular for FR responses, which vary depending on whether a species adaptation to an open or shaded environment, where they will be more or less responsive to a lower R:FR ratio, respectively (Dudley and Schmitt, 1995; Power et al., 2021). Both Basil and Coriander are Equatorial in origin, however Basil grows best in full sun and is tolerant only of light shade (Chang et al., 2008), which indicates that it would be sensitive to FR supplementation, as has been seen in other studies (Bantis et al., 2016; Larsen et al., 2020). Whereas experiments with Coriander have also demonstrated FR sensitivity but that this can differ between cultivars (Fraser et al., 2017). Differences in sensitivity between species may also be true for UV-B exposure, for which Coriander appeared to withstand the UV-B dose supplied in these experiments with less visible stress than Basil. Therefore, Coriander could be more tolerant of supplementary wavelengths altogether, which is important to note when designing spectral treatments for different species and cultivars.

5.2. The Future of Lighting Control in CEA

Overall, this thesis has shown that the application of specific light wavelengths is a valid method of altering growth parameters and the content of antioxidants for crop species grown in a CEA system. The findings of this study could be used to target the accumulation of specific compounds beneficial to human health by applying a consistent treatment throughout the life cycle of a plant, or alternatively, wavelengths could be applied as a pre-harvest treatment. Light intensity is commonly linked to yield in a variety of species (Najera and Urrestarazu, 2019), including lettuce (Zhou et al., 2022) and Basil (Sipos et al., 2021). Therefore, intensity is also an important factor when designing lighting spectra across species. However, further research would be required to find out the effective treatment length and intensity of particular wavelengths to see the desired change in nutritional content.

It is documented that the MEP, carotenoid, tocopherol and AA genes show cyclic expression, frequently peaking in the morning (Cordoba et al., 2009; Covington et al., 2008; Dowdle et al., 2007; Munné-Bosch and Alegre, 2002), that is measured in the increase of pathway products (Fukushima et al., 2009; Gao et al., 2011). Exploration into promoter-based influences on the biosynthesis genes found light-regulatory elements but also circadian regulation (Appendix Figure 6.1.5). Binding sites for morning loop circadian genes were discovered which provides a mechanism for the regulation of CCA1 and LHY found for MEP pathway genes (Pokhilko et al., 2015; Vranová et al., 2013), *PSY* (Pokhilko et al., 2015), *VTE1* (Abuelsoud et al., 2020), and VTC2 (Abuelsoud et al., 2020; Dowdle et al., 2007). Therefore, timing of sample harvesting is important for accurate comparison between species. As diurnal variation in gene transcription was observed (Figure 2.6), differences in harvesting times could be the main explanatory factor for the discrepancies seen between the published *Arabidopsis* datasets (He et al., 2015; Hu et al., 2013), and self-generated gene expression measurements by qPCR. Moreover, as circadian clock entrainment occurs via cryptochromes and phytochromes (Nagy et al., 1993; Seaton et al., 2018), expression patterns may shift under alterations of spectral qualities. Therefore, measurement of the cyclic expression pattern, in addition to the enzyme and target compound accumulation, must be measured over different R:B ratios to determine the influence of wavelengths on diurnal expression.

The next step for research in this field is to combine circadian and photoreceptor regulation findings to explore the effects of dynamic lighting schedules, varying either over development stage or over the course of a day, which would require further research into the acute impacts on gene expression (Appendix Figure 6.1.1, 6.1.2 and 6.1.7). Such research could begin with genetic and metabolic analyses in model plants, such as *Arabidopsis*, to untangle the mechanism by which light is regulating the biosynthesis and turnover of these important compounds differentially over developmental periods or over 24 h. In addition to direct regulation, there are other effects upon the flux of the biosynthesis pathway not accounted for in this study. For example, there are post-translational effects to consider which could modulate protein levels by currently unknown mechanisms (Ghassemian et al., 2006; Hemmerlin, 2013). In addition, a prominent feedback mechanism exists between up-regulation of *PSY* resulting in the post-transcriptional accumulation of *DXS* protein (Guevara-García et al., 2005; Rodríguez-Villalón et al., 2009b) indicating a mechanism in which the isoprenoid pathway is regulated by its products. Further effects could be at play that are yet to be discovered. Finally, when a potential lighting regime is found, it is important to then test the suitability of such a regime on the target crop or multiple crops to account for the different responsiveness between species as seen in this study.

As the MEP pathway and associated compounds are so central to plant health and function (Chappell, 2003; Croteau et al., 2000), when undertaking experiments that target these pathways it is crucial to be aware of unintended consequences, such as potentially compromising photosynthetic function or hormonal regulation. In addition, whilst light has a vast potential for controlling plant growth through photobiology (de Carbonnel et al., 2022), it is also important to consider plant growth as a whole. Many parameters affect plant growth and metabolism outside of light quality including species and cultivar, or environmental parameters such as temperature and nutrient availability (Hernández-Pérez et al., 2020; Ren et al., 2022; Zhu et al., 2021). Because of this, future experimentation in the CEA field will likely consist of multifactorial experiments and analyses, which is required to gain a comprehensive understanding of the CEA system and the holistic effects on plants. Indeed the sector is already moving towards utilising artificial intelligence insights, with the aim of vertical farms becoming self-learning and fully automated operations (Siropyan et al., 2022; Sung, 2018); to get to that point will require a great deal more experimentation with the collection and analysis of big

data. However, it is clear that interest in vertical farming and the potential benefits to food security, nutrition and societal health is only growing and therefore it may only be a matter of time before the required research has been conducted and such farming systems are a reality.

5.3. Conclusions

In addition to demonstrating subtle differences in species light responsiveness and therefore the validity of performing experimentation across multiple species, the main findings of this thesis are:

1. MEP pathway *DXS*, *DXR* and *HDR* gene expression is primarily driven by red wavelengths via phytochrome (Chapter 2).
2. Blue, red and UV-B wavelengths positively influence rate limiting carotenoid, tocopherol and ascorbic acid biosynthesis genes (Chapter 2).
3. FR wavelengths negatively impact on the expression of all key biosynthesis genes for antioxidant production (Chapter 2).
4. Carotenoid, tocopherol and AA rate limiting biosynthesis genes show diurnal expression patterns that have circadian influence (Chapter 2).
5. Hydroponic Basil growth parameters in addition to α-tocopherol and AA content were greatest in the light condition $B_{25}R_{75}$ (Chapter 3).
6. Carotenoids in Basil showed greatest accumulation under $B_{50}R_{50}$ or $B_{75}R_{25}$ (Chapter 3).
7. An equal proportion of blue and red wavelengths ($B_{50}R_{50}$) resulted in the best overall growth of commercially viable Coriander (Chapter 4).
8. The accumulation of carotenoids and tocopherols in Coriander were observed to primarily increase with BL content with supporting role for phytochrome in their accumulation (Chapter 4).
9. AA content in Coriander was most closely associated with BL (Chapter 4)

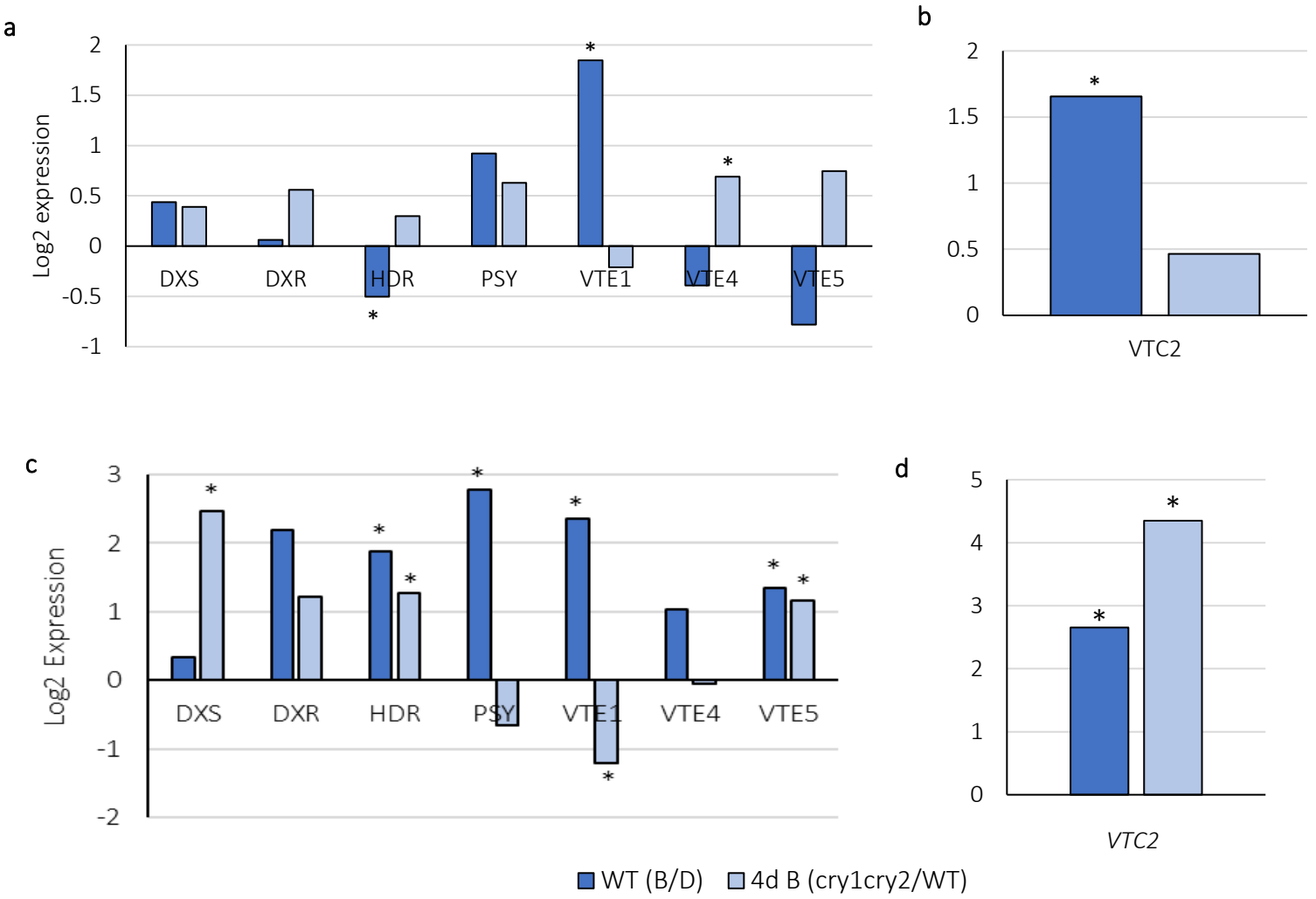
CHAPTER 6

Appendices

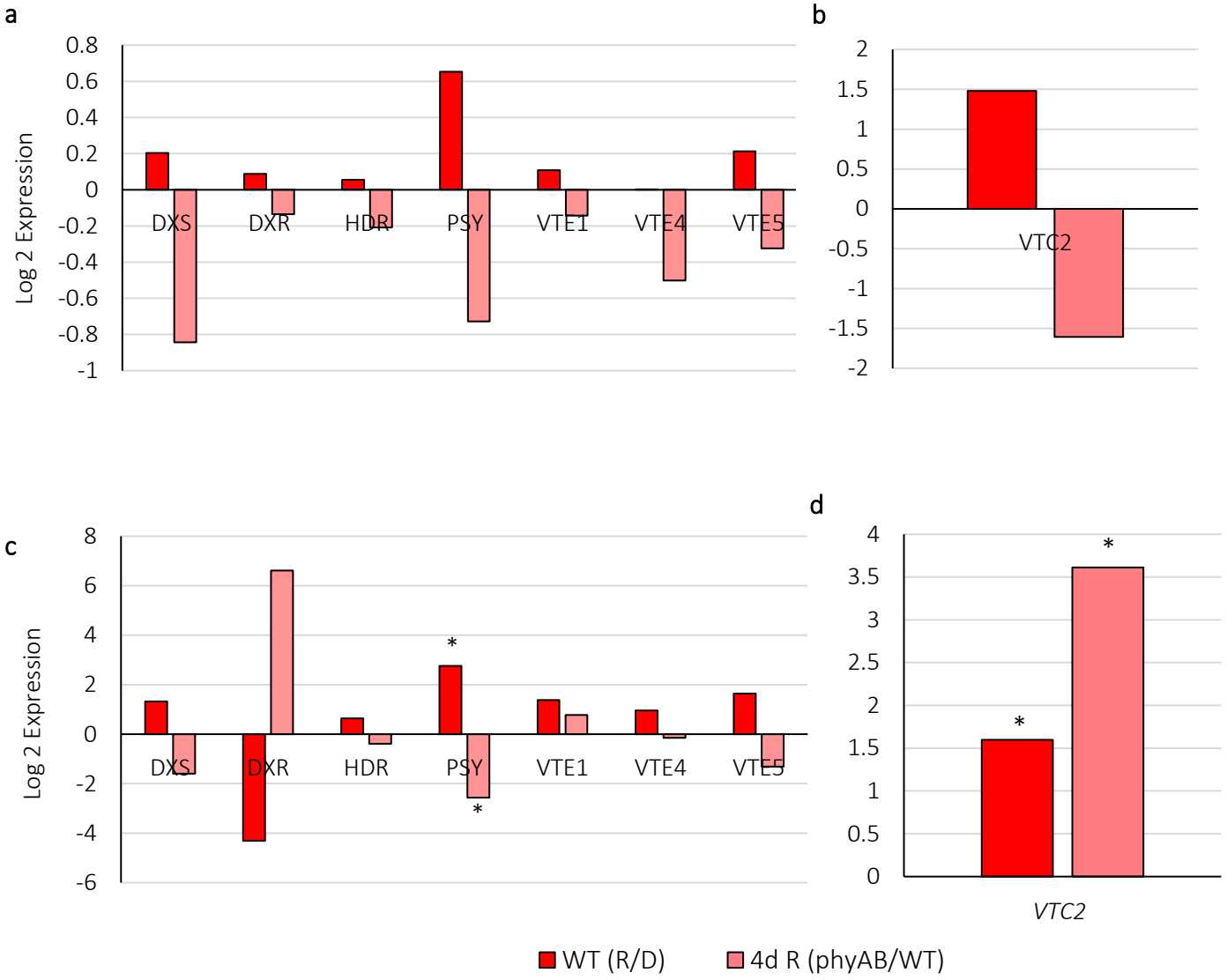
6.1. Supplementary Data

Gene	Locus	Forward or Reverse	Primer Sequence
PP2A	AT1G69960	F	TAT CGG ATG ACG ATT CTT CGT GCA G
		R	GCT TGG TCG ACT ATC GGA ATG AGA G
DXS	At4G15560	F	TCG CAA AGG GTA TGA CAA AG
		R	CAG TCC CGC TTA TCA TTC C
DXR	AT5G62790	F	ACG CTA CTC AAG AGC GAC AAG AC
		R	TCC ATC CGC CAA CCA CTA G
HDR	At4G34350	F	TGG AGA ACT GGT CGA GAA GGA
		R	GTC GAA CAC CTT CAC CAA AGC
PSY	At5G17230	F	GAC ACC CGA AAG GCG AAA GG
		R	CAG CGA GAG CAG CAT CAA GC
VTE1	At4G32770	F	GTT GCA GAT ATG GGA ACG GC
		R	TTA GTA GCT CGG GCG TGT TG
VTE4	At1G64970	F	AGA CGT TCT ATG TCC CGG CT
		R	CTC TGA CCA ATC CGC ACA CT
VTE5	At5G04490	F	GAG TTC GGC AGG AAGCAT CT
		R	GCG ACC ATT GAG ACC ATT GC
VTC2	At4G26850	F	ACTGCTGTGTTTGCCTTCAA
		R	CAATACCTCCGGGCTCACTT

Appendix Table 6.1.1. Primer sequences of rate limiting genes used for qPCR analysis. The sequences used for *DXS* and *DXR* were previously used in (Pokhilko et al., 2015).

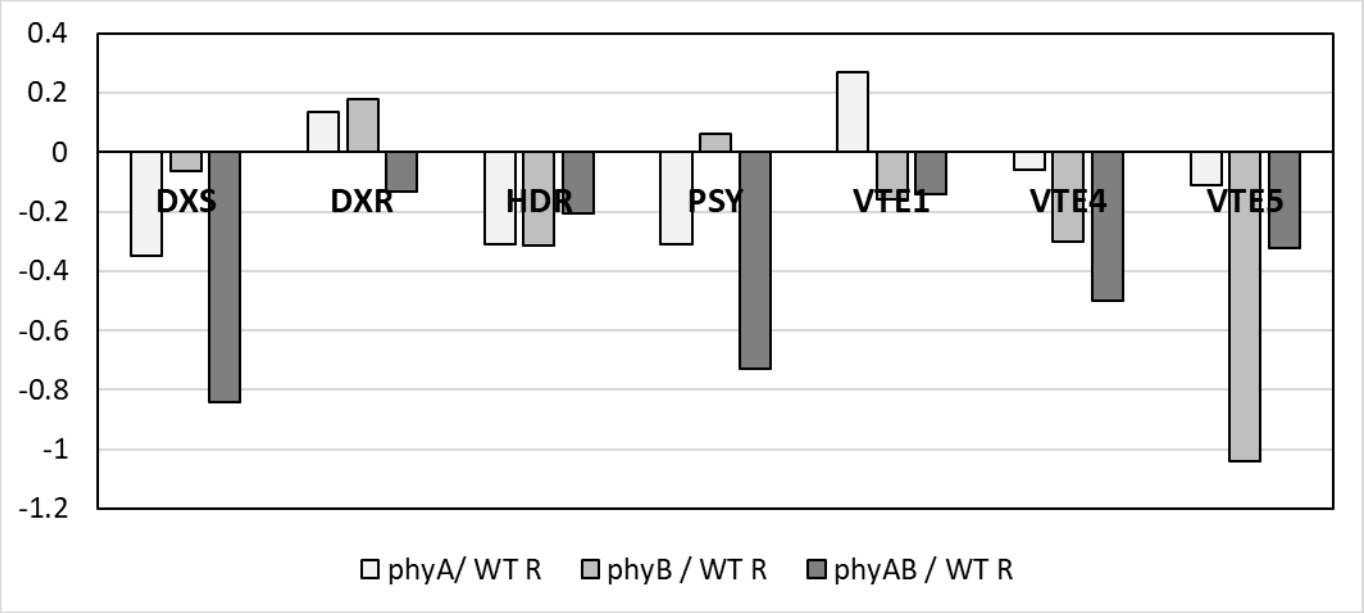


Appendix Figure 6.1.1. Acute responses of flux controlling genes to blue light (B) taken from the RNA-seq GSE80350 (Wang *et al.*, 2016) for (a) isoprenoid biosynthesis genes, and (b) rate-limiting ascorbic acid gene, *VTC2*. Wild type (WT) or *cry1cry2* light mutant 5 day-old etiolated seedlings were exposed to either 2 hrs BL (20 µmol.m^{-2}.s^{-1}) or dark (D) as a control. Experimental data was gathered using qPCR (see Methods 2.2.3) for (c) isoprenoid biosynthesis genes, and (d) rate-limiting ascorbic acid gene, *VTC2*. Bars indicate the average expression of three biological replicates where data was available, with asterisks indicating a significant difference calculated by *t*-test ($p < 0.05$) between the treatments indicated in the figure legends.

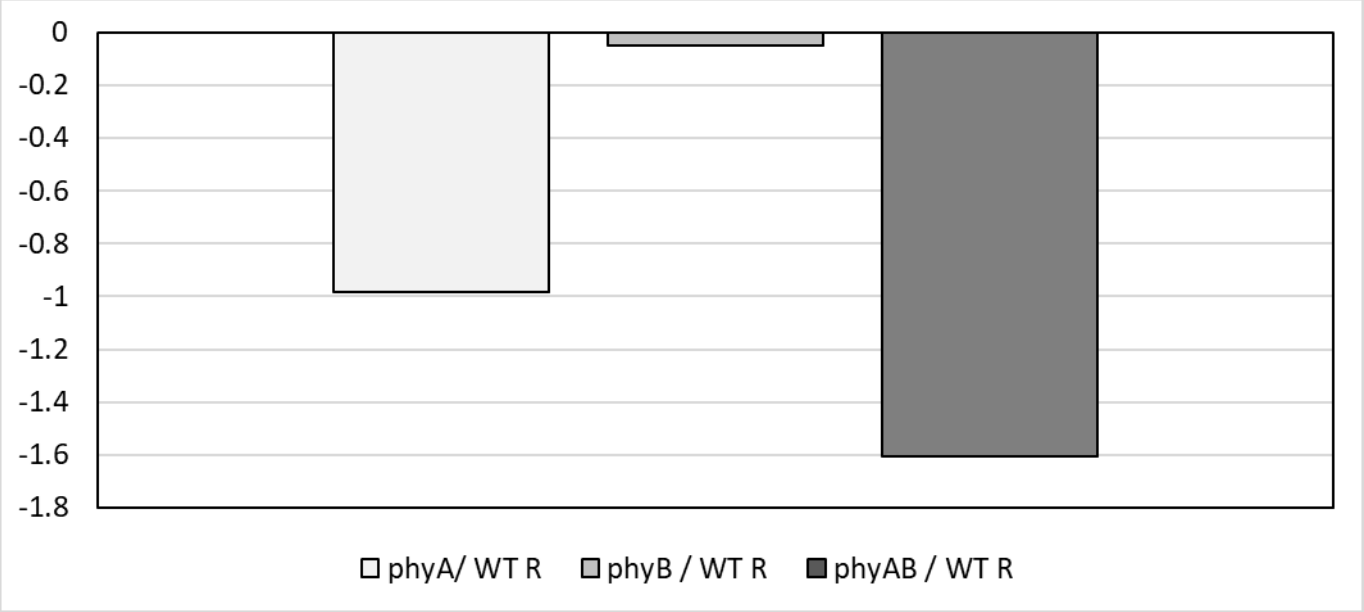


Appendix Figure 6.1.2. Acute responses of flux controlling genes to red light (B) taken from the dataset GSE3811 (Tepperman, Hwang and Quail, 2006) for (a) isoprenoid biosynthesis genes, and (b) rate-limiting ascorbic acid gene, *VTC2*. The genomic data measured 4-day old wild-type RLD and phy*A101*, phy*B1* and phy*A*phy*B* mutant etiolated seedling response to 1 h RL (680 nm, 8 µmol.m^{-2}.s^{-1}), compared to a dark (D) control. Experimental data gathered using qPCR under 2 hr of red light exposure (see Methods 2.2.3) for (c) isoprenoid biosynthesis genes, and (b) rate-limiting ascorbic acid gene, *VTC2*. Bars indicate the average expression of three biological replicates where data was available, with asterisks indicating a significant difference calculated by *t*-test ($p < 0.05$) between the treatments indicated in the figure legends.

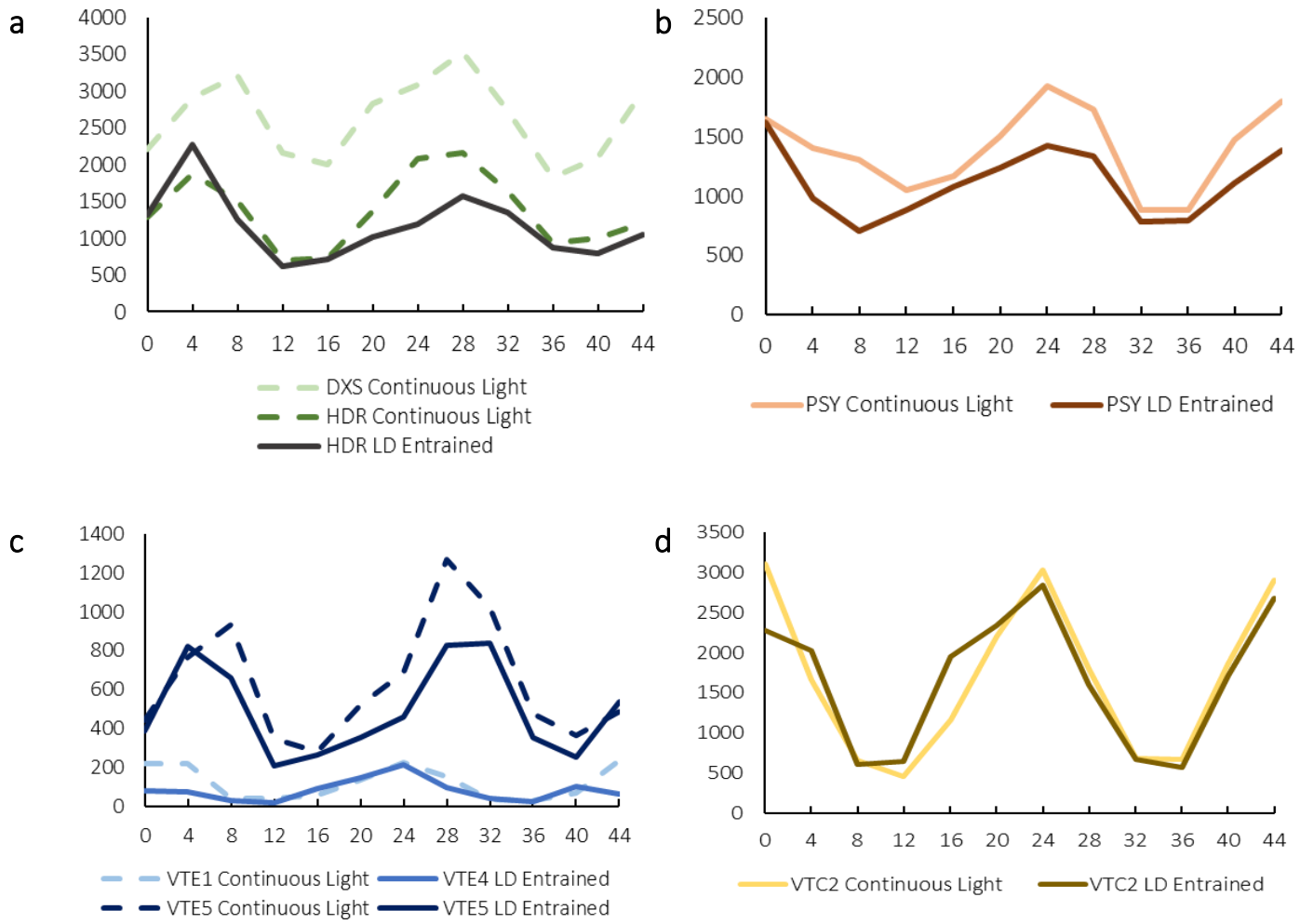
a



b



Appendix Figure 6.1.3. Log2 expression of (a) key biosynthesis genes of isoprenoid pathways and (b) *VTC2* of the ascorbic acid biosynthesis pathway in *phytochrome* (*phy*) *A*, *phyB* and *phy AB* photoreceptor mutants. Data presented is from the GSE3811 microarray (Tepperman, Hwang and Quail, 2006) using WT Arabidopsis and phytochrome mutants under 1 h RL (680 nm, 8 µmol.m^{-2}.s^{-1}).



Appendix Figure 6.1.4. Relative expression (y axis) of rate limiting genes within (a) MEP, (b) Carotenoid, (c) Tocopherol, and (d) ascorbic acid pathways across a 44 h time period (x axis). 'Continuous light' conditions constitute plants grown under a 24 h light cycle, whilst 'Entrained' plants grown under a 12 h light cycle then exposed to 44 h continuous light. Data collected from the Mockler DIURNAL database (Mockler et al., 2007).

(a) DXS (At4g15560.1)

TGATTGTATGTTGTTAGAATGGTTTATCAGAGTGCATTCGCACAACTATCCAATCTCTCGAGATCTTCTCTATTCGTTTTGATA
TTTTGAACTTTTTGAAAACCTCTCAATCTATTGATAAGACAACTTTTTTATGAAAAAATCAAGTTTGAGAACTAGGTTT[TGTG]
TTTATTGATCTAGTTTACTTA[GTCATA]TTCACTAAGTC[GTCAAT]ATGGTATGCTTTCAACAATAACATAGTAATTTGAATGC
CTCGTACATTTTAGGCGATTTTCTATTACAATGTTTTGGTAGAGAA[T{ACGT}A]TTTGTATATTTATGATGTAT[ACGT]GCATA
TACATTAATTGTTTTGTCGGTTTATTATGATCAAACAATAGTTTGTCT[GTCAGA]AGATTCAAGAAATATCTTTGAATCTGAGA
GTTTCCAAAAA[ACGT]TATGATGAAAATTTGGCATA[[T{ACGT}A]TTTCTGACAAGCTGATAATATT[GTCATC]TTGCATTTCA
AGCAGTTTCGACAAAATTTTATC[CAACTG]TTATATTCAAACTAATTGAAAATCGAAAAACCACCATTAAAAATTAGTTAAAA
ATAACATATCATTTTGAAAATGCAATATTTAAAAATTTAACATATCAATTTAACATGCTTGTTTTCTTAATAAGTTTTGATATA
TTATAAAATGAACCGCGTGATTACAATTTTTCTTTT[TGTG]ATGGAACTGTCGTTAGTTAAACACTTAAACTTAAACTTTCATG
TAAGTTTTGTTTATTCATATTTGACT[CAATTG]AAACCCAATAATCAAGAAT[GTCAAA]TATTATATGTTTTGTCGGATTTGTA[
ACGT]GATTCTCGCTCATTTAATAA[TGTG]ATAATATCATAAAATTTAATCTATGTAGTTGTTTAATATGATATTAAACTTTATT
AATGTAATAAGATCGATAATACTAAGTTACTAACCCAAATCATACACTTACGAGTTACGACACTACCGCACTATCTTGGTTAT
ATTAAATACTCCTTTTGTTTCTAATAGTTTGATGTTTATATCAACATTATTATTTACTTT[CATTTG]TTACCGATAGAAAGAGGA
GAAAATTGTTGACAAAAACAAAGAAAAAAGTAAAATTAATATTATTAAATTAATAAAAATAACAAACTGTAAAAGCTATTTT
TAAAAATTTTTCTTGTAAAACATCTAAAAATTATTCTTGTAGAAACAGAGGAATATCATTGAAGATAATAG[TGTG]AAATTAT
ATATATATATAGAAATATATAAAGTAGGATTTTTTTCTGTATACAAATAT[ACGT]TTCCAATTTTATCAAAAACTGTAAAGATT
TTTTTCTTT[GTCAGT]ACCTGCTAAACTTGTTAATTTTTTTATTAAAAAAAAAATCAAATTACAATTCTTCTATAATCATTTTAAA
TTCCATTTCTTTATACCACAAAAGATTATATTGCCTTTATCGTCTTTGGTATGTATGCGTGAATATATTTATTTATTTTCTTTTCT
TTCATTTTCTTTTTAAAGAACTTTATAAATGAAATAAGGAACAAACAATATA[CACATG]TACTA[ACGT]ATATAAATAATATC
ATCAATATCTATCCAAAACTTGGATTTCATGGTT[G{ACGT}G]GCCCAACC[AAAATCT]CAAGTTCTCTGCGGATGACGAACCA
TCTCACCATCTCTTTTTTTCTCTCTCTTTTTTTTTTTTTAATATCATCAGCACGGTTACATAAAATTCGTGATCCATGAAGTTGGCT
TTCTTGTCGTTTTACTTCATCACCCCATTTTTTTAAAGTCTCCATCTTTATACTTCTTCAACTCTCCACCACCACCATT[GTCACC]
ACCACATTTAAACACACACTTT[CACTTG]TAGTGGGATTCGAAAGTGCGTTTTATT[CATTTG]TTTTACTGTTTTTGATAACCTC
AAAATTTGCCTAAATTTTATTCTCTATAAATCCTTATATGTTTTACTTACATTCCTAAAGTTTTCAACTTTCTTGAGCTTCAAAA
AGTACCTCCAATG

(b) DXR (At5g62790)

ATATCCC[CATTTG]GAGGAAACGATCGTACTATTGTAAAGATGGCGACGCATAAAGTGACCGTAGATGATGTTATATGAGCC
AGTGTAATTGCATTCGAGGTTAGGGCAAGAGCATTGAGAGTAGTTGCATTCCTTTTCGTGACTTGACACTTTTTCATAAGAGA
CACTTTT[TGTG]CAGCCAAACTCAGTATTTTGACATGGAACAAAGGCTGATTCGAGAACCCTCTCCATTGCGAAGCATCGCTT
ATTACCAATGGGTAAATCACACCCAGGACCAGGG[CACTTG]TTACTCACTTTGGCAAAGCAAAAATTGCAAACTATATGTCCA
TCATCACACTGAAACGCATAATATACCAAACGAGATACAATCAAAGCCGTATTCAATAAAATAAATTAACAAAAGCGAAATC
AAAAT[GT{CACA]TG}AAACAAAATAAAATGCATGCAAGTTCAAAACATAACCTGAAAGGTAGGAATAGTGAGCGGCTCGAA
GCAAACCGGACA[GTCAAG]AACATCAAGATCCAACAACTTAGCCGATCGTGTTTCATTCTCCAT[ACGT]TGCTTCTTTTGCGG
GTGAGAGCTTTTTCTTTTTGATGTTTGATTTTGCGGGTGAGAGTTGCTATCCTCCATATCGATAAGGTTAACTTGAAATGGAAT
CGGAATTGGAGAGATGGCTACACCGTAATTATGGTTTTTGTTTCCATTAGTTAACGCATTAATTCAGGCTCCGATTTCCGATGT
TTAAAGAAAAAGTTGCATGCATGACTTGGGATGGAATTTGAGTGGATGCAGCAAGTAA[CACTTG]TTTA[ACGT]CTCTTTCAC
TGTTTTGC[CATGT{G]TCACT}TA[ACGT]ACAA[CACTTG]TCCT[CAGATG]ATCCATTAGCAGTAGTAAATGATTTACTGACTC
ATCTACCAATACCAACATACCATTATCTTACCATTATCATTTATCTATTGATCTCTGTATCCCCACGAAAACACTATGTAA[TGT
G]ATA[GTCAAT]CATAACAGCTTAATCACACTTAAATAAAGTAAATTATCTTTTTTCTTTTTCTTTTCTTTTTATG[GGATA]AAT
TTTATTCCTTTCCGAAGAAAGAAAAGAGAGCACCATTCAAACAACAATAATCTTTTTGAACGGATGATCTAATAGTCTACAAG
AAGACTAATTTTATTTAAACAATCGAACCTAACATGACTCTAATTAGAGATATAGAGAATTATA[T{ACGT}A]AAAAATTCAA
GTTGGTTAAAGTTACATGGCTTTTGAAATTTAAATTTACAAATTGAACATTGAGGAACATATCAAAGAAATAAGAATGATAGT
GAAAAATGTTGACAAAAAAAAAAAGAATGATAGTGAAAAATATTCAATACATAATTTTTAAAAAGAAAAGTATATTATATACT
T[GTCAAA]GATCTAAACTAAGTTTGA[CATTTG]GATTATAATTGTTACAGCATATAACATGTACTTTATAAAAAAAAAAAAAA
AAGAGAAGAAGAAAAAAAAAACATGTAACATGTTTTATTTTTGAAAAAGACAGCATACAACATGTTA[GTCAAA]ACATATTC
AATTTAAGATATTCATATCAAAAATTCATAATACGAACGAATACCATAAACTTTTTCTTTTTTATTTTGTAAGAGAGAAATCCT
TAACCATTAATCCCTTGTAATGCATATTTCTAATAATAATTATATTTGACAAACTATATAATTGATGATACAGATTCTGCTGTT
TAATACTCTAAAGAAATCAAATAGAAAATGGGAACATAGTAAGACTGGATTATTTACCACTTACAAGTTGTCGTTACCTTAAT
AAAAAGGTCCTTGTTACTAAATGCTCAGCGAAATCTTTAAAAAATGACA[AAAATCT]GTTGGGTACCATTCAAATCCAGATTC
CTTTCTTATCATCATCTCTCTCTCTCACACTGTTTATCTGATTCGTCTTCTCTGATAATCAAGAGTAGTAGTGCGGTTCTCTGGA
AAATATTCGATTTTTAAAAGACTCTGATGATG

(c) *HDR* (At4g34350)

GGTTTCAAAACCCGGTGAACTCCATCAAGGGTTGCCATGACTTTGCTAACAGTTTCTGGACGGGGATTCCAAGGATCACCAGCATC
CACAAACAGTACATCCTACAACACGAGATTAATTACAAAAAGGGACAAATCATGAGCTATCTCTCCTTTTCAAGAAAATATATAC
TTTAGAACCTCACTG[AAAAATCT]GAAGTCCTAAACAAACAATGGATCGAAAGAAAGTTGTAGCTCAGTTACAGATTTTACCATG
GTCCCTTTCTCAATAACCACATCAAAAGACTCGCT[GT{CAAA]TG}GGAGGTCTAGCAT[GTCAGC]TTGCACCACTTTTATTTCTGA
ATATATCAAAGTATCACATCAAATATTGAAC[ACGT]CGAGTGTAAGATCTAACTTTAAACCAGCAGAAACTAATAAACTTCTCAG
TATAATTAAATATAGACTATT[GTCAGT]GTCTAGTTTTAAAGAATCAGAATTCAGAAATCATTGTTAACTCTAGTTCTTCTTCATTA
CATTCATGCCTATAGAACTACTCAAACAAATTATAACTTAAGCAATACCTTTGTACCCTTTTGGTAGCAGCCGACTCTGCATCTTCT
CTACCGCCACGGAAGATAAATCAATGCAGGTAATATCAACAATCCCATCCTTATACAACTCTTCACA[CAACTG]AGAGTTCCCAC
ATCCCAATTCCAATACCTTCATTACAACAACAAGGAAAACTCAAAAATCAATTTTTTTTTTTGCAATCATCAAGAGATTAATAACAA
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GAACCATTCATAGTGTTCTTCAGAAGAGAATCTCTCGTCCCTGCATTCTTCCAAGACATTCAGAAACACAGGAAAGAGAGAGGGA
GACTACTTGTAGCTGAATGCAATTCATGATTACCAGTAGTGAGGATCGAGGTAGGTTAAAGCAGAAGGAGGACCAACGGAATTTG
GATTCTCCATAGCTGGACTTGCTCGATTCTCCTCCTTATCAGCTCAGCCAAT[AAACCCT]AAACCCCTTTTCACTAATCACACTAGT
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CATATG]GGCCTATAAAAGCAGCCGCATTTCTTAAGCC[CATGTG]ATTTTCTTACATCTACAAGTCTCTGTTTTTTTTTTTTATTC[AC
GT]CTTAACGGATTACTTTTGTCCAAATCATTTAAGATCAGCAGACTTATTGCC[TGTG]ACGATTATCAATTCATTCTATATTTTTCC
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GCGACTTCAAGAGACTTTCACTTTCGCGACCCAATTTTTTTTTTTT[TGTG]GCGTAGGTACGCTCTTTTAGTAGGATGTCTCTTCCCA
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TTGCATTTTACACTAATTCCCAAATACATTTCCTT[GTCAAT]TATCCAAAAACATATTAATTCAATTTTTTTTTTTTTTTTGACATCT
CTATTTTTTTTTTTTATTATAAA[TGTG]GAAGAGGCTCTGAGATAACTCGTGGACCAAAATCGTCCATTGAAGTGAACA[GTCAGC]
ATCGTTTTGCTTGCT[TGTG]CGTTTCTCTCGAACTCTCTCCATG

(d) *PSY* (At5g17230)

CTTTCTACCAACAATTACTTAATGACTTATATAATATTCTAATTATATTTTAATTATGTAATTAAATAACTATTGATCCCATCTC
CATAGTGACATGTCTCACTTTTTCTTCCACAACAAAATGC[GTCACT]CTTGATTCATCATGTTCGTAGCAAAATCC[ACGT]TAT
TACAAAAA[ACGT]TATACATTTTGAAAGGTAATCCATAAAATAGTGAGATGGATCAAGTTTTCTACGCAACCCTCGTAGAATT
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ACATGTAATTAACCAAACTTTCTAATTGATCAGGAATCAATTTAGCTAAATAATAAGACATAAATTTGGCCAAGAAAAAGAA
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TGGGTGAGAATTTTTTACGATAGAGGAAGAGACAGCATCATCTACT[TGTG]TTGTC[TGTG]TACATATATTACAGTAAGCGTT
GCAATATAACTTCTTGAGGATCTTCTCACATTAATGG[GTCAAA]CCTTTTGCTCTTCCTTTTGATTAATTTAGTGTTTG[ACAAT
CT]CCTCCTCCTTCTCCTTCTTCTTCAAAGTTTTGTCGCAGTATCTATTGTTCTTACAGAGAGAAAGGTAGGCTTT[TGTG]TCCAT
CACTCATTTTCGCTCGTTTGGTTAAGCTT[CATCTG]C[CA{TGTG}]GTTCACTGTTTTGATACTTTTGGGCTCTTATACCTAATGT
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ACTTGAGTATG

(e) *VTE1* (At4g32770)

TAAACAGTTTTTGTTTTTCACA[CATCTG]GAGATTAGATTGCAGTATGCCAATGTTTCTCTGGTTATGGCAACAAGGAGAAAG
AATTTCCTTCTTTTGGATTCAGAAGAATCAGCTT[CAATTG]TGTCTCATGTCTAATCATTAATTGTTCTGTTTTTATGGTAATCT
TGTAATATAAAGAAGGTGCCCAAGACAGTACGGCATTTCCACCTCCCCCATGGGAAGCTCAGCTCCAAGACTATAGTCCCTCT
GCGGA[GTCAGG]AAGTCCGTTTTCTCCTGGAATGCATCCAACTCAGACTGCCTTCA[CACATG]CTCAACCAGTTAACAACAAC
AATCCATATCCTCAAATTCCCCAAACCGGGCCACCA[GTCAAC]AATAACAGTC[CATATG]CT[CAAATG]CCCCAAACAG[GTC
AAG][CAGTTG]CCAACATCAGTCCATATCCTCAAATTCCACAAAACGGCGTGTACATGCCAAACCAACCAAATCAGGCTCTTG
G[GTCAGG]CTATCAACCACAGCAACAACAGCAGCAG[CAGATG]ATGATGGCTCAGTACTATGCCCAACAGCAACAGCTACAG
CAACAACAACAGCAACAGGCGTATGGAAAC[CAGATG]GGA[GGATA]C[GGATA]TGGCTATAATCAACAGCAACAAGGAAGC
AGCCCATATCTGGACCAG[CAAATG]TACGGTTTATCCATGAGAGACCAGACATCGCATCAGGTAGCATCATCATCATCTACCA
CATCTTATCTGCCTCCAATGAAACCTAAGAATAAACCAGAGGACAAGCTATTTGGGGATCT[TGTG]GACATCTCCAAATTCAA
GCCTACAAAACCGACTTCCGGAAGAGCTGGTAC[CA{TGTG}]AAAATTCCTCCATCCATTCATCATTTACCAGTATTCATCTCC
TCTATCCTCCTCAGCTAACTCTCTTTCTTCTCTTTGTTAAGCTTTTTTCATCATTGATTTTATTACCCTCTTGGGAGATAACATAG
ATATA[CATATG]TGTTATGTTCTTCCTTATAATTTACCGTTGGTTATGGGTTTGTATATTTCTTGTAGAGAAAAGAAAACTGG[A
AAATCT]TTTGTTGAGAACTCAGCGATTTGGGGTTTGTCTCGAGATTCTATTTTAGGACATTTTAATCTCT[CATTTG]TATTTTC
AGACCTTTTATTTGTTATATCCATTGGCAATAAAGTTTGATAT[CAATTG]GATGAGAGTTATGGTTCTTAAATTTGCCAAAAGG
ACAGTTTATGTTCTTTGTATATGATTGCAACCCTAATTCCATA[GTCAAT]GGAGACATCAAAAT[GTCAAA]CTATGCTTGCATT
ATATAATCTCTTTCGACTCAATGAT[GTCAAT]TACCGAAAAAGTAGAAAGGAAGGATTTGTTTTGTTTGCGACTGTTGTTAGTT
CTTATGTTGCTCGTCGTAAACAAGAAAAACCTGCCATTCGTTTGGGTTTTTGAAATCTAGTCTAACAGAAAGAAACAAGAGA[
CAAATG]AAGAAGAGTAAATCTAGTCCATTCATCATCATCAGTCTTTTGGATCGTGCATATATTAATTTGTATAAGAGTCTGTC
TAACAAAATT[GTCAAT]ATGTCCGAGTGGTTAAGGAGATTGACTCGAAAT[CAATTG]GGCTTTGCCCGCGCAGGTTCGAATCC
TGCTGTT[G{ACGT}A]TTCTATTTTGTTTTCTTTTCAGCTCTCTTCAGAATTTTTGTAAATATTTTAAAAGGGAA[AAAAATCT]C
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ACTGAAAACAAGACACGAGAAATTGTTAATTTAACAAAATAAAAAT[ACGT]T[ACGT]TTGGGGTTTGCC[ACGT]AACTACTTA
CCG[CCAC{A]CGT}TCTCGTCCTTTTCTTCTTCCTCTCTGCATTCTTCACAGAGTTT[GTCACC]ACCAACACCAAACACACAATT
TCACATTCTTTTGCATATTTCTTCTTCTTCTTCCATTATG

(f) *VTE4* (At1g64970)

GAAAGATCCCACCACGCAGCCTAAAGCGGTGCATTATACTCGTGGAGGGCCTTGGTTTGATGCTTGGAAGGATTGCGAGTTTG
CGGATCTTTGGCTTAATGAGATGGAAGAGTACAACAAAGAGAACAAGAAGGAAGCTGATAACGCAAAGTAGAAAGAAAGAA
GTAAGAGGTATGTTGTTACTTACCAACACGAGTAGTGAACTTCCTTTACATAGTGTTGGCTTTGTAGAGCCAGGGATCATTAG
TCT[TGTG]GAGGGCTATGGAGTTTTTTTTAATTTCATTGAATTCGTTAAATCATTCGCAAACACTATTAGTACCTATATTGAG[G
T{CATA]TG}GTATAATAACCCTATTCGTTTGTTTATTTGT[TGTG]TTTTGTACCTTTGATGGTATTTTATTTGTATAATTGATA[T
GTG]AATTTATGTAGCATTTCTTTTTAATTGATCTATAATGAGCAGTAGCTCAACATATTTAAAACATGGATGGATGAGTAATG
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TGGGACTTGTACATGTACTTACTGATAATTACCTAACTAAACGAGACTCAGTGCTAGAATATGAGCATAAATCCTCATATTGA
GGAATCACTAATCATTTTTATACTATTTACCTTTCACATCGTATTCTTATTCATTTTGATCCTTTTTTGGTATCAAACTAACAAT
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ATATTCACACCCACCATCACTATGCATCATATTAACTCATAATAAATGTTGTCGAAATCCTCTTC[TGTG]AGTTTGTAATGCAC
AACTACAAATCTT[CAAGT{G]GATA}AAAAC[ACGT]TACTATGTTTGTA[TGTG]ATTTCAAATATTATAAATTAGTAGGTGATA
CAATTATTACTTTATCTTACAACATTAATAATAGGTATCTATGTTTATTTTTATTTTCTTAAAACTTGTAATTTATATAGTTTTTA
TAAAGTATTAGGCCGCAGATTGGTCTTTTTCTAAATGGATTCAAC[CATTTG]AAATTGATGCG[TGTG]ATATGCTAATCCACAA
TATCCAAAAAAAATTCCTTCTAAAAT[TGTG]ACTTTAAAATTTTGACTGGTTTTGTTAAGAGTA[ACGT]TGAAGAGGAAGGGG
AAGGAAAA[GTCAAT]AATGAAAGAAAATAGTTTCTTCATTTTTGGATTTAAATGGTGAGCATGGTTAGAAGAAAAGAAAATT
ATCAAAGGAAACAATATCTATATATTACCCAACTATTTATCA[GTCAAA]AAAAAAGAATTGTTTCCACCCTACCAAAAAAAA
AAAAAAATATTGTTTCCAACATATTTTTTCTTTTTTCTGAATAATCCATTTTTGACCGGACAAA[CAGTTG]GGTA[GTCAGA]TT
AACTTTTGTACTTTACCATTTTAATCTTTCTCTAACCTACTCATCCGTAATAGAACGGTGTCCACGAGGCTCATAGTCCTACAA
G[CA{TGTG}]ATAGTT[GTCACA]ATTACCATATTAAGCTCTCTTTGCTTACTTCACTTCTTCTATCAATATTTGTTTGCCACTGAC
TTTTCACTAAATTACAAAACTAAAATGATGGAAGAAAAAGAAAAGTTAAACAAAAAAAAAGGAGAAAAATCAAAGTGATAA
GTGGATGTAGACGATTGGCCCTTTCCCCAAGACC[TGTG]GCAACAGAAAGTTT[TGTG]GCTCTAAAGTTAAGAAAAATGGTCC
AATGTTATATATCCAAAGTTTGATCTCACACA[GTCACA]CTGTAACAATAATCAAATAATCCCTGACTTC[GTC{ACGT}]TTCT
TTGTATCTCCA[ACGT]CCAATAAATG

(g) *VTE5* (At5g04490)

AGCGGAGACAGTGGTGGAGAGATTGAACAATTTTTAT[GTCAAA]AACTTTTAATAATTCTACTAAATTTACATTTTTCGAATTT
AATATATTTTTGTTAGGACGGTGACAATAATTTCACGCGTTTTATCACTCAAGTCCAACTTGCTTTCTTGAGCAGTAATT[GTCA
CT]ATTAGTGATAATGGAATCACTATCCTTTTCGTATATCTCTATCTAATTAT[GT{CATT}TG}TCTAATCTCTATCTAATAATCA
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TCTATAGTTGTTGTTTATCGTAACCATTTTTCATCTACATTTATTTTTGAATTGAAACTTGATGAAAAAATTAGTAGAAAATAG[
TGTG]AAACCATTGACTTCTAGCGTGTTGATTGAGTACGAATCAATCAAAATTAAATGAAAACATAAAACTGTTACTTACCAG
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T]CGCCTATGTTTGAGGGTCCATATTTATAGCTAGCTCTCATATACATGATTTTGTACTCCTTTCTTCTTTCTTTTTGTTTTCTACT
TTGTTAGATATTATCTTCGTTCGTGCAATTATTGAGAAATTAATTGCAGTACTTTTTACATTTACACCCATATTTGCATATACAC
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ATATACTATGATAATGTAGAGAACAGCTAGTTTAAAACTGGAAATTTCATC[CAAGTG]TTAGTATA[GTCAAT]CCGTTTTTTA
AAATGATGTTTTATCTCTCTTGGATTGGAGTTCAGAGTTCAGACCATGTTGAAGAATGGTAATTTATACAATGTAAAATTTCAG
ACCAAAAGAATAGTAACGGTCTTAAGTACTAAAATGTTAGGTCTTCTACAACTAGTGAAAAAGATAAAGTGACAGAAAAACT
CAAAACGATTTAATTGTTAAAAACGATTTACAATAAAAAAATCAATTCTCAGTTCAAAATATTGTAGATCGTCTAAGCTTTCA
GAATATATACTAGGTTGAGAAAAAAATAGATTCACC[GTCATA]ATTAGGGTCGGTCGGATTTTACAATAAACCCAAAAATAA
AGTACGGAAGGTAAAAAAGTAAAAAACACAATTACCCGAATAGGCCATGCTCATTGGCCATTTCCCACCACTAGGTTAGTAC
AAACTGAC[CAACTG]TCCCAATAAAGAAAGACGAAATTACAA[TGTG]AGGCAGATTTTTTGCATTCTTCTCGATCAATCAGAA
AGTGACACCTCATAGATT[ACGT]CTTTTTAGTTCTTACTAACGAAACACCCAAACAAAAAAAGATAAATTACAAAATATCATT
TTCCTTATCTTATTGACTT[GTCAAG]ATTCTCTTCTTCTTCTTCTTCCTCCTCCTCCAAACTCAGTTCCCTCCGTCCATG

(h) *VTC2* (At4g26850)

CTTTGTAACTGAGTTGAATATTTGTAACAGCAGATATCGCATTTTGATACTTGTCCTCTCGAAATTGATGTTTACTGACGCACG
ATTATTGGGAATTTGGGACTTAGTGGTGTACCAATAGCATCTCAGCTTTCATGGTTACAAAGAGGCCAGTGAAAGAGGCTTG[
GTCAAC]CGCAATTATGATCCGTTACTTGCACTGACTACCACCGATCCACCGCCGTAACTCTTTAAATCTTAATGCAATACTAA
TTCGAATTACATGTCGAGGTCTTAAAACATGATAAATGGTTGTTTAAAGTAAAAAAAGAAAAGAAACAATAAATAAAACAAA
CGGTGATGGAAGGAGG[G{ACGT}A]CGATCGTAGTCGTAGAGACTGCCAAATAAATAATGGACCACTATTA[TGTG]GTTCAAG
AACTATATCCTTTTCTTAGATTCCGAATCTTAACT[TGTG]TTTCTTGCCTTGTTTGGTTTTTTTTTTGTTTATTTTGTAGTTTTGTC
TCATAAAATAACGAACAAAAAGCAATGGTCTAAATATTTCTCAATATATGTATTTAAAAATTATATCATAGTTTCGCAAAAAA
ACGACCGAACGAACGATGGTA[GT{CATT]TG}CCCAAATTGAGCCTCGTAGTAGTTGCTACGA[CCACAC]CCCCGTCCCATGA
ATAAACACGACCATGTAAATATTATCTATAAAGACAACATAAATTTACAAATTAATGTTGAACAGAAAAA[GTCAAA]AAGAA
AATTTCGTTAATATAATAATAATATTCTATGATAATATAAA[ACGT]GGCATAA[CACATG]ACTT[CACATG]ACATCATAAGAA
GA[CATATG]C[CACATG]AACTCTTCATCGCCTCCATCCTTTTAGTCTCGTTTACATGCAGCAAACTACGATCTACGATTATATA
CAATGAAATTCAAATTCATAAT[CAATTG]GCATTAAACA[T{ACGT}A]TATCATAATTCATAAGGTTAACTAGGTTTAG[CAAA
TG]TTATTCTCTTT[GGATA]A[ACGT]TTAG[CAATTG]TTTATCTCAAATTTAATTGAAAATACTTGTAAGACACAGTTACAATTA
GTTCTCAATTTCCAATCTACACAAGATTTAAATCTTTCTTTAGTTCATTCTGATCTATTTTTCTCTCAAAGAAAATGTAGTGCTC
CAACTTTTCTCCCAAGACTAATAAAAATTTTCTTAATTGCATGCAAAAATATTTTTTCCAACTTTAATCTTATCAAACTTAGAT
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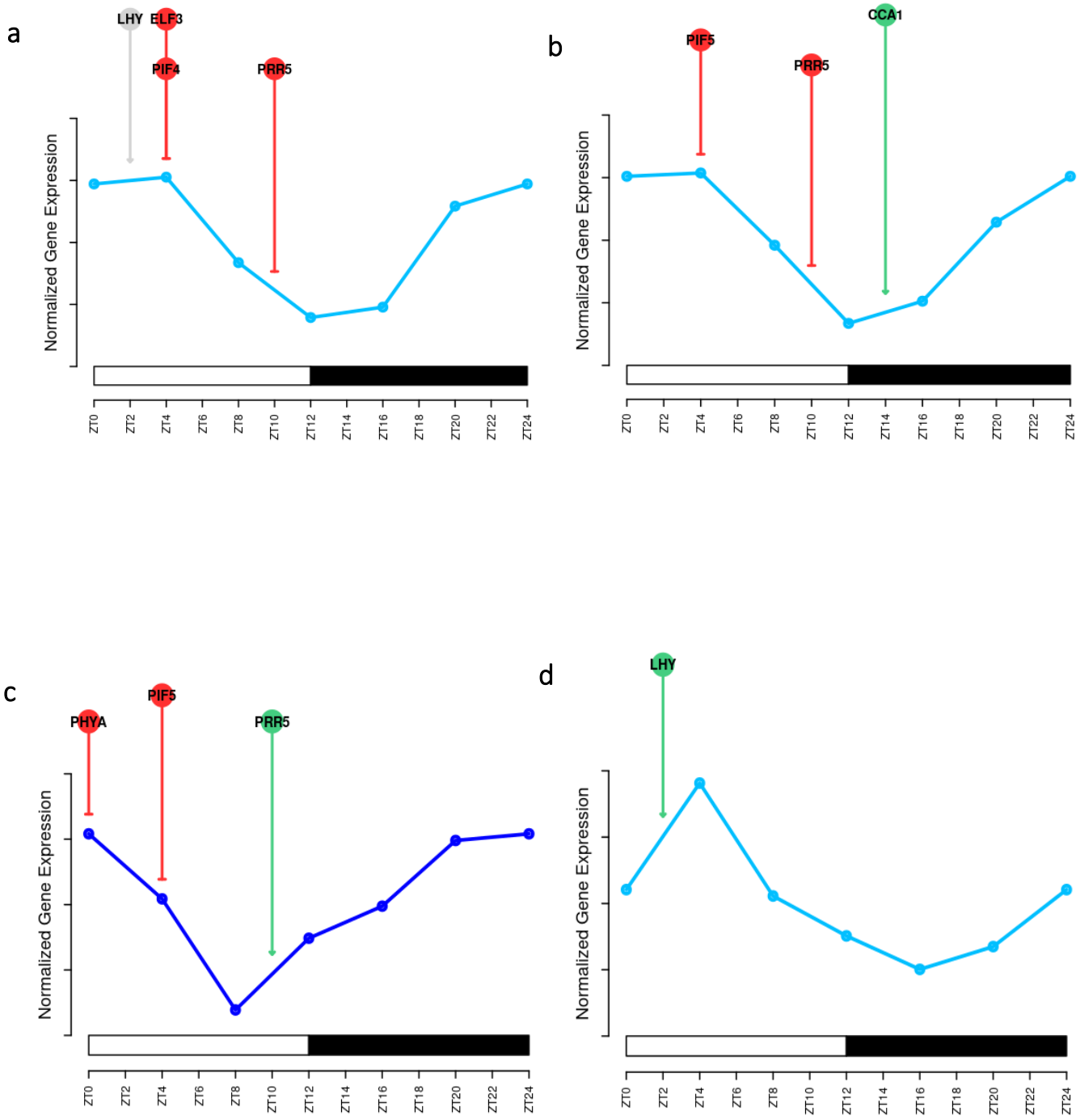
AGACAAAATAATATCGATGAAAAAATAAAATCCACAAGAAAGGACCTAAGAAATTTC[ACGT]CCGAATCACAACCACAGAA
AAAAAGAGAGAAATATTAGTATATAATTAAAATTTTGTCGTCTGTCTTCTCTGGTTCACTCATCTCCTATCTATTTA[AAGCCC]
ATTCGATATCCTAAAACACTGTATCATCAAAAAACACCTCAAAGAATTATTCATTCAGGCATCTTCTCAAATTTTTGTT[TGTG]
AAAAAAACCCACATCAAAAGATCTCTCATTTATTCGTTTCGTTTCTGCTGTTTTGAGTGTCGGGTTCGTTTTAGCTGTAATCTTT
TTTTCCGGCGTTCGATTTGAAAAAATCCGGGGAA[CAGGTG]ATCGGAATCACGGCTATACACG[GGATA]TCACGGGGTGTTA
GCT[CACATG]TCCATATTGTCCGACAGAAGGGTTGTTTAATCGAAACTAATCCTTTGCCGCACGGAG[G{ACGT}G]GAGCTCTG
CCGTCTGAAGGCGGCAGCCCTTCCGATCTCCTCTTTCTCGCCGGTGGCGGTTCCAGCTTTAACTTCTTTTCCTTTAGGTTTTAGG
AGTTAGGGTTTGTTAGTGTTTTTTCCTTCTTCTTTTTTTGGTGCTCTTGAATCGCTTTTTTCTTGGGGGAAGTTTTTTCTTTTGCTC
TTCGAAATTTGTCTTTTTTGAGAATG

(i) Key

Name	Motif	Circadian Module	Binds
G-box	CACGTG	Morning	HY5, PIFs
Morning Element (ME)	CCACAC	Morning	TOC1
HUD / PBE-box	CACATG CATGTG	Morning	PIFs, MYC
Evening Element	AAAATATCT	Evening	CCA1, LHY
GATA motif	GGATA	Evening	LHY
Starch box	AAGCCC	Midnight	
Telo-box	AAACCCT	Midnight	
Protein box (PBX)	ATGGGCC	Midnight	TOC1
CCA1, CBS	A(A/C)AATCT		CCA1
T1ME	TGTG		TOC1, PRRs
Z-box	ATACTGTGT		HY5, PIFs
C-box	GTCANN		
E-box	CANNTG		
G-box coupling elements (GCE)-box	ACGT		
A-box	TACGTA		
CA-hybrid box	GACGTA		
CG-hybrid box	GACGTG		

Appendix Figure 6.1.5. Binding motifs with light signalling associations were searched within the 2Kbp promoter region upstream of ATG in (a) *DXS*, (b) *DXR*, (c) *HDR*, (d) *PSY*, (e) *VTE1*, (f) *VTE4*, (g) *VTE5*, and (h) *VTC2*. (i) Shows the colour code key with the name and sequences of the motif searched, including any associations with circadian modules and targeted binding molecule.


a
LHY
ELF3
PIF4
PRR5
Normalized Gene Expression
ZT0
ZT2
ZT4
ZT6
ZT8
ZT10
ZT12
ZT14
ZT16
ZT18
ZT20
ZT22
ZT24
b
PIF5
PRR5
CCA1
Normalized Gene Expression
ZT0
ZT2
ZT4
ZT6
ZT8
ZT10
ZT12
ZT14
ZT16
ZT18
ZT20
ZT22
ZT24
c
PIF5
PHYA
PRR5
Normalized Gene Expression
ZT0
ZT2
ZT4
ZT6
ZT8
ZT10
ZT12
ZT14
ZT16
ZT18
ZT20
ZT22
ZT24
d
LHY
Normalized Gene Expression
ZT0
ZT2
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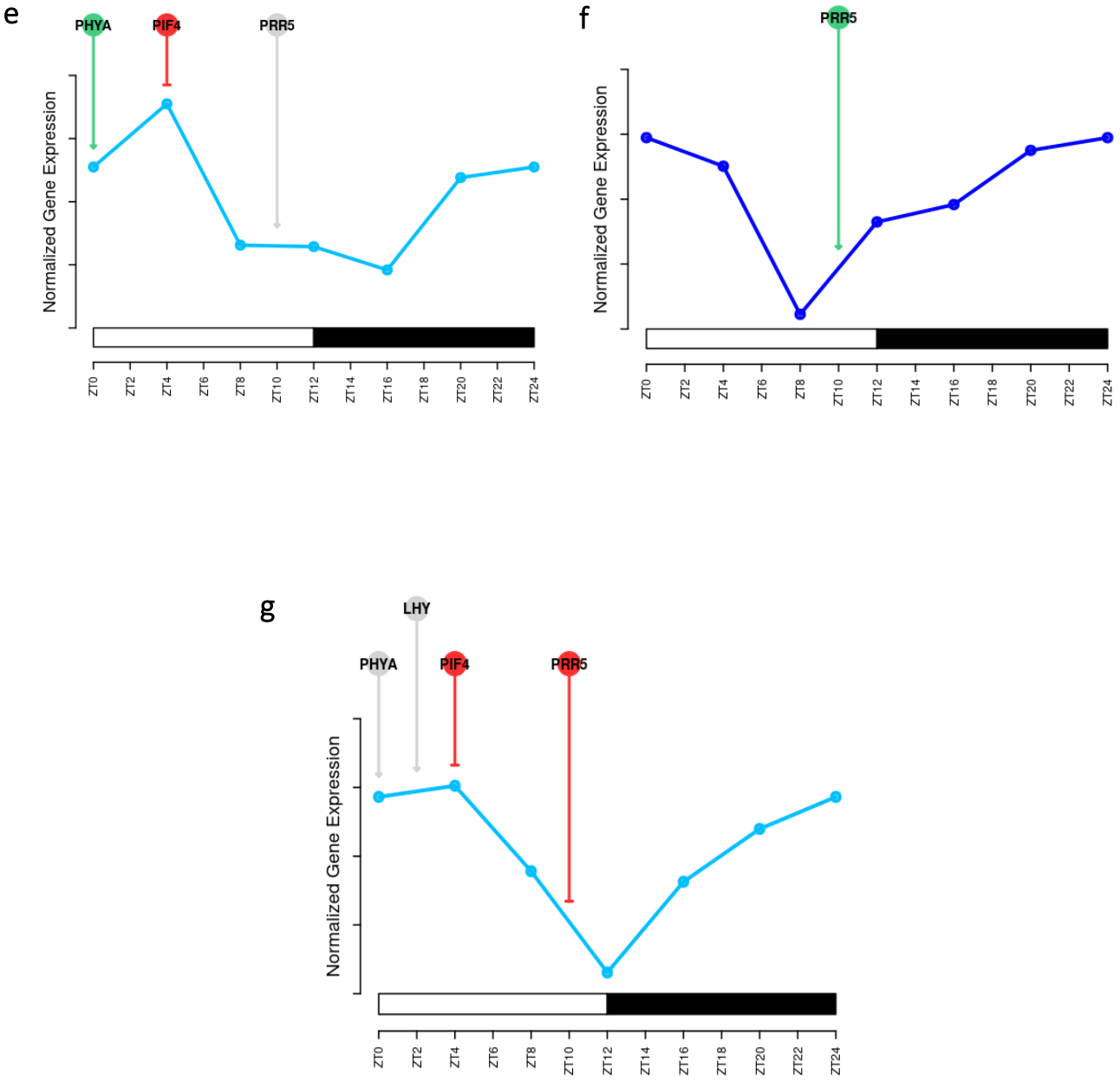
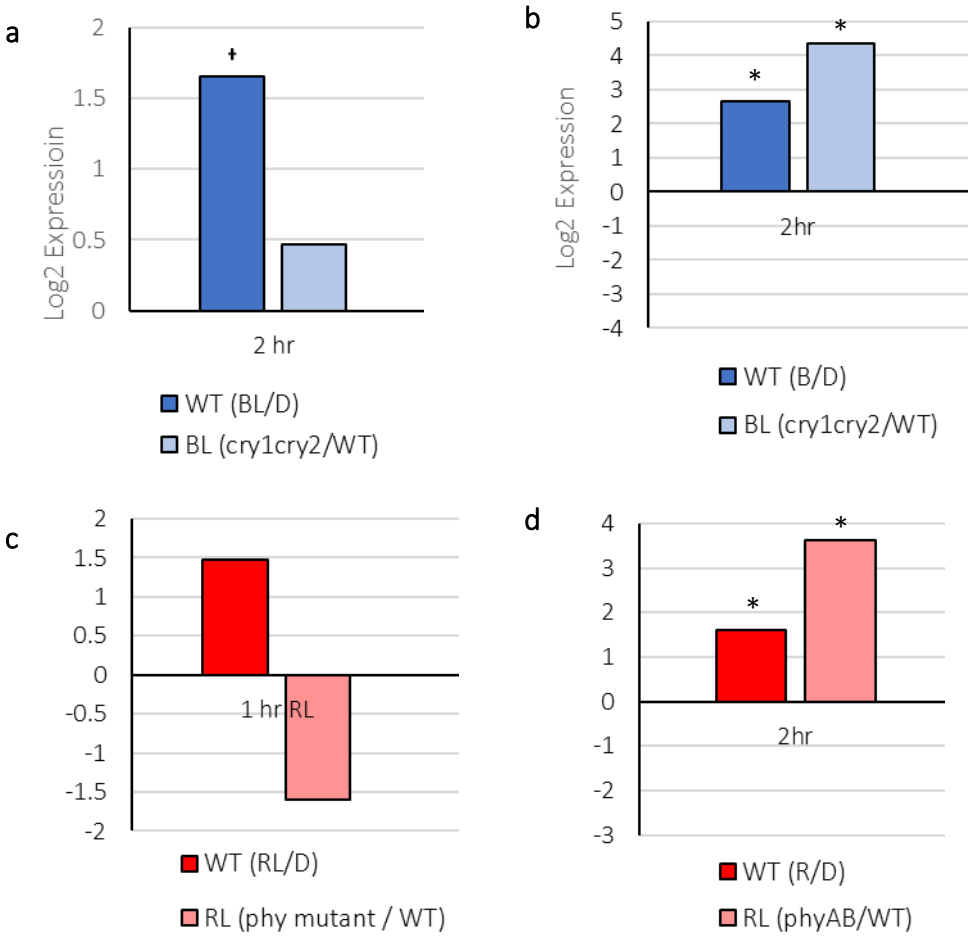
ZT24



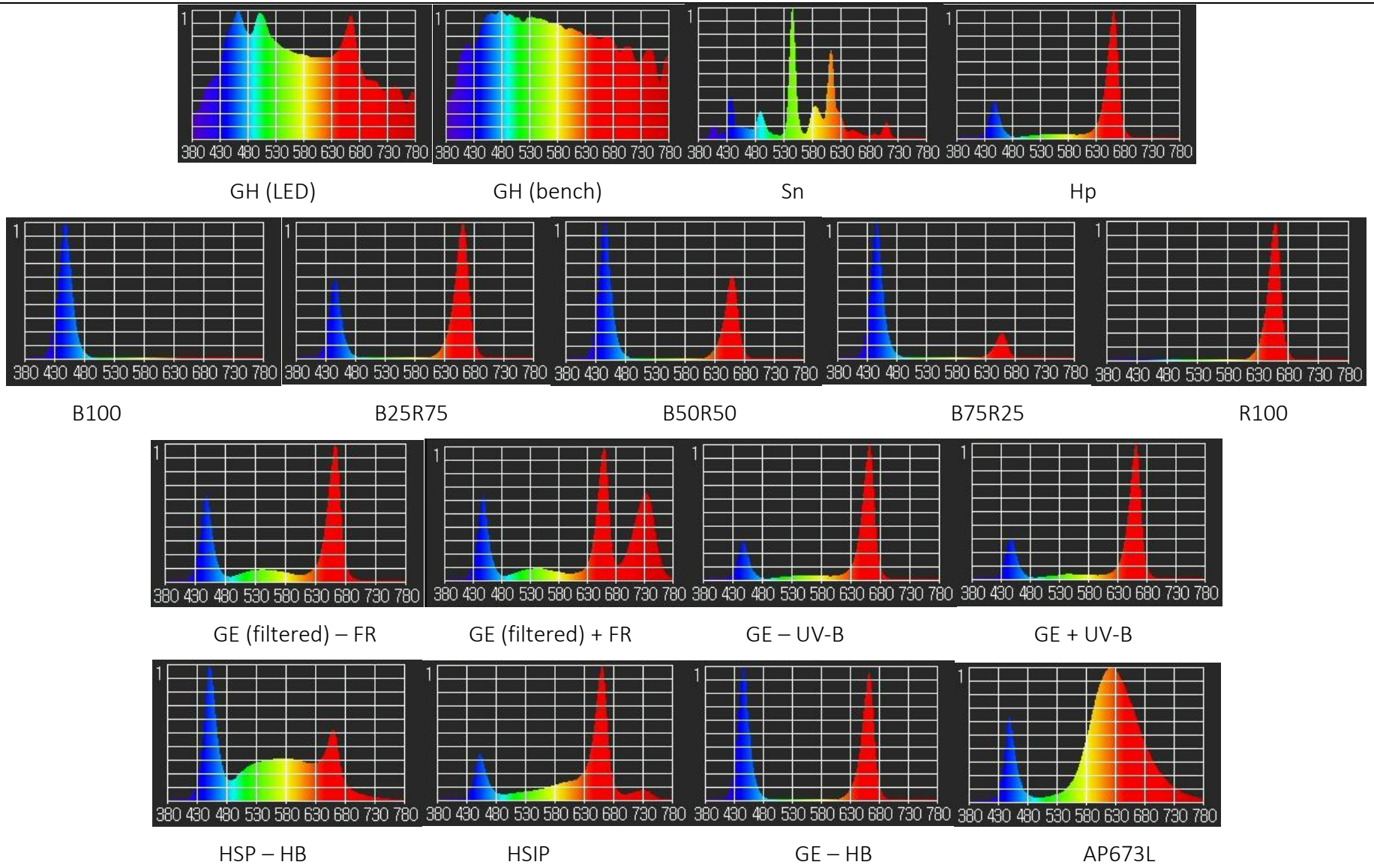
Appendix Figure 6.1.6. Results gathered from the ATTRACTOR database (https://greennetwork.us.es/ATTRACTOR/) for rate limiting genes: (a) *DXS*; (b) *HDR*, (c) *PSY*; (d) *VTE1*; (e) *VTE4*; (f) *VTE*; and (g) *VTC2*. The result displays a 24 h expression profile with the influences of associated signalling component attributed to particular time points and contributing to the circadian expression.



Appendix Figure 6.1.7. Acute responses of VTC2 to short term red and blue light. (a) Genomic data from GSE80350 and (b) qPCR data for WT and *cry1cry2* mutants grown under 2 h blue light (BL) compared to darkness (D). (c) Genomic data from GSE3811 for WT *Arabidopsis* and *phyAB* mutants grown under 1 h red light (RL), (d) qPCR data for WT *Arabidopsis* and *phyAB* mutants grown under 2 h R. For information on qPCR data see Methods 2.2.3. Bars indicate the average expression of three biological replicates where data was available, with a cross denote statistical significance as determined by FDR, and asterisks indicate a significant difference calculated by *t*-test ($p < 0.05$) between the treatments indicated in the figure legends.

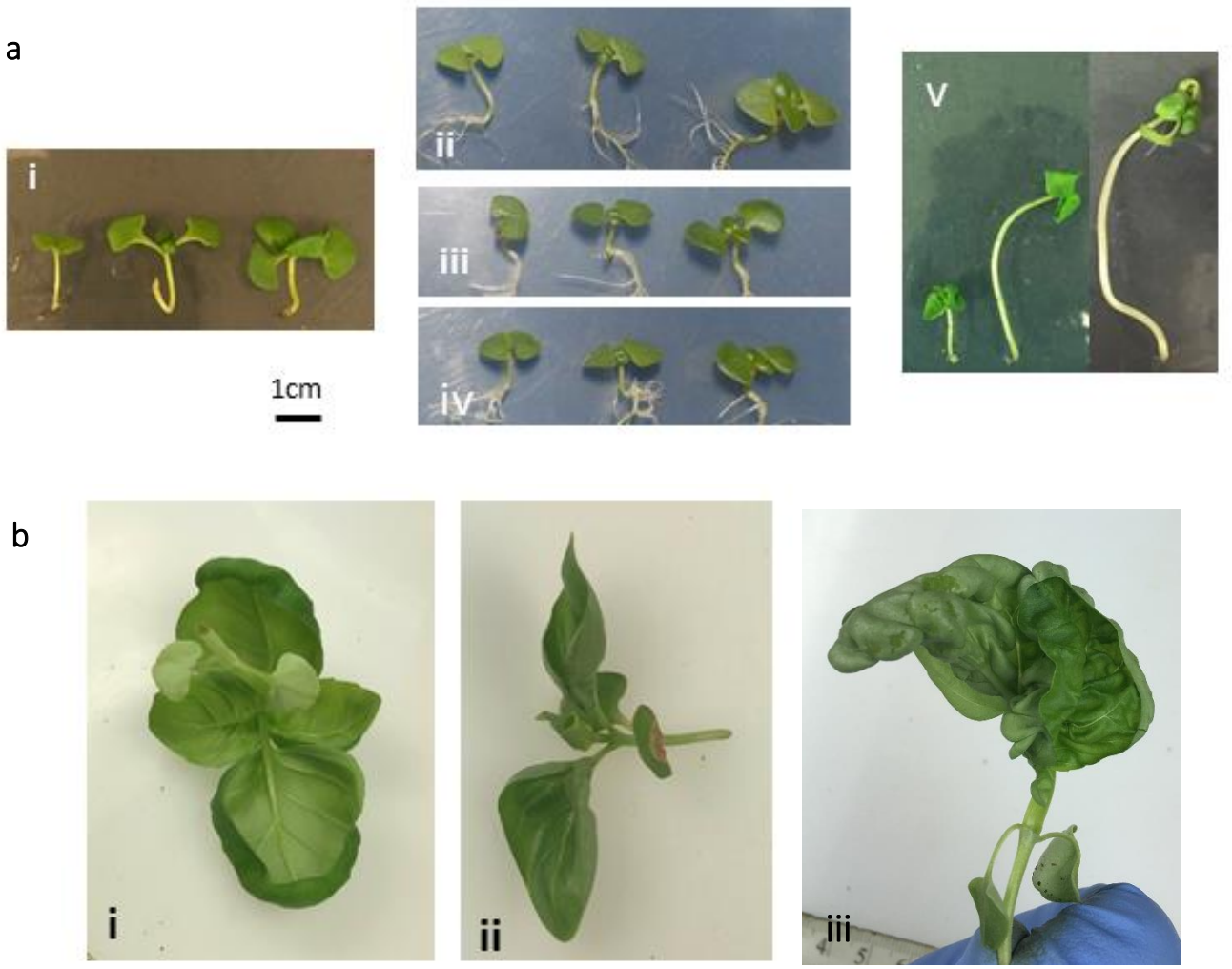
Spectra	PPFD (400 - 700nm)	Blue (400-500nm)	Green (500-600nm)	Red (600 - 700nm)	Far-Red (700-780nm)	Ultraviolet (320-400nm)	PFD (320 – 780nm)	R:B Ratio	B:G Ratio	R:FR Ratio
GH (LED)	64.30	96.8	114.1	125.1	64.30	5.407	405.7	1.3	0.8	1.9
GH (Bench)	433.0	110.7	154.4	167.9	128.60	7.434	569.0	1.5	0.7	1.3
Snijder (Sn)	162.9	33.3	72.7	57.0	10.20	0.565	173.7	1.7	0.5	5.6
Hydroponic (Hp)	150.1	22.2	11.7	116.2	1.49	0.121	151.7	5.2	1.9	78.0
B_{100}	148.7	148.7	0.0	0.0	0.00	0.132	148.9	-	-	-
$B_{75}R_{25}$	142.5	109.5	0.4	32.6	0.13	0.119	142.8	0.3	273.8	250.8
$B_{50}R_{50}$	151.5	74.7	0.6	76.2	0.58	0.142	152.2	1.0	124.5	131.4
$B_{25}R_{75}$	150.7	37.0	0.7	113.0	1.09	0.138	151.9	3.1	67.1	94.5
R_{100}	153.2	0.0	0.0	153.2	0.24	0.000	153.4	-	-	-
GE (F) -FR	156.1	38.5	21.3	96.3	2.36	0.163	158.6	2.5	1.8	40.8
GE (F) +FR	159.0	35.1	19.4	104.5	76.42	0.154	235.5	3.0	1.8	1.4
GE –UVB	145.6	35.6	19.5	88.5	1.88	0.130	145.6	2.5	1.8	47.1
GE +UVB	154.1	37.6	20.3	96.2	2.00	0.134	156.2	2.6	1.9	48.3
HSP-HB	256.8	75.1	85.2	96.6	8.65	0.239	265.7	1.3	0.9	11.2
HSIP	211.1	31.3	33.0	146.9	17.28	0.194	228.6	4.7	1.0	8.5
GE-HB	267.1	106.1	2.0	158.9	1.87	0.240	269.2	1.5	53.1	84.8
AP673L	153.8	17.1	32.0	104.7	13.70	0.136	167.6	6.1	0.5	7.6

Appendix Table 6.1.2 Values (± 10 µmol m^{-2} s^{-1}) acquired by spectrophotometer readings by PG100N (UPRtek, Taiwan) describing the spectra depicted in Appendix Figure 6.1.9 by the content of wavelengths (nm) within specified ranges. GH (LED), reading from the LED in the greenhouse; GH (Bench), reading at the bench level of the greenhouse on a sunny day; GE (F), GE-Arize LEDs filtered with SC60 filter (LEE filters); HSP-HB, Heliospectra Indoor Propogation High Blue; HSIP, Heliospectra Indoor Propagation; GE-HB, GE Arize High Blue; AP673L, Valoya AP673L.



Appendix Figure 6.1.8 Relative spectrograms taken by PG100N Spectrophotometer (UPRtek) of all the experimental light qualities tested.

For abbreviations see Appendix Table 6.1.2.



Appendix Figure 6.1.9 (a) Images of Basil seedlings grown for 14 days under (i) 100% blue light, (ii) 75% blue light and 25% red light ($B_{75}R_{25}$), (iii) $B_{50}R_{50}$, (iv) $B_{25}R_{75}$, (v) R_{100}, under low, medium, and high light intensities (from left to right). The intensities used can be found in Chapter 3 (Table 3.4). (b) Images of Basil grown for 4 weeks (i) without UV-B, showing normal downward cupping of the leaves, (ii) with UV-B supplementation, where leaves have begun to curl upwards, and (iii) Basil leaves displaying Red Light Syndrome, where leaves are crinkled and unexpanded.

Light Condition	Replica #	N	FW (g)	DW (g)	DW %
GH	1	26	0.53 ± 0.28 ^{a}	0.03 ± 0.02 ^{ab}	6.1
	2	27	0.53 ± 0.24 ^{a}	0.03 ± 0.02 ^{a}	6.0
Sn	1	22	1.13 ± 0.52 ^{bc}	0.06 ± 0.04 ^{bc}	5.7
	2	28	0.80 ± 0.21 ^{ab}	0.06 ± 0.02 ^{c}	7.1
Hp	-	19	1.54 ± 0.94 ^{c}	0.14 ± 0.08 ^{c}	9.2
B100	1	19	0.69 ± 0.37 ^{abc}	0.05 ± 0.03 ^{a}	7.6
	2	23	0.68 ± 0.24 ^{abc}	0.05 ± 0.02 ^{a}	7.1
B75R25	1	23	0.65 ± 0.34 ^{ab}	0.05 ± 0.03 ^{a}	7.3
	2	23	1.05 ± 0.50 ^{c}	0.11 ± 0.04 ^{bc}	10.6
B50R50	1	24	1.87 ± 0.56 ^{d}	0.15 ± 0.05 ^{c}	7.9
	2	15	0.50 ± 0.26 ^{a}	0.13 ± 0.08 ^{c}	25.8
B25R75	1	28	0.99 ± 0.34 ^{bc}	0.07 ± 0.03 ^{ab}	6.8
	2	19	0.94 ± 0.39 ^{abc}	0.06 ± 0.03 ^{ab}	6.7
R100	1	23	0.76 ± 0.42 ^{abc}	0.05 ± 0.02 ^{a}	6.4
	2	18	0.82 ± 0.34 ^{abc}	0.08 ± 0.02 ^{ab}	9.2
-UVB	-	26	0.89 ± 0.34 ^{c}	0.06 ± 0.03 ^{c}	6.9
+UVB	-	25	0.34 ± 0.21 ^{a}	0.02 ± 0.02 ^{a}	6.6
-FR	-	26	0.51 ± 0.29 ^{ab}	0.03 ± 0.02 ^{ab}	6.3
+FR	-	25	0.60 ± 0.40 ^{bc}	0.04 +- 0.03 ^{bc}	7.3
HSIP	-	28	4.19 ± 1.02 ^{c}	0.34 ± 0.09 ^{b}	8.2
HSP-HB	-	28	3.53 ± 0.84 ^{bc}	0.29 ± 0.08 ^{b}	8.1
GE-HB	-	28	3.31 ± 1.04 ^{b}	0.29 ± 0.07 ^{b}	8.9
AP673L	-	28	1.60 ± 0.35 ^{a}	0.16 ± 0.02 ^{a}	9.7

Appendix Table 6.1.3 Fresh (FW) and dry weight (DW) measurements, as well as percentage DW (DW %) of adult Basil shoots (4 weeks old) grown in different experiments in Chapter 3. Weights represented as mean ± standard deviation with different letters representing statistical significance as determined by sidak post-hoc test ($p< 0.05$). Experimental treatments and abbreviations are as described in materials and methods in Chapter 3.

(a)

Figure Number	Measurement	Treatment	Intensity	Treatment x Intensity
3.2	Height	<0.001	<0.05	<0.001
	FW	<0.001	<0.001	<0.001

(b)

Figure Number	Measurement	Treatment	Trial	Treatment x Trial
3.1	Height	<0.001	0.377	0.066
	FW	<0.001	0.086	0.091
	DW	<0.001	<0.001	<0.001
	Leaf Number	<0.001	0.070	<0.05
	Leaf Area	<0.001	0.109	<0.05
	Leaf Thickness	<0.001	<0.001	<0.05
3.3	Height	<0.01	0.197	<0.001
	FW	<0.001	<0.05	<0.001
	DW	<0.001	0.126	<0.01
	Leaf Number	<0.001	<0.001	<0.001
	Leaf Area	<0.001	<0.001	<0.001
	Leaf Thickness	<0.001	<0.001	<0.001

(c)

Figure Number	Treatment	Vitamin	Treatment x Vitamin
3.5d	<0.001	<0.001	<0.001
3.6a	<0.001	<0.001	<0.001

(d)

Figure Number	Treatment	Trial	Treatment x Trial
3.6b	<0.001	<0.001	<0.001
3.6c	<0.01	<0.05	0.267
3.6d	<0.001	0.099	0.202
3.7a	<0.001	<0.001	<0.001
3.7b	0.354	<0.001	0.279
3.8a	0.102	<0.001	0.324
3.8b	0.256	<0.01	0.457

(e)

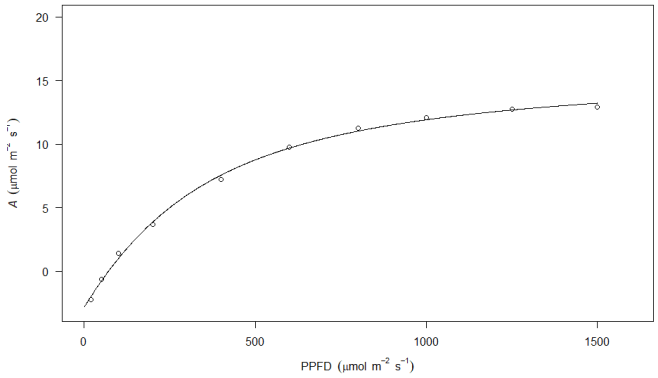
Figure Number	Treatment	Chlorophyll	Treatment x Chlorophyll
3.10a	<0.001	<0.001	0.24
3.10b	<0.001	<0.001	0.1311
3.10c	<0.001	<0.001	0.995
3.10d	<0.001	0.556	0.834

(f)

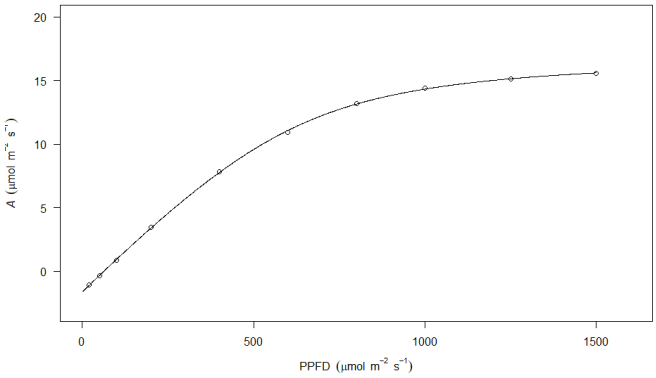
Experimental Group	Measurement	Treatment	Trial	Treatment x Trial
1	Fv/Fm	0.213	0.631	<0.01
	NPQ	<0.01	0.089	<0.001
2	Fv/Fm	<0.001	0.294	0.084
	NPQ	<0.001	<0.001	<0.001

Appendix Table 6.1.4 Group of tables displaying the calculated p-values of two-way ANOVAs conducted in Chapter 3, separated by their differing independent variables, including (f) those found in Table 3.9.

GH Rep 1



GH Rep 2



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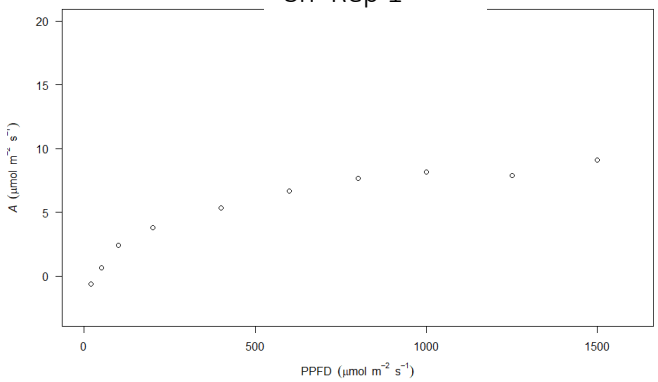

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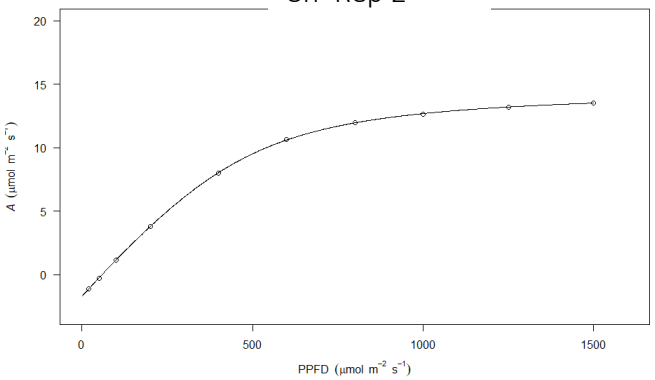

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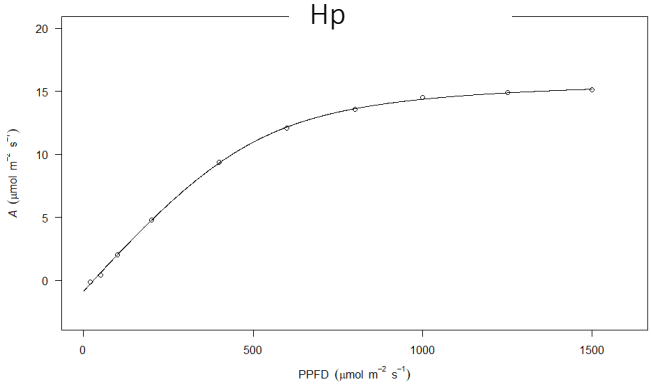

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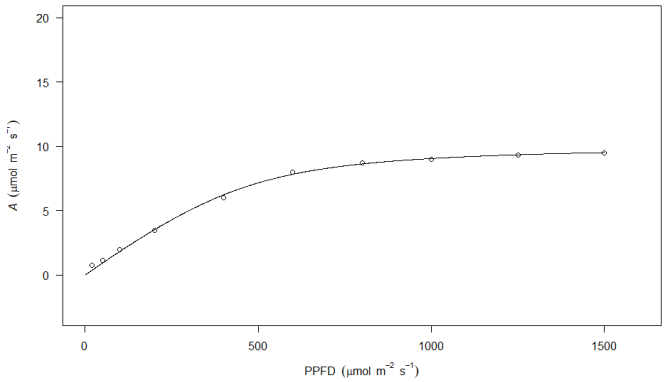

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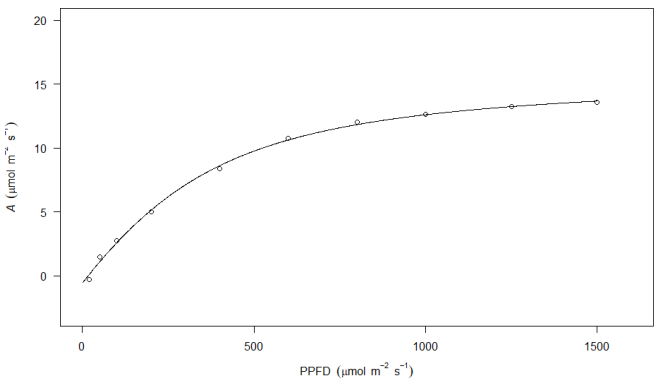

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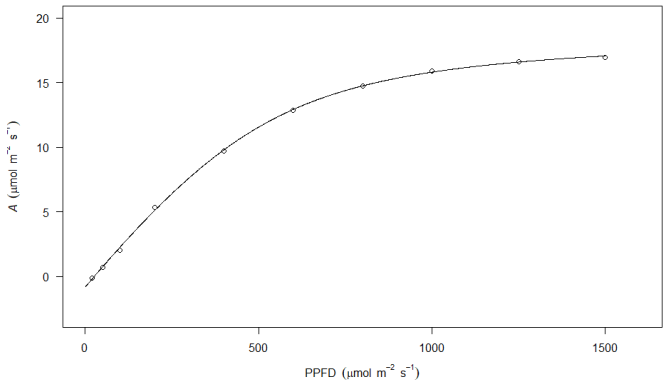

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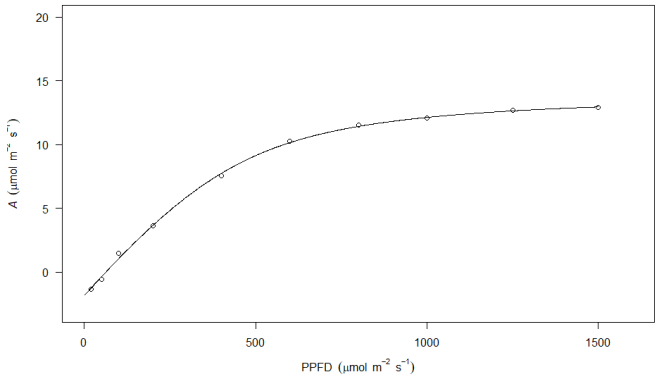

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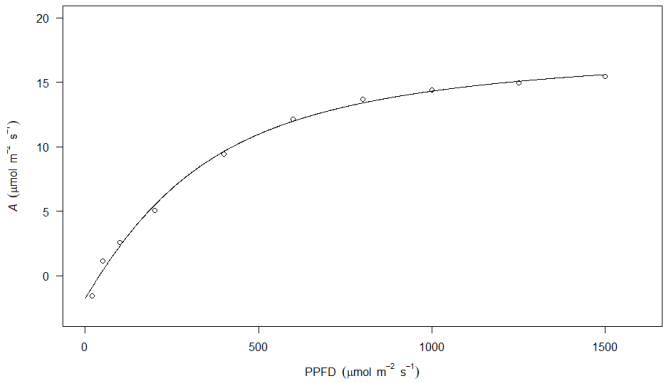

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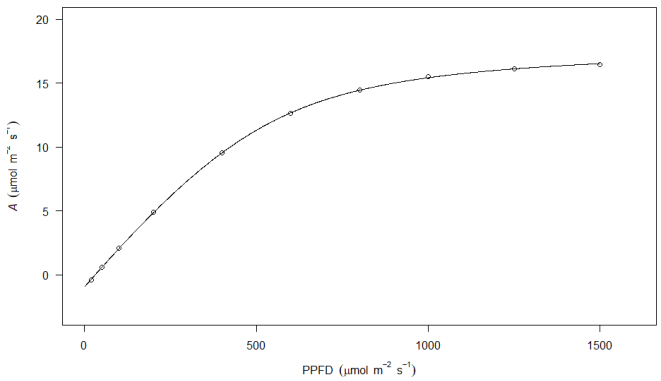

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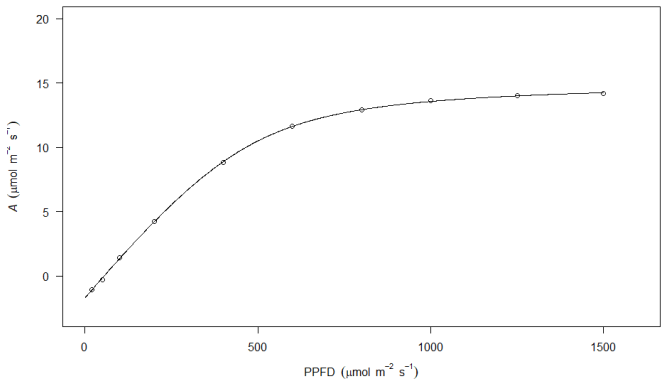

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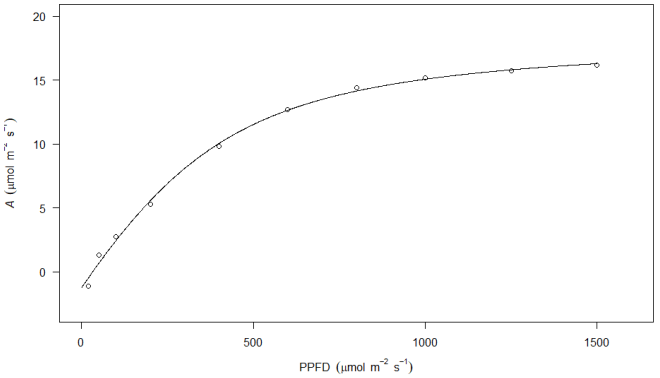
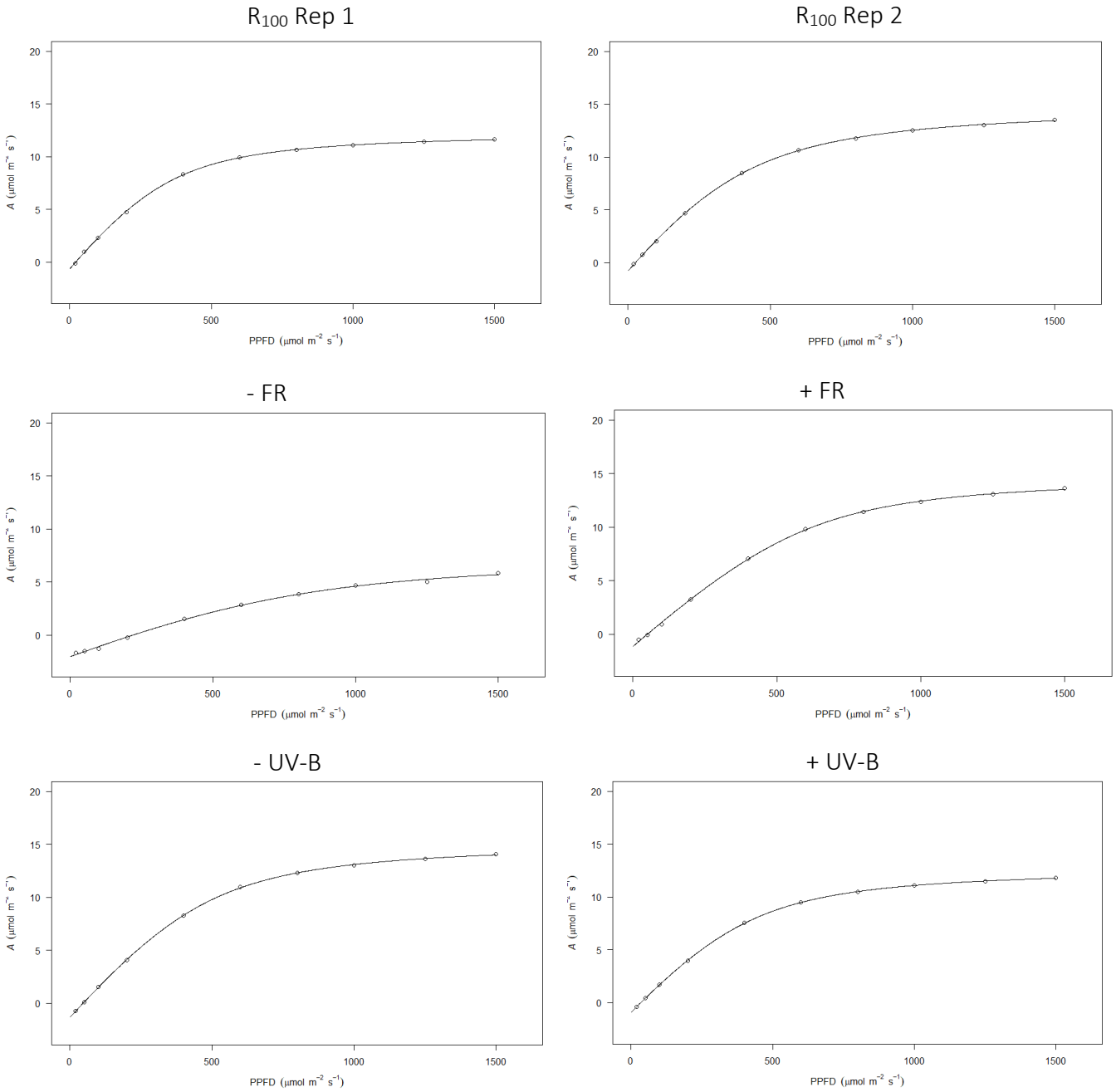




Appendix Figure 6.1.10 Rapid light Response Curves for Basil plants under experimental conditions, using non-rectangular hyperbola model (Marshall and Biscoe, 1980) fitted to data averaged across four samples. Assimilation of CO2 (µmol.m^{-2}.s^{-1}) ranging from 0 – 20 is presented on the y-axis, and PPFD (µmol.m^{-2}.s^{-1}) ranging from 0 – 1500 is on the x-axis.

Appendix Figure 6.1.11 Images of Coriander seedlings grown for 14 days under (a) 100% blue light, (b) 75% blue light and 25% red light ($B_{75}R_{25}$), (c) $B_{50}R_{50}$, (d) $B_{25}R_{75}$, (e) R_{100}, under low, medium, and high light intensities (from left to right). The intensities used can be found in Chapter 3 (Table 3.4). (

Light Condition	Replica #	N	FW (g)	DW (g)	DW %
GH	1	28	0.61 ± 0.15 $_{b}$	0.07 ± 0.13 $_{a}$	15
	2	28	0.40 ± 0.11 $_{a}$	0.05 ± 0.01 $_{a}$	15
Sn	1	28	0.81 ± 0.16 $_{c}$	0.11 ± 0.02 $_{b}$	13
	2	28	0.57 ± 0.18 $_{b}$	0.07 ± 0.09 $_{a}$	15
Hydro	1	19	0.56 ± 0.47 $_{ab}$	0.08 ± 0.07 $_{ab}$	14
B100	1	17	0.09 ± 0.04 $_{a}$	0.01 ± 0.00 $_{a}$	11
	2	20	0.24 ± 0.17 $_{ab}$	0.02 ± 0.01 $_{a}$	7
B75R25	1	24	0.18 ± 0.14 $_{a}$	0.02 ± 0.01 $_{ab}$	11
	2	22	0.39 ± 0.18 $_{bc}$	0.06 ± 0.03 $_{cd}$	15
B50R50	1	19	0.33 ± 0.24 $_{abc}$	0.04 ± 0.03 $_{bc}$	12
	2	18	0.56 ± 0.43 $_{c}$	0.11 ± 0.11 $_{d}$	19
B25R75	1	27	0.28 ± 0.19 $_{abc}$	0.03 ± 0.03 $_{a}$	12
	2	26	0.30 ± 0.35 $_{ab}$	0.07 ± 0.07 $_{a}$	23
R100	1	15	0.26 ± 0.18 $_{abc}$	0.02 ± 0.01 $_{ab}$	7
	2	26	0.26 ± 0.16 $_{ab}$	0.03 ± 0.01 $_{a}$	12
- UV-B	1	19	0.26 ± 0.15 $_{a}$	0.02 ± 0.01 $_{a}$	9
	2	21	0.26 ± 0.22 $_{a}$	0.03 ± 0.02 $_{a}$	13
+ UV-B	1	22	0.47 ± 0.33 $_{a}$	0.05 ± 0.04 $_{a}$	11
	2	17	0.38 ± 0.19 $_{a}$	0.04 ± 0.02 $_{a}$	11
- FR	1	22	0.36 ± 0.24 $_{a}$	0.04 ± 0.04 $_{a}$	13
+ FR	1	17	0.33 ± 0.28 $_{a}$	0.03 ± 0.04 $_{a}$	10
	2	12	0.40 ± 0.28 $_{a}$	0.03 ± 0.02 $_{a}$	7
HSP-HB	-	28	2.00 ± 0.58 $_{c}$	0.21 ± 0.05 $_{b}$	11
HSIP	-	28	1.71 ± 0.38 $_{bc}$	0.19 ± 0.06 $_{b}$	11
GE-HB	-	28	1.61 ± 0.31 $_{b}$	0.18 ± 0.04 $_{ab}$	11
AP673L	-	28	1.08 ± 0.26 $_{a}$	0.14 ± 0.04 $_{a}$	13

Appendix Table 6.1.5 Fresh (FW) and dry weight (DW) measurements, as well as percentage DW (DW %) of adult Coriander shoots (4 weeks old) grown in different experiments in Chapter 4. Weights represented as mean ± standard deviation with different letters representing statistical significance as determined by sidak post-hoc test ($p < 0.05$) performed within experiments which are separated by thicker lines. Experimental treatments and abbreviations are as described in materials and methods in Chapter 4.

(a)

Figure Number	Measurement	Treatment	Intensity	Treatment x Intensity
3.2	Height	<0.001	<0.001	0.237
	FW	<0.001	0.1594	0.183

(b)

Figure Number	Measurement	Treatment	Trial	Treatment x Trial
4.1	Height	<0.001	<0.001	<0.05
	FW	<0.001	<0.001	0.790
	DW	<0.001	<0.001	0.860
	Leaf Number	<0.001	<0.001	0.134
	Leaf Area	<0.001	<0.001	0.062
	Leaf Thickness (log+1)	<0.001	<0.001	<0.001
4.3	Height	<0.001	<0.001	<0.001
	FW	<0.05	<0.01	0.105
	DW	<0.001	<0.001	0.216
	Leaf Number	<0.001	0.102	0.596
	Leaf Area	<0.001	<0.001	0.171
	Leaf Thickness	<0.001	<0.05	<0.001
4.4	Height	<0.05	0.401	0.636
	FW	0.289	0.797	0.943
	DW	0.088	0.799	0.637
	Leaf Number	0.634	0.688	0.922
	Leaf Area	0.264	0.995	0.986
	Leaf Thickness	0.580	0.135	0.244

(c)

Figure Number	Treatment	Vitamin	Treatment x Vitamin
4.6a	<0.001	<0.001	<0.01
4.6b	<0.001	<0.001	<0.001
4.6c	<0.001	<0.001	<0.001
4.6d	<0.001	<0.001	<0.001

(d)

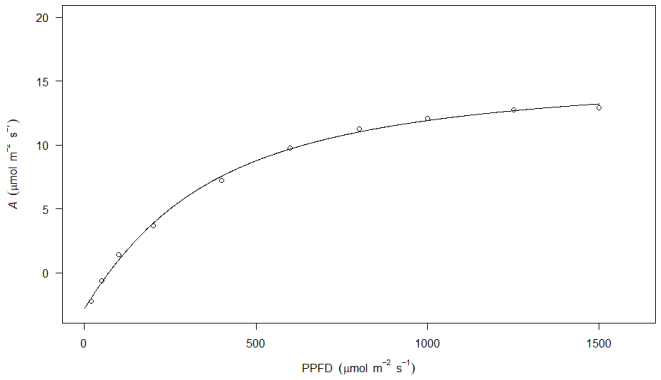
Figure Number	Treatment	Chlorophyll	Treatment x Chlorophyll
4.8a	<0.001	<0.001	0.867
4.8b	<0.001	<0.001	<0.05
4.8c	<0.001	<0.001	0.783
4.8d	<0.001	<0.001	0.986

(e)

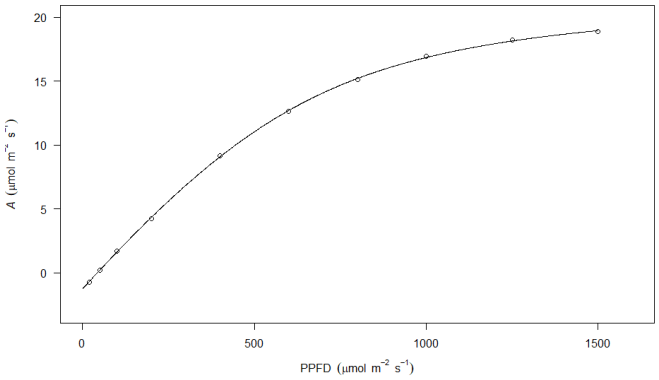
Experimental Group	Measurement	Treatment	Trial	Treatment x Trial
1	Fv/Fm	<0.001	<0.05	0.235
	NPQ	0.052	0.090	0.246
2	Fv/Fm	<0.05	0.933	0.701
	NPQ	<0.001	<0.001	<0.001
3	Fv/Fm	0.123	0.173	0.646
	NPQ	0.625	0.289	0.919

Appendix Table 6.1.6 Group of tables displaying the calculated p-values of two-way ANOVAs conducted in Chapter 4, separated by their differing independent variables, including (e) those found in Table 4.6.

GH Rep 1



GH Rep 2



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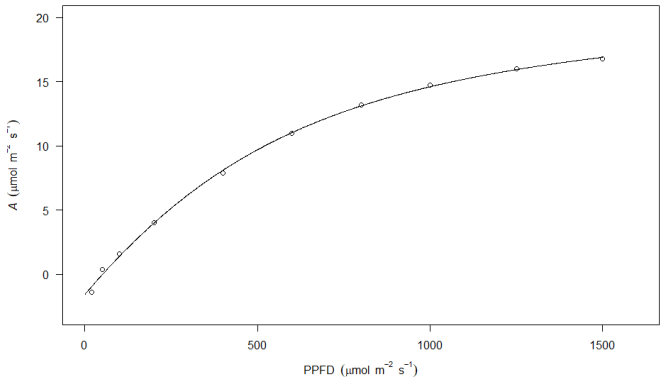

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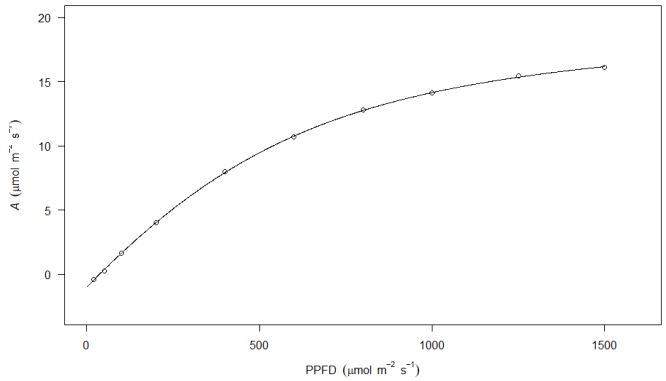

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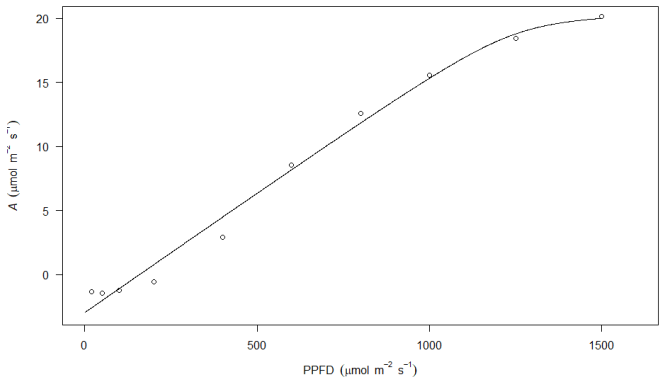
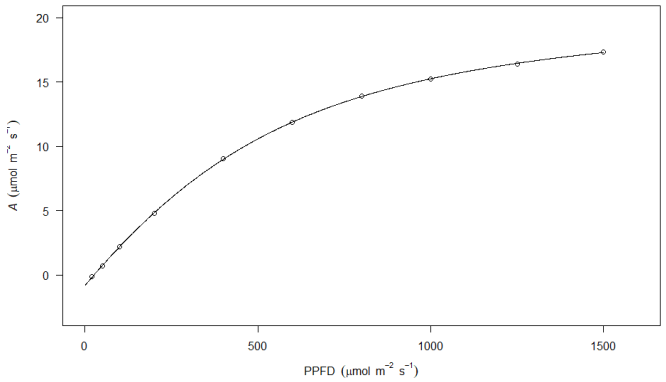
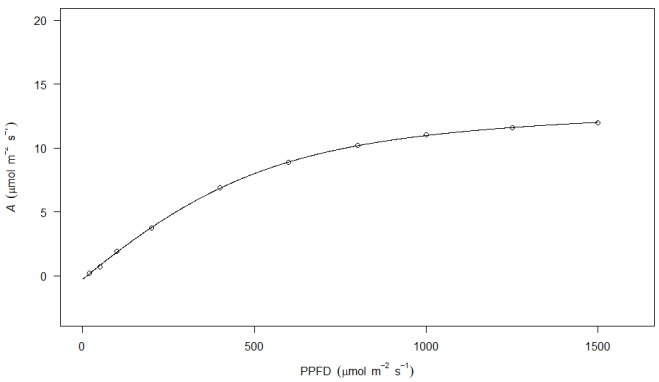


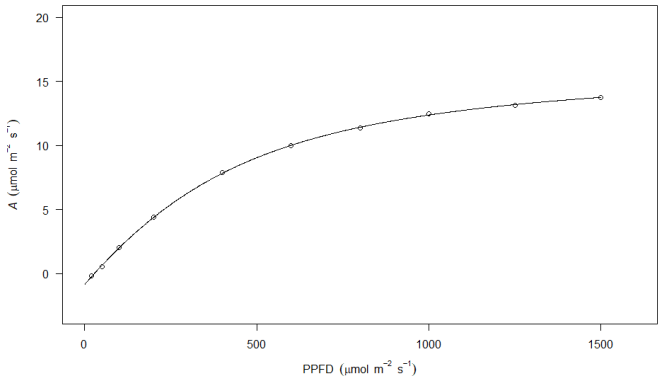
B_{100} Rep 1



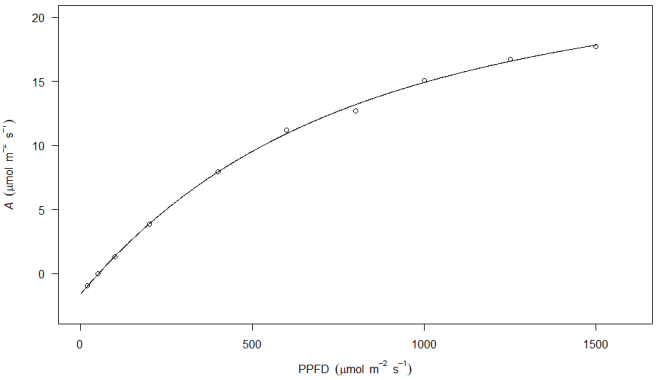
B_{100} Rep 2



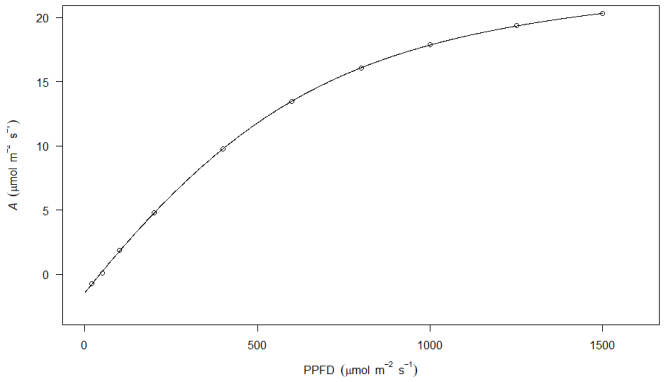
$B_{75}R_{25}$ Rep 1



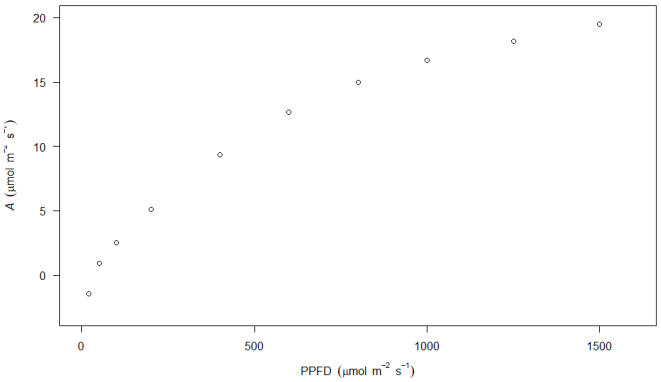
$B_{75}R_{25}$ Rep 2



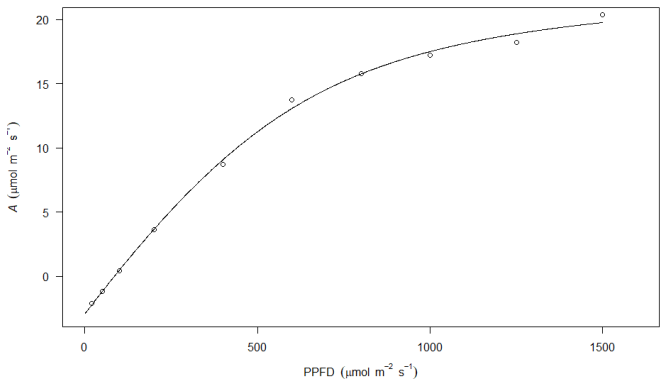
$B_{50}R_{50}$ Rep 1



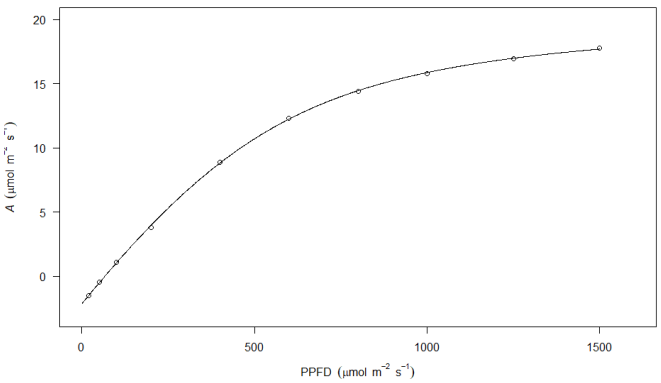
$B_{50}R_{50}$ Rep 2

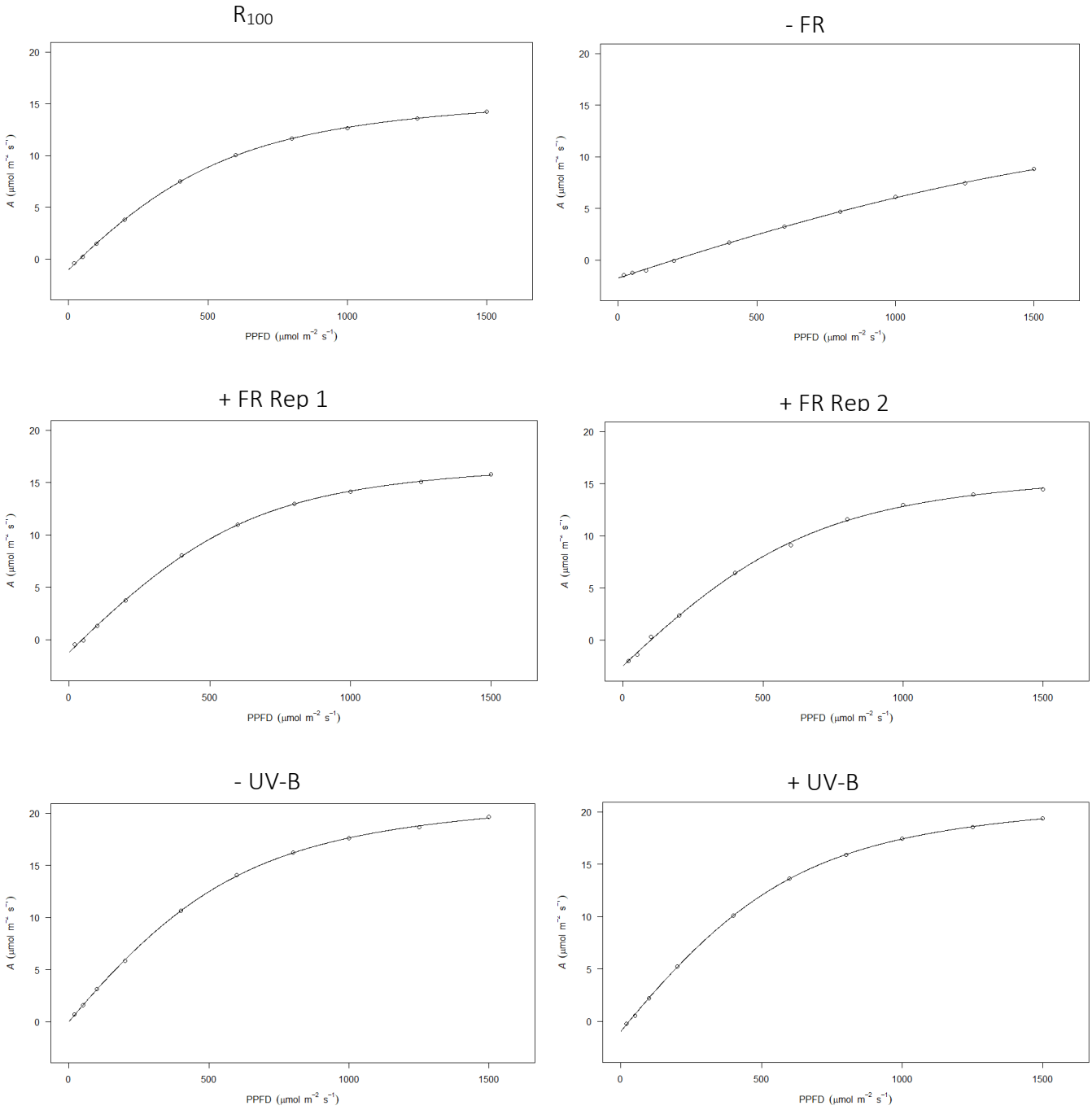


$B_{25}R_{75}$ Rep 1



$B_{25}R_{75}$ Rep 2





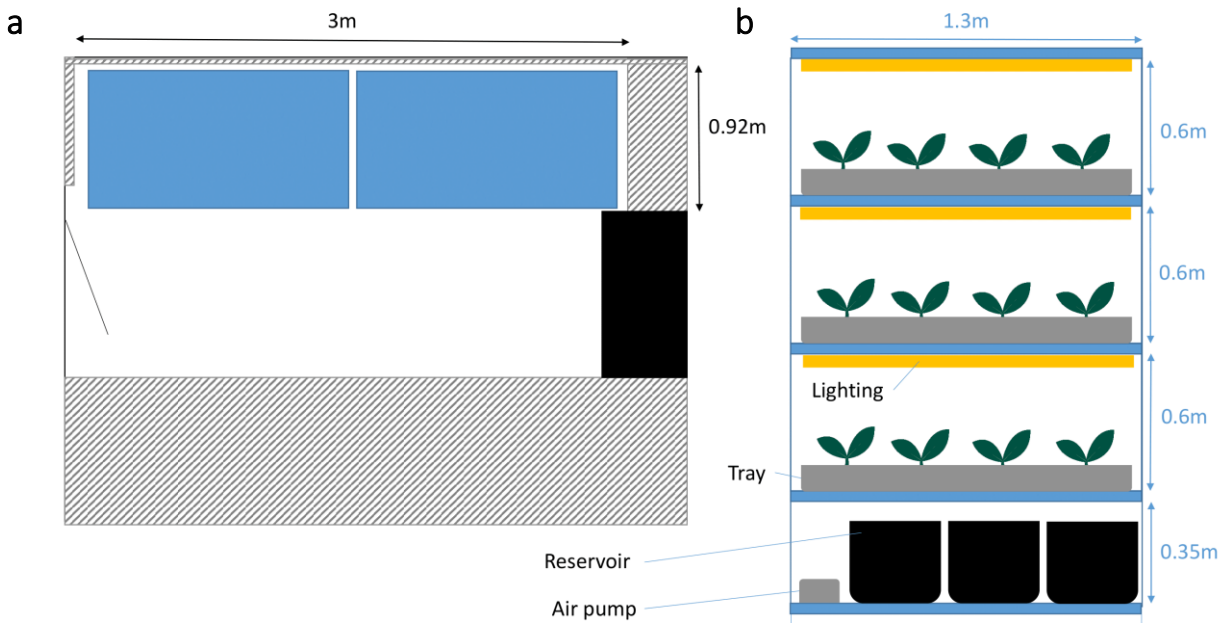
Appendix Figure 6.1.12 Rapid light Response Curves for Coriander plants under experimental conditions, using non-rectangular hyperbola model (Marshall and Biscoe, 1980) fitted to data averaged across four samples. Assimilation of CO2 ($\mu mol.m^{-2}.s^{-1}$) ranging from 0 – 20 is presented on the y-axis, and PPFD ($\mu mol.m^{-2}.s^{-1}$) ranging from 0 – 1500 is on the x-axis.

6.2. Supplementary Methods

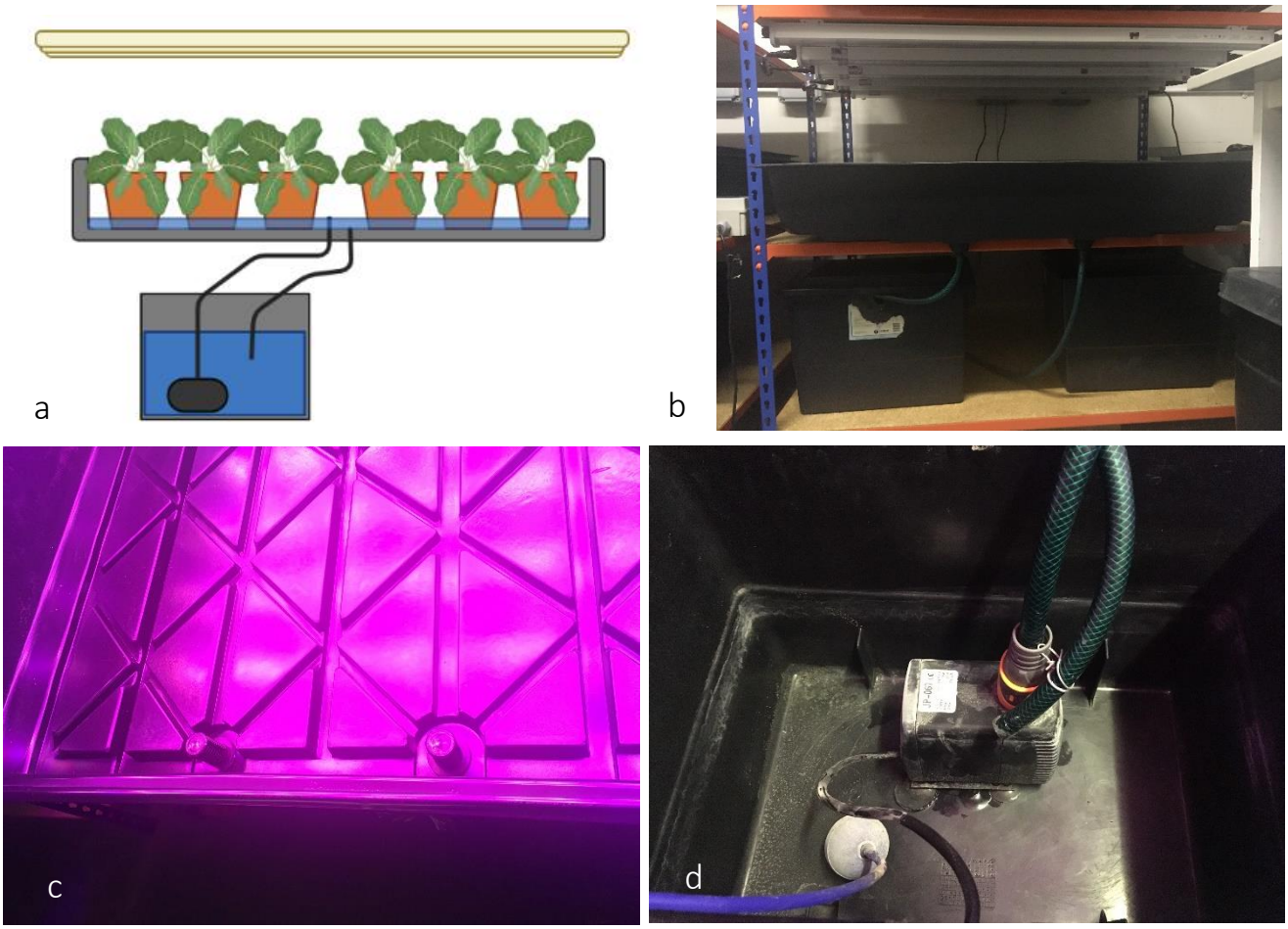
6.2.1. In-House Hydroponic Set Up

6.2.1.1. Flood and Drain System

Food grade shelving units were used to construct the vertical layers (2.2 m maximum height x 1.3 m width x 0.9 m depth) (Appendix Figure 6.2.1). The chipboard shelves were sealed with yacht varnish. Hydroponic flood and drain trays (1 m x 1 m; Hydrohobby) were placed on top of the shelves. Black plastic tubs (20L) were used for the reservoir of the flood and drain system, which was aerated by an airstone and airpump, and supplied to the trays using a submersible pump (Appendix Figure 6.2.2). The nutrient solution used was made according to the recipe supplied by May Barn Consultancy Ltd. (Appendix Table 6.2.1), and was supplied twice a day by a programmable timer (PDL 56TC Series)



Appendix Figure 6.2.1 Layout of the two racks in the Controlled Environment room with an (a) topside view, and (b) side view of a single rack, showing the shelves within each rack.



Appendix Figure 6-2.2 (a) A diagrammatic representation of a theoretical flood and drain system, created in BioRend.com, compared to (b) a photo of the constructed flood and drain system within the controlled environment room at Lancaster University. (c) Close ups the flood and drain hydroponic tray featuring grooves and holes for nutrient solution input and drainage, and (d) the pump and air stone equipment within the nutrient solution reservoir.

6.2.1.2. Nutrient Solution Provided by Dr Paul Challinor at May Barn Consultancy Ltd.

Nutrient Feed Recipe for Hydroponic Herbs: 25 May, 2018

Based on the background water analysis of 14 May, 2018

Input EC: 2,000 to 2,200 μS/cm

Tank sizes: 100 litres

Dilution: 1 in 100

A Tank		B Tank	
Compound	Weight	Compound	Weight
Calcium nitrate 19%Ca, 16% N	4.5kg	Potassium nitrate 38%K, 13% N	3.0kg
Potassium nitrate 38%K, 13% N	1.5kg	Mono-potassium phosphate 29%K, 23% P	2.5kg
Potassium chloride 50%K, 45% Cl	0kg	Magnesium sulphate 10%Mg, 13% S	2.0kg
DTPA iron chelate 6%	225ml	Manganese sulphate 32%	20g
		Zinc sulphate 23%	3g
		Borax 21%	40g
		Copper sulphate 25%	2g
		Sodium molybdate 40%	1g

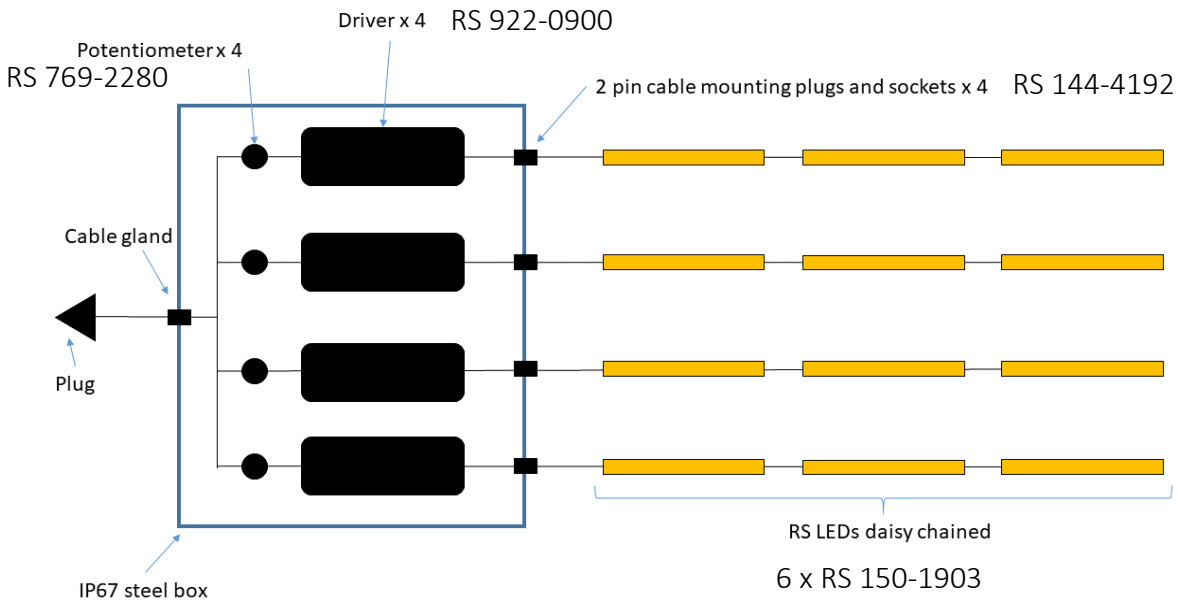
Appendix Table 6.2.1 Quantity of chemicals required to create Tank A and Tank B nutrient solutions, for mixing together for the final solution.

6.2.1.3. Growing Methods

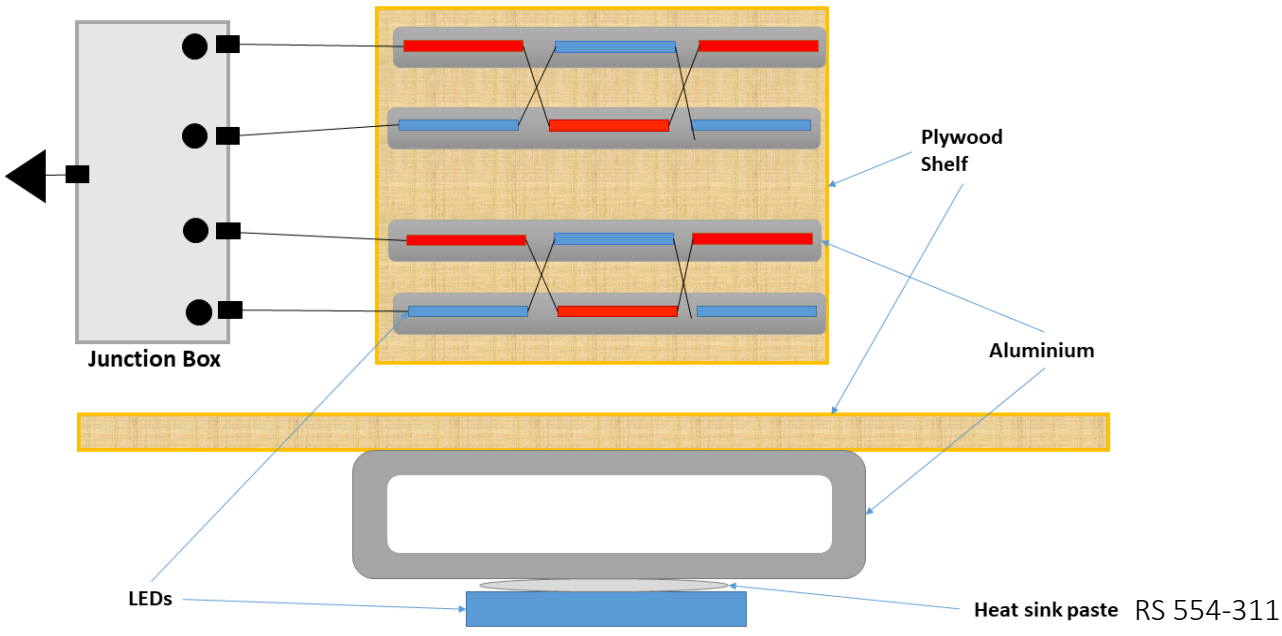
Appendix Figure 6.2.3 (a) Several methods of growing were trialled under GE lighting, including direct sowing onto (i) wool matting, (ii) rockwool sheet, (iii) 2x2 cm rockwool cubes within the tray, (iv) rockwool cubes within pot surrounded by clay pebbles, and (v) loose clay pebbles within the tray. The greatest germination success, plant survival, root growth (b) and overall growth was found with the rockwool cubes and clay pebbles in pots, over simply placing the rockwool cubes in the tray (c). Eventually, the method used involved starting growth with the rockwool cubes in germination trays (vi), before transplanting into pots with clay pebbles after 2 weeks (d).

Appendix Figure 6.2.4 Images showing the growth facilities at May Barn Consultancy Ltd. used to grow and harvest Basil and Coriander grown under different commercial lighting installations.

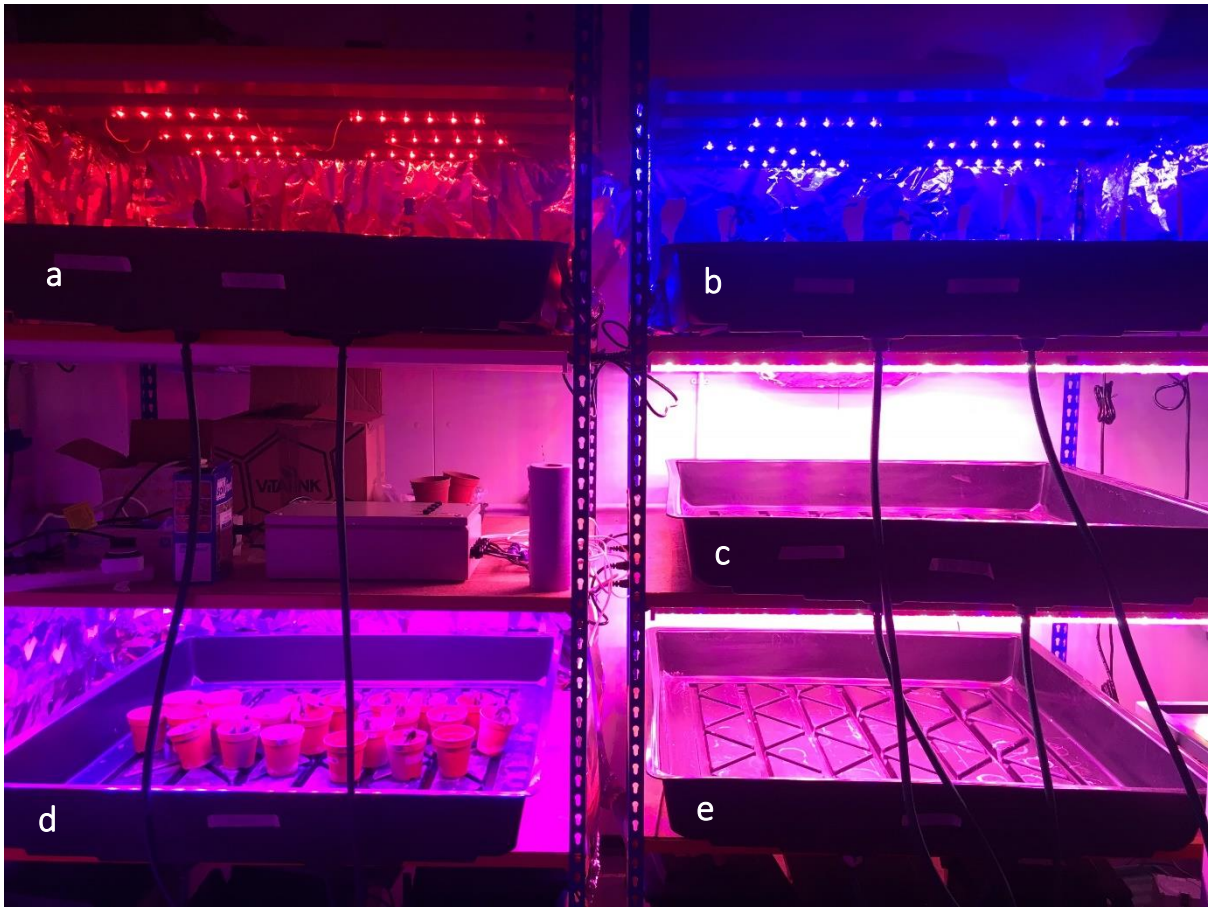
6.2.1.4. LED Lighting Systems



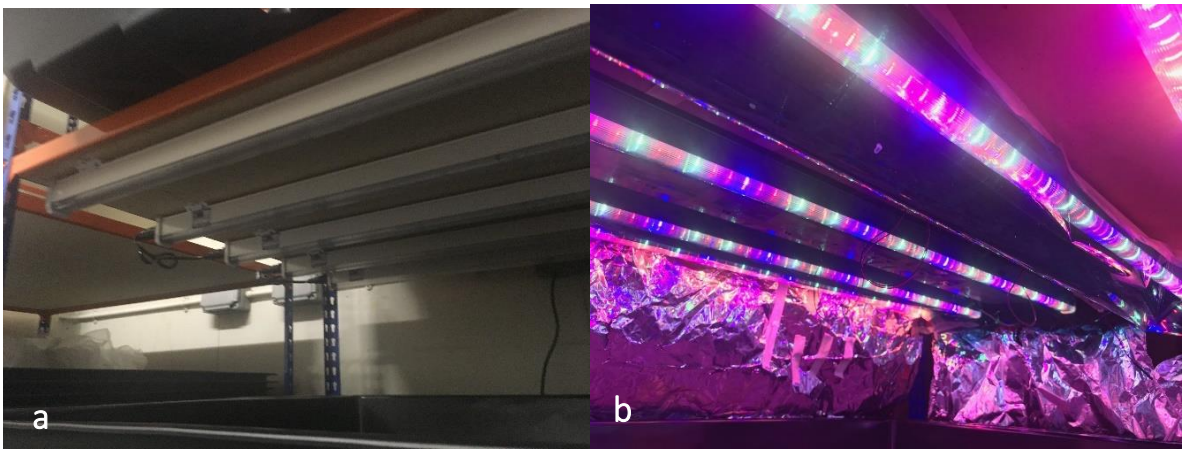
Appendix Figure 6.2.5 Diagrammatic representation of the LED lighting system created using elements purchased from RS Components (part codes listed in the figure). The resulting system was waterproof and was dimmable using the potentiometers. Constructed by Ian Edmondson and Stephen Holt.



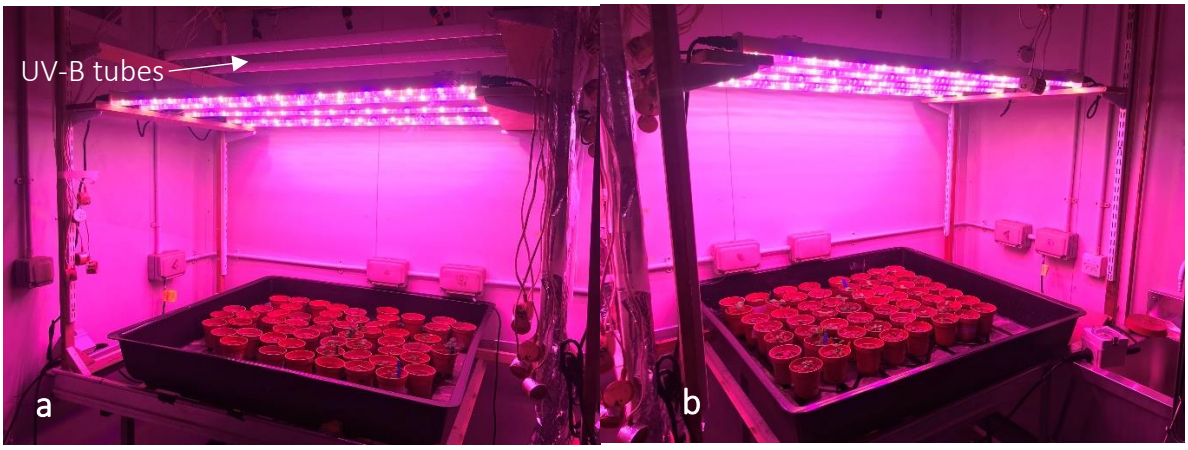
Appendix Figure 6.2.6 Diagrammatic representation of the method of LED fixture to the shelving unit in the dichromatic set up. As the heat from the LED strips was significant, they were mounted onto hollow aluminium extrusion using a heat sink compound and attached to the plywood shelf above with screws.



Appendix Figure 6.2.7 Photo showing the two racks in the controlled environment room with the racks set up with a (a) monochromatic red, (b) monochromatic blue, (d) dichromatic treatment, and GE lighting with and without FR (c and e, respectively).



Appendix Figure 6.2.8 (a) GE lighting mounted to the underside of the shelving, and (b) GE lights covered with the SC60 LEE filter.

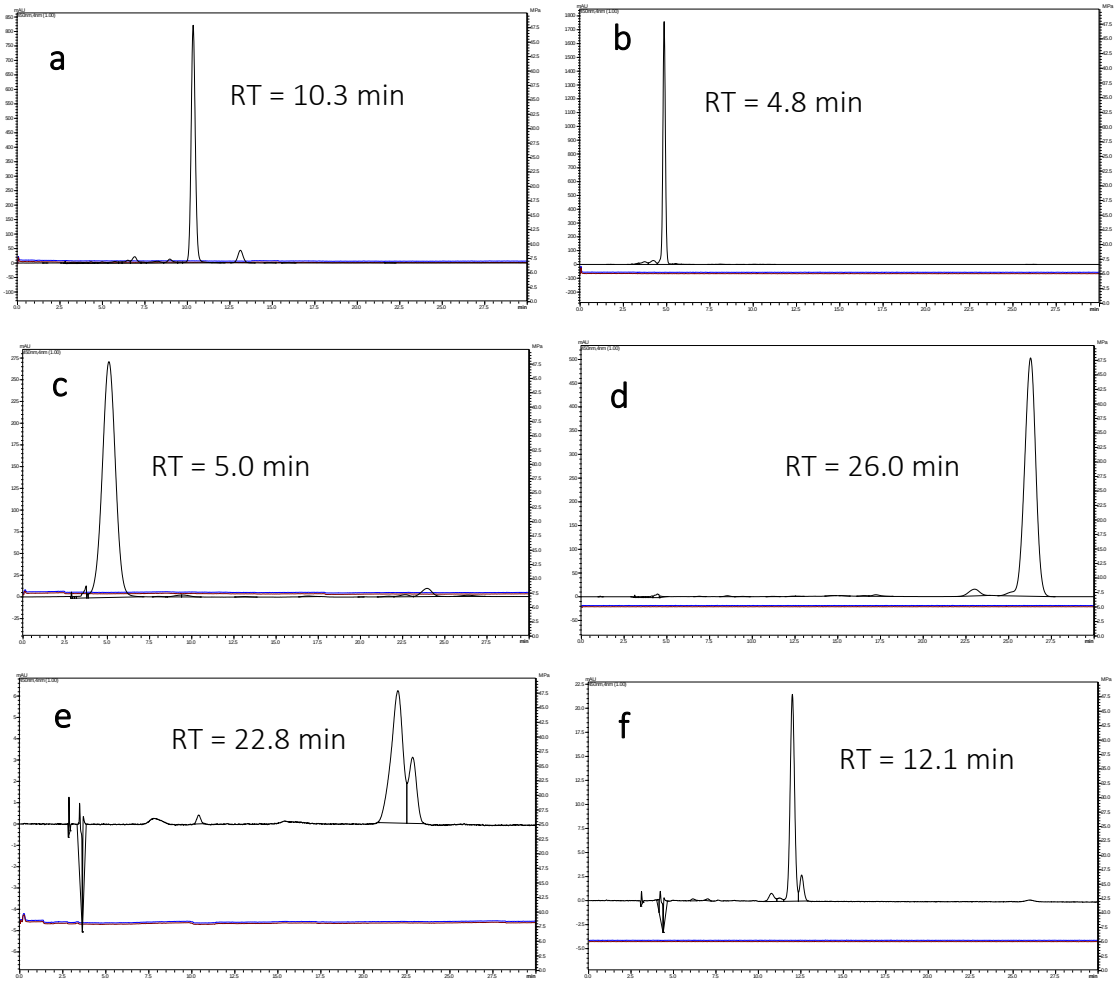


Appendix Figure 6.2.9 Plants grown under (a) + UV-B and (b) – UV-B with GE lighting (150 µmol m^{-2} s^{-1}). The UV-B fluorescent tubes can be seen mounted above the GE lighting.

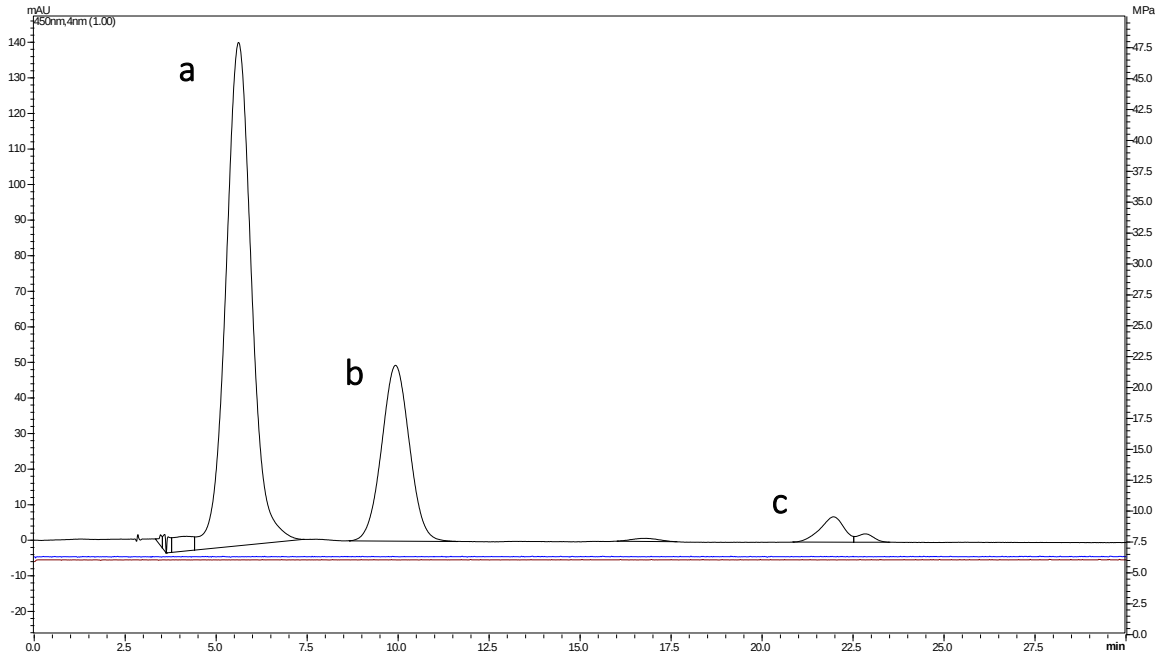
6.2.2. HPLC Method Development

6.2.2.1. Carotenoids and α-Tocopherol

For the HPLC method for Carotenoids and α-Tocopherol, two different C30 columns were tested: YMC and Acclaim columns, using samples of shop-bought Basil. The columns presented different retention times for the molecules of interest (Appendix Figure 6.2.9). However, with the YMC column, carry over of carotenoids, including β-carotene and the internal standard was detected during runs (Appendix Figure 6.2.10). A wash of acetonitrile was included between runs for the YMC column, however this increased the run time, consumables used and did not rectify the problem. Therefore, the Acclaim column was selected for the data collection.

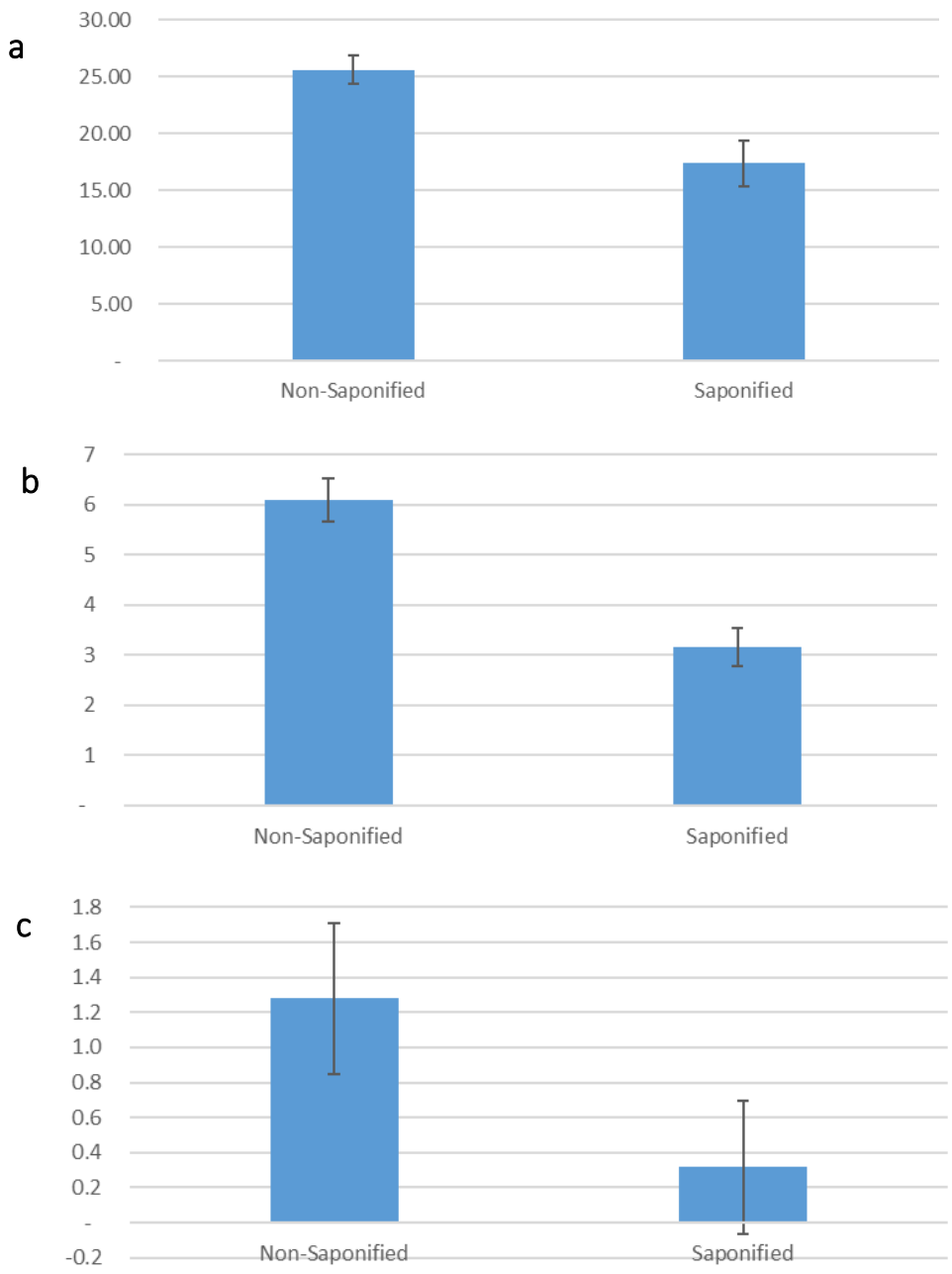


Appendix Figure 6.2.10 Chromatograms showing the standard peaks of lutein (a,b), β-carotene (c,d) and echinenone internal standard (e,f) as found in the YMC column (a,c,e) and Acclaim column (b,d,f). Retention times (RT) of the standards are displayed on the chart. Peak area is on the y-axis, and retention time on the x-axis.

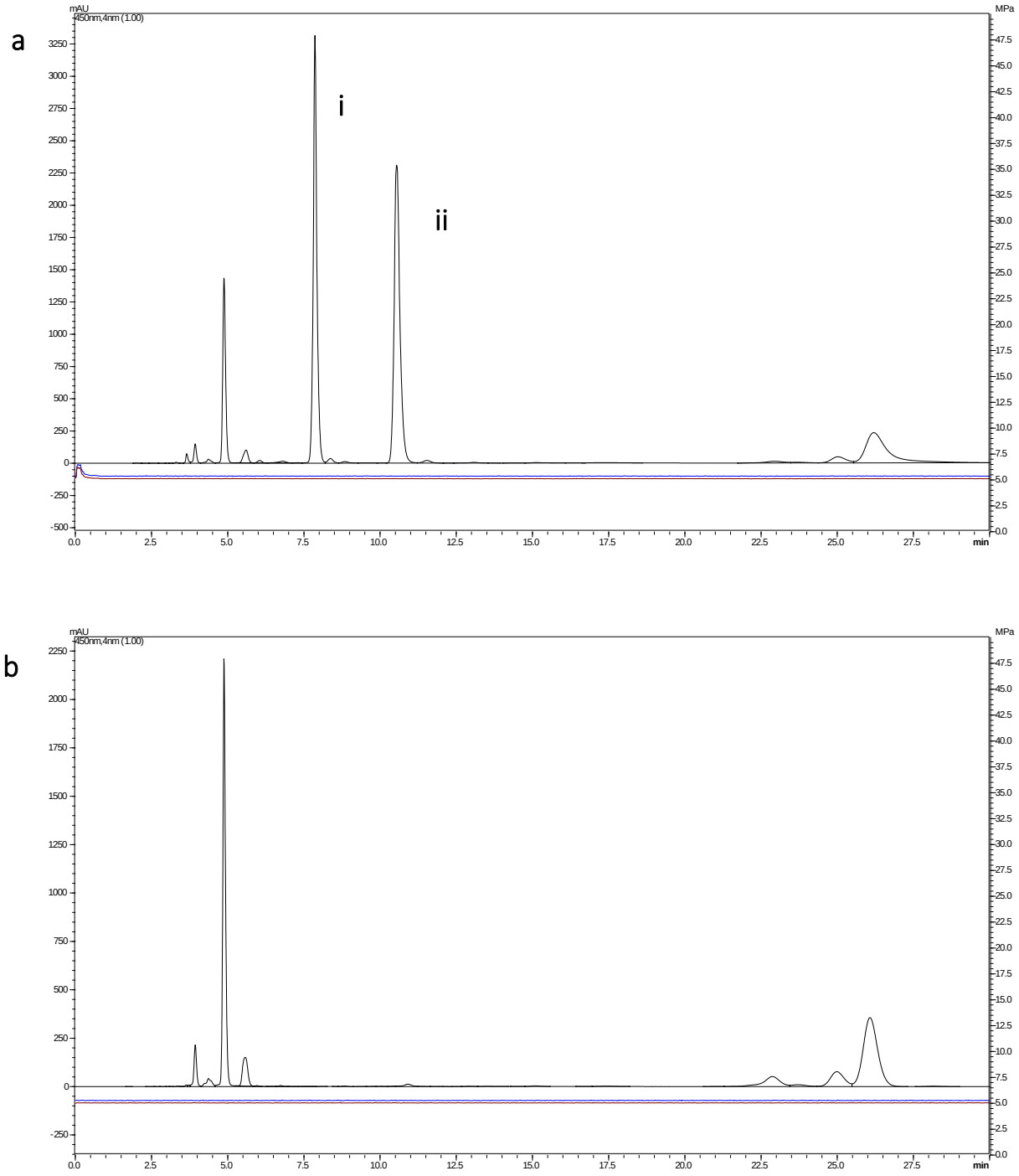


Appendix Figure 6.2.11 Chromatogram of a polluted run using the YMC column with injected echinenone standard only (c), but detection of β-carotene (a) and lutein (b) due to carry-over from previous runs.

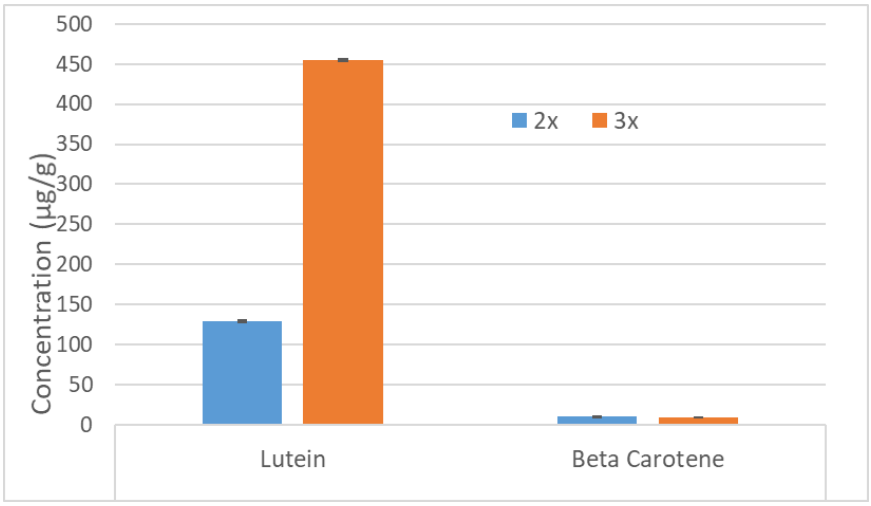
The protocol was further optimised by examining the use of saponification and the number of extractions undertaken on the sample. Saponification is a process that can remove the chlorophylls present in a sample through the use of KOH and repeated water baths at 85 °C. Saponification treatment decreased the carotenoid and tocopherol content by 50% in β-carotene, and up to 75% for α-tocopherol (Appendix Figure 6.2.12). Therefore, despite the chromatograms becoming clearer due to the lack of chlorophylls (Appendix Figure 6.2.13), it was decided to proceed without saponification and instead the internal standard echninone was used which did not co-elute with chlorophylls. Then, two vs three extractions were compared to determine the number of extractions required for exhaustion. It was found that three extractions recovered significantly more lutein (over 300 %) and β-carotene (Appendix Figure 6.2.14). Therefore, despite the additional time required, three exhaustive extractions were used in the method.



Appendix Figure 6.2.12 Extraction of samples (n=4) with and without saponification, and their quantification of (a) lutein, (b) β-carotene, and (c) α-tocopherol (mg/ 100 g).



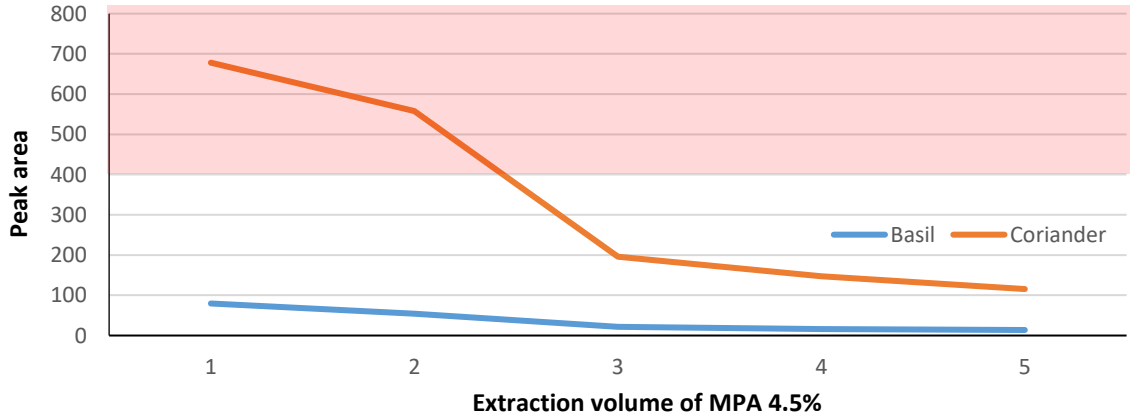
Appendix Figure 6.2.13 Spectrograms depicting (a) non-saponified sample showing peaks for (i) chlorophyll b and (ii) chlorophyll a, and (b) a saponified sample, lacking the chlorophyll peaks.



Appendix Figure 6.2.14 Comparison of extraction numbers, showing the concentration of lutein and β-carotene (µg/ g) for 2 extractions (blue) and 3 extractions (orange) (n=2).

6.2.2.2. Ascorbic Acid

A protocol sourced from the School of Food and Nutrition at Leeds University, was used to quantify the ascorbic acid content of plant leaf tissue samples. The optimal volume of MPA (4.5 % w/v) to use for extraction was determined for Basil and Coriander by performing extractions with various quantities of extraction buffer (Appendix Figure 6.2.6). Peak areas were maxed out for the Coriander samples extracted in 1 and 2 ml MPA. It was decided that the optimal extraction volume was 2 ml MPA for Basil, and 4 ml MPA for Coriander.



Appendix Figure 6.2.15 Peak areas of ascorbic acid measured in Basil and Coriander extracted in 1, 2, 3, 4, 5 ml MPA (4.5 % w/v). The shaded red area indicates the range over which the peak areas would not be read by the detector.

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