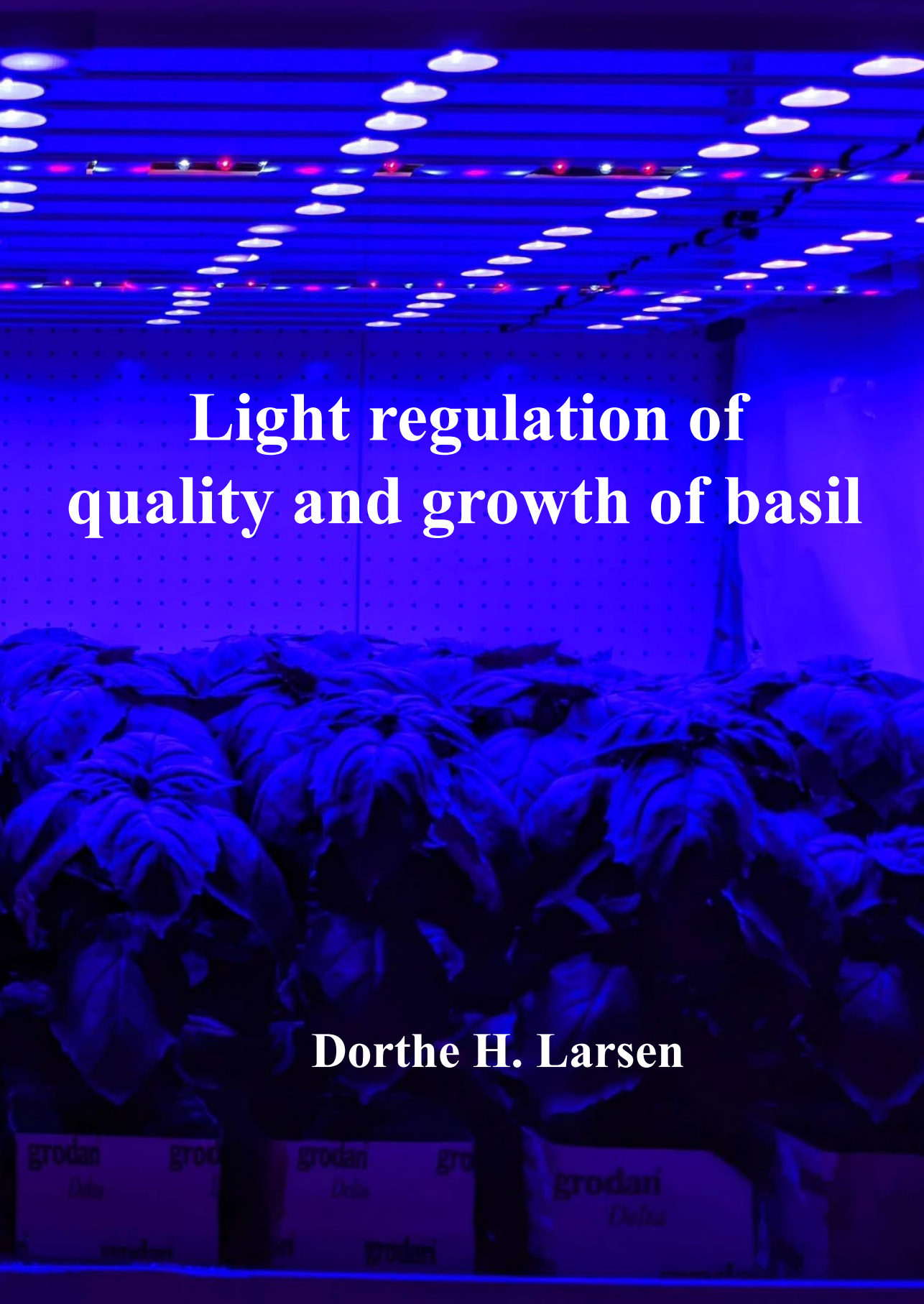

Light regulation of quality and growth of basil

Dorthe H. Larsen

Propositions

1. No individual metabolite at harvest can predict postharvest behavior.
   (this thesis)

2. Optimizing the resource use efficiency by LED lighting in a vertical farm does not go at the expense of quality in basil.
   (this thesis)

3. The contribution of technicians is undervalued in science.

4. Vertical farming produces horticultural crops with better quality than field cultivation.

5. Completing a PhD cures perfectionism.

6. Culture shocks are bigger when they are unexpected.

Propositions belonging to the thesis, entitled

Light regulation of quality and growth of basil

Dorthe H. Larsen
Wageningen, 5 July 2022

Light regulation of quality and growth of basil

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Light regulation of quality and growth of basil

Dorthe H. Larsen

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To Sigrid, my little ray of sunshine

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Chapter 1

General Introduction

1.1 Quality of fresh products

The quality of fresh products such as herbs, vegetables and fruit is a broad term that covers a range of aspects such as the sensorial quality (i.e. texture, odor and visual quality such as color, morphology), nutritional quality (i.e. content of health and flavor related compounds such as phenolic compounds, proteins and carbohydrates) and shelf life (Rouphael *et al.*, 2018; Min *et al.*, 2021). Carbohydrates both adds to the sweet taste of a product and may aid in delaying senescence during postharvest storage (Hasperué *et al.*, 2015). Phenolic compounds are often related to color and flavor (i.e. anthocyanins and volatile organic compounds (VOCs)) (Thomas-Barberan and Espin, 2001). Phenolic compounds also function as antioxidants which potentially increase shelf life. An increase in the content of carbohydrates and phenolic compounds may therefore increase quality of a product. The quality of a product is also defined by the needs, aspirations and indulgences of the consumers. For different markets, different aspects of quality (i.e. color, shape, taste) may be appreciated. For a producer of horticultural products both the likings of consumers and reproducibility of a certain quality are important for a successful horticultural products (Hewett, 2006).

1.2 Low temperature storage – Chilling injury

Herbs, vegetables and fruit are highly perishable products with a short postharvest shelf life. This can potentially result in significant postharvest loses (Liang *et al.*, 2020). Fruit and vegetables are often transported over long distances from the site of production to different locations around the world. To extend shelf life during transport and storage, low temperatures 2-7 °C, are often used. At a low temperature, both water loss and the metabolic rate slows down. Slowing down respiration and ethylene production results in a postponed senescence and ripening (Sevillano *et al.*, 2009). However, herbs, vegetables and fruit of subtropical and tropical origin suffer at temperatures below 10-12 °C resulting in chilling injury (CI). The temperature and duration of exposure together determine the degree of the CI. CI is a physiological disorder, that manifests differently in leaves and fruit. In fruit, CI manifests as pitting of the surface, internal and external discoloration, loss of aroma and flavor, failure to ripen or uneven ripening, water-soaked areas and reduced firmness. The visual symptoms of CI in fruit often appear or worsen when they are transferred to room temperature (Palma *et al.*, 1995). For leaves, symptoms of CI include dark necrotic spots, loss of glossy appearance, wilting and aroma loss (Lange and Cameron, 1994; Suzuki *et al.*, 2008). In contrast to fruit, CI in leaves appears within a few days of low temperature storage (Fratianni *et al.*, 2017). However,

damage starts to occur already before it is visible by the naked eye. Among crops sensitive to low temperature is the herb basil (*Ocimum basilicum* L.).

1.2.1 Basil

Basil (*Ocimum basilicum* L.) is an annual herb, of tropical origin from Iran and India (Özcan *et al.*, 2005; Makri and Kintzios, 2008). It is part of the *Lamiaceae* family which is one of the largest dicotyledonous families with around 240 genera, many of which are aromatic due to external glandular structures producing volatile organic compounds (VOC) (Giuliani and Maleci Bini, 2008; Makri and Kintzios, 2008). The genus *Ocimum* which comprises of 30 species often used as medicinal plants, insecticides and culinary herbs due to their aromatic profile (Grayer *et al.*, 1996). Basil includes cultivars with green leaves and white flowers and cultivars were both the leaves and flowers are red-purple due to anthocyanins in the epidermal cells (Prinsi *et al.*, 2020). Basil is mainly used as a culinary herb. It is known as the king of herbs, originating from the Greek basileus translating to "king" or basilikon translating "royal" (Makri and Kintzios, 2008). Basil is rich in secondary metabolites such as phenolic acids as rosmarinic, chicoric, cinnamic, caffeic, sinapic, caftaric and ferulic acid (Fratianni *et al.*, 2017). Furthermore, the characteristic flavour profile is provided from volatile organic compounds (VOCs) belonging to the classes of volatile phenylpropenes, monoterpenes and sesquiterpenes (Gang *et al.*, 2001). Among fresh herbs, basil is the most popular culinary herb in Europe and comprises of up to 75% of the total herb consumption based on kg. Fresh basil is sold as bunches of shoots, leaves or as potted plants. The current trend among consumers is an increase in consumption of fresh herbs as they are perceived to be healthier than dried herbs (CBI, 2020). Adding fresh herbs to the diet may have health benefits due to the high content of phenolic compounds (Pandey and Rizvi, 2009).

With the increase in consumption the awareness and demand for healthier products with high quality increases. To stay attractive for the consumer it is important for basil to maintain its freshness, green color and aroma. Basil is a highly perishable product, and it has a relatively short shelf life. High temperature storage can result in wilting. Packing in poly-ethylene cartons can reduced water loss, however a high humidity in the packing can result in soft rot caused by *Botrytis cinerea* (Aharoni *et al.*, 2010). At low temperature storage basil suffers from CI. CI appears as dark spots on the leaves, browning and collapse of the stem and loss of glossy appearance. CI also leads to a reduction in content of VOCs and content of other phenolic compounds (Lange and Cameron, 1994; Cozzolino *et al.*, 2016; Fratianni *et al.*, 2017). CI is the main reason to reduction of quality of basil as basil is often

transported with fresh products which can be kept at much lower temperatures (Aharoni *et al.*, 2010; Kenigsbuch *et al.*, 2010).

1.3 Physiological responses to low temperature

The physiological responses to low temperature show some commonalities for leaves and fruit that are sensitive to chilling. When a product sensitive to chilling is placed in low temperature the cell membranes may undergo a phase transition from a liquid crystalline to a solid gel phase (Raison and Orr, 1986). Cell membranes act as a hydrophobic barrier and protect cells and organelles from perturbation. Functioning cell membranes under non-stress conditions consist of a fluid bilayer composed of lipids and embedded sterols and proteins. The lipids consist of a hydrophilic, polar head connected to a glycerol backbone and a hydrophobic tail made of two fatty acids. The fatty acids are made up by a hydrocarbon chain with a carboxyl group (-COOH) at the end. Fatty acids are classified according to their length (i.e. number of carbon atoms) as short (up to 7 carbon atoms), medium (up to 14 carbon atoms), long (up to 22 carbon atoms or very long-chain (more than 22 carbon atoms). The fatty acids can either be saturated or unsaturated. Saturated fatty acids have no double bonds in the carbon chain whereas unsaturated fatty acids can have up to several double bonds (Reszczyńska and Hanaka, 2020). The lipids in cell membranes can be grouped into four classes phospholipids, glycolipids, sterols and sphingolipids (Reszczyńska and Hanaka, 2020). In the cell membranes sterols are structural components which reduce the fluidity. Sterols are isoprenoid-derived lipids and have a wide range of chemical structures. They can be found in free or conjugated form (i.e. linked with other molecules such as fatty acids and sugars) (Boutte and Grebe, 2009; Rogowska and Szakiel, 2020). Fatty acids and sterols play a role in membrane integrity during abiotic stress such as chilling. During chilling membranes become more rigid and lose their function. At which temp the phase change occurs is dependent on the membrane constituents, the type of phospholipids and the ratio of phospholipids and sterols. Sterols decrease the fluidity of the membranes. A decrease in sterol phospholipid ratio has been found to increase tolerance to low temperature in *Solanum comersonii* (Palta *et al.*, 1993). Membranes functioning can also be reduced due to lipid peroxidation which results in an increase in saturated fatty acids (Marangoni *et al.*, 1996). The transition to a more rigid phase affects the semi permeable barrier function and affects the functioning of embedded proteins. The loss of the function of membranes leads to loss of compartmentalization in the cell and cell death. This can be measured as ion leakage as intracellular water and metabolites leaks out of the cells due to rupture of cell membranes. Ion leakage from tissue discs is considered a marker of CI (Sharom *et al.*, 1994; Wongsheree *et al.*, 2009). Similarly, the maximal fluorescence of dark-

adapted leaves (F_v/F_m) can be used as a marker of CI. CI appears heterogeneously within the leaves and therefore imaging of chlorophyll fluorescence gives the best overview of the damage (Hogewoning and Harbinson, 2007). During chilling the thylakoid electron transport chain is one of the first parts of the plant cell to get affected. Chilling can have a negative effect on photosynthesis such as the thylakoid electron transport chain, carbon reduction cycle, regulation of stomatal conductance (Allen and Ort, 2001).

Membrane damage can be further increased by an increase in reactive oxygen species (ROS) at chilling temperatures. ROS are a major form of free radicals. Free radicals cover any molecular species that can exist independently and has at least one unpaired electron. The hydrogen atom is the simplest form of a free radical; hydrogen only has one electron hence it is unpaired. Formation of free-radicals or ROS occurs through several mechanisms such as the addition on an electron to a stable molecule (Halliwell, 2006). During non-stress conditions an equilibrium exists between ROS and antioxidants (i.e. although ROS are constantly formed as a byproduct of normal metabolism they are scavenged by the plants antioxidant system) (Foyer and Noctor, 2005). The equilibrium (i.e. redox homeostasis) between generation and scavenging of ROS is disturbed under biotic and abiotic stress conditions such as pathogen attack, salinity, drought, high light, high and low temperatures. During stress conditions excessive formation of ROS occurs which is severely damaging to the plant. ROS disturbs the functions of the cells as a high content of ROS leads to damage to proteins, DNA and lipids (Das and Roychoudhury, 2014). ROS causes lipid peroxidation through a chain reaction with unsaturated fatty acids i.e. after reaction between ROS and the lipids, new radicals are formed that can continue to attack the unsaturated fatty acids leading to membrane malfunction. The membranes lose their fluidity and semi-permeability resulting in loss of functionality which leads to cell death, flooding of intercellular spaces and desiccation (Ahmad *et al.*, 2008). The main forms of ROS are superoxide anions ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and singlet oxygen ($^1O_2$). They are a byproduct of the aerobic metabolism and formed at a number of locations such as in the mitochondria during respiration, in the chloroplast during photosynthesis, in the peroxisome, in the cell membrane through NADPH oxidases, and in the apoplast (Gill and Tuteja, 2010; Wang *et al.*, 2016*b*).

1.4 Antioxidants and their potential role in improving chilling tolerance

The antioxidant system in plants serves as a biochemical adaptation mechanism to survive in an aerobic environment (Halliwell, 2006). Antioxidants can be divided in two groups: enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants

include superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione-S- transferase (GST), and catalase (CAT), and the non-enzymatic antioxidants include ascorbic acid (AsA), reduced glutathione (GSH), α-tocopherol, carotenoids, phenolics, flavonoids, and proline (Gill and Tuteja, 2010). Phenolic compounds have shown to be stronger antioxidants than α-tocopherol and AsA (i.e. also known as vitamin E and vitamin C) (Rice-Evans *et al.*, 1997). Antioxidants have different hydrophobicity allowing them to function in more hydrophobic or hydrophilic environments. Environmental factors can increase the accumulation of antioxidants. Especially increased light intensity and modification of the light spectrum can increase the accumulation phenolic compounds.

1.4.1 Phenolic compounds

The biggest group of antioxidants are phenolic compounds. Phenolic compounds are organic compounds with at least one hydroxyl group (-OH) which is attached to a carbon atom that is part of an aromatic ring (Fig. 1). The chemical structure of phenolic compounds ranges from very simple molecules to complicated structures. The antioxidant capacity of phenolic compounds depends on their chemical structure i.e. the number of hydroxyl groups and their location in relation to the functional carboxyl group (Balasundram *et al.*, 2006). Phenolic compounds ubiquitous in plants are considered as secondary metabolites and defense compounds. They are synthesized through the shikimate pathway (de la Rosa *et al.*, 2019). During environmental stresses plants accumulate phenolics which aid plant survival. Phenolic compounds function as antioxidants in various ways. A hydroxyl group can donate a hydrogen atom to a ROS and the chain reaction of formation of new ROS can be stopped as the ROS becomes a stable molecule (Heim *et al.*, 2002). Donation of a hydrogen atom to a ROS making it a stable molecule is also called scavenging. However, after donation of a hydrogen atom the antioxidant molecule becomes unstable and exists in a radical form, however, it is much more stable than the ROS (Parr and Bolwell, 2000). The radical form of the antioxidant may be neutralized through reaction with glutathione or ascorbate and be reduced back to its original form (Yamasaki and Grace, 1998). The antioxidant radical may also react with other radicals forming a stable product. Phenolic compounds also acts as antioxidants when they chelate metal ions that are involved in production of free radicals (Croft, 1998). Phenolic compounds are stored in the vacuoles. Antioxidants can only be effective in protecting the plants against excessive ROS in the compartments of the cells that they have access to. However, the different phenolic compounds have an array of hydrophobicity and they are able to enter and act in both aqueous and lipid environments (Parr and Bolwell, 2000).

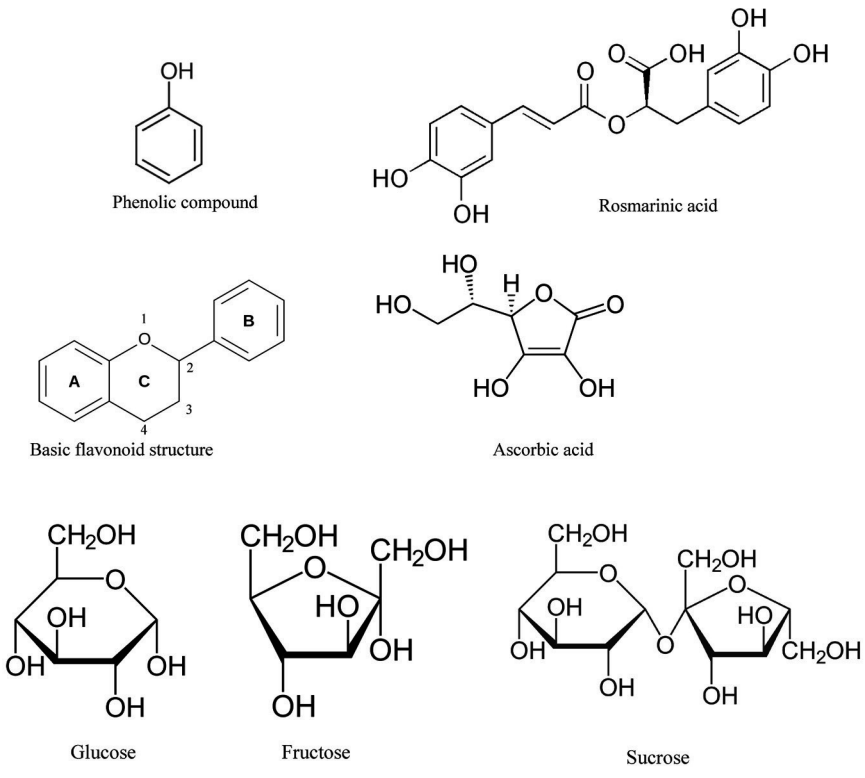

Fig. 1. Chemical structure of the simplest phenolic compound, rosmarinic acid, basic flavonoid structure, ascorbic acid, glucose, fructose and sucrose.

1.4.1.1 Phenolic acids

Phenolic acids are some of the most widespread phenolic compounds in plants. Phenolic acids are phenolic compounds with one carboxylic acid group. They are present in plants as free, conjugated-soluble and insoluble-bound forms. Phenolic acids belong to two classes i.e. hydroxybenzoic acids and hydroxycinnamic acids with either a C1-C6 or C3-C6 backbone respectively (Fig. 1) (Chandrasekara, 2019; Kumar and Goel, 2019). In basil, the dominant phenolic compounds are phenolic acids such as rosmarinic acid, chicoric acid, caftaric acid and caffeic acid, with rosmarinic acid often being found in largest amounts (Javanmardi et al., 2002; Kwee and Niemeyer, 2011). Rosmarinic acid is an ester of caffeic acid and 3,4-dihyroxyphyllactic acid. Compared to caffeic acid, rosmarinic acid contains four hydroxyl groups and not two, which in turn makes rosmarinic acid a stronger antioxidant than caffeic acid (Fig. 1) (Chen and Ho, 1997; Petersen and Simmonds, 2003). Rosmarinic acid has a strong antioxidant activity (Petersen and Simmonds, 2003). In microalgae rosmarinic acid inhibited the formation of ROS (Qiao *et al.*

2005). Thus, presence of rosmarinic acid can potentially increase membrane stabilization. Next after rosmarinic acid the most abundant phenolic acid found in basil is chicoric acid (Lee and Scagel, 2009). Although chicoric acid is often found in smaller quantities than rosmarinic acid in basil it is a stronger antioxidant (Dalby-Brown *et al.*, 2005).

1.4.1.2 Flavonoids

Flavonoids are a large group of phenolic compounds in plants existing in leaves, seeds, bark and flowers (Heim *et al.*, 2002). They consist of phenolic and pyran rings (i.e. a non-aromatic ring with five carbon atoms and one oxygen atom and two double bonds) and have a benzo-γ-pyrone structure. They have a variety of different arrangements of hydroxyl, methoxy and glycosidic side groups (Heim *et al.*, 2002). They can be divided into three sub-groups depending on which carbon of the pyran ring (the C ring) where the second phenolic ring (the B ring) is attached to (Fig. 1). The subgroups include isoflavones (i.e. the B ring is linked to position 3 on the C ring), neoflavonoids (i.e. the B ring is linked to position 4) and a large subgroup which is further divided in flavones, flavonols, flavanones, flavanonols, flavanols or catechins, chalcones and anthocyanins (i.e. the B ring is linked on position 2) (Panche *et al.*, 2016). The antioxidant function of flavonoids largely depends on the configuration of the hydroxyl groups on the B-ring (Pannala *et al.*, 2001). When comparing flavonoids from the same subgroup, the antioxidant properties increase with the number of hydroxyl groups on the B ring (Cao *et al.*, 1997).

1.4.1.3 Anthocyanins

Anthocyanins are a large subgroup of flavonoids that give rise to colorful plant tissue in the red-blue range. The difference in number of hydroxyl groups, types and number of sugars attached and the position on the molecules makes up the different anthocyanins (Kong *et al.*, 2003). Anthocyanins are stored in the vacuoles. The color and stability of the anthocyanin depends on the conditions in the vacuole such as pH, content of colorless flavonoids and metal ions to which the anthocyanin can react (Oren-shamir, 2009). Like other phenolic compounds anthocyanins function as antioxidants by donation of a hydrogen atom to free radicals or reactive oxygen species. In basil the only anthocyanins that have been identified are cyanidin and peonidin (Phippen and Simon, 1998).

1.4.2 Ascorbic acid

Ascorbic acid is also known as vitamin C or ascorbate. It exists as a reduced and oxidized form as ascorbic acid (AsA) or dehydroascorbate (DHA). AsA is constantly being oxidized to DHA and recycled to AsA; therefore, the total ascorbic acid

(TAsA) is considered the sum of AsA and DHA. AsA is formed through conversion of D-glucose through L-galactose and L-galactono-1,4-lactone (Chen *et al.*, 2003). Ascorbic acid functions as an antioxidant as it may scavenge ROS such as superoxide ($O_2^{\cdot -}$), hydroxyl radicals ($OH^{\cdot -}$), alkoxyl ($RO^{\cdot}$) and peroxyl ($ROO^{\cdot}$) though non-enzymatic reactions (Njus *et al.*, 2020). Furthermore, ascorbic acid aids aid in recycling of tocopherol (vitamin E); it reduces the radical tocopheroxyl ($TO^{\cdot}$) formed from tocopherol when tocopherol reduces free radicals in the lipid environment (Buettner, 1993). Ascorbic acid also has an enzymatic counterpart (APX) which is particularly active in the chloroplast where it scavenges H_2O_2 (Zhang, 2013). Ascorbic acid is also used in the ascorbate - glutathione cycle which reduces H_2O_2 to H_2O through a series of reactions. The ascorbate - glutathione cycle is present in most compartments in the cell (i.e. chloroplast, cytosol, mitochondria, peroxisomes, apoplast) (Garg and Manchanda, 2009). In leaves ascorbic acid levels have been associated with an extended shelf life of lettuce i.e. reducing the onset of senescence (Min *et al.*, 2021).

1.4.3 Carbohydrates

Carbohydrates (soluble sugars and starch) are generated by photosynthesis and serve as a source of energy for plant growth and carbon skeletons for organic compounds such as secondary metabolites and storage components. They also function as signalling molecules in growth, development and stress responses (Rolland *et al.*, 2006; Smeekens *et al.*, 2010; Trouvelot *et al.*, 2014).

Carbohydrates are composed of carbon, hydrogen and oxygen with the general formula $C_m(H_2O)_n$ (i.e. with m and n $\geq$3 and m always being lager than n). Carbohydrates are either polyhydroxy aldehydes or ketones. The simplest carbohydrate is glucose (Fig. 1). Carbohydrates exists in different degrees of polymerization (DP). Polymerization describes a chain like structures where units of the molecules are repeated. When classified according to their DP carbohydrates exists as monosaccharides (DP1), disaccharides (DP2), oligosaccharides (DP$\leq$10) and polysaccharides DP>10) (Ende and Peshev, 2013). Glucose and fructose are monosaccharides, whereas sucrose is a disaccharide. Starch and fructans are polysaccharides and often used for energy storage in plants (Ende and Peshev, 2013). In basil, the main carbohydrate used for storage is starch (Buchi *et al.*, 1998). In addition to their function in growth and development sugars are also involved in stress metabolism where they can act as an antioxidant. Sugars are soluble molecules with several hydroxyl groups (Fig. 1). During environmental stress sugars can protect membranes as they may scavenge hydroxyl radicals that are generated from lipid peroxidation or damaged lipids (Pommerrenig *et al.*, 2018).

1.5 Vertical farming

To improve the chilling tolerance of plants it may be possible to prepare the plant through the cultivation practices. Through cultivation practices the content of carbohydrates and antioxidants may be increased which may be beneficial for the plants to overcome cold stress. Cultivation practices include optimization of environmental factors such as light, air humidity, CO_2 concentration, airflow, temperature, nutrient concentration and water. Environmental factors can to some degree in greenhouses, whereas in a vertical farm (also known as a plant factory) environmental factors can be fully controlled (Kozai, 2018; SharathKumar *et al.*, 2020; VanDelden *et al.*, 2021). This gives an opportunity to the producer to control the quality of a year-round plant production (SharathKumar *et al.*, 2020). When all environmental factors can be controlled, the plant growth, morphology and quality can be predicted. To predict the overall plant growth, sensors that record the environmental data, plant sensors and artificial intelligence can be used to constantly update dynamic growth models. The models may help in managing the vertical farm in terms of optimal lighting strategies and fertilization, among others. A Limitation of vertical farming is the high energy input needed for cultivation. Compared to conventional (e.g. greenhouse and open field) production, vertical farms especially need a high energy input for lighting. The main light sources used in a vertical farm are light emitting diodes (LED) (VanDelden *et al.*, 2021). Currently, for a vertical farm to be financially viable, it needs to produce high quality crops that may be marketed at a higher price than products from conventional systems. Vertical farms also have the benefit that the production may occur close to the consumer which allows transportation and storage time from producer to consumer to be shorter. In turn, a fresher produce may be available for the consumer. The past two decades, consumers have had an increasing awareness and interest in fresh, high quality, nutritious products (Rouphael *et al.*, 2018). The quality of a product is affected by a number of pre-harvest factors such as environmental conditions (e.g. light, nutrients and temperature), and crop management (e.g. harvesting time, plant density) and the interaction with the genetic material (Weston and Barth, 1997). Currently, the focus of vertical farming is on high value crops for example leafy greens and herbs such as lettuce and basil, and on soft fruit (e.g. strawberry). Controlling and changing the light intensity and spectrum is a non-chemical way to optimize the plant quality (Bian *et al.*, 2015).

1.6 Light

Plants use light as a source of energy to drive photosynthesis. The light used for photosynthesis is defined as photosynthetic photon flux density (PPFD) which

includes the spectrum from 400-700 nm (McCree, 1972). Plants utilize light, ranging from Ultra Violet B (280-315 nm), UV-A (315-400 nm), blue (B) (400-500 nm), green (G) (500-600 nm), red (R) (600-700 nm) to far-red (FR) (700-800 nm). The light is used for developmental and physiological processes. Photoreceptors such as phytochromes, cryptochromes, phototropins, *ZTL/FKF1/LKPS* and UVR8 absorb light and mediate the response to the light spectrum (Huché-Thélier *et al.*, 2016). The effect of light intensity and light spectrum on plant growth, development, secondary metabolism and quality at harvest and postharvest have been studied extensively in a vast amount of plant species and cultivars (Kadomura-Ishikawa *et al.*, 2013; Ouzounis *et al.*, 2015; Thoma *et al.*, 2020).

Supplemental light for optimal year-round plant production has been used on the Northern hemisphere for over 4 decades in greenhouse cultivation where the main light source is high pressure sodium (HPS) lamps (McAvoy and Janes, 1984; Blacquiere, 1997). Light from HPS lamps contains very little blue light and is rich in red and green. HPS lamps do not allow for the light spectrum to be modified. The rise of the technology and implementation of LED lamps in plant production have especially fueled the research focusing on the effect of light spectrum on plant growth, morphology and quality. LEDs are solid-state semi-conductor devises with narrow bandwidth light. If blue LEDs are phosphor coated broadband light is produced (Bourget, 2008). The heat radiation from LEDs is low, which allows the lamps to be placed close to the plants; this is especially useful in vertical farming. The light intensity and spectrum from LED lamps can be modulated based on the need of the plants (Pattison *et al.*, 2018*b*).

1.6.1 Plant growth and morphology

The energy that plants use for growth and biomass production comes from photosynthesis. Photosynthesis generally increases with increasing light intensity until the light saturation point is reached, which may vary between species and phenotypes (Ögren and Evans, 1993). Hence, for each plant species and cultivation situation an optimal light intensity exists depending on other environmental factors such as water and nutrient availability and temperature and CO_2 concentration of the air. The optimal light intensity for basil resulting in the highest fresh mass, dry mass and dry matter percentage has been found to range from 250 µmol m^{-2} s^{-1} (Pennisi *et al.*, 2020) to 290 µmol m^{-2} s^{-1} (Dou *et al.*, 2018) to 500 µmol m^{-2} s^{-1} (Beaman *et al.*, 2009) (i.e. all with a day length of 16 hours). As an optimum of light intensity exists, a too high light intensity may lead to stunted growth. In addition to light intensity, the composition of the spectrum is also important for optimal photosynthesis. Red light is most efficient for photosynthesis (Hogewoning *et al.*, 2012). When plants are

cultivated solely under red light a so called 'red-light syndrome' may occur where plants show leaf curling and decreases in leaf thickness and leaf pigmentation. However, the addition of blue light to the spectrum ameliorates the symptoms (Trouwborst *et al.*, 2016).

Photosynthesis can be improved resulting in improved plant growth with the addition of blue light (from 6 – 12 %) in greenhouse conditions (Kaiser *et al.*, 2019). For basil there has not been a consensus on the optimal light spectrum for improved growth. Pennisi *et al.* (2019) found the optimal R:B ratio to be 3 (i.e. with 23% blue light), whereas Piovene *et al.* (2015) found 0.7 (i.e. with 37 % blue light) to be optimal. However, Lim and Kim (2021) did not find a clear effect of solely red and of R:B ratio ranging from 1.1 to 3 (from 20 to 27 % blue light) on basil growth or morphology. The spectrum of supplemental light under greenhouse conditions did not affect the fresh or dry weight of basil but the dry matter percentage was increased with a R:B ratio of 4 (i.e. with 20% blue light) (Jensen *et al.*, 2018).

In addition to biomass accumulation, plant morphology may be significantly altered by light intensity and spectrum. The plant response to a given light intensity depends on the adaptation and acclimation of the plant. Genotypes that are not adapted to low light, so called shade avoidance species, have several responses known as 'shade avoidance syndrome' (SAS) when they experience low light. This is due to a decrease in the PPFD, which increases the relative amount of FR light perceived by the plant. The addition of FR light to a given light spectrum also triggers SAS as the R:FR ratio is decreased. One of the main phenotypic response to SAS is elongation of stem and leaves (Franklin, 2008). In basil low light resulted in stem elongation, increased leaf area and reduced leaf thickness (Stagnari *et al.*, 2018). If plants are cultivated under 100% of one color, abnormalities may occur. Under 100 % blue light, SAS may also be induced due to low activity of phytochromes (Kong *et al.*, 2018; Zhang *et al.*, 2021). In basil the addition of green light to a white light background also induced elongation and SAS like response (Schenkels *et al.*, 2020)..

1.6.2 Plant quality and content of secondary metabolites

The content of secondary metabolites (i.e. phenolic compounds, carotenoids) can be increased by high light intensity. In coriander an increase in light intensity up to 300 µmol m^{-2} s^{-1} increased the content of chlorogenic acid (a phenolic acid) in coriander (Nguyen *et al.*, 2019). Often an optimum response exists to increased light intensity where a too high light intensity may have a negative effect on the nutritional content. In microgreens (red bok choy and tatsoi) an increase in light intensity from 100 up to 440 µmol m^{-2} s^{-1} increased the carotenoid content whereas 545 µmol m^{-2} s^{-1}

decreased the content (Brazaityte *et al.*, 2015). Similarly, in basil a light intensity up to 290 µmol m^{-2} s^{-1} increased the anthocyanin, flavonoid and phenolic content whereas it decreased at a light intensity of 310 µmol m^{-2} s^{-1} (Dou *et al.*, 2018). Pennisi *et al.* (2020) found that increased light intensity had no effect on the phenolic content in basil. High light intensity throughout the cultivation is associated with a high energy consumption and therefore high costs. A more efficient strategy resulting in lower energy consumption is to apply the change in light intensity in the end of the production (EOP). This may still increase the content of primary and secondary metabolites resulting in an improved plant quality. In lettuce an increase in light intensity EOP increased the content of carbohydrates and total ascorbic acid (Min *et al.*, 2021).

A change in light spectrum as EOP treatment of throughout the cultivation period can also have a significant effect on secondary metabolites. Flavonoids and anthocyanins are generally assumed to be upregulated in response to plant cultivation under UV and blue light. Cryptochromes play a major role in anthocyanin accumulation under blue light. When Arabidopsis mutants were lacking *hy4* (i.e. the *HY4* gene encodes for the blue light photoreceptor CRY1) they had a lower anthocyanin content compared to the wild types under blue light (Chen *et al.*, 2006). The addition of blue light as EOP treatment can increase the anthocyanin content in red lettuce (Owen and Lopez, 2015). Total flavonoid concentration increased in basil with an increase in blue light up to 23% blue, after which it decreased (Pennisi *et al.*, 2019). Blue light has also been shown to increase the content of rosmarinic acid in basil and chicoric acid and both lettuce and basil (Taulavuori *et al.*, 2016). Other spectra such as far-red (Schwend *et al.*, 2016) and red (Shiga *et al.*, 2009) have also been reported to increase phenolic compounds such as rosmarinic acid in basil.

1.6.3 Light and chilling tolerance

Light plays a role in tolerance to low temperature. High light in the combination with low temperature can have an adverse effect leading to a more severe CI (Allen and Ort, 2001). However, the addition on FR has been shown to improve the tolerance to low temperature in a number of species such as Arabidopsis, barley, tomato (Franklin and Whitelam, 2007; Ádám *et al.*, 2016; Affandi *et al.*, 2020). FR light can induce cold tolerance through induction of the C-repeat-binding factors (CBF) - pathway which is part of the cold acclimation response (Wang *et al.*, 2016*b*). Furthermore, light spectrum can increase the content of antioxidants which may be beneficial during chilling. In tomato, blue light improved the chilling tolerance, which was presumable due to an increase of the antioxidant lycopene (Affandi *et al.*, 2022). However, in anthurium cut flowers postharvest blue or white compared to

darkness, red or red blue (70:30) light increased the CI due to an increase in water loss (Aliniaeifard *et al.*, 2020). Similarly, addition of green light under greenhouse conditions reduced the postharvest CI in basil due to a reduced transpiration (Jensen *et al.*, 2018).

1.6.4 Bridging pre-harvest lighting with postharvest quality

Postharvest quality of products is highly affected by the pre-harvest conditions. Environmental factors during the pre-harvest such as nutrient and water availability, temperature, light intensity and spectrum can affect the quality at harvest and the postharvest quality (Hewett, 2006). The majority of studies focusing on light intensity and spectrum only investigate the at-harvest quality (Carvalho and Folta, 2014; Bantis *et al.*, 2016; Dou *et al.*, 2018, 2019; Pennisi *et al.*, 2019, 2020). Some studies have studied the effect of pre-harvest lighting on postharvest quality. In lettuce an increase in light intensity throughout the cultivation improved shelf life (Witkowska and Woltering, 2010) and pre-harvest EOP light intensity increased the carbohydrate and total ascorbic acid content resulting in an improved shelf life (Min *et al.*, 2021). In basil supplemental green light improved chilling tolerance (Jensen *et al.*, 2018).

During postharvest storage, the content of metabolites decreases due to senescence, respiration and general decay. A high initial content of metabolites may be beneficial for a prolonged quality during postharvest storage (i.e. prolonging the shelf life of the product). During postharvest storage in darkness, the product decay cannot be stopped, it can only be delayed. Thus, the pre-harvest factors play an important role in ensuring a good postharvest quality. However, the pre-harvest content of metabolites cannot always predict the postharvest content. In Chinese kale sprouts a pre-harvest treatment of 24 hours red light compared to white light had no effect on total phenolic content and total ascorbic acid content at harvest but the effects occurred during dark storage. The red light treated sprouts had an increase in total phenolic content during dark storage and the degradation of total ascorbic acid was slower than in the white light treated sprouts (Deng *et al.*, 2017). Similarly, in basil leaves the content of several phenolic compounds such as caffeic acid, rosmarinic acid, chicoric acid and catechin derivative increased during postharvest dark storage at 10 °C due to pre-harvest EOP UV-B light treatments (Nascimento *et al.*, 2020) . The at-harvest content of these phenolic compounds was not significantly increased by the EOP UV-B treatments. During low temperature storage, in a chilling sensitive species such as basil, the decrease in metabolite content may also have an interactive effect with the chilling stress. This had yet to be investigated and is addressed in the chapters of this thesis.

1.7 Outline of this thesis

The research described in this thesis was aimed at improvement of the quality of basil, both at harvest and postharvest, through pre-harvest lighting strategies. Postharvest basil quality is mainly reduced by CI thus improving the chilling tolerance would improve overall basil quality. The regulation of the nutritional content (i.e. carbohydrates and antioxidants) and hormones by light intensity and light spectrum in basil was investigated. Furthermore, the role of nutritional content and hormones in postharvest chilling tolerance of basil was studied. Light treatments were applied in a climate chamber in a vertical farming set-up where all other growth parameters were held constant. After harvest, the leaves from basil plants are stored in plastic boxes in darkness at 4 and 12 °C. The chilling symptoms, metabolite content and overall quality were evaluated for up to 15 days after harvest (Fig. 2). Improving quality without focusing on yield is not viable for basil production in a vertical farm. Hence, the yield and morphological response to light treatments have also been studied.

A schematic drawing of the outline of the research described in this thesis is presented in Fig. 3.

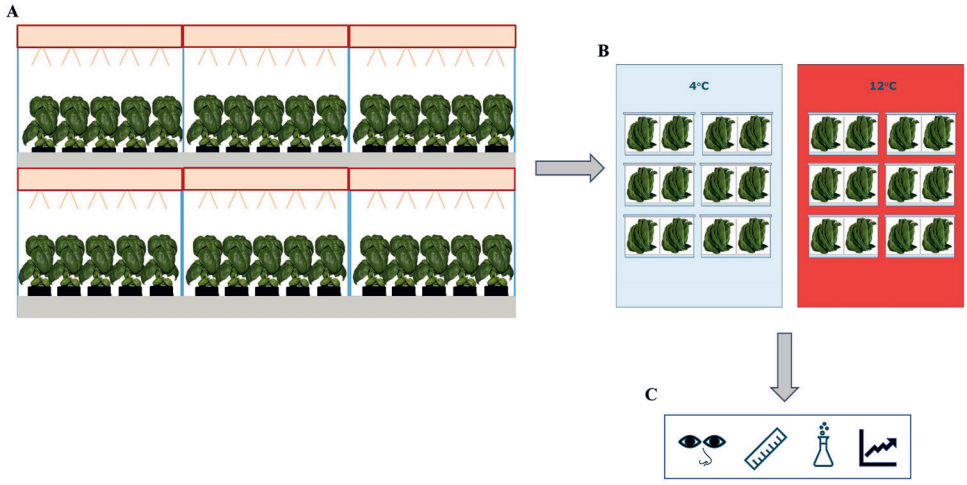

Figure 2. Overview of the experimental workflow described in this thesis. A. basil were grown as single plants in a vertical farming system under different light conditions. Individual compartments were separated by white reflective plastic to avoid light pollution between compartments. B. After harvest leaves were stored in plastic boxes at 4 and 12 °C in darkness up to 15 days. C. For postharvest evaluation of the CI symptoms and quality, leaves were scored visually, and maximal chlorophyll fluorescence of dark-adapted leaves (F_v/F_m) was measured as a marker for CI. Carbohydrates (soluble sugars and starch) and antioxidants were measured with HPLC and finally the data was analyzed.

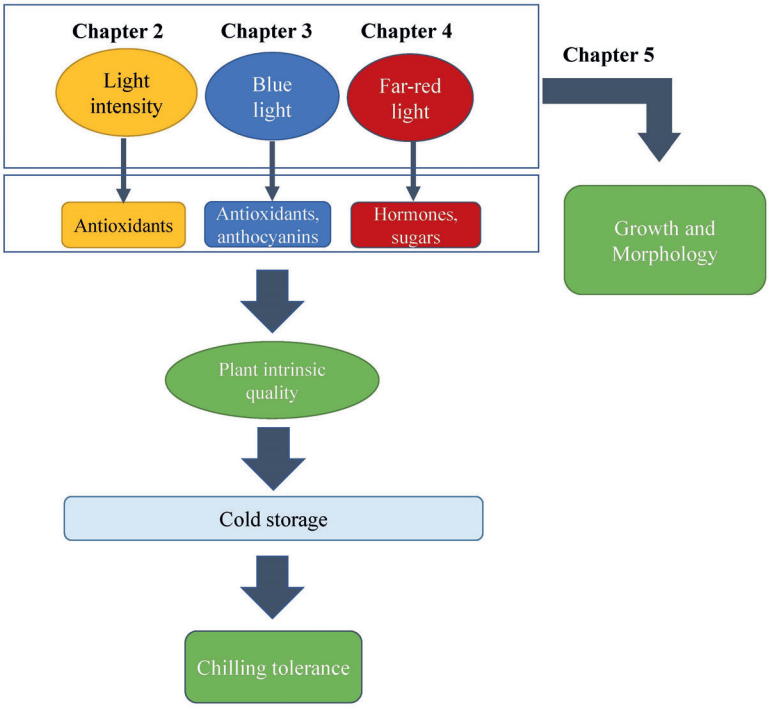

Figure 3. Schematic presentation of the work described in this thesis. The quality of basil in response to different pre-harvest lighting strategies was investigated. A series of experiments was conducted to study the regulation of nutritional content (carbohydrates and antioxidants) and hormones by light intensity and spectral composition. The possible role of nutritional content and hormones in alleviation of CI was studied. The response to EOP light intensity, blue light and far-red is presented in Chapter 2, 3 and 4, respectively. The response of growth and morphology to light intensity, blue light and far-red is presented in Chapter 5.

In **Chapter 2** the regulation of carbohydrates and antioxidants in response to high light as End-Of-Production (EOP) treatments is described in two green basil cultivars. In addition, the effect of an increase in secondary metabolites on CI was studied. It was hypothesized that increased EOP high light would increase the content of carbohydrates and antioxidants which would improve the postharvest chilling tolerance. EOP treatments (i.e. 50, 150, 300 and 600 µmol m^{-2} s^{-1}) with red-white LED lights were applied for the last five days before harvest in two green basil cultivars. After harvest leaves were stored at 4 and 12 °C in darkness. To evaluate the level of CI the overall visual quality was scored and F_v/F_m (as a marker for CI) was measured on leaves at harvest and during postharvest. The content of metabolites such as carbohydrates, antioxidants (rosmarinic acid, chicoric acid and

total ascorbic acid) and H_2O_2 were measured in leaves at harvest and in leaves stored at 4 and 12 °C.

In **Chapter 3** the regulation of carbohydrates and antioxidants in response to blue light throughout the cultivation and applied as EOP treatment in green and purple basil is described. It was hypothesized that blue light would increase the content antioxidants which would improve the postharvest chilling tolerance in green and purple basil. Two experiments were conducted. In the first experiment the effect of an increase in blue light percentage at a high light intensity (PPFD of 300 µmol m^{-2} s^{-1}) throughout the cultivation period for 25 days or as EOP treatment for 5 days was studied. In the second experiment the spectral effect in relation to light intensity treatments with a low or high blue light percentage applied at different light intensities (i.e. PPFD of 100 and 300 µmol m^{-2} s^{-1}) was studied. After harvest leaves were stored at 4 and 12 °C in darkness. To evaluate the level of CI the overall visual quality was scored and F_v/F_m (as a marker for CI) was measured on leaves at harvest and during postharvest. Metabolite content such as carbohydrates, antioxidants (rosmarinic acid, chicoric acid, total ascorbic acid, total flavonoids and total anthocyanins) and H_2O_2 were measured in leaves at harvest and in leaves stored at 4 and 12 °C.

In **Chapter 4** the effect of cultivation with additional FR on postharvest chilling tolerance of basil is described. It was hypothesized that that addition of FR would improve the CI in basil as FR would increase the content of hormones (abscisic acid (ABA) and jasmonic acid (JA)) involved in cold tolerance. It was studied if basil chilling tolerance could be improved in response to additional FR and to a lowered temperature during cultivation. FR was applied both at a high (25 °C) and low (15 °C) temperature. Plants cultivated at high temperature received either no, 1 or 3 weeks additional FR and the low temperature cultivated plants no or 3 weeks additional FR. After harvest leaves were stored at 4 and 12 °C in darkness. To evaluate the level of CI the overall visual quality was scored and F_v/F_m (as a marker for CI) was measured on leaves at harvest and during postharvest. Metabolite content such as carbohydrates, antioxidants (rosmarinic acid, chicoric acid and total ascorbic acid) and hormone content were measured in leaves at harvest and in leaves stored at 4 °C.

In addition to the intrinsic plant quality (carbohydrate and antioxidant content) the growth and morphology may also be affected by light intensity and spectrum. In **Chapter 5** the response of basil growth and morphology to light intensity, percentage of blue light and additional FR were studied, using the experiments

described in Chapters 2, 3, and 4. Light intensity was studied as EOP treatments applied for the last five days before harvest in two green basil cultivars. The effect of blue light was studied in two experiments. The effect of blue light was studied as an increase in blue light percentage at a high light intensity (PPFD of 300 µmol m^{-2} s^{-1}) throughout the cultivation period for 25 days or as EOP treatment for 5 days. Furthermore, spectral effect in relation to light intensity treatments with a low or high blue light percentage applied at different light intensities (i.e. PPFD of 100 and 300 µmol m^{-2} s^{-1}) was studied. FR was studied by applying additional FR during 1, or 3 weeks of growth compared to a treatment without FR. Plant growth parameters such as fresh and dry mass of leaves and stem and morphology parameters such as height and leaf area were measured. As a vertical farm requires a high energy input it is important to use it in the most efficient way. Therefore, the light use efficiency of the given treatments was also discussed.

In **Chapter 6** the findings in this thesis are discussed in a general discussion.

Chapter 2

High light intensity at End-Of-Production improves the nutritional value but does not affect postharvest chilling tolerance

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Abstract

Basil suffers from chilling injury (CI) when stored at temperatures below 10-12 °C which seems related to the imbalance between reactive oxygen species (ROS) and antioxidants. We hypothesized that increased light intensity applied shortly before harvest (EOP, End-Of-Production) increases nutritional value i.e. carbohydrates and antioxidants. This could improve the chilling tolerance. Two basil cultivars were grown in a vertical farming set-up at a light intensity of 150 µmol m^{-2} s^{-1}. During the last 5 days of growth, EOP light treatments ranging from 50 to 600 µmol m^{-2} s^{-1} were applied. Postharvest the leaves were stored at 4 or 12 °C in darkness. Higher EOP light intensity increased antioxidants as total ascorbic acid, rosmarinic acid as well as carbohydrate contents at harvest. During storage antioxidants decreased more rapidly at 4 than at 12 °C. However, increased EOP light intensity did not alleviate chilling symptoms suggesting a minor role of antioxidants studied against chilling stress.

2.1 Introduction

The term chilling injury (CI) is used to describe physiological disorders in plants or plant products due to exposure to low temperatures above the freezing temperature. In plants and plant products of subtropical and tropical origin CI may occur following exposure to temperatures below 10-12 °C (Lyons, 1973). Basil (*Ocimum basilicum* L.) is an herb of tropical origin and when it is exposed to chilling temperatures brown spots develop in the interveinal areas after which black necrotic lesions appear. Furthermore, CI causes a loss of glossy appearance and premature wilting (Lange and Cameron, 1994). The severity of the chilling injury depends on the duration and temperature during growth or storage. During prolonged storage under chilling temperatures basil finally may develop soft rot from fungal and bacterial attack. Often fresh basil is transported together with other herbs and leafy vegetables that greatly benefit from temperatures down to 0 °C. Such transport and storage conditions, however, are unfavorable for basil leading to severe losses.

CI negatively affect the integrity and functioning of the cell membranes (Sharom *et al.*, 1994). Under chilling conditions, parts of the membrane lipid bilayer may change from a flexible liquid phase into a solid gel phase leading to loss of semi permeability evidenced by increased ion leakage (Raison and Orr, 1986). The most sensitive organelle in the plant to chilling is the chloroplast (Kratsch and Wise, 2000) and chlorophyll fluorescence has been used as a tool to determine the severity of CI. Plants suffering from chilling injury show a decrease in the maximum quantum yield of photosystem II (PSII), quantified by the F_v/F_m value of dark-adapted leaves. Chilling injury appears heterogeneously on the leaves and therefore chlorophyll fluorescence imaging is particularly useful to estimate the severity of the injury on the whole leaf (Hogewoning and Harbinson, 2007). Furthermore, a decrease in F_v/F_m has been linked to an increase in ion leakage and an increase in reactive oxygen species (ROS) (Kasajima, 2017).

ROS are products of the aerobic metabolism; major sites to produce ROS in the cell are the chloroplasts and mitochondria but they also occur in peroxisome and apoplast (Hasanuzzaman *et al.*, 2020). ROS are constantly formed and present at low levels during normal growth conditions but counteracted by the plant's antioxidant capacity. ROS such as hydrogen peroxide (H_2O_2) at low intracellular concentrations can be beneficial to the plant where it acts as a signal molecule regulating essential plant processes e.g. senescence and growth and development (Das and Roychoudhury, 2014). At low concentrations ROS also signals to upregulate defense responses. However, under abiotic stresses such as drought, high light and chilling temperatures excessive formation of ROS may occur and the redox homeostasis is

disturbed, which can be detrimental to the plant. ROS are produced from different pathways such as photorespiration and mitochondrial respiration (Mittler, 2002). High levels of ROS cause damage to DNA, lipids and proteins. When ROS react with the unsaturated fatty acids, peroxidation of the membrane lipids occurs and this may cause changes in membrane fluidity and semi-permeability leading to increased ion leakage, cell death and desiccation (Ahmad *et al.*, 2008). To counteract the damaging cascade of ROS, both enzymatic and non-enzymatic antioxidants can come into play. The enzymatic antioxidants include superoxide dismutase (SOD), ascorbate peroxide (APX), catalase (CAT), guaicol peroxidase (GPX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Gill and Tuteja, 2010). Non-enzymatic antioxidants that scavenge ROS include ascorbic acid, reduced glutathione (GHS), α-tocopherol, carotenoids, phenolic compounds and proline (Das and Roychoudhury, 2014; Hasanuzzaman *et al.*, 2020). Of the non-enzymatic antioxidants ascorbic acid is one of the most abundant water soluble antioxidants mainly present in the leaves (Smirnoff *et al.*, 2001). Plants belonging to the *Lamiaceae* family are also rich in phenolic acids such as rosmarinic acid and other esters of caffeic acid. Particularly rosmarinic acid is present in high concentrations in basil (Kwee and Niemeyer, 2011). Rosmarinic acid has been found to have a strong ROS scavenging activity (Qiao *et al.*, 2005). An increase in antioxidants would be beneficial for the plant to minimize the damaging effect of excessive production of ROS during chilling stresses (Oh *et al.*, 2009).

One way to increase the antioxidant content either pre-harvest or postharvest. In fruit postharvest treatments with application of e.g. polyamines (Koushesh Saba *et al.*, 2012) have been successful in reducing CI and increasing antioxidants. The use of chemicals postharvest is becoming more and more restricted and a non-chemical approach is desirable. Antioxidant content in leaves can be increased through an increase in light intensity during growth. Both, ascorbic acid in leaves (Fukunaga *et al.*, 2010) and enzymatic antioxidants (Burritt and Mackenzie, 2003) can be increased by high light. In lettuce the shelf life and nutritional status were improved by increasing the light intensity before harvest (Min *et al.*, 2021). The extended shelf life was associated with an increase in ascorbic acid and carbohydrates. Furthermore, light can also increase other secondary metabolites important for quality such as volatile organic compounds (Gouinguené and Turlings, 2002). To optimize plant quality and increase the content of secondary metabolites it has been proposed to focus on the lighting strategy in the last days of cultivation (SharathKumar *et al.*, 2020). End-Of-Production (EOP) light treatments represent a non-invasive and non-chemical technique to improve postharvest quality and shelf life of fresh products.

In the present study we tested the hypothesis that increased light intensity applied 5 days before harvest would increase antioxidants such as ascorbic acid and rosmarinic acid in basil. Furthermore, we hypothesized that an increase in antioxidants would improve the chilling tolerance in fresh basil leaves.

2.2 Materials and Methods

2.2.1 Plant growth

Basil plants (*Ocimum basilicum* L.) of cvs. Emily and Dolly (Enza Zaden, NL) were grown in a climate chamber in a vertical farming set-up with compartments osf w 0. 8 x l 1.3 x h 1 m and a planting density of 123 plants m^{-2}. The compartments were separated by white reflective plastic. Plants were sown in stone wool trays with 240 plugs (Grodan Rockwool B.V., The Netherlands) with one seed per plug. After 15 days the morphologically most similar plants were selected and transplanted to 7.5 x 7.5 x 6.5 cm stone wool blocks (Grodan Rockwool B.V., The Netherlands) with one plug per stone wool block. Cultivars were grown in different compartments to maintain similar light intensities. Plants were grown under red-white light from Light Emitting Diodes (LEDs) (Green Power LED research module, Philips, the Netherlands) with an intensity of 150±5 µmol m^{-2} s^{-1} at top of the canopy with a day length of 18 hours. Throughout the plant growth the light spectrum and photoperiod was kept the same. The light spectra consisted of 9 % blue (400-500 nm) 19 % green (500-600 nm) and 70 % red (600-700 nm) and 1 % far-red (700-800 nm) light. The light intensity was regularly measured with a quantum sensor (LI-190SB quantum sensor, LI-1400 112 Datalogger, LI-COR Bioscience, Lincoln, USA) to adjust the height of the light frames throughout the cultivation. The spectral intensity for every compartment was measured with a spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, 110 Netherlands). The day/night temperature was kept at 25 °C, the relative humidity was set at 75 % and CO_2 was kept at ambient concentrations. Relative humidity and temperature in each light treatment compartment were recorded with keytag dataloggers (KTL-508, Keytag, the Netherlands) with deviations within 10 % and 1 °C from the set points. To maintain air temperature around 25 °C fans were installed in high light treatments above the lamps blowing out of the individual compartments. Plants were kept well-watered through an ebb and flood system based on plant needs and growth stage. For the first three weeks of the growth, plants were watered once every second day for 10 min and after that once every day for 10 min. High light treatments were given an extra round of watering when needed. Plants were watered with a nutrient solution

consisting of NO_3^- 8.5 mM, SO_4^{2-} 3.9 mM, HCO_3^- 1.5 mM, HPO_4^{2-} 1.5mM, NH_4^+ 1.5 mM, K^+ 5.5 mM, Ca^{2+} 4.0 mM, Mg^{2+} 1.5 mM, Cl^- 0.2 mM, Fe^{3+}/Fe^{2+} 30µM, Mn^{2+} 5 µM, Zn^{2+} 5 µM, $H_2BO_3^-$ 35 µM, Cu^+/Cu^{2+} 1 µM, MoO_4^{2-} 1 µM nutrition of pH 5.7 with EC 1.7 dS m^{-1} before transplanting and with and EC of 2.3 dS m^{-1} after transplanting. Fifteen days after transplanting the plants were exposed to 5 days of End-Of-Production light treatments of 50, 150, 300 or 600 µmol m^{-2} s^{-1} in different compartments. The experiment was carried out three times for each cultivar and each time the treatments were randomized over the compartments. Each time the experiment was repeated was considered a block in the further analysis.

The same experiments were used in **Chapter 5** to analyze the effects of the light treatments on plant growth and morphology, including plant height, leaf area fresh and dry mass at harvest.

2.2.2 Postharvest storage and sampling

Plants were harvested 35 days after sowing. At harvest three leaf pairs (excluding the oldest pair and youngest underdeveloped pair) were taken per plant and stored in plastic boxes (16 x 11 x 6 cm) with leaves from two plants per box. To maintain a high humidity in the boxes two pieces of wetted filter paper were placed on the bottom of the boxes. After that a smaller piece of plastic was placed on top of the wet filter paper to avoid direct contact between the leaves and the wet filter paper. The leaves from the two plants were separated in the boxes by a piece of plastic. Furthermore, the transparent plastic lids with 9 holes made by a 1 mm syringe needle to prevent the built up of CO_2 or ethylene in the boxes. Boxes were randomly placed in darkness at a chilling and non-chilling temperature, 4 or 12 °C. In commercial practice basil is often transported with other vegetables and herbs below 5 °C. Temperature and relative humidity in the boxes were logged with keytag dataloggers (KTL-508, Keytag, the Netherlands). The fresh mass of the leaves was determined at harvest.

Measurements and sampling were conducted on day 0 (at harvest) and 3, 6, 9 and 12 days after harvest. On each sampling day two postharvest storage boxes (i.e. each with leaves from two plants per block per light treatment) were sampled. During sampling the fresh mass of leaves was measured to determine water loss. Furthermore, an overall visual quality score was given to the leaves per sampled plant and maximum quantum yield of PSII (F_v/F_m) was measured. Afterwards the leaves were snap frozen in liquid nitrogen, ground with an IKA-A 11 basic analytical mill (im-lab, Belgium) and stored at -80 °C for further metabolic analysis.

2.2.3 PSII efficiency F_v/F_m

The F_v/F_m ratio characterizes the maximum quantum yield of the primary photochemical reactions or PSII in dark adapted leaves. For this measurement, per stored box: one leaf from the upper leaf-pair and one leaf from the middle leaf pair were measured. Leaves were dark adapted at room temperature for 20 min and chlorophyll fluorescence was measured using a PSI closed Fluorcam 800-C chlorophyll fluorescence imaging system (PSI, Czech Republic). Fluorcam software Version 7 was used to operate the fluorcam and analyze the obtained images, following the method of (Hogewoning and Harbinson, 2007). The efficiency of chlorophyll fluorescence was determined by measuring F_v/F_m ratios. Briefly, F_o, the minimal fluorescence yield, was measured with light flashes of < 0.5 µmol m^{-2} s^{-1} light after which a saturating light pulse (2500 µmol m^{-2} s^{-1}) was given to produce the image for F_m, the maximal fluorescence. The software determined an F_v/F_m image as the $(F_m-F_o)/F_m$ from the respective images of F_o and F_m.

2.2.4 Overall Visual Quality

Overall visual quality (OVQ) was evaluated using a scoring system based on visual symptoms related to chilling injury and symptoms appearing at non-chilling temperatures. Based on visual symptoms a score was given between 1 and 8, where 1 was the worst and 8 the best. The score 5 was set as limit for consumer acceptance, indicating the end of shelf life. Symptoms which reduced the score included dark spots, greenness, fungal appearance, degree of crispness, degree of wilting, leaf shininess and presence of characteristic curved leaf shape (Table S1).

2.2.5 Rosmarinic acid and Chicoric acid

Phenolic acids were extracted at room temperature from 250 ±20 mg frozen ground basil tissue with 1.5 mL of 80 % methanol with 2.5 % formic acid. Samples were further extracted for 15 minutes in an ultrasonic bath (Branson 2800). Samples were centrifuged at 10000 RCF for 15 min at 4 °C (Universal 320R, Hettich). The supernatant was filtered through a 0.45 µm cellulose syringe filter into a HPLC vial. Rosmarinic acid and chicoric acid were analyzed according to the method of Kwee and Niemeyer (2011), with modifications, on a HPLC consisting of a GS50 pump (Dionex), a 340S UV-VIS detector (Dionex) and a MIDAS autosampler (Spark Holland) equipped with a LiChrospher 100 RP-18 (5 µm), 150 x 4 mm column (Merck). The column was eluted at a flow rate of 1.6 mL min^{-1} with 2.5 % formic acid in H_2O (mobile phase A) and acetonitrile (mobile phase B) with a linear gradient of: 85 % A, 0.0 min.; 75 % A, 6.0 min.; 0 % A, 8.5 min. Detection of analytes was at 330 nm. Chromeleon 6.4 (Thermo Fisher Scientific) was used for data analysis. Calibration curves were prepared with authentic standards (Extrasynthese) ranging

from 0 to 500 mg L^{-1}. Individual phenolic acids concentrations were quantified by integration of peak areas and compared to the calibration curves. A conversion factor for each sample was made to convert to dry weight from fresh weight; 100±20 mg of fresh material for each sample were weighed into a reaction tube and vacuum dried for 120 min in a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific) at 50 °C and 5.1 mbar. Data was expressed on the base of dry weight as mg g^{-1} DW.

2.2.6 Total ascorbic acid

Vitamin C is defined as total ascorbic acid (TAsA). TAsA is the sum of ascorbic acid (AsA) and dehydroascorbic acid (DHA). TAsA is a major antioxidant group in leafy vegetables (Min *et al.*, 2021). AsA was extracted from 200 mg frozen ground basil tissue with 1 mL of 3.3 % meta-phosphoric acid (MPA) in an ultrasonic bath (BRANSON 2800) at 0 °C for 10 min in darkness. Samples were centrifuged at 21.100 RCF (Sorvall Legend Micro 21R, Thermo Fisher Scientific) at 4 °C for 10 min. The supernatant was filtered through a 0.45 µm cellulose filter into an amber HPLC vial for analysis of ascorbic acid. For total AsA analysis 100 µl of the filtered extract was transferred to another HPLC vial and 50 µl of 5 mM dithiothreitol in 400 mM Tris base was added. The vials were kept in the darkness at room temperature to convert DHA to AsA. After 15 minutes the reaction was stopped by adding 50 µl 8.5 % o-phosphoric acid. AsA was quantified using a HPLC consisting of a GS50 pump (Dionex), a 340S UV-VIS detector (Dionex) and a MIDAS autosampler (Spark Holland) equipped with a ProntoSIL 120-3 C18 AQ, 250x3mm column (Knauer). The column was eluted with 400 uL L^{-1} H_3PO_4 + 2.5 mL L^{-1} MeOH + 0.1 mM EDTA in H_2O followed by a wash step with 30 % acetonitrile in H_2O at a flow rate of 0.35 mL min^{-1} at 35 °C. AsA was detected at 243 nm. Chromeleon 6.4 (Thermo Fisher Scientific) was used for data analysis. The system was calibrated with standard AsA solution prepared in 3.3 % MPA. The TAsA amount was calculated as the sum of the AsA directly measured and the AsA measured following conversion from DHA. Data was expressed on the base of dry weight as mg g^{-1} DW.

2.2.7 Carbohydrates, total soluble sugars and starch

Carbohydrates were extracted from 300 mg frozen ground basil tissue with 5 mL of 85 % ethanol for 20 min at 80 °C in a shaking water bath. Subsequently the extracts were centrifuged for 5 min at 8500 RCF (Universal 320R, Hettich). One millilitre of supernatant was put into a 2 mL reaction tube and dried using a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific) at 50 °C and 5.1 mbar for

120 min. The pellet with remaining supernatant was stored for starch determination at -20 °C.
The dried samples were re-suspended in 2 mL of 0.01 N hydrochloric acid followed 10 min in an ultrasonic water bath (Branson 2800). The samples were centrifuged for 5 min at 21.100 RCF (Sorvall Legend Micro 21R, Thermo Fisher Scientific).
To remove amino acids and other amino compounds from the sample solution these compounds were trapped on a SPE column (UCT CLEAN-UP BCX columns, 100mg/1ml), eluted with 0.01 N hydrochloric acid.
After 10 times dilution of the samples, glucose, fructose and sucrose were quantified using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD; Dionex ICS5000, Thermo Fisher Scientific), equipped with a CarboPac PA1 column (250x2 mm) eluted with 100 mM NaOH at a flow rate of 0.25 mL min^{-1} at 25 °C. Chromeleon 7.1 (Thermo Fisher Scientific) was used for data analysis.
The stored pellet was used for starch determination. After washing three times with 80 % ethanol the pellet was dried for 20 min in a vacuum centrifuge at 50 °C and 5.1 mbar. The dried pellet was resuspended in 2 mL 1 g L^{-1} thermostable alpha-amylase (SERVA Electrophoresis GmbH) in Milli-Q water and incubated for 30 min at 90 °C. Then 1 mL of 0.5 g L^{-1} amyloglucosidase (Sigma 10115) in 50 mM citrate buffer (pH 4.6) was added and samples were incubated at 60 °C for 15 min. After centrifuging at 21.100 RCF for 5 minutes and 20-50 times dilution with Milli-Q water glucose was quantified using HPEAC-PAD as described above. Data was expressed on the base of dry weight as mg g^{-1} DW.

2.2.8 Hydrogen peroxide

For determination of hydrogen peroxide (H_2O_2) 0.1 g of frozen ground basil tissue was weighed in a 10 mL tube. To the tissue 0.4 mL of 0.1 % TCA, 0.4 mL potassium phosphate buffer (pH 7.6) and 0.8 mL of potassium iodide were added. The samples were thoroughly shaken and incubated for 10 min at 4 °C and centrifuged for 15 min at 15000 G. Samples were transferred to UV-cuvettes and the absorbance was measured at a wavelength of λ=350 nm against the blank. A calibration curve was prepared with H_2O_2 solutions with concentrations from 10 to 400 µmol L^{-1}. Samples were measured on a spectrophotometer (Genesys 50, Thermo Fisher Scientific). Each sample was prepared in triplicate for technical replicates. Data was expressed on the base of dry weight as mg g^{-1} DW.

2.2.9 Statistical and set up and analysis

The experiment was carried out in a complete randomized block design. Each light treatment was done in a separate compartment containing plants of either cv. Emily or Dolly. The border plants were not used in the analysis. The complete experiment was carried out 3 times (3 blocks). At harvest, 4 plants of each treatment were sampled for chemical analyses. The remaining plants were prepared for postharvest storage. The leaves from 2 plants, were packed in one plastic box (as described above) and the boxes were stored at 4 and 12 °C. At each postharvest sampling point, 2 boxes (leaves from 4 plants) per cultivar and light treatment were removed from the storage for visual observation and chemical analyses (4 replicate plants). An average value of each block was used as one replicate for statistical analysis. For the chemical analysis the leaves from 4 plants were analyzed as a pooled sample.

All data was analyzed with Genstat (VSN International, 19th Edition). For all parameters the assumptions of homogeneity and normality of the residuals were tested with Bartlett's test and Shapiro-Wilk test respectively. If data did not follow the assumption it was transformed with the natural logarithm. Subsequently data was analyzed by a one-way ANOVA for each time point and individual storage temperature. The statistical tests were all conducted at a probability level of α=0.05 with the posthoc test Fishers protected LSD. Furthermore, it was tested with the ANOVA if a polynomial model could explain the effect of the EOP treatment on the tested variates at harvest. Significance of the linear or quadratic component were used as proof of treatment having a significant effect (and additionally if this effect was linear or quadratic). Based on the result of the ANOVA a linear or quadratic trendline was added in Excel (Excel, Microsoft Pro Plus 2019). The correlation matrix of metabolites at harvest was created in in R-4.0.2 (http://www.R-project.org/) using the packages corrplot.

2.3 Results

2.3.1 Levels of metabolites at harvest

The levels of soluble sugars (glucose, fructose and sucrose) at harvest in cvs Emily and Dolly (Fig. 1A) increased linearly with increasing EOP light intensity. Starch increased quadratically with light intensity for cv. Dolly whereas it increased linearly for cv. Emily (Fig. 1B). The level of starch was 1 to 20 times higher than the level of sugars. In cv. Dolly starch levels at harvest were about two-fold higher than in cv. Emily at the corresponding light intensities. There were no differences between the two cultivars in the levels of total soluble sugars at harvest regardless of light intensity. In both cultivars rosmarinic acid linearly increased with increasing EOP light intensity (Fig. 1C). With respect to chicoric acid (Fig. 1D) cv. Emily did not respond to increased light intensity, whereas the chicoric acid level in cv. Dolly dropped after 300 µmol m^{-2} s^{-1}. For both cultivars total ascorbic acid (Fig. 1E) increased in response to increasing EOP light intensities up to 300 µmol m^{-2} s^{-1} with no further increase at 600 µmol m^{-2} s^{-1} i.e. indicating a saturation response. H_2O_2 (Fig. 1F) decreased with increasing light intensity for cv. Emily whereas for cv. Dolly an optimum response was found; with a low content of H_2O_2 at 50 and 600 µmol m^{-2} s^{-1}.

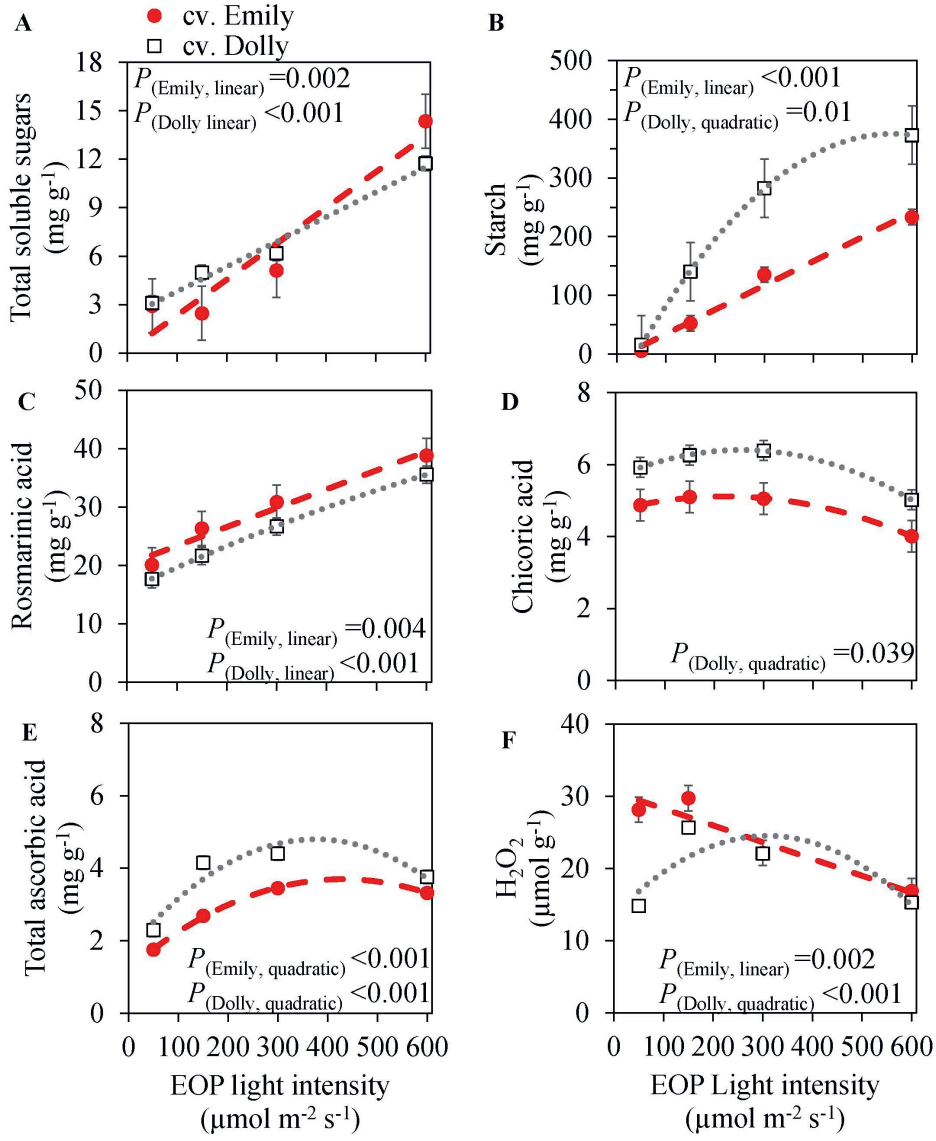

Figure 1. Contents of total soluble sugar (sum of glucose, fructose and sucrose) (A), starch (B), rosmarinic acid (C), chicoric acid (D), total ascorbic acid (E) hydrogen peroxide (F) expressed per g DW in leaves of cvs Emily (closed symbols) and Dolly (open symbols) at harvest in response to different light intensities applied as 5 days End-Of-Production (EOP) treatment. Data are means of 3 blocks (n=3) with 4 replicate plants per block. Error bars represent standard errors of means when lager than symbols. For significant linear or quadratic effects of light intensity, trendlines and the respective *p*-values (α=0.05) are depicted.

2.3.2 Changes in metabolite levels during storage at 4 and 12 °C

During the postharvest storage at 4 and 12 °C in darkness the two cultivars showed a similar response of metabolites (total sugars, starch, rosmarinic, chicoric and ascorbic acid). The changes in metabolites and chilling injury parameters for cv. Emily are shown in figure 2 to 4 whereas cv. Dolly can be found in supplementary figure 1 to 3.

During postharvest storage at 4 and 12 °C total soluble sugars increased over time (Fig. 2A, B) in cv. Emily. The increase in soluble sugars was more pronounced at 12 °C than at 4 °C. The increase in soluble sugars were most pronounced in leaves originating from plants that received high light intensities. The increase in sugars reflected the changes in starch levels. Starch was broken down during postharvest storage and this breakdown was more pronounced at 12 than at 4°C (Fig. 2C, D). At both storage temperatures, more starch was lost than the gain in sugar levels, indicating that part of the sugars was respired. In general, starch breakdown was more pronounced and sugar levels reached higher levels in cv. Dolly (Fig. S1C, D) than in cv. Emily (Fig. 2C, D).

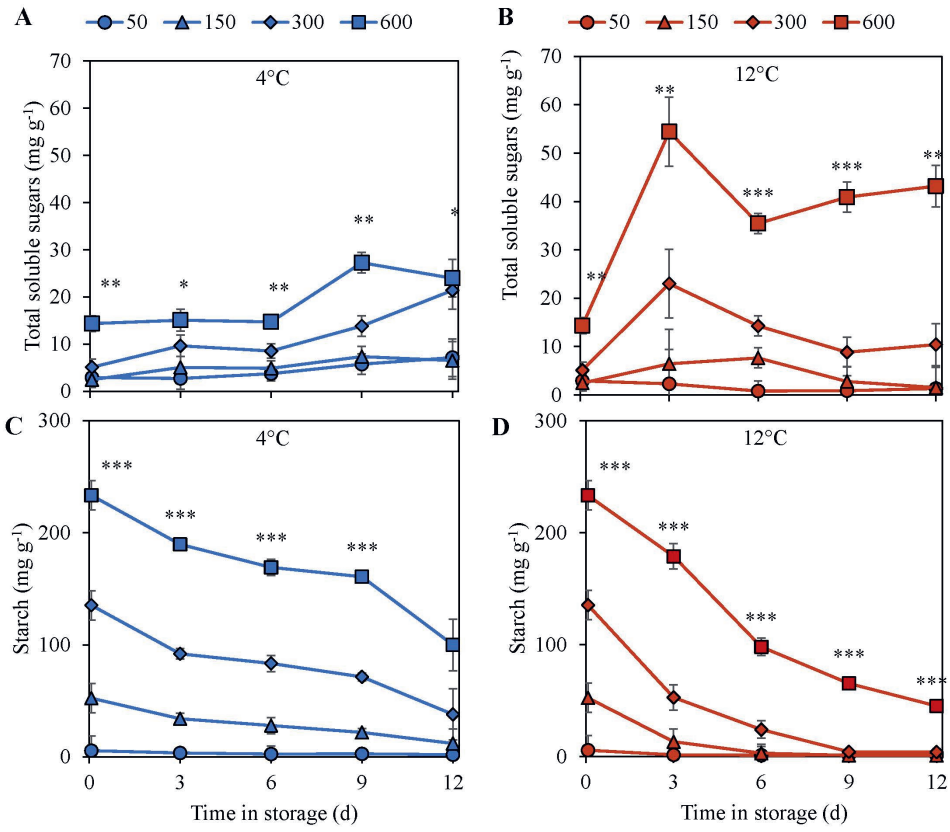

Figure 2. Content of total soluble sugar (sum of glucose, fructose and sucrose) and starch expressed per g DW in leaves of cv. Emily in response to different light intensities applied as 5 days End-Of-Production (EOP) treatment and stored in darkness at 4 °C and 12 °C for 12 days. Total soluble sugars at 4 °C and12 °C (A, B), starch at 4 °C and 12 °C (C, D). Data are means of 3 blocks (n=3) with 4 replicate plants per block. Error bars represent standard errors of means, when lager than symbols. Significance differences between EOP treatment per time point (α=0.05) with *p*-values; *p<0.05* *, *p<0.01* **, *p<0.001* *** are depicted. Pairwise comparison for each cultivar of light intensity in different temperatures and days can be found in Table S2.

Rosmarinic acid decreased over time at 4 °C whereas it showed a slight increase at 12 °C storage till day 3 after which the level was stable (Fig. 3A, B). The pattern for chicoric acid during postharvest storage was similar to that of rosmarinic acid, with a decrease at 4 °C storage (Fig. 3C). Total ascorbic acid strongly decreased over time in 4 °C (Fig. 3E). In 12 °C the decrease was less severe. Besides the effect of the storage temperature, the starting levels of the metabolites at harvest strongly influenced the postharvest pattern; a higher level at harvest a higher level at any time during the postharvest storage.

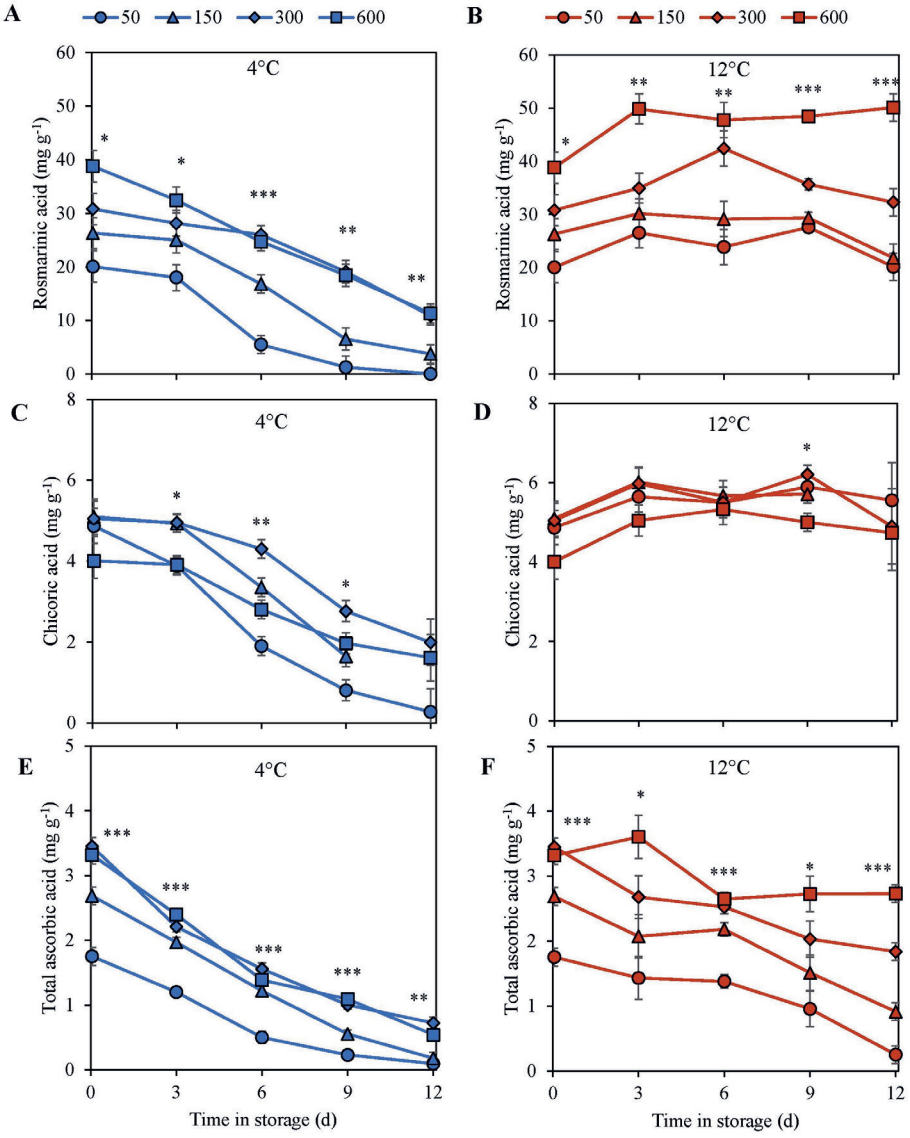

Figure 3. Content of rosmarinic acid, chicoric acid and ascorbic acid expressed per g DW in leaves of cv. Emily in response to different light intensities applied as 5 days End-Of-Production (EOP) treatment and stored in darkness at 4 °C and 12 °C for 12 days and stored in darkness at 4 °C and 12 °C for 12 days. Rosmarinic acid at 4 °C and 12 °C (A, B), chicoric acid at 4 °C and 12 °C (C, D), total ascorbic acid at 4 °C and 12 °C (E, F). Data are means of 3 blocks (n=3) with 4 replicate plants per block. Error bars represent standard errors of means, when lager than symbols. Significance differences between EOP treatment per time point (α=0.05) with *p*-values; *p<0.05 *, p<0.01 **, p<0.001* *** are depicted. Pairwise comparison for each cultivar of light intensity in different temperatures and days can be found in Table S3.

2.3.3 Chilling injury response to EOP light treatments

CI during postharvest storage was measured with chlorophyll fluorescence imaging (Fig. 4A, B). Both cvs. showed similar patterns of F_v/F_m. In leaves stored at 12 °C, F_v/F_m showed no change during most of the experimental period, irrespective the EOP light intensity. At day 12, leaves from the 50 µmol m^{-2} s^{-1} light treatment showed pronounced senescence which resulted in a lower F_v/F_m. In leaves stored at 4 °C, F_v/F_m showed a sharp decrease over time, reaching values around 0.4 after 9 days of storage. In general, there were no clear effects of the pre-harvest light intensity on the decrease of F_v/F_m. The only exception was in cv. Emily, where the 50 µmol m^{-2} s^{-1} pre-harvest light treatment performed worse than the other light treatments.

In both cultivars, OVQ of leaves stored at 12 °C was lowest for the EOP treatment with lowest light intensity (50 µmol m^{-2} s^{-1}) (Fig. 4D, Fig. S3D). In 4 °C, the 50 µmol m^{-2} s^{-1} light treatment for cv. Emily had lower scores than the other light treatments whereas for cv. Dolly OVQ of leaves from the 600 µmol m^{-2} s^{-1} light treatment first fell below the consumer acceptance limit compared to other treatments (Fig. 4C, Fig. S3C). This was due to rapid development of dark spots on the leaves.

At 4 °C, H_2O_2 decreased over time (Fig. 4E); in cv. Emily and to some extent in cv. Dolly (Fig. S3E) the decrease was more pronounced in leaves derived from plants grown at lower light intensities. At 12 °C H_2O_2 maintained a constant level in both cvs (Fig. 4F, Fig. S3F). For cv. Emily the lowest H_2O_2 content was found in leaves from the 50 µmol m^{-2} s^{-1}.

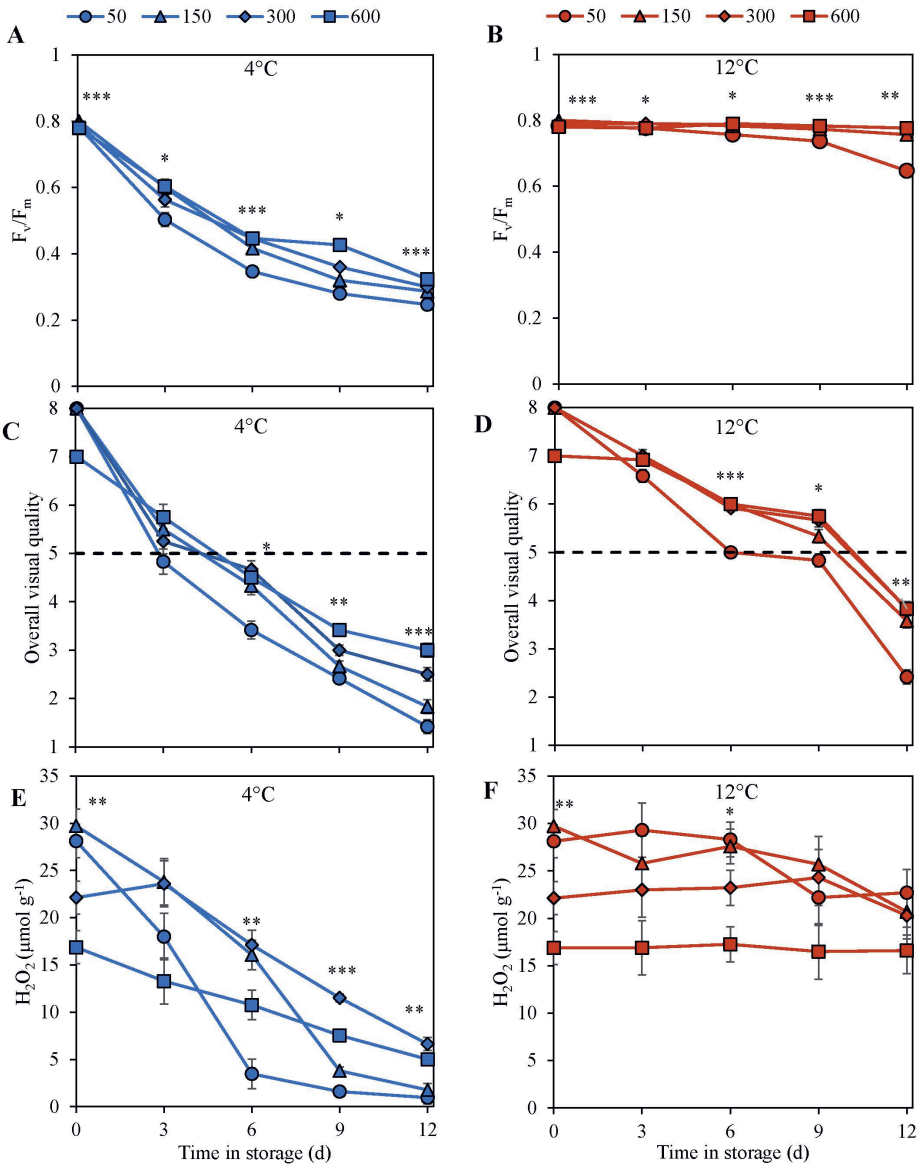

Figure 4. Maximum quantum yield of PSII of dark-adapted leaves (F_v/F_m) in leaves of cv. Emily in response to different light intensities applied as 5 days End-Of-Production (EOP) treatment and stored in darkness at 4 °C and 12 °C for 12 days (A, B), overall visual quality during storage in darkness at 4 °C and 12 °C (C, D) and content of H_2O_2 during storage in darkness at 4 °C and 12 °C expressed per g DW in leaves (E, F). Data are means of 3 blocks (n=3) each with 4 replicate plants. Error bars represent standard errors of means, when lager than symbols. Significance differences between EOP treatment per time point (α=0.05) with *p*-values; *p<0.05* *, *p<0.01* **, *p<0.001* *** are depicted. Pairwise comparison for each cultivar of light intensity in different temperatures and days can be found in Table S4.

The shelf life at 4 °C, as derived from the OVQ curves (OVQ score until 5), did not show a positive correlation with metabolite content in neither cv. Emily or cv. Dolly (Fig 5A, B). Shelf life was negatively correlated with chicoric acid in cv. Emily and with total soluble sugars and rosmarinic acid in cv. Dolly. The shelf life at 12 °C, was positively correlated with starch and total ascorbic acid in cv. Emily and cv. Dolly.

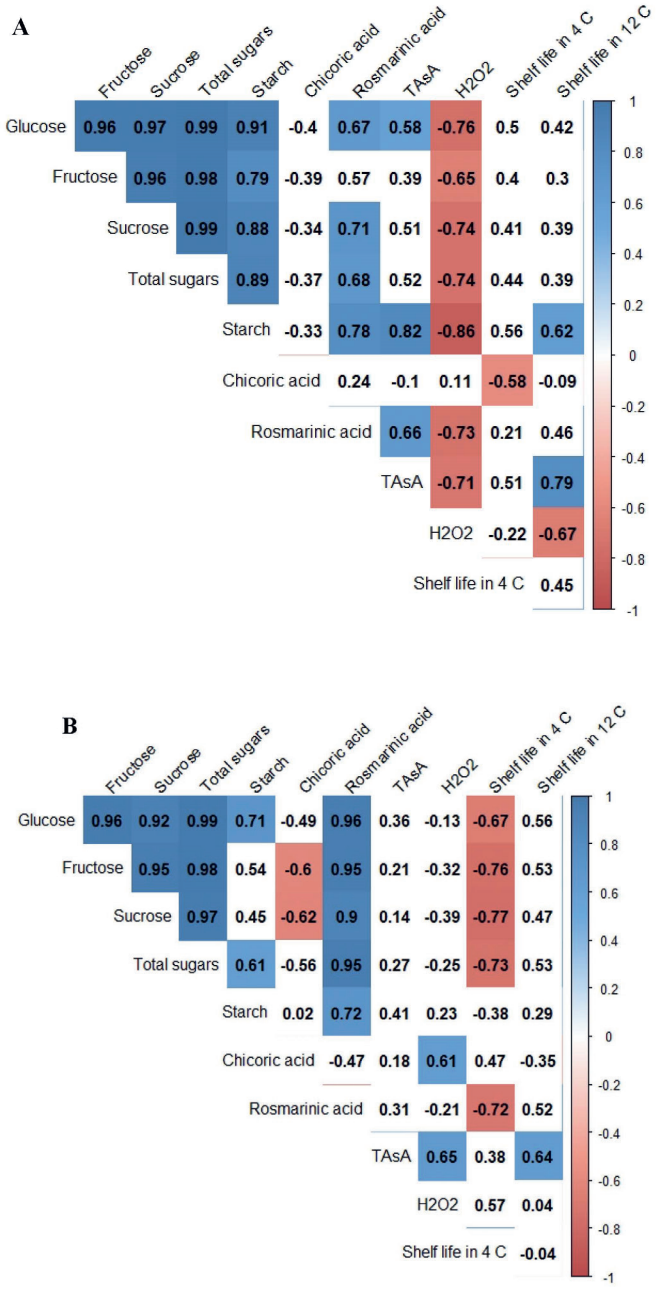

Figure 5. The correlation matrix of metabolites at harvest in response to different light intensities applied as 5 days End-Of-Production (EOP) and shelf life during storage in darkness at 4 °C and 12 °C for cv. Emily (A) and for cv. Dolly (B). Colored squares indicate a significant correlation coefficient (α=0.05) and either a positive (blue) or a negative (red) correlation between two variables.

Discussion

2.4.1 Higher light intensity at End-Of-Production increases the content of primary and secondary metabolites at harvest

High light can increase photosynthesis which in turn may increase the content of carbohydrates such as soluble sugars and starch. Here we showed that a short period of increased light intensity applied as five day End-Of-Production treatments indeed increased the total soluble sugar content and starch (Fig. 1A, B) in basil at harvest. In addition, high light increased the antioxidant content. Such an improvement in nutritional status is in line with results from Woltering and Witkowska (2016), Zhou *et al.,* (2012), and Min *et al.* (2021) in lettuce who found that an increase in EOP light levels improved nutritional quality (i.e. soluble sugars, total ascorbic acid and enzymatic antioxidants). Arnold *et al.* (2004) found a positive correlation between carbohydrate content and the phenolic compounds in hybrid poplar leaves. Similarly, with the increase in carbohydrates with increasing EOP light intensity we found a concomitant increase in rosmarinic acid (Fig.1C, Fig. 5 A, B) whereas chicoric acid was not affected (Fig. 1D). Rosmarinic acid and chicoric acid are the two most abundant phenolic acids in basil (Lee and Scagel, 2009) and they are both synthesized from the phenylpropanoid pathway (Petersen *et al.*, 2009; Lee and Scagel, 2013). However, we found that responses of these two compounds to light intensity differ. Light spectrum has been reported to have an effect on rosmarinic acid biosynthesis (Taulavuori *et al.*, 2016), whereas no reports are available regarding the effect of light intensity. In red lettuce, chicoric acid content was not affected by light intensities ranging from 225 to 410 µmol m^{-2} s^{-1} applied for two weeks (Becker *et al.*, 2013)

Kwee and Niemeyer (2011) found that the ratio of rosmarinic acid and chicoric acid is cultivar-dependent in basil. The exact enzymes involved in chicoric acid biosynthesis have not yet been determined (Lee and Scagel, 2013). Our results indicate a possible competition between the rosmarinic and chicoric acid production where rosmarinic acid gained most of the available carbon, indicating that their ratio can change with changing growth conditions.

In our experiment light intensity up to 300 µmol m^{-2} s^{-1} increased the content of total ascorbic acid (TAsA) at harvest (Fig. 1E) which corresponds to several studies determining that light is important for accumulation of ascorbic acid (Bartoli *et al.*, 2006; Min *et al.*, 2021). The effect of light intensity on TAsA accumulation might be due to the higher availability of substrate (total soluble sugars) for biosynthesis of TAsA, or mediated by the photosynthesis associated plastoquinone redox status

(Ntagkas *et al.*, 2018). Furthermore, increased light intensity may also induce increased ROS production and this may indirectly induce the production of antioxidants such as TAsA or phenolic compounds.

2.4.2 Shelf life is not extended by the increased levels of primary and secondary metabolites

During postharvest storage starch was converted into sugars presumably to provide substrate for respiration. The breakdown of starch occurred faster at 12 °C than at 4 °C, resulting in a higher soluble sugar content at 12 °C (Fig. 2C, D). Similar to our results, Costa *et al.* (2013) found that starch degraded in basil during six days of storage in darkness at 20 °C leading to an increase in soluble sugars. Generally, the metabolic rate is lower when plants are stored in the cold (Fratianni *et al.*, 2017) which in our case led to a lower starch conversion for leaves stored at 4 °C than at 12 °C. The higher content of sugars and starch at harvest due to the EOP light treatments resulted in higher levels during postharvest storage in both storage temperatures (Fig. 2).

During storage, antioxidants (rosmarinic acid, chicoric acid and total ascorbic acid) (Fig. 3, Fig. S2) steadily decreased over time, in both cvs Emily and Dolly. However, the relative differences in level of antioxidants at harvest between EOP light treatments remained (i.e. higher antioxidant levels at higher intensity of EOP light). The rapid decrease of antioxidants at 4 °C may be related to a high ROS scavenging activity (Qiao *et al.*, 2005; Hasanuzzaman *et al.*, 2020). Enzymatic antioxidants such as superoxide dismutase (SOD) and ascorbate oxidase (AO) have also been found to increase with high light up to 300 µmol m^{-2} s^{-1} (Ali *et al.*, 2005).

Although high light increases the content of desired antioxidants, it may also cause some adverse effects on plant fitness; such as increasing the content of lipoxygenase (LOX), malondialdehyde (MDA) and ROS signifying damage to cell membranes (Ali *et al.*, 2005). A high activity of LOX has been suggested to cause an increase in chilling susceptibility of basil (Wongsheree *et al.*, 2009) which also resulted in lower F_v/F_m values (Pongprasert and Srilaong, 2007). Furthermore, high light may increase the ROS content, such as H_2O_2 (Foyer and Noctor, 2003). We did not find an increase in H_2O_2 with higher EOP light intensity (Fig 1.F) or during exposure to low temperature (4 °C) (Fig. 4E, S3E). The high light intensity (600 µmol m^{-2} s^{-1}) was anticipated to have the highest H_2O_2 content at harvest. However, the EOP light treatment was applied for five days and during that time antioxidants could have come into play. In contrast to our expectations, and the general view that chilling

symptoms are accompanied by excess generation of ROS (Das and Roychoudhury, 2014), H_2O_2 markedly decreased during 4 °C storage.

A decrease in H_2O_2 during low temperature storage may indicate that chilling tolerance was improved. However, in our case the lowest F_v/F_m values and overall visual quality at 4 °C correlated with the lowest H_2O_2 values for the low light treatment (50 µmol m^{-2} s^{-1}). Thus, other species of ROS such as super oxide (O_2^-) (Asada, 1999) may play a dominant role in causing extensive membrane damage, which resulted in low F_v/F_m and overall visual quality values (Fig. 4A, S3A) in basil.

The overall visual quality and shelf life during storage at 4 °C were reduced compared to 12 °C due to the development of dark spots on the leaves, a typical symptom of chilling injury in basil (Fratianni *et al.*, 2017). The black spots might be formed by oxidation of phenolic compounds via peroxidases (PODs) or polyphenol oxidase (PPO) (Wongsheree *et al.*, 2009). Thus, a high content of phenolic compounds with increasing light intensity such as rosmarinic acid could potentially result in more pronounced black spots during chilling. Increasing the phenolic content to a certain extent may be optimal for extending shelf life in basil. Although both the carbohydrate and antioxidant levels were greatly increased by high light before harvest, the shelf life at low temperature storage (4 °C) was not positively correlated with these metabolites (Fig. 5 A, B). At 12 °C, the only metabolite which showed a consistent (positive) relation to shelf life was TAsA. This is in line with findings in lettuce where shelf life in plants from different lighting regimes was positively related to TAsA contents (Min *et al.*, 2021). Although chilling symptoms were not reduced by EOP light treatments, our results showed that a short period of high light is an effective way to improve the nutritional value (carbohydrates, TAsA, antioxidants) of basil.

2.4 Conclusion

In line with our hypothesis increased End-Of-Production (EOP) light intensity applied 5 days before harvest can increase the content of total soluble sugars, starch, rosmarinic acid, total ascorbic acid at harvest in basil. The higher content of metabolites at harvest is maintained during the postharvest storage at both 4 and 12 °C. The application of EOP light can be beneficial to improve the nutritional value of basil. The higher content of non-enzymatic antioxidants such as rosmarinic acid and total ascorbic acid did not increase chilling tolerance in basil. During cold storage the levels of antioxidants rapidly decreased, suggesting an interaction with ROS. However, no relation between the levels of antioxidants and H_2O_2 were observed. An improved nutritional value (carbohydrates, TAsA, antioxidants) at harvest was not sufficient to alleviate chilling injury in basil. This rejects our hypothesis that increased antioxidants improves chilling tolerance in fresh basil leaves.

Supplementary Material

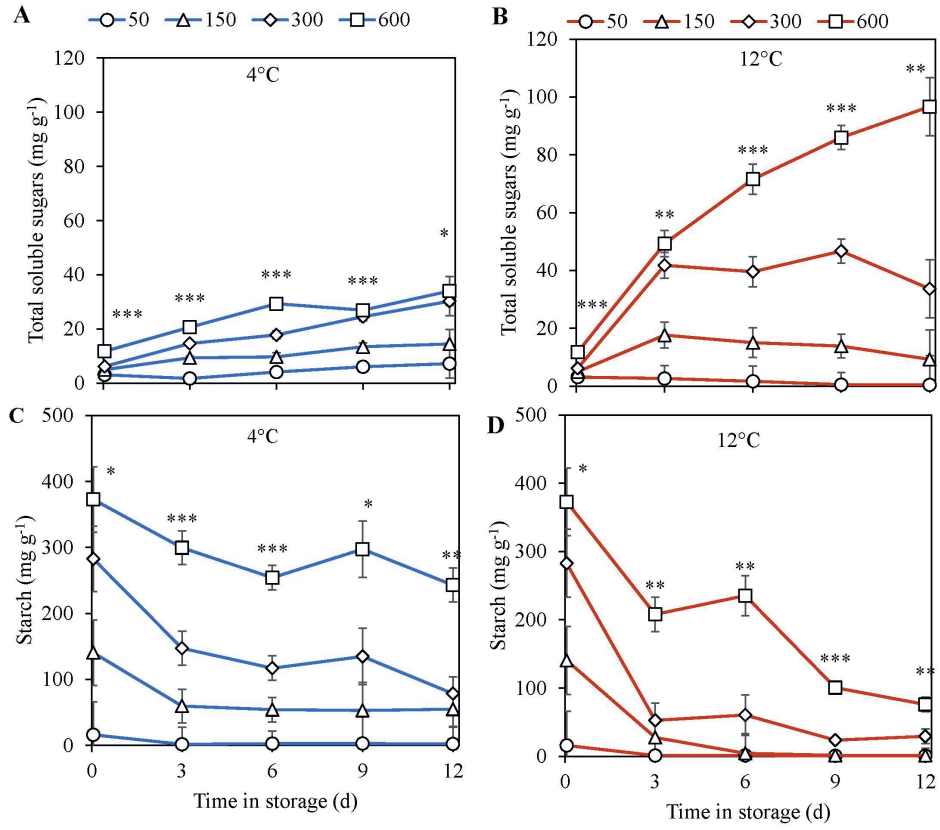

Figure S1. Content of total soluble sugar (sum of glucose, fructose and sucrose) and starch expressed per g DW in leaves of cv. Dolly in response to different light intensities applied as 5 days End-Of-Production (EOP) treatment and stored in darkness at 4 °C and 12 °C for 12 days. Total soluble sugars at 4 °C and 12 °C (A, B) , starch at 4 °C and 12 °C (C,D). Data are means of 3 blocks (n=3) with 4 replicate plants per block. Error bars represent standard errors of means, when lager than symbols. Significance differences between EOP treatment per time point (α=0.05) with *p*-values; *p<0.05* *, *p<0.01* **, *p<0.001* *** are depicted. Pairwise comparison for each cultivar of light intensity in different temperatures and days can be found in Table S2.

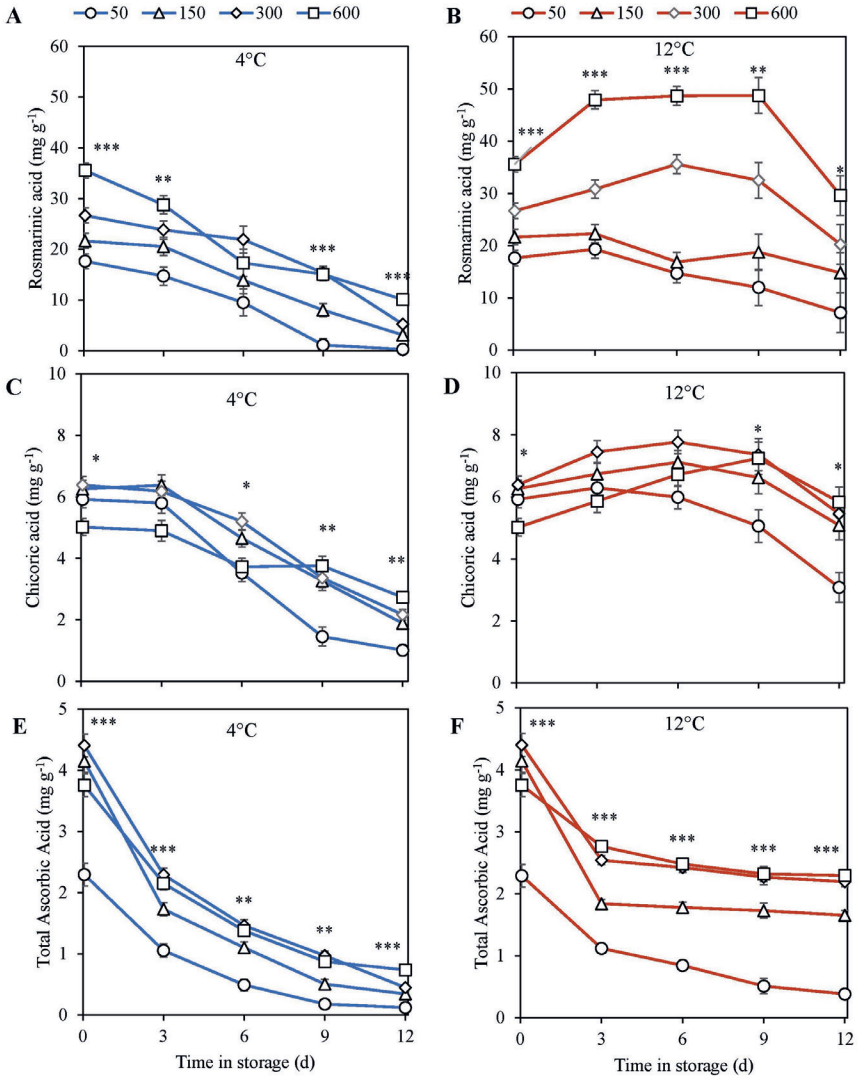

Figure S2. Content of rosmarinic acid, chicoric acid and ascorbic acid expressed per g DW in leaves of cv. Dolly in response to different light intensities applied as 5 days End-Of-Production (EOP) treatment and stored in darkness at 4 °C and 12 °C for 12 days and stored in darkness at 4 °C and 12 °C for 12 days. Rosmarinic acid at 4 °C and 12 °C (A, B), chicoric acid at 4 °C and 12°C (C, D), total ascorbic acid at 4 °C and 12 °C (E, F). Data are means of 3 blocks (n=3) with 4 replicate plants per block. Error bars represent standard errors of means, when lager than symbols. Significance differences between EOP treatment per time point (α=0.05) with *p*-values; *p<0.05 *, p<0.01 **, p<0.001 **** are depicted. Pairwise comparison for each cultivar of light intensity in different temperatures and days can be found in Table S3.

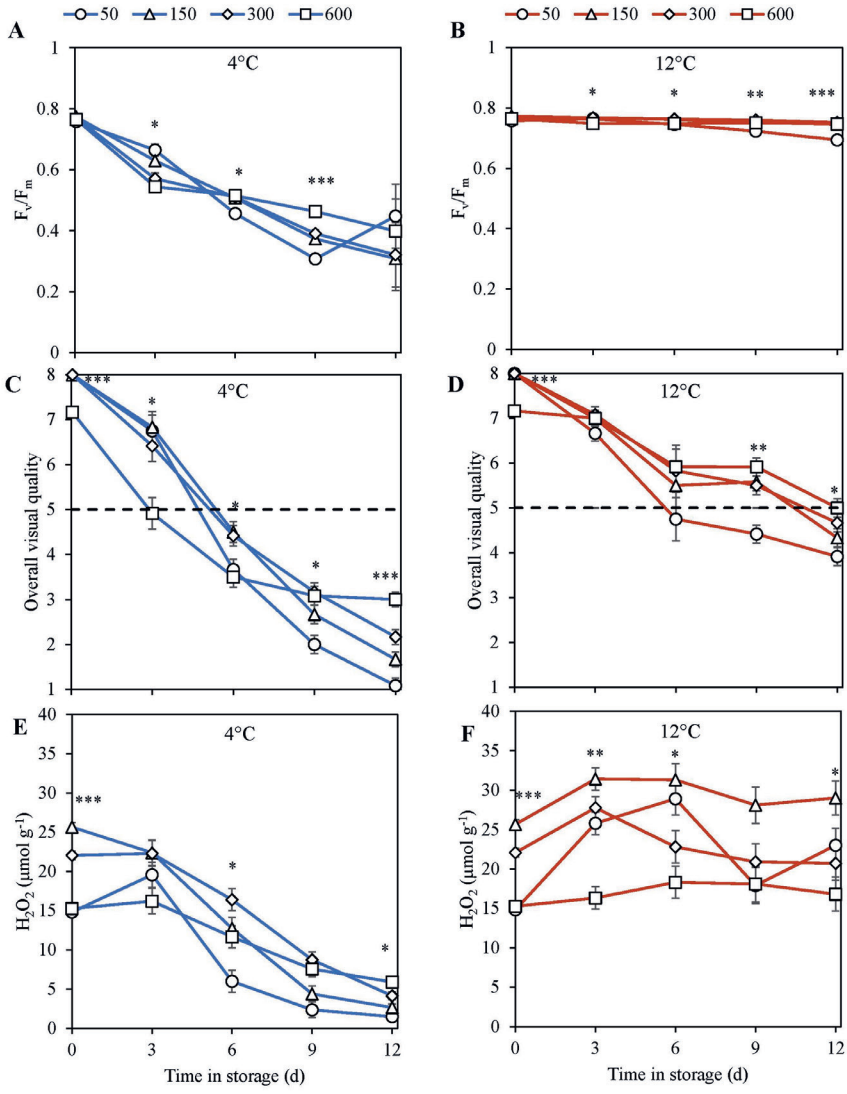

Figure S3. Maximum quantum yield of PSII of dark-adapted leaves (F_v/F_m) in leaves of cv. Dolly in response to different light intensities applied as 5 days End-Of-Production (EOP) treatment and stored in darkness at 4 °C and 12 °C for 12 days (A, B), overall visual quality during storage in darkness at 4 °C and 12 °C (C, D) and content of H_2O_2 during storage in darkness at 4 °C and 12 °C expressed per g DW in leaves (E, F). Data are means of 3 blocks (n=3) each with 4 replicate plants. Error bars represent standard errors of means, when lager than symbols. Significance differences between EOP treatment per time point (α=0.05) with *p*-values; *p<0.05 *, p<0.01 **, p<0.001 **** are depicted. Pairwise comparison for each cultivar of light intensity in different temperatures and days can be found in Table S4.

Table S1. Description of postharvest appearance of leaves and the corresponding overall visual quality score. Scores are ranging from 8-1, with 8 being the highest. The consumer acceptance limit is set at the score 5.

OVQ score	Description of postharvest appearance
8	Leaves are crisp, do not show dark spots + green color, initial leaf shininess, no wilting, curved leaf shape.
7	Slight loss of initial crispness, do not show dark spots + color change, slight loss of initial leaf shininess, no wilting and show slight loss of initial curved leaf shape.
6	Leaves are moderately crisp, show none/very limited dark spot presence + color change, moderate leaf shininess, no wilting, slight loss of curved leaf shape.
5	Indicates end of shelf life. Leaves are moderately crisp, show some dark spot presence + color change, moderately shiny, some/very limited wilting signs, moderately curved leaf shape.
4	Overall loss of crispness, obvious and widespread overall dark spot (< 50% total leaf area) presence + color change, overall loss of shininess, moderate wilting, overall loss of curved leaf shape.
3	Leaves are not crisp, obvious and widespread (>50% total leaf area) overall dark spot presence + color change, show no leaf shininess, moderate wilting, no curved leaf shape.
2	Leaves are not crisp, obvious and widespread (>50% total leaf area) overall dark spot presence + color change, show no leaf shininess, completely wilted, no curved leaf shape, ‘wet leaves’ (ion leakage).
1	Leaves are not crisp, obvious and widespread (>50% total leaf area) overall dark spot presence + color change, show no leaf shininess, completely wilted, no curved leaf shape, completely ‘wet leaves’ (ion leakage)

Table S2. Pairwise comparison of total soluble sugars and starch of cvs Emily and Dolly during 4 and 12 °C storage.

Cultivar	Storage temp (°C)	EOP treatment (µmol m^{-2} s^{-1})	Time in storage (days) 0	3	6	9	12
			Total soluble sugars (mg g^{-1})				
Emily	4	50	2.92 a	2.73 a	3.74 a	5.76 a	7.14 a
		150	2.46 a	5.06 a	4.89 a	7.36 ab	6.53 a
		300	5.12 a	9.66 ab	8.54 a	13.85 b	21.39 b
		600	14.36 b	15.06 b	14.72 b	27.26 c	23.97 b
	SEM		1.68	2.3	1.538	2.2	3.97
	p-value		0.008	0.035	0.009	0.002	0.039
Emily	12	50	2.92 a	2.30 a	0.81 a	0.87 a	1.34 a
		150	2.46 a	6.43 a	7.67 ab	2.77 a	1.53 a
		300	5.12 a	23.01 a	14.29 b	8.83 a	10.42 a
		600	14.36 b	54.42 b	35.46 c	40.92 b	43.2 b
	SEM		1.68	7.12	2.10	3.08	4.32
	p-value		0.008	0.007	<.001	<.001	0.001
Dolly	4	50	3.10 a	1.72 a	4.13 a	6.06 a	7.19 a
		150	4.99 b	9.37 b	9.70 b	13.43 b	14.48 ab
		300	6.18 b	14.62 c	17.80 c	24.54 c	30.26 bc
		600	11.75 c	20.64 d	29.32 d	26.93 c	34.03 c
	SEM		0.45	0.626	1.53	1.50	5.39
	p-value		<.001	<.001	<.001	<.001	0.036
Dolly	12	50	3.10 a	2.62 a	1.68 a	0.52 a	0.45 a
		150	4.99 b	17.62 a	15.04 a	13.85 a	9.26 a
		300	6.18 b	41.77 b	39.56 b	46.7 b	33.65 a
		600	11.75 c	49.26 b	71.55 c	85.96 c	96.65 b
	SEM		0.45	4.51	5.20	4.15	10.11
	p-value		<.001	0.001	<.001	<.001	0.002
					Starch (mg g^{-1})		
Emily	4	50	5.60 a	3.51 a	2.52 a	2.74 a	2.00
		150	52.40 b	34.02 b	27.98 b	21.87 b	12.00
		300	135.30 c	92.01 c	83.36 c	71.42 c	38.00
		600	233.30 d	189.52 d	169.06 d	160.67 d	100.00
	SEM		13.06	4.80	7.26	3.62	23.00
	p-value		<.001	<.001	<.001	<.001	0.083
Emily	12	50	5.60 a	1.52 a	1.17 a	1.35 a	1.16 a
		150	52.40 b	13.26 a	3.01 a	1.05 a	0.99 a
		300	135.30 c	52.79 b	24.14 a	4.31 b	3.96 a
		600	233.30 d	178.88 c	98.04 b	65.35 c	45.22 b
	SEM		13.06	11.32	7.81	0.455	3.2
	p-value		<.001	<.001	<.001	<.001	<.001
Dolly	4	50	16.10 a	1.80 a	2.80 a	2.90 a	2.21 a
		150	140.50 ab	59.60 ab	54.20 ab	53.00 a	54.89 a
		300	282.70 b	147.30 b	117.10 b	134.80 a	78.51 a
		600	372.70 c	299.50 c	254.20 c	297.40 b	243.02 b
	SEM		49.7	25.6	18.65	42.6	25.7
	p-value		0.01	<.001	<.001	0.012	0.003
Dolly	12	50	16.10 a	1.17 a	1.37 a	0.85 a	0.98 a
		150	140.50 ab	27.75 a	4.49 a	1.62 a	1.27 a
		300	282.70 b	52.77 a	60.69 a	23.79 b	29.39 a
		600	372.70 c	207.90 b	235.33 b	100.66 c	76.08 b
	SEM		49.7	25.5	29.1	2.69	10.84
	p-value		0.01	0.005	0.004	<.001	0.008

Table S3. Pairwise comparison of rosmarinic acid, chicoric acid and total ascorbic acid of cvs Emily and Dolly during 4 and 12 °C storage.

Cultivar	Storage temp (°C)	EOP treatment (µmol m^{-2} s^{-1})	Time in storage (days) 0	3	6	9	12
			Rosmarinic acid (mg g^{-1})				
Emily	4	50	20.08 a	18.03 a	5.52 a	1.28 a	0.06 a
		150	26.31 ab	25.03 ab	16.82 b	6.54 a	3.77 a
		300	30.82 bc	28.14 b	26.02 c	19.09 b	10.90 b
		600	38.8 c	32.46 b	24.73 c	18.44 b	11.33 b
	SEM		2.92	2.42	1.71	2.11	1.74
	p-value		0.02	0.028	<.001	0.002	0.009
Emily	12	50	20.08 a	18.03 a	5.52 a	1.28 a	0.06 a
		150	26.31 ab	25.03 ab	16.82 b	6.54 a	3.77 a
		300	30.82 bc	28.14 b	26.02 c	19.09 b	10.90 b
		600	38.8 c	32.46 b	24.73 c	18.44 b	11.33 b
	SEM		2.92	2.42	1.71	2.11	1.74
	p-value		0.02	0.028	<.001	0.002	0.009
Dolly	4	50	26.31 ab	25.03 ab	16.82 b	6.54 a	3.77 a
		150	30.82 bc	28.14 b	26.02 c	19.09 b	10.90 b
		300	38.8 c	32.46 b	24.73 c	18.44 b	11.33 b
		600	2.92	2.42	1.71	2.11	1.74
	SEM		0.02	0.028	<.001	0.002	0.009
	p-value		20.08 a	18.03 a	5.52 a	1.28 a	0.06 a
Dolly	12	50	30.82 bc	28.14 b	26.02 c	19.09 b	10.90 b
		150	38.8 c	32.46 b	24.73 c	18.44 b	11.33 b
		300	2.92	2.42	1.71	2.11	1.74
		600	0.02	0.028	<.001	0.002	0.009
	SEM		20.08 a	18.03 a	5.52 a	1.28 a	0.06 a
	p-value		26.31 ab	25.03 ab	16.82 b	6.54 a	3.77 a
			Chicoric acid (mg g^{-1})				
Emily	4	50	4.87	3.88 a	1.90 a	0.80 a	0.27
		150	5.1	4.93 b	3.35 b	1.64 ab	1.95
		300	5.05	4.95 b	4.30 c	2.76 c	1.99
		600	4.01	3.91 a	2.81 b	1.97 bc	1.61
	SEM		0.44	0.22	0.23	0.26	0.57
	p-value		0.34	0.02	0.00	0.01	0.22
Emily	12	50	4.87	5.65	5.5	5.90 b	5.56
		150	5.1	6.02	5.67	5.72 ab	4.19
		300	5.05	5.98	5.51	6.21 b	4.9
		600	4.01	5.05	5.33	5.00 a	4.74
	SEM		0.44	0.39	0.39	0.23	0.95
	p-value		0.34	0.35	0.94	0.05	0.79
Dolly	4	50	5.92 ab	5.80	3.53 a	1.46 a	1.01 a
		150	6.26 b	6.38	4.65 bc	3.27 b	1.89 b
		300	6.39 b	6.18	5.20 c	3.36 b	2.17 bc
		600	5.02 a	4.90	3.73 ab	3.75 b	2.73 c
	SEM		0.28	0.34	0.28	0.31	0.17
	p-value		0.047	0.077	0.017	0.008	0.003
Dolly	12	50	5.92 ab	6.29	5.99	5.06	3.08 a
		150	6.26 b	6.74	7.12	6.62	5.09 b
		300	6.39 b	7.45	7.77	7.36	5.46 b
		600	5.02 a	5.86	6.72	7.24	5.84 b
	SEM		0.28	0.37	0.38	0.52	0.48
	p-value		0.047	0.091	0.075	0.067	0.026

					Total Ascorbic acid (mg g^{-1})		
Emily	4	50	1.75 a	1.20 a	0.50 a	0.23 a	0.10 a
		150	2.69 b	1.97 b	1.22 b	0.55 b	0.18 a
		300	3.45 c	2.21 bc	1.56 c	1.00 c	0.72 b
		600	3.32 c	2.40 c	1.39 bc	1.09 c	0.54 b
	SEM		0.14	0.08	0.10	0.06	0.09
	p-value		<.001	<.001	<.001	<.001	0.009
Emily	12	50	1.75 a	1.43 a	1.38 a	0.96 a	0.25 a
		150	2.69 b	2.07 ab	2.18 b	1.51 ab	0.91 b
		300	3.45 c	2.68 bc	2.53 bc	2.03 bc	1.84 c
		600	3.32 c	3.60 c	2.65 c	2.73 c	2.73 d
Dolly	4	50	2.30 a	1.05 a	0.49 a	0.18 a	0.12 a
		150	4.15 bc	1.73 b	1.10 b	0.50 b	0.34 b
		300	4.41 c	2.29 c	1.46 c	0.97 c	0.45 b
		600	3.76 b	2.15 c	1.38 bc	0.87 c	0.74 c
	SEM		0.18	0.11	0.10	0.08	0.04
	p-value		<.001	<.001	0.001	0.001	<.001
Dolly	12	50	2.30 a	1.12 a	0.85 a	0.51 a	0.39 a
		150	4.15 bc	1.84 b	1.78 b	1.73 b	1.66 b
		300	4.41 c	2.54 c	2.43 c	2.27 c	2.20 c
		600	3.76 b	2.77 c	2.48 c	2.32 c	2.30 c
	SEM		0.18	0.07	0.08	0.12	0.08
	p-value		<.001	<.001	<.001	<.001	<.001

Table S4. Pairwise comparison of F_v/F_m, Overall Visual Quality, H_2O_2 for cvs Emily and Dolly during 4 and 12 °C storage.

Cultivar	Storage temp (°C)	EOP treatment (µmol m^{-2} s^{-1})	Time in storage (days) 0	3	6	9	12
					Fv/Fm		
Emily	4	50	0.78 a	0.50 a	0.35 a	0.28 a	0.25 a
		150	0.80 c	0.60 b	0.42 b	0.32 ab	0.29 b
		300	0.79 b	0.56 ab	0.45 b	0.36 b	0.30 b
		600	0.78 a	0.60 b	0.45 b	0.43 c	0.32 c
	SEM		0.002	0.021	0.009	0.014	0.004
	p-value		<.001	0.05	<.001	0.00	<.001
Emily	12	50	0.78 a	0.78 a	0.76 a	0.74 a	0.65 a
		150	0.80 c	0.79 b	0.78 b	0.77 b	0.76 b
		300	0.79 b	0.79 b	0.79 b	0.78 b	0.77 b
		600	0.78 a	0.78 a	0.79 b	0.78 b	0.78 b
	SEM		0.002	0.021	0.009	0.014	0.004
	p-value		<.001	0.05	<.001	0.00	<.001
Dolly	4	50	0.76	0.66 c	0.46 a	0.31 a	0.45
		150	0.77	0.63 bc	0.51 b	0.37 b	0.31
		300	0.77	0.57 ab	0.51 b	0.39 b	0.32
		600	0.77	0.54 a	0.52 b	0.46 c	0.40
	SEM		0.01	0.02	0.01	0.01	0.11
	p-value		0.32	0.02	0.02	<.001	0.77
Dolly	12	50	0.76	0.76 b	0.75 a	0.72 a	0.69 a
		150	0.77	0.77 b	0.76 b	0.75 b	0.75 b
		300	0.77	0.77 b	0.76 b	0.76 b	0.75 b
		600	0.77	0.75 a	0.75 a	0.75 b	0.75 b
	SEM		0.006	0.004	0.003	0.004	0.002
	p-value		0.32	0.04	0.02	0.00	<.001

				Overall Visual Quality			
Emily	4	50	8.00 b	4.83	3.42 a	2.42 a	1.42 a
		150	8.00 b	5.50	4.33 b	2.67 ab	1.83 a
		300	8.00 b	5.25	4.67 b	3.00 b	2.50 b
		600	7.00 a	5.75	4.50 b	3.42 c	3.00 b
	SEM		0.00	0.26	0.18	0.11	0.14
	p-value		0.05	0.18	0.01	0.00	<.001
Emily	12	50	8.00 b	6.58	5.00 a	4.83 a	2.42 a
		150	8.00 b	7.00	6.00 b	5.33 b	3.58 b
		300	8.00 b	7.00	5.92 b	5.67 b	3.83 b
		600	7.00 a	6.92	6.00 b	5.75 b	3.83 b
	SEM		0.00	0.13	0.07	0.14	0.15
	p-value		0.05	0.17	<.001	0.01	0.00
Dolly	4	50	8.00 b	6.75 b	3.67 ab	2.00 a	1.08 a
		150	8.00 b	6.83 b	4.50 c	2.67 ab	1.67 b
		300	8.00 b	6.42 b	4.42 bc	3.17 b	2.17 b
		600	7.17 a	4.92 a	3.50 a	3.08 b	3.00 c
	SEM		0.04	0.35	0.23	0.20	0.16
	p-value		<.001	0.03	0.05	0.02	<.001
Dolly	12	50	8.00 b	6.67	4.75	4.42 a	3.92 a
		150	8.00 b	7.00	5.50	5.58 b	4.33 ab
		300	8.00 b	7.08	5.83	5.50 b	4.67 b
		600	7.17 a	7.00	5.92	5.92 b	5.00 b
	SEM		0.04	0.18	0.48	0.20	0.20
	p-value		<.001	0.44	0.38	0.01	0.04
					H_2O_2		
Emily	4	50	28.12 bc	18	3.47 a	1.6 a	0.95 a
		150	29.73 c	23.8	16.06 bc	3.79 b	1.77 a
		300	22.13 b	23.6	17.12 b	11.52 c	6.63 b
		600	16.87 ab	13.3	10.77 a	7.55 d	5.01 b
	SEM		1.75	2.45	1.56	0.41	0.68
	p-value		0.007	0.064	0.003	<.001	0.003
Emily	12	50	28.12 bc	29.3	28.31 b	22.2	22.7
		150	29.73 c	25.8	27.58 b	25.7	20.7
		300	22.13 b	23	23.22 ab	24.3	20.3
		600	16.87 ab	16.9	17.26 a	16.5	16.6
	SEM		1.75	2.87	1.85	2.95	2.45
	p-value		0.007	0.097	0.018	0.24	0.43
Dolly	4	50	14.81 a	19.57	5.99 a	2.36	1.49 a
		150	25.66 c	22.39	12.73 b	4.39	2.65 ab
		300	22.06 b	22.29	16.39 b	8.73	4.14 bc
		600	15.26 a	16.22	11.65 ab	7.55	5.9 c
	SEM		0.56	1.62	1.39	0.99	0.48
	p-value		<.001	0.182	0.048	0.055	0.024
Dolly	12	50	14.81 a	25.79 b	28.90 c	17.90	23.00 ab
		150	25.66 c	31.42 c	31.30 bc	28.10	29.00 b
		300	22.06 b	27.76 bc	22.80 ab	20.90	20.70 a
		600	15.26 a	16.32 a	18.30 ab	18.10	16.80 a
	SEM		0.56	1.42	2.04	2.30	2.15
	p-value		<.001	0.001	0.015	0.06	0.036

Chapter 3

Lack of blue light regulation of antioxidants and chilling tolerance

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Abstract

Blue light (400-500 nm) is generally assumed to increase the content of antioxidants in plants independent of the species. Blue light stimulates the biosynthesis of phenolic compounds such as flavonoids and their subclass anthocyanins from the phenylpropanoid pathway. Flavonoids, anthocyanins and phenolic acids are strong reactive oxygen species (ROS) scavengers and may lessen the symptoms of abiotic stresses such as chilling. We tested the hypothesis that a high percentage of blue light induces the accumulation of antioxidants and that this effect depends on the photosynthetic photon flux density (PPFD, 400-700nm). The effect may be more pronounced at a lower PPFD. We investigated the changes in primary and secondary metabolites of basil in response to the percentage of blue light (9, 33, 65 and 100 %) applied either as a five day End-Of-Production (EOP) treatment or continuous throughout the growth cycle in the green cv. Dolly. We also studied if the response to percentage of blue (9 or 90 %) was dependent on the total PPFD (100 or 300 μmol m^{-2} s^{-1} PPFD) when applied as a five day EOP treatment in the green cv. Dolly and the purple cv. Rosie. For both green and purple basil, it was found that the percentage of blue light had little effect on the levels of antioxidants (rosmarinic acid, total ascorbic acid, total flavonoids and total anthocyanins) at harvest and no interactive effect with PPFD was found. Antioxidants generally decreased during postharvest storage; the decrease being more pronounced at 4 than at 12 °C. Chilling injury, as judged from a decrease in F_v/F_m values and from the occurrence of black necrotic areas, was not affected by the percentage of blue. Particularly in the purple cultivar chilling tolerance was increased in plants grown under higher PPFD. This may be related to the increased levels of soluble sugar and starch in leaves from high PPFD treated plants.

3.1 Introduction

Basil (*Ocimum basilicum* L.) is rich in antioxidants, in particular polyphenolic compounds from the phenylpropanoid pathway such as rosmarinic and chicoric acid (Kwee and Niemeyer, 2011). Basil also contains compounds from the flavonoid (sub) family such as quercetin, rutin and kaempferol. Mostly basil exists as green varieties but some varieties are purple due to anthocyanins which are a subgroup of flavonoids (McCance *et al.*, 2016). Compounds such as anthocyanins, flavonoids and phenolic acids have strong antioxidant capacity. Antioxidants can scavenge reactive oxygen species (ROS) and protect the plants from oxidative damage thus contributing to tolerance against abiotic stress such as chilling and drought (Ahmed *et al.*, 2014). Chilling injury occurs in basil when it is exposed temperatures below 10-12°C during growth, storage or transport resulting in development of dark necrotic spots (Lange and Cameron, 1994). During chilling a cascade of events occur: the lipid bilayer in the cell membranes can go from a flexible to a solid gel state which may result in membrane malfunction, ion leakage and excessive formation of ROS. ROS will further lead to damage of the DNA, membrane lipids and proteins thus being severely damaging to the plant (Sevillano *et al.*, 2009). Antioxidants, such as phenolic compounds can counteract ROS and ameliorate chilling tolerance (Das and Roychoudhury, 2014). Increasing the content of antioxidants such as flavonoids and anthocyanins may improve the tolerance to chilling temperatures. In addition, an increase in sugars, starch and antioxidants is beneficial for consumers as it improves the products' nutritional value. In the production phase, antioxidants can be increased through modulation of the growth environment (**Chapter 2**). Such modulation can be facilitated by light emitting diodes (LEDs) through which we can easily increase both the light intensity and change the light spectrum. LEDs are in particular used in greenhouses and in vertical farming (SharathKumar *et al.*, 2020). Light intensity and spectrum can affect the content of phenolic acids, flavonoids and anthocyanins. In particular blue light (400-500 nm) has been found to stimulate the biosynthesis of compounds from the phenylpropanoid pathway such as flavonoid and anthocyanin content in a number of crops: in fruit and leaves of strawberry (Piovene *et al.*, 2015; Zhang *et al.*, 2018), lettuce (Samuoliene *et al.*, 2013) and Arabidopsis (Chen *et al.*, 2006). Blue light has also been found to stimulate the biosynthesis of rosmarinic acid, chicoric acid, chlorogenic acid, *p*-OH-cinnamic acid derivative, 2-*O*-feruloyl tartaric acid and quercetin rhamnoside in green basil (Taulavuori *et al.*, 2013, 2016), and phenolic acids in red lettuce (Ouzounis *et al.*, 2015). In addition, blue light increased the content of vitamin C in pak choi with a photosynthetic photon flux density (PPFD 400-700 nm) up until 100 µmol m^{-2} s^{-1} after which it decreased at a higher

PPFD (Zheng *et al.*, 2018). Although blue light has been widely accepted to stimulate the biosynthesis of compounds from the flavonoid branch of the phenylpropanoid pathway it is not fully understood why compounds such as vitamin C should increase. The energy content of a blue light photon is higher than its red counterparts due to blue light having a lower wavelength than red. Thus, blue photons might result in a stress reaction.

Application of increased light intensity during the last phase of the growth as an End-Of-Production (EOP) treatment showed to be sufficient to increase the content of secondary metabolites without having adverse effects on plant morphology (Gomez and Jimenez, 2020; Min *et al.*, 2021, **Chapter 2** & **5**). In red lettuce an EOP treatment with 69 % blue increased anthocyanins but not flavonoids (Gomez and Jimenez, 2020). However, a change in spectrum as EOP treatment is yet to be studied in basil. We hypothesized that an increased percentage of blue light would increase the content of antioxidants; phenolic acids, flavonoids and anthocyanins and thereby improving chilling tolerance. Furthermore, we hypothesized that the effect of a high percentage of blue light might have an interactive effect with the photosynthetic photon flux density (PPFD 400-700 nm). Spectral effects may be less on accumulation of antioxidants under higher PPFD as the PPFD might dominate the overall plant response.

First, we investigated the changes in primary, secondary metabolites of basil in response to the percentage of blue light (400 to 500 nm) in the spectrum applied either as an EOP treatment or continuous throughout the growth cycle. Second, we studied if the light intensity interacts with the percentage of blue light applied as EOP treatment in a green and purple cultivar and further if this improves the postharvest chilling tolerance.

3.2 Materials and Methods

3.2.1 Experimental set-up

Two cultivars of basil (*Ocimum basilicum* L.), cv. Dolly (green leaves) and cv. Rosie (purple leaves which are rich in anthocyanin) (Enza Zaden, Enkhuizen, the Netherlands) were grown in a climate chamber. Plants were grown as described in **Chapter 5**. The seeds were sown as single seeds in stone wool plugs in trays of 240 plugs (Grodan Rockwool B.V., Roermond, The Netherlands). The morphologically most similar plants were transplanted to 7.5x7.5x6.5 cm stone wool blocks (Grodan Rockwool B.V., Roermond, The Netherlands) after 15 days. For the growth of the plants a vertical farming set-up was used. Each compartment had a dimension of 0. 8 x 1.3 x 1 m, (w x l x h) and a planting density of 123 plants m^{-2}. To maintain a similar PPFD at the top of the plants the two cvs were grown in different compartments. Throughout the experiments the height of the light frames was adjusted to maintain the desired PPFD. The light frames were kept 25 cm above the plants. In the climate chamber the day/night temperature was set at 25 °C, the relative humidity at 75 % and CO_2 was kept at ambient concentrations. The temperature and relative humidity deviated within ± 10 % (RH) and 1°C (T) from the set points and were logged with keytag dataloggers (KTL-508, Keytag, Leiderdorp, the Netherlands).

Plants were watered through an ebb and flow system. At all growth stages plants were kept well-watered. Plants were watered with a nutrient solution with pH 5.7, EC 1.7 dS m^{-1} and 8.5 mM NO_3^-, 3.9 mM SO_4^{2-}, 1.5mM HPO_4^{2-}, 1.5 mM NH_4^+, 5.5 mM K^+, Ca^{2+} 4.0 mM, 1.5 mM Mg^{2+}, 0.2 mM Cl^-, 30µM Fe^{3+}/Fe^{2+}, 5 µM Mn^{2+}, 5 µM Zn^{2+}, 35 µM $H_2BO_3^-$, 1 µM Cu^+/Cu^{2+}, 1 µM MoO_4^{2-} before transplanting. After transplanting the EC was 2.3 dS m^{-1} and the concentration of the nutrients raised correspondingly.

The response to the light treatments on plant growth and morphology, (i.e. plant height, leaf area, fresh and dry mass at harvest) from these experiments are described in **Chapter 5**.

3.2.2 Blue light duration (Experiment 1)

In Exp. 1 we investigated the response of cv. Dolly to different percentage of blue light applied either as a continuous treatment throughout the growth (i.e., for 25 days) or as End-Of-Production treatment during the last five days before harvest. Seedlings grew under red-white light from Light Emitting Diodes (LEDs) (Green Power LED production module, 120 cm, Philips Eindhoven, the Netherlands) with a PPFD of 150 µmol m^{-2} s^{-1}. The red-white light contained 9 % blue (B)

(400-500 nm), 19 % green (G) (500-600 nm), 70 % red (R) (600-700 nm) and 1 % far-red (FR) (700-800 nm) light. For the blue light treatments, the different percentage of blue light were made by using two types of pure blue LEDs (Green Power LED production module, 120 cm, Blue, Philips Eindhoven, the Netherlands), (Green Power LED research module, Blue, Philips Eindhoven, the Netherlands) and red-white LEDs. When the plants were transplanted, they were treated with 25 days of four different blue light treatments with a total PPFD of 300 µmol m^{-2} s^{-1} (Table 1). In addition, in three other treatments plants were grown under red-white light (PPFD, 300 µmol m^{-2} s^{-1}) for 20 days after which they were treated with different blue light treatments for five days (Table 1). For all light treatments the spectral intensity was measured with a spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, 110 Netherlands). Throughout the experiment the day length was 16 hours. This whole experiment with similar set-up was conducted 2 times.

3.2.3 Blue light and the interactive effect with PPFD (Experiment 2)

In Exp. 2 we investigated the response of cultivars Rosie (purple) and Dolly (green) to EOP treatments with increased percentage of blue light and the interaction with PPFD during the last five days before harvest. Seedlings grew under 200 µmol m^{-2} s^{-1} red-white LED light. Plants were transplanted and continued to grow for another 15 days under 200 µmol m^{-2} s^{-1} red-white light. The last five days before harvest plants were treated with End-Of-Production treatments with either a low (100 µmol m^{-2} s^{-1}) or high (300 µmol m^{-2} s^{-1}) PPFD in combination with a low (9 %) and high (90 %) percentage of blue light (Table 1). For the blue light treatments, the different percentage of blue light were made by using pure blue (Green Power LED production module, 120 cm, Blue, Philips Eindhoven, the Netherlands), (Green Power LED research module, Blue, Philips Eindhoven, the Netherlands) and red-white LEDs. For all light treatments the spectral intensity was measured with a spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, 110 Netherlands). Throughout the growth the day length was 18 hours. This whole experiment with similar set-up was conducted 3 times for cv. Dolly and 4 times for cv. Rosie.

Table 1. PPFD (400-700 nm) and spectra of the treatments for Exp 1. and Exp. 2. Percentages of the spectra; blue light (400-500 nm), green light (500-600 nm), red light (600-700 nm), far-red light (700-800 nm) are expressed as percentages of the total photon flux density (400-800 nm).

Treatments	Treatment duration (days)	PPFD (µmol m^{-2} s^{-1})	Blue light (%)	Green light (%)	Red light (%)	Far-red light (%)
Exp. 1						
9 %	25 and 5	300	9	19	70	1
33 %	25 and 5	300	33	14	51	1
65 %	25 and 5	300	65	7	26	0
100 %	25 and 5	300	100	0	0	0
Exp. 2						
Low PPFD, low blue	5	100	9	19	70	1
Low PPFD, high blue	5	100	90	2	8	0
High PPFD, low blue	5	300	9	19	70	1
High PPFD, high blue	5	300	90	2	8	0

3.2.4 Postharvest storage and sampling

The plants were harvested 40 (Exp. 1) or 35 (Exp. 2) days after sowing. The border plants were excluded for the sampling. The postharvest storage was done as described in **Chapter 2**. For postharvest storage and sampling three leaf pairs were taken per plant. The oldest and youngest underdeveloped leaves were excluded. The leaves were stored in plastic boxes (16 x 11 x 6 cm), which combined leaves from two plants per box. Wetted filter paper was added in the bottom of the boxes for to keep the humidity high. For the leaves to avoid direct contact with the wet filter paper, a small piece of plastic was added on top of it. The leaves from the two plants were separated by a piece of plastic. To avoid a built up of CO_2 or ethylene nine holes were made in the lids with a 1 mm syringe needle. During storage the boxes were randomized in a cold cabinet in darkness at 4 or 12 °C. In the boxes the temperature and relative humidity deviated within ±2 % (RH) and 0.3 °C (T) from the set points and were recorded with keytag dataloggers (KTL-508, Keytag, Leiderdorp, the Netherlands).

In Exp. 1 measurements and sampling were done on day 0 (at harvest) and 5,10 and 15 days after harvest for EOP treated plants. In Exp. 2 measurements and sampling were done on day 0 (day of harvest) and 3, 6 for cv. Dolly and for cv. Rosie the sampling continued on day 9 and 12. Two postharvest storage boxes (i.e. each containing leaves from two individual plants per block per light treatment) were sampled on each sampling day.

During sampling an overall visual quality score was given to the leaves of each sampled plant to determine the chilling injury level. In Exp. 2, in addition to the scoring, maximum quantum yield of PSII (F_v/F_m) was measured. Following the scoring and measuring of F_v/F_m the leaves were frozen in liquid nitrogen and ground with an IKA-A 11 basic analytical mill (im-lab, Boutersem, Belgium). Samples were stored at -80 °C for further analysis of metabolite content. Each sample consisted of leaves derived from 4 plants.

3.2.5 Carbohydrates

Carbohydrates were measured according as described in **Chapter 2**. Briefly, 300 mg of frozen ground leaves were extracted with 5 mL of 85 % ethanol in a shaking water batch at 80 °C for 20 min. Samples were centrifuged for 5 min at 8500 RCF (Universal 320R, Hettich, Sigma-Aldrich, Darmstadt, Germany) and 1 mL of the supernatant was dried with a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific, Waltham, MA, USA) for 120 min at 50 °C and 5.1 mbar. The remaining pellet with supernatant was later used for starch determination.

The dried samples were re-suspended in 2 mL of 0.01 N hydrochloric acid and sonicated for 10 min (Branson 2800, Richmond, VA, USA). The samples were vortexed and centrifuged at 21.100 RCF for 5 min (Sorvall Legend Micro 21R, Thermo Fisher Scientific, Waltham, MA, USA).

Amino acids and other amino compounds were removed from the sample solution by trapping with a SPE column (UCT CLEAN-UP BCX columns, BGB analytik Benelux B.V. Harderwijk, the Nethelands, 100mg/1ml), eluted with 0.01 N hydrochloric acid.

The samples were diluted ten times and glucose, fructose and sucrose were quantified using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD; Dionex ICS5000, Thermo Fisher Scientific, Waltham, MA, USA), with a CarboPac1 column (250x2 mm, Thermo Fisher Scientific, Waltham, MA, USA) and eluted with 100 mM NaOH at a flow rate of 0.25 mL min^{-1} at 25 °C.

For starch determination the stored pellet was used. The pellet was washed three times with 80 % ethanol, dried for 20 min in a vacuum centrifuge at 50°C and 5.1 mbar. For resuspension of the dried pellet 2 mL 1 g L^{-1} thermostable alpha-amylase (SERVA Electrophoresis GmbH, Heidelberg, Germany) was used. The samples were incubated at 90 °C for 30 min. Before further incubation at 60 °C for 15 min, 1 mL of 0.5 g L^{-1} amyloglucosidase (10115Sigma-Aldrich, Darmstadt, Germany) in 50 mM citrate buffer (pH 4.6) was added to the samples. The samples were centrifuged at 21.100 RCF for 5 minutes and diluted 50-100 times. Glucose was quantified using HPEAC-PAD (see description above).

To convert from fresh weight to dry weight a conversion factor for was made for each sample; 400±40 mg of fresh frozen was weighed into a reaction tube and oven dried for 8 hours at 70 °C. Data was expressed on the base of dry weight as mg g^{1} DW.

3.2.6 Rosmarinic and chicoric acid

Phenolic acids were extracted as described in **Chapter 2**. Briefly, 250 ±20 mg frozen ground leaves were extracted with 1.5 mL of 80 % methanol with 2.5 % formic acid for 15 minutes in an ultrasonic bath (Branson 2800, Richmond, VA, USA). The supernatant was filtered through a cellulose syringe filter 0.45 µm, and analyzed according to the method of Kwee and Niemeyer (2011), with modifications. In Exp. 1 samples were measured on a HPLC system (Waters, Knowloon, Hongkong), with a UV dual wavelength detector and autosampler using a Vydac 201TP54 (C18, 5 µm, 300 Å, 4.6 mm × 250 mm) reverse-phase (RP) column. In Exp. 2 samples were measured on a HPLC system with a GS50 pump (Dionex, Thermo Fisher Scientific, Waltham, MA, USA), a 340S UV-VIS detector (Dionex, Thermo Fisher Scientific, Waltham, MA, USA) and a MIDAS autosampler (Spark, Emmen, the Netherlands) using a LiChrospher 100 RP-18 (5 µm), 150x4mm column (Merck, Amsterdam, The Netherlands). Samples were eluted with 2.5 % formic acid in H_2O (A) and acetonitrile (B) with a linear gradient of: 85 % A, 0.0 min.; 75 % A, 6.0 min.; 0 % A, 8.5 min.; [0 % A, 9.0 min.; 85 % A, 11.5 min.; 85 % A, 14.0 min]. Analytes were detected at 330 nm.
For quantification, calibration curves were prepared with standards (Extrasynthese, Genay, France) from 0 to 500 mg L^{-1}. Data was expressed on the base of dry weight as mg g^{-1} DW.

3.2.7 Total ascorbic acid

Ascorbic acid was measured according to Min *et al.* (2021). Total ascorbic acid (TAsA) is a large antioxidant group in leafy vegetables, also defined as Vitamin C (Min *et al.*, 2021). TAsA is the sum of ascorbic acid (AsA) and dehydroascorbic acid (DHA). Extraction of ascorbic acid was done from 200 mg frozen ground leaves with 1 mL 3.3 % meta-phosphoric acid (MPA) and sonicated (Branson 2800, Richmond, VA, USA) for 10 min in darkness at 0°C. The samples were centrifuged at 21.100 RCF (Sorvall Legend Micro 21R, Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at 4°C. For analysis of AsA the supernatant was filtered through a cellulose syringe filter of 0.45 µm of cellulose into an amber HPLC vial. Furthermore, for TAsA analysis, 100 µl of the filtered extract was transferred to another HPLC vial and 50 µl of 5mM dithiothreitol in 400mM Tris base was added.

To convert DHA to AsA the vials were kept in darkness at room temperature. The reaction was stopped after 15 min by adding 50 µl 8.5 % o-phosphoric acid. AsA was measured on a HPLC consisting of a GS50 pump (Dionex, Thermo Fisher Scientific, Waltham, MA, USA), a 340S UV-VIS detector (Dionex, Thermo Fisher Scientific, Waltham, MA, USA) with a MIDAS autosampler (Spark, Emmen, the Netherlands) and a ProntoSIL 120-3 C18 AQ, 250x3mm column (Knauer, Berlin, Germany). For elution of the column 400 µL L^{-1} H3PO4 + 2.5 mL L^{-1} MeOH + 0.1 mM EDTA in H_2O was used with a wash step consisting of 30% acetonitrile in H_2O at a flow rate of 0.35 mL min^{-1} at 35 °C. The detection of AsA was done at 243 nm. A standard with AsA in 3.3 % MPA was used for calibration. The amount of TAsA was calculated as the sum of the AsA and the AsA converted from DHA. Data was expressed on the base of dry weight as mg g^{-1} DW.

3.2.8 Total anthocyanin content

Total anthocyanin content was extracted from 300 mg frozen ground basil tissue with 1.5 mL 50 %MeOH with 1% formic acid in an in an ultrasonic bath (Branson 2800, Richmond, VA, USA) for 15 min. Samples were centrifuged at 15.000 RCF (Sorvall Legend Micro 21R, Thermo Fisher Scientific, Waltham, MA, USA) at 4°C for 15 min. The supernatant was filtered through a 0.45 µm cellulose filter. Samples were diluted 5 times and measured in a cuvette at wavelength of λ=530 nm in a spectrophotometer (Genesys 50, Thermo Fisher Scientific, Waltham, MA, USA) against a blank. Total content of anthocyanins was expressed as mg/g with cyanidin chloride as standard in the range 1-25 mg L^{-1}.

3.2.9 Total flavonoid content

Total flavonoid content was determined by aluminum chloride colorimetric assay (Zhishen *et al.*, 1999). Total flavonoid content was extracted from 300 mg of frozen ground basil and 1.5 mL Methanol/H_2O/Acetone (60:30:10 v/v/v) in an in an ultrasonic bath (Branson 2800, Richmond, VA, USA) for 15 min. Samples were centrifuged at 15000 RCF (Sorvall Legend Micro 21R, Thermo Fisher Scientific, Waltham, MA, USA) at 4°C for 10 min and the supernatant was collected. Catechin was used as a quantifying standard. In a 3 mL cuvette 50 µL of the extracted sample was mixed with 1.95 mL water and 75 µL of 5 % $NaNO_2$. After 6 min 150 µL of 10 % $AlCl_3$ was added and after another 5 min 500 µL of 1M NaOH was added. The absorbance was measured at a wavelength of λ=250 nm in a spectrophotometer (Genesys 50, Thermo Fisher Scientific, Waltham, MA, USA) against a blank. Data was expressed on the base of dry weight as mg g^{-1} DW.

3.2.10 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) was determined according to Junglee *et al.*(2014) with some modifications. H_2O_2 was extracted from 0.1 g of frozen ground basil leaves with 0.4 mL of 0.1 % TCA, 0.4 mL potassium phosphate buffer (pH 7.6) and 0.8 mL of potassium iodide. After incubation for 10 min at 4 °C, the samples were centrifuged at 15000 RCF (Sorvall Legend Micro 21R, Thermo Fisher Scientific, Waltham, MA, USA) at 4°C for 10 min and the supernatant collected. Samples were measured in UV-cuvettes at a wavelength of λ=350 nm in a spectrophotometer (Genesys 50, Thermo Fisher Scientific, Waltham, MA, USA) against the blank. For quantification a calibration curve was prepared with H_2O_2 solutions with concentrations from 10 to 400 µmol L^{-1}. For each sample three technical replicates were prepared. Data was expressed on the base of dry weight as mg g^{-1} DW.

3.2.11 Maximum chlorophyll fluorescence

Chilling injury was measured as an F_v/F_m ratio as described in **Chapter 2**. F_v/F_m is the maximum quantum yield of the primary photochemical reactions or PSII in dark adapted leaves. Per stored box, containing leaves from two plants, one leaf from the upper leaf-pair and one leaf from the middle leaf pair per plant were measured. First leaves were dark adapted at 20 °C for 20 min after which the measurement of chlorophyll fluorescence was done using a PSI closed Fluorcam 800-C chlorophyll fluorescence imaging system (PSI, Drasov, Czech Republic). To operate the fluorcam and analyze the images the fluorcam software Version 7 was used, according to the method of Hogewoning and Harbinson (2007).

3.2.12 Overall visual quality

Overall visual quality (OVQ) was evaluated using a scoring system as described in **Chapter 2**. The scores were given based on visual symptoms associated with chilling injury and general symptoms appearing at non-chilling temperatures. A score between 1 and 8 was given based on the visual symptoms (i.e. 1 being the worst and 8 the best). The consumer acceptance limit was set at the score 5, which represented the end of shelf life. The scores would be reduced due to symptoms such as dark spots/discoloration, fungal appearance, degree of crispness, degree of wilting, leaf shininess and presence of characteristic curved leaf shape (Table S1, **Chapter 2**).

3.2.13 Statistical set up and analysis

The experiments were carried out in a complete randomized block design. The light treatments for the different cultivars, either the green cv. Dolly or the purple cv. Rosie were located in separate compartments. Exp. 1 was carried out two times

(2 blocks) and Exp. 2 was carried out three times (3 blocks) for cv. Dolly and four times for cv. Rosie (4 blocks). The border plants were excluded from the analysis. For the chemical analysis at harvest four replicate plants were sampled per light treatment in each block. The rest of the plants were stored for postharvest sampling. For postharvest storage at 4 and 12 °C the leaves from two plants were packed in one plastic box (see description above). Two boxes (i.e. leaves from four plants) per cv. and light treatment were sampled for overall visual quality and chemical analysis per postharvest timepoint. As one replicate an average value of each block was used for further statistical analysis. For each block the chemical analyses were done on leaves from four plants as a pooled sample. The means are based on number of blocks x four replicate plants.

The data was analyzed with Genstat (VSN International, 19th Edition). The assumptions of homogeneity and normality of the residuals were tested with Bartlett's test and Shapiro-Wilk test. In the case that the data did not follow the assumption the data was transformed with the natural logarithm, after which it followed the assumption. Thereafter, the data was analyzed using a two-way ANOVA per time point and storage temperature with the posthoc test Fishers protected LSD. For Exp. 1 the test was conducted with a probability level of α=0.10 for Exp. 1 because the experiment only had two blocks, while for Exp. 2 the probability level was α=0.05.

3.3 Results

3.3.1 Metabolite content in response to percentage and duration of blue light

Soluble sugars (glucose, fructose and sucrose) at harvest were not affected by percentage of blue light during cultivation (25 days) or five days of End-Of-Production (EOP). Starch content was reduced by 30-50 % with increasing percentage of blue light for both EOP and continuous treated plants (Fig. 1A, B). Rosmarinic acid at harvest was little affected by the percentage of blue. However, continuous blue (33, 65 and 100 % blue) treatments resulted in a 15-25 % decrease compared to the shorter duration of EOP blue treatments (Fig. 1C). Chicoric acid levels increased with increasing percentage of blue whether it was provided continuously throughout cultivation or EOP, but the increase was stronger when applied throughout cultivation (+85%) compared to EOP (+45%) (Fig. 1D). Total ascorbic acid did not respond to either percentage of blue or duration of blue light (Fig. 1E).

The changes in metabolites from the EOP treated plants were measured during postharvest storage. During storage at both 4 and 12 °C, sugars increased whereas starch decreased over time (Fig. S1). However, the patterns of the time courses were not affected by the EOP blue light treatments. During postharvest storage at 4 °C, rosmarinic acid, chicoric acid and total ascorbic acid showed a steep decrease over time (Fig. S2). At 12 °C, an initial increase was observed in chicoric and rosmarinic acids for all treatments, later followed by a decrease. Total ascorbic acid at 12 °C showed a similar pattern as at 4 °C (Fig. S2).

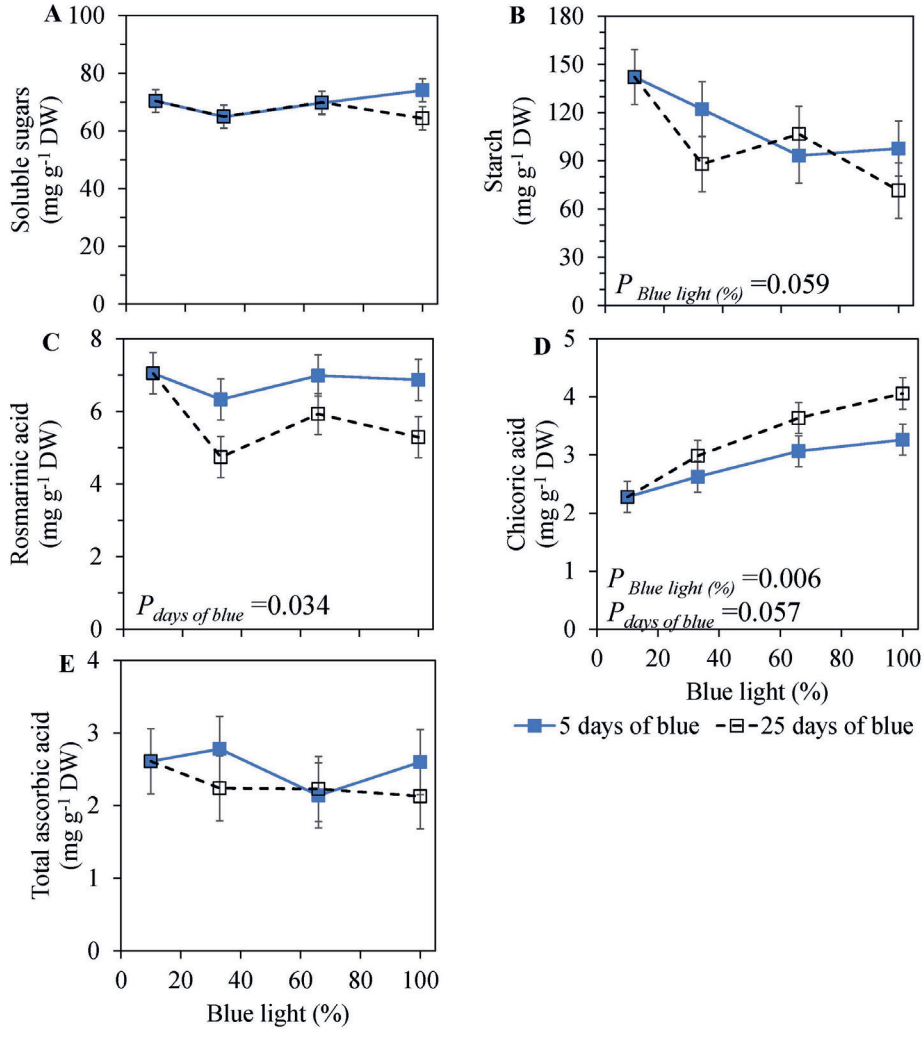

Figure 1. Metabolite levels at harvest in basil cv. Dolly exposed either continuously (25 days) (open symbols) or for five days of End-Of-Production (EOP) (closed symbols) to different percentages of blue light. Plants were harvested after 40 days of cultivation. After 15 days plants were transplanted. After transplant the continuous treated plants grew under different percentage of blue light until harvest (PPFD of 300 µmol m^{-2} s^{-1}). The EOP plants were grown under red-white (PPFD of 300 µmol m^{-2} s^{-1}, 9 % blue) light and later exposed to different percentage of blue light (PPFD of 300 µmol m^{-2} s^{-1}) for the last five days before harvest as EOP treatments. A. Soluble sugars (sum of glucose, fructose and sucrose), B. starch, C. rosmarinic acid, D. chicoric acid, E. total ascorbic acid. All values are expressed per gram dry weight in the leaves. The data are means of two blocks (n=2) (i.e. per block four replicate plants). Standard errors of means are shown as error bars. Significance of the main effects percentage of blue and days of blue (α=10 %) are depicted, (Exp. 1).

3.3.2 Metabolite content in response to percentage of blue light at different PPFD

The interactive effect between percentage of blue light (low; 9 % or high; 90 %) and PPFD (low; 100 µmol m^{-2} s^{-1}, or high; 300 µmol m^{-2} s^{-1}) applied as EOP treatment the last five days before harvest on metabolites in green cv. Dolly and purple cv. Rosie were investigated (Exp. 2). The high PPFD and the low blue were the same as in Exp. 1.

High PPFD as EOP treatment increased soluble sugars and starch content at harvest in both the green cv. Dolly (Fig. 2A, B) and the purple cv. Rosie (Fig. 3A, B). Furthermore, for both cultivars starch content was significantly higher in the high PPFD treatment combined with low blue compared to the high PPFD with high blue. A higher percentage of blue light increased the content of chicoric acid in both cultivars (Fig. 2D, 3D), while high PPFD also increased the content of chicoric acid in purple cv. Rosie. Rosmarinic acid was neither affected by PPFD nor by the percentage of blue light (Fig. 2C, 3C). Similarly, the total anthocyanin content in the purple cv. Rosie was not significantly affected by neither PPFD nor the percentage of blue light (Fig. 3G). The green cv. Dolly did not contain anthocyanins. In contrast, total flavonoid content and the level of H_2O_2 were lower at high PPFD than at low PPFD in purple cv. Rosie (Fig 3E, F). However, for neither the green nor the purple cultivar did percentage of blue light and PPFD have an interactive effect on the content of metabolites.

Sugar levels were slightly higher and starch levels were considerably lower in purple cv. Rosie compared to green cv. Dolly (Fig. 2A, 3A). Rosmarinic acid, chicoric acid and flavonoids were higher in purple cv. Rosie compared to green cv. Dolly. The purple cv. Rosie contained anthocyanin, which was absent (below the detection level) in the green cv. Dolly. Together it implies that the purple cv. Rosie had a higher level of antioxidants (secondary metabolites) but considerably lower level of carbohydrate reserves at harvest.

From Exp. 1 with the green cv. Dolly it was clear that the pre-harvest blue light had little or no effects on the metabolite changes during the postharvest phase. Therefore, in experiment 2 the green cv. Dolly was sampled only at day 3 and 6 during storage at 4 and 12 °C. The purple cultivar Rosie was sampled in addition also at day 9 and 12. During storage soluble sugars increased at both 4 and 12 °C for the green cv. Dolly (Fig. S3A, B) whereas for the purple cv. Rosie a slight decrease was observed at 12 °C (Fig. S4B). The levels of sugars in the postharvest phase were generally higher in the samples derived from plants from high PPFD EOP treatments while

percentage of blue light did not have an effect on the postharvest content. During postharvest storage at both 4 and 12 °C, the starch content remained the highest from high PPFD and low blue EOP treatments in both cvs Dolly and Rosie (Fig. S3C, D, S4C, D). The starch reserves, especially in the samples from low PPFD and high PPFD/high percentage of blue light treatments were depleted by day 3-6 in the purple cv. Rosie. At that time depletion was not complete in the green cv. Dolly had higher starch levels at harvest. Starch breakdown was generally faster at 12 than at 4 °C.

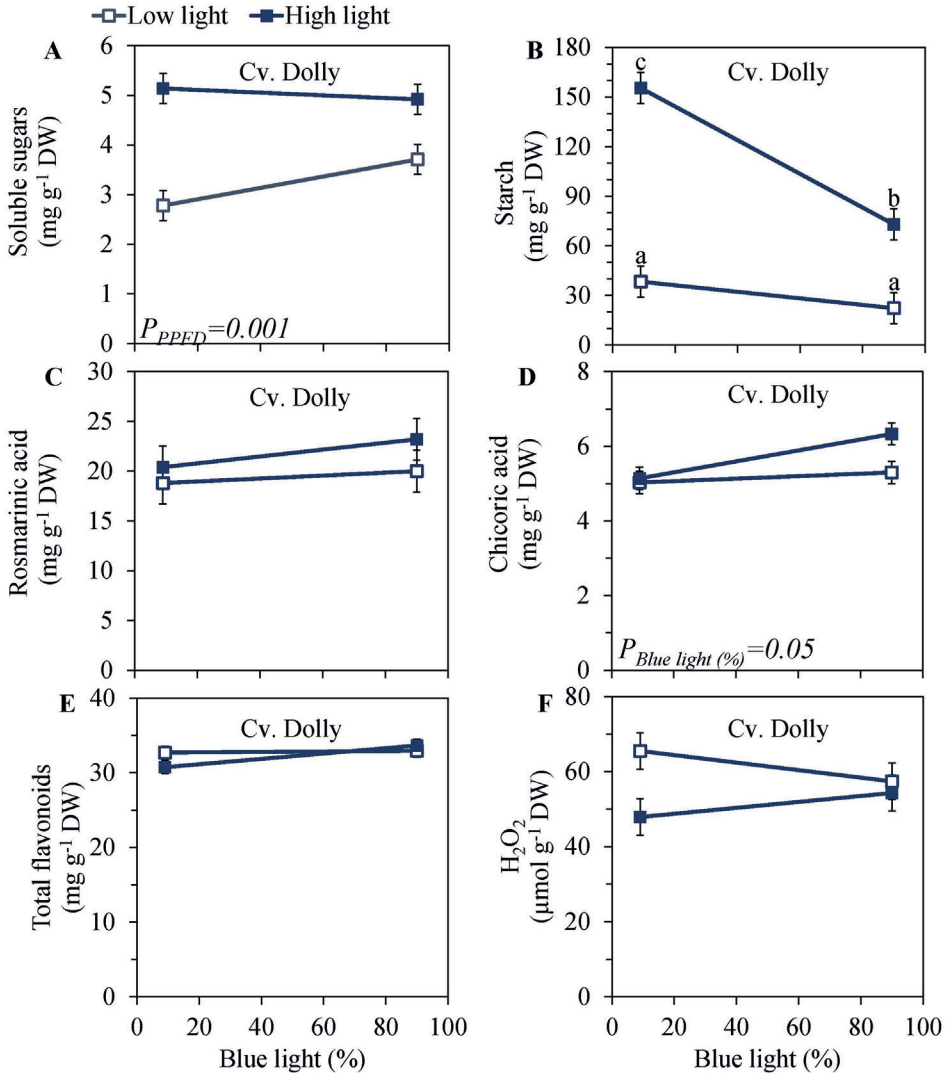

Figure 2. Metabolite levels at harvest in basil, the green cv. Dolly were exposed to either 9 % or 90 % blue light at low PPFD (100 µmol m^{-2} s^{-1}) (open symbols) or high PPFD (300 µmol m^{-2} s^{-1}) (closed symbols) applied the last five days before harvest as End-Of-Production (EOP) treatments. Before EOP treatments plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9% blue) for 30 days. A. Soluble sugars (sum of glucose, fructose and sucrose), B. starch, C. rosmarinic acid, D. chicoric acid, E. total flavonoids, F. H_2O_2. All values are expressed per gram dry weight in the leaves. The data are means of three blocks (n=3) (i.e. per block four replicate plants). Standard errors of means are shown as error bars. Significance of the main effects percentage of blue light and PPFD (α=5 %) are shown. Letters indicate and interactive effect between the two main effects (percentage of blue light and PPFD), (Exp. 2).

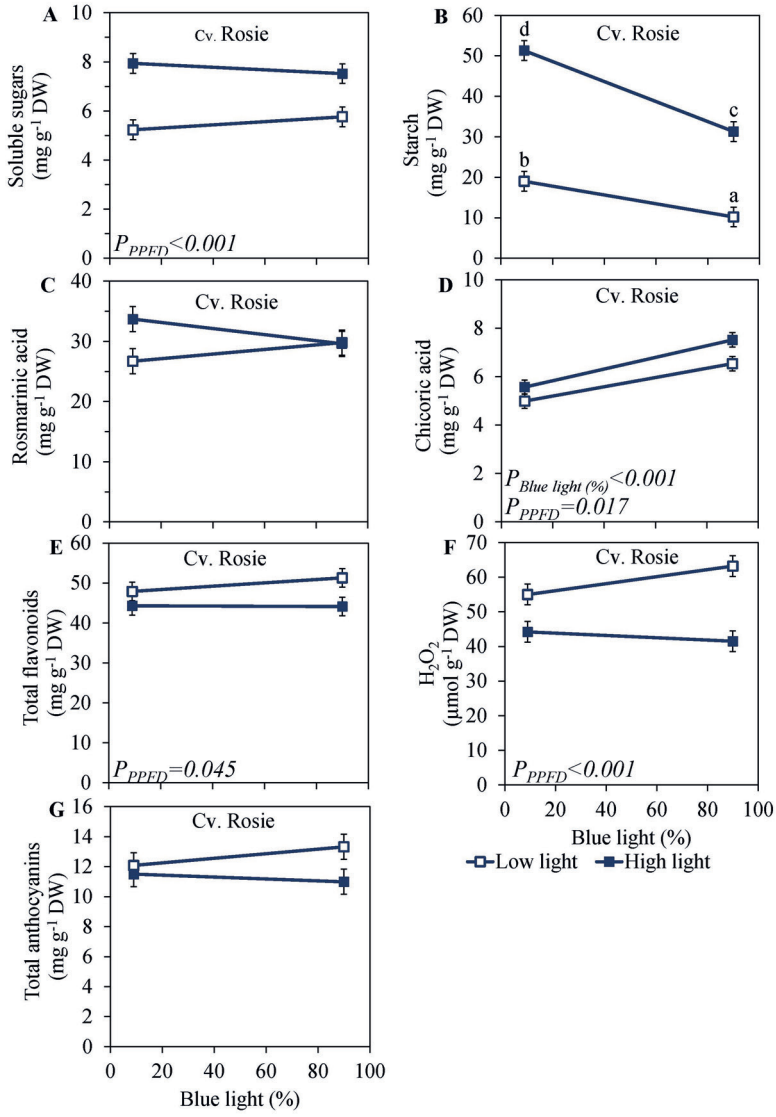

Figure 3. Metabolite levels at harvest in basil the purple cv. Rosie were exposed to either 9 % or 90 % blue light at low PPFD (100 µmol m^{-2} s^{-1}) (open symbols) or high PPFD (300 µmol m^{-2} s^{-1}) (closed symbols) applied the last five days before harvest as End-Of-Production (EOP) treatments. Before EOP treatments plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9 % blue) for 30 days. A. Soluble sugars (sum of glucose, fructose and sucrose), B. starch, C. rosmarinic acid, D. chicoric acid, E. total flavonoids, F. H_2O_2, G. total anthocyanins. All values are expressed per gram dry weight in the leaves. The data are means of four blocks (n=4) (i.e. per block four replicate plants). Standard errors of means are shown as error bars. Significance of the main effects percentage of blue light and PPFD (α=5 %) are shown. Letters indicate and interactive effect between the two main effects (percentage of blue light and PPFD), (Exp. 2).

Overall, metabolites were unchanged at 12 °C storage for both cultivars (Figs. 4, 5) but in the purple cv. Rosie a pronounced decrease in the metabolite levels was observed at 4 °C. During the 6 days of storage there was no clear effect of the EOP light treatments on the changes in metabolite levels in the green cv. Dolly. For the purple cv. Rosie, high PPFD during cultivation resulted in a slower decrease of metabolites (rosmarinic acid, chicoric acid, total flavonoid content and total anthocyanin content) during dark storage at 4 °C (Fig. 5A, C, E, G). In addition, a low percentage of blue light resulted in a slower decrease of rosmarinic acid and total anthocyanin content compared to a high percentage of blue light in the purple cv. Rosie at 4 °C (Fig. 5A, G). The reverse effect was seen for chicoric acid, where a high percentage of blue light resulted in the slowest decrease for both cvs (Fig. 4C, 5C).

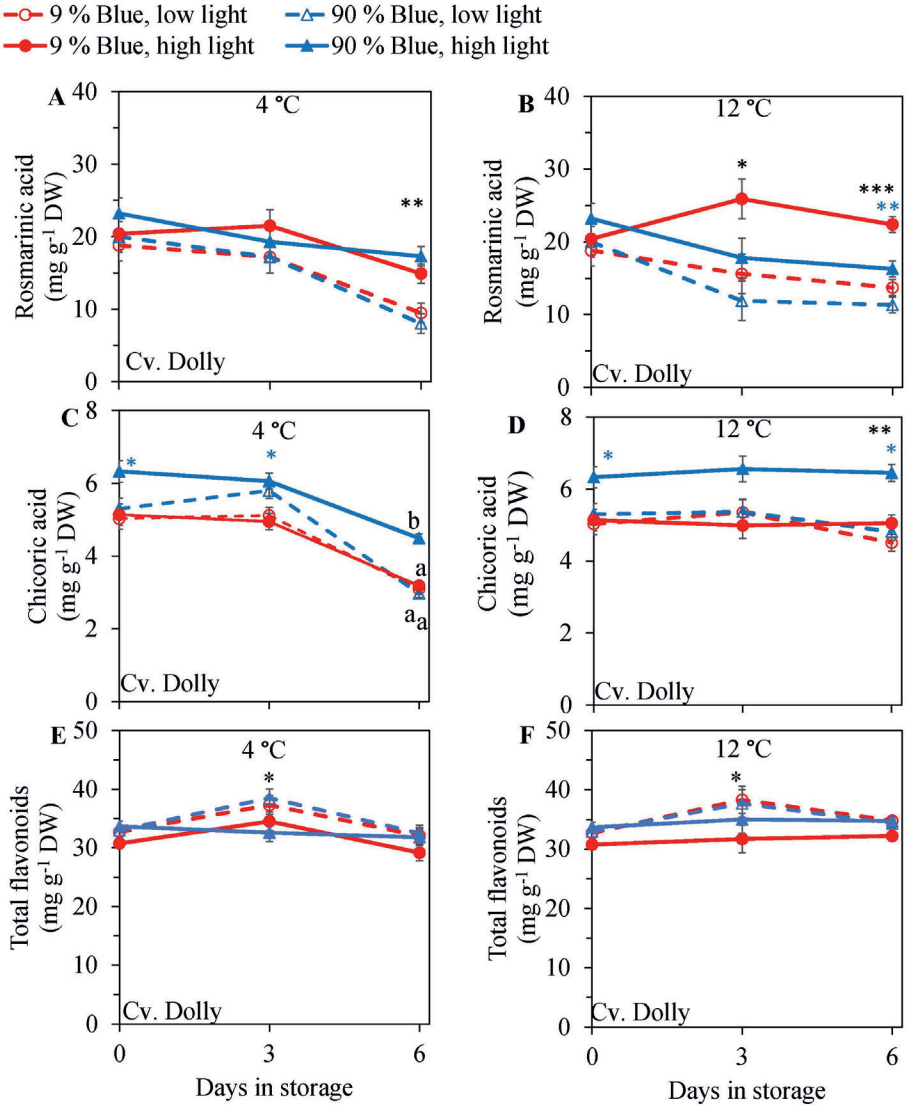

Figure 4. Changes in metabolite levels during postharvest storage at 4 (panels A,C,E) and 12° C (panels B,D,F) in basil in the green cv. Dolly exposed to End-Of-Production (EOP) treatments. Plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9 % blue) for 30 days. The last five days before harvest plants were exposed to different EOP blue light ratios 9 % or 90 % at low PPFD (100 µmol m^{-2} s^{-1}) (open symbols) or high PPFD (300 µmol m^{-2} s^{-1}) (closed symbols). A and B. change in rosmarinic acid, C and D, change in chicoric acid, E and F. change in total flavonoid content. All values are expressed per gram dry weight in the leaves. The data are means of three blocks (n=3) (i.e. per block four replicate plants). Standard errors of means are shown as error bars. If no interaction was found but only the main effects were significant the indicated with *p*-values; *p<0.05 *, p<0.01 **, p<0.001* *** are depicted with either a blue (percentage of blue light) or black asterisk (PPFD). Letters indicate and interactive effect between the two main effects (percentage of blue light and PPFD), (Exp. 2).

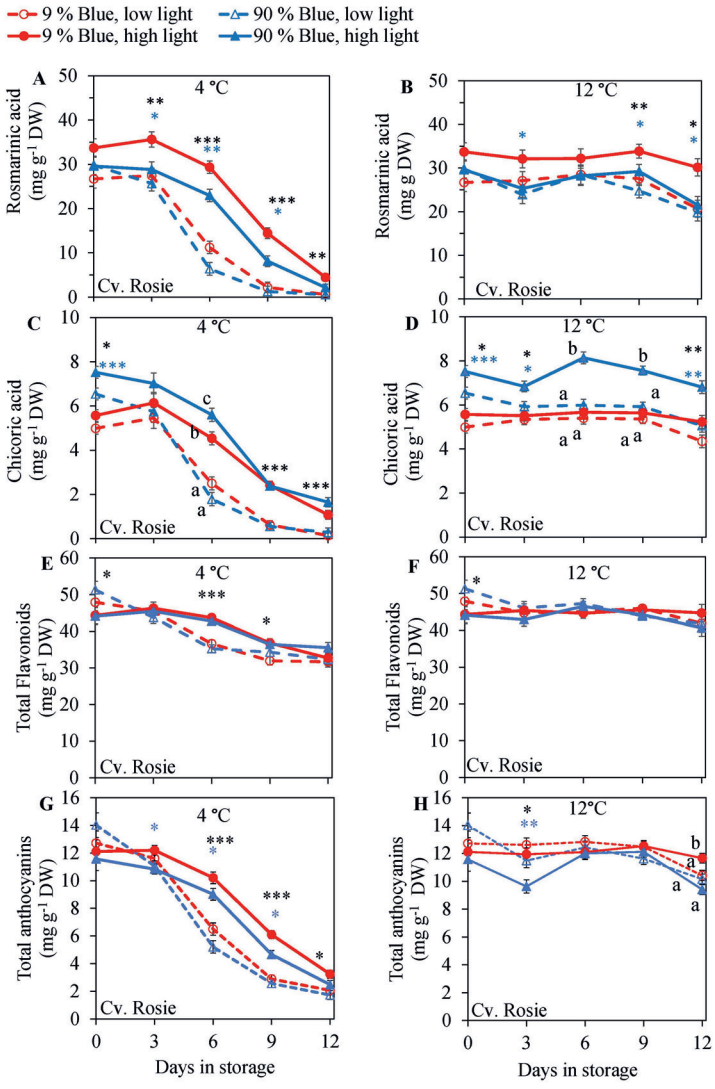

Figure 5. Changes in metabolite levels during postharvest storage at 4 (panels A, C, E, G) and 12° C (panels B, D,F, H) in basil in the purple cv. Rosie exposed to End-Of-Production (EOP) treatments. Plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9 % blue) for 30 days. The last five days before harvest plants were exposed to different EOP blue light ratios 9 % or 90 % at low PPFD (100 µmol m^{-2} s^{-1}) (open symbols) or high PPFD (300 µmol m^{-2} s^{-1}) (closed symbols). A and B change in rosmarinic acid, C and D. change in chicoric acid, E and F change in total anthocyanin content, G and H. change in total flavonoid content. All values are expressed per gram dry weight in the leaves. The data are means of four blocks (n=4) (i.e. per block four replicate plants). Standard errors of means are shown as error bars. If no interaction was found but only the main effects were significant the indicated with *p*-values; *p<0.05 *, p<0.01 **, p<0.001* *** are depicted with either a blue (percentage of blue light) or black asterisk (PPFD). Letters indicate and interactive effect between the two main effects (percentage of blue light and PPFD), (Exp. 2).

3.3.3 Chilling tolerance in response to percentage of blue light and PPFD

F_v/F_m was measured during the storage as a marker for chilling injury. During dark storage at 12 °C there was little change over time and no effect of PPFD or the percentage of blue light during cultivation (Fig. 6B, 7B). For both cultivars, high PPFD during cultivation resulted in less chilling injury during 4 °C storage (i.e. a slower decrease of F_v/F_m and a longer shelf life) (Fig. 6A, 7A). The high percentage of blue light showed a minor effect on chilling injury for both cultivars. Overall visual quality (OVQ) values were in line with F_v/F_m values. At 12 ºC, OVQ values slowly decreased and there were no clear effects of EOP PPFD or percentage of blue light (Fig. 6D, 7D). At 4 °C a high PPFD had a positive effect on OVQ values for both cultivars (Fig. 6C, 7C) whereas the effect of percentage of blue light was limited.

During chilling injury, the content of H_2O_2 (i.e. a reactive oxygen species) may increase due to chilling stress. At 12 °C the H_2O_2 content remained unchanged in the green cv. Dolly but decreased in the purple cv. Rosie (Fig. 6F,7F). H_2O_2 levels in samples from low PPFD EOP treatments were generally higher than in samples from high PPFD in both cvs. There was no effect of percentage of blue light. At 4 °C, H_2O_2 levels in both cvs rapidly decreased, the decrease seemed to be more pronounced in samples from low PPFD EOP treatments; there was no effect of percentage of blue light on the observed patterns (Fig. 6E, 7E).

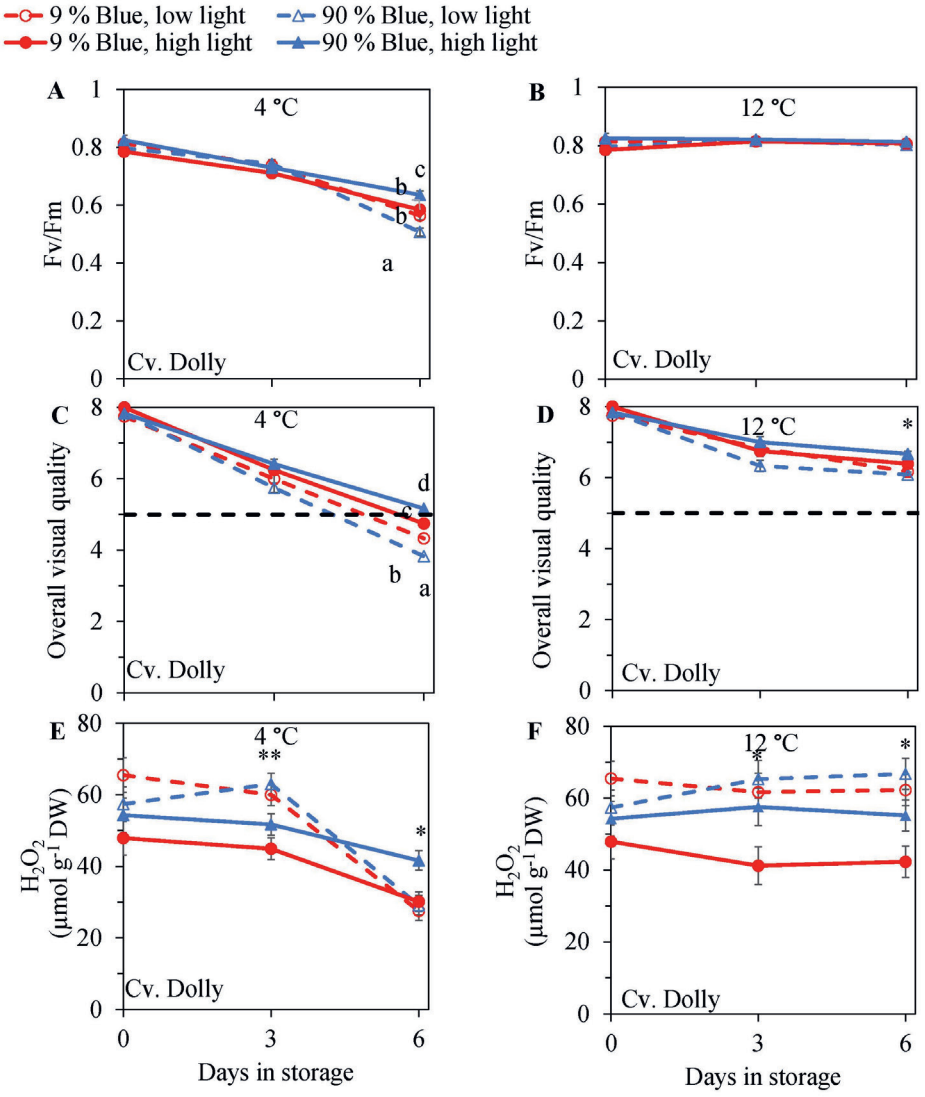

Figure 6. Changes in chilling injury parameters during postharvest storage at 4 (panels A, C, E) and 12° C (panels B, D, F) in basil in the green cv. Dolly exposed to End-Of-Production (EOP) treatments. Plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9 % blue) for 30 days. The last five days before harvest plants were exposed to different EOP blue light ratios 9 % or 90 % at low light (100 µmol m^{-2} s^{-1}) (open symbols) or high light (300 µmol m^{-2} s^{-1}) (closed symbols). A and B change in maximum quantum yield of PSII of dark-adapted leaves (F_v/F_m), C and D, change Overall Visual Quality (OVQ), E and F change in hydrogen peroxide (H_2O_2) content. All metabolite values are expressed per gram dry weight in leaves. The data are means of three blocks (n=3) (i.e. per block four replicate plants). Standard errors of means are shown as error bars. If no interaction was found but only the main effects were significant the indicated with *p*-values; *p<0.05 *, p<0.01* ** are depicted with either a blue (percentage of blue light) or black asterisk (PPFD). Letters indicate and interactive effect between the two main effects (percentage of blue light and PPFD), (Exp. 2).

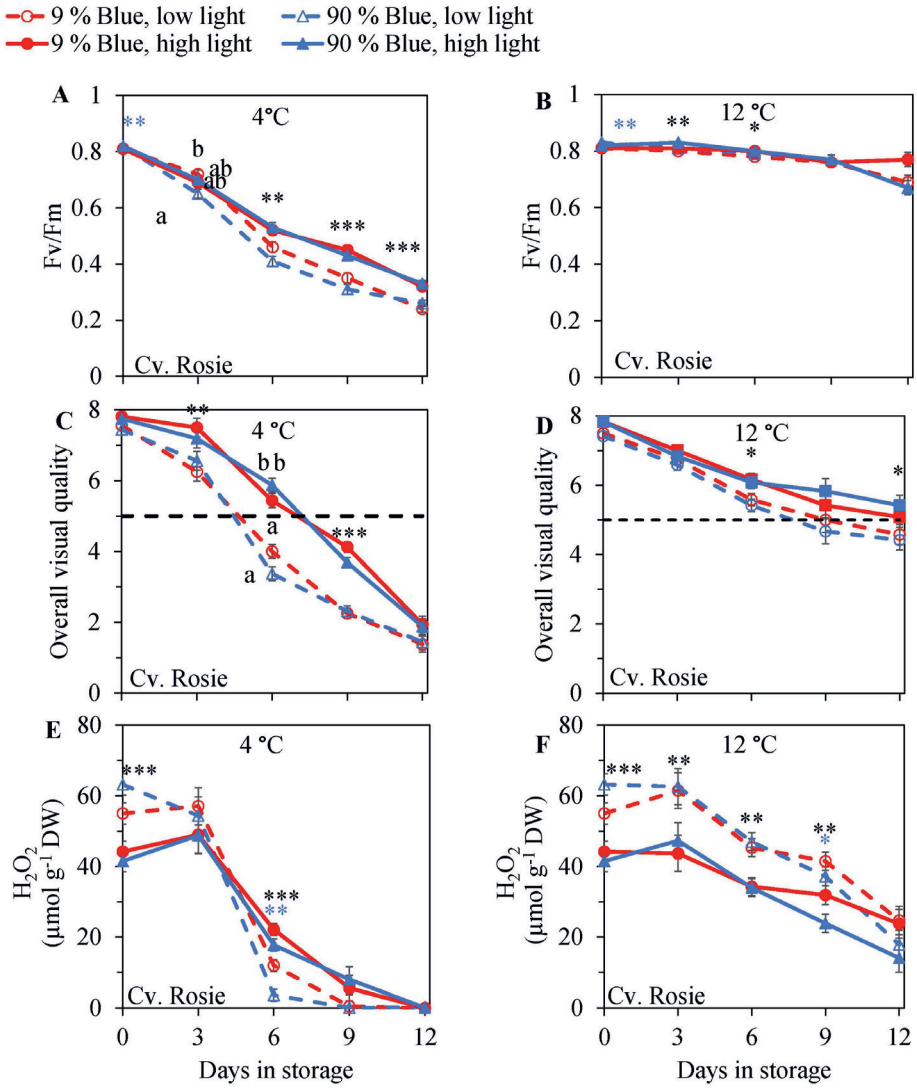

Figure 7. Changes in chilling injury parameters during postharvest storage at 4 (panels A, C, E) and 12° C (panels B, D, F) in basil in the purple cv. Rosie exposed to End-Of-Production (EOP) treatments. Plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9 % blue) for 30 days. The last five days before harvest plants were exposed to different EOP blue light ratios 9 % or 90 % at low light (100 µmol m^{-2} s^{-1}) (open symbols) or high light (300 µmol m^{-2} s^{-1}) (closed symbols). A and B change in Maximum quantum yield of PSII of dark-adapted leaves (F_v/F_m), C and D, change in Overall Visual Quality (OVQ), E and F change in hydrogen peroxide (H_2O_2) content. All metabolite values are expressed per gram dry weight in leaves. The data are means of four blocks (n=4) (i.e. per block four replicate plants). Standard errors of means are shown as error bars. If no interaction was found but only the main effects were significant the indicated with *p*-values; *p<0.05 *, p<0.01 **, p<0.001 **** are depicted with either a blue (percentage of blue light) or black asterisk (PPFD). Letters indicate and interactive effect between the two main effects (percentage of blue light and PPFD), (Exp. 2).

3.4 Discussion

3.4.1 Blue light did not stimulate the biosynthesis of secondary metabolites from the phenylpropanoid pathway

Blue light has been reported in numerous studies to increase the content of antioxidants in several plant species such as strawberry, green and red lettuce, green and red pak choi and Arabidopsis (Chen *et al.*, 2006; Samuoliene *et al.*, 2013; Ouzounis *et al.*, 2015; Zhang *et al.*, 2018; Zheng *et al.*, 2018). Thus, we hypothesized that blue light would increase the content of antioxidants at harvest in green and purple basil. Our findings indicate that a high percentage of blue light (up to 100%) during the whole cultivation period applied as a 5 day EOP treatment did not increase the content of rosmarinic acid in basil leaves (Fig. 1C, 2C, 3C). Similar to Taulavuori *et al.* (2016), we found that a continuous application of high percentage of blue light even negatively affected the content of rosmarinic acid (Fig. 1C). Spectra that earlier have been reported to result in a high content of rosmarinic acid include red with supplementary far-red (Schwend *et al.*, 2016), red light (Shiga *et al.*, 2009) and blue light under greenhouse conditions. However, these studies did not consistently compare how the different percentages of the spectra affects the biosynthesis of rosmarinic acid (i.e. percentage of blue vs, green, red and far-red). Chicoric acid generally constitutes a minor amount of the total level of phenolic compounds in the basil cultivars under study (Fig 1D, 2D, 3D). Although, chicoric acid has been found to be a stronger antioxidant than rosmarinic acid (Dalby-Brown *et al.*, 2005) and it is unknown to what extent chicoric acid contributes to the overall scavenging activity of basil antioxidants. Similar to our results, Taulavuori *et al.* (2016) found blue light to have a positive effect on the content of chicoric acid. We found different absolute levels of especially rosmarinic acid content in the two experiments. However, the response to blue light remained the same between the two experiments (i.e. no effect of blue light). The main difference between the experiments was the initial PPFD; Exp. 1 had a PPFD of 300 µmol m^{-2} s^{-1} and Exp. 2 a PPFD of 200 µmol m^{-2} s^{-1}. Similar to the high content of rosmarinic acid in Exp. 2 our previous results in basil also showed increased PPFD as EOP treatment to increase rosmarinic acid (**Chapter 2**). Furthermore, the duration of Exp. 1 was 40 days while Exp. 2 was 35. The younger leaves in Exp. 2 may have additionally contributed to a higher content of rosmarinic acid at harvest.

Blue light is absorbed by photoreceptors such as cryptochromes (cry1 and cry2) which mediates plant responses. The genes involved in the biosynthesis of flavonoids and anthocyanins though the phenylpropanoid pathway are induced through cry1 (Jenkins *et al.*, 2001). The starting point for the flavonoid biosynthesis

branch is chalcone synthase (CHS). The expression level of *CHS* have been found to increase by blue light in Arabidopsis cells (Christie and Jenkins, 1996) and increase anthocyanin and flavonoid content in a number of species. In pepper leaves increasing blue light to 75 % increased the content of anthocyanins but not flavonoids (Hoffmann *et al.*, 2016). However, in our study total anthocyanin content did not increase with increased percentage of blue light (Fig. 3G) and neither did total flavonoid content in the green cultivar (Fig. 2E) nor in the purple cultivar (Fig. 3E). Similarly, Dou *et al.* (2019) found that increasing the blue light from 16 to 24 % did not increase the anthocyanin content in green or purple basil while they did find a positive effect on flavonoids in green basil. Findings by Pennisi *et al.* (2019) indicated an optimum at 23 % blue light resulting in the highest total flavonoids content while 58 % and 19 % blue light resulted in the lowest total flavonoid content in green basil. In contrast, Piovene *et al.* (2015) found that a range of blue light from 10 to 40 % did not increase the total flavonoid content in basil. In red lettuce 47 % blue light gave the highest content of flavonoids whereas 59 % blue light yielded the highest content in green lettuce (Son and Oh, 2013). Although, it is generally assumed that blue light will increase particularly the flavonoid and anthocyanin content there is no general consistency in the results; this makes it hard to predict which percentage of blue light may be required to increase the flavonoid and anthocyanin content, if any. However, it should be noted that changes in percentage of blue light also cause changes in contribution of other wavelengths, that may contribute to the measured effects. Based on our results it seems that a low percentage of blue light (9 %) in the spectrum is already sufficient for maximal biosynthesis of phenolic compounds (phenolic acids, flavonoids, anthocyanins) in green and purple basil.

3.4.2 The content of secondary metabolites in response to percentage of blue light did not depend on PPFD

We hypothesized that the response to blue light might be reduced by high PPFD (300 μmol $m^{-2}s^{-1}$). The accumulation of antioxidants in response to blue light may be more pronounced at a lower PPFD compared to a high PPFD as a high PPFD regardless of spectrum leads to an increase in antioxidants (**Chapter 2**). In cannabis the percentage of blue light at a PPFD of 750 and 900 μmol $m^{-2}s^{-1}$ did not affect secondary metabolites which was assumed to be attributed to saturated photoreceptors (i.e. less sensitivity to spectral effects) (Westmoreland *et al.*, 2021). We tested if the effect of varying percentages of blue light was different when applied on a low or high PPFD background. We did not find such effect on the metabolite content in neither green nor purple basil. This is in contrast to findings by Zheng *et* al. (2018) in green and red leaved pak choi where the percentage of blue

light and PPFD had an interactive effect on vitamin C, carotenoids and total phenolic content.

At harvest, the dominant effect of light on metabolites came from the PPFD (Fig. 2,3) which is in line with results in **Chapter 2** where increased PPFD increased both primary and secondary metabolites in basil. In red lettuce anthocyanin and phenolic acids were not affected by PPFD whereas flavonoids increased (Becker *et al.*, 2013). This is in accordance with our results where PPFD had no effect on anthocyanin content but a small increasing effect on flavonoid content at harvest (Fig. 3E). In studies where anthocyanins have been reported to increase with an increase in PPFD, much bigger difference between light intensities were applied; from 100 to 550-650 µmol m^{-2} s^{-1} (Page *et al.*, 2012) from 50-350 to 750 µmol m^{-2} s^{-1} (Albert *et al.*, 2009). To get an overview of the whole plant we sampled all fully developed leaves at harvest except the oldest leaf pair. Young leaves contain higher amounts of anthocyanins than older leaves and by sampling young leaves along with more mature leaves we could potentially have had a dilution effect (Chalker-Scott, 1999). In addition to antioxidants we found that starch content was significantly decreased at a high PPFD and a high percentage of blue light in both green and purple basil (Fig. 1B, 2B, 3B), this could negatively affect shelf life as starch is used for respiration (Enninghorst and Lippert, 2003). Although, we found that starch was decreased in both experiments the dry mass of the leaves only decreased with percentage of blue light when continuously grown under a high PPFD (300 µmol m^{-2} s^{-1}) (**Chapter 5**).

3.4.3 High PPFD resulted in a slower breakdown of antioxidants and improved chilling tolerance postharvest

In lettuce (Min *et al.*, 2021) and in basil (**Chapter 2**) it was found that the stimulating effect of high PPFD on metabolites observed at harvest was maintained during postharvest storage. In the present experiments, levels at harvest were little affected by PPFD, but during postharvest storage at 4 °C antioxidants (rosmarinic acid, chicoric acid, total anthocyanin content and total flavonoid content) in the purple cv. Rosie from high PPFD EOP light treatments showed a slower breakdown than in samples from low PPFD treatments (Fig. 5). The effect of PPFD on the rate of the postharvest breakdown of antioxidants was less pronounced in the green cv. Dolly (Fig. 4). The slower breakdown of antioxidants during storage at 4 °C coincided with a slower decrease in F_v/F_m and Overall visual quality (OVQ) (Fig. 6A,C, 7A,C). This indicates that the plants from high PPFD were more tolerant to the cold. Although antioxidants may scavenge ROS and in turn result in maintaining high F_v/F_m values we believe that another mechanism could also be in play here. In the purple cv. Rosie

high light did increase the soluble sugar and starch content (Fig. S4) which can protect against chilling stress (Santarius, 1973). Plants with an increased chilling tolerance may have had higher levels of hormones such as ABA and JA (Lado *et al.*, 2016; Wang *et al.*, 2016*a*). However, this has yet to be studied in basil.

During excessive stress, H_2O_2 is not only formed in the mitochondria and chloroplasts but also in the peroxisomes resulting in lipid peroxidation (Corpas *et al.*, 2001). Exposure of basil to chilling temperatures was expected to increase the content of H_2O_2, however, during storage at 4 °C we observed a strong decrease of H_2O_2 in both cultivars (Fig 6E, 7E). In contrast, the level of H_2O_2 at 12 °C remained constant in the green cv. Dolly (Fig. 6F) or showed a slow decrease in the purple cv. Rosie (Fig. 7F). The low H_2O_2 concentration at 4 °C may be a result of scavenging by anthocyanins, flavonoids, rosmarinic acid and chicoric acid, which all showed comparable decreasing trends at 4 °C (Fig. 2, 3). Enzymatic antioxidants might also have been active in scavenging as the content is known to increase when plants are grown under high PPFD (Ali *et al.*, 2005; Zhou *et al.*, 2012).

3.5 Conclusion

Contrary to our hypothesis and general expectations a high percentage of blue light applied either continuous throughout the growth or as End-Of-Production (EOP) treatment did not increase antioxidants such as rosmarinic acid, total anthocyanin content or total flavonoid content at harvest. The only antioxidant which was increased by percentage of blue light was chicoric acid which is only a minor part of the total antioxidant content. The absence of effects of blue light was observed both in green and purple basil cultivars and the absence was also observed whether PPFD was high or low. Chilling tolerance is supposed to be related to the scavenging activity of antioxidants. The lack of effect of percentage of blue light on antioxidant levels is in line with the absence of percentage of blue light effects on chilling tolerance. Although, a high percentage of blue light did not improve postharvest chilling tolerance in green or purple basil, high PPFD EOP treatments did. High PPFD as EOP treatment particularly improved chilling tolerance in the purple cultivar, reflected in a slower decrease in antioxidants than in samples from in low PPFD treatments. This may not be related to the levels of antioxidants but to the higher carbohydrate levels (soluble sugars and starch) in leaves from high PPFD grown plants.

Supplementary Material

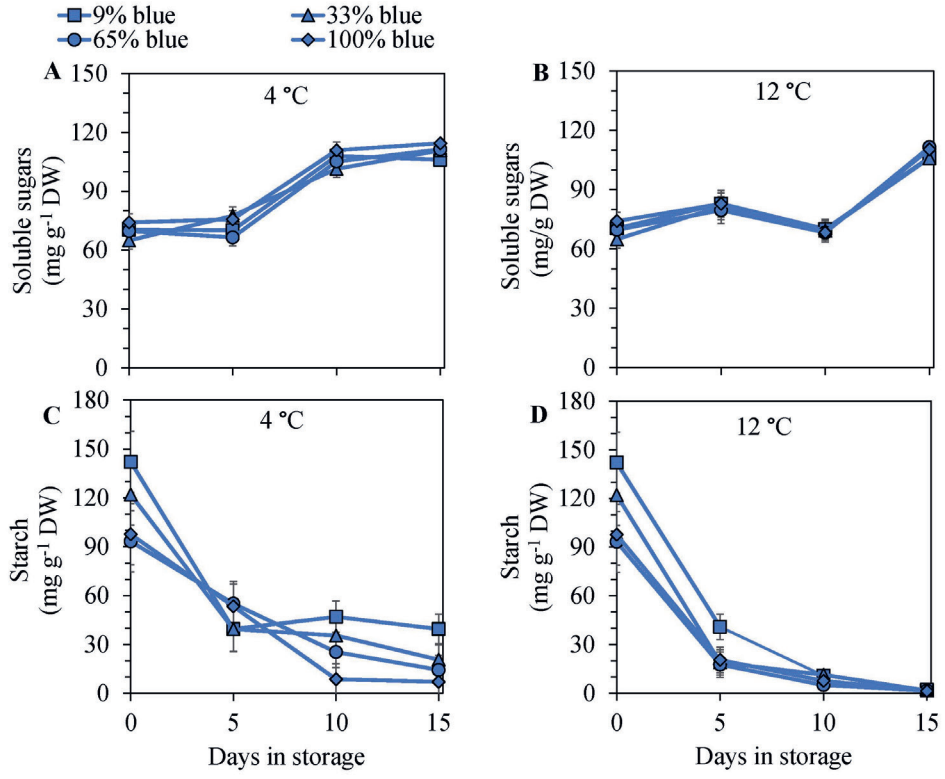

Figure S1. Changes in metabolite levels during postharvest storage at 4 (panels A,C,) and 12° C (panels B,D,) in basil cv Dolly. Plants were previously treated with different EOP to different blue ratios (Exp 1). A and B. change in soluble sugars (sum of glucose, fructose and sucrose), C and D, change in starch. Data are means of 2 blocks (n=2) with 4 replicate plants per block. Error bars represent standard errors of means, when larger than symbols. Letters indicate significant differences (α=10%) at individual time points.

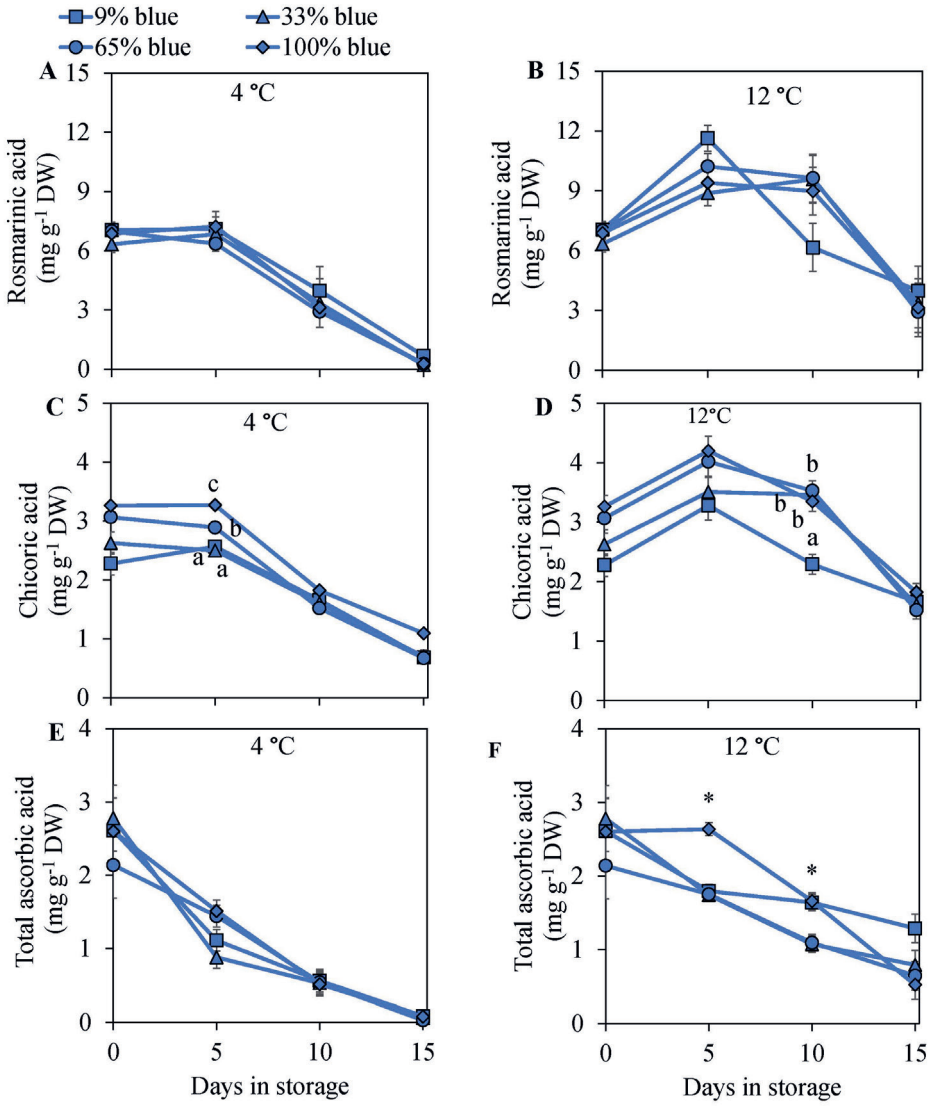

Figure S2. Changes in metabolite levels during postharvest storage at 4 (panels A, C, E) and 12° C (panels B, D, F) in basil cv Dolly exposed to End-Of-Production (EOP) treatments. Plants were harvested after 40 days of cultivation. Plants were previously grown under red-white (PPFD of 300 µmol m^{-2} s^{-1}, 9% blue) light and later exposed to different %blue light (PPFD of 300 µmol m^{-2} s^{-1}) the last five days before harvest as EOP treatment (PPFD of 300 µmol m^{-2} s^{-1}). A and B change in rosmarinic acid, C and D. change in chicoric acid, E and F. change in total ascorbic acid. All values are expressed per gram dry weight in the leaves. The data are means of two blocks (n=2) (i.e. per block four replicate plants). Standard errors of means are shown as error bars. Letters indicate significant differences (α=10%) at individual time points, (Exp. 1).

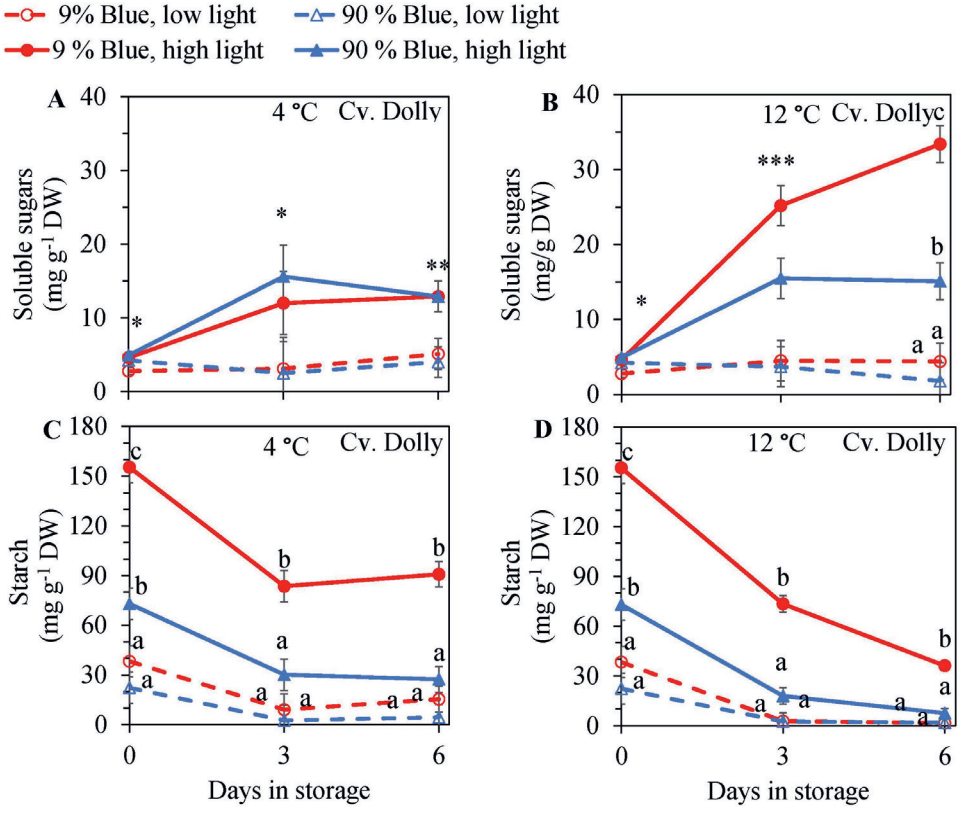

Figure S3. Changes in metabolite levels during postharvest storage at 4 (panels A, C) and 12° C (panels B, D) in basil cv Dolly. Plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9% blue). The last five days before harvest plants were exposed to different EOP blue light ratios 9 % or 90 % at low light (100 µmol m^{-2} s^{-1}) (open symbols) or high light (300 µmol m^{-2} s^{-1}) (closed symbols). A and B soluble sugars (sum of glucose, fructose and sucrose), C and D, change starch. All values are expressed per g DW in leaves. Data are means of 3 blocks (n=3) with 4 replicate plants per block. Error bars represent standard errors of means, when larger than symbols. If no interaction was found but only the main effects were significant the indicated with *p*-values; $p<0.05$ *, $p<0.01$ **, $p<0.001$ *** are depicted with either a blue (percentage of blue light) or black asterisk (PPFD). Letters indicate and interactive effect between the two main effects (percentage of blue light and PPFD), (Exp 2).

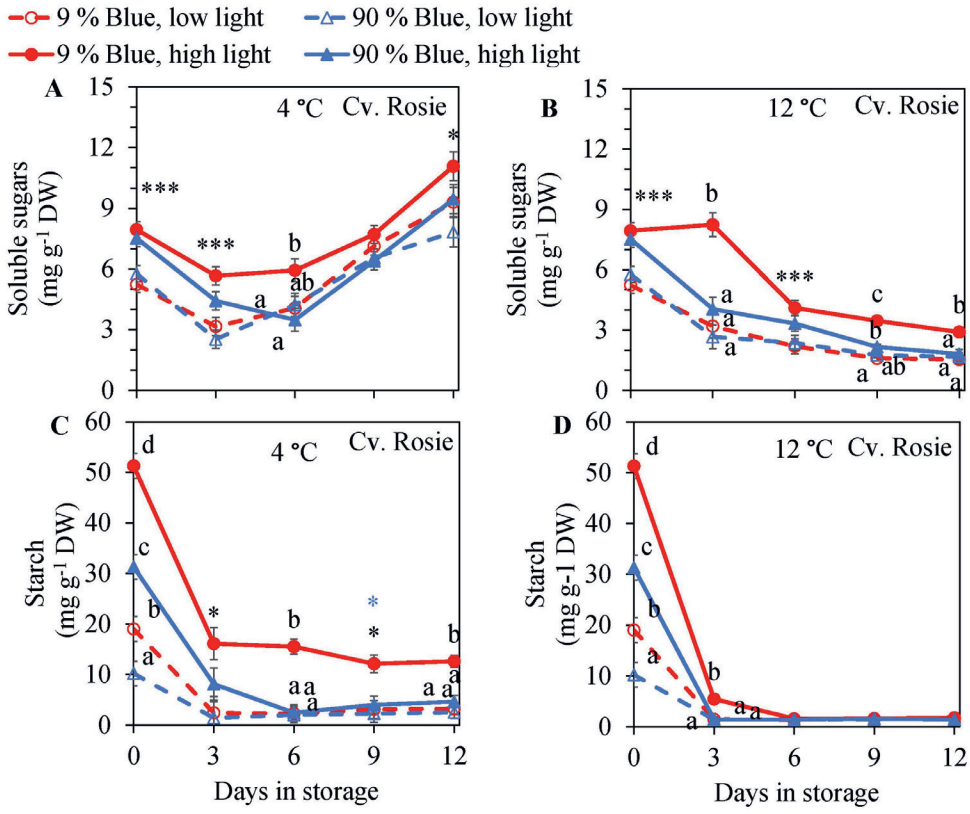

Figure S4. Changes in metabolite levels during postharvest storage at 4 (panels A, C) and 12° C (panels B, D) in basil cv Rosie. Plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9% blue). The last five days before harvest plants were exposed to different EOP blue light ratios 9 % or 90 % at low light (100 µmol m^{-2} s^{-1}) (open symbols) or high light (300 µmol m^{-2} s^{-1}) (closed symbols). A and B soluble sugars (sum of glucose, fructose and sucrose), C and D, change starch. All values are expressed per g DW in leaves. Data are means of 4 blocks (n=4) with 4 replicate plants per block. Error bars represent standard errors of means, when larger than symbols. If no interaction was found but only the main effects were significant the indicated with *p*-values; *p<0.05* *, *p<0.01* **, *p<0.001* *** are depicted with either a blue (percentage of blue light) or black asterisk (PPFD). Letters indicate and interactive effect between the two main effects (percentage of blue light and PPFD), (Exp 2).

Chapter 4

Far-red during cultivation improves postharvest chilling tolerance

Dorthe H. Larsen, Leo F.M. Marcelis, Diederick van Kempen, Wouter Kohlen, Celine C.S. Nicole, Ernst J. Woltering

Abstract

Basil (*Ocimum basilicum* L.) is a temperature sensitive plant and suffers from chilling injury (CI), especially in the postharvest phase. We investigated the effect of additional FR during cultivation at two temperatures on postharvest chilling tolerance. Basil was cultivated in a vertical farm set-up under red-white light and 25 °C. During the last 3 weeks before harvest, plants were maintained at a high temperature (i.e. 25 ºC) or exposed to a low temperature (i.e. 15 ºC). Both at high and low temperature, plants were exposed for different durations to additional FR (180 µmol m^{-2} s^{-1}). After harvest, leaves were stored at 4 and 12 ºC in darkness and overall visual quality and maximum quantum yield of PS II (Fv/Fm) as indicators of chilling injury were monitored every third day for 15 days. Furthermore, abscisic acid (ABA) and jasmonic acid (JA), carbohydrates, and antioxidants were measured at harvest and after 9 days of storage at 4 °C. Additional FR improved the chilling tolerance (i.e. reflected in overall visual quality and F_v/F_m). This effect of FR was observed at both cultivation temperatures. There was no effect of lowering the cultivation temperature on postharvest chilling tolerance. Both ABA and JA levels in basil leaves at harvest were not affected by FR. This indicates that ABA and JA are not involved in development of FR-induced chilling tolerance in basil. FR did also not affect the levels of antioxidants such as rosmarinic acid, chicoric acid and total ascorbic acid at harvest. However, the levels of soluble sugars and starch were increased under additional FR. The improved chilling tolerance in basil cultivated under a low R:FR ratio may be due to the increase in soluble sugars and starch.

4.1 Introduction

Basil (*Ocimum basilicum* L.) is of tropical origin and sensitive to temperatures below 10-12 °C during growth, transport or storage (Lange and Cameron, 1994), which results in chilling injury (CI). CI symptoms in basil are the development of dark necrotic spots on the leaf surfaces. Thus, basil cannot be transported or stored together with many other herbs and leafy greens which often occurs at about 2-7 °C. Low temperature induces the transition of the cell membrane lipid bilayer from a liquid to a solid gel phase, which leads to membrane malfunctioning and loss of membrane semi-permeability which finally is results in cell death (Raison & Orr, 1986). During CI the chloroplasts are the major organelle which is affected. CI results in a decrease in maximum quantum yield of photosystem II (PSII) which can be measured by F_v/F_m. CI does not occur homogenous in the leaves, therefore chlorophyll fluorescence imaging has been shown to be a good measurement for assessing the degree of chilling damage to the leaves (Hogewoning & Harbinson, 2007). Apart from the transition from liquid to gel phase of cell membranes, CI has been reported to cause oxidative stress with an increase in reactive oxygen species (ROS) (Mittler, 2002). However, in basil ROS have not been consistently shown to increase during cold storage (**Chapter 2**). Several methods have been attempted to improve postharvest quality in basil. Ethylene has been associated with development of CI. Postharvest application of 1-MCP blocks the ethylene sensitivity and was able to lessen CI (Berry *et al.*, 2010; Hassan and Mahfouz, 2010). Hot air treatments, where application of one stress factor should make more resistant to another stress (i.e. low temperature) were successfully applied to improve chilling tolerance (Aharoni *et al.*, 2010). Pre-harvest light treatments with high light intensity have also been investigated. Although the high light considerably increased the antioxidant content, it did not improve chilling tolerance in basil (**Chapter 2**). Light spectrum plays a role in the cold acclimation of many plants of temperate origin. Particularly the ratio between red (R, 600-700 nm) and far-red (FR,700-800 nm). In nature increased amount of FR light (i.e. resulting in a R:FR ratio<1) is a signal for plant to cold acclimate along with the induction of the C-REPEAT BINDING FACTOR (CBF) pathway (Linkosalo and Lechowicz, 2006; Franklin and Whitelam, 2007). R and FR light are sensed by the plant photoreceptors from the phytochrome family (phyA to phyE). The biologically inactive form (Pr) of phytochromes absorbs red light and converts to the physiologically active (Pfr) form which absorbs FR through chromophore isomerization when red light is absorbed. The light activated phytochromes then translocate into the nucleus where they can interact with the Phytochrome Interacting Factors (PIFs) where they control a wide range of processes (Franklin and Whitelam, 2004). The phytochrome mediated responses are induced

by the transition of Pr to Pfr. The degree and rate at which the response occurs are defined by the Pr:Pfr ratio which is mainly determined by the R:FR ratio (Duek and Fankhauser, 2005). This can also be described as the phytochrome photostationary state (PSS). The definition of PSS is the ratio of the active Pfr to the total amount (Pfr+Pr) of phytochrome at equilibrium (Sager *et al.*, 1988; Both *et al.*, 2017).

FR light improved the chilling tolerance of tomato leaves through induction of *CBF* gene expression and the accumulation of ABA and JA (Wang *et al.*, 2016*a*). We aimed at improving chilling tolerance of basil through additional FR and lowered temperature during cultivation. We investigated if basil chilling tolerance could be improved in response to additional FR and to a lowered temperature during cultivation. Basil was cultivated at either high temperature (i.e. 25 ºC) or low temperature (i.e. 15ºC) and addition of different duration of FR. Plants cultivated at high temperature received either no, 1 or 3 weeks additional FR and the low temperature cultivated plants no or 3 weeks additional FR. We show that a lowered cultivation temperature alone does not improve basil postharvest chilling tolerance, but that additional FR improved chilling tolerance independent of cultivation temperature. The improved chilling tolerance was not related to ABA, JA or antioxidant levels. We suggest that increased levels of carbohydrates in FR-treated plants are responsible for the improved chilling tolerance.

4.2 Materials and Methods

4.2.1 Experimental set-up

Basil (*Ocimum basilicum* L.) cv. Emily (Enza Zaden, the Netherlands) was grown in a climate chamber, in a vertical farming set-up as described in **Chapter 5**. The individual growth compartments had a size of 0.8 x1.3x1 m (w x l x h) and were separated from each other by with reflective white plastic. The seeds were sown as individual seeds in polystyrene trays with 240 stone wool plugs (Grodan Rockwool B.V., The Netherlands). Plants were cultivated under 150±5 µmol m^{-2} s^{-1} red-white LED light (GreenPower LED production module 120 cm, DeepRedWhite, Philips Eindhoven, the Netherlands). The red-white LED light consisted of 9 % blue (B) (400-500 nm) 19 % green (G) (500-600 nm) and 70 % red (R) (600-700 nm) and 1% far-red (FR) (700-800 nm) light. The light spectrum was measured with a spectroradiometer (SS-110; Apogee Instruments, Logan, UT, United States). Day length was 18 hours. The temperature during the day and night was 25±1°C the relative humidity was set at 75±10%, logged with Keytag dataloggers (KTL-508, Keytag, the Netherlands). The CO_2 level was at ambient concentrations. The plants with the most similar morphology were transplanted after 10 days to 7.5x7.5x6.5 cm

stone wool blocks (Grodan Rockwool B.V., The Netherlands), after which the planting density was 123 plants m^{-2}. Throughout the growth plants were well-watered with an ebb and flood system based on plant needs and growth stage with a nutrient solution consisting of NO_3^- 8.5 mM, SO_4^{2-} 3.9 mM, HPO_4^{2-} 1.5mM, NH_4^+ 1.5 mM, K^+ 5.5 mM, Ca^{2+} 4.0 mM, Mg^{2+} 1.5 mM, Cl^- 0.2 mM, Fe^{3+}/Fe^{2+} 30µM, Mn^{2+} 5 µM, Zn^{2+} 5 µM, $H_2BO_3^-$ 35 µM, Cu^+/Cu^{2+} 1 µM, MoO_4^{2-} 1 µM nutrition of pH 5.7 with EC 1.7 dS m^{-1} before transplanting. After transplanting the nutrient solution had an EC of 2.3 dS m^{-1} and the concentration of nutrients were adjusted accordingly.

4.2.2 Treatments

During the last 3 weeks before harvest, basil plants were cultivated at either a high (25 ºC) or low temperature (15 ºC). Plants cultivated at high temperature received before harvest either no, 1 or 3 weeks additional FR. Plants cultivated at low temperature received before harvest either no or 3 weeks additional FR. The light intensity of FR was 180 µmol m^{-2} s^{-1} (GreenPower Production module, 120 cm, Far Red, Philips Eindhoven, the Netherlands) (Table 1). The number of degree-days was kept the same between the high and low temperature cultivated plants. The low temperature plants were grown for a total of five weeks after transplant of which two weeks were at 25 ºC and thereafter for three weeks at 15 ºC; the high temp plants were grown for three weeks after transplant at 25 ºC. The degree-days were calculated with 10 °C as base temperature. It was estimated that below 10 °C no growth activity would occur. For the low temperature treatments, the shift in temperature from 25 ºC to 15 ºC was instant.

Morphological data and growth parameters for the far-red at high temperature are reported in **Chapter 5**. These data include plant height, leaf area, fresh and dry mass at the day of harvest.

Table 1. Overview of treatments. The last three weeks before harvest plants were cultivated at either high (25 °C) or low (15 °C) temperature at a PPFD of 150 µmol m^{-2} s^{-1} of red-white LED light. In addition, plants were treated with 180 µmol m^{-2} s^{-1} of FR light. Photosynthetic photon flux density (PPFD) from 400-700 nm. Photon flux density (PFD) from 400-800 nm. The calculation of R:FR was calculated as red (600-700 nm):far-red (700-800 nm). Phytochrome photostationary state (PSS) were calculated according to Sager et al (1988).

Temperature during the last 3 weeks (°C)	Duration of FR (week)	PPFD (µmol m^{-2} s^{-1})	Far-red (µmol m^{-2} s^{-1})	PFD (µmol m^{-2} s^{-1})	R:FR	PSS
25	-	150	2	152	61.7	0.88
25	1	150	180	330	0.6	0.62
25	3	150	180	330	0.6	0.62
15	-	150	2	152	61.7	0.88
15	3	150	180	330	0.6	0.62

4.2.3 Postharvest sampling

For postharvest storage, leaves were stored in plastic boxes, 16 x11x6 cm. Per box leaves from two plants were stored with three leaf pairs from each plant excluding the oldest and youngest (underdeveloped) pair. The boxes were mounted with two pieces of wet filter paper in the bottom, plastic on top of the paper to avoid direct contact with the leaves and a plastic separator between the leaves from the two plants. In the clear lids nine holes with a 1 mm syringe needle were made. Due to the wet filter paper a high humidity was maintained in the boxes; the holes in the lid prevented any built up of ethylene and CO_2. The boxes were randomized and stored in darkness at 4 or 12 °C. To log the temperature and relative humidity in the boxes keytag dataloggers (KTL-508, Keytag, the Netherlands) were used. The relative humidity and temperature were within 90-100 % RH and ±1 °C of the temperature (4 or 12 °C) in the boxes.

The sampling and measurements were carried out on day 0 (at harvest) and 3, 6, 9, 12 and 15 days after harvest. During each sampling day, leaves from four plants per block per light treatment were sampled (i.e. two storage boxes each containing leaves from two plants). The chilling injury was determined by an overall visual quality (OVQ) score and measurement of maximum quantum yield of dark-adapted leaves of PSII (F_v/F_m). Following the measurements, the leaves from 4 °C were frozen in liquid nitrogen and ground with an IKA-A 11 basic analytical mill (im-lab, Belgium). Before further analysis, samples were stored at -80 °C. Metabolites were analyzed on day 0, day 6 and 9. Metabolite levels at day 6 and day 9 did not differ and their values were therefore averaged (data not shown).

4.2.4 Overall visual quality

Overall visual quality (OVQ) was evaluated as described in **Chapter 2**. Based on visual symptoms a score was given from 1 to 8, with 1 indicating the worst quality and 8 the best. Symptoms which reduced the score included dark spots and discoloration, fungal appearance, degree of crispness, degree of wilting, leaf shininess and presence of characteristic curved leaf shape (Table S1, **Chapter 2**). The decrease in overall visual quality was calculated as a percentage of the initial score.

4.2.5 PSII efficiency F_v/F_m

F_v/F_m was measured as described in **Chapter 2**. Per stored box one leaf from the upper leaf-pair and one leaf from the middle leaf pair of each plant were measured. Leaves were dark adapted at 20°C for 20 min and chlorophyll fluorescence was measured using a PSI closed Fluorcam 800-C chlorophyll fluorescence imaging system (PSI, Czech Republic). Fluorcam software Version 7 was used to operate the fluorcam and analyze the obtained images, following the method of (Hogewoning and Harbinson, 2007).

4.2.6 Carbohydrates

Carbohydrates were measured as described in **Chapter 2**. Carbohydrates were extracted from 300±30 mg frozen ground basil leaves with 5 mL of 85 % ethanol for 20 min at 80°C in a shaking water bath. Extracts were centrifuged for 5 min at 8500 RCF (Universal 320R, Hettich). For further analysis of carbohydrates 1 mL of the supernatant was used and the pellet was stored at -20 °C for starch analysis. One milliliter of the supernatant was dried in a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific) at 50 °C and 5.1 mbar for 120 min.

Samples were re-suspended in 2 mL of 0.01 N hydrochloric acid, sonicated for 10 min (Branson 2800) and centrifuged for 5 min at 21.100 RCF (Sorvall Legend Micro 21R, Thermo Fisher Scientific). Amino acids other amino compounds were removed by trapping on a SPE column (UCT CLEAN-UP BCX columns, 100mg/1ml), eluted with 0.01 N hydrochloric acid.

Glucose, fructose and sucrose were quantified using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD; Dionex ICS5000, Thermo Fisher Scientific), with a CarboPac1 column (250x2 mm) eluted with 100 mM NaOH at a flow rate of 0.25 mL min^{-1} at 25 °C.

For starch determination the pellet was washed with 80 % ethanol three times and dried for 20 min in a vacuum centrifuge at 50 °C and 5.1 mbar. The dried pellet was

resuspended in 2 mL 1 g L^{-1} thermostable alpha-amylase (SERVA Electrophoresis GmbH) in MilliQ water and incubated for 30 min at 90 °C. After which 1 mL of 0.5 g L^{-1} amyloglucosidase (Sigma 10115) in 50 mM citrate buffer (pH 4.6) was added and samples were incubated at 60 ˚C for 15 min and centrifuged at 21.100 RCF for 5 minutes. Glucose was quantified using HPEAC-PAD as described above. Data was expressed on the base of dry weight as mg g^{-1} DW.

A conversion factor for each sample was made to convert to dry weight from fresh weight; 100±20 mg of fresh material for each sample were weighed into a reaction tube and vacuum dried for 120 min in a vacuum centrifuge (Savant SpeedVac SPD2010, Thermo Fisher Scientific) at 50°C and 5.1 mbar. Data was expressed on the base of dry weight as mg g^{-1} DW.

4.2.7 Total Ascorbic acid

Total ascorbic acid was measured as described in **Chapter 2**. Ascorbic acid was extracted from 200±20 mg frozen ground basil leaves with 1 mL of 3.3 % meta-phosphoric acid (MPA) in an ultrasonic bath (BRANSON 2800) at 0°C for 10 min in darkness. Samples were centrifuged at 21.100 RCF (Sorvall Legend Micro 21R, Thermo Fisher Scientific) at 4 °C for 10 min. The supernatant was filtered through a 0.45 µm cellulose filter into an HPLC vial for analysis of ascorbic acid (AsA). For total AsA analysis 100 µl of the extract was mixed with 50 µl of 5mM dithiothreitol in 400mM Tris base and after 15 minutes 50 µl 8.5% o-phosphoric acid was added. AsA was quantified using a HPLC consisting of a GS50 pump (Dionex), a 340S UV-VIS detector (Dionex) and a MIDAS autosampler (Spark Holland) equipped with a ProntoSIL 120-3 C18 AQ, 250x3mm column (Knauer). The column was eluted with 400 µL L^{-1} H3PO4 + 2.5 mL L^{-1} MeOH + 0.1 mM EDTA in H_2O followed by a wash step with 30% acetonitrile in H_2O at a flow rate of 0.35 mL min^{-1}. AsA was detected at 243 nm. The TAsA amount was calculated as the sum of the AsA directly measured and the AsA measured following conversion of dehydroascorbic acid (DHA) to AsA. Data was expressed on the base of dry weight as mg g^{-1} DW.

4.2.8 Rosmarinic acid and chicoric acid

The most abundant antioxidants in basil rosmarinic and chicoric acid were measured as described in **Chapter 2**. Rosmarinic and chicoric acids were extracted at room temperature from 400 ±20 mg frozen ground leaves with 2.5 mL of acetonitrile with 2.5 % formic acid. Samples were extracted for 15 minutes in an ultrasonic bath (Branson 2800) and centrifuged at 10000 RCF for 15 min at 4°C (Universal 320R, Hettich). The supernatant was filtered through a 0.45 µm cellulose syringe filter into a HPLC vial. Rosmarinic acid and Chicoric acid were analyzed according to the

method of Kwee and Niemeyer (2011), with modifications on a HPLC (Waters) with a UV dual wavelength detector and autosampler, and a Vydac 201TP54 column (C18, 5 µm, 300 Å, 4.6 mm × 250 mm). The compounds were eluted with 2.5 % formic acid in H_2O (mobile phase A) and acetonitrile (mobile phase B) with a linear gradient of: 85% A, 0.0 min.; 75% A, 6.0 min.; 0% A, 8.5 min.; [0% A, 9.0 min.; 85% A, 11.5 min.; 85% A, 14.0 min]. Phenolic acids were detected at 330 nm.

4.2.9 Hormones

Hormone content (jasmonic acid and abscisic acid) was measured according to Gühl *et al.* (2021). Briefly, 10 mg of ground frozen basil leaves were extracted with 1 mL of 100 % methanol (MeOH) with internal standards 1 mL of 100 % methanol (MeOH) containing stable isotope-labeled internal standards (IS, Table S1). Internal standards were used at an end concentration of concentration of 100 nM per compound per sample. All solvents were evaporated in a speed vacuum system (SPD121P, ThermoSavant, Hastings, UK) at RT and the residue stored at -20˚C until further analysis. Samples were resuspended in 100 µL of acetonitrile/water (0.1% formic acid) (20:80, v/v), and filtered through a 0.45 mm Minisart SRP4 filter (Sartorius, Goettingen, Germany). Analyses of plant hormones was performed by comparing retention times and mass transitions with those of unlabeled standards (Table S1) using a Waters XevoTQs mass spectrometer equipped with an electrospray ionization source coupled to an Acquity UPLC system (Waters, Milford, USA) as previously described (Schiessl *et al.*, 2019; Gühl *et al.*, 2021).Chromatographic separations were conducted on an Acquity UPLC BEH C18 column (100 mm, 2.1 mm, 1.7 mm; Waters, USA) by applying an acetonitrile/water (0.1 % formic acid) gradient. The column was operated at 40 °C with a flow rate of 0.25 mL min^{-1}. The acetonitrile/water (0.1% formic acid) gradient started from 20 % (v/v) acetonitrile, increasing to 70 % (v/v) acetonitrile in 17 min. The sample injection volume was 3 µL. Cone and desolvation gas flows were set to 150 and 1000 l h^{-1}, respectively. The capillary voltage was set at 3.5 kV, the source temperature at 150 °C, and the desolvation temperature at 550 °C (Waters, Milford, USA). Argon was used for fragmentation by collision-induced dissociation. Multiple reaction monitoring (MRM) was used for quantification (Gühl *et al.*, 2021). To determine sample concentrations, a 10-point calibration curve was constructed for each compound ranging from 1 µM to 190 pM and each dilution also contained a known amount of an appropriate deuterium-labelled internal standard.

4.2.10 Statistical set up and analysis

The experiment was carried out in a complete randomized block design. Each light treatment was repeated in separate compartments (each compartment was considered a block). Due to treatments with different temperature the high and low temperature treatments were sown with two weeks between them and treatments were done sequentially. First the high temperature plants were harvested after which the temperature was lowered to initiate treatment of the low temperature treated plants. The border plants were not used in the analysis. At harvest, in each block, 4 replicate plants of each treatment were sampled for chemical analyses. The remaining plants were prepared for postharvest storage. The leaves from 2 plants, were packed together in one plastic box (as described above) and the boxes were stored at 4 and 12 °C. At each postharvest sampling point, 2 boxes (leaves from 4 plants) per block were taken from the storage for visual observation and fluorescence measurements (4 replicate plants). For chemical analyses the stored leaves at 4 °C were measured. For the chemical analysis in each block the leaves from 4 plants were analyzed as a pooled sample. Each mean is based 2 blocks x 4 replicate plants.

All data was analyzed with Genstat (VSN International, 19th Edition). For all parameters the assumptions of homogeneity and normality of the residuals were tested with Bartlett's test and Shapiro-Wilk test respectively. If data did not follow these assumptions, it was transformed with the natural logarithm. Subsequently data was analyzed by a two-way ANOVA for each time point and individual storage temperature. The posthoc test Fishers protected LSD was conducted with a probability level of α=10% because the experiment had two blocks.

4.3 Results

4.3.1 Changes in hormones and metabolites

During the last 3 weeks before harvest basil plants were cultivated at either a high (25 ºC) or low temperature (15 ºC) and plants were exposed to different durations of additional FR. Plants cultivated at high temperature received either no, 1 or 3 weeks additional FR; plants cultivated at low temperature plants received either no or 3 weeks additional FR. Hormone content (abscisic acid and jasmonic acid) and metabolite content (soluble sugars and starch, antioxidants: rosmarinic acid, chicoric acid and total ascorbic acid), and volatile organic compounds (VOCs) were measured at harvest and at day 6 and 9 of storage at 4 ºC. The two time points (day 6 and day 9 of storage) were averaged as the values between day 6 and 9 did not differ (data not shown).

The ABA content at harvest was not affected by the cultivation temperature nor by the addition of FR (Fig. 1A). ABA content in low temperature cultivated plants showed a slight increase during subsequent cold storage; ABA content in high temperature cultivated plants showed a slight decrease during subsequent cold storage. This effect was independent of the FR application (Fig. 1B).

The JA content at harvest was much higher in high temperature cultivated plants than in low temperature cultivated plants (Fig. 1C). The JA content at harvest was not affected by FR application. After 9 days of cold storage, the JA content had decreased compared to the content at harvest. During postharvest storage a steeper decrease in JA content in high temperature cultivated plants that hard received FR was observed (Fig. 1D).

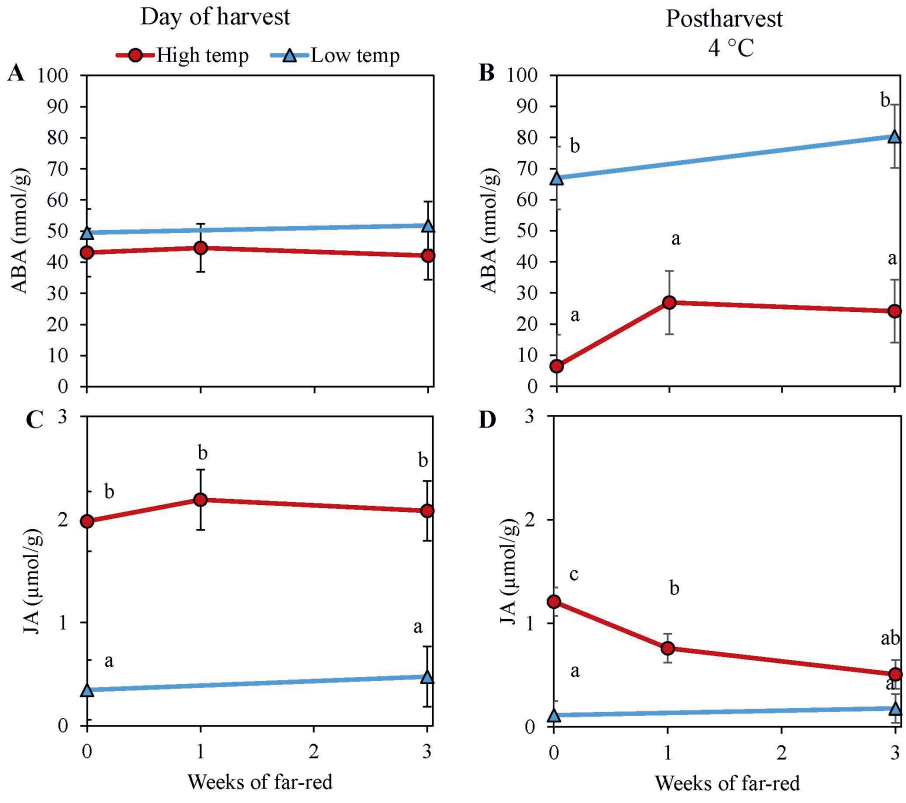

Figure 1. The content of abscisic acid (ABA) (A, B) and jasmonic acid (JA) (C, D) in basil cv. Emily at harvest and during postharvest storage. Plants were grown under different far-red and temperature treatments i.e. at a high temperature (25 ºC) and no, 1 or 3 weeks additional FR (circle, red) or at a low temperature (15 ºC) and no or 3 weeks FR (triangle, blue). During postharvest storage leaves were stored at 4 °C for 6 and 9 days and the average values are shown. Each data point is a mean of 2 blocks (n=2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences among all treatments at harvest or during postharvest storage (α=10%).

Soluble sugars and starch content at harvest increased in response to FR and to the low temperature. Interestingly, at both high and low temperature the addition of FR resulted in a doubling of the soluble sugar content (Fig. 2A) compared to the no FR treatment. At low temperature FR increased the starch content threefold, whereas FR had no effect on the starch content in high temperature cultivated plants (Fig. 2C). During postharvest storage at 4 ºC the content of soluble sugars (Fig. 2B) increased, whereas the content of starch (Fig. 2D) decreased compared to the levels at harvest (Fig. 2 A, C).

The increase in carbohydrate status at harvest in plants from low temperature cultivation and additional FR was reflected in considerable higher carbohydrate (in particular soluble sugar) levels during the postharvest storage.

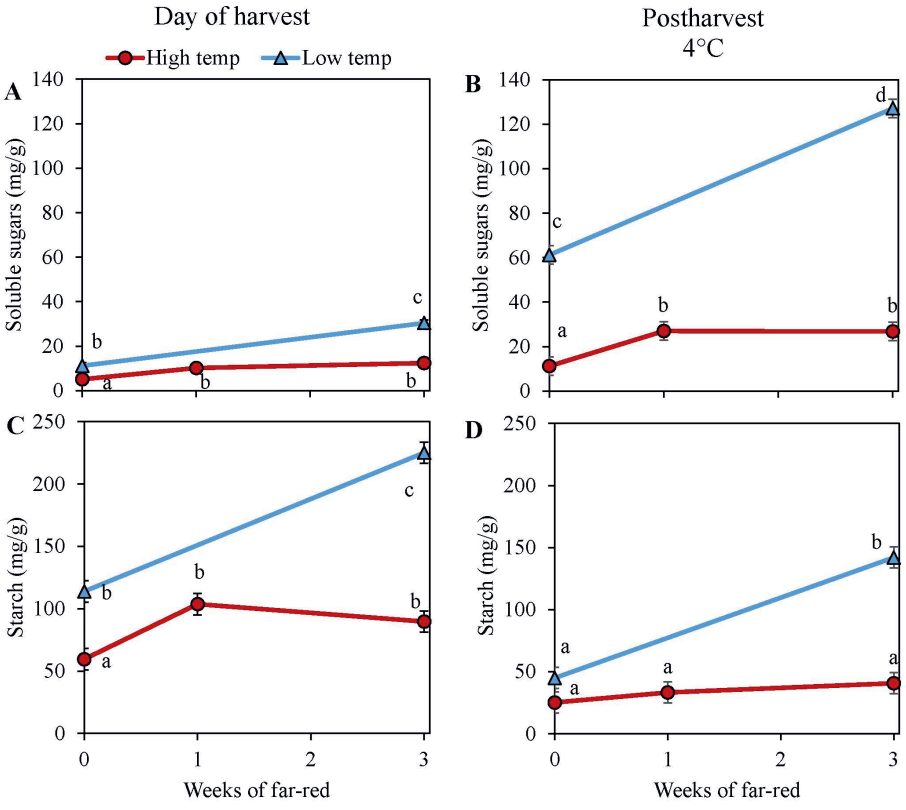

Figure 2. Soluble sugars (sum of glucose, fructose and sucrose) and starch in basil cv. Emily at harvest (A, C) and in postharvest storage (B, D). Plants were grown under different far-red and temperature treatments i.e. at a high temperature (25 ºC) and no, 1 or 3 weeks additional FR (circle, red) or at a low temperature (15 ºC) and no or 3 weeks FR (triangle, blue). During postharvest storage leaves were stored at 4 °C for 6 and 9 days and the average values are shown. A, B. Soluble sugars, C, D. Starch. The data are given per gram dry weight in the leaves. Each data point is a mean of 2 blocks (n=2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences among all treatments at harvest or during postharvest storage (α=10%).

Cultivation at low temperature increased the levels of rosmarinic acid at harvest (Fig. 3A). There was no effect on chicoric and total ascorbic acid (Fig. 3C, E). At both cultivation temperatures, there was no clear effect of FR on the levels of these antioxidants at harvest. A trend was observed towards lower levels of rosmarinic and chicoric acids at the longer FR applications. But the trend was only statistically significant for the rosmarinic acid level in low temperature cultivated plants. After 6 to 9 days (data were averaged) of storage at 4 °C, rosmarinic and chicoric acids levels were generally higher than the levels at harvest, irrespective of the cultivation temperature and FR treatments (Fig. 3B, D). The opposite was true for the total ascorbic acid levels that decreased during postharvest storage (Fig. 3F). There was little effect of FR application on the postharvest levels of these antioxidants. In general, the trends observed at harvest were still existing after storage. The levels of the main volatile organic compounds (VOCs) in the cv. Emily at harvest were not affected by cultivation temperature nor by the application of FR (Fig. S1). During postharvest storage at 4 °C, the content of volatiles did not show a significant change compared to the at harvest content (Fig. S1).

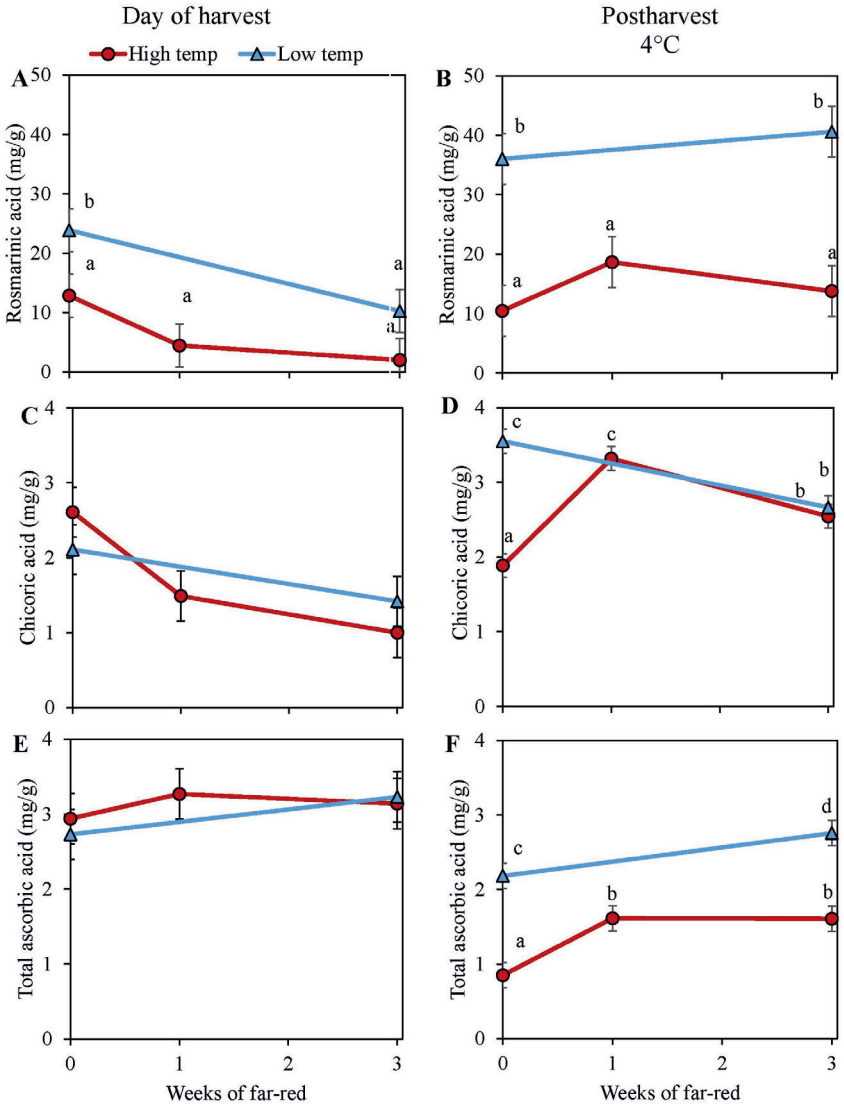

Figure 3. Antioxidants in basil cv. Emily at harvest (A ,C, E) and in postharvest storage (B,D,F). Plants were grown under different far-red and temperature treatments i.e. at a high temperature (25 ºC) and no, 1 or 3 weeks additional FR (circle, red) or at a low temperature (15 ºC) and no or 3 weeks FR (triangle, blue). During postharvest storage leaves were stored at 4 °C for 6 and 9 days and the average values are shown. A, B. Rosmarinic acid, C, D Chicoric acid, E, F Total ascorbic acid. The data are given per gram dry weight in the leaves. Each data point is a mean of 2 blocks (n=2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences among all treatments at harvest or during postharvest storage (α=10%).

4.3.2 Changes in chilling injury parameters during postharvest storage in response to FR and temperature

During postharvest storage at 4 and 12 °C overall visual quality (OVQ) and maximum quantum yield of PSII of dark-adapted leaves (F_v/F_m) as a marker for chilling injury were measured. Low temperature during cultivation resulted in a lower overall visual quality (OVQ) at harvest due to yellowing of the youngest leaves (Fig. 4). OVQ of leaves decreased faster during storage at 4 ºC than at 12 °C as the leaves showed clear signs of chilling injury at 4 ºC (i.e. dark necrotic spots). Both at 4 and 12 °C storage, the decrease in OVQ over time was not affected by cultivation temperature.

Additional FR during cultivation delayed the decrease in OVQ, especially in the leaves stored at 4 °C. This indicates that additional FR induced chilling tolerance both in plants cultivated at high and at low temperature. In leaves stored at 12 ºC the longer duration of FR treatment in high temperature cultivated plants delayed the decrease in OVQ compared to the other treatments. This indicates that additional FR for a longer duration (3 weeks) during the cultivation is beneficial in improving overall postharvest quality (i.e. both at chilling and non-chilling storage conditions). The F_v/F_m values of leaves at harvest of the low temperature cultivated plants were lower than the high temperature leaves indicating that the plants were stressed during cultivation at 15 ºC (Fig. 5). F_v/F_m values showed a decrease during storage at 4 °C and stayed at the original level during storage at 12 °C. This indicates that chilling injury was apparent at 4 °C. Additional FR, apart from increasing the value at harvest (in low temperature cultivated plants) also delayed the decrease during storage at 4 °C in both leaves from low and high temperature cultivated plants. Overall, the patterns observed in F_v/F_m were very similar to the patterns observed in QVQ.

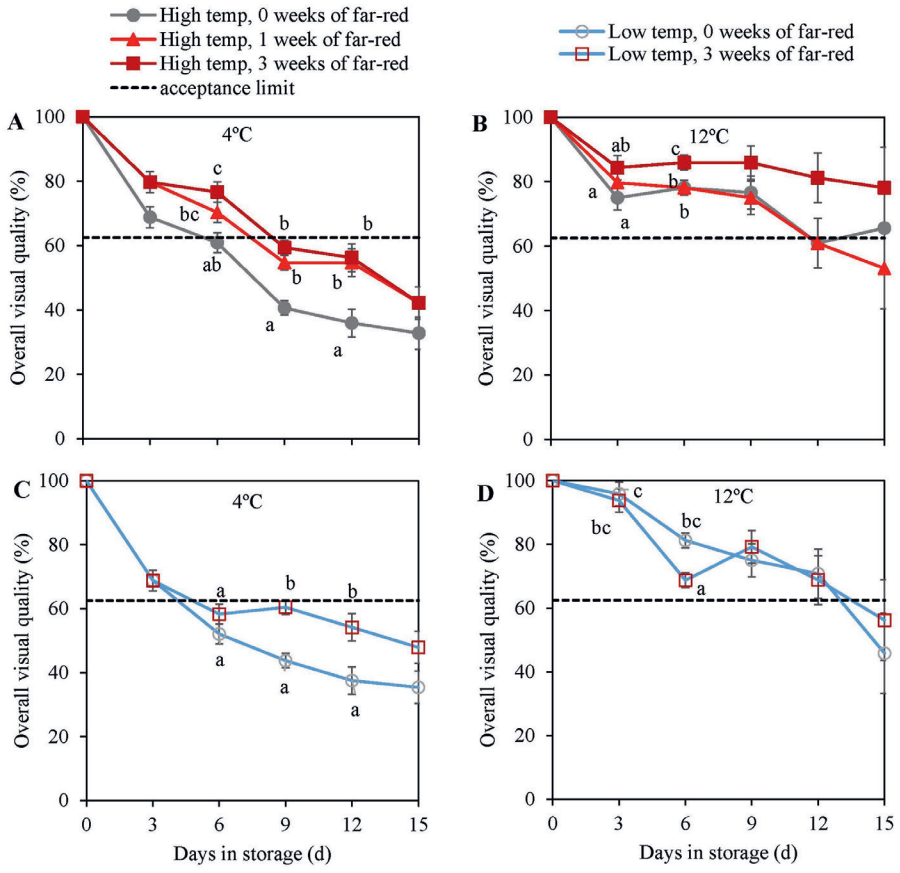

Figure 4. Changes in overall visual quality during postharvest storage at 4 (panels A,C) and 12 °C (panels B,D) in basil cv. Emily. Plants were previously grown under different far-red and temperature treatments at a high temperature (25 ºC) and no, 1 or 3 weeks additional FR (closed symbols or at a low temperature (15 ºC) and no or 3 weeks FR (open symbols). Each data point is a mean of 2 blocks (n=2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences (α=10%) for the different time points at each storage temperature.

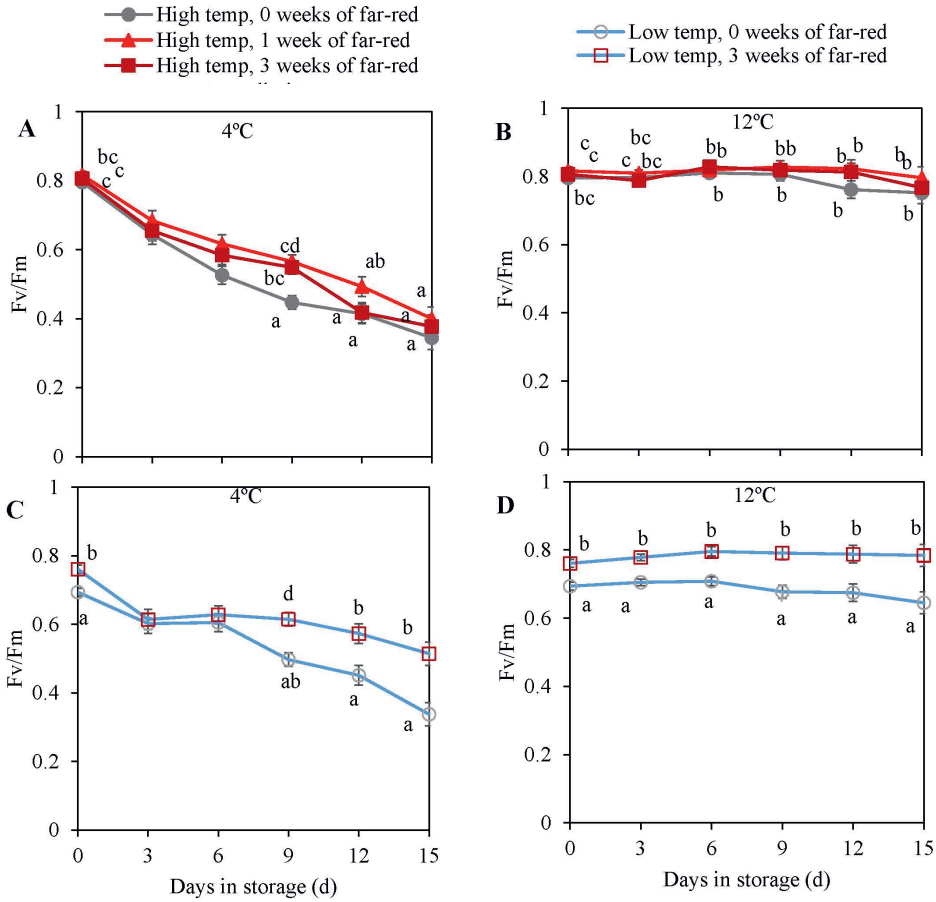

Figure 5. Changes in maximum quantum yield of PSII of dark-adapted leaves (F_v/F_m) (i.e. a marker for chilling injury) during postharvest storage at 4 (panels A, C) and 12 °C (panels B, D) in basil cv. Emily. Plants were grown under with different far-red and temperature treatments at a high temperature (25 ºC) no, 1 or 3 weeks additional FR (closed symbols) or at a low temperature (15 ºC) and no or 3 weeks FR (open symbols). Each data point is a mean of 2 blocks (n=2) and 4 replicate plants from each block. The error bars are errors of means. If letters are present, they indicate significant differences (α=10%) for the different time points at each storage temperature.

4.4 Discussion

4.4.1 Addition of FR during cultivation improves postharvest chilling tolerance in basil

Addition of FR (i.e. a R:FR ratio < 1) has been shown to increase cold tolerance in a number of plant species (i.e. Arabidopsis, tomato, barley) (Franklin and Whitelam, 2007; Ádám *et al.*, 2016; Wang *et al.*, 2016*a*). We investigated if addition of FR during cultivation would improve the postharvest chilling tolerance in basil. We found that additional FR indeed improved the chilling tolerance as based on measurements of overall visual quality and F_v/F_m (Fig. 5A, C, 6A, C). This was true for basil leaves both from plants cultivated at a low (15 °C) and a high (25 °C) temperature. Overall visual quality has previously been shown to correlate well with measurements of chilling injury (i.e. measured with chlorophyll fluorescence) (**Chapter 2**). Similar to our observations, Affandi *et al.* (2020) found that addition of FR light during cultivation improved the postharvest chilling tolerance in tomato fruit. However, Affandi *et al.* (2020) used a much lower intensity of FR (of 30-50µmol m^{-2} s^{-1}). In tomato fruit, application of FR can improve the physical and biochemical properties of the fruit i.e. through an increase of biosynthesis of the cuticle wax and ascorbic acid content eventually leading to less weight loss in chilled fruit (Cozmuta *et al.*, 2016).

In tomato leaves additional FR and chilling tolerance have been related to an increase in both ABA and JA content, which that play a role in the induction of the CBF pathway (Wang *et al.*, 2016*a*). In contrast to the findings of Wang *et al.* (Wang *et al.*, 2016*a*) we did not find an increase in ABA and JA content at harvest in response to additional FR (Fig. 1A,C). During postharvest storage at 4 °C JA content in basil leaves decreased. The strongest decrease was found in the FR treated plants at high cultivation temperature (Fig. 1). Thus, FR did not positively stimulate the hormone content in the present study. An increase in ABA and JA have been found to be beneficial for the plant to overcome abiotic stress such as cold stress (Hu *et al.*, 2017; Prerostova *et al.*, 2021). Application of exogenous ABA in basil (Satpute *et al.*, 2019) and JA in Arabidopsis (Hu *et al.*, 2013) and JA in blood oranges (Habibi *et al.*, 2019) increased chilling tolerance functioning upstream of the CBF pathway. We expected low temperature cultivation to increase ABA and JA content at harvest, however, neither ABA nor JA increased in response to low temperature cultivation. Similar to our study Liu *et al.* (2020) did not find an increase in JA in response to low temperature and cold acclimation of *Rhododendron*. In general, the optimal sampling time for studies involving hormones may be difficult to pinpoint as the peak of hormones may be transient. Based on our findings of ABA and JA (i.e. which

should activate the CBF pathway) we cannot confirm that the CBF pathway is in play in basil.

The improved chilling tolerance in basil cultivated under additional FR may have been due to the observed increase in soluble sugars and starch. Similar to our findings increases in soluble sugars were found in a number of species such as cucumber (leaves, stem and roots) (Xiong *et al.*, 2011), watermelon (leaves and stem) (Ranwala *et al.*, 2002) and tomato (leaves) (Courbier *et al.*, 2020) when additional FR was applied during cultivation. Phytochromes are highly involved in the allocation of carbon to leaves and production of biomass. When Arabidopsis mutants had a lower biomass compared to the wild type when they are missing phytochromes (Yang *et al.*, 2016). When plants are subjected to low temperature sugar and starch often accumulate in the leaves as an acclimation response (Ristic and Ashworth, 1993). This was also the case in our experiment where the low cultivation temperature also increased the soluble sugars and starch in the leaves. A high content of starch may help the plant mitigate abiotic stress such as cold stress as starch can serve as an energy reserve. The low temperature cultivation may result in limited photosynthesis resulting in limited carbon availability (Thalmann and Santelia, 2017). Under storage (in darkness) conditions starch functions as a carbon reserve that can provide energy for respiration. Yet, a delicate balance between carbohydrates and reactive oxygen species may exist. The results in **Chapter 2** showed that high light intensities increased the content of soluble sugars and starch, but it was not sufficient to improve chilling tolerance in basil as also a high light induced increase in ROS may have been in play. Sugar, in addition, can act as an osmoprotectant to protect thylakoid membranes in the chloroplast (Santarius, 1973), which in turn could improve chilling tolerance. This is in line with our results, which suggest that an increase in sugar and starch may aid in basil chilling tolerance.

4.4.2 Additional FR had no effect on antioxidants or VOCs

Antioxidants may improve chilling tolerance as they can scavenge reactive oxygen species (ROS) (Das and Roychoudhury, 2014). Additional FR has been found to increase the content of rosmarinic acid in basil (Schwend *et al.*, 2016) and ascorbic acid in lettuce (Chen *et al.*, 2016) and *Phaseolus vulgaris* (Bartoli *et al.*, 2009). In contrast, Li *et al.* (2021) found that additional FR had a negative effect on the total ascorbic acid in lettuce. We found that addition of FR had no or only minor effects on antioxidants; in combination with low temperature cultivation rosmarinic acid decreased whereas no effect on chicoric or total ascorbic acid was observed at harvest (Fig. 3). During storage at 4 °C the content of phenolic antioxidants such as rosmarinic acid is expected to decrease due to their scavenging of ROS (Fratianni *et*

al., 2017). However, we found that the content of rosmarinic and chicoric acid significantly increased during cold storage. Thus, the increase in ROS during cold storage may not have surpassed the scavenging potential of rosmarinic and chicoric acid.

Ascorbic acid decreased during cold storage which may either be related to its role in scavenging oxidants or due to the fact that the product was stored in darkness. Ascorbic acid biosynthesis is increased with light intensity (Ntagkas *et al.*, 2018). When Arabidopsis plants were stored in darkness the ascorbic acid decreased (Yoshimura *et al.*, 2014). Volatile organic compounds are important for the quality of basil as they make up the characteristic aroma and flavour profile of basil (Carvalho *et al.*, 2016). FR increased the volatiles in petunia (Colquhoun *et al.*, 2013), whereas it had no effect on sensory properties in tomato fruit (Dzakovich *et al.*, 2017). Carvalho *et al.* (2016) found an increase in sesquiterpenoids but not monoterpenoids. In accordance, we did not find an effect of additional FR or the low temperature treatments on the main volatile organic compounds (the monoterpenoids eugenol, eucalyptol and linalool) in basil at harvest (Fig. S1A, C).

4.4.3 Low temperature during cultivation did not improve chilling tolerance

In the present experiment basil was cultivated at both a high and a low temperature (i.e. 15 and 25 °C). It was expected that the cultivation at 15 °C (i.e. without FR) would improve chilling tolerance. Low temperature cultivation may have a positive effect on accumulation of carbohydrates and antioxidants. In an acclimation response to low temperature plants accumulate soluble sugars (Yuanyuan *et al.*, 2010). When plants were cultivated at 15 °C we indeed found an increase soluble sugars and starch (Fig. 2). Furthermore, we found rosmarinic acid to be higher at low temperature cultivation. Low temperatures were found to have a positive effect on rosmarinic acid content in spearmint (Fletcher et al., 2005) and in *Plectranthus scutellarioides* (Dörr et al., 2019). However, in *Dracocephalum moldavica* temperature did not have an effect on rosmarinic acid content (Khaleghnezhad et al., 2019). Different from our findings, ascorbic acid has also been found to increase in spinach cultivated at low temperature (Proietti et al., 2009) whereas we did not find a difference in total ascorbic acid between high and low temperature cultivated basil.

Plants may better tolerate low temperature if they have prior been exposed to short duration of low temperature resulting (as a priming effect) (Baier *et al.*, 2019) or during prolonged exposure (acclimation) (Yuanyuan *et al.*, 2010). In both cases we would expect leaves from plants cultivated at low temperature to have a better

postharvest chilling tolerance. However, when OVQ and F_v/F_m was measured for the plants cultivated at 15 °C we observed that the youngest leaves were light green to yellow at harvest and had a lower value of F_v/F_m indicating that the plants were stressed by the low temperature. The temperature was probably too low and/or duration too long resulting in stress rather than a priming or acclimation effect. Thus, the increase in soluble sugars, starch and rosmarinic acid in low temperature cultivated plants was probably not sufficient to overcome the stress induced by low temperature. Therefore, cultivation at low temperature (15 °C) as applied in this research, does not aid in basil chilling tolerance. Applying a more moderate decline of the temperature or applying low temperature for a shorter duration may be an interesting avenue for further research.

4.5 Conclusion

Cultivating basil at low temperature did not improve postharvest chilling tolerance. Addition of FR either applied at low or high cultivation temperature improved postharvest chilling tolerance. The improved chilling tolerance was associated with an increase in soluble sugars and starch at harvest. There was no effect of FR on the content of antioxidants (rosmarinic acid, chicoric acid and total ascorbic acid) or hormones (abscisic acid and jasmonic acid) at harvest. This suggested that antioxidants, JA and ABA do not play a role in regulating FR-induced CI, but that carbohydrates do play a role.

Supplementary material

Table S1. Multiple reactions monitoring (MRM) transitions table for all plant growth regulators and corresponding internal standards used in this study.

Number	Compound	Retention Time	Mass*	MRM transition	Cone V.	Coll. Energy
			+143.1	124.9	20	14
4	ABA	4.15	-263.25	153.15‡	30	10
				219.15	30	15
5	$^{[2H6]}$ABA	4.12	-269.25	159.15‡	30	10
				225.15	30	15
6	JA	5.78	-209.06	59.1‡	40	12
			+211.1	133.1	16	16
	$^{[2H5]}$JA	5.76	-214.25	61.9‡	40	12
			+216.2	135.29	14	12

*Mass in positive (+) or negative (-) ion mode. ‡transition used for quantification

Volatile organic compounds

Volatile organic compounds (VOCs) were determined by solid phase micro extraction (SPME) coupled with gas chromatography mass spectrometry (Trace GC with TRIPLUS, Thermo Scientific) analysis. VOCs were identified by comparing their mass spectra and retention time to the compounds in the National Institute of Standards and Technology United States Government library (http://www.nist.gov) and pure standards (Extrasynthese). For analysis 300 mg of fresh frozen ground basil leaves were weighed into a 20 mL crimp cap vial and 5 mL of 5M calcium chloride was added for headspace SPME/GC-MS detection. The peak areas of the VOCs were integrated, and the relative amounts obtained.

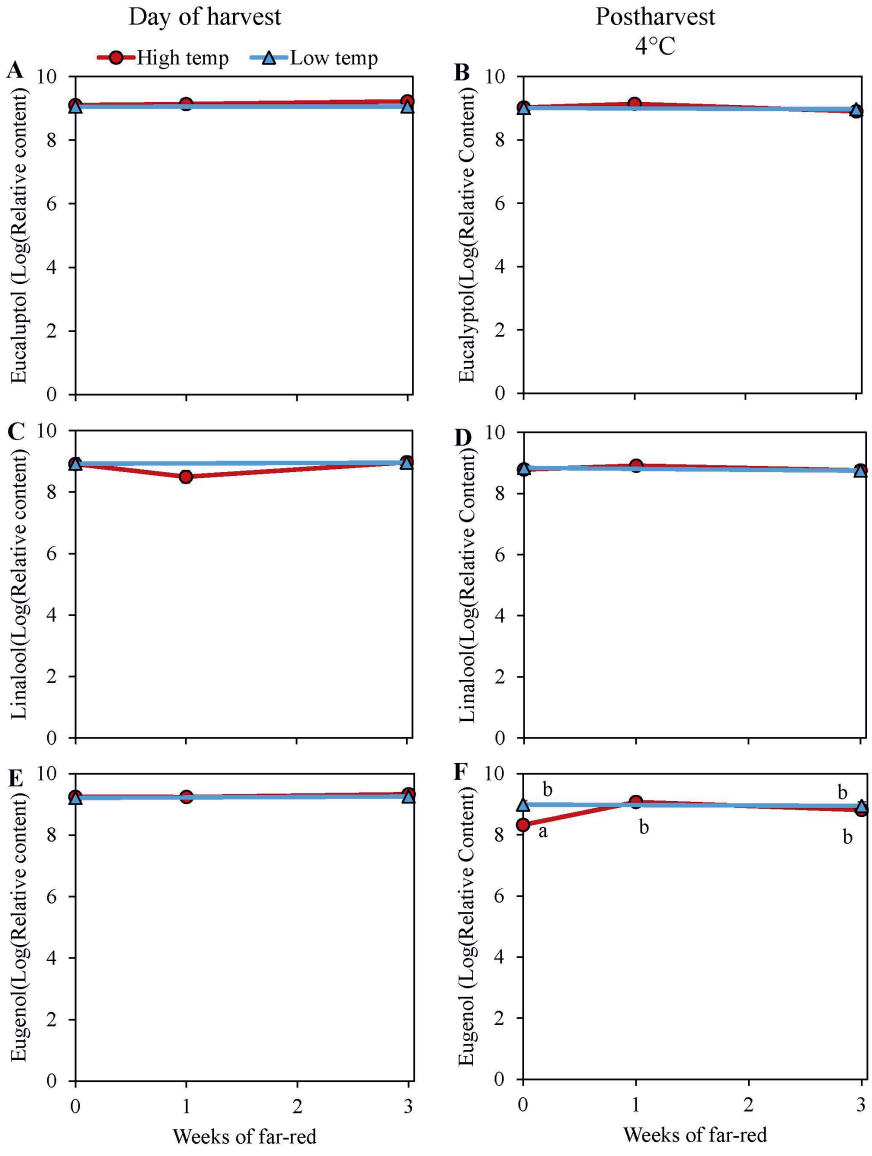

Figure S1. Volatiles in basil cv. Emily at harvest (A, C, E) and in postharvest storage (B, D, F). Plants were grown under different far-red and temperature treatments i.e. at a high temperature (25ºC) no, 1 or 3 weeks additional FR (circle, red) or at a low temperature (15 ºC) and no or 3 weeks FR (triangle, blue). During postharvest storage leaves were stored at 4°C for 9 days. A, B. eucalyptol, C, D. Linalool, E, F. eugenol. The data are given per gram dry weight in the leaves. Each data point is a mean of 2 blocks (n=2) and 4 replicate plants from each block. The error bars are errors of means. Letters indicate significant differences at harvest or during postharvest storage respectively (α=10%).

Chapter 5

Response of basil growth and morphology to light intensity and spectrum

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Abstract

Vertical farming is becoming increasingly popular for production of leafy vegetables and herbs, with basil (*Ocimum basilicum* L.) as one of the most popular herbs. In basil most research has focused on increasing secondary metabolites with light spectra. However, knowledge about the effect of light intensity (photosynthetic photon flux density, PPFD) and spectra on growth and morphology is key for optimizing quality at harvest. The impact of PPFD and spectrum on plant growth and development is species dependent and currently few studies in basil are available. Understanding the response to end-of-production (EOP) light of growth and morphology is important for successful vertical farming. We performed a comprehensive series of experiments, where the effects of EOP PPFD, fraction of blue and their interaction on the growth and morphology were analyzed in two green and one purple basil cultivar. In addition, the impact of different EOP intensities and duration of far-red were investigated. We found that increasing the PPFD increased fresh mass, dry matter content and plant height in all three cultivars. The responses were linear or quadratic depending on the cultivar. A high fraction of blue (>90%) increased plant height and decreased the dry mass partitioning to the leaves. The only interaction found between the fraction of blue and overall PPFD was on plant height in the green cultivar whereas other growth parameters and morphology responded stronger to PPFD than to the fraction of blue light. Plant dry matter production was increased with the addition of far-red. Far-red EOP intensity treatments enhanced the fraction of dry mass partitioned to the leaves, whereas a prolonged far-red treatment enhanced partitioning to the stem. Both plant fresh mass and dry matter content were improved by applying high PPFD shortly before harvest. Light spectra were found to be of less importance than PPFD with respect to plant dry matter content. Light Use Efficiency (LUE) based on fresh mass decreased with increasing PPFD whereas LUE based on dry mass increased with increasing PPFD, when given as EOP treatments. The overall physiological mechanisms of the light intensity and spectral effects are discussed.

5.1 Introduction

Vertical farming systems, also called plant factories, are indoor growth facilities with plants grown in multiple layers. In a vertical farm, plants are grown in a closed system without the use of pesticides and all climate factors can be controlled (SharathKumar *et al.*, 2020). Controlling the pre-harvest factors can have a great influence on the growth and morphology as well as postharvest quality (Mattheis and Fellman, 1999). Light is the primary source of energy for plants and the dominant light source in a vertical farm is Light Emitting Diodes (LEDs) which makes a vertical farm efficient and allows for year-round production. LEDs are energy efficient, they have a low heat emission, the light intensity can be adjusted and light spectra can be modulated (Kusuma *et al.*, 2020). Leafy vegetables and herbs are often the crops of choice in vertical farms due to fast growth, low plant height and high retail price (Touliatos *et al.*, 2016). One popular culinary herb is basil (*Ocimum basilicum* L.) that is used for its unique aroma. Besides aroma other important quality parameters include yield, plant morphology and fresh mass and dry matter content (Maness, 2003; Zhou *et al.*, 2012). However, there has been little research on elucidating the response to light intensity and spectra of yield and dry matter content in basil.

Plant development, yield and dry matter content are highly affected by light intensity. Light intensity used for photosynthesis is defined as photosynthetic photon flux density (PPFD) ranging from 400-700 nm (McCree, 1972; Poorter *et al.*, 2019). Increased light intensity generally correlates with an increase in net photosynthesis which can increase plant fresh mass and yield. Furthermore, an increase in light intensity can increase soluble sugars which are part of the dry matter. In basil, plant growth and dry matter content were found to increase under increasing light intensity but only until an optimum after which the plants might be limited by other environmental factors (Pennisi *et al.*, 2020). Yet, Kelly *et al.* (2020) found in lettuce biomass increased linearly with PPFD. In addition to PPFD, light spectrum is important for morphological features, specifically the partitioning of carbon to leaves vs stem. Some of the most studied light spectra include ratios of blue (400-500 nm) and red (600-700 nm) and addition of far-red (700-800 nm) to PPFD. While red is the most efficient color for photosynthesis and energy use, 100 % red often disturbs normal morphology (i.e. leaf curling, thin and pale leaves). It is important to add blue to the spectra for optimal morphology. Blue light plays a role in several plant processes such as photomorphogenesis, stomatal opening, and leaf photosynthetic functioning (Hogewoning *et al.*, 2010; Boccalandro *et al.*, 2012). An optimum of blue light could exist for photosynthetic capacity as well as for biomass accumulation (Kaiser *et al.*, 2019). Fresh mass (Li and Kubota, 2009) and dry matter

(Kalaitzoglou *et al.*, 2019) can also increase with the addition of far-red. Furthermore, far-red has been associated with increased leaf area and plant height in basil (Carvalho *et al.*, 2016) which could increase light interception. Plant height might also increase under 100 % blue light (Heo *et al.*, 2002; Johnson *et al.*, 2020). However, the opposite effect has been reported in several studies where a high fraction of blue light resulted in more compact plants (Hoenecke *et al.*, 1992; Islam *et al.*, 2012; Keuskamp *et al.*, 2012). In basil, contradictory reports exist with respect to the plant growth and morphology response to light spectra. Plant height was neither affected by 100% blue light compared to greenhouse grown basil (Carvalho *et al.*, 2016), nor the addition of blue to a red light spectra (20-60 % blue) in a greenhouse (Jensen *et al.*, 2018), or the response to blue light was entangled with addition of far-red (Bantis *et al.*, 2016). Piovene *et al.* (2015) reported 37 % blue had a positive effect while Pennisi *et al.* (2019) found that a fraction of blue above 30 % had a negative effect on fresh mass. However, the optimal PPFD, as well as spectra with respect to fraction of blue and addition of far-red light for plant growth have been found to be highly species dependent (Kim *et al.*, 2006; Colonna *et al.*, 2016).

Several studies have focused on increasing the secondary metabolites in basil, however, fewer studies have elucidated the effect of PPFD, light spectra and the interaction of the two on the growth and morphological features. The primary attribute of crops in a commercial production system is biomass, that is fresh and dry mass of leaves. Other relevant attributes include morphology such as short internodes and increased partitioning of carbon to the leaves. Knowledge of the response of basil to changes in light intensity and spectra will allow for a fully controlled plant production and a desired growth and morphology. To optimize production in vertical farming, it has been proposed to focus the lighting strategy during the first part of cultivation cycle on optimizing biomass increase, while the last period before harvest the lighting strategy should focus on optimizing product quality by End-Of-Production (EOP) treatments (SharathKumar *et al.*, 2020). We aimed at understanding the response of growth and morphology of basil to PPFD, fraction of blue light and far-red. In addition, we wanted to study the response to EOP light applied five to seven days before harvest. To study this, we set up a comprehensive series of (five) studies, in a vertical farming set-up with green and purple basil cultivars.

5.2 Materials and Methods

5.2.1 Growth conditions

Basil (*Oscimum basilicum* L.) was grown in a climate chamber in a vertical farming set-up with twelve compartments of the size 0.8 x1.3 m in table area and a plant density of 123 plants m^{-2}. Two green cultivars (Emily and Dolly) and one purple cultivar (Rosie) Sweet basil were used; all cultivars were derived from Enza Zaden, NL. Seeds were germinated under red-white LED light (GreenPower LED production module 120 cm, DeepRedWhite, Phillips Eindhoven, the Netherlands) varying between 150 and 200 µmol m^{-2} s^{-1} (Table 1). The spectral intensities in Experiment 1 to 4 were measured with a spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, Netherlands), and in Experiment 5 with another spectroradiometer (SS-110; Apogee Instruments, Logan, UT, USA). Phytochrome Photostationary state (PSS) values were calculated according to Sager *et al.* (1988). PPFD was regularly measured with a quantum sensor (LI-190SB quantum sensor, LI-1400 Datalogger, LI-COR Bioscience, Lincoln, USA) to adjust the height of the light frames during the growth and maintain a constant light intensity at the top of the plants throughout each experiment. The sides of each compartment were covered with white reflective plastic to increase light uniformity. Seeds were sown in trays with 240 stone wool plugs (Grodan Rockwool B.V., The Netherlands) with one seed per plug. After 10-15 days the morphologically most similar plants were selected and transplanted to 7.5x7.5x6.5 cm stone wool blocks (Grodan Rockwool B.V., The Netherlands), one outer row surrounding the plants were border plants and not used for the experiment. Day/night temperature was kept at 25 °C, the relative humidity was set at 75 % and CO_2 was ambient concentration. Relative humidity and temperature in each light treatment were recorded with either keytag dataloggers (KTL-508, Keytag, NL) or Hanwell data loggers (ML4160, Hanwell Solutions, UK) with deviations within 10% and 1°C from the set points. To maintain air temperature around 25°C fans were installed in high light treatments above the lamps blowing out of the individual compartments. Plants were kept well-watered through an ebb and flood system based on plant needs and growth stage. For the first three weeks of the growth, plants were watered once every second day for 10 min and after that once every day for 10 min. High light and high blue treatments were given an extra round of watering when needed. The nutrient solution consisted of NO_3^- 8.5 mM, SO_4^{2-} 3.9 mM, HPO_4^{2-} 1.5 mM, NH_4^+ 1.5 mM, K^+ 5.5 mM, Ca^{2+} 4.0 mM, Mg^{2+} 1.5 mM, Cl^- 0.2 mM, Fe^{3+}/Fe^{2+} 30µM, Mn^{2+} 5 µM, Zn^{2+} 5 µM, $H_2BO_3^-$ 35 µM, Cu^+/Cu^{2+} 1 µM, MoO_4^{2-} 1 µM with pH 5.7 and EC 1.7 dS m^{-1} before transplant and with an EC of 2.3 dS m^{-1} after transplant.

5.2.2 Experimental set-up

Five different experiments were performed (summarized in Table 1). In Experiment 1 the response of cultivars Emily and Dolly to different light intensities applied as EOP treatments during five days before harvest was investigated. Seeds of both cultivars germinated for 15 days under 150 µmol m^{-2} s^{-1}. After transplant, the light intensity was kept at 150 µmol m^{-2} s^{-1} for another 15 days. During the whole growth period a light spectrum with red-white LED was used and a day length of 18 hours. EOP treatments were given for five days and included light intensities of 50, 150, 300 and 600 µmol m^{-2} s^{-1} respectively.

In Experiment 2 the response of cultivar Dolly to different fractions of blue light applied either throughout the growth (25 days) or as EOP treatment during five days before harvest were investigated. The different fractions of blue light were created by using different ratios between pure blue (GreenPower LED production module, 120 cm, Blue, Phillips Eindhoven, the Netherlands) and red-white LEDs. Seeds germinated for 15 days under 150 µmol m^{-2} s^{-1} red-white LED light. After transplant the plants were exposed for 25 days to four different blue light (400-500 nm) treatments of 9%, 33%, 65% and 100 % out of the total PPFD of 300 µmol m^{-2} s^{-1}. In three other treatments the plants were grown under red-white light of 300 µmol m^{-2} s^{-1} for 20 days after which they were exposed for five days to different blue light treatments of 33%, 65% and 100%. Day length was 16 hours.

In Experiment 3 the response of cultivars Rosie and Dolly to EOP treatments with increased fractions of blue light and the interaction with PPFD during five days before harvest were investigated. Seeds of both cultivars germinated under 200 µmol m^{-2} s^{-1} red-white LED light for 15 days. After transplant the plants were grown for another 15 days under red-white light of 200 µmol m^{-2} s^{-1}. Five day before harvest plants were exposed to treatments of 100 µmol m^{-2} s^{-1} PPFD with 9% blue, 100 µmol m^{-2} s^{-1} PPFD with 90% blue, 300 µmol m^{-2} s^{-1} PPFD with 9% blue and 300 µmol m^{-2} s^{-1} PPFD with 90% blue. The different fractions blue light was created by using different ratios between pure blue (GreenPower LED production module, 120 cm, Blue, Phillips Eindhoven, the Netherlands) and red-white LEDs. Day length was 18 hours.

In Experiment 4 the response of cultivar Emily to EOP treatments with increasing intensities of far-red in addition to the PPFD during five days before harvest were investigated. Seeds germinated under 150 µmol m^{-2} s^{-1} red-white LED light for 15 days. After transplant the PPFD was increased to 300 µmol m^{-2} s^{-1} of red-white LED light for 15 days. EOP treatments were applied five days before harvest with 0, 50

or 100 µmol m^{-2} s^{-1} far-red (GreenPower Production module, 120 cm, Far Red, Phillips Eindhoven, the Netherlands) added to the 300 µmol m^{-2} s^{-1} of red-white LED light. This resulted in treatments with a total photon flux density (PFD) of 303, 350, 400 µmol m^{-2} s^{-1} (400-800 nm). Day length was 16 hours.

In Experiment 5 the response of cultivar Emily to different durations of far-red before harvest were investigated. Seeds germinated under 150 µmol m^{-2} s^{-1} red-white LED light for 10 days. After transplant the plants continued to grow under 150 µmol m^{-2} s^{-1} red-white light for another 21 days. No far-red was applied or additional far-red (GreenPower Production module, 120 cm, Far Red, Phillips Eindhoven, the Netherlands) (180 µmol m^{-2} s^{-1}) was applied during one week (as EOP treatment) or three weeks (throughout the growth). This resulted in treatments with a total PFD of 152, 330, 330 µmol m^{-2} s^{-1} (400-800 nm). Day length was 18 hours.

5.2.3 Measurements of growth and morphological parameters

Plant height was measured from the surface of the stone wool block to the height of the apex. Leaves of a minimum size of 1 cm^2 were counted as true leaves, leaf area was measured with a leaf area meter LI-3100C (LICOR, Lincoln, NE, USA). Leaves and stem were separated and weighed for fresh mass and dry mass. Dry mass was measured after drying for 48 hours at 80°C.

Daily light integral ($mol_{400\text{-}700nm}$ m^{-2} d^{-1}) was calculated as:

$$\boldsymbol{PPFD\left(\mu mol\, m^{-2}\, s^{-1}\right) \times day\ length\ (h)\ \times\ 0.0036} \quad (1)$$

Daily radiation integral ($mol_{400\text{-}800nm}$ m^{-2} d^{-1}) was calculated as:

$$\boldsymbol{PFD\left(\mu mol\, m^{-2}\, s^{-1}\right) \times day\ length\ (h) \times\ 0.0036} \quad (2)$$

Light use efficiency (g $mol_{400\text{-}700\ nm}^{-1}$) was calculated as:

$$\frac{\boldsymbol{plant\ mass(g) \times plant\ density(plants\ m^{-2})}}{\boldsymbol{Daily\ Light\ Integral_{(400-700\ nm)}\ (mol\ m^{-2}\ d^{-1}) \times days\ of\ cultivation(d)}} \quad (3)$$

Radiation use efficiency (g $mol_{400\text{-}800\ nm}^{-1}$) was calculated as:

$$\frac{\boldsymbol{plant\ mass(g) \times plant\ density(plants\ m^{-2})}}{\boldsymbol{Daily\ Radiation\ Integral_{(400-800\ nm)}\ (mol\ m^{-2}\ d^{-1}) \times days\ of\ cultivation(d)}} \quad (4)$$

Table 1. Overview of the experiments carried out. PPFD (400-700 nm) and PFD of far-red (700-800 nm) during the initial phase (i.e. from transplant until start of treatments) and treatments, and spectral composition during the treatments. Fractions of the spectra; blue (400-500 nm), green (500-600 nm), red (600-700 nm) and far-red (700-800 nm) are percentages of the total Photon flux density (PFD) (400-800 nm), treatment duration, the Daily light integral (DLI) from 400-700 nm for the initial phase, DLI and Daily radiation integral (400-800 nm) and treatments are given. Phytochrome photostationary state (PSS) were calculated according to Sager et al. (1988).

Experiment	Cultivar	PFD (µmol m^{-2} s^{-1})			Spectra during treatments (%)				Treatment duration (d)	Day length (hours)	DLI Initial phase (mol m^{-2} d^{-1})	DLI Treatment (mol m^{-2} d^{-1})	DRI Treatment (mol m^{-2} d^{-1})	PSS treatment
		Initial phase	Treatment		Blue	Green	Red	Far-red						
		PPFD		Far-red										
Experiment 1 PPFD	Dolly and Emily	150	50	0	9	19	70	1	5	18	9.7	3.2	3.2	
			150	2								9.7	9.9	0.88
			300	3								19.4	19.6	
			600	6								38.9	39.3	
Experiment 2 Blue light fraction	Dolly	300	300	3	9	19	70	1	5 and 25	16	17.3	17.3	17.5	0.88
				2	33	14	51	1					17.4	0.86
				1	65	7	26	0					17.3	0.82
				0	100	0	0	0					17.3	0.49
Experiment 3 Blue light interaction PPFD	Rosie and Dolly	200	100	1	9	19	70	1	5	18	13.0	6.5	6.5	0.88
			100	0	90	2	8	0				6.5	6.5	0.70
			300	3	9	19	70	1				19.4	19.6	0.88
			300	0	90	2	8	0				19.4	19.4	0.70
Experiment 4 Far-red PFD	Emily	300	300	3	9	19	70	1	5	16	17.3	17.3	17.5	0.88
			300	50	9	17	60	14					20.2	0.82
			300	100	8	15	53	25					23.0	0.78
Experiment 5 Far-red duration	Emily	150	150	2	9	19	70	1	21	18	9.7	9.7	9.7	0.88
			150	180	5	9	32	55	7				21.4	0.62
			150	180					21				21.4	0.62

Specific leaf area (cm^2 g^{-1}) was calculated as:

$$\frac{\boldsymbol{Leaf\ area\ (cm^2)}}{\boldsymbol{leaf\ dry\ mass\ (g)}} \quad (5)$$

Dry matter content (%) was calculated as:

$$\frac{\boldsymbol{dry\ mass\ (g)}}{\boldsymbol{fresh\ mass\ (g)}} \times \mathbf{100}\ \% \quad (6)$$

5.2.4 Statistical set-up and analysis

The experiments were carried out as complete randomized block designs. Each experiment was repeated in time, which represented the blocks. In each experiment six small compartments were used for plant growth. Each light treatment was done in one compartment and repeated in time. For each repetition the position of the light treatments in the six compartments were randomized. Generally, 5 or 6 representative plants from the light compartment were sampled for the analyses. For statistical analyses, the average values of each block were used as one replicate. Experiment 1 and 3 (cv. Dolly) were carried out three times, experiment 3 (cv. Rosie) 4 times and experiment 2, 4 and 5 and two times.

Data was analyzed with Genstat (VSN International, 19th Edition). Experiments on light intensity, blue light and far-red were analyzed with One-way Analysis of Variance (ANOVA), while the blue and blue light x light intensity experiment were analyzed with a Two-way ANOVA, followed by posthoc LSD test. Treatment effects were tested at a probability level of 5%, unless an experiment had only two blocks in which case probability level of 10% was applied (Ott and Longnecker, 2010). Furthermore, it was tested with the ANOVA if a polynomial model could explain the effect of the light treatment on the tested variates. Significance of the linear or quadratic component were used as proof of treatment having a significant effect (and additionally if this effect was linear or quadratic). Based on the result of the ANOVA a linear or quadratic trendline was added in Excel (Excel, Microsoft Pro Plus 2019). When no interaction was found in the two-way ANOVAs the overall means were shown. Assumptions of homogeneity and normality were met as tested with Bartlett's and Shapiro-Wilk test, respectively.

5.3 Results

5. 3.1 Response to End-Of-Production PPFD (Experiment 1)

The PPFD during the last five days had a significant effect on all growth parameters in cultivars Emily and Dolly. Plant height, plant fresh mass, leaf area and partitioning of dry mass to the leaves all increased with an increase in PPFD (Fig.1). The response to PPFD was linear or quadratic depending on the different parameters and cultivars. Plant fresh mass displayed a significant linear response to light intensity for cv. Emily while it was a quadratic response for cv. Dolly, indicating that within this range an optimum PPFD exists for cv. Dolly (Fig. 1B). A similar trend was found for plant height between the two cultivars (Fig. 1A), whereas both cultivars had a quadratic response to light for leaf area (Fig. 1C). Plant dry matter content and partitioning to the leaves increased linearly with increase in PPFD for both cultivars (Figs. 1E, F). Specific leaf area (SLA) decreased due to a strong increase in dry mass of the leaves for both cultivars (Fig. 1D). Plants from both cultivars grown under 600 µmol m^{-2} s^{-1} displayed very brittle leaves that easily broke at the petiole and broke easily when handled. For both cultivars light use efficiency (LUE) based on dry mass increased with increasing PPFD but decreased when based on fresh mass (Table 2).

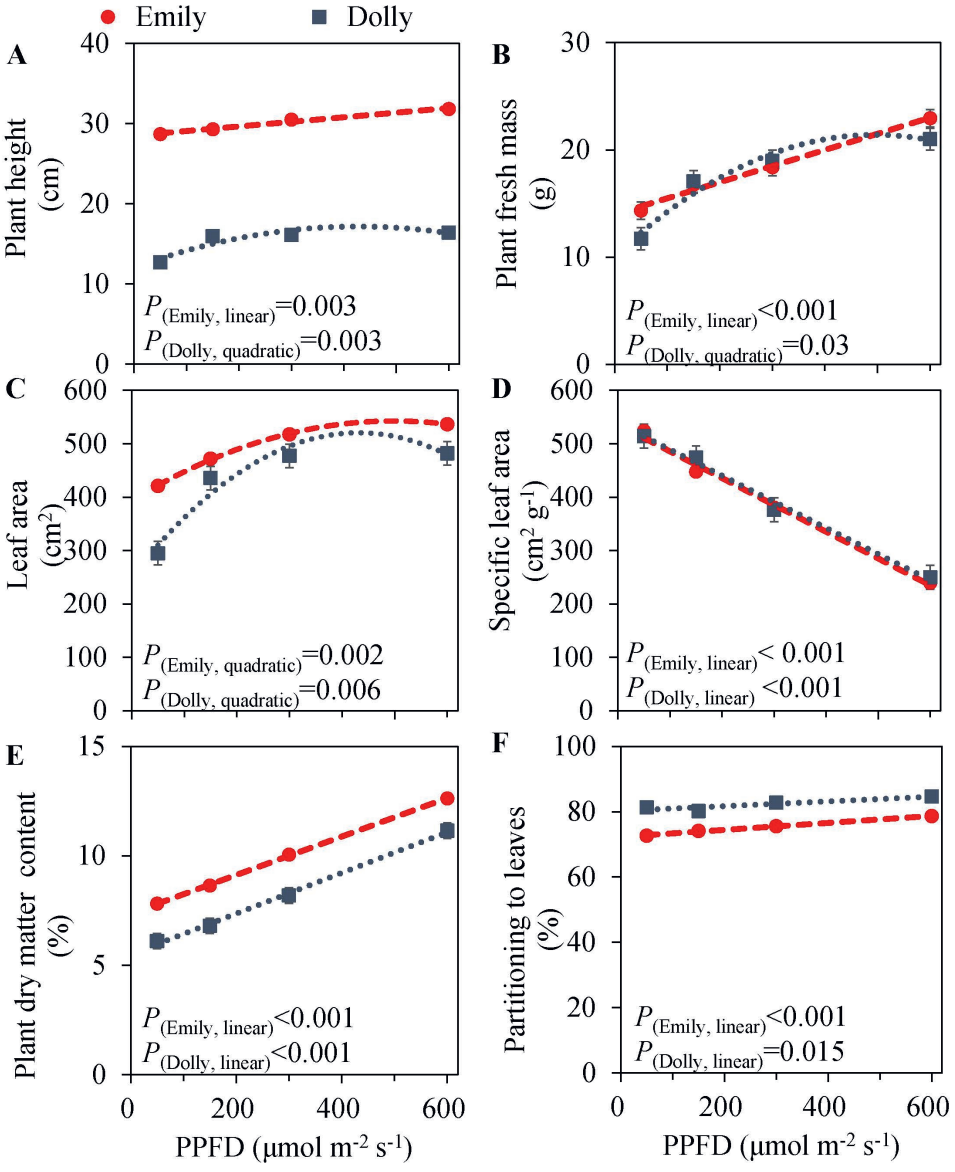

Figure 1. Response of basil cvs. Emily (red circles) and Dolly (grey squares) to different End-Of-Production PPFD (Experiment 1). Plants were grown for 30 days under 150 µmol m^{-2} s^{-1} after which they were exposed to different PPFD (i.e. 50, 150, 300 and 600 µmol m^{-2} s^{-1}) during 5 days before harvest. A. Plant height, B. plant fresh mass, C. leaf area, D. specific leaf area, E. plant dry matter content, F. dry mass partitioning to leaves. Data are means of 3 blocks (n=3) each with 6 replicate plants. Error bars represent standard errors of means, when larger than symbols. For significant quadratic or linear effects of PPFD, trendlines together with the respective *p*-values (α=0.05) are depicted.

Table 2. Overview of Light Use Efficiency (LUE) for plant fresh and dry mass in response to PPFD and fraction blue (Experiment 1-3) and. LUE is based on PPFD incident on the plants accumulated over the initial (i.e. from transplant until start of treatments) and treatment phase. Letters indicate significant differences.

Experiment	Cultivar	Treatment		LUE (g mol^{-1})	
				plant fresh mass	plant dry mass
Experiment 1 PPFD		**PPFD (µmol m^{-2} s^{-1})**			
	Emily	50		10.92 ±0.39 c	0.84 ±0.03a
		150		10.63 ±0.39 bc	0.91 ±0.03a
		300		9.33 ±0.39 ab	0.92 ±0.03a
		600		8.31 ±0.39 a	1.04 ±0.03b
	Dolly	50		8.92 ±0.26 b	0.55 ±0.03 a
		150		10.82 ±0.26 c	0.74 ±0.03 b
		300		9.62 ±0.26 b	0.79 ±0.03 bc
		600		7.60 ±0.26 a	0.83 ±0.03 c
Experiment 2 Blue light fraction		**days**	**Blue light (%)**		
	Dolly	5	9	9.01 ±0.65	0.71 ±0.06
			33	8.40 ±0.65	0.65 ±0.06
			65	7.52 ±0.65	0.61 ±0.06
			100	7.73 ±0.65	0.56 ±0.06
		25	9	9.01 ±0.65	0.71 ±0.06
			33	9.27 ±0.65	0.69 ±0.06
			65	9.40 ±0.65	0.70 ±0.06
			100	8.12 ±0.65	0.62 ±0.06
Experiment 3 Blue light interaction PPFD		**PPFD (µmol m^{-2} s^{-1})**	**Blue light (%)**		
	Rosie	100	9	5.12 ±0.40	0.35 ±0.04
		100	90	6.01 ±0.40	0.42 ±0.04
		300	9	5.09 ±0.40	0.37 ±0.04
		300	90	5.75 ±0.40	0.41 ±0.04
	Dolly	100	9	10.8 ±0.27	0.66 ±0.02a
		100	90	11.31 ±0.27	0.68 ±0.02a
		300	9	11.03 ±0.27	0.78 ±0.02b
		300	90	11.00 ±0.27	0.73 ±0.02b

5.3.2 Response to increasing fraction blue light in the light spectrum (Experiment 2)

The response to varying fractions of blue light was studied in a five day EOP treatment and as a throughout the growth treatment (25 days) in cv. Dolly. Plants showed a fairly similar response to the fraction of blue light, both to the EOP and throughout the growth treatments. The largest difference was found between the 100% blue treatment and the other treatments which also included red and green light. Plant height increased quadratically while the fraction of dry mass partitioned into the leaves decreased quadratically with increasing blue light (Fig. 2); in fact, it only showed a strong response when the fraction of blue was raised to 100%. The leaf area (Fig. 2 C), leaf fresh and leaf dry mass (Figs. S2A, C) decreased linearly with increasing fraction of blue light. There was no appreciable effect on the dry matter content of the leaves. LUE based on both dry and fresh mass did not significantly change with neither fraction of blue light or number of treatment days (Table 2). The only difference found between 5 or 25 days of application of blue light was on leaf area with increases in SLA when grown under 25 days of increased fraction of blue light.

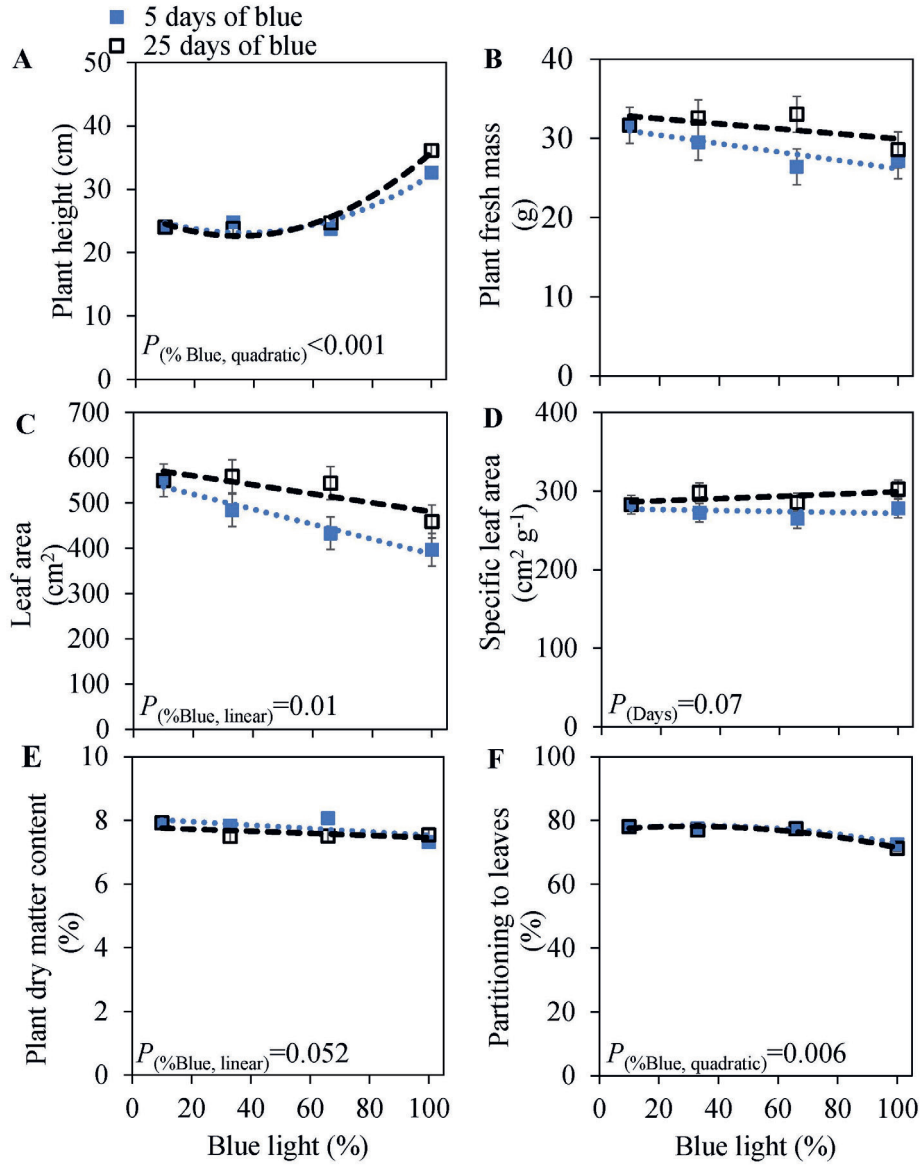

Figure 2. Response of basil cv. Dolly to different blue fractions out of a total PPFD of 300 µmol m^{-2} s^{-1} either applied throughout the growth for 25 days (open squares) or as 5 days End-Of-Production treatments (closed squares) (Experiment 2). The data point 9% blue is shared between 5 days and 25 days as 9% blue light also was the initial phase before EOP treatments. A. Plant height, B. plant fresh mass, C. leaf area, D. specific leaf area, E. plant dry matter content, F. dry mass partitioning to leaves. Data are means of 2 blocks (n=2) each with 6 replicate plants. Error bars representing standard errors, when larger than symbol size. For significant quadratic or linear effects of increasing fraction of blue, trendlines together with the respective *p*-values (α=0.10) are depicted.

5.3.3 Interaction between fraction of blue light and PPFD (Experiment 3)

PPFD could play an important role in the response to blue light. Therefore, the interaction of PPFD and fraction of blue light was studied in a purple (Rosie) and a green cultivar (Dolly) (Fig. 3, 4). The green leaved cultivar (Dolly) showed only a limited response to blue light in Experiment 2, therefore we here extended the experiment with a cultivar with purple leaves. In this way we could test if the response to the light depended on the color (i.e. content of anthocyanins) of the leaves. Plant height (Figs. 3A, 4A) was higher at high PPFD compared to low PPFD (100 vs. 300 µmol m^{-2} s^{-1}) and height was higher at 90% compared to 9% blue light for both cultivars. These results were similar as in the experiments where either the PPFD or the fraction blue were studied separately (Figs. 1, 2). For cv. Dolly the response of plant height to blue light was greater at a lower PPFD (about 20 % increase) than at higher PPFD (10%) whereas for the purple cultivar Rosie no interaction between blue light and PPFD was found. The increase in plant height corresponded to an increase in fresh mass of stems (Figs. S3B, S4B) and a lower dry mass partitioning to the leaves with higher light intensity and fraction of blue (Figs. 3F, 4F). Moreover, cv. Rosie responded mainly to the increase in PPFD while cv. Dolly had an increase in plant dry matter content and dry mass of leaves with an increase in both fraction of blue and PPFD. The LUE based on plant dry mass increased for cv. Dolly when PPFD was increased from 100 to 300 µmol m^{-2} s^{-1} whereas no change in LUE was found for cv. Rosie (Table 2).

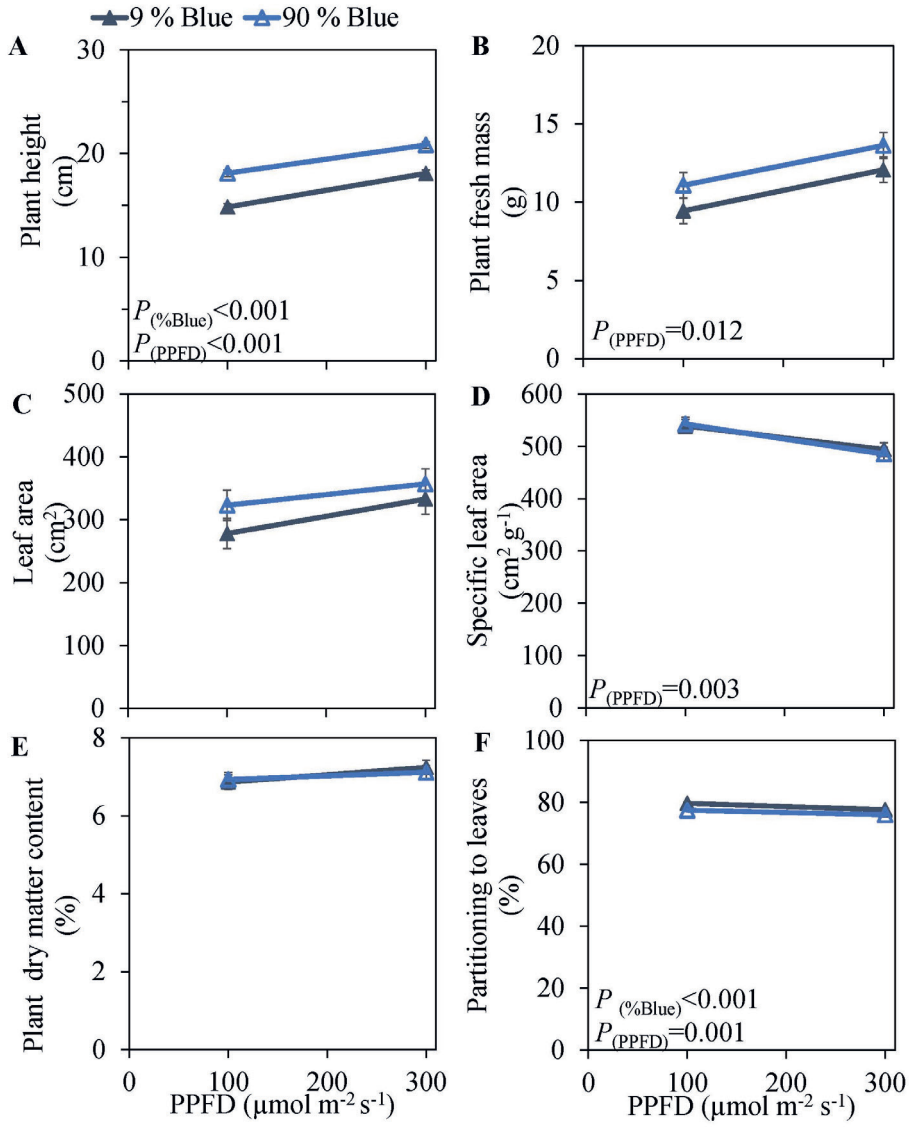

Figure 3. Response of basil cv. Rosie to End-Of-Production blue light and PPFD. Plants were grown for 30 days under red-white light (9% blue) and PPFD of 200 µmol m^{-2} s^{-1} (Experiment 3). EOP treatments were applied five days before harvest blue light and PPFD were changed to 100 µmol m^{-2} s^{-1} red white with 9 % blue or 90 % blue, and to 300 µmol m^{-2} s^{-1} red white with 9 % blue or 90 % blue. Closed triangle 9% blue and open triangle 90% blue A. Plant height, B. plant fresh mass, C. leaf area, D. specific leaf area, E. plant dry matter content, F. dry mass partitioning to leaves. Data are means of 4 blocks (n=4) each with 6 replicate plants. Error bars representing standard errors, when larger than symbol size. *P*-values of main effects %Blue and PPFD (α=0.05) are depicted.

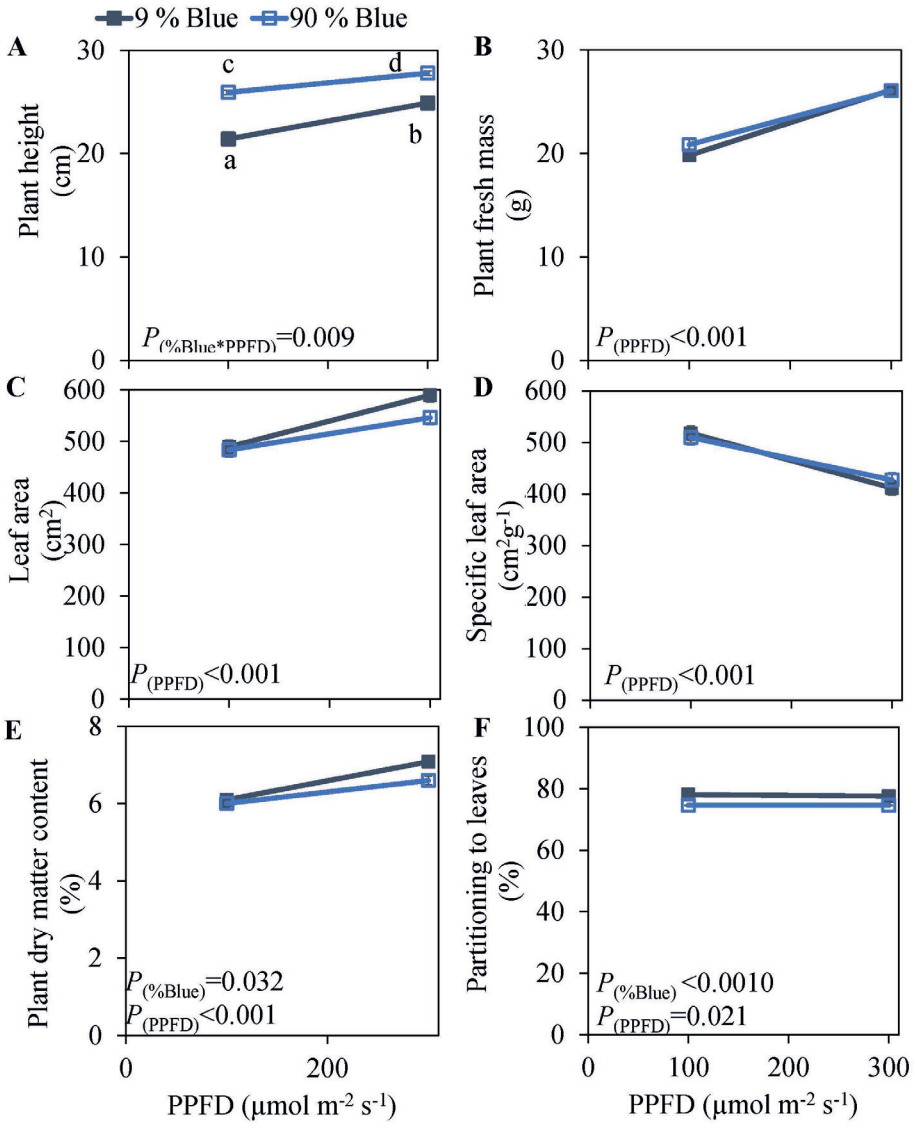

Figure 4. Response of basil cv. Dolly to End-Of-Production blue light and PPFD. Plants were grown for 30 days under red-white light (9% blue) and PPFD of 200 µmol m^{-2} s^{-1} (Experiment 3). EOP treatments were applied five days before harvest blue light and PPFD were changed to 100 µmol m^{-2} s^{-1} red white with 9 % blue or 90 % blue, and to 300 µmol m^{-2} s^{-1} red white with 9 % blue or 90 % blue. Closed squares 9% blue and open squares 90% blue. A. Plant height, B. plant fresh mass, C. leaf area, D. specific leaf area, E. plant dry matter content, F. dry mass partitioning to leaves. Data are means of 4 blocks (n=3) each with 6 replicate plants. Error bars representing standard errors, when larger than symbol size. P-values of main effects %Blue and PPFD (α=0.05) are depicted.

5.3.4 Response to increasing far-red intensities and duration (Experiment 4 and 5)

In an experiment with cv. Emily, five day EOP treatments were applied adding 0, 50 or 100 µmol m^{-2} s^{-1} far-red to the PPFD of 300 µmol m^{-2} s^{-1} red-white light (Fig. 5). In another experiment different durations of (180 µmol m^{-2} s^{-1}) far-red were applied for 0, 1 and 3 weeks (Fig. 6) on top of 150 µmol m^{-2} s^{-1} red-white light. Plant height increased by 15 % with the addition of 100 µmol m^{-2} s^{-1} of far-red and 7 and 36% with the one and three weeks duration of far-red respectively. The increase in plant height was significant as the linear component of the statistical analysis was significant. Other responses to duration and intensity of far-red in terms of fresh, dry mass, dry matter content and specific leaf area differed greatly. Increased intensity of EOP far-red on top of 300 µmol m^{-2} s^{-1} red-white light resulted in a significant decrease in leaf area and specific leaf area while a small increase in plant dry matter content was observed. Plant fresh mass and partitioning to leaves did not respond to increased intensity of far-red when given on top of 300 µmol m^{-2} s^{-1} red-white light during five days. Interestingly plant dry matter content increased with the addition of 100 µmol m^{-2} s^{-1} far-red (Fig. 5E) due to an increase in dry matter content of both leaves and stem (Figs. S5 E, F). Neither LUE nor Radiation Use Efficiency (RUE) based on fresh or dry mass were affected by EOP far-red treatments (Table 3).

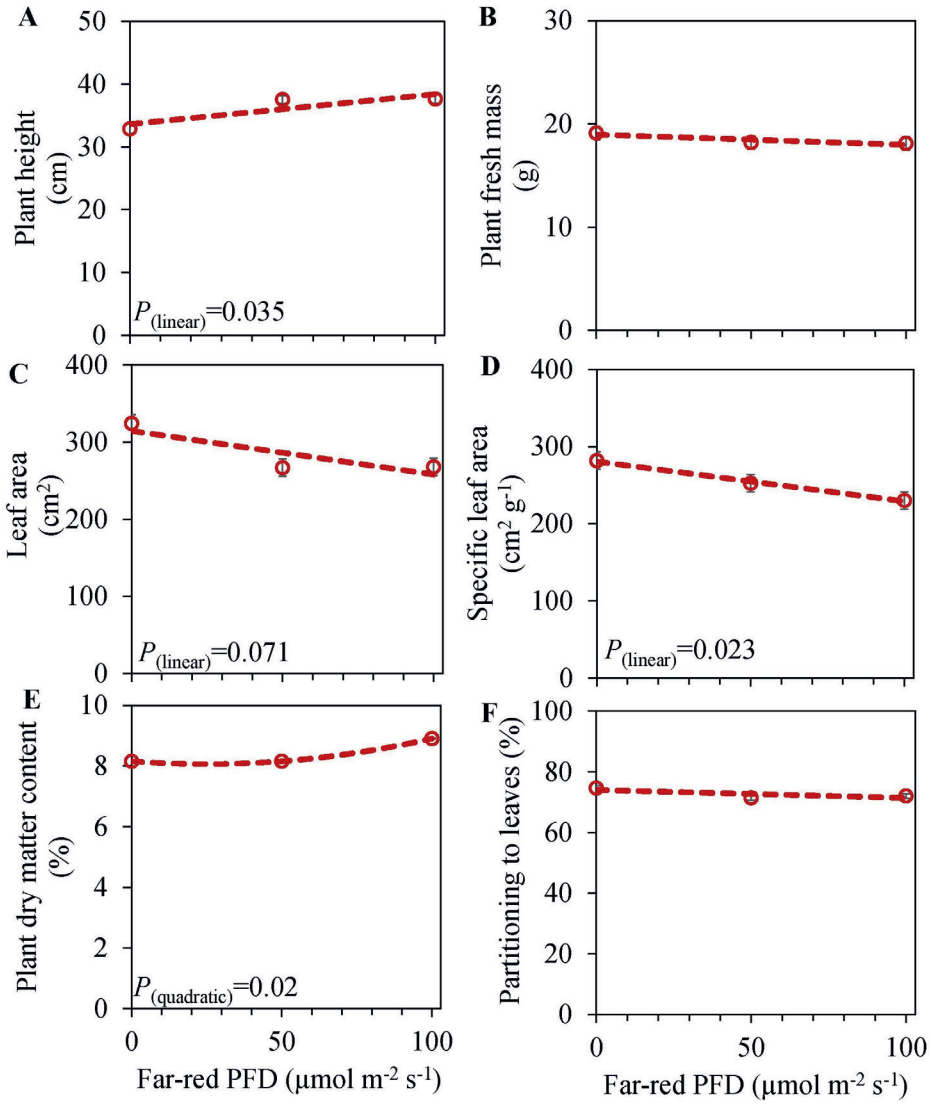

Figure 5. Response of basil cv. Emily to End-Of-Production increased far-red PFD (Experiment 4). Plants were grown for 15 days under PPFD 150 µmol m^{-2} s^{-1}, after transplant for another 15 days of PPFD 300 µmol m^{-2} s^{-1} red-white light and exposed to different far-red intensities (i.e. 0, 50, 100 µmol m^{-2} s^{-1}) in addition to 300 µmol m^{-2} s^{-1} red white light applied during 5 days before harvest. A. Plant height, B. plant fresh mass, C. leaf area, D. specific leaf area, E. plant dry matter content, F. dry mass partitioning to leaves. Data are means of 2 blocks (n=2) each with 5 replicate plants. Error bars representing standard errors, when larger than symbol size. For significant quadratic or linear effects of increasing far-red intensity, trendlines together with the respective *p*-values (α=0.10) are depicted.

Table 3. Overview of Light Use Efficiency (LUE) for plant fresh and dry mass in response to far-red (Experiment 4 and 5). LUE is based on PPFD incident on the plants accumulated over the initial (i.e. from transplant until start of treatments) and treatment phase, and the Radiation Use Efficiency (RUE), which is based on PFD incident on the plants. Letters indicate significant differences.

Experiment	Cultivar	Treatment	LUE (g mol^{-1})		RUE (g mol^{-1})	
			plant fresh mass	plant dry mass	plant fresh mass	plant dry mass
Experiment 4		**PFD of far-red (µmol m^{-2} s^{-1})**				
Far-red PFD	Emily	3	6.79 ± 0.23	0.55 ± 0.02	6.66 ± 0.21	0.54 ± 0.02
		50	6.47 ± 0.23	0.53 ± 0.02	6.22 ± 0.21	0.51 ± 0.02
		100	6.44 ± 0.23	0.57 ± 0.02	6.07 ± 0.21	0.54 ± 0.02
Experiment 5		**duration of far-red (d)**				
Far-red duration	Emily	0	11.63 ± 0.37 b	0.98 ± 0.10	11.63 ± 0.17 c	0.98 ± 0.06 b
		7	9.78 ± 0.37 a	0.90 ± 0.10	6.99 ± 0.17 b	0.65 ± 0.06 a
		21	13.33 ± 0.37 c	1.36 ± 0.10	6.06 ± 0.17 a	0.62 ± 0.06 a

Plants grown with one week of added far-red did not show an increase in plant fresh mass while plant fresh mass increased after three weeks (Fig. 6B). A similar response was found for leaf area (Fig. 6C), where a quadratic response to duration of far-red was found; after one week leaf area decreased while it increased after three weeks. Specific leaf area did not change when far-red was applied on top of 150 µmol m^{-2} s^{-1} red-white light (Fig. 6D). The response of LUE based on fresh mass followed the pattern of leaf area (Table 3). No differences were found for LUE based on dry mass whereas RUE based on dry and fresh mass decreased when far-red was added (Table 3). The dry mass partitioning to the leaves had an overall linear decrease with duration of far-red (Fig. 6F).

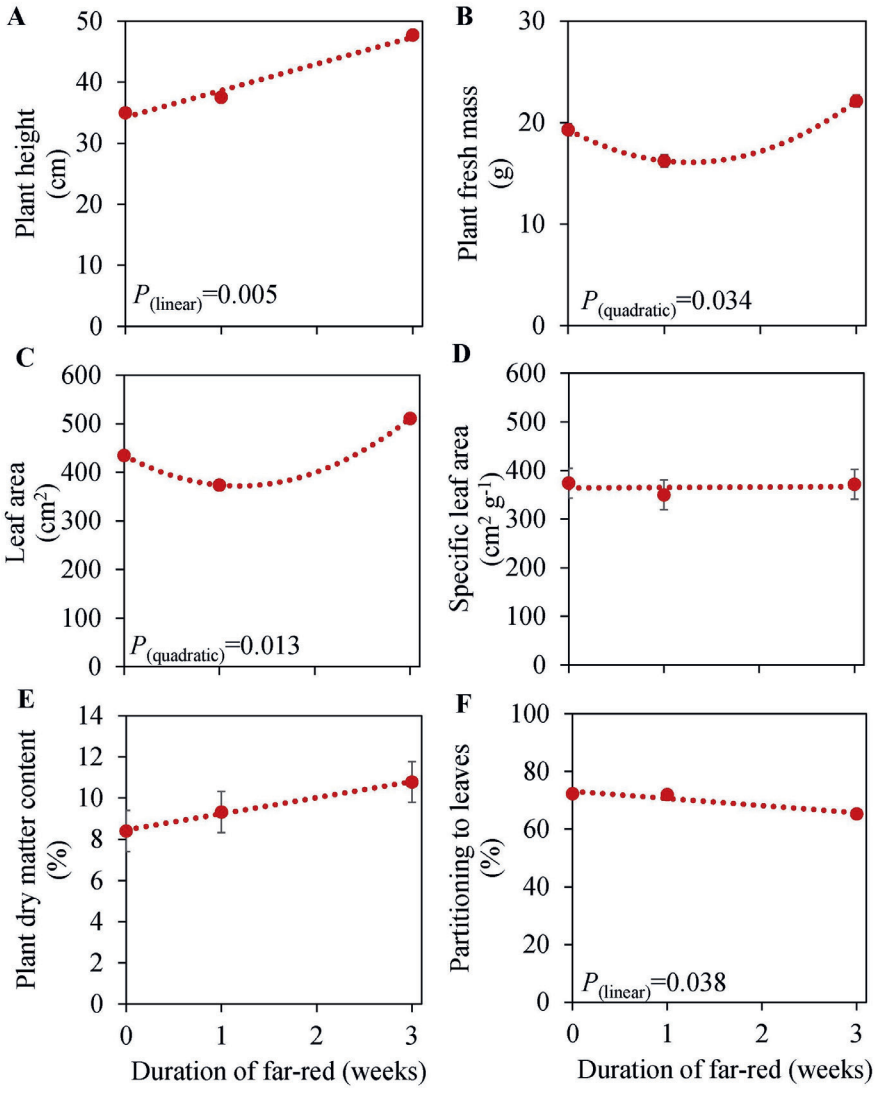

Figure 6. Response of basil cv. Emily to different duration of far-red treatments either throughout the growth for three weeks or as one week End-Of-Production treatment (Experiment 5). Plants were grown for 31 days under 150 µmol m^{-2} s^{-1} red-white light, and additional far-red light (180 µmol m^{-2} s^{-1}) was applied during 0, 1 and 3 weeks before harvest. A. Plant height, B. plant fresh mass, C. leaf area, D. specific leaf area, E. plant dry matter content, F. dry mass partitioning to leaves. Data are means of 2 blocks (n=2) each with 5 replicate plants. Error bars representing standard errors, when larger than symbol size. For significant quadratic or linear effects of duration of far-red, trendlines together with the respective *p*-values (α=0.10) are depicted.

5.4 Discussion

5.4.1 Increased PPFD applied as End-Of-Production treatment increases plant fresh mass and dry matter content

The effect of LED light on plant growth has previously been investigated in species such as lettuce (Li and Kubota, 2009), spinach, rocket, microgreens (Colonna *et al.*, 2016) and basil (Carvalho *et al.*, 2016; Jensen *et al.*, 2018; Pennisi *et al.*, 2019). However, the effects of both light spectra and PPFD have been found to be species dependent (Cope and Bugbee, 2013; Colonna *et al.*, 2016), and in lettuce and tomato even cultivar dependent (Ouzounis *et al.*, 2015, 2016; Gomez and Jimenez, 2020). This was also found in the present study where certain responses to PPFD and spectra were shown to be cultivar dependent. While we found that plant dry matter content in two sweet basil cvs., Emily and Dolly (Fig. 1) increased linearly with PFFD, a saturation response was found for the fresh mass of leaves (Fig. S1a) in cv. Dolly whereas in cv. Emily a linear response to the increase in PPFD was observed. This is in line with results from Pennisi *et al.* (2020) where fresh and dry mass of both lettuce and basil saturated at a light intensity of 250 µmol m^{-2} s^{-1}. A light saturation response occurs when plant growth gets limited by other factors e.g. CO_2, temperature or nutrients (Osmond, 1983). Under high light, photosynthesis becomes CO_2 limited and thus the growth is hampered (Long and Bernacchi, 2003). However, the light intensity at which net photosynthesis gets light limited is species dependent and dependent on the growth environment. Basil, grown under increasing light intensities from 160-310 µmol m^{-2} s^{-1} showed a saturating net leaf photosynthesis at above 220 µmol m^{-2} s^{-1}, yet shoot fresh mass and dry matter content increased linearly with light intensity (Dou *et al.*, 2018). A high dry matter content, as observed at higher PPFD, implicates higher levels of carbohydrates. In postharvest storage carbohydrates are used for respiration. Therefore, having a large reserve of carbohydrates are beneficial for shelf-life and quality (Dorais *et al.*, 2002; Caleb *et al.*, 2016). This has also been shown in lettuce (Woltering and Witkowska, 2016) and broccoli (Finger *et al.*, 1999). Consequently, basil with a higher dry matter content might have a better postharvest quality.

Optimal PPFD for basil growth (i.e. highest LUE for plant dry mass) has been suggested to be 250 µmol m^{-2} s^{-1} (DLI 14.4 mol m^{-2} d^{-1}) (Pennisi *et al.*, 2020), 224 µmol m^{-2} s^{-1} (DLI 12.9 mol m^{-2} d^{-1}) (Dou *et al.*, 2018) and 500 µmol m^{-2} s^{-1} (DLI 28.8 mol m^{-2} d^{-1}) (Beaman *et al.*, 2009). In our study, the LUE based on dry mass was the highest at 600 µmol m^{-2} s^{-1} for both cv. Emily and cv. Dolly. For growers the LUE based on fresh mass is probably more interesting, however, at 600 µmol m^{-2} s^{-1} LUE based on fresh mass was the lowest for both cultivars (Table

2). Furthermore, at 600 µmol m^{-2} s^{-1} the leaves were brittle and broke easily; this high light level can therefore not be considered optimal. The combination of initially raising the plants at a PPFD of 150 µmol m^{-2} s^{-1} and an EOP of 300 µmol m^{-2} s^{-1} resulted in an increase in dry mass partitioning to the leaves for both cvs. Emily and Dolly. Whereas an initial PPFD > 150 µmol m^{-2} s^{-1} for cv. Dolly resulted in a slightly higher dry mass partitioning to the stem. Therefore, we overall consider the combination of initially raising the plants at a PPFD of 150 µmol m^{-2} s^{-1} and an EOP of 300 µmol m^{-2} s^{-1} (DLI 19.4 mol m^{-2} d^{-1}) the optimal growth conditions. This consideration is based on both LUE parameters and growth and morphological parameters (e.g. dry matter content and leaf area).

5.4.2 Plant biomass does not respond to the fraction of blue light in the spectrum

Blue light and red:blue ratios have been intensely studied. Red light is the most efficient color driving photosynthesis but adding blue to a red background improves the overall photosynthesis (Hogewoning *et al.*, 2010). Yet, the optimal fraction of blue light to a growth light spectrum has had varying conclusions. A blue light optimum was found of 12% blue, in tomato, with respect to leaf dry mass (Kaiser *et al.*, 2019). In basil, Pennisi *et al.* (2019) found that an increase in blue light up to 58% blue reduced plant fresh mass. However, opposite results were found where an increase in blue fraction up to 37% increased plant fresh mass Piovene *et al.* (2015) and Jensen *et al.* (2018) found that a fraction of blue of 60% increased the leaf dry matter content. In our experiments, the fraction of blue mostly affected plant height in cv. Dolly as well as in the purple cv. Rosie. In line with this finding, stem fresh mass and dry mass increased at higher fraction of blue. This may have happened at the expense of leaf fresh mass and dry mass (Fig S2). There were no significant effects of fraction of blue light (range 9-100%) on overall plant fresh mass (Figs. 2B, 3B, 4B) nor on plant dry mass (Figs. S2, S3, S4) or plant dry matter content (Figs. 2E, 3E). Previous findings by Snowden *et al.* (2016) indicated that an interaction between light intensity and fraction of blue light existed. In the current experiment we found a limited interaction of blue light and light intensity i.e. only on the dry mass of the leaves (Fig. S4C) in the green cv. Dolly. For experiment 3 the purple cv. Rosie was chosen a long with the green cv. Dolly and as in line with results from Dou *et al.* (2019) the purple cultivar had a lower plant fresh and dry mass of leaves than the green cultivar (Figs. 3B, 4B, S3C, S4C). However, Dou *et al.* (2019) found a negative effect of blue light in the purple cultivar and not in green basil whereas blue light did not affect biomass in cv. Rosie in experiment 3.

Based on our findings a spectrum with 9% blue while the remaining part of the spectrum being 70% red and 19 % green can be maintained throughout the growth of basil as an increased amount of blue did not improve the plant fresh mass nor the dry matter content. Furthermore, no differences were found between the EOP and throughout the growth blue light treatment.

5.4.3 A high fraction of blue light induces SAS like responses

Amongst morphological parameters plant height is one that has been recorded in numerous studies. Blue light usually suppresses elongation (Laskowski and Briggs, 1989) but in a number of cases a promotion of stem elongation has been observed (Johnson *et al.*, 2020) depending on the species and fraction of blue in the spectra (90-100%) (Kong *et al.*, 2018). Blue light is sensed by photoreceptors such as cryptochromes, phototropins and Zeitlupes (Huché-Thélier *et al.*, 2016). However, phytochromes also absorb blue and consequently blue light can affect the PSS value which indicates the active phytochromes out of the total phytochromes (Sager *et al.*, 1988; Casal, 2013; Meng *et al.*, 2019). A low PSS value results in shade avoidance syndrome (SAS). Hundred % blue light has been found to increase stem elongation due to low phytochrome activity (PSS 0.49) (Kong *et al.*, 2018; Johnson *et al.*, 2020). In accordance with Kong *et al.* (2018), we also observed increased stem elongation under both 90% and 100% blue light (Figs. 2A, 3A, 4A). This was likely because of the reduced PSS values of 0.7 at 90% blue and 0.49 at 100% blue. Furthermore, the response to blue light on plant height was more pronounced under a low light intensity compared to the high light intensity in cv. Dolly. This finding was also reported by Johnson *et al.* (2020) although the response was found to be species dependent. In addition, Shade avoidance syndrome (SAS) can lead to an increase in leaf area (Demotes-Mainard *et al.*, 2016) but the response is not universal but rather species dependent. Although, 100% and 90% blue induced stem elongation, we found 100% blue to reduce leaf area while 90% blue had no effect. In addition, leaf area decreased overall with increasing fraction of blue light (Fig. 2C) which is in accordance with Kaiser *et al.* (2019).

5.4.4 Far-red increases plant height while effects on biomass depend on duration of far-red application

SAS like responses were also found when we grew plants under additional far-red light (Figs. 5A, 6A) where plant height increased with far-red intensity and duration. However, leaf area, similar as in the experiments with blue light EOP, decreased when far-red was applied EOP for five days or one week (Figs. 5C, 6C). Increased leaf area in response to far-red has been found to be more pronounced in the early growth stage (Kalaitzoglou *et al.*, 2019) which is in agreement with the increase in

leaf area when far-red was added throughout the growth period (Fig. 6C). Specific leaf area decreased with increasing far-red PFD during the five-days EOP treatment due the decrease in leaf area and no difference in the dry mass of leaves (Fig. S5C). Specific leaf area which is an indicator of the leaf thickness and would be expected to increase with increasing far-red and decreasing PSS values as found in other species (Ji *et al.*, 2019; Zou *et al.*, 2019). Interestingly, cv. Emily grown with a PSS of 0.62 did not have any change in specific leaf area (Fig. 6D). Therefore, we suggest that SAS response in basil is mostly linked to stem elongation. While plant dry matter content increased quadratically under five days of EOP far-red (Fig. 5E) no increase was found in the longer duration far-red (Fig. 6E). However, for the plant fresh mass the longer duration of far-red had a significant effect, mainly due to an increase in stem fresh mass (Fig. S6B) which also resulted in a lower dry mass partitioning to the leaves. This is in accordance with previous findings, where stem dry mass increased with far-red (Ji *et al.*, 2019).

Recently, Zhen and Bugbee (2020) suggested far-red photons to be photosynthetically active. They found the magnitude of the increase in net photosynthesis to be species dependent where basil was one of the less responsive species. An increase in net photosynthesis is expected to be reflected in an increase in biomass. This was not the case in either of our experiments as cv. Emily did not increase in plant dry mass after five days (total PFD 350-400 µmol m^{-2} s^{-1}) (Fig. S5) or after one week of added far-red (total PFD 330 µmol m^{-2} s^{-1}) (Fig. S6). However, when the PPFD increased from 150 to 300 µmol m^{-2} s^{-1} dry mass did increase (Fig. S1). The decrease in fresh mass after one week of added far-red and subsequent increase after three weeks could indicate an acclimation period (Fig. 6B). This is supported by our results with increased far-red intensity where no increase in plant fresh mass was found (Fig. 5B). Although, the radiation use efficiency for plant fresh mass increased by three weeks of added far-red (Table 3) it decreased based on dry mass with increasing duration of far-red in Experiment 5 while far-red did not affect radiation use efficiency in Experiment 4. Thus, additional far-red, in a small dosage added five days before harvest may be beneficial to improve leaf dry matter content whereas a higher dosage throughout the growth does not yield a desired plant morphology as the stem is not a used organ from basil plants.

5.6 Conclusion

We showed that growth (plant fresh mass, plant dry matter content and dry mass partitioning to the leaves) and morphology (plant height and leaf area) were significantly affected by EOP increase in PPFD. Interestingly, LUE based on fresh mass decreased with increasing PPFD whereas LUE based on dry mass increased. The plant fresh mass did not respond to the fraction of blue light while plant dry matter content was reduced at the combination high fraction of blue and a high PPFD. When the spectrum consisted of either 90% or 100% blue, either applied as EOP treatments or throughout the growth shade avoidance syndrome was induced and plants grew taller resulting in more fresh and dry mass partitioned to the stem. Therefore, a high fraction of blue in the spectrum is not desirable for basil growth as the leaves are the consumed part.

Addition of far-red for basil during growth is most beneficial when added as EOP treatment before harvest and only in a lower dosage at a high PPFD as it increases dry matter content of both leaves and stem.

Supplementary material

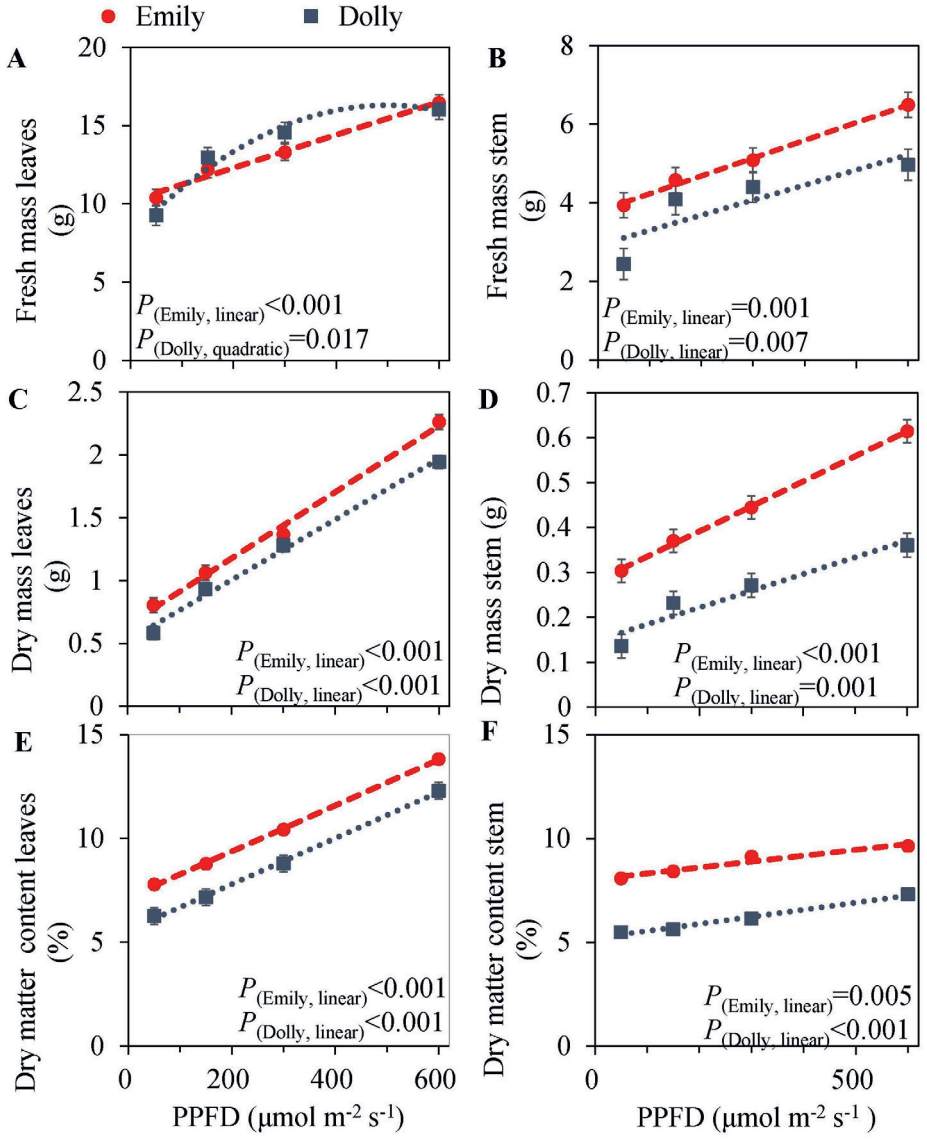

Figure S1. Response of basil cvs. Emily (red circles) and Dolly (grey squares) to different End-Of-Production PPFD. Plants were grown for 30 days under 150 µmol m^{-2} s^{-1} after which they were exposed to different PPFD (i.e. 50, 150, 300 and 600 µmol m^{-2} s^{-1}) during 5 days before harvest. A. Fresh mass leaves, B. fresh mass stem, C. dry mass leaves D. dry mass stem, E. dry matter content leaves, F. dry matter content stem. Data are means of 3 blocks (n=3) each with 6 replicate plants. Error bars represent standard errors of means, when larger than symbols. For significant quadratic or linear effects of PPFD, trendlines together with the respective p-values (α=0.05) are depicted.

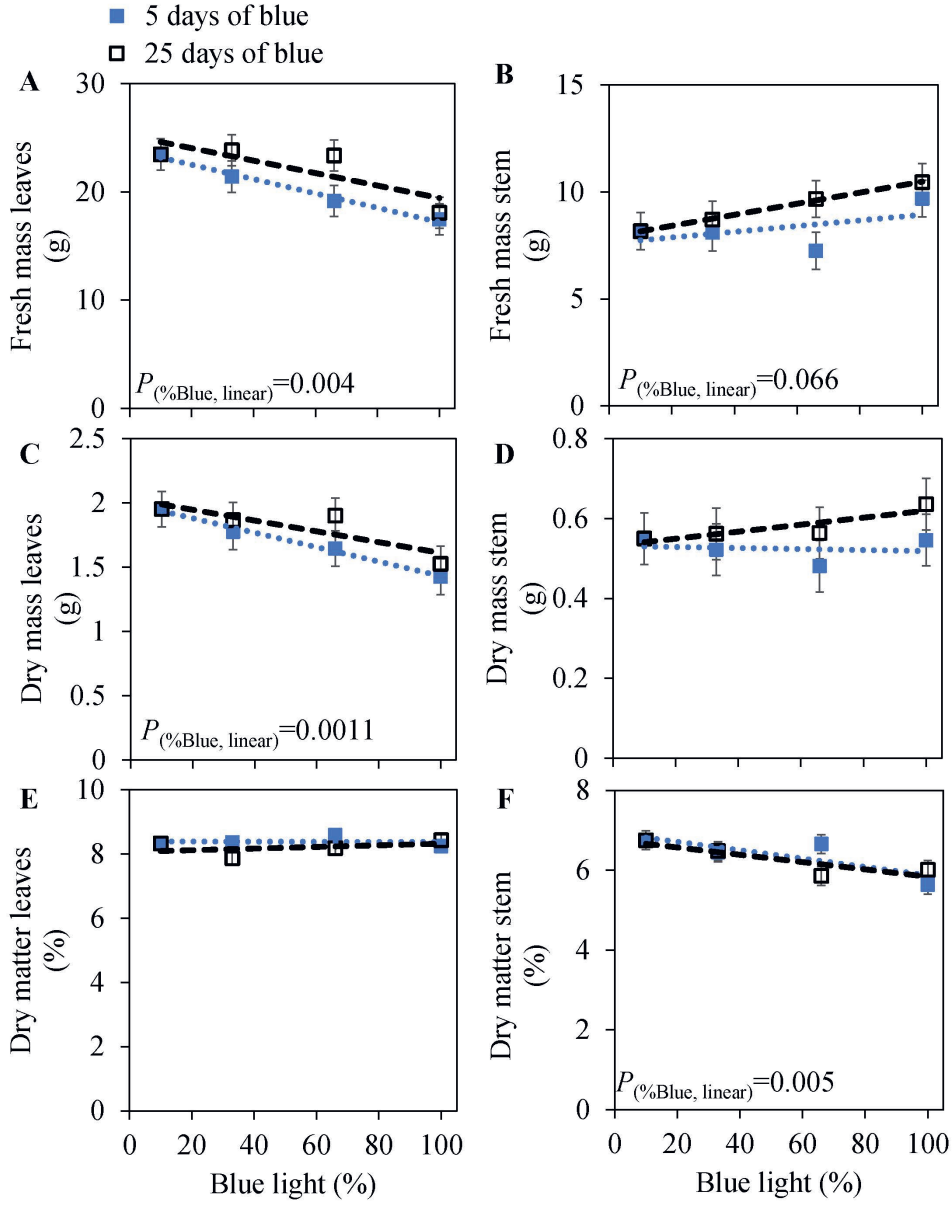

Figure S2. Response of basil cv. Dolly to different blue fractions out of a total PPFD of 300 µmol m^{-2} s^{1} either applied throughout-the-growth for 25 days (open squares) or as 5 days End-Of-Production treatments (closed squares). The data point 9% blue is shared between 5 days and 25 days as 9% blue light also was the initial phase before EOP treatments. A. Fresh mass leaves, B. fresh mass stem, C. dry mass leaves D. dry mass stem, E. dry matter content leaves, F. dry matter content stem. Data are means of 2 blocks (n=2) each with 6 replicate plants. Error bars representing standard errors, when larger than symbol size. For significant quadratic or linear effects of increasing fraction of blue, trendlines together with the respective p-values (α=0.10) are depicted.

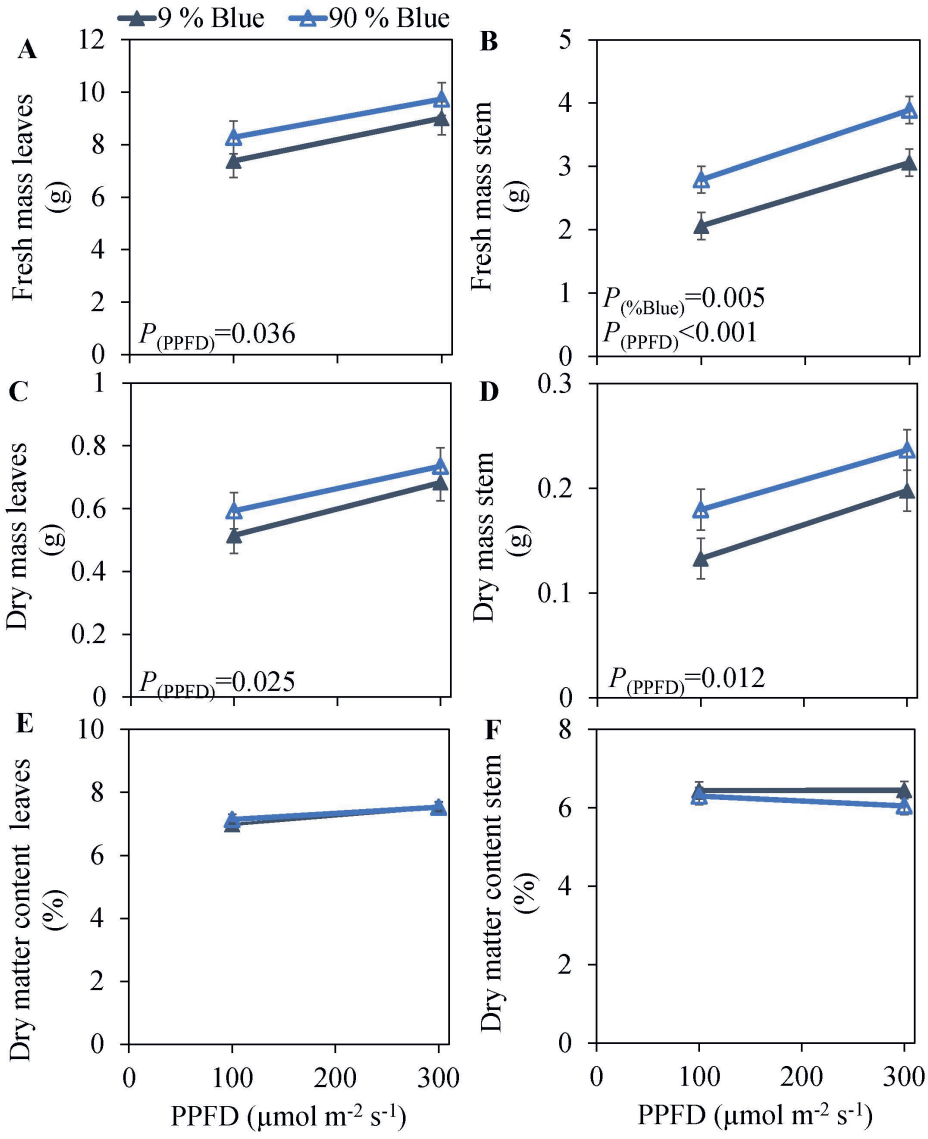

Figure S3. Response of basil cv. Rosie to End-Of-Production blue light and PPFD. Plants were grown for 30 days under red-white light (9% blue) and PPFD of 200 µmol m^{-2} s^{-1}. EOP treatments were applied five days before harvest blue light and PPFD were changed to 100 µmol m^{-2} s^{-1} red white with 9 % blue or 90 % blue, and to 300 µmol m^{-2} s^{-1} red white with 9 % blue or 90 % blue. Closed triangle 9% blue and open triangle 90% blue. A. Fresh mass leaves, B. fresh mass stem, C. dry mass leaves D. dry mass stem, E. dry matter content leaves, F. dry matter content stem. Data are means of 4 blocks (n=4) each with 6 replicate plants. Error bars representing standard errors, when larger than symbol size. *P*-values of main effects %Blue and PPFD (α=0.05) are depicted.

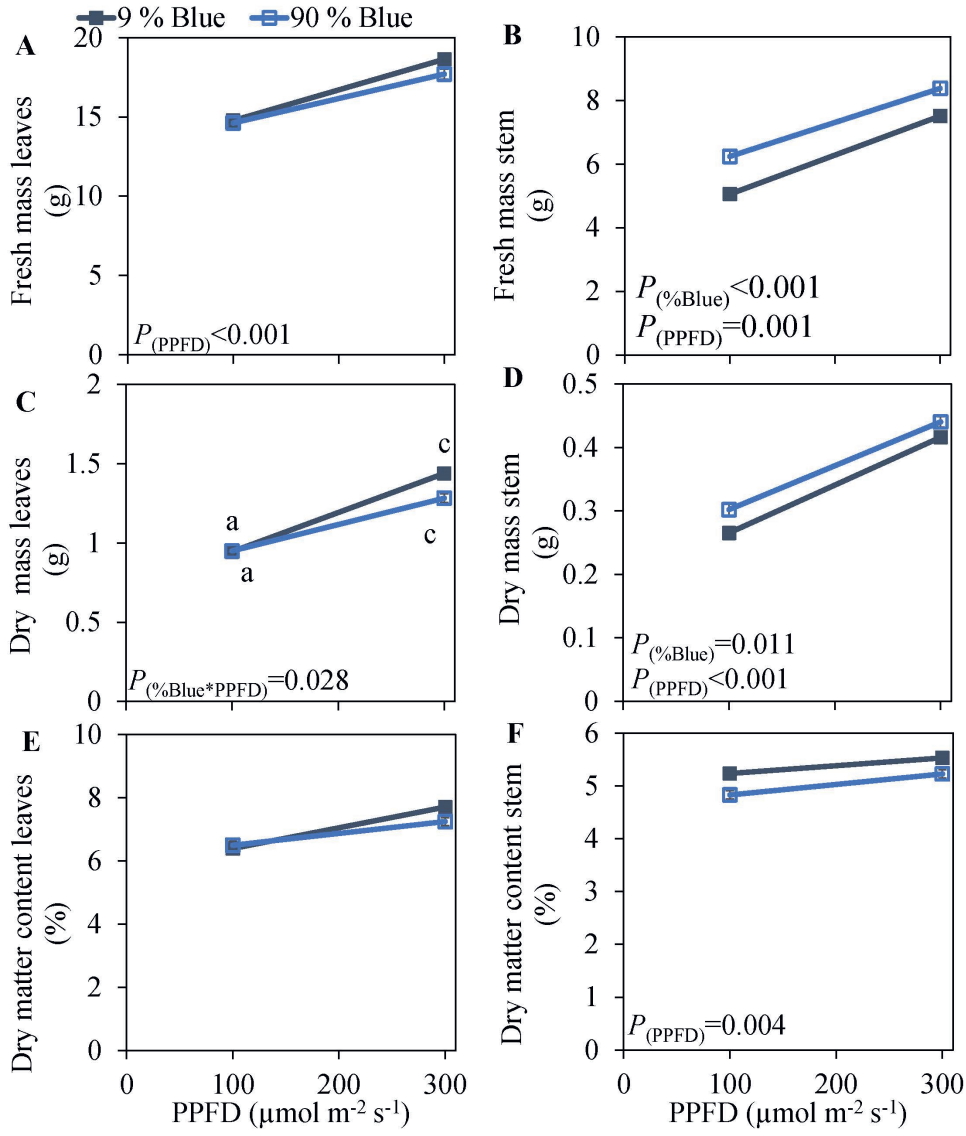

Figure S4. Response of basil cv. Dolly to End-Of-Production blue light and PPFD. Plants were grown for 30 days under red-white light (9% blue) and PPFD of 200 µmol m^{-2} s^{-1}. EOP treatments were applied five days before harvest blue light and PPFD were changed to 100 µmol m^{-2} s^{-1} red white with 9 % blue or 90 % blue, and to 300 µmol m^{-2} s^{-1} red white with 9 % blue or 90 % blue. Closed squares 9 % blue and open squares 90% blue. A. Fresh mass leaves, B. fresh mass stem, C. dry mass leaves D. dry mass stem, E. dry matter content leaves, F. dry matter content stem. Data are means of 4 blocks (n=3) each with 6 replicate plants. Error bars representing standard errors, when larger than symbol size. *P*-values of main effects %Blue and PPFD (α=0.05) are depicted.

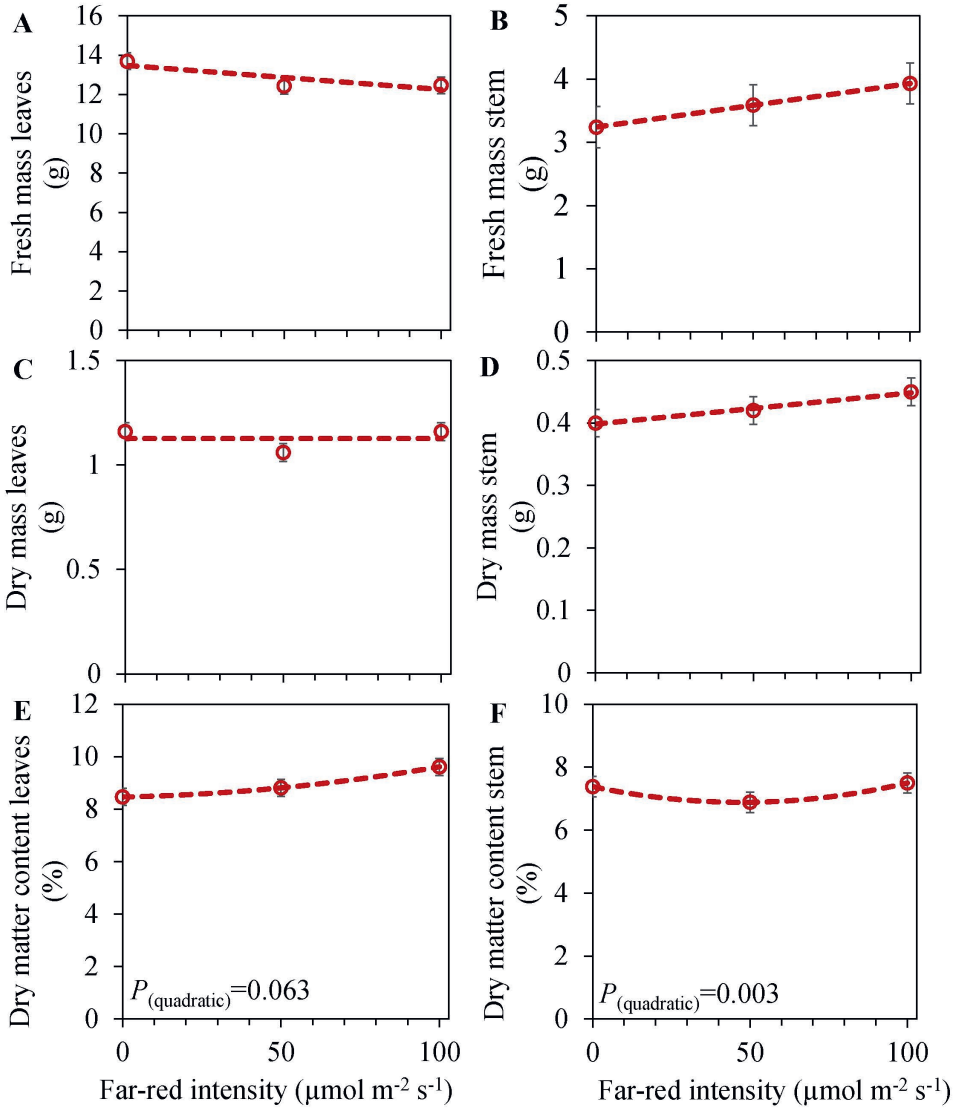

Figure S5. Response of basil cv. Emily to End-Of-Production increased far-red PFD. Plants were grown for 15 days under PPFD 150 µmol m^{-2} s^{-1}, after transplant for another 15 days of PPFD 300 µmol m^{-2} s^{-1} red-white light and exposed to different far-red intensities (i.e. 0, 50, 100 µmol m^{-2} s^{-1} in addition to 300 µmol m^{-2} s^{-1} red white light applied during 5 days before harvest. A. Fresh mass leaves, B. fresh mass stem, C. dry mass leaves D. dry mass stem, E. dry matter content leaves, F. dry matter content stem. Data are means of 2 blocks (n=2) each with 6 replicate plants. Error bars representing standard errors, when larger than symbol size. For significant quadratic or linear effects of increasing far-red intensity, trendlines together with the respective p-values (α=0.10) are depicted.

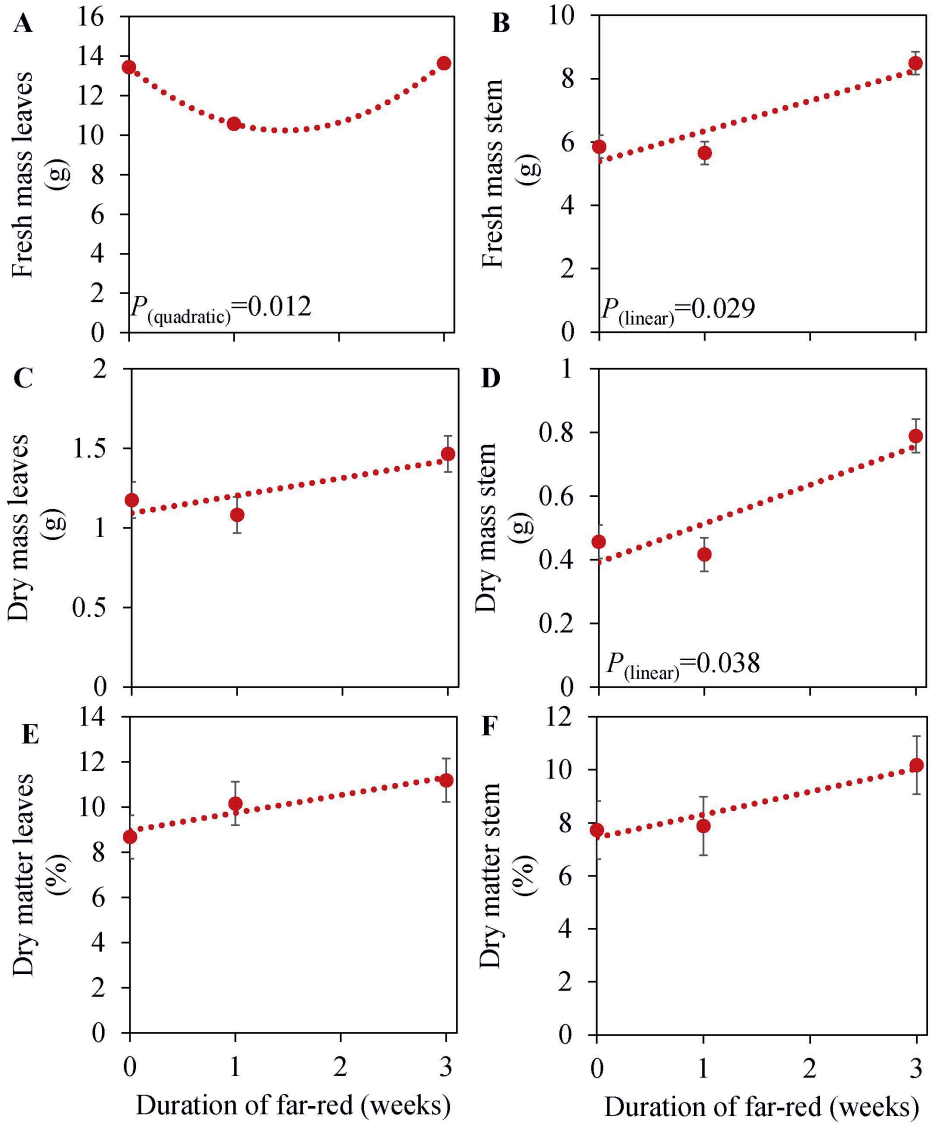

Figure S6. Response of basil cv. Emily to different duration of far-red treatments either throughout-the-growth for three weeks or as one week End-Of-Production treatment. Plants were grown for 31 days under 150 µmol m^{-2} s^{-1} red-white light, and additional far-red light (180 µmol m^{-2} s^{-1}) was applied during 0, 1 and 3 weeks before harvest. A. Fresh mass leaves, B. fresh mass stem, C. dry mass leaves D. dry mass stem, E. dry matter content leaves, F. dry matter content stem. Data are means of 2 blocks (n=2) each with 6 replicate plants. Error bars representing standard errors, when larger than symbol size. For significant quadratic or linear effects of duration of far-red, trendlines together with the respective *p*-values (α=0.10) are depicted.

Chapter 6

General discussion

6.1 General discussion

The aim of this thesis was to contribute to the understanding of how light can regulate the at-harvest and postharvest quality of basil (*Ocimum basilicum* L.). The main loss of quality during postharvest storage of basil is due to chilling injury. Quality is also determined by the appearance of the plant i.e. color of the leaves and morphology of the plant. Light during cultivation can greatly affect and growth, morphology and the content of metabolites (such as carbohydrates and antioxidants) of plants. Light represents a non-invasive, non-chemical approach to improving quality. It was investigated if increased light intensity, increased percentage of blue light and additional FR during pre-harvest could improve the postharvest chilling tolerance (**Chapter 2, 3, 4)**. In addition, the response of growth and morphology to the increased light intensity, increased percentage of blue light and additional FR treatments was also studied (**Chapter 5**).

Basil was grown in a climate chamber in a vertical farming set-up and the effect of light intensity and spectra on at-harvest and postharvest quality were studied. It was investigated if an increase in carbohydrates (soluble sugars and starch), antioxidants and hormones could improve chilling tolerance. In a vertical farm all environmental factors can be controlled which allows for year-round production with a uniform quality independent of season. Production in a closed system without external input such as light requires a high energy input for lighting and temperature control (i.e. in particular for cooling). This is accompanied by high costs and it is therefore important to understand how light treatments affect the light use efficiency, morphology and growth parameters including the biomass allocation to the leaves and stems. In addition, this thesis also adds to the sparse literature investigating the effect of pre-harvest conditions on postharvest quality.

6.2 Are more antioxidants beneficial for chilling tolerance?

Many studies in plant physiology investigate the increase in antioxidants under abiotic stress such as chilling. The antioxidant system in chilling resistant species is more effective compared to chilling sensitive species (Walker and Mckersie, 1993). In plants antioxidative systems exists in all subcellular compartments to protect cells against oxidative stress (Pandhair and Sekhon, 2006). Improving the antioxidant content may have the potential to help plants overcome abiotic stress such as chilling. During chilling excessive ROS are formed which disturbs the redox homeostasis of the plant and may be detrimental to the plants (Das and Roychoudhury, 2014).

The scavenging and formation of ROS by enzymatic and non-enzymatic antioxidants during chilling stress is a dynamic process. The process consists of a range of ROS

and antioxidants depending on plant species and cellular compartment. At a given sampling time only a snapshot of the process can be identified. Most studies focus on a few types of ROS and antioxidants such as H_2O_2 and either enzymatic or non-enzymatic antioxidants (i.e. phenolic compounds). In the present thesis the focus was on non-enzymatic antioxidants such as rosmarinic acid, chicoric acid, total ascorbic acid, flavonoids and anthocyanins (**Chapters 2, 3 and 4**). Phenolic compounds are effective scavengers of ROS and increase in phenolic compounds can therefore improve stress tolerance of the plant (Rice-Evans *et al.*, 1997). The presence of non-enzymatic antioxidants may increase the activity of enzymatic antioxidants (Ashraf *et al.*, 2019). The application of caffeic acid (a phenolic acid) on cucumber plants improved their chilling tolerance through an increase in several enzymatic antioxidants such as SOD, catalase (CAT) and ascorbate peroxidase (APX). Furthermore, the content of proline, soluble sugars and the overall content caffeic acid also increased with the application of caffeic acid (Wan *et al.*, 2015). Similarly, the activity of superoxide dismutase (SOD) increased in soybean when the plants were treated with caffeic acid which improved the tolerance to salinity (Klein *et al.*, 2013). Caffeic acid, is a phenolic acid similar to rosmarinic and chicoric acid. The antioxidant scavenging activity of rosmarinic acid is stronger than that of caffeic acid (Chen and Ho, 1997). Thus, a high content of rosmarinic acid in basil potentially increases the activity of enzymatic antioxidants. However, a high endogenous content of phenolic compounds on its own has also been found to be beneficial for the plant to overcome abiotic stress. In grapevine leaves, a high content of phenolic acids resulted in a higher chilling tolerance (Król *et al.*, 2015). Similarly, in eggplant an improved chilling tolerance correlated with a slower decrease of total phenolic content during cold storage (Shi *et al.*, 2018). In contrast, we showed that in basil a high content of phenolic acids was not sufficient to improve chilling tolerance (**Chapter 2**). Despite several studies show that high levels of phenolic compounds protect against ROS and chilling symptoms, some studies show that the effect of phenolic compounds may depend on the initial ROS content. For an increase in phenolic compounds to be beneficial the factors inducing phenolics should not also cause an increase in ROS. For example, high light intensity may upregulate phenolic compounds as well as ROS. In this case the increase in phenolic compounds will not be beneficial to the plant as there also is an increase in ROS. A high content in phenolic content due to the genotype itself may be beneficial and aid the plant in chilling tolerance.

6.2.1 Scavenging of H_2O_2

During chilling stress plants accumulate H_2O_2 which is scavenged by CAT (Prasad *et al.*, 1994). After SOD, CAT is one of the first enzymes that gets activated during cold stress (Sevillano *et al.*, 2009). The content of both CAT and H_2O_2 are expected to increase during cold storage. Yet, in basil CAT did not increase in five red and green cultivars when stored at low temperature (Kalisz *et al.*, 2016). Similarly, we observed no increase in the H_2O_2 in basil stored at 4 °C, although symptoms of CI were present (**Chapters 2 & 3**). This indicates the complexity of the ROS and antioxidants network. For phenolic compounds to scavenge ROS during chilling stress they must encounter each other. While ROS are formed in most cellular compartments such as the chloroplast, mitochondria and peroxisomes phenolic compounds are mostly synthesized in the intracellular endoplasmic reticulum of plants and stored in the vacuoles (Das and Roychoudhury, 2014; Gan *et al.*, 2019). There are great differences in the mobility of ROS. H_2O_2 can easily cross membranes and move a long distance from the site of the production whereas $^1O_2$, $O_2^{\cdot-}$ and $OH^{\cdot}$ are considered not very mobile (Gechev *et al.*, 2006). Thus, H_2O_2 may easier be scavenged by phenolic compounds than other ROS species. Especially anthocyanins have been shown to scavenge H_2O_2 in the leaves (Gould *et al.*, 2002).

6.3 Can pre-harvest light improve postharvest chilling tolerance?

To improve postharvest chilling tolerance several studies have investigated the use of pre-harvest light treatments such as modifying the light spectrum to increase the percentage of blue (400-500 nm) and far-red (FR, 700-800 nm) light (Affandi *et al.*, 2020, 2022; Kovács *et al.*, 2020; Ahres *et al.*, 2021). In this thesis it was investigated if light intensity and spectra influenced chilling tolerance. Specifically, the increase in percentage of blue light and addition of FR.

6.3.1 Increase in antioxidants due to increased light intensity

If plants are subjected to high light it can result in light stress which can increase the content of reactive oxygen species (ROS). However, high light also activates the antioxidant system (Szyma *et al.*, 2017). An increase in light intensity can therefore upregulate the antioxidant content which may be beneficial for the plant to overcome other stress factors. When high light was applied only in the last phase of the cultivation, as EOP treatments the content of carbohydrates and antioxidants such as carotenoids, anthocyanins and total ascorbic acid increased in lettuce (Gómez and Jiménez, 2020; Min *et al.*, 2021). This prolonged the shelf-life (Min *et al.*, 2021). Similarly, we found that an increase in light intensity as EOP light resulted in a

higher content of carbohydrates and antioxidants in basil (**Chapter 2**). Carbohydrates can improve chilling tolerance as they act as an antioxidant, scavenging hydroxyl radicals. Sugars can thereby protect the cell membranes (Pommerrenig *et al.*, 2018). In rice, chilling tolerance was improved as they over accumulated sugars during cold stress (Garg *et al.*, 2002). However, we found that the increase in carbohydrates due to increased EOP light intensity in green basil did not improve chilling tolerance (**Chapter 2**). Surprisingly, in a comparable experiment with both a green and purple basil cultivar, increased light intensity did improve chilling tolerance (**Chapter 3**). The discrepancy between these results may be related to the plants cultivation history. In addition to the increase in light intensity as EOP treatments, the light intensity before the EOP treatments may have an effect. The light intensity used for cultivation in the experiment described in **Chapter 2** was 150 µmol m^{-2} s^{-1} whereas in the f experiment described in **Chapter 3** it was 200 µmol m^{-2} s^{-1}. The higher light level during cultivation (**Chapter 3**) may have resulted in a higher photosynthesis rate and more carbohydrates (Dou *et al.*, 2018). At the day of harvest, we found the absolute soluble sugar content of the EOP treatment with a light intensity of 300 µmol m^{-2} s^{-1} described in **Chapter 2** to be slightly higher (i.e. about 6 mg g^{-1} DW) than the content found in **Chapter 3** (i.e. about 4 mg/g DW) for cv. Dolly. Thus, the absolute values of soluble sugar cannot indicate whether a plant will be chilling tolerant. The optimum content of soluble sugar which results in improved chilling tolerance is presumably affected by the level of ROS induced by the light treatments, as increased light intensity can lead to an increase in ROS (Das and Roychoudhury, 2014). The at-harvest level of ROS may have been high when a high light intensity is used as EOP treatment. To understand the full extent of increased light intensity on chilling tolerance it is therefore necessary to study the content of ROS induced by the light treatments at-harvest as well as during cold storage.

6.3.2 The effect of blue light on chilling tolerance

Photoreceptors are highly involved in the regulation of growth and development of plants. The biosynthesis of phenolic compounds such as flavonoids and antioxidants is thought to be regulated by the photoreceptor cryptochrome (Fox *et al.*, 2012). Cryptochromes absorb light in the range from UV-A to blue light (340 and 520 nm). Therefore, an increase in UV and blue light is generally expected to increase the content of phenolic compounds especially flavonoids and anthocyanins.

Several authors have shown that blue light can increase flavonoids and anthocyanins (Chen *et al.*, 2006; Zhang *et al.*, 2018; VanDelden *et al.*, 2021). For instance, an increase in blue light from 13 to 69 % for four days as EOP treatment increased the

anthocyanin content in lettuce (Gómez and Jiménez, 2020). However, in our experiments, an increase of the percentage of blue light in the light spectrum did not increase the content of antioxidants compounds such as anthocyanins, flavonoids and rosmarinic acid in green or purple basil (**Chapter 3**). In tomato, an optimum of 12 % blue light (compared to 0 and 24 % blue) as supplementary light in greenhouse conditions improved the postharvest chilling tolerance of fruits. Blue light did not affect the activity of CAT or content of total ascorbic acid. The chilling tolerant tomatoes lost the red color faster which may indicate that the antioxidant lycopene scavenged ROS (Affandi *et al.*, 2022). In basil, we found that both a low (9 %) and a high (90 %) percentage of blue light in combination with increased light intensity improved the postharvest chilling tolerance. This was not attributed to an increase in antioxidants but rather an increase in soluble sugars and starch (**Chapter 3**). While we found that blue light had no effect on postharvest chilling tolerance Jensen *et al.* (2018) found supplementary blue light in greenhouse cultivated basil to have a negative effect on postharvest chilling tolerance. In contrast, green light improved the chilling tolerance in basil, and this was correlated with a decrease in stomatal density and stomatal pore area per leaf area. The improved chilling tolerance in response to modified light spectra and increased light intensity can therefore be due to several mechanisms such as sugar metabolism, antioxidants, and stomatal regulation characteristics.

6.3.3 The effect of far-red light on chilling tolerance

The involvement of far-red (FR) light in acclimation to chilling has gained increasing interest. Most authors relate the proposed mechanism of FR to the induction of the C-repeat-binding factors CBF pathway which function upstream of the cold responsive (*COR*) genes (Franklin and Whitelam, 2007; Wang *et al.*, 2016*a*; Ahres *et al.*, 2021). *COR* genes have a vast function in plants such as inducing enzymes for the biosynthesis of osmoprotectants and proteins related to lipid metabolism (Liu *et al.*, 2019). FR has also been found to have an effect on the physical properties of tomato such as increased firmness which led to an increase in postharvest chilling tolerance (Affandi *et al.*, 2020). Schwend *et al.* (2016) found that FR increased the content of rosmarinic acid in basil and suggested phytochromes to be involved in the regulation. In barley, additional FR improved freezing tolerance, reflected in a lower ion leakage (Ahres *et al.*, 2021). Additional FR at moderate temperature (15 °C) also upregulated the content of abscisic acid (ABA) (Ahres *et al.*, 2021). An increase in ABA and jasmonic acid (JA) are expected when the CBF pathway is induced (Wang *et al.*, 2016*a*). We found that in basil cultivated at both high (25 °C) and low (15 °C) temperature, additional FR improved chilling tolerance (**Chapter 4**). In basil, FR did not increase ABA or JA (**Chapter 4**). Thus, although FR did not

increase ABA or JA and presumably did not induce the CBF pathway in basil FR can still improve chilling tolerance. The addition of FR light increased the content of carbohydrates in basil (C**hapter 4**) up to 12 mg/ g DW when cultivated at a high temperature (25 °C) and up to 30 mg/g DW when cultivated at a low temperature (15 °C). FR-induced improvement of chilling tolerance. This was most likely due to an increase in carbohydrate content as no effect on antioxidants (i.e. rosmarinic, chicoric and total ascorbic acid) or hormones (ABA and JA) was found. Furthermore, FR has an effect on photochemistry. For optimal photochemistry both photosystem II and photosystem I should be equally excited. PSII is generally excited by shorter wavelengths (400-670 nm) and additional FR may improve the excitation balance (Zhen and Bugbee, 2020). The combination of high light and low temperature can induce photoinhibition of PSII (Allen and Ort, 2001) and cause damage to PSI (Scheller and Haldrup, 2005). The addition of FR could potentially aid the plant in overcoming light and temperature stress during cultivation.

6.3.4 Light and chilling tolerance in leaves

Light can improve chilling tolerance in basil if a small increase in carbohydrates is achieved before harvest. This can be done through an increase in light intensity (**Chapter 3**) or through moderation of the light spectra, specifically under cultivation of additional FR light (**Chapter 4**). If the CBF-pathway is present FR may improve chilling tolerance (Wang *et al.*, 2016*a*). The CBF-pathway has yet to be identified in basil and we have no indications that FR changes levels of associated plant hormones (ABA and JA). Phenolic compounds are strong antioxidants and have been suggested to improve stress tolerance (Rice-Evans *et al.*, 1997). From the studies described in this thesis (**Chapter 2, 3, 4**) it appears that an increase in antioxidants such as rosmarinic acid, chicoric acid and total ascorbic acid does not improve chilling tolerance. Thus, increasing the content of antioxidants in basil is not an efficient strategy to improve chilling tolerance. Basil generally is rich in antioxidants (Kwee and Niemeyer, 2011) and based on our results, the antioxidant level present at regular cultivation conditions (i.e. without an increase in light intensity or increase in blue or FR) is sufficient.

6.4 Can lowered temperature improve chilling tolerance?

Low temperature cultivation may increase antioxidants such as rosmarinic acid. During low temperature the activity of phenylalanine ammonia-lyase (PAL) was up-regulated in jojoba leaves (Gao *et al.*, 2019). PAL is a key enzyme in the phenylpropanoid pathway from which rosmarinic acid is synthesized. In petunia, rosmarinic acid was found to increase in response to cold acclimation (Pennycooke *et al.*, 2005). In accordance, a chilling tolerant inbred line of maize had an increased

phenolic content (i.e. phenolic acids and flavonoids) when subjected to cold stress (Yu *et al.*, 2022). Similarly, we found that the content of rosmarinic acid increased in response to low temperature cultivation (**Chapter 4**). However, the low temperature cultivation did not improve chilling tolerance in basil. Low temperature during cultivation can have a priming function i.e. preparing plants for chilling stress and improving their tolerance (Baier *et al.*, 2019). During priming of peaches enzymes related to lipid and carbohydrate metabolism were up-regulated which added to the improved chilling tolerance (Tanou *et al.*, 2017). We found low temperature during cultivation to increase the carbohydrate content (**Chapter 4**). This, is a common response to low temperature (Yuanyuan *et al.*, 2009). Although an increase in carbohydrates may be beneficial and improve chilling tolerance, we did not observe an improved chilling tolerance in basil (**Chapter 4**). Thus, the low temperature presumably caused an increase in ROS. The increase in carbohydrates during low temperature cultivation was not sufficient to improve chilling tolerance.

The tolerance to chilling can be improved through exposure to a stress event (e.g. low temperature) which can result in a stress memory and improve the tolerance in the future. This is also called priming, acclimation, conditioning or hardening (Hossain *et al.*, 2018). Priming is defined as a process where the duration of the low temperature is not long enough induce cold acclimation and a lag phase takes place between the priming event and the sustained stress (Baier *et al.*, 2019). We cultivated basil for three weeks at low temperature (15 °C) before harvest (**Chapter 4**). This had a negative effect on the plants resulting in chlorotic leaves. To attain a priming effect the temperature should have been raised to the regular cultivation temperature (25 °C) before the adverse postharvest storage. Three weeks of low temperature could have resulted in cold acclimation. However, chilling sensitive plants such as basil may experience cold stress during cultivation at 15 °C resulting in chilling injury (Mckersie and Leshem, 1994). Light intensity also plays a role in the plant response to cultivation at low temperature. The combination of low temperature and high light can increase chilling injury. Increased light intensity (up to 600 µmol m^{-2} s^{-1}) in cucumber kept at 4 °C for 15 hours increased chilling injury (Ashrostaghi *et al.*, 2022). This indicates that even short durations of low temperature can result in unwanted increase in chilling injury.

Thus, lowering the temperature during cultivation for a short period may result in a priming effect which can improve the later tolerance to chilling. However, it is important to take other environmental factors such as light intensity and spectrum into consideration as it can have an interactive effect with the temperature.

Furthermore, the duration of the low temperature period and a potential lag phase needs to be investigated in basil.

6.4.1 Postharvest temperature effects on chilling tolerance

Chilling tolerance can also be improved through different temperature treatments during postharvest storage. An example of temperature treatments during postharvest storage are low temperature conditioning i.e., the product is exposed shortly (hours to days) to temperatures just above the temperature that can lead to chilling injury. This is a useful strategy especially in fruit such as avocado (Woolf *et al.*, 2003). It has also been shown to function in leaf discs of cucumber cotyledons that were kept at 12.5 °C for six hours before being transferred to 2.5 °C (Lafuente *et al.*, 1991). In basil, the recommended non-chilling temperature is between 10-12 °C (Lange and Cameron, 1994). Thus, low temperature conditioning would be around 8-10 °C. Temperature conditioning of basil cuttings for one day at 10 °C before transfer to 5 °C increased the shelf life with 5 days (Lange and Cameron, 1997). We found that basil stored at 8 °C during 12 days in darkness did not show chilling injury (i.e. based on F_v/F_m values) (Fig. S1). Temperature conditioning of basil could potentially be carried out at 8 °C. Another temperature strategy is intermittent warming. When intermittent warming is used the product is placed in room temperature before onset of irreversible chilling injury (Wang, 1994). This has been shown to improve chilling tolerance in tomato fruit (Biswas *et al.*, 2012) and bell pepper (Liu *et al.*, 2015). The onset of severe chilling injury is much slower in fruit compared to leaves. Symptoms of chilling injury are visible in basil leaves after 3-6 days of storage (**Chapter 2, 3 & 4**). Intermittent warming would therefore not be a useful strategy in leaves as the period before the transfer from low temperature to warm temperature would be too short. In contrast to low temperature treatments, treatments with hot air (i.e. 38-40 °C for 4 to 8 hours) had some effect on chilling tolerance in basil (Aharoni *et al.*, 2010). However, the effect of the hot air was affected by the harvesting time of basil. Basil harvested late in the afternoon or evening has an improved shelf life (Kenigsbuch *et al.*, 2010). Carbohydrate content of the plants are the highest in the late afternoon and evening as photo-assimilates are produced during the day (Scialdone and Howard, 2015). This indicates that the metabolite status (especially the carbohydrate content) is important for chilling tolerance. Treatments with hot water of 45 °C for 10 min of kiwi fruit induced the CBF pathway and improved chilling tolerance (Ma *et al.*, 2014). Thus, short durations with either low temperature (for a few hours) or hot temperature (for few minutes) may be viable strategies to improve chilling tolerance in basil. However, this remains to be investigated further.

6.5 Application of chemicals to improve chilling tolerance

Chilling tolerance can also be improved by chemical priming where application of chemical components during cultivation induce stress tolerance (e.g. through an increase in ROS scavenging and increase in osmoprotection) (Savvides *et al.*, 2016). Numerous of natural and synthetic chemical components have been investigated to improve tolerance to low temperature. Examples of application of chemical components during cultivation include; ascorbic in tomato plants (Elkelish *et al.*, 2020), treatments of tomato roots with H_2O_2 (İşeri *et al.*, 2013), proline in chickpea (Kaur *et al.*, 2011), ABA in basil (Satpute *et al.*, 2019). Chemical components can also improve chilling tolerance when they are applied during the postharvest phase. In peaches application of melatonin improved the chilling tolerance (Cao *et al.*, 2018), and in bell pepper application of glutathione improved chilling tolerance (Yao *et al.*, 2021). These chemical components may have a scientific relevance aiding in the understanding of the underlying mechanism of chilling tolerance, however, application in practice does not appear feasible. Consumers prefer products without application of chemicals. Furthermore, applications of these chemicals during growth or postharvest is often not allowed by law. Depending on the mode of action and purpose of the chemical they may be regulated as plant protection products (also known as pesticides) (Commission, 2022). Approval of pesticides are a lengthy and costly process. Therefore, these potential solutions have not been studied nor discussed in the framework of this thesis.

6.6 What is good production quality?

6.6.1 Visual quality

Quality is composed of several characteristics such as the visual quality (color, morphology, lack of brown spots or decay), flavor (taste and aroma), texture and nutritional value. A product is first judged on its visual quality after which the flavor, texture and nutritional value is taken into consideration (Barrett *et al.*, 2010). Part of the visual quality of herbs and leafy vegetables is related to their color which is provided by pigments i.e. chlorophyll (green) and anthocyanin (red-purple) content. Pigment content can be affected by light intensity and spectrum. Chlorophyll concentration increased with increasing percentage of blue light in leaves of tomato, cucumber radish and pepper when cultivated under a high light intensity (i.e. 500 µmol m^{-2} s^{-1}) (Snowden *et al.*, 2016). The chlorophyll concentration also correlated with the intensification of the visually green color. In basil an increase in light intensity from 160-310 µmol m^{-2} s^{-1} was negatively correlated with an increase

in chlorophyll content (Dou *et al.*, 2018). During senescence leaf color can change from green to yellow due to the degradation of chlorophyll. A high content of chlorophyll at-harvest is desired to maintain the green color during postharvest. In our experiment with blue light, we found no effect on chlorophyll content (Fig. S2), whereas an increased light intensity resulted in a moderate increase. Additional FR to a R:B background reduced the relative chlorophyll content in lettuce whereas FR only decreased the chlorophyll content in basil when cultivated without blue light (Meng and Runkle, 2019). Anthocyanin content can also be increased with an increase of blue light in the spectra (VanDelden *et al.*, 2021) or with an increase in light intensity (Dou *et al.*, 2018). However, in our experiments neither intensity nor increase in blue light affected the anthocyanin content (**Chapter 3**).

During storage of basil at low temperature dark spots can form on the leaves (Wongsheree *et al.*, 2009). Browning of leaves can be a result of enzymatic reactions by peroxidases (POD) and polyphenol oxidases (PPO). Reactions with POD and PPO uses phenolic compounds as substrate (Nokthai *et al.*, 2010) thus a high content of phenolic compounds can have a negative effect resulting in a lower quality. We found that a high light intensity (600 µmol m^{-2} s^{-1}) applied as EOP treatment stimulated the development of black spots when the leaves were stored at low temperature (**Chapter 2**), this was also the treatment with the highest content of phenolic compounds. Thus, although increased light intensity has been reported to increase pigment content a high light intensity can also have adverse effects. We did not find high light intensity as EOP treatments to improve the visual quality of basil (**Chapter 2**).

6.6.1.1 Shelf life

Shelf life is an important parameter for quality. An increase in dry matter content is an indicator of storability for fruit (Velemis *et al.*, 1997). Similarly, for spinach an increase in dry matter content up to 6.5 % increased the shelf life (Gertsson and Olsson, 2006). An increase in dry matter content may also have been beneficial up to an optimum in our studies. When plants were treated with an increase in light intensity as EOP treatment the dry matter content generally increased (**Chapter 5**). We found that an increase in EOP light intensity (i.e. from 50 to 600 µmol m^{-2} s^{-1}) increased the dry matter content from 7 - 12 % (**Chapter 5**). The shelf life of basil treated with the lowest light intensity (i.e. 50 µmol m^{-2} s^{-1}) had the lowest dry matter content and the shortest shelf life at both a chilling or non-chilling temperature (**Chapter 2**). However, a further increase in dry matter content did not improve shelf life (**Chapter 2**). In the experiments described in **Chapter 3** the increase in shelf life correlated with an increase in dry matter content (**Chapter 5**). This was also reflected

in the carbohydrate content which accounts for a large part of the dry matter content. A high dry matter content may come at an expense of a high yield (fresh weight). In baby leaves belonging to the Brassicaceae, Asteraceae and Amaranthaceae families a high dry matter content was negatively correlated with a high yield (i.e. based on fresh weight) as the crops had less water (Takahama *et al.*, 2019). Total ascorbic acid has also been associated with an increase in shelf life in spinach and lettuce (Gertsson and Olsson, 2006; Min *et al.*, 2021). EOP high light intensity (i.e. up to 300 μmol m^{-2} s^{-1}) is a very useful strategy to increase the total ascorbic acid content (**Chapter 2**). Thus, high EOP light intensity can improve shelf life of leafy greens and herbs when stored in optimal conditions and is only effective in modulating chilling sensitivity when a moderate (100 μmol m^{-2} s^{-1}) increase in light intensity occurs (**Chapter 3**).

6.6.2 Morphology

Appearance of a product is determined by its color and morphology such as the leaf shape (i.e. leaf area, thickness, shape, length, width, curling), stem shape (i.e. length and thickness and number of leaves (Barrett *et al.*, 2010). A desired morphology of herbs such as basil for fresh cut or potted plants would include compact plants and a high allocation of biomass to the leaves rather than the stems. Compact plants are easier to transport which is important for the growers and transporters in the supply chain (Mcausland *et al.*, 2020). Stem elongation is often a reflection of the shade avoidance syndrome (SAS). SAS is one of the most common responses to a lowered R:FR (Franklin, 2008). The addition of FR light to basil cultivation did indeed resulted in a linear increase with increased FR intensity and duration in plant height in basil (**Chapter 5**). A similar response can be obtained with a high percentage of blue light (≥90 % blue) (Kong *et al.*, 2018; Zhang *et al.*, 2021). This is in accordance with our findings where 90 % and 100 % blue light resulted in an increased plant height in green and purple basil (**Chapter 5**). When the blue light in the spectrum is ≥90 % the activity of the phytochromes is low (PSS 0.7-0.49) resulting in a similar response as when the spectrum contains FR. Both spectrum with low PSS value and high temperature may increase SAS such as plant height. In Arabidopsis SAS is increased when cultivated in a warm climate (Romero-Montepaone *et al.*, 2020). Thus, the increase in plant height in basil cultivated under a high percentage of blue light and additional FR may have further been increased due to cultivation at 25 °C (**Chapter 5**).

For the consumers the leaves of herbs are the main consumed part and an increase in leaf area and leaf biomass is a desired morphology (Mcausland *et al.*, 2020). For fresh-cut basil big leaves are desired (i.e. a large leaf area). We found, fresh mass

and leaf area to increase with an increase in EOP light intensity up to 300 µmol m^{-2} s^{-1} at both a high and low percentage of blue in green basil (**Chapter 5**). Leaf area may also increase with an increase in FR light as a SAS response (Demotes-Mainard *et al.*, 2016). In this thesis, FR light only increased the leaf area when applied throughout the cultivation (for three weeks). FR did not increase the leaf fresh mass (**Chapter 5**). FR has been reported to increase the leaf fresh mass of baby leaves lettuce in comparison to white light without FR (Li and Kubota, 2009). Additional FR to a 89:11 red:blue background also increased the plant dry mass of lettuce at three planting densities (Jin *et al.*, 2021). FR had the largest effect on plant biomass at a low plant density (23 plants m^{-2}) compared to a high plant density (51 plants m^{-2}). Thus, the spectral effects on plant growth and morphology may depend on plant density.

We found that FR throughout the cultivation also resulted in an increase in stem length which is not desirable for quality. A high intensity of FR (180 µmol m^{-2} s^{-1}) also reduced the dry mass partitioning to the leaves (**Chapter 5**). Optimal morphology and other aspects of quality such as flavor may not correlate. In coriander, a trait off between desired morphology (compact plants and low stem biomass) and aroma existed under different supplementary light treatments with light emitting diodes (LED) light (Mcausland *et al.*, 2020). This indicates that when studying quality all aspects of the plant response to a given environmental treatment is important. We showed that increased light intensity (up to 300 µmol m^{-2} s^{-1}) as EOP treatments did not have an adverse effect on morphology and could be beneficial for improvement of shelf-life and chilling tolerance (**Chapter 5, 3**). The use of FR had undesirable effects on morphology but improved chilling tolerance (**Chapter 5, 4**). To find one light regime which will optimize growth, morphology, taste and chilling tolerance may be difficult. The potential trait off between different growth and quality parameters still needs to be further investigated in basil. Furthermore, in the research described in this thesis the effect of plant density was not considered. At a high plant density, the captured light per plant is lower than at a low plant density. An increase in plant density decreases the total vegetative plant mass, decreases the allocation leaves, and increases specific leaf area. Plant density may also affect plant height (Poorter *et al.*, 2016). Thus, plant density should be taken into consideration when the effect of light intensity and spectrum on plant growth and morphology is studied. Another important aspect to take into consideration is the consumer preferences which was not studied in the chapters of this thesis.

6.6.3 Consumer preferences

Optimal quality is driven by consumer preferences. Basil is consumed as a culinary herb for its flavor. Flavor is determined by the content and ratios of volatile organic compounds (VOC) that can be affected by the environmental conditions such as light intensity and spectrum (Carvalho *et al.*, 2016; Walters *et al.*, 2021). As basil is a high value crop it is important to study the effect of cultivation on flavor and consumer liking. An increase in daily light integral (DLI) increased the content of VOCs (Chang *et al.*, 2008). Similarly, Walters *et al.* (2021) found the content of VOCs to increase with an increase in light intensity. Thus, the VOC content of basil cultivated under EOP high light intensity (**Chapter 2**) may also been increased, but this was not measured. Interestingly, the consumer preference was not correlated with an increase in VOCs but reached an optimum in basil cultivated under 200 µmol m^{-2} s^{-1} (Walters *et al.*, 2021). The content of different volatile classes can be altered by the composition of the light spectrum. Carvalho *et al.* (2016) found monoterpenoids to be dominant when plants were grown under red/blue/green or red/blue/yellow whereas the red/blue/FR increased the content of sesquiterpenoids. Pennisi *et al.* (2019) found a high percentage of blue light in the spectra reduced the content of linalool (i.e. one of the main VOCs in basil. As we did not measure the VOCs in the experiments described in **Chapter 3** the effect of blue light on VOCs in the cannot be ruled out. However, in contrast to Pennisi *et al.* (2019) we did not find an effect of percentage of blue light had on total flavonoid content. Hence, the response of secondary metabolites (antioxidants and VOCs) may be cultivar dependent in basil. For further studies it is important to include analysis of VOCs and sensory panels to establish quality in relation to consumer preferences.

6.6.4 Is light important for quality?

Light is important for plant growth and morphology. Modifying the light spectra and increasing the intensity can increase photosynthesis leading to an increase in growth, leaf area, leaf thickness, increase or reduction in stem length. We only found light spectra with an increase in the percentage of blue blight or duration of additional FR had a moderate impact on plant secondary metabolites (phenolic acids, total ascorbic acid, flavonoid content, anthocyanin content and VOCs) (**Chapter 3, 4**). Compared to a modification in light spectra an increase in light intensity had a bigger effect on secondary metabolites. However, only a moderate increase in light intensity (up to 300 µmol m^{-2} s^{-1}) improved chilling tolerance (**Chapter 2, 3**). Thus, based on the results described in this thesis a light intensity of 150-300 µmol m^{-2} s^{-1} during growth is sufficient for optimal growth and postharvest quality. However, when considering the optimal light intensity, it is important to take the plant density into account as this can change the light available per plant. A low light intensity (i.e. EOP light

intensities of 50 µmol m^{-2} s^{-1} and 100 µmol m^{-2} s^{-1}) resulted in a decreased overall visual quality (**Chapter 2, 3**). Contrary to findings by Min *et al.* (2021) increased light intensity as EOP light only increased the postharvest quality until an optimum (i.e. 300 µmol m^{-2} s^{-1}) (**Chapter 2**). The pigment content is important for the visual quality. Although light intensity and spectra may have an effect on chlorophyll and anthocyanin content in basil (Dou *et al.*, 2018; Pennisi *et al.*, 2019, 2020) this was not the case in the studies described in this thesis (**Chapter 3,** Fig. S2). Additional FR to a red blue background had no effect on relative chlorophyll content in basil (Meng and Runkle, 2019). Thus, the light spectra do not need to be modified to achieve an optimal leaf coloration of basil.

6.6.4.1 Light and nutrients

Light is not the only environmental factor which can affect quality. Most studies focus on one environmental factor. It is important to further investigate light in combination with other environmental factors such as temperature, nutrient concentrations and CO_2. Basil cultivated with a high potassium concentration (5.0 mM K) had a higher content of phenolic compounds such as rosmarinic and chicoric acid (Nguyen *et al.*, 2010). The fertilizer composition may also increase biosynthesis of some compounds. In hydroponically grown lettuce, an increase in nitrogen application from 60-180 mg/L decreased the ascorbic acid whereas it increased the content of chicoric acid in a green and red cultivar (Mampholo *et al.*, 2019). The electrical conductivity (EC) of the nutrient solution used in hydroponically cultivated basil can also affect plant growth and quality. An optimum EC of 3.0 dS m^{-1} was found for growth resulting in increased leaf fresh and dry mass and increased leaf area. Lowering the EC to 0.5 dS m^{-1} as EOP treatment 5 days before harvest also improved quality through an increase in total phenolic content and antioxidant capacity (Ren *et al.*, 2022). In the research described in this thesis a standard solution of nutrients was used. The EC was increased after transplant from1.7to 2.3 dS m^{-1}, the pH was maintained at 5.7 throughout the cultivation period. The effect of the nutrient solution and nutrient availability on plant growth and quality may also depend on the light intensity and composition of light spectrum. Pennisi *et al.* (2019) found the red:blue ratio to affect the nutrient use efficiency (i.e. the ratio between fresh mass and the total concentration such as N, P, K, Ca, Mg, and Fe). The effect of nutrients and their interaction with light intensity and spectra on basil quality needs to be further investigated.

6.6.4.2 Light and temperature

The plant response to light treatments can potentially interact with other environmental factors such as temperature. We found low temperature to increase

the content of carbohydrates and antioxidants. Low temperature in combination with additional FR light further increased the content of carbohydrates (**Chapter 4**). Most studies cultivate basil at a temperature range of 22-26 °C with a light intensity range of 150-310 µmol m^{-2} s^{-1} (Dou *et al.*, 2018, 2019; Jensen *et al.*, 2018; Pennisi *et al.*, 2019, 2020). These authors studied the effect of light without studying the potential effect of temperature. Most studies focus on one environmental factor. It is important to further investigate light in combination with other environmental factors such as temperature, nutrient concentrations and CO_2. In a study focusing on the effect temperature on basil growth, the optimal temperature for basil growth to be 29 °C under 366 µmol m^{-2} s^{-1} and ambient CO_2 concentrations (Walters and Currey, 2019). In gerbera, cultivation at suboptimal temperature (both high and low temperature) combined with supplemental lighting with high pressure sodium lamps increased undesirable morphology of stem bending and reduced the shelf life (Davarynejad *et al.*, 2008). The effect of light intensity and spectrum with an increase in percentage of blue light on growth and quality have yet to be combined with temperature studies in basil.

6.7 Efficiency of cultivation system in a vertical farm

Production of herbs, vegetables and fruits in closed production systems such as verticals farms requires a large energy input especially for lighting and temperature regulation (in particular cooling). In traditional greenhouses the energy from the sun is largely used for both light and heating, however supplementary lighting and heating or cooling may be needed depending on the latitude and season (Graamans *et al.*, 2018). As the energy input to a closed production system has high economic and environmental costs the resource must be utilized most efficiently. Part of this is to improving the light use efficiency (LUE) of the plants (VanDelden *et al.*, 2021). LUE has several definitions such as based on the incident radiation (LUE_{inc}), on the total absorbed light (LUE_{abs}) (Gitelson and Gamon, 2015) and can be based on the fresh mass or the dry mass of the whole plant or the marketable product (g mol^{-1}) .

LUE can be optimized in several ways by for instance plant density, light spectra and intensity, photoperiod, climate. With the addition of FR, the LUE can be increased as FR increases the leaf area and thereby increasing the capture of light. In lettuce, LUE was increased with the addition of FR (Jin *et al.*, 2021). Similarly, we found additional FR applied throughout the growth improved LUE in basil whereas FR as EOP treatment did not (**Chapter 5**). Here, LUE (for both fresh and dry mass) was calculated based on the incident light in the range of 400-700 nm which is considered the photosynthetic photon flux density (PPFD). FR light ranges from 700-800 nm, thus the radiation from FR should be included to evaluate the

efficiency. When FR is included it is called the radiation use efficiency (RUE) (based on light from 400-800nm). Indeed, we found that additional FR of 180 µmol m^{-2} s^{-1} applied for one or three weeks reduced the RUE based on both fresh and dry mass. This is in contrast to the findings by Jin *et al.* (2021) where the addition of FR in lettuce cultivation increased the RUE based on dry mass. Furthermore, we found that the LUE basil was not affected by percentage of blue light throughout the growth or as EOP (**Chapter 5**) as the fresh mass of basil was not affected by the percentage of blue light. Light intensity also highly affects the LUE. Pennisi *et al.* (2020) found the light intensity 250 µmol m^{-2} s^{-1} resulted in the highest LUE based on harvested dry mass for basil after which the LUE decreased at higher light intensity. A similar response was seen for the basil cv. Dolly where LUE based on dry mass reached an optimum with a light intensity of 300 µmol m^{-2} s^{-1} (**Chapter 5**). The LUE is based on the fresh or dry mass of the whole plant (i.e. both leaves and stem). The edible part of basil is only the leaves i.e. although a light intensity or spectra results in an increased LUE it may not result in the desired morphology. In the end the financial viability of the vertical farming system is affected by the energy use efficiency (EUE). EUE also takes the accumulated power consumptions of the lamps into account. This depends on the efficacy of the lamps which is the lamps efficiency in converting electricity to photon flux density (400-800 nm) (µmol J^{-1}) (Kusuma *et al.*, 2020). LEDs have had a large increase in efficacy the last decade and may continue to have a moderate increase. The efficacy is affected by the light spectra of the fixture (Pattison *et al.*, 2018*a*). Piovene *et al.* (2015) found an increase in blue light (up to 37% blue) to improve the energy use efficiency (EUE). An increase in blue light is often expected to lower the EUE as the efficacy of blue LEDs is lower than the efficacy of red LEDs (Kusuma *et al.*, 2020). Thus, besides LUE, the efficacy of the fixture needs to be taken into consideration for the energy use efficiency of the whole production system. Based on the results in **Chapter 3** where an increase in percentage of blue light did not improve quality it is possible to optimize the EUE of a basil production system without compromising the quality.

However, to optimize the EUE of the whole production system climate control (i.e. cooling and ventilation) is an important parameter. With an increase in LUE less fixtures are needed which will result in a smaller need for cooling (VanDelden *et al.*, 2021). Thus, a holistic view (i.e. incorporating plant growth, morphology, metabolite content and shelf-life) is needed before a light intensity or spectrum used during cultivation can be considered optimal.

6.8 Perspective for further research

Based on the collective results described in this thesis the following recommendations were derived for future research in basil:

Breeding of cultivars for vertical farming: The current cultivars have been bred for greenhouse or field cultivation where the environmental conditions potentially change more rapidly. The response of growth and morphology to an environment without abiotic or biotic stress may be different to that of a more dynamic climate in field or greenhouse. However, this depends on the management strategies of a vertical farm. The content of secondary metabolites which gives the color and flavor of horticultural products are often increased when plants are subjected to environmental stress. With a vertical farm it is easy to apply a controlled environmental stress such as changes in light intensity or spectrum, temperature, water and nutrients, or air flow (VanDelden *et al.*, 2021).

Effect of multiple environmental factors: The effect of a single factor may be enhanced or diminished if combined with another factor. Current research mainly focuses on one environmental factor.

Temperature conditioning: Temperature conditioning can be carried out either during the pre-harvest phase or during postharvest. Lowering the temperature for some hours can potentially improve chilling tolerance in basil (Lange and Cameron, 1997). However, the underlying mechanism has not been determined in basil.

Postharvest light: This thesis adds to the literature on chilling injury of leaves stored in darkness. Light can also be applied during the storage. During storage of broccoli 12 µmol m^{-2} s^{-1} of continuous light improved the shelf life at 20 °C (Büchert *et al.*, 2011). In lettuce, 7 and 30 µmol m^{-2} s^{-1} light from fluorescent tubes improved the shelf life of fresh cut lettuce stored at 10 °C (Woltering and Witkowska, 2016). However, this was in chilling tolerant species. The use of postharvest light in the non-tolerant species basil has yet to be studied. The combination of high light and low temperature has a negative effect on chilling tolerance (Ashrostaghi *et al.*, 2022). However, it remains to be studied if low light and low temperature can have a positive effect of chilling tolerance.

Chemical priming: Chemical priming can be carried out in the last phase of the pre-harvest period or in the beginning of the postharvest. It may be a useful tool for research. Exogenous application of antioxidants or hormones can help us to

understand which compound may be important for chilling tolerance. However, chemical priming is not a viable strategy as the focus of vertical farming should be to move away from chemical applications on products. Thus, after understanding which compounds may be beneficial for chilling tolerance, we need to find a way to increase it without external application. Chilling tolerance in basil is still not fully understood and chemical priming may help to solve a piece of the puzzle.

Combining pre-harvest and postharvest: To improve quality and chilling tolerance it is important to combine pre- and postharvest factors. Studies of plant growth should always include at least a test of shelf life before conclusions can be made on the effect of pre-harvest factors on quality.

6.9 Conclusion

Based on the experiments described in this thesis (**Chapter 2-5**) and the current literature it is concluded that:

- Phenolic compounds may increase the activity of enzymatic antioxidants. This might have a moderate positive effect on chilling tolerance.
- An increase in phenolic compounds through light treatments does not improve chilling tolerance.
- An increase in soluble sugars can improve chilling tolerance. Increased light intensity can lead to more soluble sugars but also a simultaneous increase in ROS hence this may result in no net effect on chilling tolerance.
- Decision on the lighting strategy can mainly be based on improving the growth and morphology of basil as within a wide range the effects of spectrum and intensity on carbohydrates and antioxidants are relatively small.
- Increase in percentage of blue light (>9 %) during cultivation does not improve basil quality.
- Additional FR during cultivation improves chilling tolerance of basil.
- To improve quality, it is important to study the effect of the pre-harvest factors on postharvest quality.

Supplementary material

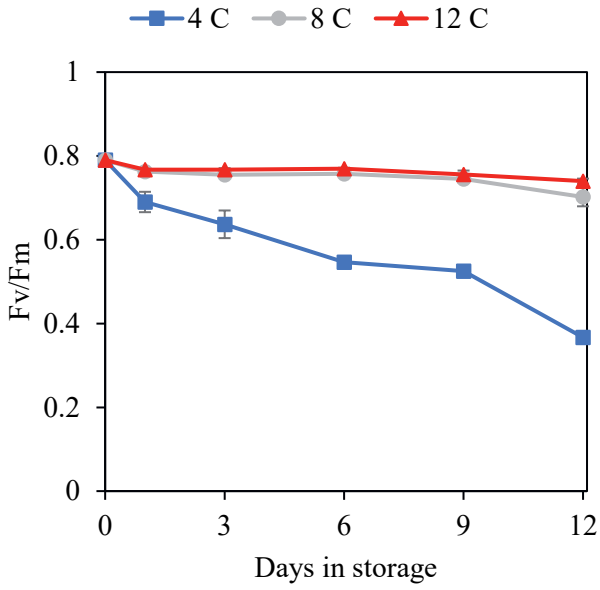

Figure S1. F_v/F_m of basil leaves stored in darkness at 4 °C (blue, square), 8 °C (grey, circle), 12 °C (red, triangle) for 12 days. Basil cv. Emily was cultivated under 300 µmol m^{-2} s^{-1} red-white LED light for 40 days.

Chlorophyll extraction

Chlorophylls was extracted according to Wellburn (1994) with modifications. Extraction was done on 100±10 mg of frozen ground basil leaves with 1.1 mL of 80% acetone. The mixture was vortexed and centrifuged at 21300 rcf for 10 min at 4°C (UNIVERSAL 320R Centrifuge, Hettich Zentrifugen). Then, 0.9 mL of the supernatant was collected into a 3 mL cuvette. The extraction was carried out two more times and the supernatant was collected in the same 3 mL cuvette. The absorbance was measured at λ= 470, 646.8, and 663.2 nm with a UV/Vis spectrophotometer (GENESYS Family spectrophotometer, ThermoFisher Scientific) against a blank.

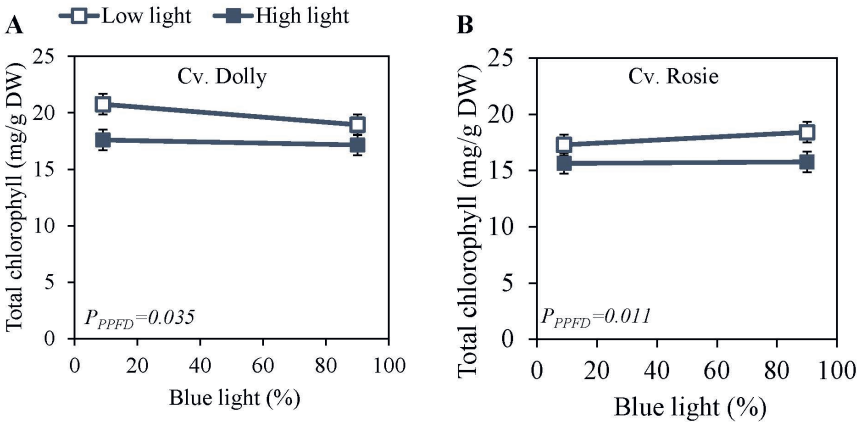

Figure S2. Total chlorophyll content in A. the green. cv Dolly, B. the purple cv. Rosie. The plants were exposed to either 9 % or 90 % blue light at low PPFD (100 µmol m^{-2} s^{-1}) (open symbols) or high PPFD (300 µmol m^{-2} s^{-1}) (closed symbols) applied the last five days before harvest as End-Of-Production (EOP) treatments. Before EOP treatments plants were grown under red-white light (PPFD of 200 µmol m^{-2} s^{-1}, 9% blue) for 30 days. All values are expressed per gram dry weight in the leaves. The data are means of three blocks (n=3) for cv. Dolly and means of four blocks (n=4) for cv. Rosie (i.e. per block four replicate plants). Standard errors of means are shown as error bars. Significance of the main effect percentage of blue light and PPFD (α=5 %) are shown. Experiment 2, **Chapter 3**.

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Summary

Quality of fresh products is determined by genotype and environmental practices. Light intensity and spectrum are environmental factors which have significant effects on quality. Light can increase the content of metabolites, such as carbohydrates and antioxidants. To maintain shelf life of fresh products low temperature storage is often used during the postharvest phase as the water loss and metabolic rates slows down which will delay senescence. However, crops of tropical origin are sensitive to temperatures below 10-12 °C. One of such crops is basil (*Ocimum basilicum* L.). Basil suffers chilling injury (CI) when stored at low temperature. CI is visible within a few days as dark spots on the leaves. Thus, there is a large potential to improve the chilling tolerance of basil. Chilling tolerance may be improved by an increase in carbohydrates and antioxidants such as phenolic compounds. They may scavenge the reactive oxygen species produced during chilling. In this thesis it was investigated if an increase in light intensity and modification of the light spectrum (i.e. an increase in percentage of blue light) could increase the content of carbohydrates and antioxidants resulting in improved chilling tolerance. Furthermore, it was investigated if the addition of far-red (FR) during cultivation would increase the levels of hormones related to chilling tolerance and in turn improve the postharvest chilling tolerance. Light also affects the morphology which is an important quality parameter. Thus, response of growth and morphology to light intensity and spectra was also studied. In a number of experiments basil was grown in a climate chamber in a vertical farming set-up and the effect of light intensity and spectra on at-harvest and postharvest quality were studied.

A general introduction is presented in **Chapter 1**. Quality of fresh products is described; it is generally accepted that an increase in carbohydrates and phenolic compounds can increase the quality of a product. Carbohydrates add to the sweet taste of a product whereas phenolic compounds include the flavor related volatile organic compounds and color. Furthermore, carbohydrates and phenolic compounds can act as antioxidants. The effect of low temperature and physiological responses of chilling sensitive plants such as basil are reviewed. An increase in carbohydrates and antioxidants may be beneficial for the plants to improve chilling tolerance, the function of phenolic compounds, phenolic acids, flavonoids, anthocyanins, total ascorbic acid and carbohydrates as antioxidants are reviewed.

Chilling tolerance of fresh products may be improved through modification of the cultivation environment. The cultivation environment in a vertical farm can be fully controlled. In particular, the light intensity and light spectrum may influence the quality of fresh products. The effect of light intensity and spectrum on plant growth

and morphology, content of secondary metabolites and quality, and chilling tolerance is described. Finally, the gap in the literature is described and an overview of this thesis is given.

In **Chapter 2** the regulation of carbohydrates and antioxidants in response to high light as End-Of-Production (EOP) treatments is described in two green basil cultivars. In addition, the effect of an increase in secondary metabolites on CI was studied. It was hypothesized that increased EOP high light would increase the content of carbohydrates and antioxidants which would improve the postharvest chilling tolerance. EOP treatments with different light intensities with red-white LED lights were applied for the last five days before harvest in two green basil cultivars. After harvest leaves were stored at 4 and 12 °C in darkness. To evaluate the level of CI the overall visual quality was scored and F_v/F_m (as a marker for CI) was measured on leaves at-harvest and during postharvest. The content of metabolites such as carbohydrates, antioxidants (rosmarinic acid, chicoric acid and total ascorbic acid) and H_2O_2 were measured in leaves at-harvest and in leaves stored at 4 and 12 °C. EOP high light intensity increased the content of carbohydrates and antioxidants at-harvest. However, high light intensity did not improve chilling tolerance. This was likely due to an increase in the level of ROS due to the high light intensities.

In **Chapter 3** the regulation of carbohydrates and antioxidants in response to blue light throughout the cultivation and applied as EOP treatment in green and purple basil cultivars is described. It was hypothesized that blue light would increase the content antioxidants which would improve the postharvest chilling tolerance. Two experiments were conducted. In the first experiment the effect of an increase in blue light percentage at a high light intensity (PPFD of 300 µmol m^{-2} s^{-1}) throughout the cultivation period for 25 days or as EOP treatment for 5 days was studied. In the second experiment the spectral effect in relation to light intensity treatments with a low or high blue light percentage applied at different light intensities (i.e. PPFD of 100 and 300 µmol m^{-2} s^{-1}) was studied. After harvest leaves were stored at 4 and 12 °C in darkness. To evaluate the level of CI the overall visual quality was scored and F_v/F_m (as a marker for CI) was measured on leaves at-harvest and during postharvest. Metabolite content such as carbohydrates, antioxidants (rosmarinic acid, chicoric acid, total ascorbic acid, total flavonoids and total anthocyanins) and H_2O_2 were measured in leaves at-harvest and in leaves stored at 4 and 12 °C. Contrary to the hypothesis the percentage of blue light did not affect the antioxidants such as rosmarinic acid, total ascorbic acid, total flavonoid content and total anthocyanin content. The content of soluble sugars was not affected by the

percentage of blue light whereas the starch content decreased with an increase in percentage of blue light. The effect of blue light on metabolite content was not affected by the light intensity. An increase in the percentage of blue light did not improve chilling tolerance. However, an increase in light intensity did improve chilling tolerance in green and purple basil presumably due to an increase in carbohydrates.

In **Chapter 4** the effect of cultivation with additional FR on postharvest chilling tolerance of basil is described. It was hypothesized that the addition of FR would improve the CI in basil as FR would increase the content of hormones (abscisic acid (ABA) and jasmonic acid (JA)) involved in cold tolerance. It was studied if basil chilling tolerance could be improved in response to additional FR and to a lowered temperature during cultivation. FR was applied both at a high (25 °C) and low (15 °C) temperature. Plants cultivated at high temperature received either no, 1 or 3 weeks additional FR and the low temperature cultivated plants no or 3 weeks additional FR. After harvest leaves were stored at 4 and 12 °C in darkness. To evaluate the level of CI the overall visual quality was scored and F_v/F_m (as a marker for CI) was measured on leaves at-harvest and during postharvest. Metabolite content such as carbohydrates, antioxidants (rosmarinic acid, chicoric acid and total ascorbic acid) and hormone content were measured in leaves at-harvest and in leaves stored at 4 °C. Additional FR did not affect the antioxidant content nor the hormone content. Additional FR increased the carbohydrate content which presumably the improved chilling tolerance. FR improved chilling tolerance was at both high and low temperature.

In addition to the intrinsic plant quality (carbohydrate and antioxidant content) the growth and morphology may also be affected by light intensity and spectrum. In **Chapter 5** the response of basil growth and morphology to light intensity, percentage of blue light and additional FR were studied, using the experiments described in Chapters 2, 3, and 4. Light intensity was studied as EOP treatments applied for the last five days before harvest in two green basil cultivars. The effect of blue light was studied in two experiments. The effect of blue light was studied as an increase in blue light percentage at a high light intensity (PPFD of 300 µmol m^{-2} s^{-1}) throughout the cultivation period for 25 days or as EOP treatment for 5 days. Furthermore, spectral effect in relation to light intensity treatments with a low or high blue light percentage applied at different light intensities (i.e. PPFD of 100 and 300 µmol m^{-2} s^{-1}) was studied. FR was studied by applying additional FR during 1, or 3 weeks of growth compared to a treatment without FR. Plant growth parameters such as fresh and dry mass of leaves and stem and morphology parameters such as

height and leaf area were measured. As a vertical farm requires a high energy input it is important to use it in the most efficient way. Therefore, the light use efficiency of the given treatments was also discussed. Plant height was increased by EOP light intensity, a high percentage of blue light >90 % and additional FR. Leaf area increased quadratically with increasing EOP light intensity. In contrast an increased percentage of blue light decreased the leaf area when cultivated at a high light intensity. Plant fresh mass was increased with increasing EOP light intensity and with FR throughout the cultivation. Increased EOP light intensity increased partitioning of dry mass to the leaves whereas increased percentage of blue light and additional FR throughout the growth decreased the partitioning.

Chapter 6 consists of a general discussion. The role of antioxidants and their potential benefit is discussed. Furthermore, it is discussed if pre-harvest light intensity and spectra can affect chilling tolerance. The potential of low temperature during cultivation and postharvest to improve chilling tolerance is reviewed. Quality of fresh products is discussed with respect to the influence of light and other environmental factors. Lastly the efficiency of cultivation in a vertical farm is discussed and a conclusion is given.

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About the Author

Dorthe H. Larsen was born in Denmark in 1988. After traveling in South America she started her studies in 2009 at University of Copenhagen. She studied a BSc in Natural resources with a specialization in environmental science. However, during her elective courses and her bachelor thesis she found out that plant science was what caught her interests. She changed specialization and studied a MSc in agronomy with specialization in plant science also at University of Copenhagen. After her studies she continued to work as a research assistant at University of Copenhagen. When the opportunity came she applied for a PhD position at Wageningen University which she began in 2017.

List of publications

Larsen DH, Li H, van de Peppel AC, Nicole CCS, Marcelis LFM, Woltering EJ. 2022. High light intensity at End-Of-Production improves the nutritional value of basil but does not affect postharvest chilling tolerance. Food Chemistry **369**, 130913.

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PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS
(= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Preharvest light for postharvest quality of basil

Post-graduate courses (12.6 ECTS)

- Applied methods in crop physiology; Aarhus University (2016)
- Lighting in greenhouses and vertical farms; HPP, WUR (2017)
- Design of experiments; PE&RC (2017)
- Introduction to R for statistics; PE&RC (2018)
- Plant nutrients in terrestrial ecosystems, acquisition and turnover; University of Copenhagen (2018)
- Plants and metabolomics workshop; Leiden University (2019)
- Environmental signalling in plants; Leiden University (2019)
- VOC´s : from volatile sampling to data processing; German Centre for Integrative Biodiversity Research (iDiv) (2020)

Deficiency, refresh, brush-up courses (1.5 ECTS)

- Basic statistics; PE&RC (2017)

Invited review of journal manuscripts (1 ECTS)

- Frontiers in Crop and Product Physiology: preharvest UV light, secondary metabolites, basil (2018)

Competence strengthening / skills courses (4.5 ECTS)

- Workshop carousel; PE&RC (2017)
- Brain training; WGS (2018)
- Scientific writing; WGS (2018)
- Project and time management; WGS (2018)
- Teaching and supervision of BSc and MSc students; WGS 92019)

Scientific integrity/ethics in science activities (0.9 ECTS)

- Ethics in plant and environmental sciences; WGS (2018)
- Scientific integrity; WGS (2019)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC First year weekend (2017)
- PE&RC Day (2019)

Discussion groups / local seminars or scientific meetings (6.45 ECTS)

- Symposium plant control by LED light; WUR (2017)
- Energy and light in greenhouses; WUR (2017)
- FLOP; WUR (2017-2021)
- EPS Meeting; WUR (2017, 2019)
- Postharvest seminar; WUR (2018-2020)

International symposia, workshops and conferences (5.1 ECTS)

- Greensys, ISHS conference; Angers, France (2019)
- VertiFarm workshop on vertical farming; Wageningen, the Netherlands (2019)
- Vertical farming in a Nordic context; Copenhagen, Denmark (2020)

Lecturing/supervision of practicals/tutorials (4.7 ECTS)

- Physiology and development (2017)
- Advanced methods (2018)
- Physiology and development (2018)
- Research methods in crop science (2020)

BSc/MSc thesis supervision (23 ECTS)

- Preharvest far-red light for alleviation of chilling injury in basil; Xinyuan Ma
- Preharvest far-red light for alleviation of chilling injury in basil; Diederick van Kempen
- Postharvest light for alleviation of chilling injury in basil; Elena Argyri
- Postharvest light for alleviation of chilling injury in basil; Xin Zhang
- Increase in preharvest light intensity for alleviation of postharvest chilling injury in basil; Ruiyun Zhang
- Effect of preharvest blue light and light intensity on red and green basil; Jooseop Park
- Interaction between blue light spectrum and light intensity for postharvest chilling tolerance in basil; Xiaojing Zhang
- Effect of preharvest blue light and light intensity on red basil; Sarah Carton
- Effect of preharvest blue light and light intensity on metabolites in red and green basil; Samikshya Shrestha

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