The Invisible Light Stimulus

Physiological Mechanisms of Yield Improvement by Far-red Radiation in Tomato

The Invisible Light Stimulus: Physiological Mechanisms of Yield Improvement by Far-red Radiation in Tomato Yongran Ji 2021

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The invisible light stimulus: physiological mechanisms of yield improvement by far-red radiation in tomato

Yongran Ji

Thesis

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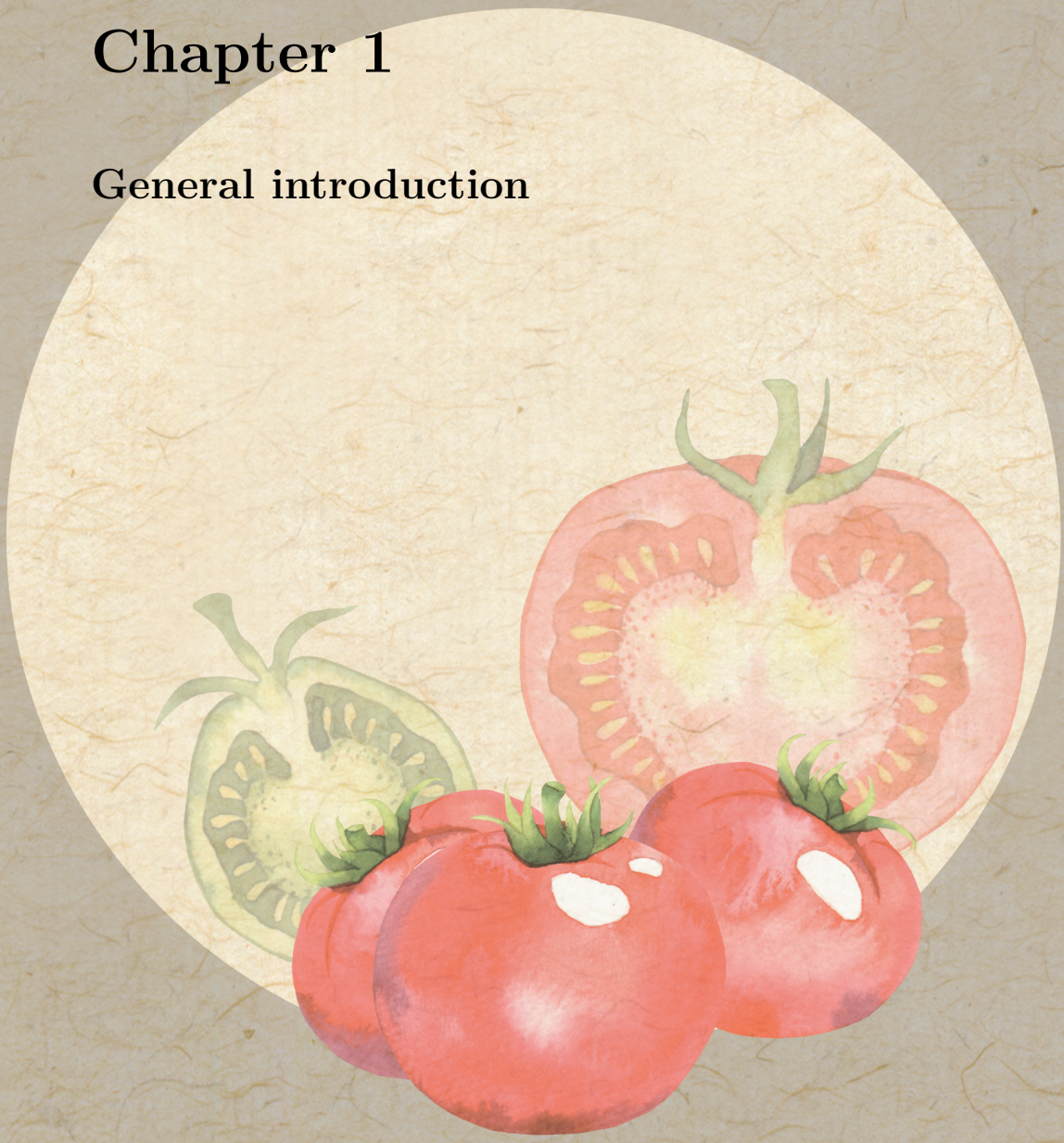
dedicated to
Jiadong Ji
1944-2008
farmer, inspirer
grandfather

Contents

Chapter 1

General introduction



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1.1 Tomato production and its supplementary lighting

Tomato (*Solanum lycopersicum*) is a member of the *Solanaceae* family and, like two other members of this family such as potato (*Solanum tuberosum*) and tobacco (*Nicotiana tabacum*), belongs to the world' s most important crops. Globally, tomato is grown on over 4.7 million hectares and its annual yield is more than 180 million tonnes (FAOSTAT, 2018). Although tomatoes for fresh consumption are mainly grown in the open field, greenhouse tomato production rapidly expanded in the past decades. Compared to open-field production, the greenhouse production system allows for optimized climate control, protection from pests, and year-round production. In the Netherlands, for example, greenhouses only represent 0.7% of the overall agricultural land but represent 20% of the annual agriculture income (Stanghellini et al., 2019). Over the last three decades, greenhouse tomato yield (kg m^{-2}) has more than doubled thanks to the rapid development of greenhouse technologies (Marcelis and Heuvelink, 2019). Recently, more sustainable greenhouse production has been increasingly demanded by society. Compared to conventional open-field systems, greenhouse production is quite energy demanding, and this energy consumption is increasing globally due to the more intense use of climate control and lighting (Katzin et al., 2021). Light is one of the most important environmental factors in greenhouse tomato production. It is common to apply supplemental lighting in countries at higher latitude to maintain a year-round production as natural light is limited from late autumn to early spring in such regions. In the Netherlands, electricity use, especially for assimilation lighting, has been increasing yearly (van der Velden and Smit, 2019). Typically, gas discharge-type lamps (*e.g.*. high-pressure sodium lamp, HPS) are used in greenhouses. Regardless of their relatively high efficiency in converting electric energy into light, HPS lamps typically emit much radiative heat, thus requiring a relatively long distance to the plants to avoid heat damage to the crop. Solid-state lighting such as light-emitting diodes (LEDs) has brought exciting new possibilities to the crop production systems. Compared to HPS lamps, LEDs have hardly any radiative heat production, higher energy efficiency, higher flexibility in spectrum output, and longer longevity (Pattison et al., 2018). LEDs can also facilitate precise spectral and intensity control (Pattison et al., 2018). These features enable researchers and growers to manipulate their light source in a much more precise way than before. Compared to the LEDs which hardly emit any radiative heat, the heat emission from the HPS lamps does contribute to the heating of the greenhouse, especially during the winter seasons. Model simulation showed that, despite a 9-49% increase in energy consumption of heating, replacing HPS (efficacy 1.8 μmol J^{-1}) with LEDs (efficacy 3 μmol J^{-1}) reduced annual total energy consumption by 13-27% (Katzin et al., 2021). The de-coupling of heat source from light source also allows flexible

placement of the light source (closer to the canopy, or within the canopy) and the possibilities of using heat sources that are more economically and environmentally friendly. This is even more exciting if electricity is provided by green energy instead of fossil fuel. Despite that the high investment cost of LED lighting systems may limit the application of LEDs in greenhouse lighting, technological development and increased production is expected to reduce the operating and capital cost per unit of production in the future. Considering the increasing popularity of LED lighting in greenhouse production, knowledge of growth responses of important crops such as tomato is urgently needed.

1.2 Plant responses to far red

One unique advantage of using LED lighting is the possibility to fully customize the light spectrum for different crops. In addition to providing photosynthetically active radiation (PAR, 400-700nm) that drives photosynthesis, developments of LEDs also stimulated research on light quality to increase crop productivity and quality (Pattison et al., 2018). The shade avoidance syndrome (SAS), which is a set of responses plants deploy when exposed to a low red to far red (R: FR) ratio, is one of the most extensively studied plant responses towards changes in light quality. SAS can be triggered not only by natural shading but also by lowering the R: FR ratio in the growth light conditions. Plants have a cassette of photoreceptors to sense changes in the spectrum in their light environment. Phytochrome is the photoreceptor family responsible for perceiving changes in the R: FR ratio. Phytochromes exist as two photo-interconvertible isoforms: the biologically inactive red-absorbing form (Pr) and the biologically active far-red-absorbing form (Pfr), and an equilibrium between the two isoforms is established depending on the R: FR ratio (Figure 1.1, see Casal, 2012). Upon being activated, the Pr isoform turns into the active Pfr isoform, which is then translocated into the nucleus and mediates different photomorphogenic responses (Ruberti et al., 2012).

The most distinct SAS response is stem elongation, which was not only reported in *Arabidopsis thaliana* (Devlin et al., 1998; Franklin and Quail, 2010; Huber and Wiggerman, 1997), but also in crop species such as tobacco (Kasperbauer, 1971), cucumber (Shibuya et al., 2019) and tomato (Kalaitzoglou et al., 2019). Other typical SAS responses such as leaf hyponasty (Michaud et al., 2017), reduced chlorophyll content and increased apical dominance (Smith and Whitelam, 1997) and accelerated flowering (Devlin et al., 1998) have been studied extensively in the model plant *A. thaliana* (Casal, 2012). The development of LEDs stimulated many studies that explored SAS responses in ornamental crops (Park and Runkle, 2017), leafy vegetables (Li and Kubota, 2009; Zhen and van Iersel, 2017), and fruit crops (Hao et al., 2017; Kalaitzoglou et al., 2019). FR may also increase the

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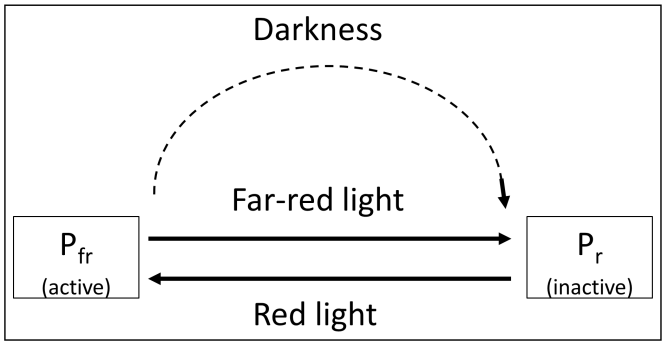


Figure 1.1: *The photo-interconversion between the two isoforms (Pr and Pfr) of phytochrome.*

above-ground dry mass because of both elevated total biomass production and a concomitant increase in shoot: root ratio (Keiller and Smith, 1989; Page et al., 2009). Considering that FR may regulate the SAS responses via FR-increased auxin biosynthesis and transportation (Iglesias et al., 2018; Li et al., 2012), the response of shoot: root ratio may also be regulated via auxin. However, the detailed mechanism of how FR affects dry mass production and partitioning is largely unknown.

Interestingly, it seems that additional FR also increases fruit yield in tomato (Kalaitzoglou et al., 2019; Zhang et al., 2019), but the main reason for this increase is largely unknown. One explanation may be that the addition of FR in the supplementary lighting often resulted in a significant increase in total plant dry mass production (Cao et al., 2018; Kalaitzoglou et al., 2019; Kim et al., 2019; Zhang et al., 2019). Some authors reported that FR may enhance the quantum yield of PAR photons (Zhen and Bugbee, 2020; Zhen and van Iersel, 2017), which is the reverse interpretation of the "Emerson enhancement effect" that described the enhancement of quantum yield of longer wavelength radiation by shorter wavelength radiation (Emerson et al., 1957; Emerson and Rabinowitch, 1960; Govindjee et al., 1964). However, the long-term effect of FR on photosynthesis of the plants adapted to FR varied between studies (Cao et al., 2018; Kalaitzoglou et al., 2019; Zhang et al., 2019). FR also induces morphological changes that may increase light interception (Kalaitzoglou et al., 2019) as well as the improved light distribution within the canopy (Zhang et al., 2019).

To date, the physiological and molecular mechanism in which FR affects dry mass production and fruit yield in tomato remains unknown. Furthermore, considering that contradicting results exist in the plants' response to FR even within the same species, it is also crucial to evaluate and explain a possible genotypic variation in the FR responses. Besides the growth responses, there is also evidences that FR may affect plant immunity. For example, both salicylic acid mediated and jasmonic acid

mediated responses were downregulated in *A. thaliana* (de Wit et al., 2013). FR was also reported to reduce constitutive defenses and jasmonic acid mediated defense in tomato (Cortés et al., 2016). *Botrytis cinerea* is one of the major pathogens in tomato production and, despite that no effect is known in tomato, FR was shown to reduce resistance against *B. cinerea* in *A. thaliana* (Cargnel et al., 2014; Cerrudo et al., 2012). Thus, it is crucial to not only understand the growth responses to FR but also study whether there is a trade-off between growth and defense in response to FR.

1.3 Uploading and transport of photosynthetic assimilates

In addition to the increased overall dry mass production, a higher fruit yield in tomato may also be due to an increased fraction of dry mass being partitioned to fruits. Photosynthetically active "source" organs like mature leaves export the fixed carbon to "sink" organs like fruits, tubers, and meristems (Koch, 2004). Tomato fruits are capable of photosynthesizing as they initially contain photosynthetically active chloroplasts. Earlier experiments estimated that 10%-15% of total carbon skeletons required in fruit growth originated from fruit photosynthesis of its own (Hetherington et al., 1998; Obiadalla-Ali et al., 2004; Tanaka et al., 1974). However, it is generally agreed that tomato fruits are rarely net assimilators of CO_2 (Carrara et al., 2001) and fruit photosynthesis appeared unimportant for fruit energy metabolism throughout their development (Lytovchenko et al., 2011; Okello et al., 2015; Tanaka et al., 1974).

Sucrose is, in most higher plants, the major transported photosynthetic assimilate (Geigenberger and Stitt, 2000; Salerno and Curatti, 2003), while starch may be considered as an overflow product in source organs that is synthesized when CO_2 fixation exceeds sucrose synthesis (Osorio et al., 2014). Interestingly, manipulating sucrose biosynthesis pathways in the leaf provides possibilities to increase the amount of assimilates available for sink organs. For example, sucrose-6-phosphate synthase (SPS) and sucrose-6-phosphate phosphatase (SPP) are two major enzymes which each catalyzes a key step in sucrose synthesis. While SPP shows a limited influence on sucrose biosynthesis (Chen et al., 2005), the reaction catalyzed by SPS represents a major regulatory step in the pathway (Huber and Huber, 1996). SPS plays a crucial role in carbohydrate metabolism by regulating carbon partitioning between starch and soluble sugars (Maloney et al., 2015). Micallef et al. (1995) found a considerable increase in the partitioning of ^{14}C into water-soluble carbohydrates and a decrease in partitioning into starch in the leaves of tomato *SPS* overexpression lines. These plants also had higher total fruit numbers and fruit weight (Micallef et al., 1995). Tomato and *A. thaliana* transformants overexpressing heterologous maize *SPS*

also showed increased rates of sucrose synthesis and decreased rates of starch synthesis, increased pools of soluble sugars, and reduced starch content (Galtier et al., 1995; Laporte et al., 1997; Signera et al., 1998). Similarly, overexpressing a sugarcane *SPS* gene increased plant height, total dry mass as well as sucrose content in *Brachypodium distachyon* (Falter and Voigt, 2016). In consistence with these overexpression experiments, reduced SPS activity resulted in decreased sucrose synthesis and increased leaf starch accumulation in soybean (Huber and Israel, 1982), but contradicting results exists in *A. thaliana* (Strand et al., 2000). Overall, sink organs and their photosynthetic capacity determine the availability of sucrose to the sink organs and may help increase overall biomass production and indirectly increase the yield of harvestable organs.

Another important factor determining yield in a harvestable organ, such as fruits, is the fraction of dry mass partitioning to this organ. Dry mass partitioning to fruits is determined by the sink strength of both vegetative organs and the fruits, the latter being the integral of individual fruit sink strength of all fruits (Heuvelink, 1997). Despite some reports on the FR effect on flowering and fruit numbers (Hao et al., 2016; Kalaitzoglou et al., 2019), it is unknown whether FR affects fruit sink strength. The sink strength of an organ is the intrinsic capacity of that organ to attract assimilates and it is quantified as the growth rate of the organ under non-limiting assimilate supply (Marcelis, 1996). In tomato fruit, the capacity of unloading assimilates from the transportation system as well as metabolizing the imported assimilates can influence the fruit sink strength (Osorio et al., 2014). Sucrose is the main assimilate transported to sink organs in tomato and its transporter activity, either at expression level or translation level, positively correlates with sugar accumulation in sink organs (Gottwald et al., 2000; Lu et al., 2020; Slewinski et al., 2009). Sugar transporter such as SWEETs exports sucrose from phloem parenchyma cells and feed the secondary active transporter SUT in *A. thaliana* (Chen et al., 2012). Antisense expression of *SUT1* in tomato leaves resulted in early senescence and chlorosis, reduced photosynthesis rate, accumulation of soluble sugars, inability to mobilize transitory starch, and blockage in phloem loading, while reduced *SUT2* expression reduced fruit seed development and pollen germination (Hackel et al., 2006). Ruan et al. (1997) reported that maximal activities of hexose/H^+ symporters were significant determinants of hexose content in tomato fruits of different varieties. Besides, RNAi knockdown of three hexose symporters led to a decrease in fruit hexose accumulation while assimilate production by source leaves and phloem transport capacity remained unaffected (McCurdy et al., 2010). Upon transportation into the fruit, sucrose can be downgraded into glucose, fructose, and its derivatives (Ruan, 2014) or can be accumulated in the form of starch (Osorio et al., 2014). Hence, the metabolism of imported sucrose is crucial in preventing negative feedback in sucrose transport into the fruits. In agreement with this, the inclusion of *LIN5* allele from a wild accession (Fridman et al., 2004;

Gur and Zamir, 2004; Zanor et al., 2009) or silencing the inhibitor of *LIN5* (Jin et al., 2009), increased fruit soluble sugar content in tomato. Similarly, fruit soluble sugar content positively correlated with enzyme activity in starch biosynthesis (Petreikov et al., 2006), suggesting that a higher starch synthesis rate may positively affect the import of soluble sugars into the fruits. Based on this, it is logical to speculate that FR may upregulate these pathways to stimulate sucrose import and storage in the fruits. Indeed, many key genes that are involved in sugar transport and metabolism in the fruit including *LIN, AGPase, STS, SBE* were reported to be phytochrome-regulated (Ernesto Bianchetti et al., 2018; Fridman and Zamir, 2003; Kocal et al., 2008). Thus, it is intriguing to quantify the effect of FR on the fruit growth, fruit sugar content, and fruit sink strength and evaluate its contribution in the determination of dry mass partitioning as well as fruit yield.

1.4 About this thesis

In this thesis, I aimed to understand the effect of adding FR on the responses of growth and development of both young and fruit-bearing tomato (*Solanum lycopersicum*) plants. The main research questions addressed in this thesis are

- What are the underlying physiological and/or morphological components that can explain the genotypic variation of plant dry mass production in response to FR?
- Does FR affect the dry mass partitioning between shoot and root in young tomato plants? If so, how is it regulated?
- Does FR lead to any trade-off between plant growth and plant immunity?
- What is the driving force of such yield increase and what are the physiological and molecular pathways by which FR regulates this response?

For all experiments in this thesis, the focus was to compare the differences between plants grown with or without additional FR added in an otherwise common light background. This thesis combines growth analysis with model simulation and quantification of various physiological parameters as well as a molecular biology approach to provide a thorough explanation of FR induced changes in tomato growth and insights into its application in modern greenhouse production.

1.5 Thesis outline

This thesis consists of six chapters: general introduction (**Chapter 1**, this chapter), four research chapters (**Chapter 2-5**) and a general discussion (**Chapter 6**)

In **Chapter 2**, I aimed to examine the similarities and differences in the growth responses of young tomato plants. A climate chamber experiment was conducted with 33 tomato genotypes and grown under different levels of additional FR. In this experiment, growth parameters such as plant height, dry mass of leaf, stem and root, leaf area as well as other physiological parameters such as chlorophyll content and stomatal conductance were measured. This experiment provided a detailed understanding of the genotypic similarities and differences in the growth parameters towards increasing FR in the background light. Also, it provided further explanation of the causes of the observed genotypic variation.

In **Chapter 3**, I aimed to understand the role of phytochrome B1/B2 in the control of shoot: root ratio of young tomato plants in response to FR. Further, I aimed to test whether auxin transport was involved in facilitating the phytochrome-mediated control of shoot: root ratio. A loss-of-function double mutation of *phyB1/B2* led to a strong increase in shoot: root ratio, similar to the wildtype phenotype observed when grown with additional FR. Interestingly, *phyB1/B2* double mutant plants were still able to respond to additional FR in shoot: root ratio, suggesting that other phytochromes may regulate this response in the absence of phyB. These results suggest that the response of shoot: root ratio to additional FR is the result of a phytochrome-mediated effect on auxin transport.

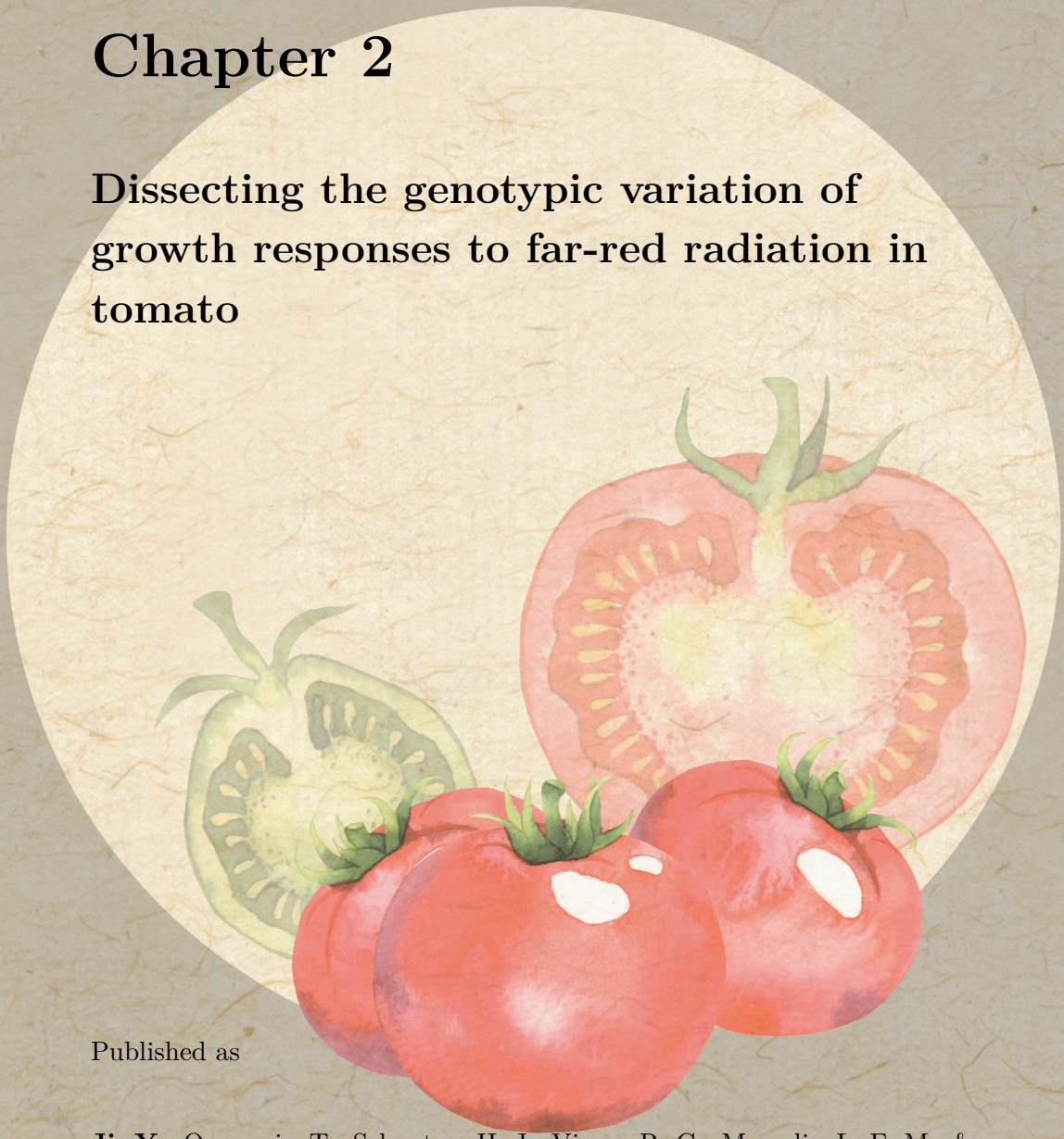
In **Chapter 4**, I focused on fruit-bearing tomato plants. I aimed to quantify the effect of FR on tomato yield and the physiological explanation for any observed effect. A greenhouse experiment was conducted to compare fruit yield between the plants grown with and without additional FR. To explain the yield differences, the FR effect on photosynthesis, light interception, as well as dry mass partitioning between organs were analyzed. Only a slight increase in total dry mass production was observed, hence improved dry mass partitioning to fruits was the main component by which additional FR improved tomato yield. FR also reduced resistance against *Botrytis cinerea* in tomato leaves.

In **Chapter 5**, I continued the work in the previous chapter and aimed to further explain how FR affected dry mass partitioning in tomato. Here, plants were grown with or without FR, and fruit sink strength was quantified under the two conditions. Using model simulation, the consequence of measured FR-increased fruit sink strength on dry mass partitioning to fruits under different fruit load scenarios was simulated and compared with experimentally observed data. Also, contents of sucrose, glucose, fructose, and starch was measured in samples ranging from flowering to fully ripe fruits on plants grown with or without FR. FR radiation increased fruit sink strength by 38%, which, in model simulation, led to an increased dry mass partitioned to fruits that quantitatively agreed very well with measured partitioning. FR radiation increased fruit sugar concentration and upregulated the expression of genes associated with both sugar transport and metabolism.

In **Chapter 6**, I discussed the findings of this thesis. A comprehensive overview of the FR effect on tomato growth was presented by relating the findings of this thesis to the existing literature. I also provided insights on targeting the FR-mediated responses and the control mechanisms of sink strength for yield improvement in modern crop production. Lastly, I addressed unsolved research questions in this thesis and provided perspectives for future research on this topic.

Chapter 2

Dissecting the genotypic variation of growth responses to far-red radiation in tomato



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Abstract

Recent development of light-emitting-diodes (LEDs) and their application in modern horticulture stimulated studies demonstrating that additional far-red radiation (FR, 700-800 nm) increases plant dry mass. This effect of FR has been explained by-improved photosynthesis and/or plant architecture. However, the genotypic variation in this response is largely unknown. Here, we aim to explore and explain the genotypic variation in growth responses to additional FR. We expected genotypic variation in responses of plant dry mass to additional FR. Further, we hypothesized that a significant improvement of both net assimilation rate (NAR) and leaf area ratio (LAR) are responsible for a strong dry mass increase under additional FR, while some genotypes respond only marginally or even negatively in NAR or LAR under FR, thus resulting in a weak FR effect on plant dry mass. To test these hypotheses, we grew 33 different tomato genotypes for 21 days with 0, 25 or 100 μmol m^{-2} s^{-1} of FR added to a common white + red LED background lighting of 150 μmol m^{-2} s^{-1}. Genotypes responded similarly with respect to plant height, stem dry mass and shoot: root ratio, *i.e.* they all increased with increasing FR. However, the response of total plant dry mass varied among genotypes. We categorized the genotypes into three groups (a strongly, moderately, and weakly responding group) based on their relative response in total plant dry mass to FR. Growth component analysis revealed that, the strongly responding genotypes increased strongly in net assimilation rate (NAR) rather than leaf area ratio (LAR). The weakly responding genotypes, however, showed a substantial increase in LAR but not NAR. The increase in LAR was due to the increase in specific leaf area. Leaf mass fraction, which is the other component of LAR, decreased with FR and did not differ between groups. In conclusion, tomato genotypes that increased strongly in NAR in response to FR were able to achieve a more substantial increase in dry mass compared to other genotypes. This is the first study to explain the differences in growth responses of a large number of tomato genotypes towards FR in their light environment.

Keywords: Far red, genotypic variation, growth analysis, LED lighting, *Solanum lycopersicum*.

2.1 Introduction

Far-red radiation (FR, 700-800nm) is an important light signal perceived by plants via the phytochrome photoreceptor family. Phytochromes exist as two photo-interconvertible isoforms, that is, the red (R)-absorbing biologically inactive Pr and the FR absorbing active Pfr (Chen et al., 2005). A low red (R): FR ratio causes the equilibrium between the two isoforms of phytochromes to shift towards Pr, resulting in a set of morphological and physiological changes collectively known as the shade avoidance syndrome (SAS). SAS responses such as stem elongation, leaf hyponasty, as well as flowering acceleration enable the plant to compete for more light capture and to secure a reproductive success as decreased R:FR ratio occurs naturally when plants are shaded (Devlin et al., 1998; Huber and Wiggerman, 1997; Michaud et al., 2017; Yang et al., 2016).

The past decades light-emitting diodes (LEDs) gained popularity in modern horticulture, a development which stimulated the study of spectral effects on plant growth and development. Plant photosynthesis is driven by photosynthetically active radiation (PAR, 400-700 nm). FR is not commonly considered to be part of PAR as monochromatic FR drives neither CO_2 assimilation nor O_2 evolution from photosynthesis (Kono et al., 2020). When added to PAR radiation, however, FR radiation may not only increase yield but also total plant biomass production (Li and Kubota, 2009; Park and Runkle, 2017; Zhen and van Iersel, 2017). Much effort has been made to explain FR enhanced plant growth. It has been found that FR-induced changes in plant architecture increase light interception (Kalaitzoglou et al., 2019). Since long, FR effect on leaf photosynthesis has been described as the Emerson enhancement effect: radiation at shorter wavelength enhances the quantum yield of radiation at longer wavelength (Emerson et al., 1957; Emerson and Rabinowitch, 1960; Govindjee et al., 1964). Several recent studies revisited this concept and proposed the reverse interpretation: FR radiation enhances quantum yield of PAR (Zhen and van Iersel, 2017). Furthermore, Zhen and Bugbee (2020) demonstrated in an experiment with 14 species of both C_3 and C_4 crops that FR can be as efficient in driving photosynthesis as PAR radiation not by itself, but when provided in addition to PAR radiation.

Modern horticultural production can benefit from a deeper understanding of plants' responses to different light spectra. More importantly, it is crucial to explore the genotypic variation in such responses. For example, Ouzounis et al. (2016) showed genotypical differences in growth and physiological parameters when plants were grown in a red LED background with or without 12% of blue LED lighting. Plant' s response to FR is a new way to increase crop production and resource use efficiency (Demotes-Mainard et al., 2016). However, the genotypic variation in plants' responses to additional FR is largely unknown due to the often-limited numbers of genotypes used in FR related research. Here, we aim to evaluate and explain the similarities and differences between tomato genotypes in growth responses under additional FR. We hypothesize that not all genotypes respond the same way in their dry mass production under additional FR. Further, we hypothesize that this variation is the result of different morphological or physiological responses in the components of dry mass production under additional FR. To test these hypotheses, we conducted a climate chamber experiment where 33 tomato genotypes were grown for 21 days with 0, 25 or 100 μmol m^{-2} s^{-1} of FR added to a common white + red LED lighting background of 150 μmol m^{-2} s^{-1}. Growth component analysis, which subdivides growth into underlying morphological and physiological components (Jolliffe and Courtney, 1984), is a useful tool to dissect the effect of FR on dry mass production (Higashide and Heuvelink, 2009). Here, growth components such as relative growth rate, net assimilation rate, leaf area ratio, specific leaf area and leaf mass fraction were determined and the contribution of the different growth components to the genotypic variation in growth response was evaluated.

2.2 Material and methods

Plant materials and growth conditions

The experiment was conducted in a fully controlled climate chamber at Wageningen University (Wageningen, the Netherlands). The air temperature was maintained at 22°C and the relative humidity was 70%. In this climate chamber, seeds of 33 tomato (*Solanum lycopersicum*, Table 2.1) genotypes, varying in genetic background and morphological traits (Aflitos et al., 2014), were germinated under white fluorescent light

(Philips, Eindhoven, The Netherlands) with 16 h photoperiod. Ten days after sowing, eight uniform seedlings of each genotype were individually transplanted into 10.5 cm diameter plastic pots filled with sterilized river sand and placed onto the experimental bench equipped with ebb-and-flow system. The plants were irrigated daily with nutrient solution (electrical conductivity 2.0 dS m^{-1}, pH 5.5) containing 1.2 mM NH_4^+, 7.2 mM K^+, 4.0 mM Ca^{2+}, 1.8 mM Mg^{2+}, 12.4 mM NO^{3-}, 3.3 mM SO_4^{2-}, 1.0 mM PO_4^{2-}, 35 μM Fe^{3+}, 8.0 μM Mn^{2+}, 5.0 μM Zn^{2+}, 20 μM B, 0.5 μM Cu^{2+}, 0.5 μM MoO_4^{2-}.

Light treatments

A deep red + white light at 150 μmol m^{-2} s^{-1} with 0.16 W m^{-2} UV-B was used as the control light treatment and two light treatments were applied from transplanting (10 d after sowing). There were three FR treatments: 0, 25 or 100 μmol m^{-2} s^{-1} of FR radiation was added to a common background of red + white LED light of 150 μmol m^{-2} s^{-1} with 0.16 W m^{-2} UV-B. The UV-B radiation was included to mimic the UV dosage in natural solar radiation. All lighting was provided by LED modules (Control: 3x GreenPower LED-TL-DR/W-MB-VISN; FR: 15 or 60x GreenPower LED-RM-FR, Philips, Eindhoven, the Netherlands) except for UV-B (2x TL 20W/12 RS Ultraviolet-B, Philips). Light modules were placed 1.3 m above the experimental bench. Spectral distribution (Figure S2.1) and photon flux density (PFD) of the LED lighting (Table 2.2) was measured at canopy height at transplanting with a spectroradiometer (USB 2000 + UV-VIS, Ocean Optics, Duiven, the Netherlands) on 30 evenly distributed spots on the experimental bench. Based on these measurements, values of phytochrome photostationary state (PSS) were calculated as described in Sager et al. (1988).

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Table 2.1: *List of genotypes used in the experiment and their relative response in total dry mass to increasing far red and their corresponding growth response groups.*

No.	Code	Name	Source/identification[1]	Relative response (μmol^{-1} m^2 s^1)	Group
1	RF-1	Moneymaker	LA2706/EA00840	0.0073	Strong
2	RF-102		LA4133/TR00026	0.0092	Strong
3	RF-15	Momotaro	TR00003	0.0075	Strong
4	RF-16	Rote Beere	LYC11/EA01965	0.0177	Strong
5	RF-2	Alisa Craig	LA2838A/EA01101	0.0094	Strong
6	RF-23		PI272654/EA05170	0.0104	Strong
7	RF-29	Black Cherry	LA4451/EA00027	0.008	Strong
8	RF-3	Gardeners delight	EA06086/PI406760	0.0109	Strong
9	RF-7	Katinka Cherry	EA00375	0.0083	Strong
10	RF-94	Marmande	TR00022	0.0089	Strong
11	RZ-CAP	Cappricia	Rijk Zwaan	0.0093	Strong
12	BJ-HB1	Hybrid-1	Bejo Zaden	0.0073	Moderate
13	RF-11	Allround	LA2463/LYC1365	0.005	Moderate
14	RF-20		LYC3153/EA03221	0.0055	Moderate
15	RF-22		PI129097/EA04710	0.005	Moderate
16	RF-226		EA05721	0.007	Moderate
17	RF-27	Cal J Tm VF	EA02054/CGN20815	0.0039	Moderate
18	RF-34	Tiffen Mennonite	EA01088	0.0038	Moderate
19	RF-40	ES 58 Heinz	LYC1410/EA02655	0.0063	Moderate
20	RF-43		LYC2910/EA03058	0.0071	Moderate
21	RF-89	Brandywine	EA01019	0.0053	Moderate
22	RF-97	Watermelon beefsteak	EA01640	0.0073	Moderate
23	BJ-HB2	Hybrid-2	Bejo Zaden	-0.0014	Weak
24	N-9008	Foundation	Nunhems	0.0037	Weak
25	N-9098	9098	Nunhems	0.0004	Weak
26	N-FM001	FM001	Nunhems	0.0009	Weak
27	RF-103		LA1421/TR00027	-0.0021	Weak
28	RF-206		EA00915	0.0034	Weak
29	RF-229		EA05979	0.0026	Weak
30	RF-4	Rutgers	LA1090/EA00465	0.0003	Weak
31	RF-91	Giant Belgium	EA01037	-0.0006	Weak
32	RF-93	Kentucky Beefsteak	TR00021	0.0037	Weak
33	RZ-CAL	Caldino	Rijk Zwaan	0.0036	Weak

[1]Identification starting with "EA" , "LA" , "LYC" , "PI" , and "TR" are genotypes registered by "EU-SOL tomato core collection database" (Aflitos et al., 2014) while others are provided by the corresponding company.

Table 2.2: *Photosynthetic photon flux density (PPFD), photon flux density of far red, red: far red ratio (R: FR) and phytochrome photostationary state (PSS) value of the light measured at the top of canopy in each light treatments.*

Light treatment	PPFD[1] μmol m^{-2} s^{-1}	Far red μmol m^{-2} s^{-1}	R:FR	PSS
White + Red	151 ± 22[2]	3 ± 0.2	35 ± 1.3	0.87
White + Red + 25FR	152 ± 3	28 ± 0.9	3 ± 0.1	0.80
White + Red + 100FR	155 ± 5	95 ± 3.6	1 ± 0.1	0.69

[1]For the calculation of ratios, PFD was integrated over 100 nm intervals for red(600–700 nm) and far red (700–800 nm).
[2]All values are means ± standard error of means (s.e.m.). s.e.m of PSS was very small (<0.001) and therefore not shown.

Data collection

Nondestructive measurement

After 14 days of growth, stomatal conductance and chlorophyll index on the first fully expanded leaf of each experimental plant was determined. Stomatal conductance was measured with a SC-1 leaf porometer (Decagon Devices, Inc., Pullman, WA, USA) and chlorophyll index was measured using a Dualex leaf-clip sensor (Force-A, Orsay, France). For the chlorophyll measurement, the values measured from both sides of the leaf were averaged.

Destructive measurement

After 21 days from transplanting a final destructive harvest was carried out. Each experimental plant was carefully cleaned to remove any remaining river sand from the roots. Excess water was wiped clean with tissue paper and the plant height was measured immediately, after which the plant was separated into roots, stem, and leaves. Total leaf area was measured using an area meter (LI -3100, Li-Cor Biosciences, Lincoln, NE, USA). Leaves, stem, and roots were dried in a ventilated oven for 72 h at 105 °C to obtain the dry mass. For each genotype, the initial dry mass at transplanting was measured using seedlings of each genotype germinated in the same conditions as the experimental plants.

Growth component analysis

A linear relation was fitted between the total dry mass and PFD of FR for each genotype. Then, the relative response of each genotype was calculated as the ratio between the slope of this line and the absolute total plant dry mass in the control light treatment. All 33 genotypes were then ranked by their relative response to increasing FR in total dry mass and three response groups were distinguished, *i.e.*, the strongly, moderately, and weakly responding groups, with 11 genotypes in each group. Effects of additional FR on relative growth rate (RGR) were analyzed using a growth component analysis (Figure 2.1)s, which separates RGR into its underlying components (Hunt et al., 2002).

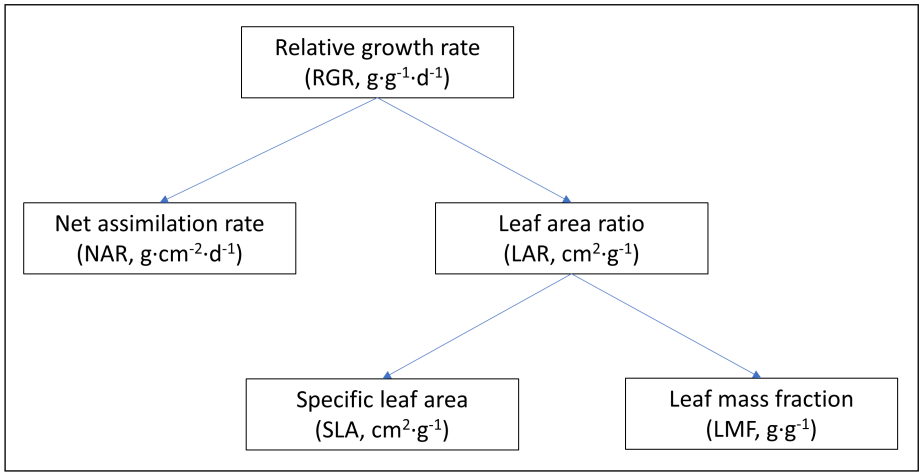

Figure 2.1: *General scheme of a growth component analysis of relative growth rate. Abbreviations and units are included in brackets. RGR is the product of NAR and LAR, and LAR is the product of SLA and LMF.*

RGR is the product of net assimilation rate (NAR) and leaf area ratio (LAR), as shown in equation 2.1. NAR was calculated by dividing RGR by LAR.

$$NAR = \frac{RGR}{LAR} \tag{2.1}$$

RGR was calculated according to equation 2.2 using the initial plant dry mass ($DW_{initial}$) and the final plant dry mass (DW_{final}) of each plant after

21 days of growth.

$$RGR = \frac{ln(DW_{final)} - ln(DW_{initial})}{21} \tag{2.2}$$

Further, LAR was analyzed as the product of specific leaf area (SLA) and leaf mass fraction (LMF) as indicated by equation 2.3.

$$LAR = \frac{SLA}{LMF} \tag{2.3}$$

LAR, SLA and LMF were calculated from the measured total leaf area (LA_{plant}), final plant dry mass (DW_{final}) and leaf dry mass per plant (DW_{leaf}) using equations 2.4-2.6.

$$LAR = \frac{LA_{plant}}{DW_{final}} \tag{2.4}$$

$$SLA = \frac{LA_{plant}}{DW_{leaf}} \tag{2.5}$$

$$LMF = \frac{DW_{leaf}}{DW_{final}} \tag{2.6}$$

Experimental set-up and statistical analysis

Each experiment with one light treatment was conducted consecutively in the same fully-controlled climate room. For each light treatment eight blocks were designed according to the light distribution over the bench and one plant per genotype was randomly placed in each block. The experiment with 25 μmol m^{-2} s^{-1} FR was repeated in time for one extra time (again with eight blocks). To prevent border effects, *S. lycopersicum* cv. "Moneymaker" plants were grown around the experimental plants as border plants. Responsiveness of plant dry mass and relative growth rate to additional FR was quantified as the slope of a linear regression with the FR PFD as the regressor. For the growth component analysis, statistical differences for the FR effect in each group was tested with paired sample t-test (genotypes defining the pairs). All statistics were performed in

Genstat (18th Edition, VSN International Ltd., Hemel Hempstead, UK) at $\alpha = 0.05$.

2.3 Results

Effect of FR radiation on growth parameters

Effects of additional FR varied among genotypes and among growth parameters studied (Figure 2.2). Plant height, stem dry mass and shoot root ratio increased in all genotypes with increasing FR. Chlorophyll index showed a minor decrease by adding 25 μmol m^{-2} s^{-1} FR and a stronger and universal decrease in all genotypes by adding 100 μmol m^{-2} s^{-1} FR. Responses of plant dry mass, leaf dry mass, root dry mass, and leaf area to increasing FR varied among genotypes. For plant dry mass, 58% of the genotypes showed a positive response under 25 μmol m^{-2} s^{-1} FR and this percentage increased to 70% under 100 μmol m^{-2} s^{-1} FR. For leaf dry mass and root dry mass, about 30-40% of the genotypes responded positively to increasing FR, most of which belong to the strongly responding group (genotypes whose total dry mass increased relatively strong with FR). For stomatal conductance, half of the genotypes responded positively to 25 μmol m^{-2} s^{-1} additional FR while this fraction decreased to 21% under 100 μmol m^{-2} s^{-1} additional FR. Absolute numbers of each parameters are shown in Table S2.1.

Growth component analysis

In order to explain the variation in the FR effect on plant dry mass production, we categorized the genotypes into three groups (*i.e.*, a strongly, a moderately, and a weakly responding group; 11 genotypes in each group) based on their relative response to increasing FR in total plant dry mass (Figure 2.3A, Table 2.1). Relative growth rate (RGR), which is a common parameter used for growth component analysis, showed a similar pattern as total plant dry mass in response to increasing FR (Figure 2.3B). Slopes of the regression models fitted for both total dry mass and relative growth rate showed significant differences between the three groups.

This similarity facilitates using a growth component analysis of RGR to explain the genotypic variation in the FR effect on total dry mass (Figure 2.4). When 25 μmol m^{-2} s^{-1} of FR was provided, RGR and net

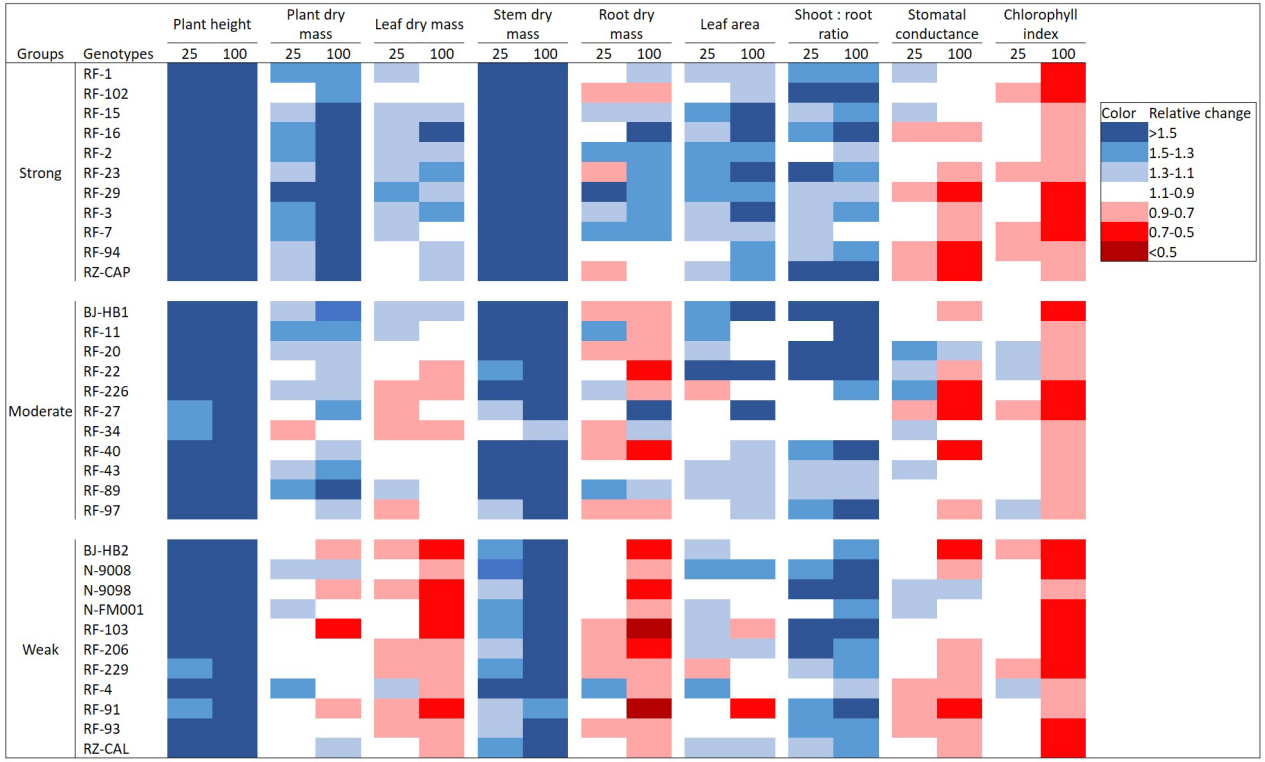

Figure 2.2: *Effects of adding 25 or 100 µmol m^{-2} s^{-1} FR radiation on plant height, plant dry mass, leaf dry mass, stem dry mass, root dry mass, leaf area, shoot: root ratio, stomatal conductance and chlorophyll index in 33 tomato genotypes. Genotypes were categorized into three groups (a strongly, a moderately, and a weakly responding group) based on their relative responses in total plant dry mass to FR. Color scales represent relative changes of parameters when compared to the control light treatment without FR, with blue indicating an increase under FR and red representing a decrease.*

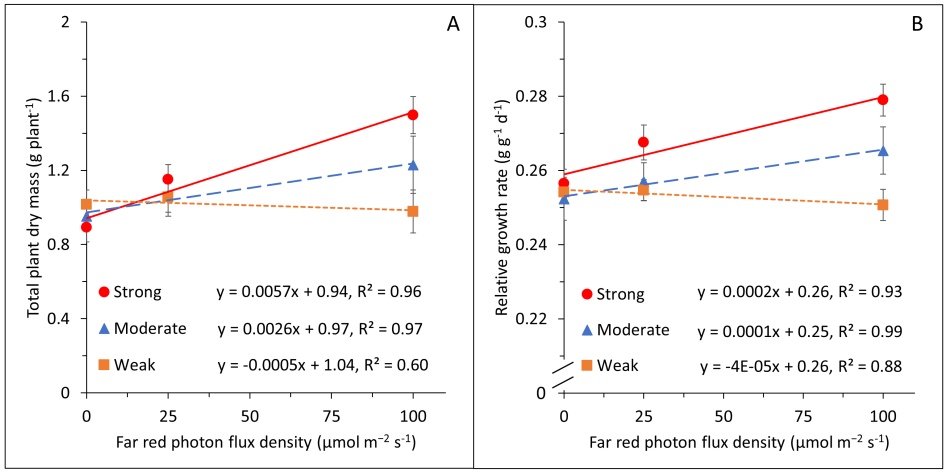

Figure 2.3: *Effects of adding 25 or 100 µmol m^{-2} s^{-1} of FR radiation on total plant dry mass (A) and relative plant growth rate (B) in the strongly (red circle), moderately (blue triangle) and weakly (orange rectangle) responding groups of genotypes. Lines represent linear regression. Error bars represents standard error of means (n=8 for 0 and 100 µmol m^{-2} s^{-1} FR and n=16 for 25 µmol m^{-2} s^{-1} FR).*

assimilation rate (NAR) increased in the strongly responding group while both were not significantly affected in the moderately and weakly responding groups. Leaf area ratio (LAR) showed an opposite response to FR with a decrease in the strongly responding group and an increase in the weakly responding group. LAR was further divided into specific leaf area (SLA) and leaf mass fraction (LMF). LMF decreased in all three groups by a comparable magnitude, while SLA was increased by FR with the weakly responding group showing the strongest increase, followed by moderately and strongly responding group. Similar responses of the growth components were observed when additional FR increased from 25 μmol m^{-2} s^{-1} to 100 μmol m^{-2} s^{-1}. Here, additional 100 μmol m^{-2} s^{-1} FR resulted in a significant increase in RGR, NAR in the strong and moderate groups while that in the weak group was not statistically significant. Also, 100 μmol m^{-2} s^{-1} FR decreased the LAR in the strong and moderate groups while increasing that in the weak group. This was due to the difference in the increasingly large response in SLA from strong to weak group. LMF was strongly reduced by FR with only marginal differences between the three groups. For all parameters, there was a clear dosage effect as the responses became more substantial as FR increased from 25

to 100 μmol m^{-2} s^{-1}. The absolute numbers of the parameters used in the component analysis are presented in Table S2.2.

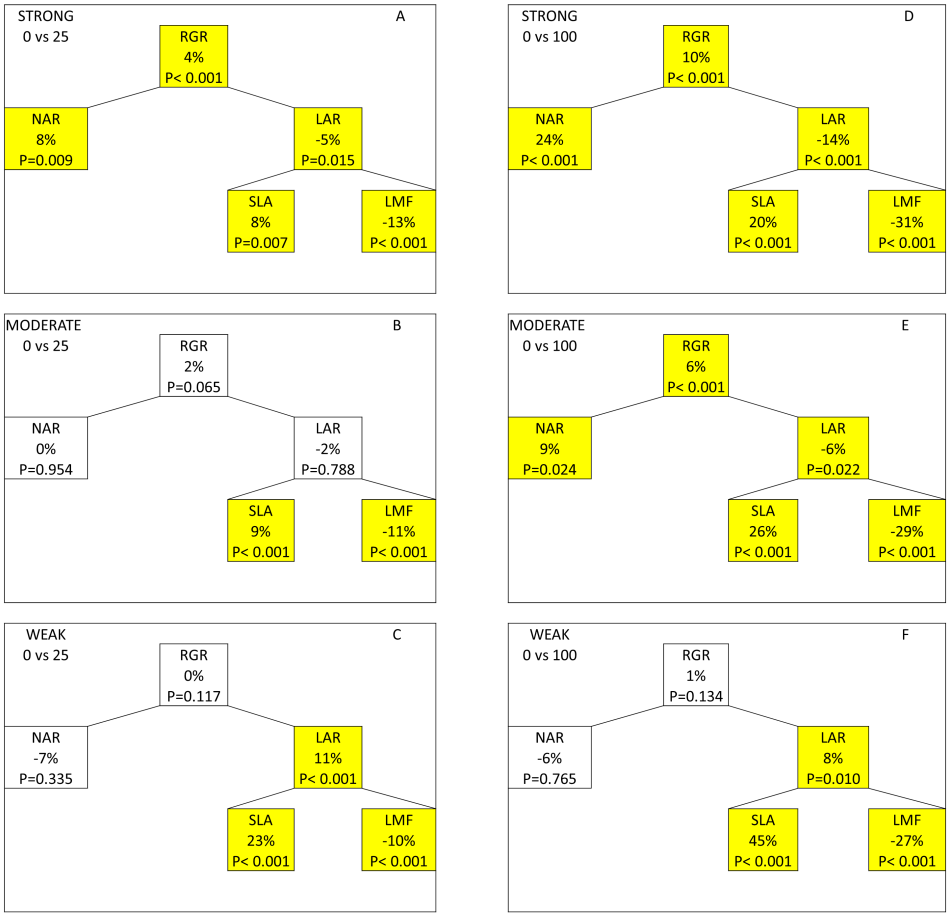

Figure 2.4: *Effects of adding 25 (A-C) or 100 μmol m^{-2} s^{-1} of FR radiation (D-F) on the growth components in the strongly, moderately and weakly responding groups of genotypes. Abbreviations in this figure: RGR (relative growth rate), NAR (net assimilation rate), LAR (leaf area ratio), SLA (specific leaf area), and LMF (leaf mass fraction). The percentage represents the relative change in the components when compared between the FR treatment and the control treatment. P-value of the paired t-test is indicated in each component with a significant difference (P<0.05) being highlighted yellow.*

2.4 Discussion

Genotypic similarities and variations in growth response to FR

This study is the first to analyze the differences in growth responses of a large number of tomato genotypes towards FR in their light environment (Figure 2.2). The most distinct response to FR in many species is stem elongation, which has been reported in many species (Franklin and Quail, 2010; Kalaitzoglou et al., 2019; Kasperbauer, 1971; Shibuya et al., 2019). In agreement with this, we observed a universal increase of plant height in all 33 genotypes and this increase in plant height was dosage dependent. Corresponding to the FR-induced stem elongation, stem dry mass was also increased by FR in all genotypes and this agreed well with other studies (Zhang et al., 2019). In general, responses of leaf growth to FR may vary between species and genotypes (Casal and Aphalo, 1989). Also in tomato both positive (Cao et al., 2018; Zhang et al., 2019) and negative (Kim et al., 2019) effects of FR on leaf dry mass have been reported. Similarly, we observed that the response of leaf dry mass to FR varied among genotypes, ranging from negative to positive response when grown with FR, with a negative response being more frequent. FR stimulates the dry mass to be distributed more to the above ground, thus increasing the shoot: root ratio (Cao et al., 2018; Kasperbauer, 1987; Lee et al., 2016). In line with these results, we observed that all genotypes responded positively to increasing FR in shoot: root ratio, which may be a combined result of higher shoot (mainly stem) dry mass and a lower root dry mass. In this study, we noticed that the increase in shoot: root ratio for the strongly responding genotypes was likely due to an increase in shoot dry mass that was stronger than the increase in root dry mass. For moderately and weakly responding genotypes, this was a result of an increase in shoot dry mass combined with a decrease in root dry mass. Interestingly, FR decreased the chlorophyll index, which indicates that FR reduces chlorophyll content and suggests that photosynthetic capacity may be reduced. Similarly, decrease in chlorophyll content was also reported both in young tomato and fruiting tomato plants (Cao et al., 2018; Kalaitzoglou et al., 2019; Kim et al., 2019) as well as other crops (Casal and Aphalo, 1989; Li and Kubota, 2009; Tucker, 1981). Furthermore, despite a trend of increased total plant dry mass (TDM) due to FR, the genotypic variation

in the response was very noticeable when comparing the magnitude of this FR effect.

Genotypes achieved a stronger increase in dry mass production by the increase in net assimilation rate

We categorized the genotypes into three groups (*i.e.*, strongly, moderately, and weakly responding group) based on their relative response in total plant dry mass to FR (Table 2.1) to conduct a growth component analysis based on the break-down of RGR (Hunt et al., 2002). RGR is the product of net assimilation rate (NAR) and leaf area ratio (LAR). The strongly responding genotypes substantially increased their RGR under additional FR, followed by the moderately responding genotypes, while the weakly responding genotypes showed no significant changes in their RGR under FR (Figure 2.4). The increase in RGR of the strongly responding genotypes under FR was the result of an increase in NAR, but not in LAR as it was decreased by FR. FR was reported by Kalaitzoglou et al. (2019) to increased specific leaf area (SLA). Here, we found that the weakly responding genotypes showed a stronger increase in SLA compared to other genotypes. Leaf mass fraction, the other component of LAR, was significantly decreased for all groups and the response did not differ between groups and was only dependent on the amount of FR. The dry mass partitioning between organs is regulated by the relative sink strength of the organs (Marcelis, 1996). The decreased LMF may be due to the strong enhancement of stem sink strength under FR, causing less dry mass to be partitioned to the leaves. For both the strongly and weakly responding groups, their responses to FR were in accordance with the known SAS responses. Our result suggests that when grown under additional FR, tomato plants are not likely to be able to increase NAR and LAR simultaneously, and that the genotypes with a strong increase in NAR under FR allowed them to achieve a stronger increase in RGR compared to other genotypes.

Possible mechanism of FR enhancement in NAR

One explanation for the FR-increased NAR maybe that the morphology of plants grown with FR contributed to better vertical distribution of light. FR increases the internode length in tomato, which may lead to a more

open plant architecture. Indeed, up to 10% increase in canopy photosynthesis was achieved in a model simulation by increasing internode length in tomato (Sarlikioti et al., 2011). Also, NAR represents largely the net carbon gain from photosynthesis (Poorter and Remkes, 1990). FR enhances quantum yield of photosynthetically active radiation (PAR, 400-700 nm) in various species (Zhen and Bugbee, 2020; Zhen and van Iersel, 2017). Such improvement in photosynthesis agrees with our finding that FR increases NAR. However, their studies focused on short-term light treatments. Experiments with plants grown or adapted to additional FR showed varying results. For example, Kalaitzoglou et al. (2019) found that four-week growth period with additional FR resulted in a higher net leaf photosynthesis rate (A) when 50 μmol m^{-2} s^{-1} FR was added to 150 μmol m^{-2} s^{-1} PAR. Cao et al. (2018), however, reported no significant differences in A using a comparable spectrum. In addition, no significant FR effect on A was reported for tomato plants grown with prolonged exposure to additional FR until fruiting stage (Zhang et al., 2019). This may indicate that the short-term FR enhancement in photosynthesis cannot fully explain the increase in NAR either, especially when considering the decrease in chlorophyll index (Figure 2.2; Cao et al., 2018; Kalaitzoglou et al., 2019; Li and Kubota, 2009) and a decreased photosynthetic capacity. To date, there is still insufficient evidence to fully dissect the effect of FR on the NAR due to the complex interaction between the underlying morphological and physiological components. We do, however, speculate that the effect of FR (positive, neutral, or negative) on net photosynthesis rate, light interception and light distribution varies and that it is the combined effect that determines the NAR.

2.5 Conclusions

Genotypes responded similarly with respect to plant height, stem dry mass and shoot: root ratio. However, the response of total plant dry mass varied among genotypes. Here, we demonstrated that it was the differences in genotype' s responses in net assimilation rate (NAR) and leaf area ratio (LAR) that explains the genotypic variation in response of total dry mass. Genotypes with a strong increase in RGR with increasing FR showed a strong increase in NAR rather than LAR. The weakly responding genotypes, however, showed a substantial increase in LAR but not NAR. The genotypic

differences in the increase in LAR was mainly due to the genotypic difference in the increase in specific leaf area, while responses of leaf mass fraction to FR were conserved between genotypes.

Contributions

YJ wrote the manuscript. YJ, TO conducted the experiment and conducted the analysis. LFMM, EH initiated this work and obtained funding for this research, provided guidance in the experimental design and data analysis and provided critical comments on the manuscript. HJS and RGFV provided critical comments to the overall structure of the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgments

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Supplementary information

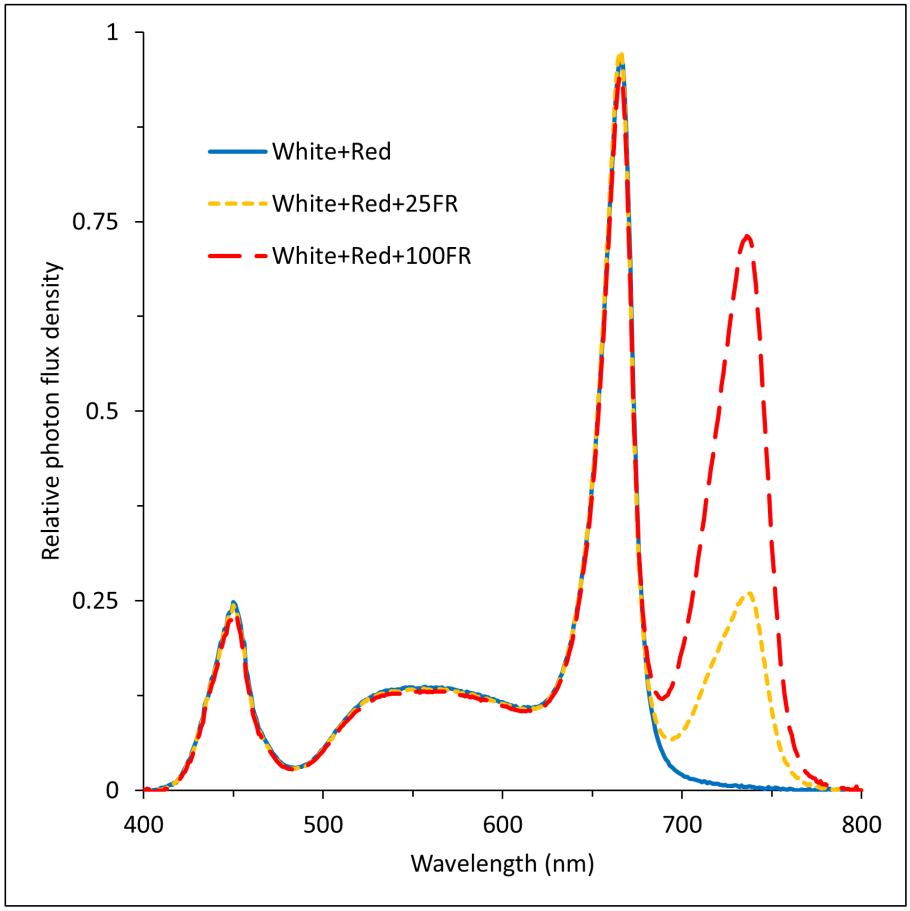


Figure S2.1 Spectral composition of light treatments provided by light-emitting diodes (LEDs) measured at the top of the canopy.

Table S2.1 Plant height, plant dry mass, leaf dry mass, stem dry mass, root dry mass, leaf area, shoot: root ratio, stomatal conductance and chlorophyll index of all genotypes grown with 0, 25 or 100 µmol m^{-2} s^{-1} of far red measured after 21 days of growth.

Group	Genotype	Plant height (cm)			Leaf area (cm2)			Stomatal conductance (mmol $m^{-2}s$-1)			Chlorophyll index (µg cm^2)		
	Far red	0	25	100	0	25	100	0	25	100	0	25	100
Strong	RF-1	15 ± 0.6	31 ± 1	49 ± 2.4	246 ± 16.7	276 ± 34	276 ± 21.6	7 ± 0.5	10 ± 0.8	10 ± 1.6	414 ± 47.4	532 ± 34.7	402 ± 40.5
	RF-102	19 ± 0.6	29 ± 1.7	50 ± 1.2	228 ± 10.5	226 ± 26.2	281 ± 20.3	6 ± 0.7	11 ± 1.9	10 ± 0.7	478 ± 96.6	478 ± 53.2	438 ± 19.8
	RF-15	20 ± 0.7	32 ± 1	52 ± 1.7	242 ± 11.6	341 ± 26.6	384 ± 19.6	8 ± 0.5	9 ± 0.7	11 ± 1.1	431 ± 67.8	517 ± 31.1	461 ± 36.5
	RF-16	20 ± 0.7	35 ± 1.2	59 ± 1.3	300 ± 20.3	381 ± 42	560 ± 44.3	6 ± 0.6	8 ± 0.5	9 ± 1.1	735 ± 79.9	604 ± 29.8	578 ± 87.2
	RF-2	17 ± 0.6	34 ± 1	60 ± 2.2	277 ± 21.4	372 ± 30.5	404 ± 15.3	9 ± 1.2	9 ± 1	12 ± 0.6	519 ± 67	516 ± 53.4	470 ± 59.2
	RF-23	19 ± 0.4	36 ± 1.5	54 ± 1.1	316 ± 19	443 ± 30.1	527 ± 33.8	3 ± 0.3	5 ± 0.4	4 ± 0.4	547 ± 42.8	502 ± 48.9	426 ± 51
	RF-29	20 ± 0.8	38 ± 0.7	54 ± 2.2	283 ± 11.1	424 ± 23.7	412 ± 21.8	8 ± 0.5	9 ± 1.1	9 ± 0.6	558 ± 70.1	500 ± 62.1	384 ± 40
	RF-3	21 ± 0.6	37 ± 1.2	51 ± 5	330 ± 14.4	382 ± 41.1	500 ± 54.3	6 ± 0.4	7 ± 0.8	9 ± 0.6	465 ± 66.8	462 ± 31.3	371 ± 80.5
	RF-7	14 ± 0.5	28 ± 1.2	45 ± 1.3	233 ± 16.3	264 ± 25.9	282 ± 15.8	7 ± 0.4	9 ± 2	7 ± 0.9	451 ± 66.5	469 ± 47.6	395 ± 21.3
	RF-94	18 ± 0.5	32 ± 1.4	53 ± 1.7	324 ± 15.2	343 ± 16.5	425 ± 35.1	7 ± 0.4	9 ± 1	11 ± 1.2	571 ± 44.4	462 ± 58.7	346 ± 40.3
	RZ-CAP	18 ± 0.5	34 ± 1.8	56 ± 1.3	250 ± 16.2	297 ± 32.6	354 ± 12.9	6 ± 0.2	11 ± 2.4	10 ± 0.4	643 ± 73.3	461 ± 55.7	418 ± 43.5
Moderate	BJ-HB1	17 ± 0.4	34 ± 1.1	52 ± 1.5	261 ± 14.3	343 ± 27.6	443 ± 38.9	3 ± 0.2	6 ± 0.8	7 ± 0.4	532 ± 44.9	554 ± 36.5	428 ± 69.5
	RF-11	16 ± 1.2	32 ± 1.4	54 ± 1.2	260 ± 21	348 ± 37	284 ± 43.5	7 ± 1	7 ± 0.8	13 ± 1	425 ± 41.2	463 ± 53.8	435 ± 47
	RF-20	20 ± 0.5	38 ± 1	49 ± 1	295 ± 17.3	339 ± 45.5	322 ± 11.8	4 ± 0.3	9 ± 2.2	8 ± 0.6	344 ± 52.8	477 ± 40.4	416 ± 36.6
	RF-22	20 ± 0.6	33 ± 1.2	58 ± 1.4	141 ± 45	405 ± 33.4	371 ± 34.7	5 ± 0.5	8 ± 3	10 ± 0.7	387 ± 75.4	474 ± 49.6	298 ± 74.7
	RF-226	15 ± 0.5	26 ± 2.2	43 ± 0.6	210 ± 10.9	176 ± 35.6	204 ± 19.3	8 ± 0.3	9 ± 1.5	12 ± 0.9	451 ± 98.1	588 ± 70.7	261 ± 32.8
	RF-27	16 ± 0.7	23 ± 1.3	50 ± 2	332 ± 13.2	323 ± 34.1	502 ± 47.9	5 ± 0.3	5 ± 0.4	5 ± 0.5	531 ± 44.5	476 ± 53.3	371 ± 43.9
	RF-34	19 ± 0.8	28 ± 2.8	43 ± 0.9	242 ± 31	231 ± 46.3	254 ± 12.5	7 ± 0.4	7 ± 0.8	7 ± 1	335 ± 40.1	435 ± 45.5	357 ± 21.3
	RF-40	17 ± 0.6	30 ± 1.4	54 ± 2.3	267 ± 29.3	282 ± 30.9	329 ± 16.4	5 ± 0.8	7 ± 0.7	10 ± 0.7	527 ± 46	482 ± 76.2	352 ± 50.5
	RF-43	15 ± 0.4	30 ± 2	47 ± 1.4	261 ± 26.2	296 ± 44	325 ± 20.4	6 ± 0.3	7 ± 0.8	7 ± 0.7	467 ± 71.2	575 ± 48.1	427 ± 57.8
	RF-89	18 ± 0.5	32 ± 1.1	51 ± 1.6	238 ± 18.2	271 ± 23.9	272 ± 19.4	7 ± 0.7	8 ± 1.2	8 ± 1	432 ± 49.9	423 ± 41.4	438 ± 44.5
	RF-97	19 ± 0.7	31 ± 0.8	50 ± 2.1	328 ± 16	335 ± 34.8	378 ± 26.9	4 ± 0.3	6 ± 0.7	7 ± 0.5	537 ± 95.3	532 ± 53.8	408 ± 57.6
Weak	BJ-HB2	18 ± 0.6	30 ± 1.3	39 ± 1.6	228 ± 9.2	251 ± 26	239 ± 23.5	5 ± 0.5	5 ± 0.6	7 ± 0.6	613 ± 84.1	661 ± 55	421 ± 57.6
	N-9008	18 ± 0.5	34 ± 1.4	55 ± 1.4	267 ± 17.5	348 ± 31.5	366 ± 27.2	5 ± 0.1	7 ± 0.9	10 ± 2.2	532 ± 60.2	538 ± 18.2	398 ± 46.9
	N-9098	19 ± 0.6	30 ± 2.2	50 ± 0.9	212 ± 8.5	232 ± 32.4	229 ± 10.2	5 ± 0.4	9 ± 2.5	9 ± 1.3	379 ± 42.3	493 ± 47.3	422 ± 50.3
	N-FM001	21 ± 0.8	33 ± 0.6	50 ± 1.3	187 ± 20.6	242 ± 21.4	189 ± 23.4	7 ± 0.4	7 ± 0.7	10 ± 1.3	366 ± 53.1	434 ± 49.9	371 ± 41.5
	RF-103	12 ± 0.3	18 ± 1.2	25 ± 2.9	236 ± 47.9	304 ± 44.7	173 ± 44.7	4 ± 0.2	9 ± 4	7 ± 0.7	382 ± 46.1	417 ± 77	345 ± 51.5
	RF-206	19 ± 0.4	35 ± 0.9	53 ± 1.8	336 ± 46.6	407 ± 39.7	406 ± 32.4	5 ± 0.4	9 ± 1.8	8 ± 0.5	580 ± 68.2	615 ± 81.5	446 ± 72
	RF-229	16 ± 0.7	22 ± 0.9	35 ± 1.9	300 ± 7.9	270 ± 24.6	318 ± 19	4 ± 0.1	5 ± 0.6	6 ± 0.4	458 ± 41.6	452 ± 78.7	390 ± 34.8
	RF-4	19 ± 0.3	34 ± 1	51 ± 2.2	318 ± 6.2	426 ± 29.8	302 ± 25.1	6 ± 0.3	6 ± 0.7	7 ± 0.4	573 ± 49.9	487 ± 40.9	493 ± 54.6
	RF-91	21 ± 0.5	32 ± 2.1	48 ± 3.1	271 ± 9.1	249 ± 23.9	143 ± 26	5 ± 0.5	7 ± 0.8	9 ± 0.7	488 ± 42.9	367 ± 34.9	316 ± 42.4
	RF-93	20 ± 0.5	30 ± 1.3	48 ± 1.4	302 ± 21.3	302 ± 33.3	326 ± 29.9	5 ± 0.3	7 ± 1.4	7 ± 0.9	482 ± 54.3	433 ± 51.2	387 ± 32.9
	RZ-CAL	19 ± 0.6	32 ± 1	51 ± 1.6	274 ± 12.8	314 ± 20.5	306 ± 33.8	6 ± 0.3	7 ± 0.5	9 ± 0.5	490 ± 67.7	528 ± 39.5	377 ± 60.5

Data are mean ± s.e.m (n=8 for 0 and 100 µmol m^{-2} s^{-1} of far red and n=16 for 25 µmol m^{-2} s^{-1} of far red)

Table S2.1 Plant height, plant dry mass, leaf dry mass, stem dry mass, root dry mass, leaf area, shoot: root ratio, stomatal conductance and chlorophyll index of all genotypes grown with 0, 25 or 100 µmol m^{-2} s^{-1} of far red measured after 21 days of growth. (continued).

Group	Genotype	Plant dry mass (g)			Leaf dry mass (g)			Stem dry mass (g)			Root dry mass (g)			Shoot: root ratio (g g-1)		
	Far red	0	25	100	0	25	100	0	25	100	0	25	100	0	25	100
Strong	RF-1	0.7 ± 0.07	0.9 ± 0.08	1 ± 0.1	0.4 ± 0.04	0.5 ± 0.05	0.4 ± 0.05	0.1 ± 0.01	0.3 ± 0.02	0.4 ± 0.03	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	7 ± 0.5	10 ± 0.8	10 ± 1.6
	RF-102	0.5 ± 0.05	0.6 ± 0.05	0.8 ± 0.06	0.3 ± 0.03	0.3 ± 0.03	0.3 ± 0.03	0.1 ± 0.01	0.1 ± 0.01	0.3 ± 0.02	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0	6 ± 0.7	11 ± 1.9	10 ± 0.7
	RF-15	0.9 ± 0.05	1.1 ± 0.1	1.4 ± 0.11	0.6 ± 0.03	0.6 ± 0.06	0.7 ± 0.06	0.2 ± 0.01	0.3 ± 0.02	0.6 ± 0.05	0.1 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	8 ± 0.5	9 ± 0.7	11 ± 1.1
	RF-16	0.9 ± 0.07	1.2 ± 0.13	2.2 ± 0.23	0.5 ± 0.04	0.7 ± 0.09	1.1 ± 0.16	0.2 ± 0.01	0.3 ± 0.03	0.8 ± 0.07	0.14 ± 0.01	0.14 ± 0.02	0.21 ± 0.01	6 ± 0.6	8 ± 0.5	9 ± 1.1
	RF-2	0.9 ± 0.07	1.2 ± 0.11	1.6 ± 0.12	0.6 ± 0.06	0.7 ± 0.07	0.8 ± 0.06	0.1 ± 0.01	0.4 ± 0.03	0.7 ± 0.04	0.09 ± 0.01	0.12 ± 0.01	0.12 ± 0	9 ± 1.2	9 ± 1	12 ± 0.6
	RF-23	1.1 ± 0.06	1.3 ± 0.1	1.9 ± 0.11	0.6 ± 0.03	0.7 ± 0.05	0.8 ± 0.05	0.2 ± 0.01	0.4 ± 0.03	0.7 ± 0.04	0.28 ± 0.03	0.22 ± 0.03	0.39 ± 0.03	3 ± 0.3	5 ± 0.4	4 ± 0.4
	RF-29	0.9 ± 0.04	1.4 ± 0.08	1.4 ± 0.12	0.6 ± 0.02	0.8 ± 0.05	0.7 ± 0.06	0.2 ± 0.01	0.4 ± 0.02	0.6 ± 0.04	0.1 ± 0.01	0.17 ± 0.03	0.14 ± 0.01	8 ± 0.5	9 ± 1.1	9 ± 0.6
	RF-3	0.9 ± 0.06	1.2 ± 0.1	1.6 ± 0.43	0.6 ± 0.04	0.7 ± 0.06	0.8 ± 0.23	0.2 ± 0.01	0.4 ± 0.03	0.6 ± 0.14	0.12 ± 0.01	0.15 ± 0.02	0.17 ± 0.05	6 ± 0.4	7 ± 0.8	9 ± 0.6
	RF-7	0.5 ± 0.07	0.7 ± 0.11	0.8 ± 0.03	0.4 ± 0.04	0.4 ± 0.06	0.3 ± 0.02	0.1 ± 0.01	0.2 ± 0.03	0.3 ± 0.01	0.07 ± 0.01	0.1 ± 0.03	0.1 ± 0.01	7 ± 0.4	9 ± 2	7 ± 0.9
	RF-94	1 ± 0.07	1.2 ± 0.07	1.5 ± 0.15	0.6 ± 0.05	0.6 ± 0.05	0.7 ± 0.08	0.2 ± 0.01	0.3 ± 0.01	0.6 ± 0.05	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	7 ± 0.4	9 ± 1	11 ± 1.2
	RZ-CAP	1 ± 0.09	1.2 ± 0.17	1.7 ± 0.1	0.6 ± 0.06	0.6 ± 0.09	0.8 ± 0.05	0.2 ± 0.01	0.3 ± 0.05	0.7 ± 0.03	0.15 ± 0.01	0.12 ± 0.02	0.15 ± 0.01	6 ± 0.2	11 ± 2.4	10 ± 0.4
Moderate	BJ-HB1	0.9 ± 0.08	1.2 ± 0.1	1.4 ± 0.16	0.5 ± 0.04	0.7 ± 0.06	0.7 ± 0.08	0.1 ± 0.01	0.3 ± 0.02	0.5 ± 0.05	0.22 ± 0.03	0.18 ± 0.02	0.19 ± 0.02	3 ± 0.2	6 ± 0.8	7 ± 0.4
	RF-11	0.9 ± 0.11	1.3 ± 0.13	1.3 ± 0.11	0.6 ± 0.07	0.7 ± 0.08	0.6 ± 0.06	0.1 ± 0.01	0.3 ± 0.03	0.6 ± 0.04	0.12 ± 0.02	0.17 ± 0.02	0.09 ± 0	7 ± 1	7 ± 0.8	13 ± 1
	RF-20	1 ± 0.06	1.2 ± 0.16	1.3 ± 0.07	0.6 ± 0.04	0.6 ± 0.09	0.6 ± 0.05	0.2 ± 0.01	0.3 ± 0.04	0.5 ± 0.01	0.19 ± 0.01	0.15 ± 0.03	0.13 ± 0.01	4 ± 0.3	9 ± 2.2	8 ± 0.6
	RF-22	1.2 ± 0.1	1.3 ± 0.14	1.4 ± 0.13	0.7 ± 0.07	0.7 ± 0.07	0.6 ± 0.07	0.2 ± 0.02	0.3 ± 0.03	0.6 ± 0.05	0.19 ± 0.01	0.2 ± 0.04	0.13 ± 0.01	5 ± 0.5	8 ± 3	10 ± 0.7
	RF-226	0.5 ± 0.04	0.6 ± 0.09	0.6 ± 0.03	0.3 ± 0.02	0.3 ± 0.06	0.2 ± 0.01	0.1 ± 0	0.1 ± 0.03	0.2 ± 0.01	0.05 ± 0	0.06 ± 0.01	0.04 ± 0	8 ± 0.3	9 ± 1.5	12 ± 0.9
	RF-27	1 ± 0.06	0.9 ± 0.09	1.5 ± 0.08	0.6 ± 0.03	0.5 ± 0.06	0.7 ± 0.05	0.2 ± 0.01	0.2 ± 0.01	0.5 ± 0.03	0.18 ± 0.01	0.17 ± 0.02	0.29 ± 0.02	5 ± 0.3	5 ± 0.4	5 ± 0.5
	RF-34	0.9 ± 0.17	0.8 ± 0.17	0.9 ± 0.08	0.5 ± 0.07	0.4 ± 0.08	0.3 ± 0.02	0.2 ± 0.09	0.2 ± 0.05	0.3 ± 0.01	0.11 ± 0.02	0.1 ± 0.03	0.14 ± 0.04	7 ± 0.4	7 ± 0.8	7 ± 1
	RF-40	0.9 ± 0.09	0.9 ± 0.11	1.1 ± 0.07	0.5 ± 0.07	0.5 ± 0.07	0.5 ± 0.03	0.1 ± 0.02	0.2 ± 0.02	0.5 ± 0.02	0.15 ± 0.01	0.12 ± 0.02	0.1 ± 0.01	5 ± 0.8	7 ± 0.7	10 ± 0.7
	RF-43	0.7 ± 0.1	0.9 ± 0.13	1 ± 0.09	0.4 ± 0.07	0.5 ± 0.08	0.4 ± 0.04	0.1 ± 0.01	0.2 ± 0.04	0.4 ± 0.04	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	6 ± 0.3	7 ± 0.8	7 ± 0.7
	RF-89	0.7 ± 0.06	1 ± 0.11	1.1 ± 0.08	0.4 ± 0.04	0.6 ± 0.06	0.5 ± 0.04	0.1 ± 0.01	0.3 ± 0.02	0.5 ± 0.03	0.1 ± 0.01	0.14 ± 0.02	0.13 ± 0.02	7 ± 0.7	8 ± 1.2	8 ± 1
	RF-97	1.2 ± 0.09	1.1 ± 0.12	1.4 ± 0.11	0.7 ± 0.05	0.6 ± 0.07	0.7 ± 0.06	0.2 ± 0.01	0.3 ± 0.03	0.5 ± 0.04	0.22 ± 0.02	0.15 ± 0.02	0.17 ± 0.02	4 ± 0.3	6 ± 0.7	7 ± 0.5
Weak	BJ-HB2	0.8 ± 0.06	0.8 ± 0.1	0.7 ± 0.06	0.5 ± 0.04	0.4 ± 0.06	0.3 ± 0.03	0.1 ± 0	0.2 ± 0.02	0.2 ± 0.02	0.14 ± 0.01	0.13 ± 0.02	0.09 ± 0.01	5 ± 0.5	5 ± 0.6	7 ± 0.6
	N-9008	1.2 ± 0.08	1.5 ± 0.12	1.4 ± 0.12	0.8 ± 0.05	0.8 ± 0.08	0.6 ± 0.06	0.2 ± 0.01	0.4 ± 0.03	0.6 ± 0.04	0.18 ± 0.01	0.19 ± 0.03	0.14 ± 0.02	5 ± 0.1	7 ± 0.9	10 ± 2.2
	N-9098	1 ± 0.06	0.9 ± 0.16	0.9 ± 0.04	0.6 ± 0.04	0.5 ± 0.08	0.3 ± 0.02	0.2 ± 0.01	0.3 ± 0.05	0.4 ± 0.02	0.16 ± 0.01	0.14 ± 0.03	0.09 ± 0.01	5 ± 0.4	9 ± 2.5	9 ± 1.3
	N-FM001	0.8 ± 0.07	0.9 ± 0.08	0.7 ± 0.1	0.4 ± 0.04	0.5 ± 0.05	0.3 ± 0.04	0.2 ± 0.01	0.3 ± 0.02	0.3 ± 0.04	0.1 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	7 ± 0.4	7 ± 0.7	10 ± 1.3
	RF-103	0.6 ± 0.12	0.6 ± 0.07	0.4 ± 0.08	0.4 ± 0.08	0.4 ± 0.05	0.2 ± 0.05	0 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.11 ± 0.02	0.1 ± 0.02	0.05 ± 0.01	4 ± 0.2	9 ± 4	7 ± 0.7
	RF-206	1.4 ± 0.08	1.3 ± 0.13	1.3 ± 0.12	0.9 ± 0.05	0.7 ± 0.07	0.6 ± 0.06	0.3 ± 0.02	0.4 ± 0.03	0.5 ± 0.04	0.21 ± 0.01	0.16 ± 0.03	0.13 ± 0.01	5 ± 0.4	9 ± 1.8	8 ± 0.5
	RF-229	0.8 ± 0.02	0.7 ± 0.06	0.7 ± 0.05	0.5 ± 0.02	0.4 ± 0.04	0.3 ± 0.02	0.1 ± 0	0.1 ± 0.01	0.2 ± 0.02	0.14 ± 0	0.12 ± 0.01	0.1 ± 0	4 ± 0.1	5 ± 0.6	6 ± 0.4
	RF-4	1.1 ± 0.11	1.5 ± 0.09	1.2 ± 0.09	0.7 ± 0.08	0.8 ± 0.06	0.5 ± 0.05	0.2 ± 0.01	0.4 ± 0.01	0.5 ± 0.03	0.16 ± 0.01	0.22 ± 0.03	0.13 ± 0	6 ± 0.3	6 ± 0.7	7 ± 0.4
	RF-91	0.9 ± 0.02	0.8 ± 0.1	0.7 ± 0.08	0.5 ± 0.01	0.4 ± 0.04	0.3 ± 0.04	0.2 ± 0	0.2 ± 0.02	0.3 ± 0.03	0.15 ± 0.01	0.13 ± 0.05	0.06 ± 0.01	5 ± 0.5	7 ± 0.8	9 ± 0.7
	RF-93	1 ± 0.11	0.9 ± 0.12	1.1 ± 0.01	0.6 ± 0.07	0.5 ± 0.07	0.5 ± 0.01	0.2 ± 0.02	0.2 ± 0.03	0.4 ± 0.01	0.15 ± 0.02	0.12 ± 0.02	0.13 ± 0.01	5 ± 0.3	7 ± 1.4	7 ± 0.9
	RZ-CAL	1.1 ± 0.05	1.2 ± 0.11	1.3 ± 0.08	0.7 ± 0.03	0.7 ± 0.07	0.6 ± 0.05	0.2 ± 0.01	0.3 ± 0.02	0.5 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.12 ± 0	6 ± 0.3	7 ± 0.5	9 ± 0.5

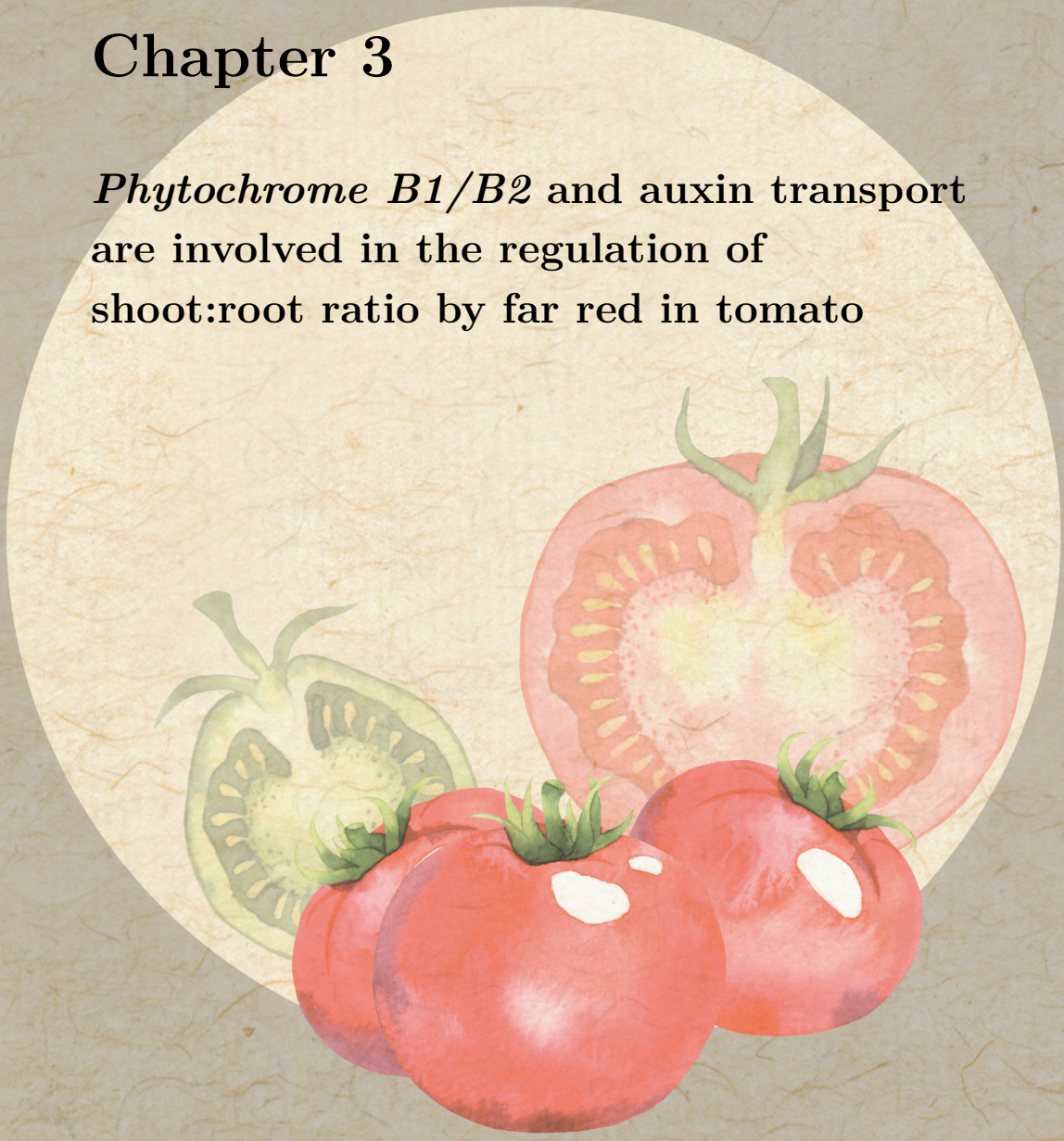
Data are mean ± s.e.m (n=8 for 0 and 100 µmol m−2 s−1 of far red and n=16 for 25 µmol m^{-2} s^{-1} of far red)

Table S2.2 Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf mass fraction (LMF) used in growth components analysis measured after 21 days of growth.

Parameter	Light treatment	Group		
		Strong	Moderate	Weak
RGR ($g\ g^{-1}\ d^{-1}$)	White + Red	0.26	0.25	0.25
	White + Red + 25FR	0.27	0.26	0.25
	White + Red + 100FR	0.28	0.27	0.26
NAR ($g\ cm^{-2}\ d^{-1}$)	White + Red	8.3E-04	8.9E-04	9.8E-04
	White + Red + 25FR	9.0E-04	9.0E-04	9.0E-04
	White + Red + 100FR	1.0E-03	9.7E-04	9.2E-04
LAR ($cm^2\ g^{-1}$)	White + Red	326.7	301.9	275.7
	White + Red + 25FR	310.7	296.3	305.5
	White + Red + 100FR	281.9	282.6	298.7
SLA ($cm^2\ g^{-1}$)	White + Red	504.4	488.3	440.2
	White + Red + 25FR	545.4	531.3	539.7
	White + Red + 100FR	603.4	617.0	636.5
LMF ($g\ g^{-1}$)	White + Red	0.66	0.63	0.63
	White + Red + 25FR	0.57	0.56	0.57
	White + Red + 100FR	0.46	0.45	0.46

Chapter 3

Phytochrome B1/B2 and auxin transport are involved in the regulation of shoot:root ratio by far red in tomato



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(to be submitted).

Abstract

Plants possess a set of photoreceptors to perceive changes in light spectrum. Phytochromes B1/B2 sense changes in red: far red ratios and are involved in mediating the shade-avoidance responses in tomato (*Solanum lycopersicum*). Far red (FR) increases the fraction of dry mass partitioning between shoot and root in tomato, but the control of this response has not been explained. Here we aimed to study the role of phytochromes and auxin transport in the regulation of shoot: root ratio at young plant stage. We hypothesized that a loss-of-function mutation in *phyB1/B2* leads to a strong increase in shoot: root ratio, similar to the effect of reduced R: FR ratio in wildtype plants. We also hypothesized that the *phyB1/B2* double mutant may still respond to FR in shoot: root ratio. Furthermore, we hypothesized that the increased shoot: root ratio is linked to the auxin transport between shoot and root. To test these hypotheses, we conducted an experiment in a climate chamber where both wildtype and *phyB1/B2* double mutant tomato plants (*S. lycopersicum* cv. Moneymaker) were grown for 21 days with 0, 55 or 85 μmol m^{-2} s^{-1} FR in a background of white + red light at 150 μmol m^{-2} s^{-1}. On 14th day, auxin polar transport inhibitor 1-N-naphthylphthalamic acid (NPA) was applied at the shoot-root junction. *PhyB1/B2* mutant showed higher shoot: root ratio compared to the wildtypes. *PhyB1/B2* double mutant still responded to FR. Blocking auxin transport from shoot to root led to an increase in shoot: root ratio for both genotypes under all light conditions. These results suggest that the response of shoot: root ratio to additional FR involves the regulation of phytochromes, possibly via affecting auxin transport.

Keywords: auxin transport, dry mass partitioning, far red, phytochrome, tomato (*Solanum lycopersicum*)

3.1 Introduction

Light is one of the most important environmental factors influencing growth and development of plants by not only serving as the driving force for photosynthesis, but also as a signal alerting plants for changes in the growth environment. Shading by neighboring plants, which means a decrease in light intensity and a decrease in red: far red (R:FR) ratio, is one of the most intensively studied environmental signals. Upon the detection of shading, which suggests competition for light, plants impose a set of morphological and physiological responses to maximize their light capture and to ensure reproductive success (Franklin, 2008; Yang et al., 2016). These responses, collectively termed shade avoidance responses, typically involve stem elongation (Huber and Wiggerman, 1997), changes in leaf angles (Michaud et al., 2017), increased apical dominance (Finlayson, 2007), and accelerated flowering (Devlin et al., 1998). In addition to the well documented elongation responses to low R: FR ratio, many authors also reported responses in dry mass production and partitioning. Cao et al. (2018) and Kalaitzoglou et al. (2019) reported that a low R: FR ratio increased the total plant dry mass in young tomato (*Solanum lycopersicum*) plants. Similarly, a higher biomass was reported for ornamental crops such as geranium (*Pelargonium × hortorum*) and petunia (*Petunia × hybrida*) (Park and Runkle, 2017), leafy vegetables such as lettuce (Li and Kubota, 2009), as well as fruiting tomato (Hao et al., 2016; Kalaitzoglou et al., 2019).

A low R: FR ratio led to changes in the partitioning of dry mass between organs, often favoring the growth of shoots over roots (Keiller and Smith, 1989; Page et al., 2009). In the vegetative phase of plant growth, the dry mass partitioning between the above- and below-ground parts has be related to the activities of the organs, as suggested by the "functional equilibrium" theory (Brouwer, 1963). Based on this, it is reasonable to speculate that changes in the light spectrum sensed by the shoot organs may cause shifts in the functional equilibrium between shoot and root, thus influencing the dry mass partitioning between the two parts. The detection of changes in R: FR ratio is mediated by the phytochrome photoreceptor family, which exists as two photo-interconvertible isoforms: the red-light absorbing form Pr (biologically inactive) and the far-red absorbing form Pfr (biologically active) (Chen et al., 2005). The

biologically active Pfr translocates to the nucleus to mediate the downstream shade avoidance responses (Ruberti et al., 2012). Auxin signaling is heavily involved in the regulation of shade avoidance responses in *Arabidopsis thaliana* (Iglesias et al., 2018).Shading or a low R: FR ratio affects not only biosynthesis of auxin in *A. thaliana* (Li et al., 2012), but also its transport and perception (Keuskamp et al., 2010; Kohnen et al., 2016). Furthermore, other phytochromes such as phytochrome E may be required to mediate tomato' s elongation responses to FR in the absence of phytochrome B1 and B2 (Schrager-Lavelle et al., 2016). These results suggest that also the impact of FR on dry mass partitioning between shoot and root may be mediated by multiple phytochromes, possibly via affecting auxin biosynthesis and transport.

Despite the intensive studies on *A. thaliana*, little is known about this interaction in tomato, which has substantial and genotypically-conserved responses of shoot: root ratio to increasing FR (this thesis, Chapter 2). In the present study, we aim to understand the role of phytochrome B1/B2, which are mainly responsible for sensing R: FR ratio in tomato (Weller et al., 2000), in controlling the shoot: root ratio of young tomato plants. Further, we investigated the involvement of auxin transport in facilitating the phytochrome-mediated control of shoot: root ratio in tomato. We hypothesized that a loss-of-function mutation in *phyB1/B2* leads to a strong increase in shoot: root ratio similar to the effect of reduced R: FR ratio on wildtype plants. We also hypothesized that expected that the *phyB1/B2* double mutant may still increase in shoot: root ratio in response to FR. Furthermore, we hypothesized that the increased shoot: root ratio is linked to the auxin transport between the shoots and roots. To test these hypotheses, we conducted a climate chamber experiment where both wildtype and *phyB1/B2* double mutant tomato plants (*S. lycopersicum* cv. Moneymaker) were grown with 0, 55 or 85 μmol m^{-2} s^{-1} FR in a background of white + red lighting at 150 μmol m^{-2} s^{-1}. In half of the plants an auxin polar transport inhibitor 1-N-naphthylphthalamic acid (NPA) was applied at the shoot-root junction. Growth component analysis was conducted to evaluate the effects of treatments on the components determining shoot: root ratio.

3.2 Material and methods

Plant materials and growth conditions

The experiment was conducted in a fully-controlled climate chamber of Wageningen University (52°N, 6°E, Wageningen, the Netherlands). Wildtype tomato (accession LA2706, *S. lycopersicum* L. cv Moneymaker) and a *phyB1/phyB2* double mutant (accession LA4364 in the background of cv. Moneymaker) were used in this experiment. The seeds of these genotypes were obtained from the UC Davis/C.M. Rick Tomato Genetics Resource Center (Davis, CA, USA) and maintained by Wageningen University (Wageningen, the Netherlands). The seeds were treated with 70% ethanol for 1 min and then 28 min in 3% sodium hypochlorite before being sown in trays containing vermiculite. The trays were kept in darkness for three days, after which they were placed under fluorescence tubes (Philips, Eindhoven, The Netherlands) with an intensity of 120 μmol m^{-2} s^{-1} and a photoperiod of 16 hours. Two weeks after sowing, uniform seedlings were transplanted individually into plastic pots (11x11x12 cm) containing expanded clay grid (Ø=6 mm) and grown for three weeks. A day temperature of 20˚C, night temperature of 18˚C, relative humidity of 70% and photoperiod of 16 hours was maintained during the whole experimental period. Plants were irrigated with nutrient solution (electrical conductivity 2.1 dS m^{-1}, pH 5.5) containing 1.2 mM NH_4^+, 7.2 mM K^+, 4.0 mM Ca^{2+}, 1.8 mM Mg^{2+}, 12.4 mM NO^{3-}, 3.3 mM SO_4^{2-}, 1.0 mM PO_4^{2-}, 35 μM Fe^{3+}, 8.0 μM Mn^{2+}, 5.0 μM Zn^{2+}, 20 μM B, 0.5 μM Cu^{2+}, 0.5 μM MoO_4^{2-}.

Light treatments

There were three light treatments: white + red (WR) without additional far red (FR), WR with 55 μmol m^{-2} s^{-1} FR and WR with 85 μmol m^{-2} s^{-1} FR (Table 3.1, Figure S3.1). All lighting was provided by overhead LED modules (WR: Greenpower PM-DR/W-120, FR: Greenpower PM-FR-120, Philips, Eindhoven, the Netherlands). The photosynthetic photon flux density (PPFD) of WR was maintained at around 150 μmol m^{-2} s^{-1} at plant height for all light treatments by adjusting the height of the LED fixtures. The spectral distribution and photon flux density (PFD) was measured using a spectroradiometer (USB 2000 + UV-VIS; Ocean Optics, Duiven, the

Netherlands). Phytochrome photostationary state (PSS) in each treatment was calculated based on the measured spectra as the ratio of Pfr to the total of Pfr and Pr according to (Sager et al., 1988).

Table 3.1: *Photosynthetic photon flux density (PPFD), photon flux density of far red, red: far red ratio (R: FR) and phytochrome photostationary state (PSS) value of the light measured at the top of canopy in treatments with low, medium or high far red.*

Light treatment	PPFD μmol m^{-2} s^{-1}	Far red μmol m^{-2} s^{-1}	R: FR[1]	PSS
No FR	151 ± 3[2]	1 ± 0.1	100 ± 2.1	0.88
Medium FR	151 ± 4	55 ± 1.3	2 ± 0.1	0.77
High FR	148 ± 4	85 ± 2.1	1 ± 0.1	0.73

[1]For the calculation of ratios, PFD was integrated over 100 nm intervals for red(600–700 nm) and far red (700–800 nm).
[2]All values are means ± standard error of means (s.e.m.). s.e.m of PSS was very small (<0.001) and therefore not shown.

1-N-naphthylphthalamic acid treatments

Auxin polar transport inhibitor 1-N-naphthylphthalamic acid (NPA, Sigma-Aldrich, Zwijndrecht, the Netherlands) was applied 14 days after transplanting. NPA 1% (w/w) was prepared by dissolving NPA in warm lanolin (Dražeta et al., 2004). Then, it was applied in a 3-5 mm thick ring around the stem at the root-shoot junction using a syringe. Plants used as control were applied with only lanolin using the same method.

Data collection

Plants were destructively measured 21 days after transplanting. Each plant was cleaned off any remaining growth medium and was separated into leaves, stem, and root. Leaf area of each plant was measured with a leaf area meter (LI-3100 area meter, Li-Cor Biosciences, Lincoln, USA)). Each plant part was dried in a ventilated oven for 48 hours at 105 °C and weighted for dry mass.

Growth component analysis

Effect of different treatments on shoot: root ratio was analyzed by separating shoot: root ratio into its underlying components (Figure 3.1).

Shoot: root ratio is determined as shoot dry mass divided by root dry mass. Shoot dry mass was determined as the sum of leaf dry mass and stem dry mass.

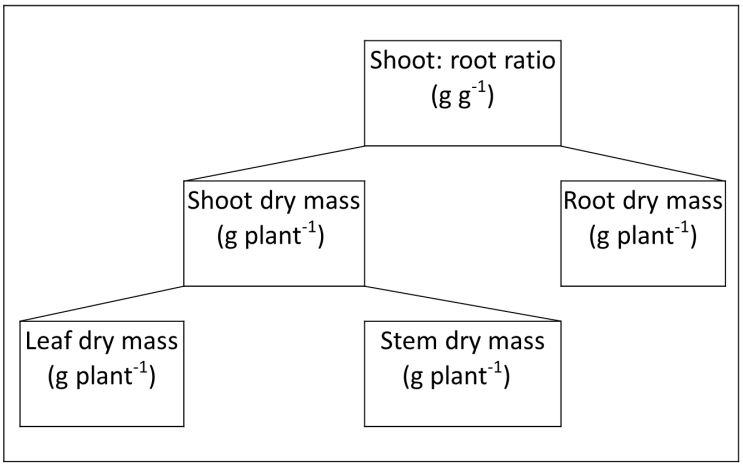

Figure 3.1: *General scheme of a top-down growth component analysis of shoot: root ratio. Units for each component are included in brackets*

Experimental set-up and statistical analysis

The experiment was a split-plot design with light and genotype as main factors and NPA application as sub-factor. Each combination of light condition and genotype was repeated 4 times. Within each light-genotype plot, three randomly selected plants were applied with only lanolin and three other plants were applied with lanolin containing NPA. Treatment effect on root: shoot ratio was analyzed using analysis of variance (ANOVA). Assumptions of homogeneity and normality of residuals were satisfied as tested by Bartlett' s test and Shapiro-Wilk test at α=0.05, respectively. Fisher' s unprotected least significant difference (LSD) test was used for mean separation. Unprotected, because we also applied this test for separating interaction means when the F-test for interaction was not significant at $\alpha = 0.05$. For the growth component analysis, each component was compared between treatments using Student' s t-test. All statistical analyses were performed in Genstat (18^{th} Edition, VSN International Ltd., Hemel Hempstead, UK) at $\alpha = 0.05$.

3.3 Results

Effect of far red and auxin transport inhibitor on the shoot: root ratio

Shoot: root ratio increased in loss-of-function *phyB1/B2* mutant compared to that in the wildtype (Figure 3.2). In both wildtype and mutant, adding 55 μmol m^{-2} s^{-1} of FR seemed to slightly increase the shoot: root ratio (not statistically significant), while adding 85 μmol m^{-2} s^{-1} of FR increased shoot: root ratio significantly. Also, the local application of NPA at the root-shoot junction significantly increased S:R ratio for both the wildtype and mutant in each light treatment.

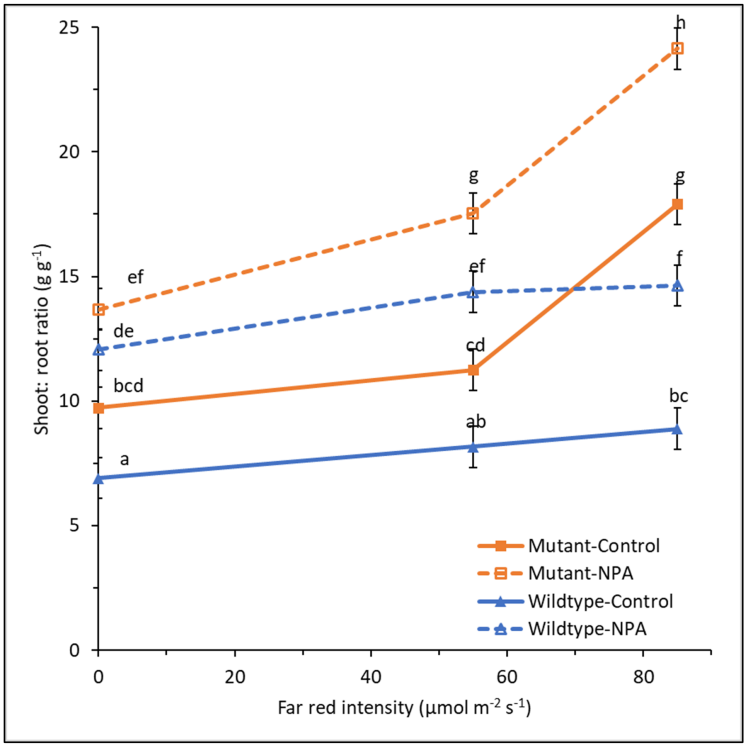

Figure 3.2: *Effects adding 0, 55, or 85 μmol m^{-2} s^{-1} of far red on the shoot: root ratio in a white + red background light. Dashed and solid lines, respectively, represent plants with or without the application of 1% (w/w) N-1-naphthylphthalamic acid (NPA) at the root-shoot junction in wildtype (blue lines) and phyB1/B2 mutant (orange lines). Error bar represents standard error of means (n=4) and different letters denote significant differences according to Fisher' s unprotected LSD test (α = 0.05).*

Growth component analysis

The increased shoot: root ratio due to loss-of-function mutation of *phyB1/B2* was the result of more substantial decrease in root dry mass and to a lesser extent also the result of a decrease in shoot dry mass (Figure 3.3). Decreased shoot dry mass resulted from decreased leaf dry mass and stem dry mass, although stem dry mass was not significantly reduced by the mutation when no FR was present (Figure 3.3A). All components showed a dosage dependent effect to the increasing FR intensity with a stronger response under higher FR intensity.

The addition of FR increased shoot: root ratio in both wildtype and *phyB1/B2* mutant (Figure 3.4). In the wildtype, FR increased shoot: root ratio by increasing shoot dry mass, with no significant effect on root dry mass. Increased stem dry mass was the main reason for the increase in shoot dry mass while the increase in leaf dry mass was only statistically significant at 85 μmol m^{-2} s^{-1} FR. In the *phyB1/B2* mutant, FR increased shoot: root ratio due to a stronger reduction of root dry mass than that of shoot dry mass. The reduction of shoot dry mass in *phyB1/B2* mutant was the result of reduced leaf dry mass, stem dry mass was hardly affected. Responses were all dosage-dependent: stronger response at higher FR intensity.

In both wildtype and *phyB1/B2* mutant, NPA application increased shoot: root ratio due to a stronger decrease in root dry mass than in shoot dry mass (Figure 3.5). In the wildtype, both leaf dry mass and stem dry mass were decreased by NPA application while only leaf dry mass was affected by NPA application in the *phyB1/B2* mutant. The wildtype was more responsive to NPA treatment compared to the *phyB1/B2* mutant.

3.4 Discussion

Phytochrome regulation of shoot: root ratio

Wildtype tomato plants showed a significant increase in shoot: root ratio with increasing FR intensity (Figure 3.2). This increase is not only in accordance with the conserved FR-induced increase in shoot: root ratio observed among various tomato genotypes (Chapter 2, this thesis), but also with previous studies of (Cao et al., 2018; Kasperbauer, 1987; Lee et al.,

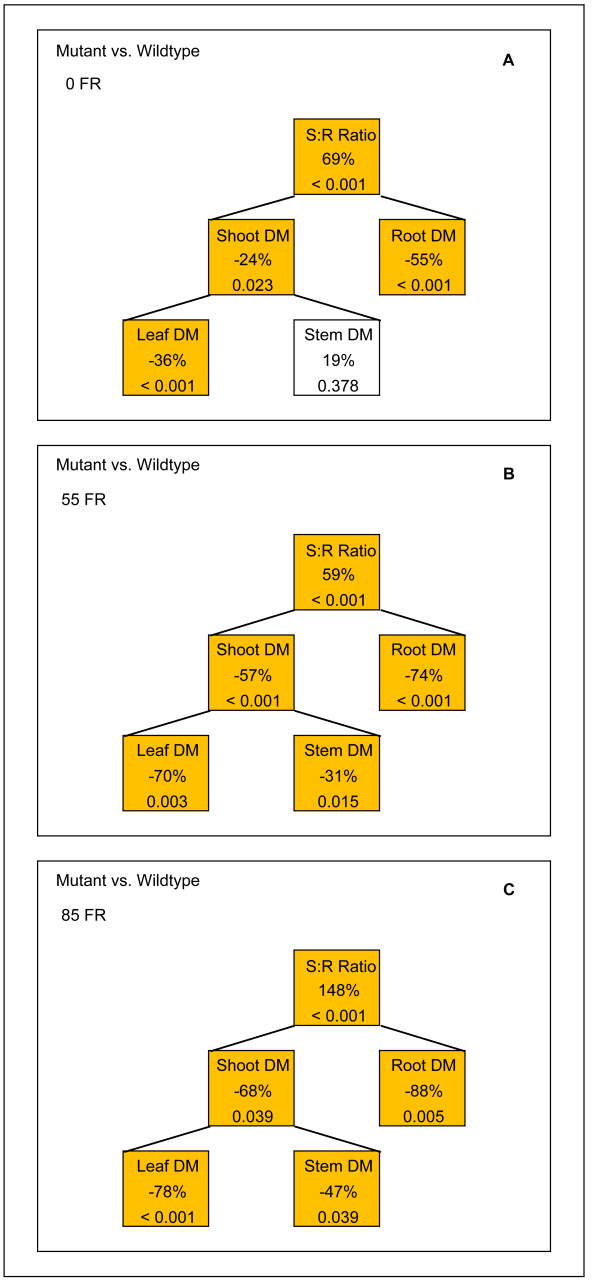

Figure 3.3: *Effect of loss-of-function mutation of phyB1/B2 on the components determining shoot: root ratio when 0(A), 55(B) or 85 $\mu mol\, m^{-2}\ s^{-1}$ of far red (C) was added in a white + red background light. The percentages represent the relative changes in the components when comparing mutant with wildtype. P-value of the t-test is indicated in each component with a significant difference (P<0.05) being highlighted in yellow.*

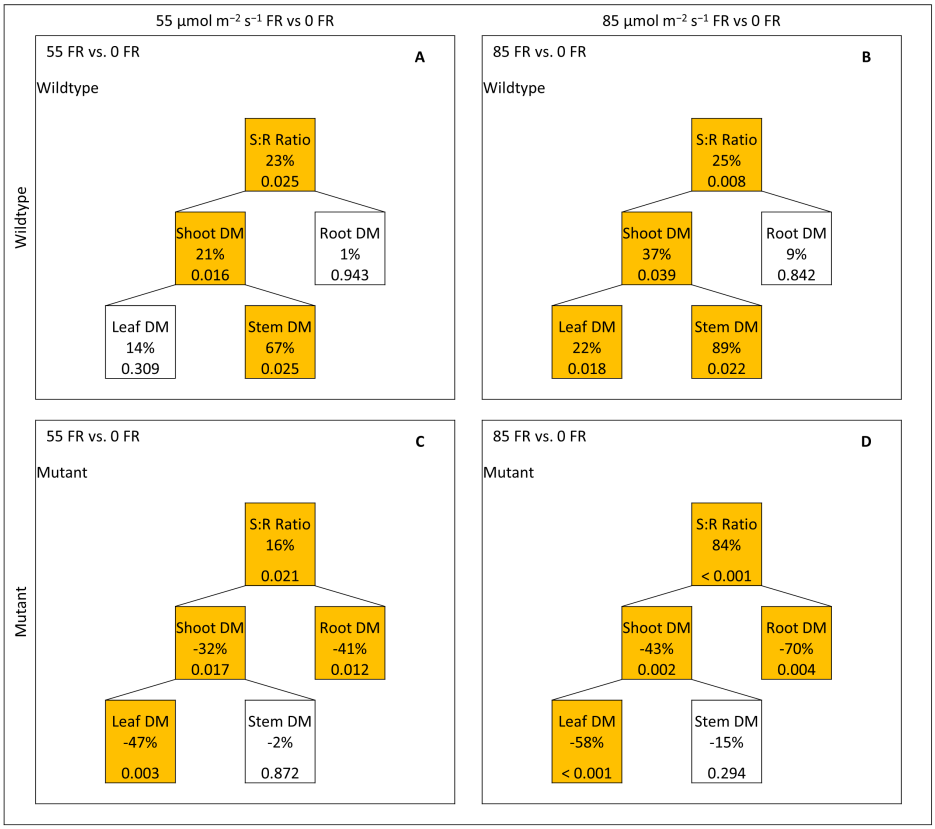

Figure 3.4: *Effect of adding 55, or 85 µmol m^{-2} s^{-1} of far red in a white + red background light on the components determining shoot: root ratio in wildtype (A, B) and phyB1/B2 mutant (C, D). The percentage represents the relative change in the components when compared with no FR. P-value of the t-test is indicated in each component with a significant difference (P<0.05) being highlighted.*

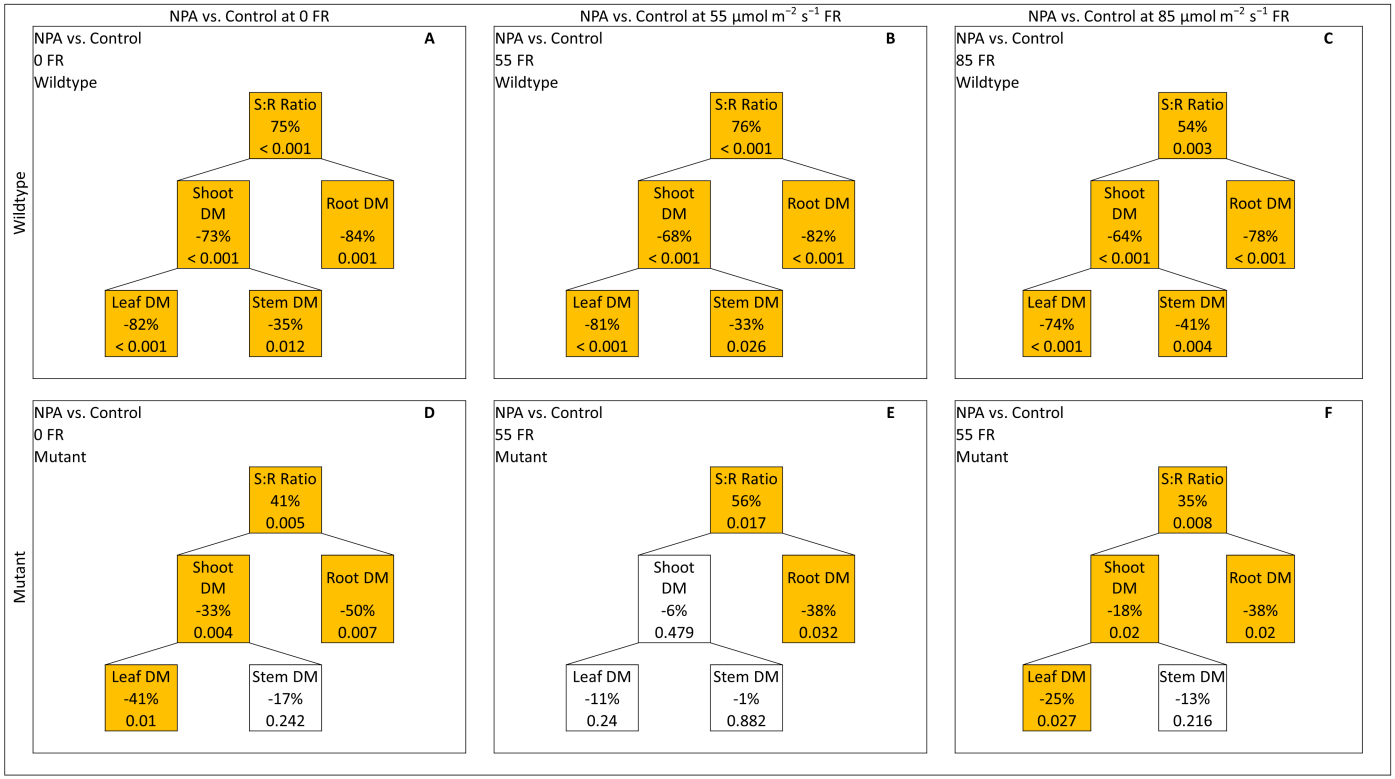

Figure 3.5: *Effect of 1% (w/w) N-1-naphthylphthalamic acid (NPA) applied in a ring of lanolin at the root-shoot junction on components determining shoot: root ratio in wildtype (A, B, C) and phyB1/B2 mutant (D, E, F) under different light conditions. For the control treatment, a mock ring of lanolin without NPA was applied at the root-shoot junction. The percentage represents the relative change in the components when compared between the genotypes. P-value of the t-test is indicated in each component with a significant difference ($P<0.05$) being highlighted.*

2016). This increase was mainly a consequence of higher shoot dry mass due to higher stem dry mass (Figure 3.4), possibly caused by FR induced stem growth. Phytochromes are the main photoreceptors responsible in sensing R: FR ratio, and a loss-of-function phytochrome mutant grown without FR is similar to the wildtype grown with FR (Devlin et al., 1999). *PhyB1/B2* double mutant increased shoot: root ratio compared to the wildtype (Figure 3.2) as a result of a stronger reduction in root dry mass than that in shoot dry mass (Figure 3.3). This reduction in plant mass as a result mutation in *phyB1/B2* was also reported by Weller et al (2000). This may result from a strong stem and petiole elongation in the double mutant, together with a reduction in the establishment of leaf area (Figure S3.2, Devlin et al. 1999). Plant response in biomass to FR seems to be dosage-dependent and we suggest that there is an optimum FR dosage or optimum R: FR ratio for the biomass production. Before reaching the optimum dosage, increase of leaf area (and consequently light interception) is more favorable for biomass production. Further increase of FR (or decrease of R: FR ratio), which strongly favors stem elongation, leads to limitation of biomass partitioning to leaves and the establishment of leaf area. This limitation, combined with the reduction in photosynthetic capacity and chlorophyll content, may lead to the reduction on biomass production that contradicts the positive effect of FR. This may explain the reduced biomass observed in the *phyB1/B2* mutants grown with FR.

When looking at biomass in absolute terms, it is difficult to determine whether changes in stem dry mass is the cause or the consequence of changes in processes such as stem elongation. On relative terms, however, it is known that dry mass partitioning between organs are mainly determined by the relative sink strength of the organs (Marcelis, 1996). A higher fraction of dry mass partitioning to the stem suggests a higher relative sink strength in the stem. No previous study touched the FR effect on the sink strength of vegetative organs. However, the expression of genes related to source-to-sink sugar transport, and sugar metabolisms in the sink, are subject to phytochrome regulation (Ernesto Bianchetti et al., 2018; Fridman and Zamir, 2003; Kocal et al., 2008). Considering the direct relationship between sink strength and sugar metabolism as well as transportation (Osorio et al., 2014), we reason that FR may increase the shoot: root ratio by increasing shoot relative sink strength. Whether this increase is a result of higher shoot sink strength, lower root strength, or

more complicated changes in the sink strength of each organ, requires dedicated studies.

In *A. thaliana*, the *phyB/phyD* double mutant showed little response to R:FR ratio (Aukerman et al., 1997; Devlin et al., 1999). This was not the case in tomato as earlier studies showed that *phyA/B1/B2* triple mutant still retain phytochrome responses (Weller et al., 2000). The *phyB1/B2* double mutant was still capable in responding with elongation to additional FR, showing that other members of the phytochrome family, such as phytochrome E, are able to mediate the elongation responses to FR in the absence phytochrome B (Schrager-Lavelle et al., 2016). Tomato *phyB1/B2* double mutant also responded to additional FR with increased shoot: root ratio (Figure 3.2), which suggest that the changes in shoot: root ratio is also a phytochrome-regulated response to FR in tomato that can be regulated by other phytochrome in absence of phytochrome B1 and phytochrome B2 in tomato.

Interaction between phytochrome and auxin in regulating shoot: root ratio under FR

Loss-of-function *phyB1/B2* mutant responded to FR with increasing shoot: root ratio just as wildtype plants (Figure 3.2). The perception of FR by phytochrome B leads to the accumulation of phytochrome interaction factors (PIFs) and induces growth responses via upregulated auxin synthesis (Courbier et al., 2020; Li et al., 2012; Pantazopoulou et al., 2017). This suggests that the *phyB1/B2* mutant may have a constitutively elevated auxin level in the shoots, which stimulates increase in shoot: root ratio and stem elongation. Auxin is transported from the shoot apex towards the base and from shoot to root. The application of auxin transport inhibitor NPA at the shoot-root junction has been shown to effectively block the transport from shoot to root and substantially reduce the auxin level in the root (Reed et al., 1998). Hence, blocking shoot-to-root auxin transport should increase shoot: root ratio just as wildtype grown under FR, or as *phyB1/B2* mutant grown without FR. Indeed, we observed significant increase in stem elongation (Figure S3.3) and in shoot: root ratio (Figure 3.2, Figure 3.5) when NPA was applied at shoot-root junction. Especially, the NPA application strongly reduced root growth relative to that of shoot growth. This is in agreement with reports demonstrating that blocking auxin transport with NPA suppressed

the development and growth of root (Casimiro et al., 2001; Reed et al., 1998). More interestingly, the effect of NPA application was less substantial in the mutant (Figure 3.5D-3.5F) than that in the wildtype (Figure 3.5A-3.5C), and it was also less substantial in treatments with high FR (Figure 3.5C, 3.5F) than that with no FR (Figure 3.5A, 3.5D). This may suggest that under high FR conditions and/or without functioning phytochrome B, the auxin level in the shoot is already elevated to a point where blocking auxin transport has little effect on. Collectively, these results demonstrated the interacting roles between phytochrome and auxin in controlling shoot: root ratio in tomato.

3.5 Conclusions

FR increases shoot: root ratio in tomato. This increase involves the regulation by phytochrome B1/B2, as shown by the strong increase of shoot: root ratio *phyB1/B2* double mutant. The *phyB1/B2* double mutant still showed an increase in shoot: root ratio when FR increased, hence providing evidence for the involvement of other phytochromes in the regulation of shoot: root ratio in tomato. Phytochrome regulates shoot: root ratio in response to FR possibly via affecting auxin transport.

Acknowledgments

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Supplementary information

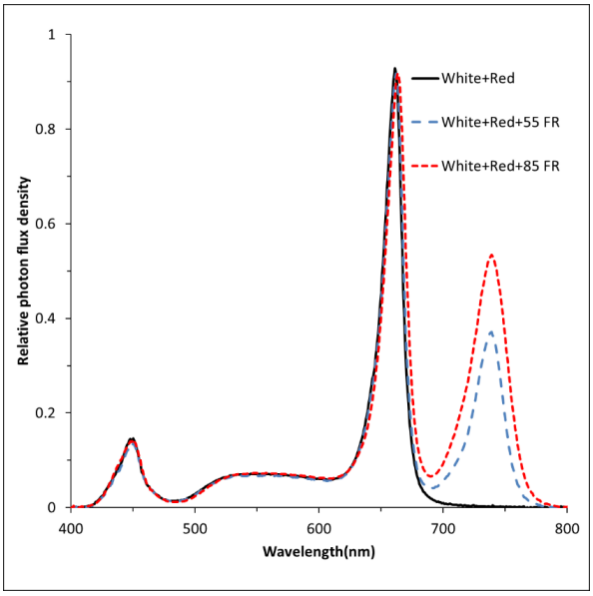


Figure S3.1 Spectral composition of light treatments provided by light-emitting diodes (LEDs) measured at the top of the canopy.

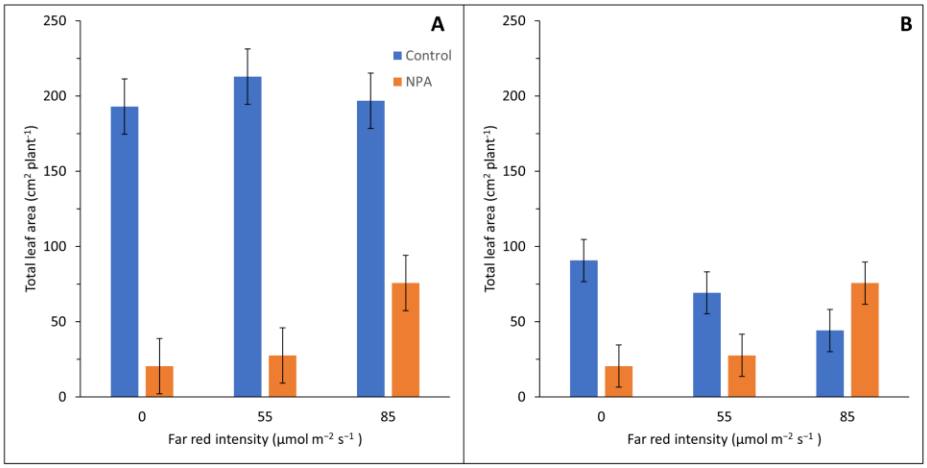


Figure S3.2 Effect of adding 0, 55, or 85 µmol m^{-2} s^{-1} of far red in a white + red background light, and application of N-1-naphthylphthalamic acid (NPA) in a ring of lanolin at the root-shoot junction on plant leaf area in wildtype (A) and phyB1/B2 mutant (B). Orange represents plants applied with NPA and blue represents control plants without NPA. For the control treatment, a mock ring of lanolin without NPA was applied at the root-shoot junction. Error bar represents standard error of means (n=4).

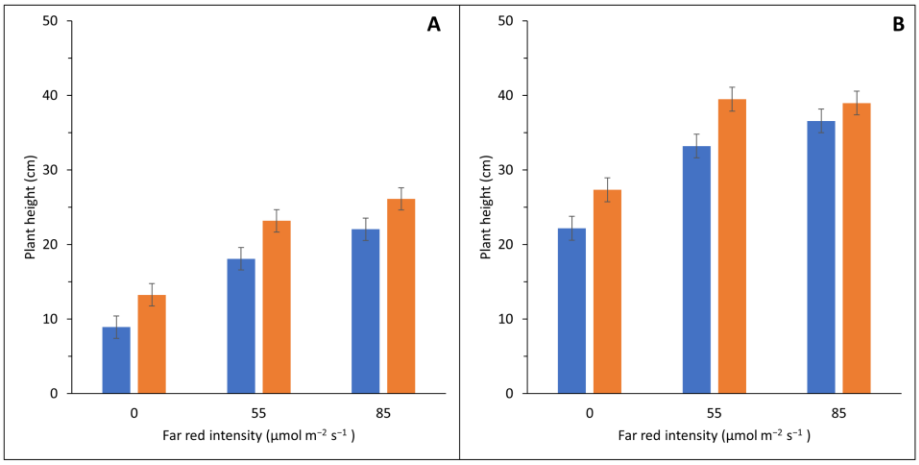
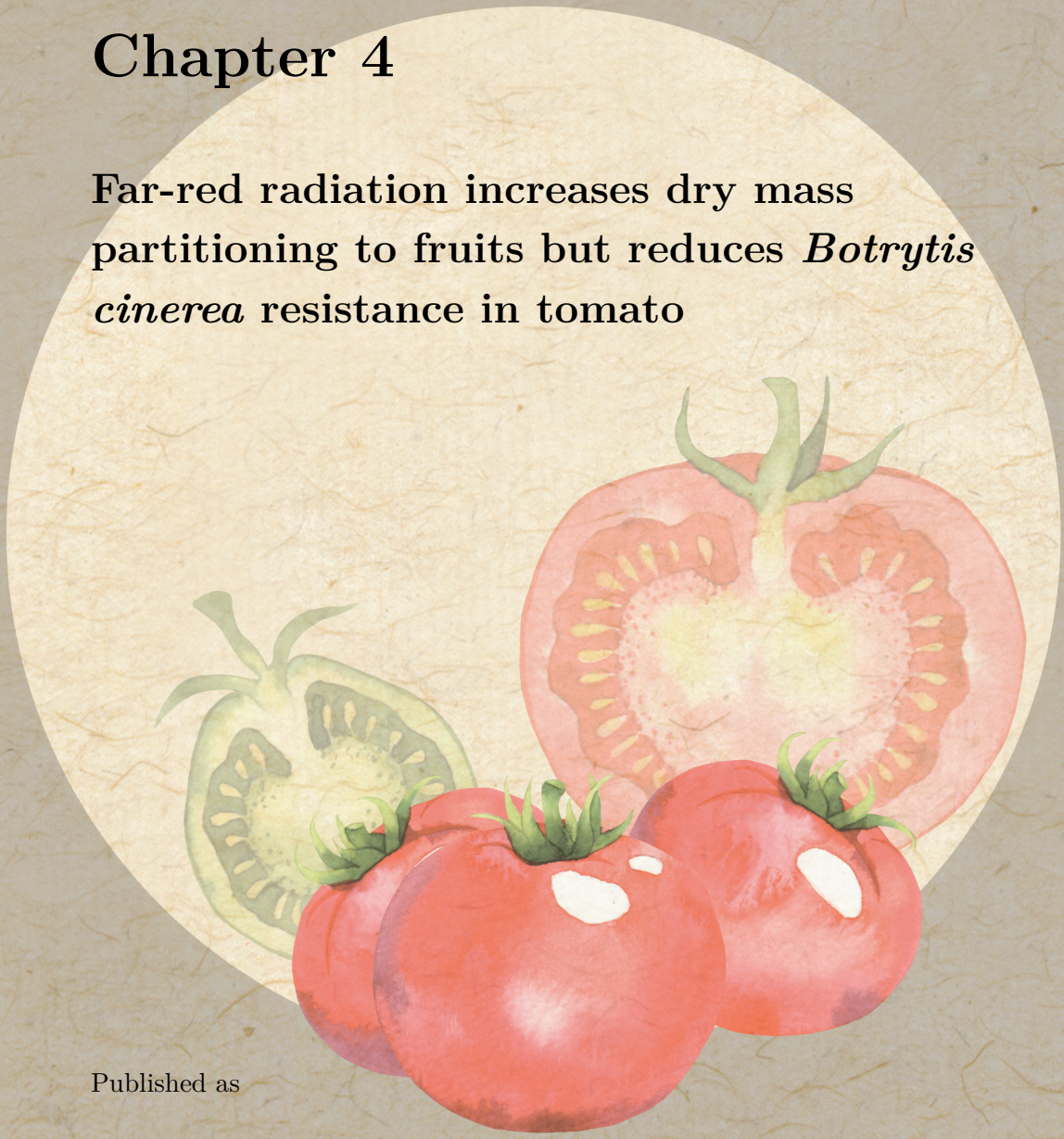


Figure S3.3 Effect of adding 0, 55, or 85 µmol m^{-2} s^{-1} of far red in a white + red background light, and application of N-1-naphthylphthalamic acid (NPA) in a ring of lanolin at the root-shoot junction on plant height in wildtype (A) and phyB1/B2 mutant (B). Orange represents plants applied with NPA and blue represents control plants without NPA. For the control treatment, a mock ring of lanolin without NPA was applied at the root-shoot junction. Error bar represents standard error of means (n=4).

Chapter 4

Far-red radiation increases dry mass partitioning to fruits but reduces *Botrytis cinerea* resistance in tomato



Published as

Ji, Y., Ouzounis, T., Courbier, S., Kaiser, E., Nguyen, P. T., Schouten, H. J., Visser,R. G., Pierik, R., Marcelis, L. F., and Heuvelink, E. (2019). Far-red radiation increases dry mass partitioning to fruits but reduces *Botrytis cinerea* resistance in tomato. *Environmental and Experimental Botany*, 168, 103889.

Abstract

The addition of far-red (FR, 700-800 nm) radiation to standard growth light triggers a set of photomorphogenic responses collectively termed shade avoidance syndrome. Recent research showed that additional FR increased fruit yield in greenhouse tomato production. However, the mechanism behind this increase is not clear; nor is it known whether there is a trade-off between growth and defense against plant diseases in tomato under additional FR. The aims of this study were 1) to quantify the effect of additional FR on tomato fruit growth, 2) to explain this effect based on underlying growth components and 3) to examine the FR effect on resistance against the necrotrophic fungus *Botrytis cinerea*. Tomato (*Solanum lycopersicum* 'Moneymaker') plants were grown for four months with 30 or 50 μmol m^{-2} s^{-1} of FR added to 150 μmol m^{-2} s^{-1} red + blue or white background LED lighting. Growth and development parameters were recorded, and a growth component analysis was conducted. Bioassays for resistance against *B. cinerea* were conducted on leaf samples collected from each light treatment. Additional FR increased total fruit dry mass per plant by 26-45%. FR affected multiple growth components, among which the fraction of dry mass partitioned to fruits was the most prominent with a 15-35% increase. Truss appearance rate was increased 11-14% by FR while instantaneous net photosynthesis rate was not affected. FR also resulted in more severe disease symptoms upon infection with *B. cinerea*. In conclusion, additional FR increases tomato fruit production mainly by increasing dry mass partitioning to fruits, rather than improving photosynthesis or increasing total plant biomass. However, FR also reduces resistance of tomato leaves against *B. cinerea*.

Key words: *Botrytis cinerea*; dry mass partitioning; far red; growth component analysis; LED lighting; *Solanum lycopersicum*.

4.1 Introduction

The shade avoidance syndrome (SAS), a set of adaptive changes that plants deploy when exposed to light with a low red (R, 600-700 nm) to far-red (FR, 700-800 nm) ratio, is among the most intensively studied set of plant responses to changes in their light environment. Plants perceive changes in the R: FR ratio via a family of photoreceptors named phytochromes, which exist as two photo-interconvertible isoforms: the biologically inactive red-light absorbing form (Pr) and the biologically active far-red-absorbing form (Pfr) (Chen et al., 2005). Pfr translocates to the nucleus and mediates different photomorphogenetic responses (Ruberti et al., 2012). Typical SAS responses such as stem elongation (Huber and Wiggerman, 1997), leaf hyponasty (Michaud et al., 2017; Pantazopoulou et al., 2017), reduced branching (Finlayson, 2007), and accelerated flowering (Devlin et al., 1998) have been studied intensively in *Arabidopsis thaliana* (for review, see Casal, 2012). Unlike in nature, where a low R: FR ratio often coincides with a decrease in photosynthetic photon flux density (PPFD), recent research typically features the addition of FR in a defined background light. The development of efficient light-emitting diodes (LEDs) in the past decade also stimulated the study of FR responses in a wide range of crop species including ornamental crops (Park and Runkle, 2017), leafy vegetables (Li and Kubota, 2009; Zhen and van Iersel, 2017) and fruit crops (Hao et al., 2017). Frequently, a positive FR effect on plant dry mass production is reported. To explain this, some authors demonstrated that additional FR may alter plant architecture to increase light interception (Kalaitzoglou et al., 2019), while in other studies additional FR was shown to increase leaf net photosynthesis rates (A) (Cao et al., 2018; Zhen and van Iersel, 2017) or whole-plant photosynthesis (Park and Runkle, 2017). However, it is also worth noting that the FR effect on photosynthesis varies between studies (Kim et al., 2019; Zhang et al., 2019). Additional FR was also reported to affect dry mass partitioning among plant organs, often increasing partitioning to shoot over root (Keiller and Smith, 1989; Page et al., 2009). In contrast to the abundance of research on relatively young plants, detailed studies of FR effects during the fruiting stage of crops such as tomato (*Solanum lycopersicum*) are less frequent. Recently, Kalaitzoglou et al. (2019) reported that additional FR increased total dry mass of tomato plants in the vegetative growth stage, as well as the fruit number per plant, fruit

fresh weight per plant and average fruit fresh weight. Similarly, Zhang et al. (2019) reported higher total plant dry mass and higher fruit yield in tomato under additional FR radiation. However, neither study provided sufficient insights on how additional FR increases fruit growth in the fruiting stage of the crop, which is a key step in understanding the FR induced yield improvement in fruit crops like tomato.

Besides the increase of plant growth under additional FR, the promotion of SAS may negatively impact plant immunity (Izaguirre et al., 2006; McGuire and Agrawal, 2005). FR has been reported to downregulate both salicylic-acid and jasmonic-acid-induced plant defense responses in *A. thaliana* (de Wit et al., 2013). *Botrytis cinerea*, a necrotrophic fungal pathogen causing gray mold disease in many plant species, has been studied intensively due to its destructive effects on crop production (reviewed by van Kan 2006). In *A. thaliana*, exposure to a low R:FR light reduced plant resistance against *B. cinerea* (Cargnel et al., 2014; Cerrudo et al., 2012). Although no direct FR effect on tomato resistance against *B. cinerea* is known, reduced constitutive defenses and reduced jasmonic-acid-induced direct defenses have been reported under low R:FR light conditions (Cortés et al., 2016). Taken together, it is reasonable to expect that additional FR may reduce the resistance of tomato against *B. cinerea*.

Growth component analysis, which is an analysis that subdivides growth into underlying morphological and physiological components (Jolliffe and Courtney, 1984), can be a useful tool to evaluate the contribution of these processes to fruit growth (Higashide and Heuvelink, 2009). Here, we aimed to identify and quantify the key components of tomato fruit growth as affected by additional FR and study whether additional FR affects resistance against *B. cinerea* in fruiting tomato plants grown with supplemental LED lighting. We hypothesized that additional FR would accelerate plant development (flowering, truss appearance rate), increase total fruit dry mass and fraction of dry mass partitioned to fruits and decrease plant defense against *B. cinerea*. To test these hypotheses, we conducted an experiment with tomato plants for four months in a greenhouse with different levels of FR added to different LED light combinations. Growth components were monitored and bioassays were conducted to evaluate plant resistance against *B. cinerea*.

4.2 Material and methods

Plant materials and growth conditions

The experiment was conducted at Wageningen University (52°N, 6°E, Wageningen, the Netherlands.). Tomato (*S. lycopersicum* 'Moneymaker') seeds were sown in the greenhouse (20 °C, relative humidity 80%) on 28 Nov. 2016 and germinated under natural light. On 15 Dec. 2016, uniform seedlings were transplanted into black 7.5-liter plastic pots filled with river sand and moved into another greenhouse. The greenhouse was divided into 15 compartments separated by double-sided light-impermeable white plastic sheet. In each compartment, 14 plants were placed on two gutters, including two border plants at both ends. Plant density was four plants m^{-2}. Climate in the greenhouse was controlled by greenhouse climate control computer (Hoogendoorn, Vlaardingen, the Netherlands). Measured day/night temperature was 22 ± 0.5/18 ± 0.3 °C (mean ± standard deviation) until plants started flowering (35 days after transplanting). Thereafter, temperatures were adjusted to 20 ± 0.7/16 ± 0.3 °C to facilitate fruit set. Temperature was recorded every 10 minutes with PT500 temperature sensors (Hoogendoorn) placed in the center of each plot. Measured daily average relative humidity was 78 ± 5 % and CO_2 partial pressure was 408 ± 11 μbar. The plants were irrigated with nutrient solution (electrical conductivity 2.1 dS m^{-1}, pH 5.5) containing 1.2 mM NH_4^+, 7.2 mM K^+, 4.0 mM Ca^{2+}, 1.8 mM Mg^{2+}, 12.4 mM NO^{3-}, 3.3 mM SO_4^{2-}, 1.0 mM PO_4^{2-}, 35 μM Fe^{3+}, 8.0 μM Mn^{2+}, 5.0 μM Zn^{2+}, 20 μM B, 0.5 μM Cu^{2+}, 0.5 μM MoO_4^{2-}. The EC and pH level of the nutrient solution were measured twice a week and the nutrient solution was refreshed frequently. Manual pollination with an electronic bee (Vibri Vario, Royal Brinkman, Gameren, the Netherlands) was applied three times per week. Side shoots and same number of old leaves were removed weekly from all plants.

Light treatments and experimental set-up

Five overhead light treatments were applied: white (W), white + 30 μmol m^{-2} s^{-1} FR (WFR30), red + blue (RB), red + blue + 30 μmol m^{-2} s^{-1} FR (RBFR30) and RB + 50 μmol m^{-2} s^{-1} FR (RBFR50) (Figure 4.1, Table 4.1). The spectral distribution and photon flux density of the supplementary

Table 4.1: *Photosynthetic photon flux density (PPFD), photon flux density (PFD) of blue, green, red, and far red, ratios of red: blue, red: far red and phytochrome photostationary state (PSS) value of the LED supplementary light in the five light treatments measured at the canopy level in the absence of solar radiation.*

Parameter	Unit	Light tretment				
		W	WFR30	RB	RBFR30	RBFR50
PAR PPFD[1]	μmol m^{-2} s^{-1}	153 ± 8[2]	144 ± 9	155 ± 9	146 ± 9	146 ± 8
Blue	μmol m^{-2} s^{-1}	25 ± 3	23 ± 3	9 ± 2	8 ± 3	9 ± 1
Green	μmol m^{-2} s^{-1}	38 ± 4	34 ± 3	3 ± 0.2	4 ± 0.1	3 ± 0.2
Red	μmol m^{-2} s^{-1}	86 ± 4	79 ± 7	141 ± 8	133 ± 6	136 ± 8
Far red	μmol m^{-2} s^{-1}	1 ± 0.1	30 ± 3	3 ± 0.3	28 ± 1	49 ± 2
Red: blue		3 ± 0.3	3 ± 0.1	16 ± 0.4	17 ± 0.3	16 ± 0.4
Red: far red		79 ± 4	3 ± 0.1	49 ± 5	5 ± 0.1	3 ± 0.1
PSS		0.87	0.80	0.88	0.84	0.80

[1]For the calculations of ratios, PFD was integrated over 100 nm intervals for blue (400-500 nm), green (500-600 nm), red (600-700 nm) and far red (700-800 nm).
[2]All values are means ± standard error of means (s.e.m.). s.e.m of PSS was very small (0.001-0.002) and is therefore not shown.

light was measured with a spectroradiometer (USB 2000 + UV-VIS, Ocean Optics, Duiven, the Netherlands), on 6 evenly distributed locations in each plot at the top of the canopy. Phytochrome photostationary state (PSS) in each treatment was calculated based on the measured spectra as the ratio of Pfr to the total of Pfr and Pr according to (Sager et al., 1988). The RBFR50 treatment was included because it had the same PSS value as WFR30 (Table 4.1). The blue, red and far-red spectra in this experiment peaked at 453 nm, 666 nm and 735 nm, respectively. Photoperiod was set to 16 hours (0400h - 2000h). On average, solar daily photosynthetic photon flux density (PPFD, 400-700 nm) contributed 12% to the total daily PPFD integral during the whole experiment at canopy level (Figure S4.2).

All supplementary lighting was provided by LED modules (W: GreenPower LED-TL-DR/W-MB-VISN, RB: GreenPower LED -TL-DR/B-150, FR: GreenPower LED -PM-FR-150, Philips, Eindhoven, the Netherlands). The height of the LEDs was adjusted weekly to maintain the desired PPFD at the top of the canopy (150 μmol m^{-2} s^{-1}, Table 4.1). When the LEDs reached the maximal height of the greenhouse, the top of the canopy was lowered weekly, as is usual in a modern high wire cultivation system. A spectroradiometer was used to ensure that both PPFD and PSS values were kept constant every time the

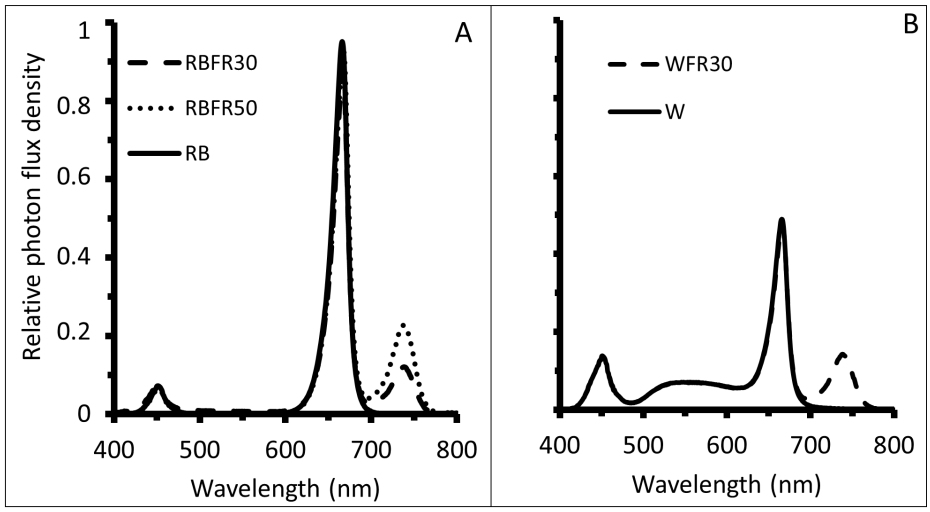


Figure 4.1: *Spectral composition of light treatments provided by light-emitting diodes (LEDs) measured at the top of the canopy. (A) 150 µmol m^{-2} s^{-1} background light of red/blue (RB) without far red (FR), with 30 or 50 µmol m^{-2} s^{-1} FR. (B) 150 µmol m^{-2} s^{-1} background light of white (W) LEDs, without FR, or with 30 µmol m^{-2} s^{-1} FR.*

height of lamps or that of the plants was adjusted. The experiment was set up as a randomized complete block design, where the five light treatments were repeated in three blocks.

4

Non-destructive measurements

Growth and development parameters

Numbers of leaves (length $\geq$ 1 cm) per plant, flowering buds per truss, fully opened flowers per truss and set fruits (diameter $\geq$ 5 mm) per truss were recorded weekly for three plants per block.

Leaf net photosynthesis rate

Measurements of leaf net photosynthesis were performed using the LI-6400XT photosynthesis system (Li-Cor Biosciences, Lincoln, NE, USA). Acclimation of leaf net photosynthesis rate (A) to FR was assessed using CO_2 (A/Ci) and light response curves (A/PPFD), by means of the leaf chamber fluorometer (leaf area: 2 cm^2) with the built-in RB LED

light source. Instantaneous A under the treatment spectra was additionally assessed using a transparent leaf chamber (leaf area: 6 cm^2). Conditions in the chamber were: 400 μbar CO_2 partial pressure, 0.9-1.2 kPa leaf-to-air vapor pressure deficit, 25 °C cuvette temperature, and 400 μmol s^{-1} air flow rate, unless described otherwise. The third or fourth leaflet of the fifth leaf (counting from the top of plant, the first leaf being longer than 5 cm) of one plant per plot was used for measurements. Measurements were conducted for two time periods (1) from 16 to 23 Jan. 2017 (32-39 days after transplanting) and (2) from 19 to 24 Feb. 2017 (66-71 days after transplanting).

A/Ci curves: Leaves were first adapted to 500 μbar CO_2 and 1500 μmol m^{-2} s^{-1} PPFD for 10 - 15 minutes, after which CO_2 partial pressure was increased to 2000 μbar. Then, CO_2 was reduced to 1500, 1000, 800, 600, 400, 200, 100, 50 and 40 μbar, with each step taking ∼5 min. Data was logged every five seconds, and averages of six stable values at each CO_2 step were calculated. PPFD was maintained at 1500 μmol m^{-2} s^{-1}. Parameters of a biochemical photosynthesis model (Farquhar et al., 1980), namely the maximum rate of carboxylation of Rubisco (Vcmax), maximum electron transport rate at 1500 μmol m^{-2} s^{-1} PPFD (J_{1500}), and maximum rate of triose phosphate use (TPU) were estimated by using the fitting procedure by (Sharkey et al., 2007).

A/PPFD curves: Directly after A/Ci curve measurements, CO_2 partial pressure was adjusted to 400 μbar, and PPFD remained at 1500 μmol m^{-2} s^{-1} until A and stomatal conductance were stable. Then, PPFD was reduced stepwise to 1000, 800, 600, 400, 200, 150, 100, 50 and 30 μmol m^{-2} s^{-1}. Data were logged as described above. Quantum yield (QY), a curvature parameter (Θ) and maximum A (A_{max}) were determined by fitting a non-rectangular hyperbola (Ögren and Evans, 1993).

Instantaneous A: Opposite leaflets of those used in A/Ci and A/PPFD curves were clamped into the transparent leaf chamber. After waiting for approximately two minutes for CO_2 partial pressures and water vapor to equilibrate, data were logged for 30 seconds, as described above. This was repeated at 1000h, 1200h and 1400h on the same leaflets and the same day. Instantaneous A was later expressed as a function of PPFD inside the transparent leaf chamber, which was 88.4% of PPFD measured above the transparent leaf chamber.

Destructive measurements

On 30 Jan. 2017 (46 days after transplanting), 6 Mar. 2017 (81 days after transplanting) and 24 Apr. 2017 (130 days after transplanting), three replicate plants per compartment (experimental unit) were destructively measured. Total leaf area per plant (LI -3100 area meter, Li-Cor) and fruit number were measured. Leaves, stem, fruits and roots were separated and dried in the oven for 72 hours at 105 °C to obtain the dry mass. The number and dry mass of harvested ripe fruits and removed old leaves was recorded and included in the calculation of total fruit dry mass and total plant dry mass.

Growth component analysis

Effects of light treatment on plant growth were analyzed by separating growth in its underlying components (Figure 4.2). Fruit dry mass (FDM) was analyzed as the product of total plant dry mass (TDM) and fraction of dry mass partitioned to fruits (F_{fruit}). F_{fruit} was further divided into fruit number (FN_{plant}) and fruit relative sink strength (FSS, fruit sink strength relative to total sink strength of fruit and vegetative organs). FSS was not directly measured but can be evaluated by examining individual ripe fruit dry mass (IFDM). FN_{plant} can be explained by truss appearance rate (TAR) and fruit number per truss (FN_{truss}). All comparisons were based on the destructive harvests 46 and 130 days after transplanting, unless described otherwise. Instantaneous A was the average of the two measurement periods in January and February. Leaf area index (LAI), total leaf number per plant (LN_{plant}), and average leaf area per leaf (LA_{leaf}) were calculated from daily values estimated from linear interpolation of the data measured 46, 81 and 130 days after transplanting. TAR was calculated based on leaf appearance rate assuming that after formation of every three leaves a truss was formed.

4.3 Results

Growth component analysis

During the generative growth period (46–130 days after transplanting), adding 30 μmol m^{-2} s^{-1} of far red (FR) to white (W) or red + blue (RB) background light resulted in 33% and 26% higher total fruit dry mass,

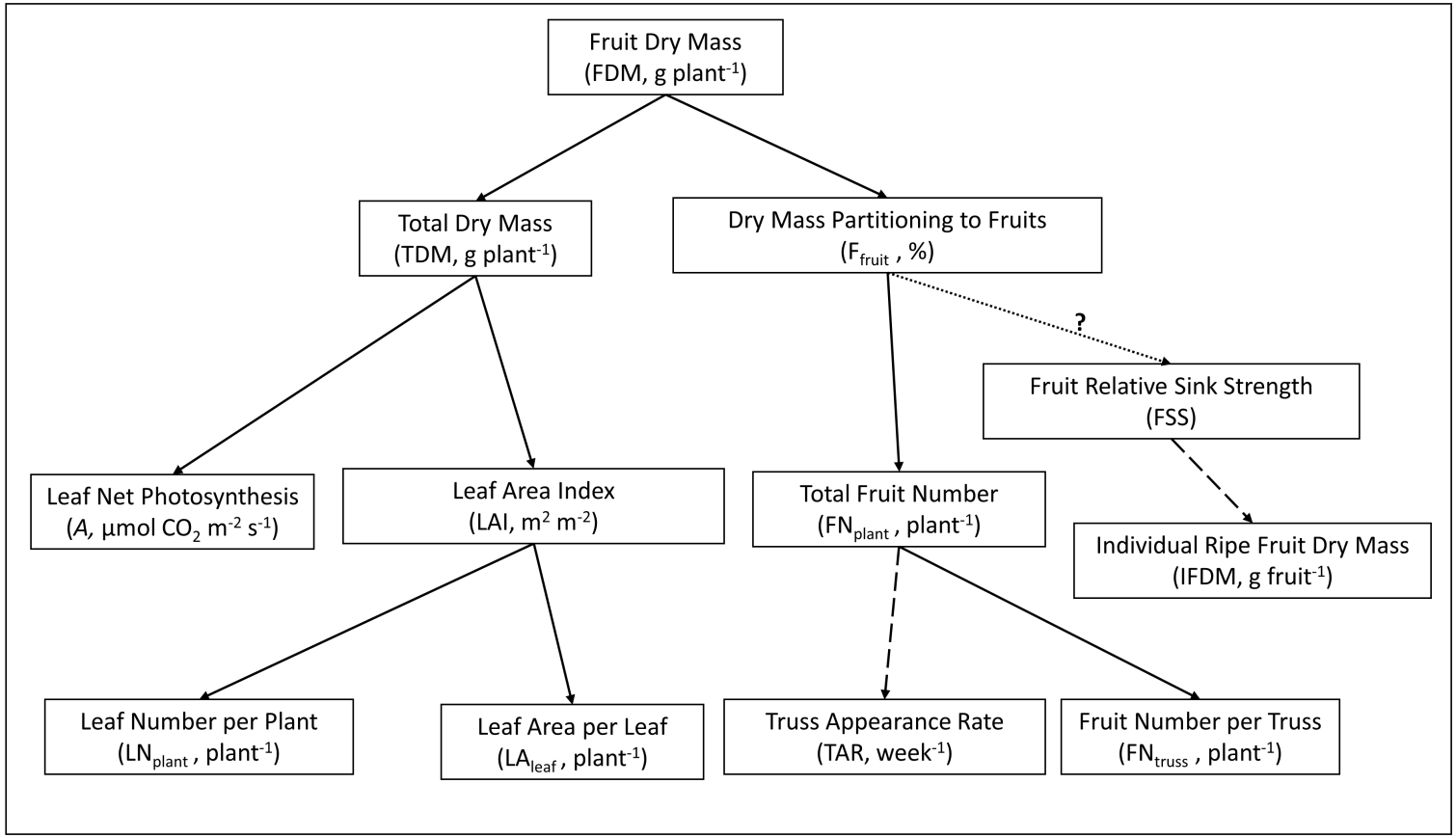


Figure 4.2: *General scheme of a top-down growth component analysis of total fruit dry mass. Abbreviations and units are included in brackets. Dashed lines indicate that the component was estimated instead of being directly measured.*

respectively (Figure 4.3A). Adding 50 μmol m^{-2} s^{-1} of FR in an RB background increased total fruit dry mass by 45%. Similar effect was observed for the individual ripe fruit dry mass (Figure 4.3B).

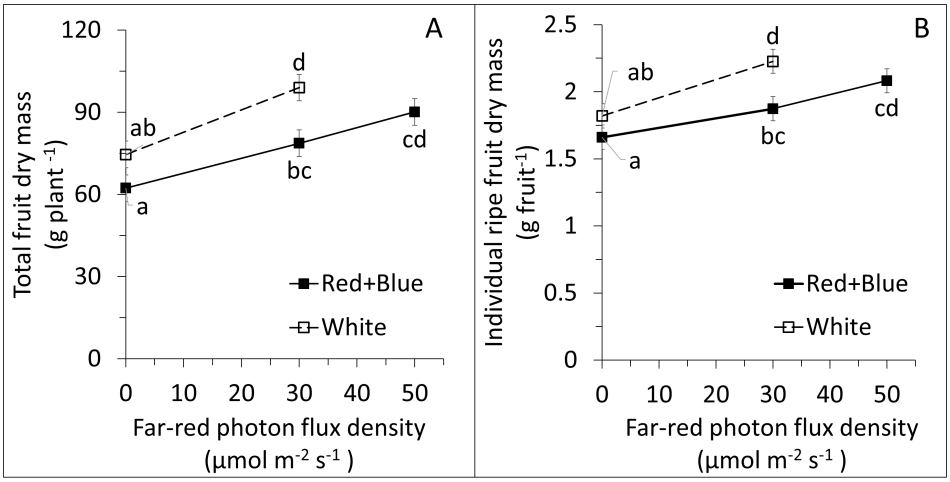


Figure 4.3: *Effects of additional far red on cumulative fruit dry mass (A) and individual ripe fruit dry mass(B) in white or red + blue background light during generative growth (46–130 days after transplanting). Error bars represent s.e.m. Different letters denote significant differences according to Fisher' s protected LSD test (α=0.05).*

In both RB and W backgrounds, adding 30 μmol m^{-2} s^{-1} of FR significantly increased the fraction of dry mass partitioned to fruits (F_{fruit}), and truss appearance rate (TAR, Figure 4.4A, 4.4B). These two components further increased when FR was increased from 30 to 50 μmol m^{-2} s^{-1} (Figure 4.4C). FR increased significantly (9–10%) the leaf number per plant (LN_{plant}) during this period, however only in the RB background (Figure 4.4). No significant effect of additional FR was found for other components. Absolute values of all components are shown in Table S4.1.

Additional FR shifts dry mass partitioning towards fruits and stem at the expense of leaves

After 130 days of growth with additional FR in a W or RB background, the fraction of dry mass partitioned to fruits and stem increased, whereas that partitioned to the leaves was reduced (Figure 4.5). There were no significant differences in the fraction of dry mass partitioned to roots between light

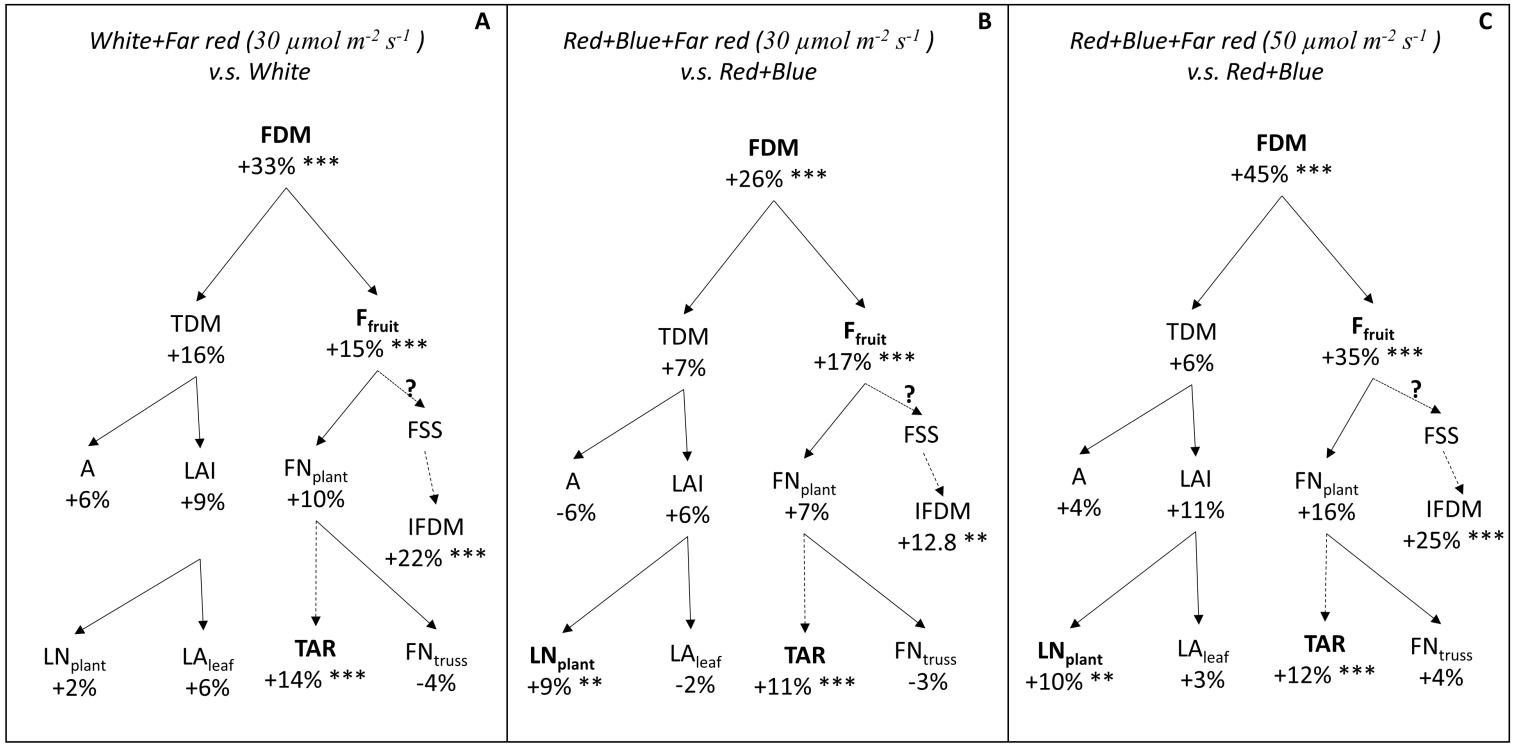


Figure 4.4: *Effects of additional far red in white (A) or red + blue (B, C) background light on the growth components 46–130 days after transplanting. Abbreviations in this figure: FDM (fruit dry mass), TDM (total dry mass), F_{fruit} (dry mass partitioning to fruits), A (leaf net photosynthesis), LAI (leaf area index), FN_{plant} (total fruit number per plant), IFDM (individual ripen fruit dry mass), LN_{plant} (leaf number per plant), LA_{leaf} (leaf area per leaf), TAR (truss appearance rate), FN_{truss} (fruit number per truss). Dashed lines indicate that the parameter was based on estimation. Asterisks denote significant effects of additional FR as tested by linear regression (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Fruit sink strength was not determined.*

treatments (Figure 4.5D). Similar patterns were observed 46 and 81 days after transplanting (Figure S4.1).

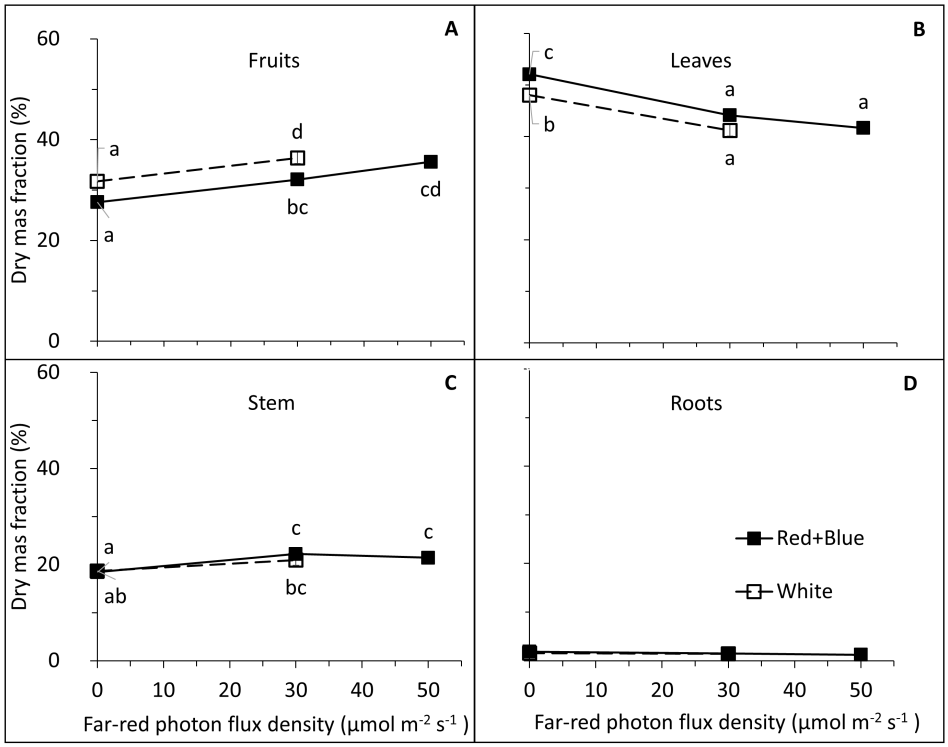


Figure 4.5: *Effects of additional far red in white or red + blue background light on the fraction of dry mass partitioned to fruits (A), leaves (B) stem (C) and roots (D). Error bars represent s.e.m. Different letters denote significant differences for the dry mass fraction of the same organ according to Fisher' s protected LSD test ($\alpha = 0.05$).*

Acclimation to additional FR tends to reduce leaf photosynthetic capacity

No difference was observed in net photosynthesis rate (A) measured with a RB light source in the A/Ci or the A/PPFD curve when comparing plant grown in W and RB without FR, while plants grown under additional FR showed lower A (Figure 4.6). This difference was most clear at higher light intensities (Figure 4.6A) and CO_2 partial pressures (Figure 4.6B). Quantum yield (QY) was not significantly affected by growth under additional FR, except that compared to RB, RBFR30 resulted in a significantly lower QY

(Figure 4.6A, inset). Additional FR significantly reduced the maximum electron transport rate in W background (J_{1500}, Figure 4.6B, inset). Other parameters derived from the fitted model were not significantly different, although reduction in A_{max} ($P = 0.056$) and TPU ($P = 0.052$) when grown under additional FR was close to significant. Instantaneous A, which is the net photosynthesis rate measured under actual growth light, was not significantly affected by the light treatments, neither as absolute rates nor when normalized for PPFD during the measurement (Table S4.1).

Plants grown with additional FR show reduced resistance against *B. cinerea*

In the RB background, additional FR resulted in significantly larger lesion size, which further increased as FR increased from 30 to 50 μmol m^{-2} s^{-1}. In the W background, lesion size was not significantly affected by the addition of 30 μmol m^{-2} s^{-1} FR, although there was a tendency for lesion size to increase with increased FR.

4.4 Discussion

Additional FR increases fruit growth mainly by increasing dry mass partitioning to fruits

Additional FR in both W and RB backgrounds increased total fruit dry mass (Figure 4.3A), confirming earlier findings in experiments that used either continuous or end-of-day FR treatments (Hao et al., 2016; Kalaitzoglou et al., 2019; Zhang et al., 2019). FR has been shown to increase leaf area, especially in the early stage of plant growth (Cao et al., 2018; Kalaitzoglou et al., 2019). In the present study we observed a similar but smaller FR effect (Figure 4.4), which could be a result of the difference in developmental stage and the fact that we used a less extreme FR treatment in this compared to previous experiments. Despite a negative FR effect on photosynthetic capacity, total plant dry mass was not significantly affected by additional FR (Figure 4.4). This may be the result of an improved light distribution within the plant canopy as the elongated internodes under additional FR led to a more open plant architecture, which allowed radiation to penetrate deeper into the canopy (Zhang et al., 2019). This is further supported by a model simulation

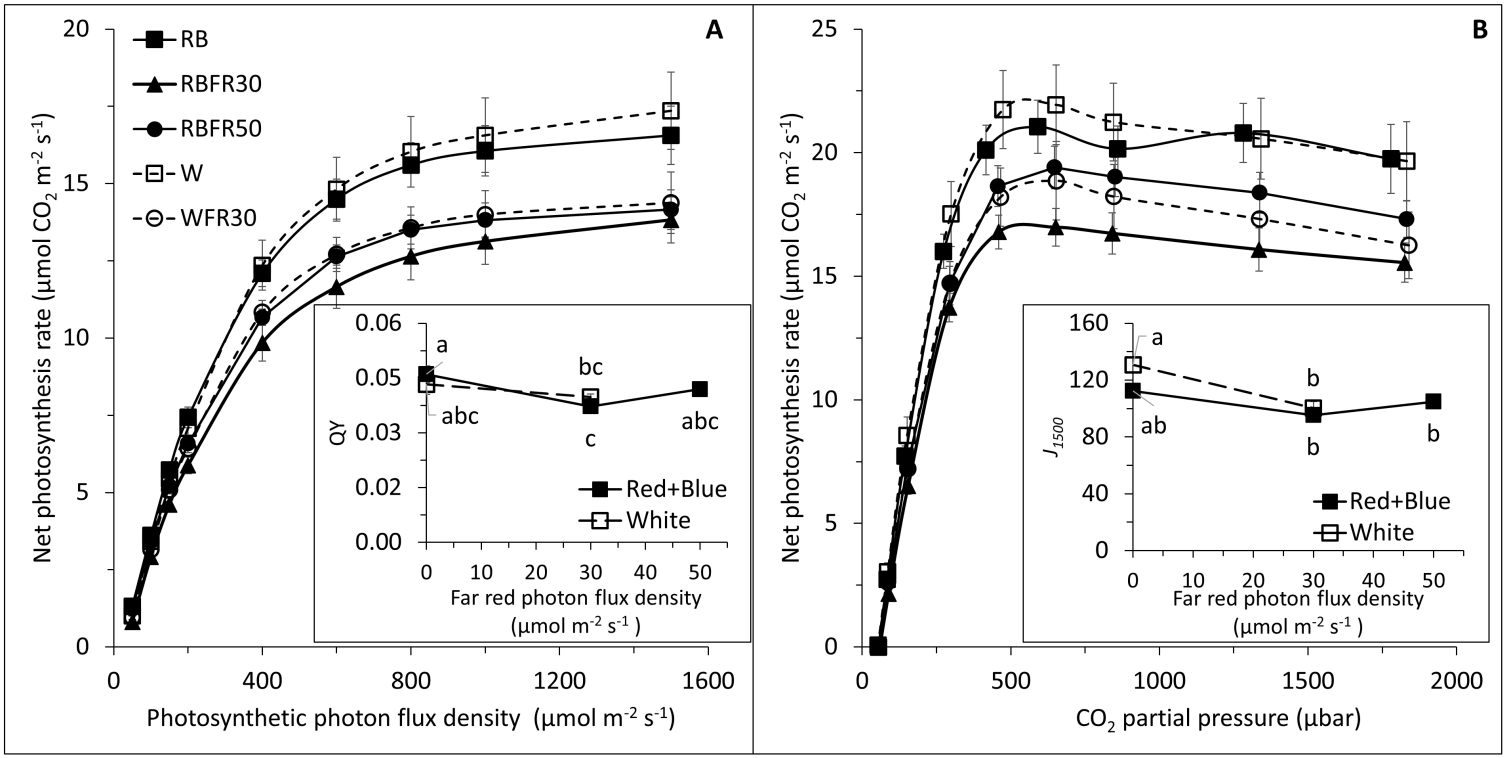


Figure 4.6: *Light (A) and CO_2 (B) response curves of tomato leaves grown in white (W) or red + blue (RB) background, with 0, 30 or 50 µmol m^{-2} s^{-1} far-red (FR) radiation. The insets show the effects of additional FR on quantum yield (QY) and electron transport rate (J_{1500}) with W or RB background light. During measurements, all leaves were illuminated by RB light. Error bars represent s.e.m. Different letters denote significant differences according to Fisher' s protected LSD test ($\alpha = 0.05$).*

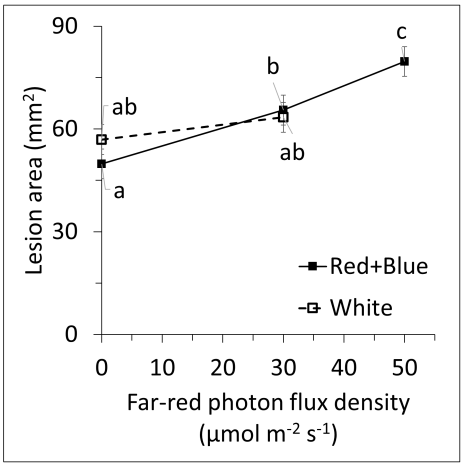


Figure 4.7: *Effects of additional far red in white or red + blue background light on lesion area after B. cinerea infection. Error bars represent s.e.m. Different letters denote significant differences in lesion area between growth conditions according to Fisher' s protected LSD test (α = 0.05).*

showing that increasing internode length indeed increases canopy light absorption in the upper part of the canopy and increased canopy photosynthesis rate by up to 10% (Sarlikioti et al., 2011).

Exposure to FR may increase the efficiency of photosystem II electron transport by balancing the excitation of both photosystems, thereby increasing net photosynthesis rate and decreasing non-photochemical quenching in the short term; this has been described as the Emerson enhancement effect (Emerson et al., 1957; Pettai et al., 2005; Zhen and van Iersel, 2017). Here, we observed a tendency towards a decreased photosynthetic capacity and a reduced efficiency for CO_2 fixation per unit leaf area in FR acclimated leaves when these were measured using a light source containing only RB light (Figure 4.6). This negative effect may be related to a lower chlorophyll content per unit leaf area, which was often reported in plants grown with additional FR (Héraut-Bron et al., 2001; Kalaitzoglou et al., 2019; Tucker, 1981). When measured with a transparent chamber exposed to the different treatment spectra, we found no significant effect of FR on A (Figure 4.4, Figure S4.1), which was similar to the results reported by (Zhang et al., 2019). Previous FR acclimation studies showed positive (Kalaitzoglou et al., 2019), negative (Barreiro et al., 1992) or no effects on A (Bonnett et al., 1975; Chow

et al., 1990). In the present study, we observed a higher specific leaf area under additional FR (Table S4.2), which is in line with decreased photosynthetic capacity per unit leaf area. Here, we argue that the FR effect on total plant growth via affecting photosynthesis, on the long run, is rather limited. A low R:FR is a signal for light competition and plants may respond by optimizing their architecture for better light interception. For young tomato plants, the increase in total plant dry mass under additional FR mainly resulted from an increased light interception due to a higher total leaf area index (Kalaitzoglou et al., 2019). In a fruiting tomato canopy, which usually has a leaf area index higher than three and typically intercepts about 90% incident light (Heuvelink and Dorais, 2005), total light interception can only be improved marginally. This is in accordance with our finding that total plant dry mass was not significantly affected by FR during generative growth. Further, we speculate that the positive short-term effect (Emerson enhancement effect) and slightly negative long-term acclimation effect of additional FR on A may cancel each other out.

Additional FR significantly increased the fraction of dry mass partitioned to fruits during generative growth (Figure 4.4), which was at the expense of partitioning to leaves. This reduced partitioning to leaves is in accordance with a meta-analysis on young plants by (Poorter et al., 2012), which associated low R:FR with a decreased leaf mass fraction, in that case accompanied by increased stem mass fraction. Similar changes of partitioning patterns were also reported by Kalaitzoglou et al. (2019). Recently, Kim et al. (2019) showed a tendency that an increased fraction of dry mass was partitioned to tomato fruits when additional FR was provided in an intra-canopy R lighting, however the effect was not statistically significant. In contrast, Zhang et al. (2019) showed small negative effects of additional FR on dry mass partitioning to fruits when overhead additional FR was provided, while RB was provided as intra-canopy lighting. In that research, additional FR increased leaf dry mass remarkably and hence influenced the partitioning pattern. Also, for a fully-grown tomato canopy, whose uppermost leaf layer is responsible for most of the light capture (Acock et al., 1978), intra-canopy lighting may result in different responses to FR compared to that of a plant grown solely under overhead supplemental lighting. To our knowledge, this is the first study to demonstrate a FR induced increase in dry mass partitioning

to fruits in an overhead LED crop production system.

Possible mechanisms of how FR may increase dry mass partitioning to fruits

In the present study, we show that during generative growth, additional FR significantly increases the fraction of dry mass partitioned to fruits in both light backgrounds (Figure 4.5). In tomato, fruit load may influence fraction of dry mass partitioned to fruits (Heuvelink, 1997). In this study, fruit number per plant tended to increase by 7–16% (Figure 4.4, Table S4.1). Following the concept of sink strength as a determinant of dry mass partitioning (Heuvelink, 1997; Marcelis, 1996), this could increase the fraction of dry mass partitioned to fruits by up to 11% (Table S4.3). Hence, increased fruit number can only partly explain the observed 22–42% increase in fraction of dry mass partitioned to the fruits (Figure 4.4, Table S4.1). Collectively, this points to the possibility that additional FR may affect dry mass partitioning by increasing individual fruit sink strength, which is the intrinsic capacity to compete for photosynthetic assimilates (Heuvelink, 1997). To our knowledge, no direct effect of additional FR on fruit sink strength has been reported, but we may derive some clues from existing studies. For example, individual fruit size or mass, which is an indication for fruit sink strength, were shown to be increased by additional FR (Hao et al., 2016; Kim et al., 2019). In the present study, we have also observed a significant increase in individual ripe fruit dry mass under additional FR (Figure 4.3B). Further, phytochromes in tomato fruits have been shown to be involved in the transcriptional regulation of genes crucial for sink activity (Ernesto Bianchetti et al., 2018; Fridman and Zamir, 2003; Kocal et al., 2008). Taking these lines of evidence together, we argue that higher individual fruit sink strength may be an important reason for improved fruit growth under FR. Indeed, a decreased leaf sink strength may also partly contribute to the increase in fraction of dry mass partitioned to fruits. However, should that be the case, we would expect the dry mass partitioning to stem and roots to be increased to a similar extent as that in fruits, which was not observed in the present study. These results warrant further investigation into the direct effects of additional FR on fruit sink strength.

Additional FR alters the trade-off between growth and defense

The FR-induced SAS is a strategy that FR-sensitive plants use to ensure reproductive success. The deployment of such a strategy implies a change in the allocation of resources between different physiological processes. The trade-off between growth and defense, often titled "the dilemma of plants" (Herms and Mattson, 1992), is one of the most well-studied allocation trade-offs. In our study, we observed that tomato plants grown under additional FR showed reduced resistance against *B. cinerea* (Figure 4.7). Early works on the effects of canopy density on plant defense against fungal diseases already provided hints for a decreased resistance under high density (low R:FR, for review, see Ballaré, 2014). However, a decreased R:FR is only part of the consequences of a higher planting density. Further work demonstrated the effect of light spectrum, including additional FR, on growth and sporulation of *B. cinerea* (Schumacher, 2017). Research on *A. thaliana* showed that exposure to low R:FR reduced resistance against *B. cinerea* (Cargnel et al., 2014; Cerrudo et al., 2012; de Wit et al., 2013). Also, it has been reported that inactivation of *phyB* in tomato downregulates direct defenses via the jasmonic acid pathway (Cortés et al., 2016), which is closely linked to plant defense against fungal diseases like *B. cinerea*. These results imply that phytochrome mediated FR responses may play important roles in regulating plant' s balance between growth and defense.

4.5 Conclusions

Our results demonstrate that additional FR in supplementary lighting significantly increases tomato fruit dry mass production. This increase is mainly due to the increase in the fraction of dry mass partitioned to fruits, rather than increased photosynthesis or a higher plant biomass. However, additional FR also comes with some negative effects. The larger lesion size observed on leaf samples collected from plants grown under additional FR suggests that additional FR during growth can reduce tomato resistance against *B. cinerea*.

Contributions

YJ and TO wrote the manuscript. YJ, TO, and PTN, collected and analyzed the growth component analysis data. SC collected and analyzed the bioassay data against *Botrytis cinerea*. EK collected and analyzed the photosynthesis data. LFMM, EH., and RP. provided guidance in the experimental design and provided critical comments on the manuscript. HJS, and RGFV, provided critical comments to the overall structure of the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgments

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Supplementary information

Table S4.1. Absolute values of component variables used in growth components analysis for the generative growth period of 46-130 days after transplanting.

Parameter[1]	Unit	Light treatment					s.e.m.[3]	P-value
		RB	RBFR30	RBFR50	W	WFR30		
Total Fruit Dry Mass	(g plant^{-1})	62	79	90	75	99	5.0	0.006
Total Plant Dry Mass	(g plant^{-1})	193	207	206	203	235	8.8	0.08
Fruit Dry Mass Fraction	(g g^{-1})	0.32	0.38	0.44	0.36	0.42	0.01	0.002
Instantaneous *A* (Absolute)	(µmol CO_2 m^{-2} s^{-1})	4.8	4.5	5.0	4.5	4.8	0.31	0.8
Instantaneous *A* (Relative[2])	(µmol CO_2 µmol PPFD^{-1})	0.06	0.06	0.07	0.04	0.04	0.005	0.3
Leaf Area Index	(m^2 m^{-2})	3.8	4.0	4.2	3.8	4.2	0.19	0.4
Leaf Area per Leaf	(cm$^{2)}$	411	402	424	405	430	21.8	0.8
Total Leaf Number	(plant^{-1})	23	25	25	24	24	0.26	0.002
Total Fruit Number	(plant^{-1})	56	60	65	50	66	3.5	0.3
Truss Appearance Rate	(plant^{-1} week $^{-1}$)	0.9	1.0	1.0	0.9	1.0	0.01	0.001
Fruit Number per Truss	(truss^{-1})	4.3	4.1	4.4	4.6	4.4	0.28	0.8
Individual Ripe Fruit Dry Mass	(g fruit^{-1})	1.7	1.9	2.1	1.8	2.2	0.09	0.002

[1]Total fruit dry mass, total plant dry mass, fruit dry mass fraction, total leaf number, total fruit number and individual ripe fruit dry mass were calculated as the average increase over the generative growth period. Other parameters were average values over the period.
[2]Instantaneous *A* (relative) was calculated by dividing instantaneous *A* (absolute) by PPFD at measurement leaf position.
[3]s.e.m and P-value of each parameter were calculated by ANOVA.

Table S4.2. Specific leaf area of plants (cm^2 g^{-1}) grown in the five light treatments measured on 46, 81, 130 days after transplanting and weighted average during the period of 46-130 days after transplanting.

Measurement time (d)	Light treatment					s.e.m.	P-value
	RB	RBFR30	RBFR50	W	WFR30		
46	259	349	332	263	352	13.4	0.002
81	238	261	287	245	291	11.0	0.03
130	215	267	255	214	263	17.3	0.16
46-130	236 a	281 b	287 b	240 a	295 b	6.8	<0.001

s.e.m and P-value of each parameter were calculated by ANOVA. Different letters denote significant difference according to Fisher's protected LSD test (α=0.05)

Table S4.3. Effect of increased fruit number on fruit sink strength and fraction of dry mass partitioned to fruits.

	Reference	16% More Fruits
Fruit sink strength[1] (g day^{-1})	0.3	0.348
Vegetative sink strength[2] (g day $^{-1}$)	0.7	0.7
Dry mass partitioning to fruits (%)	30	33.2

[1]Fruit sink strength was assumed proportional to fruit number.

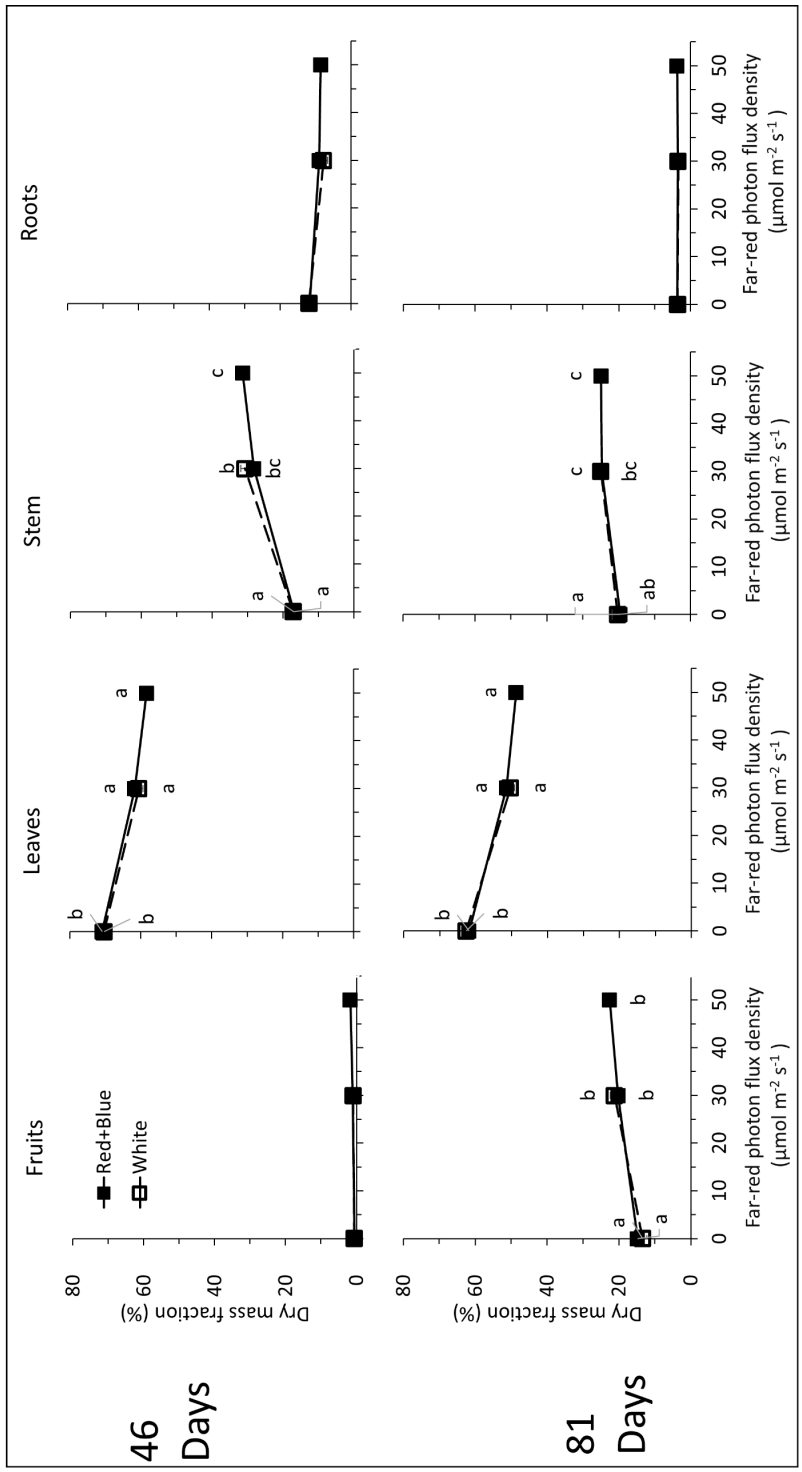


Figure S4.1. Fraction of dry mass partitioned to fruits, leaves, stem and roots measured 46 and 81 days after transplanting. Open symbols represent white background and closed symbols represent red + blue background. Different letters denote significant differences for the dry mass fraction of the same organ according to Fisher's protected LSD test (α=0.05).

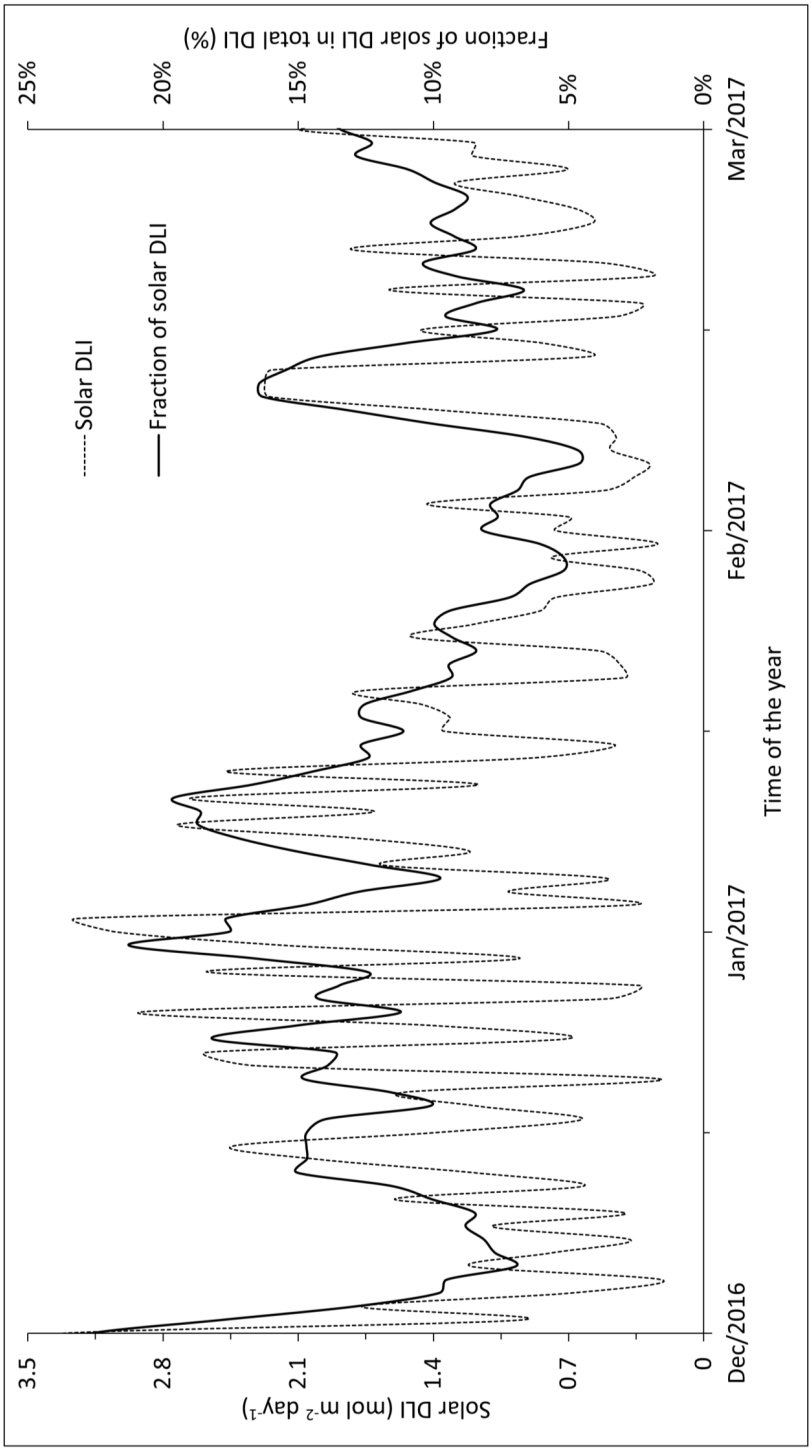
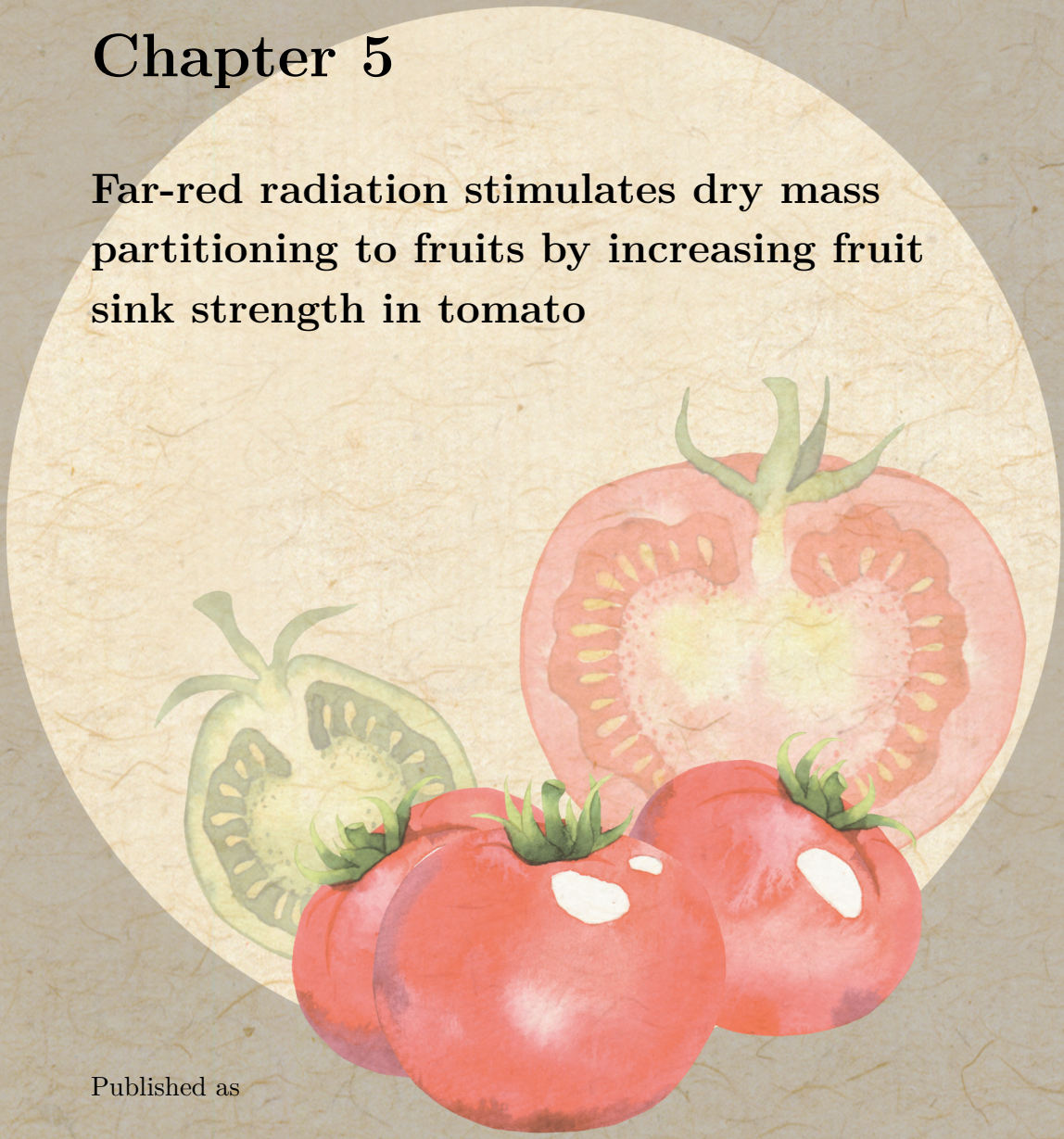


Figure. S4.2. Time course of solar daily light integral (DLI) of photosynthetically active radiation and its fraction of total DLI of photosynthetically active radiation measured at the canopy level during the experiment. The dashed line represents solar DLI reaching the canopy, calculated with solar radiation measured at the roof of greenhouse and the transmissivity and shading coefficient of the greenhouse. The solid line represents a 5-day average of fraction of solar radiation in the total radiation at the canopy level.

Chapter 5

Far-red radiation stimulates dry mass partitioning to fruits by increasing fruit sink strength in tomato



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Abstract

Far-red (FR) promotes fruit growth by increasing dry mass partitioning to fruits, but the mechanism behind this is unknown. We hypothesize that it is due to an increased fruit sink strength as FR enhances sugar transport and metabolism. Tomato plants were grown with or without 50-80 μmol m^{-2} s^{-1} of FR added to a common background 150-170 μmol m^{-2} s^{-1} red + blue LED lighting. Potential fruit growth, achieved by pruning each truss to one remaining fruit, was measured to quantify fruit sink strength. Model simulation was conducted to test whether the measured fruit sink strength quantitatively explains the FR effect on dry mass partitioning. Starch, sucrose, fructose, and glucose content were measured. Expression levels of key genes involved in sugar transport and metabolism were determined. FR increased fruit sink strength by 38%, which, in model simulation, led to an increased dry mass partitioned to fruits that quantitatively agreed very well with measured partitioning. FR increased fruit sugar concentration and upregulated expression of genes associated with both sugar transport and metabolism. This is the first study to demonstrate that FR stimulates dry mass partitioning to fruits mainly by increasing fruit sink strength via simultaneous up-regulation of sugar transport and metabolism.

Key words: dry mass partitioning, far red, LED lighting, sink strength, *Solanum lycopersicum* (tomato), sugar metabolism, sugar transport.

5.1 Introduction

Recent development of light emitting diodes (LEDs) stimulated research on light quality to achieve higher crop productivity with more health benefit but less energy consumption (Pattison et al., 2018). Far-red (FR) radiation promotes plant growth and development (Li and Kubota, 2009; Park and Runkle, 2017; Zhen and Bugbee, 2020). In tomato, which is a crop of both economical and scientific importance, FR significantly increased fraction of dry mass partitioned to fruits and this increase was shown to be the main explanation of the yield increase under additional FR (Chapter 4, this thesis).

Fraction of dry mass partitioned to fruits is strongly influenced by the vegetative sink strength and the fruit sink strength, the latter being the product of the sink strength of individual fruits and total fruit number (Heuvelink, 1997). Sink strength, quantified as the growth rate of an organ under non-limiting assimilate supply (potential growth), is the intrinsic capacity of an organ to attract assimilates (Marcelis, 1996). Unlike fruit sink strength, the vegetative sink strength cannot be easily measured experimentally as leaves may get deformed when exposed to prolonged low sink-source ratio (Heuvelink, 1996; Ho et al., 1982; Nederhoff et al., 1992). Sink strength of a fruit is directly linked to its capability to 1) unload assimilates into the fruit and 2) optimize the utilization and metabolism of imported assimilates (Osorio et al., 2014). Photosynthetic assimilates (sucrose) unloading from the phloem to the sink is usually symplasmically, facilitated by sugar transporters (Carpaneto et al., 2005; Chen et al., 2012). For example, sugar transporter activities positively correlate with sugar accumulation in *Arabidopsis thaliana* (Gottwald et al., 2000), pea (Lu et al., 2020), tobacco (Bürkle et al., 1998) and maize (Slewinski et al., 2009). Upon transportation into sinks, sucrose can be degraded into glucose, fructose, or other derivatives (Ruan, 2014). An enhanced hydrolysis of sucrose in sink organs may increase yield (Baroja-Fernández et al., 2009) by increasing the gradient of sucrose concentration from source to sink (Fridman et al., 2004; Koch, 2004; Tortora et al., 2009) and enhancing cell growth and sugar accumulation (Jin et al., 2009). In tomato, the inclusion of the more-efficient wild allele of *Lycopersicum Invertase 5* (*LIN5*) increases soluble solids in fruits (Fridman et al., 2004; Gur and Zamir, 2004; Zanor et al., 2009). Silencing *INVINH1*, which

encodes a putative inhibitor of *LIN5*, increases seed weight and fruit hexose level (Jin et al., 2009). Starch is also an important part of sugar metabolism. APGase, for example, is a key regulatory enzyme of starch biosynthesis its activity positively correlates with fruit sugar content in tomato (Petreikov et al., 2006).

To our knowledge, there is no report on the effect of FR on the sink strength of fruits. However, there are some results hinting towards a positive FR effect on fruit sink strength. For example, FR increases the size and dry mass of individual fruits in tomato (Chapter 4, this thesis, Fanwoua et al. (2019); Kalaitzoglou et al. (2019)). Furthermore, key genes (*e.g. LIN, AGPaseL1, AGPaseS1, STS1, STS2, SBE1*) involved in regulating fruit sugar transport and metabolism are associated with phytochromes, which are photoreceptors sensing FR (Ernesto Bianchetti et al., 2018; Fridman and Zamir, 2003; Kocal et al., 2008). Collectively, these results warrant further study into the application of FR to lift sink strength of fruits, as was recently suggested to have great potential in yield increase (Fernie et al., 2020).

In this research, we aim to identify the mechanism by which FR affects dry mass partitioning to fruits. We hypothesize that FR affects partitioning by increasing sink strength of individual fruits. Furthermore, we hypothesize that FR stimulates sugar transport into the fruits as well as sugar metabolism in the fruit resulting in a higher sink strength. Tomato plants were grown in a glasshouse with or without additional FR. Potential fruit growth, achieved by pruning each truss to one remaining fruit, was measured to quantify fruit sink strength. We used model simulation and concluded that the measured FR-enhanced fruit sink strength could quantitatively explain the FR-enhanced dry mass partitioning to fruits. In a climate chamber experiment, we observed that FR increased fruit sugar concentration and upregulated expression of genes associated with both sugar transport and metabolism. We conclude that FR stimulates dry mass partitioning to fruits mainly by increasing fruit sink strength via simultaneous up-regulation of sugar transport and metabolism.

5.2 Material and methods

Plant material and growth conditions

To determine the effect of FR on fruit sink strength an experiment was conducted from in a glasshouse of Wageningen University (52° N, 6° E, Wageningen, the Netherlands). Tomato (*Solanum lycopersicum* L. cv. Moneymaker) seeds were sown in potting soil and seedlings were transplanted two weeks later into Rockwool blocks. After four weeks, uniform young plants with 11 visible leaves were transplanted into another glasshouse and grown with the high wire system. This glasshouse was divided into eight compartments (5.0m x 2.5m) separated by white plastic film. Two double gutters were placed in each compartment, 12 plants (planting density 3.4 plants m^{-2}) were placed on each double gutter, including two border plants placed at each end of a gutter. The day/night temperature was maintained at 19.2 ± 0.5/17.1 ± 0.5 °C. Daily average CO_2 partial pressure and relative humidity were 680 ± 80 μbar and 79 ± 5%, respectively. Plants were irrigated with nutrient solution (electrical conductivity 2.1 dS m^{-1}, pH 5.5) containing 1.2 mM NH_4^+, 7.2 mM K^+, 4.0 mM Ca^{2+}, 1.8 mM Mg^{2+}, 12.4 mM NO^{3-}, 3.3 mM SO_4^{2-}, 1.0 mM PO_4^{2-}, 35 μM Fe^{3+}, 8.0 μM Mn^{2+}, 5.0 μM Zn^{2+}, 20 μM B, 0.5 μM Cu^{2+}, 0.5 μM MoO_4^{2-}. The EC and pH level of the nutrient solution in the drainage were monitored twice a week. In addition to the use of bumblebees, manual pollination with a Vibri Vario electronic bee (Royal Brinkman, 's Gravenzande, the Netherlands) was applied three times per week. For all plants, side shoots were pruned when visible. In the second half of the experiment, four to six old leaves per plant were removed for four times. The dry mass of the removed parts was recorded and included in the calculation of total dry mass and dry mass partitioning.

A climate chamber experiment was conducted to determine the sugar concentration and expression level of key genes in sugar transport and metabolism. Two climate chambers were each divided into four compartments, separated by white plastic film and six uniform plants were placed in each compartment. The day/night temperature was maintained at 19.9 ± 0.6/18.4 ± 0.4°C. Daily average CO_2 partial pressure and relative humidity were 488 ± 31 μbar and 75 ± 6%, respectively. Same irrigation and plant management practices were applied as for the glasshouse experiment except that only manual pollination was applied in

the climate chamber experiment.

Experimental treatments

There were two light treatments: red + blue (RB, R:B 95:5) without far red (FR) and RB + 80 μmol m^{-2} s^{-1} far red (RB+FR) (Figure 5.1, Table 5.1). The spectral distribution and photon flux density (PFD) of the supplementary light was measured with a spectroradiometer (USB 2000 + UV-VIS, Ocean Optics, Duiven, the Netherlands), on eight evenly distributed locations in each plot at the top of the canopy. Phytochrome photostationary state (PSS) in each treatment was calculated based on the measured spectra as the ratio of Pfr to the total of Pfr and Pr according to Sager et al. (1988). Photoperiod was 16 hours. Hence, in the glasshouse experiment, the photoperiod was longer than the natural photoperiod, *i.e.*, LED lamps were switched on before sunrise and switched off after sunset. On average, solar daily photosynthetic photon flux density (PPFD, 400-700 nm) contributed ∼18% to the total daily PPFD integral at canopy level in the glasshouse experiment (Figure S5.1). All supplementary lighting was provided by overhead LED modules (RB: Greenpower TL-DR/B-150, FR: Greenpower PM-FR-150, Signify, Eindhoven, the Netherlands). The blue, red, and far-red spectra peaked at 453 nm, 666 nm, and 735 nm, respectively. Height of the LED frames was adjusted weekly to maintain the desired PPFD at the top of the canopy (∼170 μmol m^{-2} s^{-1} in the glasshouse experiment and ∼150 μmol m^{-2} s^{-1} in the climate chamber experiment, Table 5.1). When the LED frames reached the maximum height, top of the plants were lowered weekly (high wire cultivation system). A spectroradiometer was used to ensure that both PPFD and PSS values were maintained at the desired level every time the LED frame or the plant was adjusted.

In the glasshouse experiment, within each light treatment, plants were either unpruned, or pruned to one, two, or five remaining fruits per truss (four plants per treatment in each of the four repetitions). Plants without fruit pruning were used to test the overall effect of FR on partitioning to fruits while those pruned to five fruits per truss were used to test whether partitioning was affected by FR, independent of potential effects of FR on fruit number. Plants with two fruits per truss were included to test whether one fruit per truss reflected potential growth. If there was no significant difference between fruit growth when fruit load was doubled

Table 5.1: *Photosynthetic photon flux density (PPFD), photon flux density (PFD) of far red, red: far red ratio and phytochrome photostationary state (PSS) value of the LED supplementary light measured at the top of canopy.*

Experiment	Light treatment	PPFD[1] (μmol m^{-2} s^{-1})	Far red (μmol m^{-2} s^{-1})	R:FR	PSS
Glasshouse	RB	167 ± 5[2]	4 ± 0.5	42 ± 5.1	0.87
	RB+FR	170 ± 4	80 ± 4.2	2 ± 0.1	0.77
Climate chamber	RB	152 ± 1	2 ± 0.2	46 ± 5.2	0.88
	RB+FR	150 ± 3	51 ± 1.8	2 ± 0.1	0.78

[1]For the calculation of ratios, PFD was integrated over 100 nm intervals for red(600–700 nm) and far red (700–800 nm).
[2]All values are means ± standard error of means (s.e.m.). s.e.m of PSS was very small (<0.001) and therefore not shown.

(one fruit per truss vs. two fruits per truss), then it can be assumed that observed fruit growth was potential fruit growth. The proximal fruit on each truss was removed at anthesis for all plants except those that received no fruit pruning. In the climate chamber experiment, there were five to six experimental plants in each of the four repetitions and all trusses were pruned at anthesis to one remaining fruit per truss to achieve potential fruit growth.

Measurement of growth parameters

Leaf number (width > 1 cm) was determined weekly and number of flower buds, flowers (fully open flower) and fruits (visible set fruit with a diameter > 5 mm) on the second, third and fourth trusses were recorded three times a week for the unpruned plants and those with five fruits per truss. Fruit ripening was monitored three times per week with a hand-held pigment analyser (PA1101, CP, Potsdam-Golm, Germany). Two readings on the equatorial region of each tomato fruit were taken to estimate the normalized difference vegetation index (NDVI) and the normalized anthocyanin index (NAI). When a fruit reached a NDVI value lower than -0.65 and a NAI value higher than 0.4, it was harvested as fully ripe fruit (Farneti et al., 2013) and the date was recorded. At the end of the experiment, two plants with five fruits per truss and two plants with no pruning were destructively measured from each of the eight main plots to determine dry mass of fruits, stem, and leaves (ventilated oven for 24 hours at 70 °C and then 36 hours at 105 °C). For plants with two or five fruits per truss, two ripe fruits from 2nd,

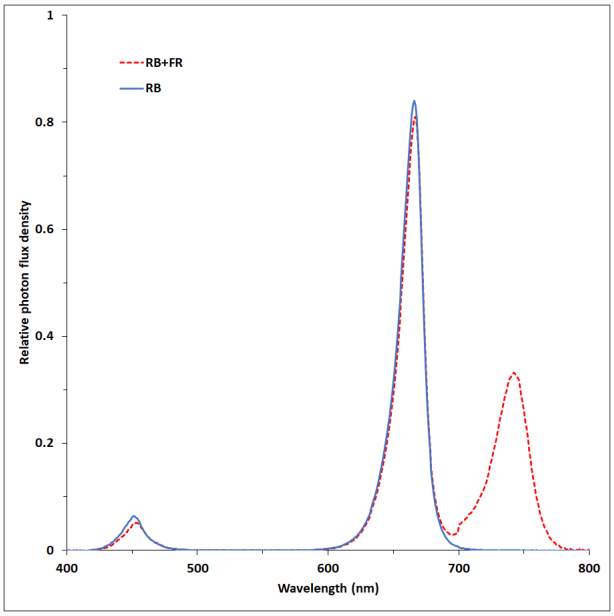

Figure 5.1: *Spectral composition of red + blue (RB) and RB + far-red (FR) light treatment provided by the LEDs measured at the top of the canopy.*

3rd and 4th truss were randomly selected per plot for counting of number of seeds per fruit as seed number may also influence fruit growth. The dry mass of these fruits was also measured and added to the calculation of dry mass partitioning.

Determination of potential fruit growth

Potential growth rate (g day^{-1}) was calculated from non-destructive measurement of height (h) and diameter (d) of the remaining proximal fruits on the 2nd, 3rd and 4th truss of the plants with one or two fruits per truss (the first fruit was removed at anthesis). Measurements were performed twice per week and the volume (v) of a tomato fruit was calculated with the following formula assuming the shape to be a spheroid (Li et al., 2015).

$$v = \frac{4}{3} \cdot \pi \cdot (\frac{d}{2})^2 \cdot \frac{h}{2} \tag{5.1}$$

Forty fruits varying in size were randomly collected from each light treatment to establish a light-specific linear regression between fruit volume and fruit fresh mass (R^2=0.99 for both RB and RB+FR). In accordance with (Wubs et al., 2012) a fourth-order polynomial function was established between fruit age and fruit dry matter content under each light treatment (R^2=0.78 for RB and R^2=0.90 for RB+FR) by randomly sampling of two fruits every 3-4 days. These samples were collected from a separate set of plants grown alongside the experimental plants. With this function, fruit fresh mass was converted to fruit dry mass. The dry mass of each individual fruit was fitted as a function of fruit age (DAA) by a Gompertz function:

$$W(t) = W_{max} \cdot e^{-e^{-k(t-t_m)}} \tag{5.2}$$

where W(t) is the dry mass (g) of a fruit at age t (days after anthesis, DAA), W_{max} is the upper asymptote of fruit dry mass (g), k is the growth-rate coefficient, and t_m is the fruit age (DAA) at maximum growth rate. The derivative of this Gompertz function gives the fruit growth rate (FGR, g day^{-1}) as a function of fruit age (t, DAA).

$$FGR(t) = W(t) \cdot k \cdot e^{-k(t-t_m)} \tag{5.3}$$

Fitting of the growth curve was done for each individual fruit using a non-linear mixed model, which considered that the measurements on one fruit are grouped while assuming lower variation between measurements of one fruit than between different fruits (Li et al., 2015).

Model simulation of sink strength and dry mass partitioning

A crop growth model was used to determine whether an observed effect of FR on fruit sink strength could quantitatively explain observed effects on partitioning. First, the growth model was used to simulate the daily dry mass production of a whole tomato plant during the experimental period. Following the LINTUL approach, which describes dry mass accumulation as a function of light interception and light use efficiency (Haverkort et al., 2015), daily plant dry mass (GR_{plant}, g day^{-1}) production was calculated

as:

$$GR_{plant} = 0.33 \cdot (1 - e^{-0.004 \cdot CO_2}) \cdot DPAR_{tot} \cdot (1 - e^{-0.7 \cdot LAI}) \quad (5.4)$$

where CO_2 is the daily average CO_2 partial pressure (μbar) during photoperiod, $DPAR_{tot}$ is total daily PAR integral on top of the canopy (mol m^{-2} day^{-1}) and LAI is leaf area index. LAI was assumed to increase linearly from 0.7 to 3.4 in 30 days after transplanting (DAP) and was maintained at 3.4. The values of 0.7 and 3.4 used here were based on the LAI measured at transplanting and the start of leaf pruning. 0.33 was a constant calculated from the calibration process to fit the reference treatment (RB light, 5 fruits/truss).

The daily fraction of dry mass partitioned to fruits (F_{fruits}) was calculated as:

$$F_{fruit} = \frac{SS_{fruit}}{SS_{fruit} + SS_{veg}} \quad (5.5)$$

where SS_{fruit} is fruit sink strength (g day^{-1}) and SS_{veg} is vegetative sink strength (g day^{-1}). SS_{fruit} was calculated as the sum of measured potential growth rate of every fruit present at a given day. As suggested by previous studies, a constant SS_{veg} can be assumed at constant daily average temperature for the simulation of F_{fruits} (Heuvelink, 1996; Kano and van Gavel, 1988). In this simulation, SS_{veg} was calibrated to be constant at a value of 3.4 g day^{-1} to fit the observed final fruit dry mass fraction in the reference treatment (RB light, 5 fruits/truss). According to previous study, a SS_{veg} of 3.4 g day^{-1} was within the reasonable range for tomato plants (Heuvelink, 1996). A sensitivity analysis was conducted to test the effect of changes in SS_{fruit} and SS_{veg} on F_{fruits}. Values of SS_{fruit} and SS_{veg} calibrated for the treatment with RB light pruned to 5 fruits/truss was used as reference. Then we simulated the F_{fruits} when the SS_{fruit} was set to -50%, -30%, -10%, +10%, +30% and +50% of the reference value while maintaining the SS_{veg} reference value. Finally, we simulated F_{fruits} with the same changes made to SS_{veg} while maintaining SS_{fruit} at the reference value.

For simulation of plants where no fruits were pruned, SS_{fruit} was increased

by 1.5 times because the fruit number on plants with no pruning was on average 7.3-7.5 fruits per truss, which was 1.5 times that of plants pruned to five fruits per truss. The product of GR_{plant} and F_{fruits} gave the daily fruit dry mass production, which was then accumulated over the whole growth period to calculate total fruit dry mass. The ratio between the accumulated fruit dry mass and accumulated plant dry mass gave the simulated fraction of dry mass partitioned to fruits and was compared to the final fraction of dry mass partitioned to the fruits in plants with five fruits per truss or no pruning, grown under RB and RB+FR. With this approach, we ensured that the simulated fraction of dry mass partitioned to fruits at the end of the growth period was only a result of the differences in SS_{fruit}. If this would result in differences between the simulated and observed F_{fruits} under a given treatment, these differences would then suggest that SS_{veg} was also affected.

Carbohydrate analysis

In the climate chamber experiment, flowering time of each fruit was registered. One flower/fruit per plant (5-6 plants per treatment repetition) was harvested at the stages of 0 (fully open flower), 10, 20, 30, 40, 50, 60 DAA. Additionally, two fully ripen fruits, determined as described before, were harvested from each plant. At harvest, fruits were detached from the plant and were quickly sliced into two halves. One half was immediately frozen in liquid nitrogen for later use. The other half was weighed for fresh weight before being transferred to a ventilated oven for drying (24 hours at 70 °C and then 36 hours at 105 °C), after which the dry weight was measured.

Frozen tissue of each individual sample was grinded mechanically into fine powder with liquid nitrogen. Then, equal weights of powdered tissues harvested at the same developmental stage from six replicate plants of the same compartment were pooled into one sample and mixed well. Glucose, fructose, sucrose and starch concentrations were measured as described by Plantenga et al. (2019) with an adaptation that 300 mg ground frozen fresh material from each pooled sample was weighed and mixed with 5 ml of 85% ethanol in a shaking water bath for 20 min at 80 °C. After centrifugation at 8500 rcf for 5 min, 1 ml of the supernatant containing soluble sugars was vacuum dried using Savant SpeedVac (SPD2010, Thermo Fisher Scientific, Waltham, MA, USA) and then dissolved in 1 ml

Milli-Q water and diluted 50x for the analysis of soluble sugars. Sucrose, fructose and glucose quantification was conducted using a high-performance ion chromatograph (ICS-5000, Thermo Fisher Scientific) with an anion CarboPac 2x250 mm exchange column (PA1, Thermo Fisher Scientific) at 25 °C with 100nM NaOH as eluent at the flowrate of 0.25 ml min^{-1}. Pulsed amperometry was used for the detection and Chromeleon (Thermo Fisher Scientific) was used for the analysis of the chromatograms and the quantification of sugar concentrations. The remaining pellet after sugar extraction was used for starch determination. After discarding the supernatant that contains soluble sugars, the remaining pellet was washed three times with 80% ethanol, each time followed by 5 min centrifugation and removal of the supernatant. The remaining pellet was dried for 20 min in SpeedVac and resuspended in 2 ml 1 mg ml^{-1} thermostable α-amylase solution (SERVA Electrophoresis, Heidelberg, Germany) and incubated for 30 min at 90 °C. Then, 1 ml of 0.5 mg ml^{-1} amyloglucosidase (10115, Sigma-Aldrich, St. Louis, MO, USA) in 50 mM citrate buffer (pH 4.6) was added and incubated for 15 min at 60 °C. By now, the starch in the sample was converted into glucose. After centrifugation for 5 min at 8500 rcf, 1 ml of the supernatant was diluted 50x and was used for the quantification of glucose content as described above.

Gene expression analysis

Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to determine expression levels of target genes involved in sugar transport (*SUTs, HTs, SWEET*s), sucrose synthase (*SUS*), invertase (*LINs, INVINH*), starch synthesis (*STSs, SBE, AgpL1, AgpS1*) and starch catabolism (*pGlcT1, pGlcT3*). Further information and primer sequences of the target genes are provided in Table S5.1. Fine powders of the pooled samples of the fresh frozen fruit material was used for RNA isolation using the CTAB method (Schultz et al., 1994). RNA quality was examined with 2% agarose gel. Furthermore, quality and concentration of the isolated RNA was tested with a spectrophotometer (DS-11, DeNovix Wilmington, DE, USA). 200 ng of RNA was treated with RQ1 RNAse-free DNAse kit (Promega, Madison, WI, USA) and synthesized to cDNA with the MultiScribe kit (Applied Biosystems, Foster City, CA, USA). The DNAse treatment and cDNA synthesis were conducted according to manufacturers' manuals. The synthesized cDNA was diluted 5 times

before use. The expression of target genes was analyzed in a 10 μl system of 5 μl SYBR-green master mix (Bio-rad, Hercules, CA, USA), 0.5 μl forward primer (10μM), 0.5 μl reverse primer (10 μM), 3 μl Milli-Q water and 1 μl cDNA with a thermal cycler (CFX96, Bio-rad) set at 95 °C for 2 min followed by 40 cycles of 95°C for 5 s, 60 °C for 20s, 72 °C for 15s, and for a melt curve from 55 °C to 95 °C in a 0.5 °C steps every 5 s. Absolute fluorescence data was analyzed using LinRegPCR (Ruijter et al., 2009) and was normalized against two references genes (*ACTIN* and *EFα1*).

Experimental set-up and statistical analysis

For the glasshouse experiment, each light treatment was repeated four times while the four pruning treatments were applied within the light treatments. Hence, the experimental set-up was a split-plot design with light as the main factor and pruning as sub-factor. The climate chamber experiment was a randomized complete block design with each light treatment repeated four times. Growth parameters were analyzed with Genstat (18th Edition, VSN International, London, UK). The assumptions of homogeneity and normality of the residuals were tested with Bartlett's test and Shapiro-Wilk test, respectively. Data satisfied the assumptions and were further analyzed with Analysis of Variances (ANOVA). Parameters of the Gompertz growth function of each individual fruit were estimated with package "Saemix" in R (R Core Team, 2013) and were subsequently analyzed for differences between treatments with ANOVA in Genstat. Statistical differences between light treatments in sugar content and relative expression levels of target genes were tested at each sampling stage with Student' s t-test in Genstat. All statistical tests were conducted at a probability level of α=0.05.

5.3 Results

Far red promotes dry mass partitioning to fruits

To study the effect of FR on fruit number and dry mass partitioning to fruits, plants were unpruned or pruned to five remaining fruits per truss. The plants without fruit pruning were used to test the overall effect of FR on partitioning to fruits while those pruned to five fruits per truss were used

to test whether partitioning was affected by FR, independent of potential effects of FR on fruit number. FR significantly increased fraction of dry mass partitioned to fruits and stems at the expense of that partitioned to leaves (Figure 5.22). Also, FR increased dry mass of individual ripe fruits (Table 5.2). Pruning the trusses to five fruits per truss reduced the fraction of dry mass partitioned to fruits. Unpruned plants produced 42-43% more fruits and 20-30% more total fruit dry mass per plant while total plant dry mass was not affected. FR significantly accelerated fruit ripening but had no effect on flowering time (Table 5.2). Total seed number per fruit, which may also influence fruit development, was not affected by either light or pruning treatment. Leaf appearance rate was not affected either (Figure S5.2).

Far red increases sink strength of individual fruits

In order to quantify fruit sink strength, we pruned the tomato plants to one and two remaining fruits per truss to achieve a potential fruit growth condition in which fruit growth was not limited by the assimilate supply from the source leaves. Comparison between plants with one and two fruits per truss showed no difference in fruit growth, which means fruit growth was not affected even when fruit load was doubled (one fruit per truss vs two fruits per truss). Hence, we showed that fruit growth with one fruit per truss was potential fruit growth (Figure S5.3). In the glasshouse experiment, FR radiation significantly promoted potential fruit growth (Figure 5.3a). Potential fruit growth rate was higher under RB + FR throughout the whole growth period and reached a higher maximal growth rate at an earlier fruit age (Figure 5.3b; Table S5.2). Similar FR radiation effect was also observed in the climate chamber experiment (Figure S5.4).

Increased sink strength of individual fruits explains the increase in fraction of dry mass partitioned to fruits

Fruit dry mass fraction was simulated with a model assuming proportionality between fruit dry mass fraction and the ratio between fruit sink strength and the sum of fruit and vegetative strength (equation 5.5). Using the measured data on flowering time and the potential growth rate of individual fruits, we calculated the total sink strength of all fruits together for plants with five fruits per truss grown with or without

Table 5.2: *Effects of adding far red (FR) to red + blue (RB) light and fruit pruning on total fruit number, individual ripe fruits dry mass, total fruits (ripe and unripe fruits) and total plant dry mass, seed number of ripe fruits, flowering time of the first truss (days after transplanting, DAP), fruit ripening time (days after anthesis, DAA) in tomato (Solanum lycopersicum).*

Light	Fruit pruning	Total fruit number[1] (plant^{-1})	Individual ripe fruits dry mass (g fruit^{-1})	Total fruit dry mass (g plant^{-1})	Total plant dry mass (g plant^{-1})	Seed number (fruit^{-1})	Flowering time (DAP)	Fruit ripening time (DAA)
RB	5 Fruits/Truss	62	2.7	92	277	154	22.2	71.6
	No Pruning[2]	88	2.2	123	302	155	22.6	71.7
RB+FR	5 Fruits/Truss	65	3.6	117	289	157	21.4	67.4
	No Pruning	93	2.9	140	300	151	21.5	67.0
s.e.m (n=4)		2.9	0.07	3.6	5.7	7.7	0.5	2.1
Light effect[3]		n.s.	**	**	n.s.	n.s.	n.s.	*
Pruning effect		*	**	*	n.s.	n.s.	n.s.	n.s.
Light x Pruning		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1]The average number of fruits per truss ranged from 7.32-7.47 in the treatments without pruning, while the number of trusses per plant ranged from 12.0-12.4 for plants without pruning and 12.4-13.0 for those with 5 fruits/truss.
[2]Total fruit number, dry mass of individual ripe fruits, total fruits (ripe and unripe fruits) and total plant dry mass measured 90 days after transplanting.
[3]Asterisk denotes statistical significance tested with ANOVA (* $P<0.05$, ** $P<0.01$, n.s. not significant).

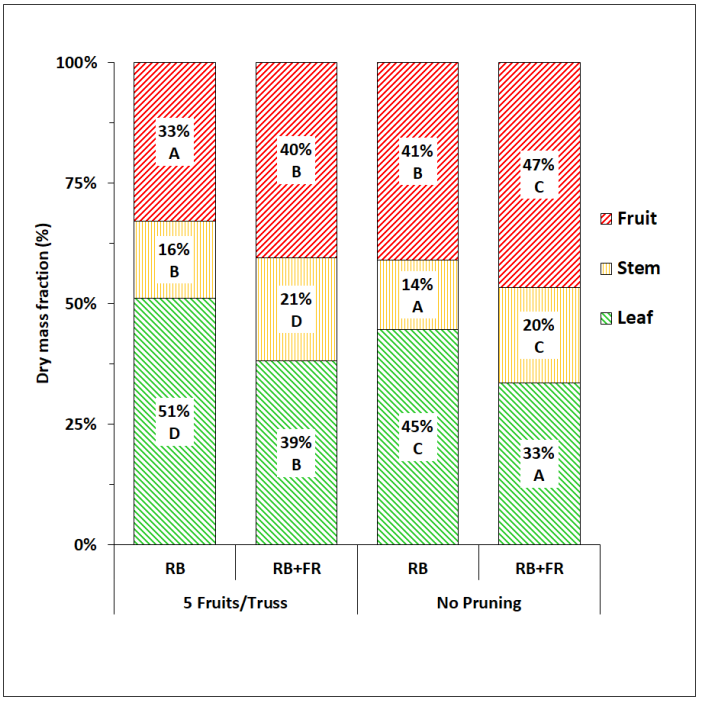

Figure 5.2: *Effects of adding far-red (FR) to red + blue (RB) light on the fraction of dry mass partitioned to fruits, stem and leaves in tomato (Solanum lycopersicum) plants where fruits were pruned to five fruits per truss or not pruned (7–8 fruits per truss). Data were based on cumulative dry mass of plants 90 d after transplanting. Different letters denote significant differences between treatments according to Fisher' s protected least significant difference (LSD) test conducted independently for fruit, stem, and leaf ($n = 4$, $\alpha = 0.05$).*

additional FR. The total fruit sink strength was increased by ∼38% by FR radiation and started to increase rapidly after flowering of the 1st truss (∼10 DAP) (Figure 5.4a). The simulated fruit dry mass fraction was increased by ∼21% with additional FR radiation in both pruning scenarios (Figure 5.4b). Simulation results agreed very well with the measured fraction of dry mass partitioned to fruits (Figure 5.2). Using the scenario of plants with five fruits per truss grown with RB as reference, sensitivity analysis of the simulated dry mass partitioning to fruits showed that changes in both vegetative and fruit sink strength influenced dry mass partitioning to fruits (Table 5.3).

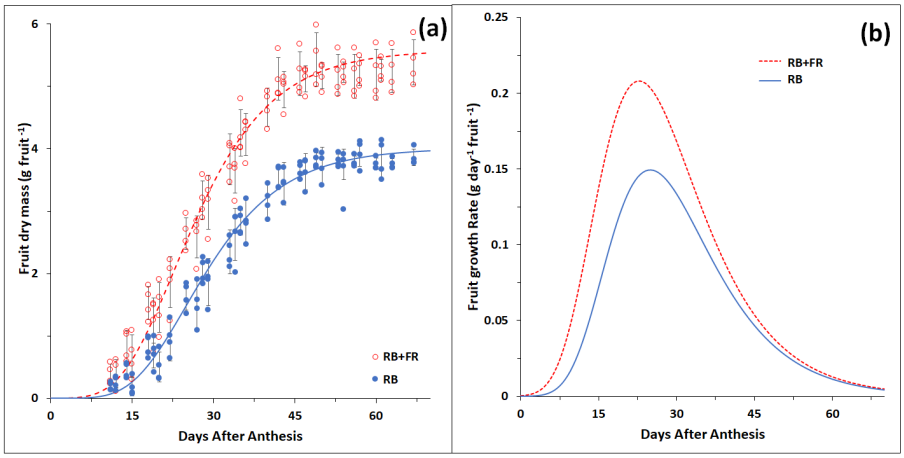

Figure 5.3: *Effects of adding far-red (FR) radiation to red + blue (RB) light on potential fruit growth (a) and potential fruit growth rate (b) in tomato (Solanum lycopersicum). Curves represent Gompertz function (a) and its derivative (b) fitted for RB + FR (dashed lines) and RB (solid lines) light conditions. Symbols represent measured fruit dry mass for RB + FR (open symbols) and RB (closed symbols) and error bar represents standard error of means (n = 4).*

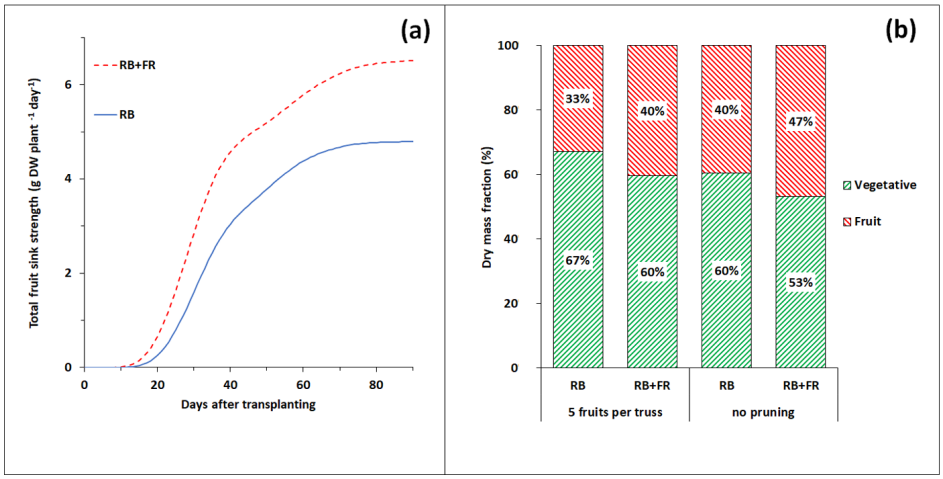

Figure 5.4: *Simulated total fruit sink strength per plant for plants with five fruits per truss (a) and dry mass fraction of fruits and vegetative parts (b) of tomato (Solanum lycopersicum) plants grown with or without additional far-red radiation. Curves represent fruit sink strength simulated for a fruit load of five fruits per truss. Both simulations were conducted for a period of 90 d after transplanting. RB, red + blue; FR, far red.*

Table 5.3: *Simulated fraction of dry mass partitioning to fruits in tomato (Solanum lycopersicum) when fruit sink strength (SS_{fruit}) or vegetative sink strength (SS_{veg}) was changed by −50, −30, −30, +10, +30 or +50%.*

Parameter	Changes in input parameter relative to the control						
	−50%	−30%	−10%	Control	+10%	+30%	+50%
SS_{fruit}	21%	26%	31%	33%	34%	38%	41%
SS_{veg}	45%	39%	35%	33%	31%	28%	26%

Far red elevates fruit sugar content and sugar metabolism

To further understand the cause of the increase in fruit sink strength, we measured the concentrations of starch, sucrose, fructose and glucose throughout the growth of the fruits. Starch concentration increased rapidly until 20 DAA and then decreased almost linearly afterwards (Figure 5.5a). Both rates of starch accumulation and break-down were significantly accelerated by FR. Sucrose concentration gradually decreased during fruit development and no significant FR effect was observed (Figure 5.5b). Both fructose and glucose concentrations increased during fruit growth and concentrations of both sugars were significantly higher in fruits grown with additional FR radiation after 10 DAA (Figure 5.5c, 5.5d). Same FR effect was observed when concentrations were expressed on dry weight basis (Figure S5.5).

Far red upregulates the expression of genes responsible for sugar transport and metabolism in tomato fruit

To further explain the elevated sugar concentration, we measured the expression of key genes involved in sugar transport and sugar metabolism at different growth stage of the fruits. At flowering stage (0 DAA), expression of genes encoding sucrose transporters, sucrose synthase and starch synthase were significantly upregulated by additional FR radiation (Figure 5.6). During early fruit growth at 10–20 DAA, FR radiation significantly upregulated genes of sucrose synthase *SUS1*, invertase *LIN7* as well as ADP-Glc pyrophosphorylase *AgpL1* and *AgpS1*. However, expression of sugar transporter *SUT2* decreased with FR. At 30 DAA, FR radiation significantly increased the expression of genes of sucrose synthases *SUS1* and *SUS3*, the invertases *LIN5* and *LIN7* and starch

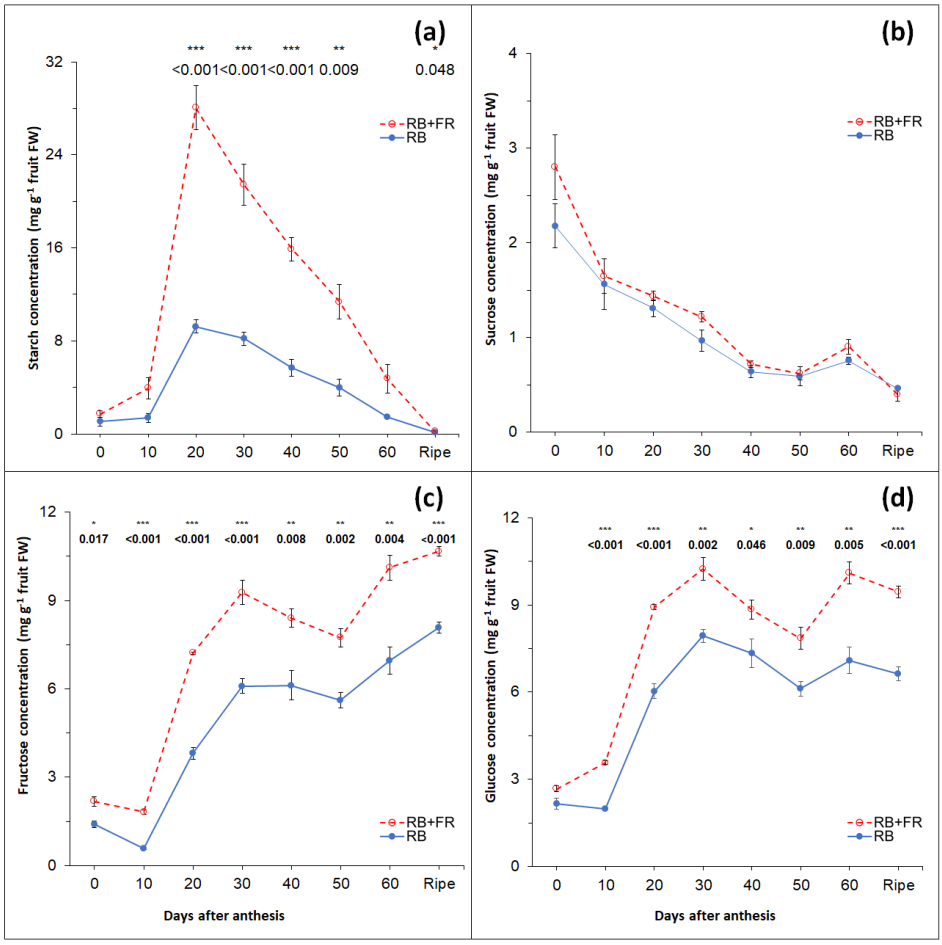

Figure 5.5: *Effects of adding far-red (FR) radiation to red + blue (RB) light on concentration of starch (a), sucrose (b), fructose (c) and glucose (d) in tomato (Solanum lycopersicum) fruits measured every 10 d after anthesis until fully ripe. Starch concentration is expressed as equivalent glucose concentration. Error bar represents standard error of means (n = 4). Asterisks denote statistically significant effects of FR radiation as tested with Student' s t-test (n = 4; *, P < 0.05; **, P < 0.01; ***, P < 0.001).*

catabolism *pGlcT1* and *pGlcT3*. Interestingly, in addition to the increased expression of invertase genes, FR radiation also significantly increased the expression of *INVINH1*, which encodes a putative invertase inhibitor. In this developmental stage, FR radiation resulted in the downregulation of sugar transporter *HT2* and invertase *LIN4*.

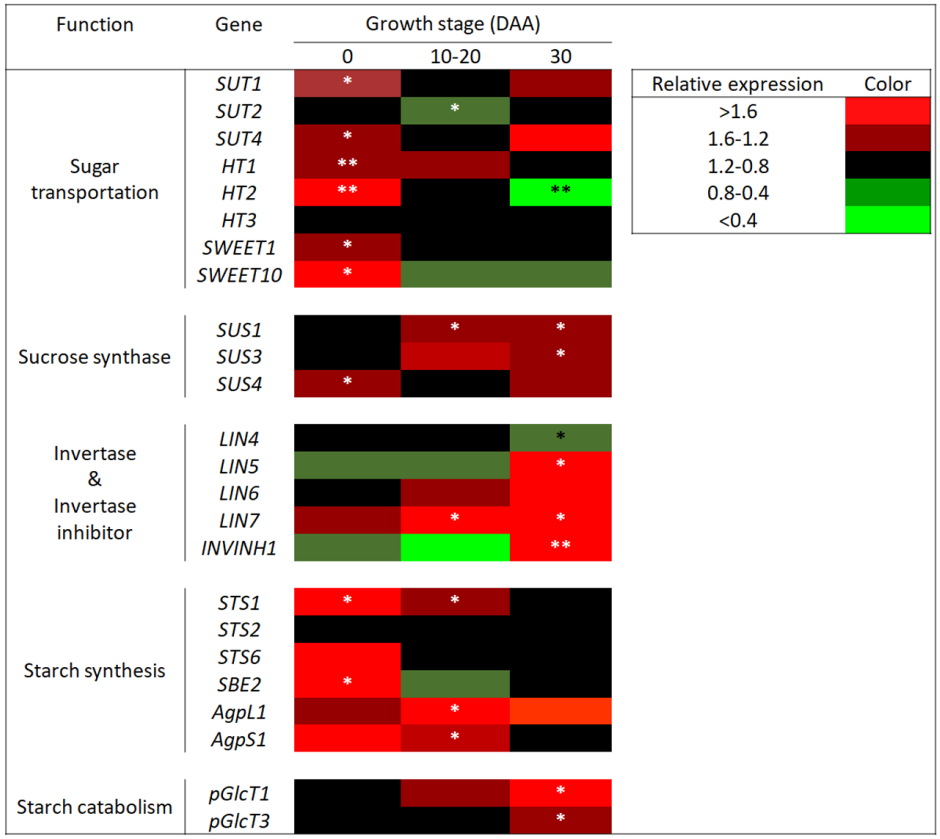

Figure 5.6: *Effects of adding far-red (FR) radiation to red + blue (RB) light on relative expression of genes related to sugar transport, sucrose synthase, invertase, starch synthesis and starch catabolism in tomato (Solanum lycopersicum) flowers/fruits at 0, 10–20 and 30 d after anthesis (DAA). Different colors represent expression levels of each gene under RB + FR relative to that under RB. Asterisks denote statistically significant effects of FR radiation as tested with Student' s t-test (n = 4; *, P < 0.05; **, P < 0.01).*

5.4 Discussion

Far red increases fruit sink strength and hence stimulates dry mass partitioning to fruits

FR radiation increased dry mass partitioning to fruits (Figure 5.2) and total fruit dry mass per plant (Table 5.2), while PPFD was kept constant

(Table 5.1). This finding agreed with recent reports on the effects of additional FR radiation on tomato growth and development (Chapter 4, this thesis, Kalaitzoglou et al. (2019); Zhang et al. (2019)). Sink strength of individual fruits and total fruit number collectively determined total fruit sink strength and consequently influenced the fraction of dry mass partitioned to fruits (Heuvelink, 1997; Marcelis, 1994). FR radiation was reported to significantly increase size or mass of individual fruits (Chapter 4, this thesis, Kim et al. (2019)). Indeed, we observed significantly higher individual fruit dry mass under additional FR radiation (Table 5.2). FR radiation significantly increased the potential growth rate and hence the sink strength of individual fruit (Figure 5.3b). Model simulation, taking into account the observed increase in fruit sink strength, resulted in an increase in simulated dry mass partitioning to fruits (Figure 5.4b). The sensitivity analysis of the model showed that both a higher fruit sink strength and a decreased vegetative sink strength can lead to increased dry mass partitioning to fruits (Table 5.3). Vegetative sink strength cannot be measured experimentally, hence it is difficult to evaluate directly whether it is affected by FR. However, in a separate experiment when 100 m^{-2} s^{-1} FR radiation was added to 150 m^{-2} s^{-1} RB LED lighting, soluble sugars in the leaves of 3-wk-old tomato plants increased from 7.9 mg g^{-1} DW to 19.1 mg g^{-1} DW. This FR-increased soluble sugar was also reported by (Courbier et al., 2020) and may suggest that FR radiation could affect vegetative sink strength. If that was true, we should have found significant differences between simulated and observed dry mass partitioning to fruits, as the simulation only took into account the observed increase in fruit sink strength while assuming a constant vegetative sink strength. However, the simulated results agreed very well with the observed result. Therefore, we reasoned that the FR radiation enhancement of fruit sink strength alone could explain the increase in fruit dry mass fraction.

Total plant dry mass, which reflected the source strength (dry matter production of the plant), was not influenced by FR radiation (Table 5.2). In a study with younger plants, an increase in source strength of the plants was observed and this increase was due to increased light interception (Kalaitzoglou et al., 2019). Here we started the light treatments when plants had already formed sufficient number of leaves and established an LAI of 3 within 4 wk. For a fruiting tomato canopy, an LAI of 3 usually means that 90% of the incident light will be intercepted

(Heuvelink and Dorais, 2005), leaving little room for FR radiation to affect total light interception (Chapter 4, this thesis). Total fruit number may also influence total fruit sink strength. FR radiation does not have a strong effect on total fruit number per plant (Chapter 4, this thesis, Kalaitzoglou et al. (2019); Kim et al. (2019)), Table 5.2). Accelerated flowering is part of typical shade avoidance responses in many species (Ballaré, 2017; Yuan et al., 2017) and may lead to a higher fruit number and consequently a larger total fruit sink size at a given time. We observed a trend of accelerated flowering of about 1–2 d caused by additional FR radiation, but this effect was not statistically significant (Table 5.2). Model simulation for plants grown under RB showed that advancing flowering time by 1 or even 2 d resulted in minor increase in the cumulative fraction of dry mass partitioned to fruits at the end of the experiment (Table S5.3). Hence, we argued that the FR effect on flowering time does not contribute significantly to the increase of dry mass partitioning to fruits in tomato.

Taken together, we concluded that the increase in fraction of assimilates partitioned into fruits under additional FR radiation is mainly due to an increase in fruit sink strength. To the best of our knowledge, this is the first study to demonstrate a strong FR effect on fruit sink strength and its contribution to dry mass partitioning.

Far red increases fruit sink strength by upregulating sugar transport and metabolism in fruit

Sink strength is closely linked to the sugar metabolism in the sink organs (Osorio et al., 2014)). Considering that the fruits in the present study were grown under non-limiting assimilate supply, we reason that the sugar content in the fruits was mainly determined by the metabolic activities in the fruit. Tomato fruit typically switches from starch accumulation to hexose-accumulation at around 15–20 d after anthesis (Ruan and Patrick, 1995). In agreement with this, starch concentration in the fruit kept increasing until 20 DAA (Figure 5.5a). Starch may be considered as an overflow product when sugar concentration (especially sucrose) increases in the sink organ (Osorio et al., 2014). The substantial increase of starch concentration and upregulation of starch synthesis genes suggests a substantially increased amount of sugars are being transported into the fruit, and this was indeed supported by the upregulation of sugar

transporters genes (Figure 5.6). FR radiation significantly increased the fructose and glucose content in tomato fruits across the whole growth period, accompanied by an enhanced break-down of starch (Figure 5.5), which was also reported by (Fanwoua et al., 2019). Indeed, after the fruit switched to hexose-accumulation, we observed that FR radiation increased the expression of genes encoding sucrose synthase and invertases in the fruits (Figure 5.6), suggesting that FR radiation induced a stronger metabolism of the imported sucrose, thus allowing more sucrose to be imported into the fruit (Fridman et al., 2004; Koch, 2004; Tortora et al., 2009). In accordance with our findings, specifically knocking-down fruit-localized phytochromes (major FR-sensing photoreceptors) also upregulated *LIN5* and *LIN6*, which encode cell-wall invertases crucial for sink activity in tomato fruits (Ernesto Bianchetti et al., 2018; Fridman and Zamir, 2003; Kocal et al., 2008). Interestingly, we also found that FR radiation increased the expression of *INVINH1*, which encodes an inhibitor of the above-mentioned invertase *Lin5* in tomato (Jin et al., 2009). Upregulation of *INVINH1* allows the tomato to regulate fruit development by capping cell-wall invertase activity (Jin et al., 2009), thus further supporting that FR radiation elevated the invertase activities in tomato fruit. The sucrose concentration was not significantly affected by FR radiation (Figure 5.5b). We observed a higher expression of sucrose transporters at flowering but not in later stages (Figure 5.6). However, considering that FR radiation enhanced sugar metabolism and increased carbohydrate content, it is reasonable to argue that the import rate of sucrose should have been increased to maintain the same sucrose concentration in the fruit. This is further supported by the finding that *HY5*, a transcription factor known to directly enhance the expression of sucrose transporters *SWEET11* and *SWEET12* (Chen et al., 2016), was shown to be activated by FR radiation (van Geest et al., 2016). Interestingly, FR radiation also accelerated fruit ripening (Table 5.2). This is in agreement with studies in which knocking out *phyB1, phyA/B1, phyB1/B2* and *phyA/B1/B2* significantly reduced fruit ripening time by accelerating transition from mature green to breaker stage and that from breaker to red ripe stage (Gupta et al., 2014). Furthermore, phytochrome-interacting factors (PIFs) have key regulatory roles in fruit ripening (Gramegna et al., 2019; Rosado et al., 2019). These results collectively suggested that phytochromes are important regulators of not only fruit growth but also fruit ripening.

5.5 Conclusions

Additional FR radiation upregulated key genes involved in fruit sugar transport and sugar metabolism, which resulted in a substantial elevation of fruit sink strength. When this FR effect on fruit sink strength was used in a model simulating dry mass partitioning, the model predicted very well the measured increase in dry matter partitioning to the fruits by FR radiation. Hence, we concluded that FR-enhanced dry mass partitioning to fruits is primarily the result of an increased fruit sink strength.

Contributions

YJ, EH and LFMM conceptualized the research plan. YJ and DC designed and conducted the glasshouse experiment and analyzed the data. YJ and DNO and DHL designed and conducted the climate chamber experiment and analyzed the data. YJ wrote the manuscript. LFMM and EH initiated the project, acquired funding and guided the experimental design, data processing and interpretation and critically reviewed and edited the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgments

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Supplementary information

Table S5.1 List of genes and corresponding forward and reverse primers used in the expression analysis

Locus	Gene	Forward primer	Reverse primer	Reference
Solyc01g109790	*AGPaseL1*	CGCTCACACAAGAGTTTCCA	CTAAGCGCGATCTTTCACCC	Bianchetti et al. 2018
Solyc07g056140	*AGPaseS1*	GCCCCAATCTACACCCAACC	CTGCCCCCATCAAAAGTGAG	Bianchetti et al. 2018
NM_001321306.1	*EFα1*	CGGCCACAGGGATTTCATCA	GGGTGGTAGCATCCATCTTGT	Expósito-Rodríguez et al. 2008
NM_001247106.2	*ACTIN*	ACTGGAATGGTGAAGGCTGG	CCCAGTTGCTGACAATACCG	Zhang et al. 2013
Solyc02g079220	*HT1 / STP1*	ATTGGAATTTCCGGGGGTGT	GGTGACAGTAGATGCCACCA	Shen, et al. 2019
Solyc09g075820	*HT2 / STP2*	CCTAGCTGGTTTAACGGCGA	GAACAGCCTGATTGGCGAAA	Shen, et al. 2019
Solyc07g006970	*HT3 / STP3*	ATTTGGCAACCAGGCAGTTC	TCATAACGAAAGCCGGTGCT	Shen, et al. 2019
Solyc03g083910	*LIN4 (TIVI)*	ACCATCCTACCCGATGGTCA	GTCCAAGCAGTAGTCGGGTC	Zhang et al. 2013
Solyc09g010080	*LIN5*	GGATGGGCTGGAATTCAAGGTA	AGCACTTCAACATCAGCCTGT	Zhang et al. 2013, Fridman & Zamir 2003, Liu et al. 2016
Solyc10g083290	*LIN6*	GCTCGAACCCGCTATCTACC	CGGGCTTGATCCACTTACGA	Zhang et al. 2013, Fridman & Zamir 2003, Liu et al. 2016
Solyc09g010090	*LIN7*	ATAATGCGAAGGGATGGGCT	CATCAGCCTGTGCTGGTGTG	Zhang et al. 2013, Fridman & Zamir 2003, Liu et al. 2016
Solyc03g112180	*INVINH1*	ACAGCAAGTATGCCAGAAGCA	ACAATGGCTCTACCAACATCAGA	Jin et al. 2009, Zhang et al. 2013, Shen et al. 2019
Solyc02g086160.2	*pGlcT1*	GCGGTCACTGGAGGAGATAG	TTCATGAAGCCGGTGTGTAA	Reuscher et al. 2014
Solyc07g020790.2	*pGlcT3*	GGTTGGATTGCTGATGGAGT	GCAGGAGAAACCTCTGCAAC	Reuscher et al. 2014
Solyc04g082400	*SBE2*	GTAAGCCAGCCATTCCACAC	TGGAAGGGCGAGGGTATTTG	Bianchetti et al. 2018
Solyc08g083320	*STS1*	TGAATGCGATGTTGTTGACCC	GCTCCTAAGCCCAATAGCAAC	Bianchetti et al. 2018
Solyc03g083090	*STS2*	CTGTTGACCTTGATGTGCGGG	GAGAAAGCAAAGTGAAGCGAAAC	Bianchetti et al. 2018
Solyc02g088000	*STS6*	ATGTGGTGGGGTCTGCTATG	AAAGGACCACGACCCTGATG	Bianchetti et al. 2018
Solyc12g040700	*SUS1*	CTTGTCGACGGAGCCTCAAA	TAATGGTGAGCGCGGAGAAA	Goren et al., 2011
Solyc07g042520	*SUS3*	TCACCACTACAATGGAAAGTCAA	TCTCTAGAACACGCTCTGCG	Goren et al., 2011
Solyc09g098590	*SUS4*	TCCTTGTTGTCACCCGACTG	CCTGCCACGTCCTCAGTAAA	Goren et al., 2011
Solyc11g017010	*SUT1*	GTACACTCATGGGCCCGAC	GACACCAACATCTGTGGTACA	Shen, et al. 2019
Solyc05g007190	*SUT2*	CTCTTCCTCCACCGCCTGTA	GGTTGTACCACAAGGCCAGT	Shen, et al. 2019
Solyc04g076960	*SUT4*	GGGATCCCACTAGCTATAACG	ACAAAGGCTGGTGAATTGCC	Shen, et al. 2019
XM_004237674.4	*SWEET1*	TGCCTTCTTTCTGCATGGTATG	AAACAAGGGCTACAGCAGCA	Shen, et al. 2019
Solyc03g097600	*SWEET10b*	CGTGGACAAGTTGTTGGATGG	TTGGAGCCGCGATGTTAATG	Shen, et al. 2019
Solyc03g097870	*SWEET11a*	CTACGCACCAAAGAAAGCCAG	TGACTTGTCTCACAATGCCT	Shen, et al. 2019
Solyc03g097590	*SWEET12a*	CAAAGCCAGGGTCCATACTATAA	CCACACTCTTTGTTTTGATGACT	Shen, et al. 2019

Table S5.2 Effects of adding far red (FR) to red + blue (RB) light on the parameters of the Gompertz fruit growth curve.

	Unit	RB+FR	RB	P-value
k		0.10	0.10	n.s.
t_m	days	22.76	24.91	P<0.01
W_{max}	g fruit^{-1}	5.59	4.01	P<0.01

P-value was calculated by ANOVA (n=4, α=0.05).

Table S5.3 Effect of earlier flowering on simulation of dry mass partitioning to fruits for a period of 90 days after transplanting.

Pruning	Simulated dry mass partitioning		
	Reference	1 day early	2 days early
5 fruits per truss	32.9%	33.4%	33.9%
No pruning	39.5%	39.6%	40.2%

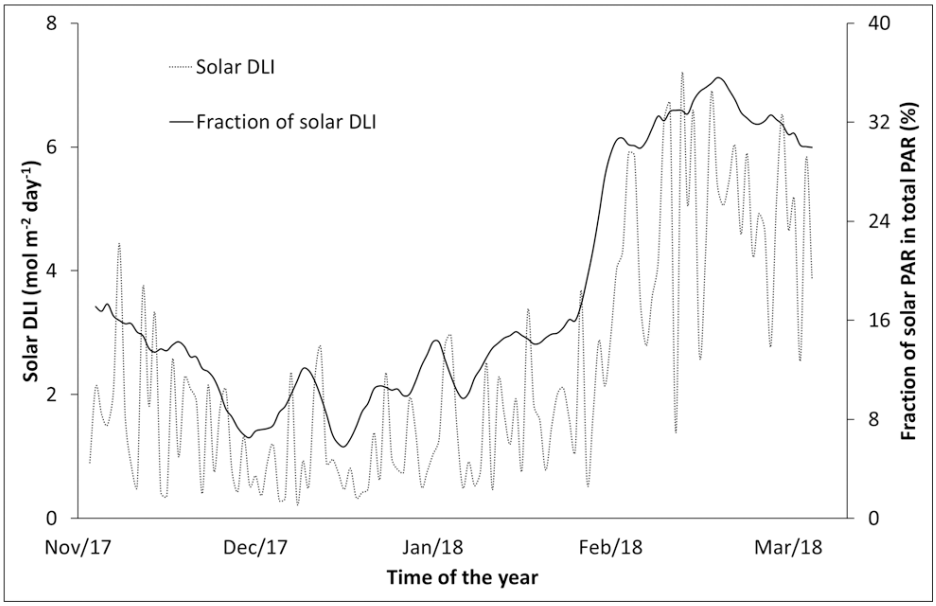


Figure.S5.1 Time course of solar daily light integral (DLI) of photosynthetically active radiation (PAR) and its fraction of total DLI of photosynthetically active radiation measured at the canopy level during the experiment. The dashed line represents solar DLI reaching the canopy, calculated with solar radiation measured at the roof of greenhouse and the transmissivity and shading coefficient of the greenhouse. The solid line represents a 5-day average of fraction of solar DLI in the total DLI of PAR at the canopy level.

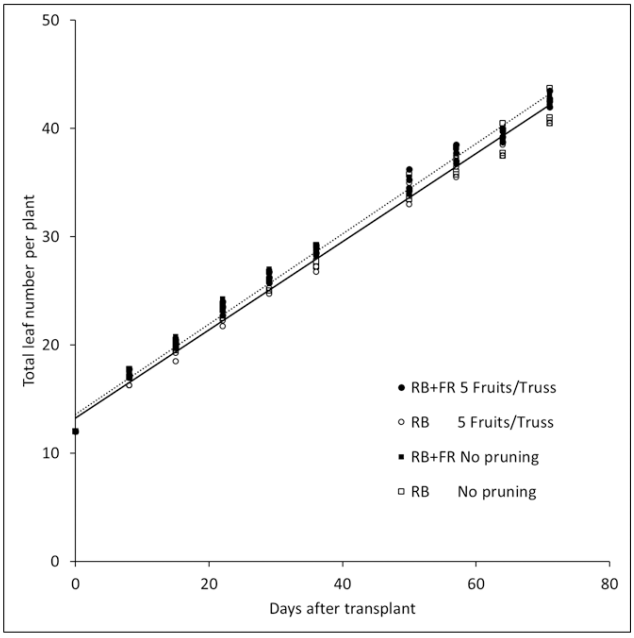


Figure.S5.2 Leaf number of plants of tomato (Solanum lycopersicum) plants grown with or without additional far red with 5 fruits/truss or without fruit pruning. Symbols represent actual measured leaf number and lines (dashed for RB+FR, solid for RB) were fitted with linear regression.

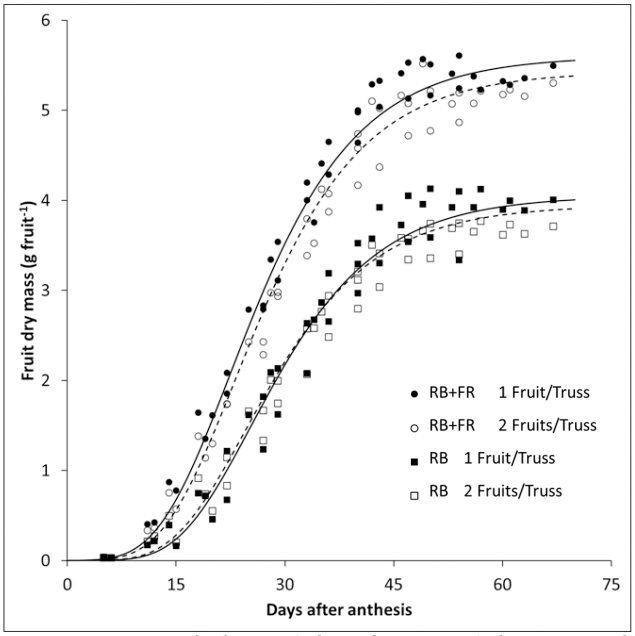


Figure.S5.3 Fruit growth of tomato (Solanum lycopersicum) plants grown with or without additional far red with one or two fruits per truss. Symbols represent actual measured fruit growth data and lines represent Gompertz growth curve fitted for the measured data points.

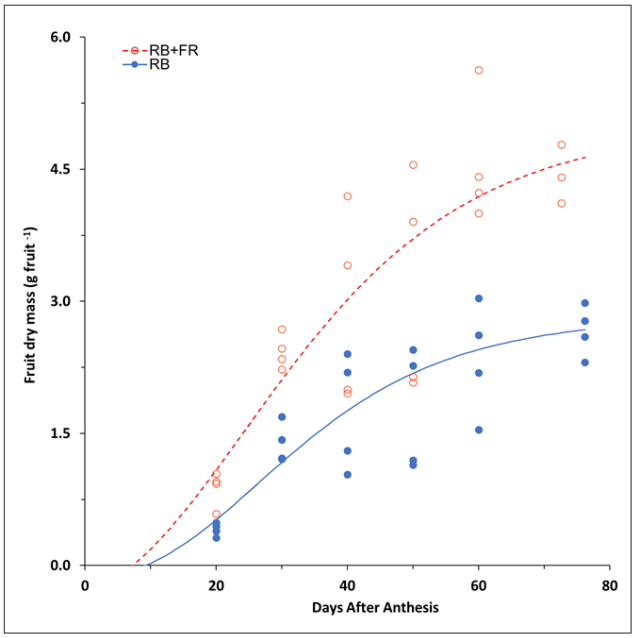


Figure.S5.4 Effects of adding far red (FR) to red + blue (RB) light on potential fruit growth in tomato (Solanum lycopersicum) in the climate chamber experiment. Curves represent Gompertz function fitted for RB+FR (dashed lines) and RB (solid lines) light conditions.

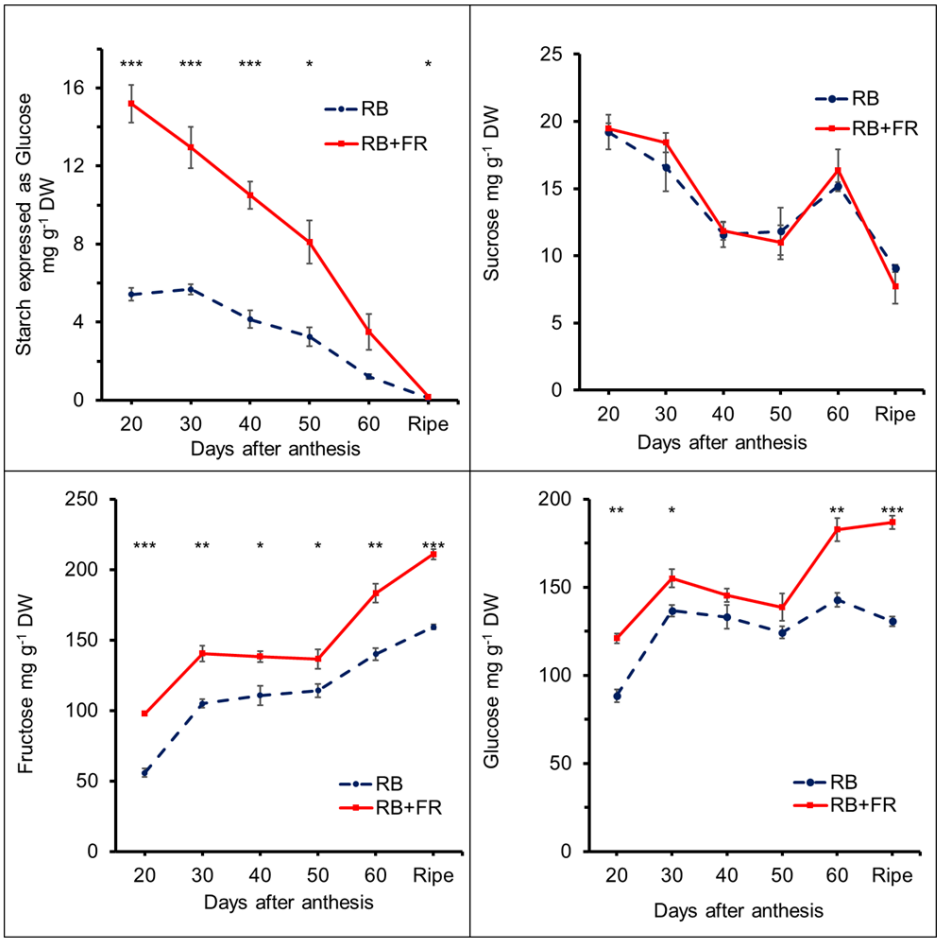
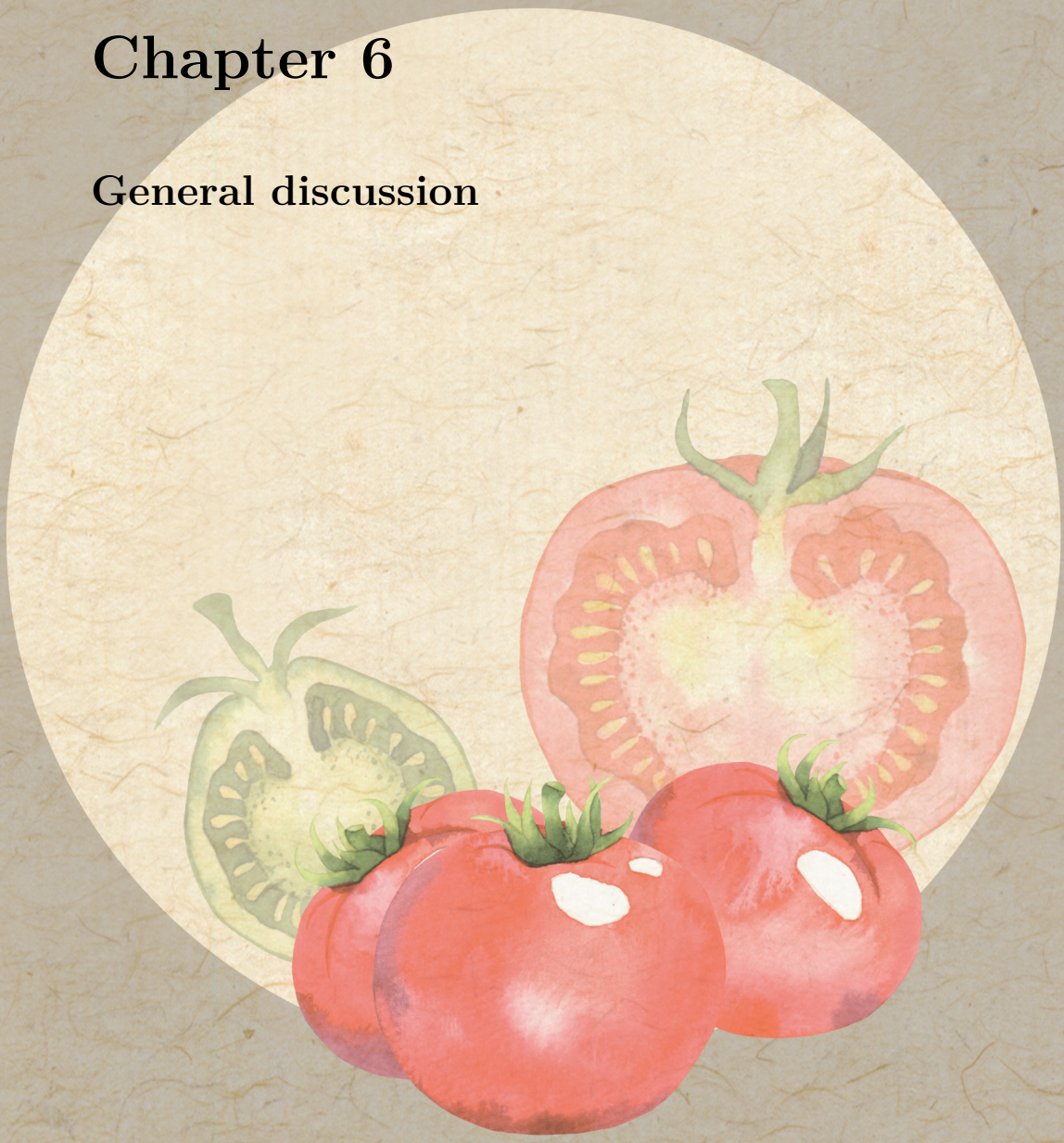


*Figure.S5.5 Effects of adding far red (FR) to red + blue (RB) light on concentration (mg g^{-1} fruit dry weight) of starch, sucrose, fructose, and glucose in tomato(Solanum lycopersicum) fruits measured every 10 days after anthesis(DAA) until fully ripe. Starch concentration is expresses as equivalent glucose concentration. Error bar represents standard error of means (n=4). Asterisks denote statistically significant effects of FR as tested with Student's t-test (n=4, * P<0.05, ** P<0.01, *** P<0.001).*

Chapter 6

General discussion



In this thesis, I aimed to understand the effect of adding FR on the responses of growth and development of both young and fruit-bearing tomato (*Solanum lycopersicum*) plants. First, I explored the various growth responses of young tomato plants when grown with different levels of FR and evaluated the genotypic variation in these responses (**Chapter 2**). Similar to the commonly known elongation response, the increase in shoot: root ratio under additional FR was also conserved among genotypes. This suggests that this change in dry mass partitioning patterns between organs may also be considered as a typical response to additional FR. To better understand this regulation, we conducted experiments using *phyB1/B2* double mutant and demonstrated that the change in shoot: root ratio was mainly mediated by phytochromes (**Chapter 3**). Interestingly, the *phyB1/B2* double mutant was still able to respond to FR, suggesting that other phytochromes may also regulate shoot: root ratio in the absence of phyB. Furthermore, it was also demonstrated that auxin transport between shoot and root was involved in the regulation of dry mass partitioning between the shoot and the root. In **Chapter 4**, I demonstrated that an increased dry mass partitioning to fruits was the main reason for FR-simulated tomato fruit yield. This increase, however, resulted in a trade-off where plant' s resistance against *Botrytis cinerea* was reduced. By means of experimentation and model simulation, we proved that the FR-simulated dry mass partitioning to fruits was the result of FR-increased individual fruit sink strength (**Chapter 5**). This was further supported by the corresponding changes observed in fruit sugar concentrations and the expression levels of genes involved in sugar transport and metabolism. In this general discussion, I first extend the discussion on the positive and the negative consequences of additional FR in tomato and other species. Secondly, I focus on the findings related to the enhancement of sink activities and discuss the potential of targeting the sink activities for yield improvement. Thirdly, I discuss the possibilities of incorporating FR as part of standard growth light in modern greenhouse production. Lastly, I present the perspectives for future research on this topic.

6.1 FR induced trade-offs during plant growth

The addition of FR results in a reduction of R: FR ratio, similar to natural shading, but without a reduction in photosynthetically active radiation. The reduction of R: FR ratio functions as a signal for competition and triggers responses in morphology (stem elongation, leaf hyponasty, etc.) and physiology (accelerated flowering, change in resource allocation) (Casal, 2012). Often, adding FR leads to an increase in growth such as higher dry mass production, higher yield of harvestable parts, etc. (Hao et al., 2016; Kalaitzoglou et al., 2019; Li and Kubota, 2009). It is intriguing to consider whether such promotion of growth would come at costs of resistance or tolerance against biotic or abiotic stress. Previously, it was reported that plants grown under high density (shading, with low R: FR ratio) expressed a reduced resistance against not only pathogens but also herbivory (for review, see Ballaré, 2014). We observed that additional FR during growth reduced tomato' s resistance against *B. cinerea* (**Chapter 4**), suggesting that the positive effect on yield may come at the cost of reduced plant immunity. This result was further explained in the work of Courbier et al. (2020) demonstrating that the additional FR reduced jasmonic-acid mediated defense response against *B. cinerea*, resulting in more severe disease symptoms. FR also increased sugar content in tomato fruits, which was a positive FR effect in the fruits (**Chapter 5**). FR also increased the sugar content in the leaves and this was shown to help support pathogen growth in tomato leaves (Courbier et al., 2020). Not only the jasmonic-acid-mediated resistance was reduced by additional FR, the salicylic acid pathways, which were predominately activated against biotrophic microbial pathogens (Glazebrook, 2005), were also downregulated by additional FR (Ballaré, 2014; Moreno et al., 2009; Wang et al., 2010). Taken together, it is crucial to further understand the regulatory mechanism of FR-induced reduction of plant resistances against biotic stress such as pathogens and pests to secure the yield of modern crop production.

Abiotic stress, such as salt stress, may also have a negative impact on plant growth and development. Salinity stress reduces water potential in the soil and may lead to reduced water uptake by the plants and consequently limit plant growth. Earlier studies showed that a low R: FR ratio triggers the production of salinity-responding molecules such as

pinitol, suggesting the involvement of phytochromes in the transduction of salt stress signals (Cockburn et al., 1996; Guo and Oosterhuis, 1997). Indeed, the addition of FR increased the tolerance of tomato seedlings against salinity (Cao et al., 2018). Further, these authors demonstrated that a lower R: FR ratio resulted in a decrease in electrolytic leakage, an increase in proline and soluble protein contents, and reduced accumulation of H_2O_2 and O^{2-}. Similarly, mutation in phytochromes led to increased salt tolerance in *Nicotianum tabacum* (Yang et al., 2018) and *Oryza sativa* (Kwon et al., 2018). Taken together, it may be suggested that FR increases the tolerance against salinity by inactivating phytochromes. This increase in salt tolerance may be a result of regulation via phytochrome-interaction-factors (PIFs), which were suppressed by phytochromes and hence were upregulated under additional FR. For example, the expression of maize *ZmPIF3* in rice significantly increased its tolerance against salt stress (Gao et al., 2015). In addition, FR was also shown to promote the SPA1-COP1-PIF1 kinase regulatory complex to promote the expression of *STO* (*SALT TOLERANCE-RELATED*) in *Arabidopsis thaliana* to improve root growth under salt stress (Indorf et al., 2007; Junior et al., 2020). Plant hormones such as auxin and abscisic acid may also be involved in this regulation (Hayes et al., 2019). The responsive network of FR-induced increase in salt tolerance still requires further investigation.

Clearly, FR has different, even opposite effects on plants' capacities in dealing with various stress conditions. Our understanding of this complex response network is still far from complete; thus, further research is needed to not only quantify the FR effect on plants' responses towards different stress stimuli, but also the regulatory mechanisms. These studies should also extend from model species to important crops for a further yield improvement achieved by increased stress tolerance/resistance.

6.2 Targeting FR responses and sink activities for yield improvement

The global human population is expected to reach 10 billion in the coming 30 years, posing an urgent demand for further yield improvement. The yield of major crops increased substantially in the past decades (Table 6.1). Such yield improvement is a result of not only advances in

cultivation techniques but also in breeding for new varieties. In tomato, for example, modern varieties showed significantly higher yield compared to that of old cultivars when grown under current greenhouse conditions (Higashide and Heuvelink, 2009; van der Ploeg et al., 2007). Yield is a complex trait with multiple components and the cause for the yield improvements varies between crops. For example, modern rice cultivars increased in harvest index (Morrison et al., 1999; Saitoh et al., 1990) and some modern maize varieties showed improved light use efficiency (Hay, 1995; Tollenaar and Aguilera, 1992). In tomato, yield improvement of cultivars released from 1950 to 2000 was correlated with higher light use efficiency and leaf photosynthetic rate (Higashide and Heuvelink, 2009). In recent years, the development of climate control technologies in horticultural production enables precise and tailor-made conditions to maximize crop production. Such precision should be matched with varieties selected for these growth conditions. The rapid development of genetic and molecular tools allowed for a precise breeding approach that targets specific pathways or components of yield. Therefore, the knowledge of how crop yield is affected in response to different environmental cues such as light quality, as well as the underlying mechanism of such effects, becomes increasingly crucial.

Table 6.1: *Global average yield of tomato potato, maize, rice, wheat and soybeans in the 1968 and 2018.*

Crop	Yield hg ha^{-1}		Increase %
	1968	2018	
Tomatoes	192879	382694	98
Potatoes	143266	209441	46
Maize	22894	59237	159
Rice	22328	46789	110
Wheat	14534	34254	136
Soybeans	14345	27914	95

FAOSTAT: http://www.fao.org/faostat (accessed October 2020)

FR increases yield in not only leafy crops (Li and Kubota, 2009; Zhen and van Iersel, 2017) but also in fruit crops (Hao et al., 2016; Kalaitzoglou et al., 2019; Zhang et al., 2019). We demonstrated that FR may increase the total plant dry mass of young tomato plants by increasing net assimilation rate (**Chapter 2**). Furthermore, FR increased dry mass

partitioning to the shoot in all tested genotypes, which contributes to the increase in shoot dry mass (**Chapter 2**). This promotion of dry mass partitioning to the shoots was later demonstrated to be subject to the regulation of phytochrome B and auxin (**Chapter 3**). These results suggest that increasing dry mass partitioning harvestable organs may be a new approach for yield improvement. For example, whether increasing dry mass partitioning to fruits can also yield in tomato. Indeed, FR increased tomato fruit yield, and such increase was mainly due to enhanced dry mass partitioning to the fruits (**Chapter 4**). Environmental factors such as temperature and irradiance may affect dry mass partitioning due to their indirect effects on traits like fruit number, but such effect was usually limited (Heuvelink, 1995; Marcelis, 1993). Sink strength, which is quantified by the potential growth rate of an organ under non-limiting assimilate supply, is the intrinsic capacity of an organ to attract assimilates (Marcelis, 1996). Dry mass partitioning to fruits is determined by the sink strength of both vegetative organs and the fruits, the latter being the integral of individual fruit sink strength of all fruits (Heuvelink, 1997). In **Chapter 5**, we proved that FR significantly increased the individual fruit sink strength of a tomato fruit and that this increase quantitatively explained the FR increased dry mass partitioning to fruits. These results hint at the potential in targeting the elevation of sink strength for yield improvement. Sink strength of an organ is directly linked to its capacity to unload, utilize, and metabolize assimilates. When delivered to sinks, the assimilates (mainly sucrose) are unloaded symplasmically from the phloem, facilitated by sugar transporters such as SWEETs and SUTs (Carpaneto et al., 2005; Chen et al., 2012). We observed an elevated expression of sugar transporter genes under FR, suggesting that an enhanced sugar transport is indeed responsible for the increased sink strength (**Chapter 5**). Over-expression of *SUC* in pea successfully increased source-to-sink assimilate transport (Lu et al., 2020). In accordance with this, repressing the expression of sucrose transporters in *A. thaliana* (Gottwald et al., 2000), tobacco (Bürkle et al., 1998) and maize (Slewinski et al., 2009) led to the reduction in growth as well as starch accumulation in source leaves. Efficient unloading of the assimilates is also needed to avoid negative feedback of sugar signals to source leaves in their photosynthetic capacity (Franck et al., 2006). Similarly, to maintain the efficiency of assimilate unloading into the sinks, rapid hydrolysis of sucrose is also needed. Several enzymes are known to be

involved in this process. For example, invertases are responsible for the degradation of imported sucrose into glucose and fructose (Renz and Stitt, 1993), and sucrose synthase for degradation into fructose and UDP-glucose (Huber and Akazawa, 1986). Indeed, we demonstrated that FR increased the post-transport metabolism of imported sucrose in the fruits, shown by the increase in glucose and fructose content, as well as the elevated expression of genes encoding sucrose synthase and invertases (**Chapter 5**). Others attempted to manipulate these processes, such as the inclusion of a more efficient allele of invertase *LIN5* (Fridman et al., 2004; Gur and Zamir, 2004; Zanor et al., 2009), or silencing the inhibitor of *LIN5* (Jin et al., 2009) led to increased sugar content in tomato fruits. Many of the above-mentioned genes and pathways were demonstrated to be regulated by FR or FR-induced regulatory networks. For example, expression of *LIN5* and *LIN6*, which encodes cell-wall invertases, were significantly upregulated in fruits when phytochromes were locally knocked down in the fruits (Ernesto Bianchetti et al., 2018). Expression of *SWEET11* and *SWEET12*, which encode sucrose transporters, was increased by *HY5* (Chen et al., 2016). As *HY5* was directly regulated by FR (van Gelderen et al., 2018), it may be concluded that sugar transporters are also subject to the FR regulation (**Chapter 5**).

Collectively, these results shed light on the potential of targeting FR responses and sink activities for further yield improvement. First, FR-induced enhancement of dry mass partitioning to the fruits warrants the possibility to include FR in the supplementary lighting provided in the greenhouse. Second, the regulatory mechanism, key genes, and enzymes, as well as hormones involved in the FR-enhancement of sink strength can be specifically selected or engineered to achieve a higher fraction of dry mass partitioning to the fruits. The genotypic variation in FR responses provides the foundation for such specific breeding programs. Third, the development of powerful tools such as efficient gene editing by CRISPR/Cas9 facilitates the targeted manipulation of multiple genes and pathways at the same time, while speed-breeding (Ghosh et al., 2018) greatly shortens the breeding cycle by using specific growth conditions. Altogether, it can be expected that breeding for yield-promoting FR responses, and elevated sink activities, possess great potential in further yield improvement for modern agricultural production (Fernie et al., 2020).

6.3 Potential of incorporating FR in modern agricultural production

The recent development of light-emitting diodes (LEDs) as a light source provides exciting opportunities to secure a year-round production of greenhouse crops, especially at high latitudes where light is limited during winter periods. Currently, high-pressure sodium (HPS) lamps are the most used source of supplementary lighting in greenhouses around the world. LEDs, however, are progressively becoming popular and used either as an addition or as a replacement of the HPS lamps (Marcelis and Heuvelink, 2019; Pattison et al., 2018). One of the advantages of LEDs, compared to HPS lamps, is that they have a higher efficacy in converting electrical power to light. HPS lamps typically have an efficacy of ∼1.7 μmol J^{-1} (Nelson and Bugbee, 2014) while that of LEDs used in horticultural production exceeds 3 μmol J^{-1} (Kusuma et al., 2020). In addition to the high efficacy, LEDs are also able to provide more flexibility compared to HPS lamps in the spectral composition. Unlike HPS lamps that have a pre-determined spectrum with little capability to adjust either its intensity or its spectrum, LED light modules can be dimmable and can be designed to provide a wide range of wavelengths with desired spectral composition. Plants use the photons in the 400-700nm range to drive photosynthesis; hence light within this range is termed as photosynthetic active radiation (PAR). FR radiation has a wavelength of 700-800 nm and is generally considered as unable to directly drive photosynthesis. However, it can increase photosynthetic rate of PAR (Zhen and Bugbee, 2020). The addition of FR to PAR light results in positive effects on plant growth and development in various crop species. In pot flowers, for example, additional FR promotes leaf expansion and whole-plant net assimilation as well as flowering (Park & Runkle, 2017). For leafy vegetables such as lettuce, additional FR increased dry mass production (Li and Kubota, 2009; Zhen and Bugbee, 2020; Zou et al., 2019). The promotion of biomass production by FR (Cao et al., 2018; Kalaitzoglou et al., 2019) was also observed in **Chapter 2**. FR increased yield of tomato (Hao et al., 2016; Kalaitzoglou et al., 2019; Zhang et al., 2019),which was also observed in **Chapter 4** and **Chapter 5**. Such yield increase does not have to compromise product quality, as demonstrated by the increased sugar content in **Chapter 5** and in the results of Fanwoua et al. (2019) and

Kim et al. (2019). Furthermore, FR during cultivation improved tolerance to chilling injury in post-harvest storage (Affandi et al., 2020) and improved nutritional content and taste (Kim et al., 2020).

One frequently asked question when considering supplying FR as supplementary light is whether it is more efficient to invest the electricity consumption in the PAR range (*e.g.* red or white light). For major greenhouse-grown crops like cucumber, tomato, lettuce, rose etc., a general rule of thumb is that 1% increment in light leads to 0.5-1% yield increase (Marcelis et al., 2006). In **Chapter 4**, adding 30-50 μmol m^{-2} s^{-1} of FR to a 150 μmol m^{-2} s^{-1} supplementary background (20-33% extra photon) resulted in 26-45% increase in fruit yield, which was slightly higher than the expected yield increase according to Marcelis et al. (2006). Further, with more research being published about FR effects on yield, the total dosage of FR required for a significant yield increase may even be lowered. For example, Hao et al. (2016) showed that only 8 μmol m^{-2} s^{-1} FR added to 165 μmol m^{-2} s^{-1} HPS lighting (5% extra supplementary photons) was sufficient to trigger a yield increase of 9%. This can be further accompanied by a smart application of FR in terms of time (*e.g.* end-of-day lighting) and space (*e.g.* inter-canopy lighting).

These results demonstrate that the addition of FR improves yield and product quality in modern agriculture production, especially greenhouses or vertical farms. FR also proves to be a new tool to fine-tune the balance between vegetative and generative growth. These advantages may become more prominent as more research accumulates, accompanied by a further increase in efficacy of the LED modules as technology develops.

6.4 Perspectives for future research

The results presented in this thesis provide exciting advances in the understanding of how FR increases the growth and development of tomato. These findings also bring up new questions that warrant future research. First, it is not clear how the long-term application of FR increases dry mass production. While factors such as short-term Emerson enhancement effect (Zhen and Bugbee, 2020), higher net assimilation rate (**Chapter 2**, Park and Runkle 2017), higher whole-plant light absorption (Kalaitzoglou et al., 2019), and improved light distribution (Zhang et al., 2019) were suggested to explain the increase in total plant biomass by FR,

the explanations remain inconclusive. Using a study combining growth analysis and model simulation, FR effects on both plant architecture and leaf photosynthesis can be measured and their contribution to final total plant biomass can be quantified. Second, despite that FR upregulates sugar transport into the fruits (**Chapter 5**), it is not known if this is a consequence of 1)more sugar transporters are translated, 2) sugar transporters are working at a higher efficiency, or a combination of both. The number of transporter proteins can be quantified with well-established methods such as western blot. Using more advanced techniques such as FRET (fluorescence resonance energy transfer), it would be possible to monitor the transport rate of sugar molecules through individual sugar transporters (Chen et al., 2010). Third, further research should be conducted on the location where FR signals are perceived and study whether the FR signals works locally or systemically. Especially, the role of fruit-localized FR perception should be studied. Fourth, from a practical point of view, the dosage, timing, and location of FR application should be studied in more detail. It is not yet known what the minimal amount of FR is needed to trigger a significant yield improvement, or when the optimal timing is during the diel cycle to apply FR. Also, this study should be conducted under different backgrounds such as HPS, or different LED lighting like white or red + blue. Effects of FR on other major fruiting crops such as cucumber, pepper, strawberry, etc. are still lacking. It would be also interesting to extend this research to cereal crops such as wheat, maize, and rice and study whether targeting phytochrome-regulated sink activities can lead to further yield improvement. Lastly, breeding traits based on the regulation of sink activities should be identified and, with the help of efficient tools such as CRISPR/Cas9, attempts should be made to target sink activities for further yield improvement. This breeding approach aiming at FR related responses should be conducted under LED lighting conditions, rather than in the greenhouse with only HPS lighting or even without any supplemental lighting, to ensure that selected genotypes are suitable for growth in a fully controlled environment with LED lighting.

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Summary

Tomato (*Solanum lycopersicum*) is not only one of the world' s most important horticultural crops but also one of the main crops for greenhouse production. Modern greenhouse production is not only expected to increase yield and product quality but also to achieve that sustainably. The horticultural sector has long been at the front of technological advances in crop production. Light is one of the most important environmental factors in crop production, and it is a common practice to apply supplementary lighting in greenhouses at locations where low daily light integral is low during the growing season to ensure a year-round production. In these greenhouses, the high-pressure sodium (HPS) lamps are the most used light source due to their low price and decent efficacy in converting electric power to light photons. Despite their popularity, HPS lamps also have disadvantages such as excessive heat emission and inflexibility in the light spectrum. Recently, light-emitting-diodes (LEDs) emerge as an exciting alternative to HPS lamps. LED lighting has over 60% higher efficacy, low heat emission, and can be customized to provide different intensities and spectra. The popularity of LED lighting in horticultural production also stimulated research on the spectral effects of supplementary lighting on plant growth and development, even extending the research to the spectrum that is beyond photosynthetically active radiation (400-700 nm). Far-red radiation, which has a wavelength between 700-800 nm, has been extensively studied due to its role in plant' s neighbor detection and sensing of shading. Interestingly, several studies point towards yield increases as a result of additional FR in several crops. This thesis aims to understand the effect of adding FR on the responses of growth and development of both young and fruit-bearing tomato plants. Specifically, 1) to evaluate and explain genotypic variation in dry mass production of young tomato plants in response to FR, 2) to quantify the FR effect on dry mass partitioning between shoot and root in young tomato plants and explain the regulatory mechanisms, 3) to evaluate whether FR leads a trade-off between growth and plant immunity, and 4) to quantify the FR effect on tomato fruit yield and study the physiological and molecular pathways by which FR regulates this response.

Chapter 1 described the status of greenhouse tomato production, and what is

known about plants' responses to FR. The development of supplementary lighting used in greenhouse tomato production was described, and a comparison was made between the more efficient LED lighting and conventional HPS lighting. Here, known effects on the perception of FR and its regulation of shade avoidance responses were summarized. The latest studies were summarized, and they pointed towards a positive effect of FR on tomato yield. However, these studies did not reveal a clear mechanism for this yield improvement. Moreover, there was contradiction and variation between the results, suggesting that species and even different genotypes within the same species may respond differently to FR. FR may also alter the partitioning of photosynthetic assimilates between organs. Findings in the regulation of dry mass partitioning in plants were summarized and they demonstrated a knowledge gap between a well-studied regulatory network downstream the perception of FR and a set of FR-induced growth responses. Lastly, the research of this thesis was introduced and the different approaches for each of the research questions were outlined.

Chapter 2 demonstrated the genotypic variation within 33 different tomato genotypes in their responses to FR at the young plant stage. Genotypes responded similarly in plant height, stem dry mass, and shoot: root ratio, *i.e.* they all increased with increasing FR. However, the response of total plant dry mass varied among genotypes. Then, genotypes were categorized into three groups (a strong, moderate, and weak responding group) based on their relative response in total plant dry mass to FR. Growth component analysis revealed that tomato genotypes that increased strongly in growth response to FR, compared to the moderate and weak responding ones, were characterized by a strong increase in net assimilation rate.

Chapter 3 continued from the finding in the previous chapter that young plants of all genotypes increased in their shoot: root ratio with FR. Using both wild type and loss-of-function double mutant of *phyB1/B2*, it was demonstrated that the FR-induced increase in shoot: root ratio involved phytochrome B, as the *phyB1/B2* double mutant also showed a strong increase in shoot: root ratio when grown without FR. Interestingly, the *phyB1/B2* double mutant still responded in shoot: root ratio when FR increased, providing evidence for the involvement of other phytochromes in the regulation of shoot: root ratio in tomato. Lastly, it was demonstrated that the phytochrome-regulated response of shoot: root ratio to FR may be mediated by affecting auxin transport.

Chapter 4 focused on fruit-bearing tomato plants and demonstrated the FR effect on tomato fruit growth, and the main cause for this effect. FR significantly increased total fruit dry mass. Growth component analysis revealed that FR increased tomato fruit production mainly by increasing the fraction of dry mass partitioned to fruits, rather than improving photosynthesis and total plant dry mass. Furthermore, FR also reduced the resistance of tomato leaves against

Botrytis cinerea.

Chapter 5 explained how FR increased the fraction of dry mass partitioned to fruits. Sink strength, quantified as the growth rate of an organ under non-limiting assimilate supply, is the intrinsic capacity of an organ to attract assimilates and the main determinant of dry mass partitioning. FR increased fruit sink strength by 38% in the glasshouse experiment. Based on this measurement, the model simulation showed that such an increase in sink strength resulted in increased dry mass partitioned to fruits, and the simulation result quantitatively agreed very well with experimentally measured partitioning fractions. FR also increased fruit sugar concentration and upregulated the expression of genes associated with both sugar transport and metabolism. Taken together, it was concluded that FR stimulates dry mass partitioning to fruits mainly by increasing fruit sink strength via simultaneous upregulation of sugar transport and metabolism.

Chapter 6 summarized the findings of the experimental chapters and extended the interpretation and discussion of these findings. The promotional effect of FR in tomato growth comes at a cost in the form of reduced resistance against pathogens. However, FR may increase plants' tolerance against abiotic stress such as salt stress. The understanding of these trade-offs is far from complete. Also, I discussed the potential of targeting FR responses and sink activity in yield improvement. It was demonstrated that there is genotypic variation in tomato' s growth responses to FR, thus providing the foundation for breeding. FR increased fruit growth in tomato by increasing fruit sink strength, and genes responsible for sink strength were extensively studied. Therefore, it was intriguing to attempt to specifically select varieties with higher fruit sink strength. Powerful tools such as CRISPR and speed breeding are expected to further accelerate this breeding process. Furthermore, I discussed the possibility of integrating FR in modern greenhouse production based on the effects of not only yield improvement but also an enhancement in product quality. Finally, I provided perspectives for future research which will not only help complete our understanding of the plant' s response to FR but also assist the application of FR in modern agriculture production.

Acknowledgments

I did it!

Obviously, I did not do it all by myself. Newton said that he saw further because he was standing on the shoulders of Giants. Well, in my case, I am a little bit taller than Newton. But that was not the point. The point is, I was also standing on the shoulders of Giants, except that there were **A LOT** of Giants, probably so many more what Newton could ever have wished for in his wildest dreams. I know that it is impossible to express my appreciation to you in words, but I will try!

Let me begin with the usual suspects: Leo and Ep. Leo, I still remember the evening we first met in SHOT when you came to watch the table tennis matches of your son, who I happened to be coaching. I was desperately looking for PhD positions around the world at that time, and it was just a few days ago that I came across the news about LED-it-Be project being granted. I was bold enough to approach you about the project and you were nice enough to brief me about it, and later you even offered me a chance to join the project as a research assistant. Ep, it was during my time as a research assistant that I met you for the first time. I was so nervous as I have heard from others that you were extremely "harsh" and "strict" with your students. It turned out that you were just keeping your standards (sky-)high. I could not have wished for a better supervision team because not only do both of you have so much knowledge in this project but also that you have always been supportive and available for me. Our discussions and decision-making were conducted in such an open and equal way that I felt less of like a "student", but more like a "colleague" in our three-men team. Through this, I learned to be confident and to express my opinion even when we did not agree with each other. You have inspired me with not only your knowledge and experience in science but also with your personality. I have been and will always be looking up to you. I cannot be any more excited to keep working with you for my postdoc.

I worked within a large project with 11 PhDs and Postdocs, and I felt like we bonded like a big family. I would like to express my love and gratitude to the LED-it-Beers. Aina, you have always kept me humble with your German seriousness and kept me positive with your German sense of humor. Dalia, who would have

thought that we would be working happily together after fighting so hard for a PCR machine during our times in plant breeding! David, I could not imagine that mathematicians and engineers could be this cool, especially with the cats! Haris, boss, we started working together from day one of the whole project, and indeed it was the beginning of such a beautiful collaboration and friendship. Kiki, it was nice to also have a fellow competition-sport player in a scientific t eam. And yes, I still need to meet your dog! Priscilla, thank you so much for all the small talks in the office and your (E)scientific inspirations. Also, that you have provided me with so much encouragement and (E)support, (E)specially during the drinks! Rachel, you really inspired me to be brave enough to make my PhD life fun and enjoyable with all my hobbies. Sarah, I enjoyed our shared interest in food, drinks, spontaneous gatherings, and of course our collaboration! Victor, you were so good with your work and you were certainly one of the most productive ones of us all. But you inspired me most to be true to myself and follow my heart in making any decisions. Wouter, I may never be able to understand your projects, but you convinced me that they are absolutely cool! You also proved to me that it is possible to balance the relationship between PhD research and other interesting projects as, somehow, you are just that good in both! To all of you, I have enjoyed the chemistry between us and all the fun days and nights we spent together not only in the project meetings, the excursions, but also in our activities in Croatia, Bosnia, and Maastricht. Thank you all for being there for me and for your support all along.

Wageningen is my home far away from home. I am grateful to have many Chinese colleagues and friends who helped me fight the loneliness and homesickness. My Chinese colleagues (and the significant halves) from work: 何立中老师, 朱竹老师, 邱栋梁老师, 涛哥, Didi, 刘老师, 老司机, 希曦, 星哥, 点爷, 宁一, 金总, 包总, 小寒, 张雪, 波哥, 忆菲, 思佳, 旭光, 李桦, 玉琪, 老杨, 王娜, 韩总, thank you for all the enjoyable discussions, jokes, gossips, food & drinks and your support and inspiration for me to keep moving. My Chinese corridor mates in Dijkgraaf 宇哥, 文昭, 玉婕, 清华哥, 成然, 陈思, 姐夫, 羽翔, 童昕, Cece, 严寒, 张鑫, 佳欣, 梦迪, 桃军, 小东北, and roommates in Schaepmanstraat 陈老师, 马老师, 郭老师, 思奇, 盼盼, 图南, 老薛, 老韩, thank you for taking care of me. All those who I studied with during my MSc time 小熊君, 王总, 邱总, 佳哥, 佳姐, 费博, 沂君, 梦茹, 李昂, 刘钺, 亦儒, 翰舒, 老姜, 曹老板, just to name a few, also, 杨其长老师, 戴剑锋老师, 朱丹老师, 张晓勇老师, 张钊老师, 张海林老师, 群峰, Joyce, 季方, 朱峰, 舒航, 晓雪, 凯乐, 小卷丹, 晓梅, 材哥, 栋哥, 文皓, 大熊, 凡哥, 贝勒爷, 小麦, 瑞洲, 青青, 大鑫, 志纯, 露露, 苗苗 and many others who I run into for all kinds of reasons, I cannot name every single one of you, but I would like to say that it was these little pieces of memory that kept me strong and positive in this journey. Thank you. 谢谢!

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Life as a PhD is stressful, and I am glad that I have many (maybe too many?) hobbies that allow me to take a break and recharge. My dear Dr. Beer's, 主席, 老王, 教授, 夏厂长, 燕子, 大肠杆君, 墨船长, 大湿, Cob, 辉哥, 螂王, 孟哥, 中原, 肥猫, 狸小羊, 亦午, who would have thought that our drinking group will evolve into one of the most influential beer-related self-media in China! We started it when I was still doing my MSc study, and thank you all for cheering up for me (pun intended) during my PhD time. Aad, Anatolj, Eloy, Frans, Hans, Helma, Johan (Frederiks), Johan (van de Pol), Marco, Ric, Roland, Sebastian, Sietse, Wim (van Breenen) and many others from TTV SHOT Wageningen, and Chinese table tennis players in the Netherlands 佼姐, 李洁, 董指导, 小晗, 彦儒, 丁铭, 颖洁, 张宇, 金文, 秦指导, 关哥, 宽哥, 侯指导, 根哥, 牛指导, 常指导, 强哥, 徐指导, Flora, 润玮, 谭指导, thank you all for keeping my passion for table tennis alive. 星月, 章老板, 小吴学长, 小七, thank you for all the hours we spent together in Erangel, Miramar, Sanhok

and Vikendi. This thesis may have been finished earlier had I used this time on my research instead of having all the fun with you. Maybe. But I know if I would have done that, the journey would have been with much more pain and suffering. So, I guess it is still time well spent, and I thank you all for keeping my life balanced between work and fun.

Some have been in so many aspects of life that I cannot categorize into a single group. Cathy, you were the one taking all the negativity from me. Whenever I was in doubt of myself, or when I was feeling hopeless, you were there all the time to listen, to support, and to carry away the burden on my shoulders. Thank you for being there. Shazia, you are warm, caring, funny, inspiring, in your own "strange" ways. You kept me fed with your amazing food, kept me motivated with your life stories, kept me laughing with your terrible jokes, kept me thinking with your scientific inputs, kept me "jealous" with your excellent thesis and publications, and most importantly, you kept me running towards my dreams. 旭哥, you helped me get used to the Netherlands when I first arrived, inspired me to pursue my PhD, taught me how to excel in both scientific and social activities, and how to handle all of the obstacles I faced in my life. 培成,白溪, thank you for supporting and caring for me. We may only know each other for a few years, but it feels like we are close since childhood. I am blessed to have amazing friends like you and I look forward to many other important occasions to share in our future friendships. Virginia, we pretty much grew up together and you even managed to visit me in the Netherlands. Thank you very much for being there with all the support and inspiration. And yes, I want to be your first trainee once you get your IFBB pro card. 话不多说,就是加油。大佬, 嘉慧, 正仪, 佳伦, 刘畅, 文博, 嘉莉, 鹤鸣, 老板, 老板娘, thank you for constantly checking up on me and always trying to brighten up my life with your positive energy, your company, and the amazing food and drinks. Some of you have seen the worst of me, and have been the victim of my mistakes, yet still decided to reach out and help. You made my time in Wageningen so much more colorful. Young-lim, thank you for being a nice friend, a nice corridor mate, and a nice fellow PhD student. We should definitely do more of those beer nights with Korean chicken! Jarst, Luuk, Galina, Tim, and Jaap, we know each other since almost day one in Wageningen during our studies and I love how we have always just randomly decided to do a BBQ, grab a kebab, get a drink, or go to a Star Wars movie. It was so much fun and I hope they keep making those burgers, kebabs, drinks, and most importantly, Star Wars movies for us! Last but not least, Josephine, thank you for all of the moments that you allowed me to hang around in your kitchen (even though you always tell me to "f**k off"). Your personality is as spicy as your curry, as warm as your soup, and as sweet as your cheesecake (second-best cheesecake in Wageningen!).

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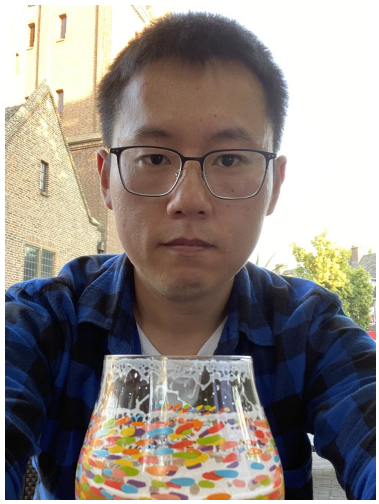
I am extremely lucky to have the support and help from so many people. And I will always be grateful, and I will carry that with me in my future.

Yongran

About the author



Yongran Ji was born in Nanchang, P. R. China on January 15th, 1991. Both of his parents studied agronomy and they both became professors in an agricultural university. Hence, it was not surprising at all that he went to China Agricultural University in Beijing in 2008 to pursue his bachelor' s degree in, you guessed it, agronomy.

After obtaining a BSc degree in agronomy (specialization of crop breeding and genetics) and a double degree in English literature, he applied for the MSc program of Plant Sciences at Wageningen University with a specialization in plant breeding. During his MSc study, he worked mainly with *brassica* crops. For his major thesis, he worked with Prof. Edith Lammerts van Bueren and Prof. Paul Struik on the breeding of cabbage (*Brassica oleracea*) for organic production with limited nitrogen availability. This work was a combination of field experiments, lab analysis, grower interviews, and the weekly 3-hour transportation between Wageningen and the experimental field. Later, he continued to work with Dr. Guusje Bonnema and Prof. Richard Immink to study the molecular mechanism in which increased ambient temperature delayed the flowering of cauliflower (*B. oleracea*). It was safe to say that his love and interests for a scientific career grew substantially at the cost of his love to eat cabbages and cauliflowers.

After finishing his MSc study, he started his "search year" to search for a PhD position. He accidentally came across the news about LED-it-Be 50% program being granted and immediately he was impressed and attracted by the topic. Even more accidentally, he met Prof. Leo Marcelis, the leader of the program, in his table tennis club. After several talks and, he was lucky enough to start to work

as a research assistant in the LED-it-Be program in the Horticulture and Product Physiology (HPP) group. Later, he was offered a PhD position in the LED-it-Be program under the supervision of Prof. Leo Marcelis and Dr. Ep Heuvelink. Without hesitation, he happily accepted the offer and started his journey as a PhD candidate in October 2015.

At this moment, he is working in HPP group as a Postdoc researcher in the Sky-High program, which is a large research program focusing on crop production in vertical farms.

Publications in peer-reviewed journals

- **Ji, Y**., Ouzounis, T., Schouten, H. J., Visser, R. G., Marcelis, L. F. M., & Heuvelink, E. (2020). Dissecting the genotypic variation of growth responses to far-red radiation in tomato. *Frontiers in Plant Science*, 11, 2172.
- **Ji, Y**., Nuñez Ocaña, D., Choe, D., Larsen, D. H., Marcelis, L. F. M., & Heuvelink, E. (2020). Far-red radiation stimulates dry mass partitioning to fruits by increasing fruit sink strength in tomato. *New Phytologist, 228(6)*, 1914-1925.
- Affandi, F. Y., Verdonk, J. C., Ouzounis, T., **Ji, Y**., Woltering, E. J., & Schouten, R. E.(2020). Far-red light during cultivation induces postharvest cold tolerance in tomato fruit. *Postharvest Biology and Technology,159*, 111019.
- **Ji, Y.**, Ouzounis, T., Courbier, S., Kaiser, E., Nguyen, P. T., Schouten, H. J., Visser, R. G.,Pierik, R., Marcelis, L. F. M., & Heuvelink, E. (2019). Far-red radiation increases dry mass partitioning to fruits but reduces *Botrytis cinerea* resistance in tomato. *Environmental and Experimental Botany,168*, 103889.
- Sun, X., Bucher, J., **Ji, Y**., van Dijk, A. D., Immink, R. G., & Bonnema, G. (2018). Effect of ambient temperature fluctuation on the timing of the transition to the generative stage in cauliflower. *Environmental and Experimental Botany,155*, 742–750.

Other publications

- **Ji, Y**., Hendriks, A., Heuvelink, E., & Kierkels, T. (2020). Verrood licht zorgt ervoor dat tomaten de suikers meer naar zich toetrekken. Andere verdeling leidt tot meer productie. *Onder Glas,17*, 46–47.(in Dutch).
- Ouzounis, T., Heuvelink, E., **Ji, Y.**, Schouten, H., Visser, R., & Marcelis, L.F. M., (2016). Blueand red led lighting effects on plant biomass, stomatal conductance, and metabolite con-tent in nine tomato genotypes. *Acta Horticulturae, 1134*, 251–258.

- Hospers-Brands, A., **Ji, Y**., Struik, P. C., & Lammerts van Bueren, E. (2014). Perspectieven voor veredeling op stikstofefficientie bij sluitkool: Literatuuroverzicht. Louis Bolk Instituut.(in Dutch).

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 / writing of project proposal (4.5 ECTS)

- Control of Assimilate Partitioning in Tomato as Affected by LED Light.

Post-graduate courses (5.2 ECTS)

- Participatory Plant Breeding & Resilient Seed Systems; WUR (2020)
- International Workshop on Vertical Farming (Vertifarm 2019); WUR (2019)
- Introduction to R for Statistical Analysis; WUR (2016)
- Basic statistics; WUR (2016)

Invited review of journal manuscripts (1 ECTS)

- New Phytologist: Light signalling in plants (2019)

Competence strengthening / skills courses (3.2 ECTS)

- Presenting with Impact; Wageningen in'to Language (2016)
- Efficient Writing Strategies; Wageningen in'to Language (2016)
- Pitch Perfect; Wageningen in'to Language (2016)

Scientific integrity / ethics in science activities (0.3 ECTS)

- Ethics in Plant and Environmental Sciences; WUR (2018)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC Day (2019, 2017, 2015)
- PE&RC PhD Weekend Last Year (2019)
- PE&RC PhD Weekend First Year (2015)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)

- FLOP (Frontier Literature Of Plant Physiology) meeting (2016-2020)
- International Symposium on Environment Control Technology for Value-added Plant Production (2019)

International symposia, workshops and conferences (7.5 ECTS)

- GreenSys 2019; oral presentation; Angers, France (2019)
- GreenSys 2017; oral presentation; Beijing, China (2017)
- LightSym 2015; poster presentation; East Lansing, USA (2016)

Lecturing / supervision of practicals / tutorials (10.5 ECTS)

- Research Methods in Crop Science (2016-2020)
- Concepts in Environmental Plant Physiology (2016-2020)

Societally relevant exposure (0.6 ECTS)

- Famelab Wageningen Heat (2020)
- Science Slam Wageningen (2019)

Supervision of 5 MSc / BSc students

- Diego Nuñez Ocaña: Far-red effect on sugar status and molecular control of fruit sink strength in tomato (2020)
- Daegeun Choe: Far red increases fruit sink strength in tomato (2018)
- Jonas Driessen: Expression of phytochrome and ethylene genes under far-red light in Tomato (2017)
- Jarno Mooren: Far-red effect on shoot: root partitioning in young tomato plants (2017)
- Liying Gao: Spectral effect on growth and development of tomato (2016)

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