Study on Periodic Alteration of Plant Density and Inter Supplemental Lighting to Enhance Plant Growth and Fruit Production of Single-Truss Tomato

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移動式トマト一段密植栽培における栽植密度と群落内補光の研究

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蒋程瑶

Chapter 1

General Introduction

1-1 Light condition in greenhouse crop production

Light condition plays a crucial role in plant's whole life physiology reaction. Providing energy source for photosynthesis, light also acts as signal regulator of numerous processes such as seed germination (Assmann et al., 1985; De Villers et al., 1994; Fan et al., 2004; Zohar et al., 1975), leaf development (Evans et al., 2001; Fu et al., 2011), flowering (Cerdán et al., 2003; Goto et al., 1991), stomatal regulation (O'Carrigan et al., 2014; Shimazaki et al., 2007) and membrane transport of cells (Mullineaux et al., 2002) to extensively regulate growth and development of plants, largely determines greenhouse crop productivity (Kubínová, 1991; Lee et al., 2007; Shimazaki et al., 2007; Talbott et al., 2006; Talbott et al., 1993). In general, growth responses to light environment are influenced by both quantity (cumulative light or light sum or light integral; light intensity × light period; the number of photos intercepted per m^2 per unit of time) and quality (spectral distribution) of light, as well as interaction with temperature and cultural practice. The quantity of light is affected by a combination of day period, solar angle, atmospheric cover, plant density, canopy structure, and so on. As for greenhouse crops, it also includes greenhouse structure, and cover materials, while the spectral distribution of light received at a given point depends on solar angle, atmosphere, transmission through leaves, and reflection from nearby plants and other objects, including the soil surface (Heuvelink et al., 2005).

In greenhouse crop production, intensive cultivation is often applied to achieve high

annul yield and such high plant density usually induce light insufficient stress on plant consequently. And the situation is especially serious in the low solar irradiation climate such as cloudy, rainy, and snowy day in the winter. The shortage of light would leads to damage of plant morphogenesis and photosynthesis with variable effects among species (Hogewoning et al., 2010; Terfa et al., 2013) and cause many light stress responses via photoreceptors, such as phytochromes, cryptochromes, and phototropins, which alter the expression of a large number of genes (Barnes et al., 1997; Walters et al., 2003).

Light insufficiency in greenhouse tomato cultivation often originates from decrease of light vertical distribution along the plant profile as well as mutual shading (Talbott and Zeiger, 1993; Walters et al., 2003; Zhang et al., 2015). The light irradiation at leaf decreases rapidly with the depth of canopy: in high trees like oak the light intensity could decrease 10% by canopy layer (Kull et al., 1999) and in single-truss tomato leaf under fruit truss only received less than 35% of total intercepted solar light (Lu et al., 2012a). With low incident light, the understory leaves present an extremely low net photosynthetic rate and premature senescence (Acock et al., 1978; Xu et al., 1997), which declined the plant growth and restrict productive capacity (Frantz et al., 2000; Shimazaki et al., 2007; Steinger, 2003). Generally, it is considered that a decrease of 1% in cumulative daily light leads to a loss of 1% yield under greenhouse cultivation (Cockshull et al., 1992).

However, in summer cultivation period, the situation of light insufficient to canopies usually reverses. In Japan, the average photosynthetic photon flux density (PPFD) at plant top canopy exceeds 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the midday period of sunny days. Excessive light irradiation leads to changes ranging from macroscopic whole-plant level to microscopic ion

investment (Evans et al., 2001).

Surplus irradiation can break the biomass balance and decrease allocation to leaves and stem while increase the fraction to roots (Brouwer 1962; Poorter et al., 2000) to maintain a constant transpiration rate per unit root mass (Sims et al.,1994). Plants grown in high light generally have thick leaves with a low specific leaf area (SLA, leaf area per unit leaf dry mass) (Björkman, 1981) but more chloroplasts and photosynthetic enzymes, thereby the photosynthetic capacity per unit leaf area is enhanced. However, by having more biomass in a given area, the increase in photosynthetic capacity of the high-light leaves comes at a cost of having less light capture per unit biomass. Consequently, high light only stimulates half on photosynthesis per unit area, compared to appropriate irradiation (Poorter et al., 2000).

Meanwhile failure to dissipate or avoid excessive light often leads to oxidative damages in plant, which impair the photosynthetic apparatus and induce bleaching, chlorosis and bronzing to leaves (Karpinski et al., 1999; Mullineaux et al., 2002), largely decrease leaf photosynthetic rate. In order to confront such oxidative damage, plants have to develop a series of protective strategies, such as chloroplast avoidance (Kagawa et al., 2001), photosynthetic reaction centres decrease (Walters et al., 1999), stomatal behaviour alternation (Willmer et al., 1996), leaf curling (Neuner et al., 1999) and even leaf epidermis wax increase (Horton et al., 1996), which however, weaken electron transport rates, obstruct photosynthesis quantum yield, and generate plant growth delay even death in extreme case (Jiang et al., 2006).

1-2 Tomato production in Japan and single truss tomato production system (STTPS)

Traditional greenhouse tomato production is always labor intensive and has a long production period and variable yield (Fisher et al., 1990; Kozai et al., 1996; 2005; Govindasamy, 1996).Although with advanced cultivation techniques, the annual yield of tomato in Japan has remained at 30 kg·m^{-2} without obvious increase since 1980s, which is about half of the amount in Netherland and the USA. The modern Dutch tomato cultivars usually show higher light use efficiency and dry matter production rate, consequently with an enhanced of total yield (Higashide et al., 2009; van de Ploeg et al., 2007). However, higher dry matter accumulation will decrease soluble solid content and fruit pericarp coloration, which is unappeasable for Japanese market requirements (Sasaki, 2008). It is difficult for Japanese popular tomato cultivars, such as "Momotato" series, to obtain yield as high as that of Dutch cultivars.

Meanwhile, within a greenhouse, tomato plants are grown at fixed location, and multiple clusters of fruit are harvested from each plant during a production season. Though simple to use, this production system is difficult to achieve high utilization efficiency of space, light, materials, and labor, and possibility of continuous and predictable year round production. Therefore, it is necessary to develop a more appropriated culture system that uses simple management to improve labor efficiency and can realize year round production of tomato with high yield.

Researchers have developed an innovative greenhouse tomato production system, in which each plant is allowed to produce only one truss of fruit making the growth and

production cycle for each bath of plants very short. This single truss tomato production system (STTPS) is result of an integrated system design to achieve year round, continuous, predictable production of uniform quality greenhouse tomato (Giacomelli et al., 1994; Ting et al., 1993). Additionally, the short production period of single truss plant allows the cultivator to choose suitable cultivars to meet the changeable market requirements, and the production system may also alleviate the damage to growth of tomato caused by the summer higher temperature, which usually occur in long-term cultivation in Japan.

Okano et al. (2001) used a wet-sheet culture system for the cultivation of single-truss tomato to improve the STTPS. They also listed several merits of this system over the conventional multi-truss tomato, such as labor requirement for training and pruning is markedly reduced, working posture is improved by the use of elevated growing benches, few diseases and pest problems because of short growing cycle and few needs for using agrochemicals, the year round cropping schedule can be optimized to reduce seasonal labor peaks without decreasing the crop value, fruit quality would be easily improved by applying salinity stress, and so on. The fruit productivity can be improved by increasing plant density and cropping cycles per year. Kobayashi (1997) reported that it was possible to achieve the 36 $kg·m^{-2}$ of annual yield of "Momotaro" at a density of 10 $plant·m^{-2}$ with STTPS. The yield of unit area could be further enhanced with higher density (Kobayashi, 1999).

1-3 Plant density and inter-plant light condition

Plant density (PD) is one of the most important cultural practices determining crop yield, which affects plant architecture, alters growth and developmental patterns and influences carbohydrate production and partition (Bleasdale et al., 1960; Casal et al., 1985; Sangoi,

2001). Though the sensitivity response to variation in PD various in different species, it is well documented that low densities usually trigger out low leaf area and small number of reproductive units by branching (Gardner et al., 1985; Sangoi, 2001) while super high densities heightens interplant competition for light, water and nutrients, leading to detrimental to final yield and affect the fruit quality (Dong et al., 2005; Sangoi, 2001; Verheul, 2012).

Usually, as plants grow, there is inevitable mutual shading caused by branches and leaves, which leads to light deficiency in the lower canopy (Lu, 2012; Steinger, 2003). Previous research has shown that insufficient light causes both morphological and physiological changes in plants, such as an increase in specific leaf area and plant height, which maximizes the capture of available light to meet the demand for photosynthesis but results in more drastic shading. Additionally, the competition for light within the canopy during intensive crop cultivation can trigger premature leaf senescence (Steinger, 2003; Rousseaux et al., 1996), which weakens the plants' reproductive ability, leading to yield decreases. This situation is exacerbated in the STTPS because the PD is much higher than in common commercial tomato production, especially in the winter production period when solar light interception is limited in both upper and lower canopy leaves (Gunnlaugsson et al., 2006).

However, plants before anthesis are often still short and have a small leaf area so there is no mutual shading. This means the cultivation area at the immature plant stage in greenhouses has wasted space in a fixed bench system because plant and bench distances are decided by the final plant size. And these abundant research achievements mainly focus on fixed PD conditions throughout the whole production process and rarely investigate effects of changeable PD on plant development, given the fact that young plant before anthesis should

be rational close planting for their small profile while mature plants need more space in case of excessive shading.

1-4 Application of supplemental lighting in STTPS

In winter production period of Tokyo region of Japan, from late November to early March, more than 40% of weather condition will be cloudy, raining and snowy, making the irradiation level cannot meet the requirement for plant development. And tomato plants in STTPS are under high density and lower canopies are suffered more serious light insufficiency problem at this season. Therefore, it is important to improve the light environment in the lower canopy in STTPS to enhance the fruit yield. Usually, cultivators use supplemental lighting (SL) to compensate for the shortage of lighting in leaves.

SL, using artificial light resource, is considered an efficient method to relieve low-light stress on plants. Numerous studies of SL application effect have been conducted on various species via aspects of canopy layer (Hovi et al., 2004; 2008; Pettersen et al., 2010), light source (Lu et al., 2012a; 2012b; Piringer et al., 1960), light intensity (Dorais, 2003; Demers et al., 1998), light quality (Lu et al., 2012b; Ni et al., 2009; Li et al., 2009; Okamoto et al., 1996) and light period (Lu et al., 2012a; Mc Avoy et al., 1989; Piringer et al., 1960; Tewolde et al., 2016).

Early researchers applied lamps to above canopy, so called top lighting, and nowadays this SL method still wildly used in high-latitude regions and arctic countries such as Norway and Finland, because of extremely low solar irradiation (Hovi et al., 2004). However, this provides unequal irradiation distribution, within which lower canopies receive much less irradiation. It is well known that without enough irradiation, leaves only contribute to net

respiratory carbon loss other than carbon gain. In Japan, the solar light condition is better than high-latitude regions and the daily average illumination in lowest irradiation months could be 29 $mol \cdot m^{-2} \cdot day^{-1}$ (Kobayashi, 1998) and considered enough for top leaves develop. Thereby utilizing suitable SL to lower canopy is considered effective in improving leaf photosynthetic accumulation and biomass production.

It is certain to increase canopy photosynthesis with the application of SL during the whole plant growth stage, with a requirement of large initial capital investment and continuing operation budget. That means the SL must be efficient enough to cover the input cost. Mc Avoy et al. (1989) divided plant development period into four stages: Stage 1, after transplanting to anthesis; Stage 2, anthesis to initial fruit set; Stage 3, fruit set to mature green; Stage 4, mature green to red-ripe. And Lu et al. (2012a) identified the efficiency of SL was equally higher when applied to Stage 3 and whole growth stage.

Photoperiod is also an important factor to affect the tomato plant growth. Extended photoperiod to 18 hours (h) has been proven no significant effect on the leaf area of greenhouse tomato but increased their dry weight (Dorais et al., 1996), and 24 h continuous lighting not only decreased leaf photosynthesis but also induced visual leaf injury (Matsuda et al., 2014). Night SL (from 10:00 pm to 10:00 am) was experimentally proven useful in enhancing tomato yield compared to day SL (from 4:00 am to 4:00 pm) in winter production season but harmful in summer (Tewolde et al., 2016).

Light sources such as fluorescent lamps, metal-halide lamps, and high-pressure sodium lamps are generally used for plant cultivation. Fluorescent lamps emit light high in the blue range and excellent for starting seedlings. High frequency electric ballast fluorescent lamps

can achieve electrical efficiency values from typically around 20% to 30%, where more than 90% of the emitted photons are inside the photosynthetically active radiation (PAR) region with typical life times of around 12000 hours. The metal halide lamps belongs to the group of high-intensity discharge lams and the inclusion of metal halides during manufacture allows to a certain extent the optimization of the spectral quality of radiation emitted. The high-pressure sodium lamps have advantages of high radiant emission, low price and long life, but their spectral quality is not optimal for photomorphogenesis (Tibbitts et al., 1983; Wheeler et al., 1991). All these lamps are used to increase the PPFD, but they also provide wavelengths that are not used efficiently or at all to support photosynthesis and plant growth (Mc Cree, 1972; Björkman, 1981). In comparison, light-emitting diodes (LEDs) lighting systems have several advantages, including greater wavelength specificity (i.e., an arrow band width), long operating lifetimes, and less heating. The spectral output of an LED lighting system can be matched to plant photoreceptors and optimized to provide maximum production without wasting energy (Dougher et al., 2001).

The selection of optimized light wavelength is more complex and often reported with mixed results (Lu et al., 2012b; Ni et al., 2009; Li et al., 2009; Okamoto et al., 1996). For example, blue light suppresses hypocotyl elongation in wheat (Goins et al., 1997) and tomato (Massa et al., 2008), but improves dry matter production and the photosynthetic capacity in pepper (Brown et al., 1995), wheat (Goins et al., 1997), and spinach (Matsuda et al., 2007). In contrast, red light seems to be most effective in biomass assimulation of lettuce (Yanagi et al., 1996; Kim et al., 2006), but not for spinach and radish (Okamoto et al., 1996; Yorio et al., 2001). Similarly, different red/far-red ratios (R/FR) show contrary results in phytochemical

concentration (Alokam et al., 2002) and plant photomorphogenesis (Brown et al., 1995; Kirdmanee et al., 1993; Runkle et al., 2001). Those results have shown the viability of optimizing light quality in promoting plant morphology and productivity to eventually improve greenhouse economic benefit.

However, the investigation of structure-function relationships in leaf photosynthesis shows that internal maximum photosynthesis rates were not near the leaf surface where light intensity was highest, but occurred in the middle and lower palisade layers (Nishio et al., 1993; Evans, 1995, 2003; Sun et al., 1998, 2001). These deeper layers have higher electron transport activities and greater amounts of photosynthetic proteins (Terashima et al., 1985, 1988; Sun et al., 2001). This indicated that SL from underneath the canopy (USL, with light orientation to the abaxial epidermis) might function better in improving leaf and plant development than using inner canopy SL (ISL, with light orientation to the adaxial epidermis).

1-5 Photosynthesis characteristic and stomatal regulation

Photosynthesis is essential for plant growth and development, and improved leaf photosynthesis would enhance crop yield (Hovi et al., 2004, 2008; Long et al., 2006; Pettersen et al., 2010; Zhu et al., 2010). Researches on regulations of steady-state photosynthesis in response to variations in light intensity (Evans et al., 1993; Ögren et al., 1993), CO_2 concentration (Alonso et al. 2008; Farquhar et al. 1980), temperature (Alonso et al., 2008; Bernacchi et al., 2001), and humidity (Bunce, 1997; Rawson et al., 1977) have been extensively examined under controlled laboratory conditions. Predicting the environmental responses of the steady-state photosynthetic rate is central to many models of changes in the future global carbon cycle and terrestrial biosphere (Bernacchi et al., 2013; Groenendijk et al.,

2011; Zhu et al., 2004).

Light intensity is the most variable factor in natural environments; during the day, it changes accompanying with changes in leaf angle, cloud cover, and overshadowing canopy. Previous researches on photosynthetic responses to fluctuating light have focused on their mechanisms and interspecific variations (Pearcy, 1990; Pearcy et al., 2012). Photosynthesis starts with the absorption of light by the light-harvesting systems, which drive photosynthetic electron transport through the thylakoid membranes of the chloroplasts. And photosynthetic responses to sunflecks differ among and within species depending on sunfleck duration, frequency, and intensity (Chazdon et al., 1986; Leakey et al., 2004; Sims et al., 1993; Watling et al., 1997; Yin et al., 2000). A sudden increase in light intensity typically leads to a hyperbolic increase in the leaf photosynthetic rate (Bai et al., 2008; Han et al., 1999; Pearcy, 1990; Valladares et al., 1997), while a longtime insufficiency in light intensity causes extreme low even minus leaf photosynthetic rate (Chen et al., 2011; Valladares et al., 1997). Because of biochemical and stomatal limitations, there is a time lag from onset of light to achievement of the maximum rate of photosynthesis (Allen et al., 2000; Bai et al., 2008; Chen et al., 2011; Han et al., 1999; Pearcy, 1990; Rijkers et al., 2000).

Stomata are microscopic structures formed by two guard cells flanking a central pore in plants. Stomatal regulation, highly correlated with leaf photosynthesis, governs overall carbon dioxide (CO_2) assimilation and water loss from plants (Casson et al., 2010; Araújo et al., 2011), and can be affected by numerous biotic and abiotic factors, including hormones, humidity, and CO_2 concentration (Fan et al., 2004; Mott et al., 2008; Chen et al., 2012). Among those, light wavelength and intensity can regulate stomatal behavior through energy

conversion, membrane ion transport, and metabolic activity in guard cells (Shimazaki et al., 2007; Araújo et al., 2011; O'Carrigan et al., 2014). For instance, numbers of stomata, rate of photosynthesis and transpiration, and stomatal conductance increased progressively with increasing PPFD in plantlets of *Withania somnifera* L. (Lee et al., 2007) and *Solanum lycopersicum* L. (O'Carrigan et al., 2014).

It was reported that raising the PPFD from 25 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased the average number of stomata and stomatal length and reduced stomatal frequency in barley (Kubínová, 1991). Stomatal pore length, stomatal density and index were all influenced by the irradiance signal and were reversible upon changing irradiance except for stomatal pore length (Thomas et al., 2003). Generally, stomatal density is higher in plants grown in full sunlight or at high light intensities than in plants grown in shade and also the stomatal index and density of wild type and transgenic plants have also been shown to increase with irradiance (Baroli et al., 2008; Lake et al., 2001; Schoch et al., 1980; Thomas et al., 2003). The geometry of guard cells including length, width and volume represent some of the most important characteristics for stomatal movement (Khazaei et al., 2010; Meckel et al., 2007). Increase in guard cell volume is driven by the uptake of ions, solutes and water and intracellular solute production (Fan et al., 2004; Hills et al., 2012). It was reported that the stomatal length of barley increased from 40.7 to 42.5 μm when PPFD was increased from 25 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kubínová, 1991). In W. somnifera L. plantlets, light levels did not cause any significant effect on width and length of stomata particularly up to 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PPFD. However, both width and length of stomata declined at 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of PPFD (Lee et al., 2007).

Plant growth and yield depend largely on photosynthesis (Long et al. 2006; Pettersen et

al., 2010; Zhu et al. 2010). And further correlation analysis show a close relationship among stomata regulation, gas exchange, and plant growth parameters. Understanding photosynthetic characteristics and stomatal behaviors under fluctuating environments is needed for using biotechnological strategies to escape photoinhibition and improve photosynthetic performance under stressful conditions.

1-6 Background and objectives of this thesis work

In winter STTPS production, cultivators usually use SL to compensate for the shortage of lighting in lower leaves. Though traditional SL to inner canopy has been proven effective, the investigation of structure-function relationships in leaf photosynthesis, internal maximum photosynthesis rates were not near the leaf surface where light intensity was highest, but occurred in the middle and lower palisade layers (Nishio et al., 1993; Evans, 1995, 2003; Sun et al., 1998, 2001). These deeper layers have higher electron transport activities and greater amounts of photosynthetic proteins (Terashima et al., 1985, 1988; Sun et al., 2001). All these indicated that SL from underneath the canopy (USL) might function better in improving leaf and plant development than using inner canopy SL (ISL).

However, the application of SL adds extra electricity consumption cost and equipment spoilage, unavoidably adding production costs. And plants before anthesis are often still short and have a small leaf area so there is no mutual shading. This means the cultivation area at the immature plant stage in greenhouses has wasted space in a fixed bench system because plant and bench distances are decided by the final plant size. To deal with this conflicting situations, a periodic alteration PD schedule might be effective, through which an optimized inter-plant light condition established to meet different light needs for plants at different physiological

stages.

Thereby, the overall aim of this thesis work is to investigate the applicability of periodic alteration of PD and SL oriented from underneath and inner canopy to optimize the inter-plant light condition of the STTPS to enhance tomato plant vegetative accumulation and reproductive production. The objectives included investigating the effects of periodic alteration of PD on optimizing inter-plant irradiation, understanding how tomato plant physiological characters respond to changes in plant density at different growth stages, exploring the action mechanism of critical factors, identifying the most effective plant density change for tomato development and reproduction, as well as investigating the effects of different orientated SL to the low canopy on intensive cultivated tomato young seedlings growth, photosynthesis, and stomatal regulation and mature plant reproductive characteristics and analyzing the economic benefits of this SL technique.

Chapter 2

Responses of Leaf Photosynthesis, Plant Growth and Fruit Production to Periodic Alteration of Plant Density in Winter Produced Single-truss Tomato

2-1 Introduction

Traditional greenhouse tomato production is always labor intensive and has a long production period and variable yield (Fisher et al., 1990; Kozai et al., 1996; 2005; Govindasamy, 1996). To solve these problems, the single-truss tomato production system (STTPS), which has a shortened production cycle, uniform fruit, potential for automation and labor saving, has been developed and drawn more and more attention worldwide (Giniger et al., 1988; Janes et al.,, 1989; Logendra et al., 1999; Ting et al., 1993). In Japan, the annual average yield of tomato in STTPS can reach 36 kg·m^{-2} at a plant density (PD) of 10 plants·m^{-2}, and it is hoped that this number will be enhanced at higher densities and light use efficiency (Higashide and Heuvelink, 2009; Kobayashi, 1997; 1999).

As plants grow, there is inevitable mutual shading caused by branches and leaves, which leads to light deficiency in the lower canopy (Lu, 2012; Steinger, 2003). Previous research has shown that insufficient light causes both morphological and physiological changes in plants, such as an increase in specific leaf area and plant height, which maximizes the capture of available light to meet the demand for photosynthesis but results in more drastic shading. Additionally, the competition for light within the canopy during intensive crop cultivation can trigger premature leaf senescence (Steinger, 2003; Rousseaux et al., 1996), which weakens the

plants' reproductive ability, leading to yield decreases. This situation is exacerbated in the STTPS because the PD is much higher than in common commercial tomato production, especially in the winter production period when solar light interception is limited in both upper and lower canopy leaves (Gunnlaugsson et al., 2006). In winter production period of Tokyo region of Japan, from late November to early March, more than 40% of weather condition will be cloudy, raining and snowy, making the irradiation condition of lower canopy worse. Therefore, it is important to improve the light environment in the lower canopy in STTPS to enhance the fruit yield.

Usually, cultivators use supplemental lighting to compensate for the shortage of lighting in lower leaves, which adds extra cost of electricity consumption and equipment maintenance, unavoidably adding to production costs. However, plants before anthesis are often still short and have a small leaf area so there is no mutual shading. This means the cultivation area at the immature plant stage in greenhouses has wasted space in a fixed bench system because plant and bench distances are decided by the final plant size. These conflicting situations inspired us to develop a new STTPS in which young and mature tomato plants are cultivated together to realize space-saving and yield enhancement. In this system, we adopted movable cultivation benches to manipulate the PD by adjusting the bench distance to meet light needs for plants at different developing stages. We assumed that a high PD in the vegetative stage would without affect plant reproductive potential as long as the PD were alternated to a relative lower level in the fruit development stage. However, a suitable PD to satisfy the physiological and reproductive needs for plant development at certain stages and maximize land usage needs to be determined. Therefore, the present experiment was carried out to

investigate how tomato plant morphological and physiological characters respond to changes in PD at different growth stages, and identify the most effective PD change for tomato development and reproduction.

2-2 Materials and methods

Plant material and growth conditions

Tomato ('Momotaro York', Takii Seed Co., Ltd., Kyoto, Japan) was cultivated hydroponically with a STTPS in a greenhouse in Kashiwa-no-ha, Kashiwa, Chiba, Japan ($34°53'29''$ N, $139°65'14''$ E) from November 2014 to March 2015. Seeds were sown in plug trays filled with a commercial substrate (Best Mix, Nippon Rockwool Co., Tokyo, Japan) and germinated in darkness for 3 days, then grown in a temperature-controlled chamber equipped with fluorescent tubes (Nae Terrace, MKV Dream Co., Ltd., Tsukubamirai, Japan) for 21 days. The chamber was operated at a photosynthetic photon flux density (PPFD) of 350 $\mu mol·m^{-2}·s^{-1}$, a 16-h photoperiod, 23/18 $°C$ day/night temperatures and an 800 $\mu mol·mol^{-1}$ CO_2 concentration. The trays were sub-irrigated every other day with a commercial nutrient solution at an electrical conductivity (EC) of 1.5 $dS·m^{-1}$.

On 24th day after sowing, the seedlings were transplanted to foam block cultivation benches in the greenhouse. A drip irrigation nutrient solution (Nakano et al., 2010) adjusted to 1.8 $dS·m^{-1}$ EC was automatically supplied with a feeding rate of 100-120 mL per irrigation event per plant from 06:00 until 18:00. The EC of the nutrient solution was gradually increased from 1.8 to 6.5 $dS·m^{-1}$ depending on plant growth and development. Three leaves were left under the fruit truss from anthesis, the first top pinching was performed on the 35th

day after transplanting, and three leaves were left above the fruit truss. Other maintenance, including pruning of lower leaves and removal of side shoots, was performed weekly and a 4-chlorophenoxy acetate-containing solution (Tomato Tone, ISK Biosciences K.K., Tokyo, Japan) was sprayed on completely blooming flowers and then the five most productive fruits were selected for future fruit production. During the experiment, the daytime mean air temperature was 24-30 °C, the night-time mean air temperature was 17-22 °C, and the daily mean relative humidity was maintained above 60%. Although the CO_2 concentration in the canopy was not measured, it was assumed to be close to the outside level, based on measurements in the same season in another year (data not shown).

PD treatment

Seedlings were transplanted into foam containers on a cultivation bench. In each row of benches, the plant distance was fixed at 0.1 m according to commercial production routine. Two fixed bench distances were set as controls, with row distances of 0.7 and 1.0 m, which gave PDs of 14.3 plants·m^{-2} (Lu et al., 2012a) and 10 plants·m^{-2} (Kobayashi, 1997; 1999), respectively (abbreviated as F14.3 and F10).

In the movable bench group (MB) (Figure 1), the bench distance was adjusted to minimize shading between rows. Previous literatures usually care about light interception extent (light received by the lower canopy / light received by the upper canopy), not mutual shading extent. In this study, to identify the light irradiation loss caused by mutual shading among rows of plants at a certain bench distance, we defined the bench shading index and calculated as [1- (light received by the second leaf under the fruit) / (light received by the first leaf above the fruit)] × 100%. Integrated solar radiation on the leaf surfaces was measured

with Quantum Sensors (SQ/MQ-200, Apogee Instruments, Logan, UT, USA).

According to previous research, we divided plant development period into four stages (Lu et al., 2012a; McAvoy et al., 1989): Stage 1, after transplanting to anthesis, 21 days; Stage 2, anthesis to initial fruit set, 21 days; Stage 3, fruit set to mature green, 42 days; Stage 4, mature green to red-ripe, 14 days. In this experiment, we adjusted the bench distance to give an average bench shading index of around $40 \pm 3\%$ for each stage to maintain nearly 60% of light could reach lower canopy, which equally to previous research results that when applied with supplemental lighting, the light irradiated on the lower canopy could reached 60% compared with the amount irradiated on upper canopy (calculated according to Lu et al., 2012a). Correspondingly the row distances were 0.4 m (Stage 1), 0.6 m (Stage 2), 0.8 m (Stage 3) and 0.9 m (Stage 4), with corresponding PDs of 25, 16.6, 12.5 and 11.1 plants·m^{-2}.

Leaf gas-exchange measurements

Leaf gas-exchange measurements were made on the third fully mature leaflets of leaves on the trusses both above and under the fruit truss essentially as described in work of Matsuda et al. (2014), using a portable gas-exchange measurement system (LI-6400, LI-COR Inc., Lincoln, NE, USA) between 9:00 and 14:00 on the 21st, 42nd, 84th and 98th days after transplanting. A light photosynthesis curve was made to determine the response of the net photosynthetic rate (NPR) to PPFD, and the measurement was conducted with a leaf temperature setting of $25 \pm 1°C$, ambient CO_2 concentration setting of 370 ± 10 μmol·m^{-2}, relative humidity setting of $60 \pm 2\%$, leaf-to-air vapor pressure deficit setting of 1.1 ± 0.1 kPa, and PPFD settings of 1500, 1200, 1000, 800, 600, 400, 200, and 0 μmol·m^{-2}·s^{-1}. The actual NPR of the leaf under growth conditions was measured in the same position of leaves with

actual PPFD of each treatment and the same setting of leaf temperature, ambient CO_2 concentration, relative humidity and leaf-to-air vapor pressure deficit as shown above.

Plant growth and development analyses

The lengths and diameters of stems were measured every 7 days after transplanting and the rates of stem elongation (SER) and stem diameter enlargement (DER) of each plant were calculated as the slopes of the first-order regression equations for time courses of stem length and diameter, respectively. On the 21st, 42nd, 84th and 98th days after transplanting, randomly selected samples from each group and destructively harvested. Each plant shoot was divided into leaves and stem parts, the fresh weight and total leaf area were measured, and the materials were oven dried at 100°C for 1 h followed by 80°C for 3 days to measure dry weight. Leaf area was measured with an area meter (LI-3000C, LI-COR Inc.). The roots were not subjected to measurements because of the difficulty of retrieving them from the substrate.

Growth analysis of shoots (leaves and stem) was carried out using the dry weight of each organ and total leaf area. The relative growth rate (RGR, increase in dry weight per unit time), net assimilation rate (NAR, increase in dry weight per unit of leaf area and time), leaf area ratio (LAR, leaf area/total dry weight) and leaf area index (LAI, leaf area/total area) of shoots were calculated according to the standard time-averaged equations as below (Radford, 1967; Poorter et al., 1996; Peterson, et al. 2004), except that shoot DW instead of whole-plant DW including roots was used for the calculation.

Fruit yield and quality analyses

The fruit was harvested from 99th day after transplanting, and fruit diameter, and fresh

and dry weight were measured. The soluble solids content of tomato fruit was determined using a refractometer (PAL-1, Atago Co., Ltd., Tokyo, Japan), ascorbic acid content was determined with a RQ Flex plus (Merck Co., Ltd., Darnstadt, Germany), and fruit hardness was determined with a fruit hardness tester (FR-5120, Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan). Fifty fruits were collected for fruit diameter and fresh weight determination and the relationship between with a regression equation as follows: $y = 0.0588x^2 - 1.7325x - 13.444$, with $R^2 = 0.9918$ (y = fruit fresh weight and x = fruit diameter). The fruit diameter of each group was measured every 7 days from the fruit sizing stage to harvest, and the fruit fresh weight in each stage was estimated using the equation above.

Economic performance analyses

The net profit of each treatments was calculated to analyze to economic performance. The gross profit was calculated as total yield × wholesale price. The labor cost was calculated as total labor hour × labor hourly cost, as labor hour was calculated as total working hour/cultivation area. The tomato wholesale price was collected from data published by Tokyo Metropolitan Central Wholesale Market and labor hourly cost was based on local survey. Since the moving bench system used in this study was primitive and the bench cost was almost the same as commonly used fixed bench. Therefore, we evaluated the economic performance based on the running costs. The running cost included cultivation materials input (e.g. seed price, nutrient solution), greenhouse and instruments maintenance was general estimated according to local surveys and previous production data from MKV Dream Co.

Statistical analyses

Statistical software (SPSS 11.0, SPSS Inc., Chicago, USA) was used for data analysis. Mean separations were conducted using a Tukey's HSD test protected by ANOVA (Analysis of Variance) at p < 0.05.

2-3 Results

Light conditions for plants in different treatments

Obvious shading occurred as the tomato plants grew (Figure 2). In the vegetative stages (Stages 1 and 2), the shading index was maintained at approximately 45% in all three treatments. However, the shading index in the F14.3 treatment drastically increased to 68.68% in Stage 3 and 70.4% at harvest time. There was a slight but steady increase trend in both the F10 and MB treatments, but the shading index was kept below 43% most of the time. However, F10 showed an obvious increase at Stage 4.

Leaf photosynthesis characteristics

The light response curve of leaves assess the leaf photosynthetic capacity in different treatments at each stage (Figure 3A-D). Under low light irradiation (PPFD < 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$), NPR (the net photosynthetic rate at a certain PPFD) was almost the same in all treatments. However, as the light intensity increased, differences in NPR were observed. In Stage 1 (Figure 3A), the NPR was highest in F10 (PPFD=1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and was 1.9% and 6% higher than in F14.3 and MB, respectively. From Stage 2 (Figure 3 B), the NPR values of MB and F10 approached and then became obviously higher than that of F14.3. This phenomenon was significant in Stages 3 and 4 (Figure 3 C, D). The actual NPRs in all three treatments at Stages 3 and 4 were higher than in the previous two stages (Figure 3 E). In each

stage, among the three treatments, NPR in F10 was higher than data in the other two treatments, and data in F14.3 was lowest, while MB was in the middle throughout the whole production period but was close to F10.

Whole plant growth and development

The final stem diameter, shoot DW and total leaf area were significantly lower in F14.3 (Table 1), although the plants in this treatment presented a significantly higher stem height. There were no significant differences in these indexes between F10 and MB. Table 2 shows the results of growth analysis. There was a peak of plant morphology development at Stage 1, and the speed obviously declined since then, considering the variation trends of SER, DER, RGR and NAR. In Stages 1 and 2, F14.3 presented the lowest RGR, but highest LAR among treatments, while MB and F10 showed a similarly higher RGR and NAR. In stages 3 and 4, a relatively higher RGR was observed in F14.3. Although there was no significant difference to F10, a higher NAR was observed in MB, especially in Stage 3. The correlation coefficients of these indexes are shown in Table 3. Overall, SER was negatively correlated to DER. RGR was positively correlated with NAR, but not with LAI and LAR. NAR was negatively correlated with LAR, but not with LAI.

Tomato fruit development and yield

The fruit weight increased the most in Stage 3 and the cumulative fresh weight of fruit was higher in MB and F10 than in F14.3, especially in Stage 4 (Figure 4). In terms of the harvest yield of a single plant, fresh fruit weight in MB increased 13.2% and 2.4%, and dry weight increased 13.4% and 1.7% compared with F14.3 and F10, respectively (Table 5). The

differences between F14.3 and the other two treatments were significant. To calculate the total yield of the whole winter production period, we calculated the actual calculated plant density (ACPD) of MB instead of using the plant density of MB in stage 4. Because plants were transplanted into the greenhouse and cultivated for 98d, and MB was transplanted every 14 days because of the commercial annual production schedule, thus seven benches were included in the calculation (Supplemental Figure 1). In the bench distance arrangement described above, the final distance from the 1st to 7th bench was 4.3m. Forty plants were cultivated on each bench with a distance of 4 m. Thus, the ACPD was 16.27 plants·m^{-2}, as a result of the calculation: (40 plants × 7 benches)/ (4.3 m × 4 m). Therefore, the total yield in MB was the highest, followed by F14.3 and F10, and the differences among three treatments were significant. The soluble solids content in MB and F10 was significantly higher than in F14.3, while the ascorbic acid content and fruit hardness were not significantly different among the treatments.

Economic value of different treatments

The Table 5 shows that the F14.3 and MB presented significantly higher labor cost compared with F10, but the difference within these two treatments was similar. The running cost was nearly the same among treatments. However, the net profit of MB was significantly highest, followed by F14.3 and F10.

2-4 Discussion

Previous researches has shown that high PD would aggravate mutual shadings, cause large light interception along plant profile and lead to suppressed photosynthetic activity in

lower canopy, which consequently limit plant growth and yield (Heuvelink et al., 2005; Okano et al., 2001). In this experiment, although the PDs in MB were obviously higher than those in F10 and F14.3 in Stage 1 and Stage 2, the actual net photosynthesis rates were not significantly different among treatments (Figure 3). This results indicates that a higher PD in vegetative stage would not cause a significant decrease in leaf photosynthetic capacity development. However, after fruit set, the drastically increased shading index in F14.3 (Figure 2) lead to significantly lower photosynthetic rate in this treatment, while there was no significant difference between MB and F10 (Figure 3). This indicates that the PD at least needs to be adjusted to 12.5 plant·m^{-2} at the fruit development stage. According to the FvCB photosynthesis model of C$_3$ plants (Farquhar et al., 1980; 1982; Sharkey, 1985; von Caemmerer, 2000), with normal CO$_2$ concentration, a reduced PPFD limits photosynthesis through light-harvesting and electron-transport capacities. This explained that a relatively higher PD (14.3 plant·m^{-2}) in the reproductive stage significantly affect leaf photosynthetic production in the reproductive stage. The data of SER, DER, RGR, and NAR were gradually declined as plant developed, which indicates a plant morphological development peaked at Stage 1(Table 2). Generally, light interception increased drastically as LAI increased and lead to plant growth limitation (Heuvelink et al., 2005). However, there were no significant difference in RGR and NAR between MB and F10, even with obvious LAI differences in vegetative stage, indicating a higher PD in this stage would not affect plant morphological development. The plant growth is often evaluated through analysis of parameters above (Poorter et al, 1996), and correlation coefficient between growth parameters showed a positive relationship between RGR and NAR, but a negative relationship between NAR and

LAR (Tables 2 and 3), which was in accordance with work done by Matsuda et al. (2014). Other studies has been reported that RGR, NAR and LAI has positive relationship (Heuvelink et al., 2005, Higashide et al., 2009). Higashide et al., (2009) explained that NAR could be determined by LAI, due to the influence of light distribution and leaf photosynthetic and respiration rate. But this phenomenon was divers from tomato cultivars and general cultivation condition, and tomato could grow equally when light distribution changes accompanied with changes of PD and LAI (Gunnlaugsson et al., 2006). In this study, NAR was diversly related to LAI in different stages (Table 3), and NAR was not significantly different within MB and F10 (in which LAI significantly different especially in vegetative stage, Table 2), indicating that LAI was not the only limit factor for plant morphological development.

Given the higher PD in vegetative stage would not cause a significant decrease in both leaf photosynthetic capacity and plant morphological development, it should not affect the plant reproductive potential. Meanwhile the MB treatment with relatively less shading had better light irradiation of the lower canopy in reproductive stage (Figure 2), this improved light environment should lead to enhancement of fruit yield (McAvoy and Janes, 1988; Hovi et al., 2004; Pettersen et al., 2010; Trouwborst et al., 2010). This hypothesis was confirm by the significantly higher fruit development speed and both fresh and dry yield observed in MB, compared with F14.3 (Figure 4, Table 4). Previous studies have reported that the tomato fruit assimilation rate is highest in Stage 3 and fruit is the highest priority for plant assimilate partitioning and this partition fraction increases until a steady-state occurs before harvest (Scholberg et al., 2000; Heuvelink et al., 2005). With the lower PDs in MB and F10 since

Stage 3, better light irradiation of the lower canopy (Figure 2) and higher leaf NPR was observed, compared with F14.3 (Figure 3). However, the NAR in MB and F10, was significantly lower than data in F14.3 (Table 2). This indicates that the enhancement of leaf photosynthesis rate directly augment net accumulation in fruit in this stage, contributing to more fruit yield. In addition, the strategy of periodic alteration of PD was also land saving. Cultivating young and mature plant together making the ACPD of MB was as high as 16.27 plants·m^{-2} (Supplemental Figure 1).This combined with the enhanced fruit yield of single plant, consequently significant enhanced total yield in MB. The total soluble solids content determines fruit taste, which is an important index for commercial tomato quality analysis. Insufficient light can decrease the total soluble solids content of tomato fruit (McCollum, 1944; Yanagi et al., 1995). In our experiment, the fruit in MB and F10 had significantly higher levels of soluble solids than F14.3, indicating a relatively smaller PD at the fruit development stage could increase the fruit soluble solids content. However, though there were differences in the ascorbic acid content and fruit hardness among treatments, the differences caused by PD changes were not significant. Previous research on the relationship between light irradiation and fruit ascorbic acid content has shown that ascorbate synthesis and metabolism in fruit are significantly affected by fruit irradiance in addition to leaf irradiance (Gautier et al., 2009). Additionally, the production of pectin and cellulose, which determine fruit hardness, in fruit cells is primarily controlled by the expression of genetic traits (Hadfield et al., 1998; Rose, 1997).

Labor cost is consider as highest expense in greenhouse production (Frantz et al., 2010), and the treatment of periodic alteration of PD need extra labor in PD adjusting and this may

added more labor input compared with fixed PD cultivation. Therefore, the PD strategies should be economic efficient for greenhouse production. Although the labor hour in F10 was significantly lower, there was no significantly difference between MB and F14.3 (Table 5). This suggests the treatment of periodic alteration of PD would not cause extra labor cost compared with regular intensive cultivation with high PD. Moreover, though without significant difference among treatments, the other cost was generally at the similar level of labor cost, which reconfirmed the great proportion of labor input in production costs. In this study, the net profit of MB was highest, indicating the highest economic efficiency of the treatment of periodic alteration of PD in STTPS.

2-5 Conclusion

MB could obviously remit the low light stress in lower canopies especially in reproductive stage. The tomato plant leaf photosynthesis rate in MB and F10 was generally significantly higher than in F14.3. Most of the vegetative growth occurred in Stage 1; F14.3 presented the highest stems but lowest leaf area and shoot dry weight, while MB and F10 were not significantly different. Fruit developed mostly in Stage 3, and MB showed highest total yield, followed by F14 and F10. The soluble solids content was increased in MB and F10 compared with F14.3, while no significant differences in ascorbic acid content or fruit hardness were observed among treatments. The net profit of MB was highest, followed by F14.3 and F10. Thus, a high PD in the vegetative stage but relatively lower PD in the fruit development stage was highly economically efficient.

Table 1 The final stem height, stem diameter, total leaf area and shoot dry weight (DW) of plant with fixed and movable bench treatments in single-truss tomato production system.

Treatment	Stem height (cm)	Stem diameter (mm)	Total leaf area (m^2)	Shoot DW (g)
F10	118 b	14.8 a	0.57 a	52.2 a
F14.3	124 a	13.4 b	0.41 b	50.8 b
MB	117 b	14.9 a	0.53 a	53.1 a

Means (n=12) with different letters within each row are significantly different by Tukey's HSD test at $P < 0.05$.

Table 2 Stem elongation rate (SER) and diameter enlargement rate (DER), relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR) and leaf area index (LAI) of tomato plants

Growth stage	Treatment	SER (cm·d^{-1})	DER (mm·d^{-1})	RGR (mg·g^{-1}·d^{-1})	NAR (g·m^{-2}·d^{-1})	LAR (cm^2·g^{-1})	LAI (m^2·m^{-2})
	F10	3.17 a	0.11a	184 a	5.44 a	330 c	3.3 b
Stage 1	F14.3	3.00 a	0.13a	180 b	5.46 a	356 a	3.4 b
	MB	2.46 b	0.13a	185 a	5.48 a	346 b	5.9 a
	F10	1.13 b	0.10a	110 ab	3.51 ab	307 b	4.4 b
Stage 2	F14.3	1.25 a	0.10a	106 b	3.47 b	338 a	4.8 b
	MB	1.16 b	0.10a	116 a	3.57 a	301 b	5.5 a
	F10	0.11 b	0.10a	49 b	1.92 b	274 b	5.7 b
Stage 3	F14.3	0.40 a	0.07b	53 a	2.15 a	291 a	5.9 a
	MB	0.26 b	0.07b	50 b	2.02 b	276 b	5.6 b
	F10	0.04 b	0.03a	21 a	1.89 b	248 a	5.7 a
Stage 4	F14.3	0.14 a	0.01b	22 a	1.93 a	277 a	5.9 a
	MB	0.07 b	0.01b	21 a	1.89 b	193 b	5.9 a

Means (n=12), within the same stage, with different letters within each row are significantly different by Tukey's HSD test at $P < 0.05$.

Table 3 Matrix of correlation coefficients (r) among stem elongation rate (SER), diameter enlargement rate (DER), relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR) and leaf area index (LAI) of tomato plants at different stages.

	SER	DER	RGR	NAR	LAR		SER	DER	RGR	NAR	LAR
Stage1						Stage2					
DER	-0.68*					DER	-0.28				
RGR	-0.10	-0.65*				RGR	-0.96*	0.75			
NAR	0.84*	-0.97*	0.45			NAR	-0.99*	0.17	0.99*		
LAR	-0.36	0.92*	-0.89*	-0.81*		LAR	0.99*	-0.36	-0.93*	-0.98*	
LAI	-0.97*	0.48	0.35	-0.68*	0.11	LAI	-0.58	0.94*	0.34	0.49	-0.66*
Stage3						Stage4					
DER	-0.87*					DER	-0.73*				
RGR	-0.51	0.87*				RGR	0.73*	-0.69*			
NAR	-0.58	0.91*	0.99*			NAR	0.77*	-0.99*	0.99*		
LAR	0.91*	-0.59	-0.11	-0.18		LAR	0.54	0.17	-0.17	-0.12	
LAI	-0.61*	0.16	-0.36	-0.29	-0.89*	LAI	0.98*	-0.83*	0.83*	0.86*	0.40

r value are given for all plants and asterisk (*) means significantly different at P < 0.05.

Table 4 The fresh and dry yields of plants and tomato fruit quality in different treatments.

Treatment	Fresh yield (g·plant^{-1})	Dry yield (g·plant^{-1})	Total yield (kg·m^{-2})	Soluble solid content (Bix%)	Ascorbic acid content (mg·kgFW^{-1})	Fruit hardness (kg·(LB·Newton)$^{-1}$)
F10	932 a	35.8 a	9.32 c	6.7 b	146 a	4.21 a
F14.3	843 b	32.1 b	12.05 b	5.3 c	143 a	4.13 a
MB	954 a	36.4 a	15.52 *a	6.8 a	147 a	4.16 a

* calculated as fresh yield ×actual calculated plant density (Supplemental Figure 1).

Means (n=12) with different letters within each row are significantly different by Tukey's HSD test at P < 0.05.

Table 5 Economical calculations of different treatments.

Treatment	Gross profit (JPY·m^{-2})	Labor hour (h·m^{-2})	Labor cost (JPY·m^{-2})	Running costs (JPY·m^{-2})	Net profit (JPY·m^{-2})
F10	4790.5 c	1.75 b	1662.5 b	2651.6 a	476.4 c
F14.3	6193.7 b	2.50 a	2375.0 a	2746.9 a	1071.8 b
MB	7977.3 a	2.71 a	2574.5 a	2790.1 a	2712.7 a

Based on data published by Tokyo Metropolitan Central Wholesale Market, the wholesale price of tomato was 514 JPY· kg^{-1} (March, 2015, when fruit harvest) and the labor hourly cost was 950 JPY· h^{-1} based on local survey. The gross profit was calculated as total yield × wholsale price. The labor hour was calculated as total working hour/cultivation area. The labor cost was calculated as total labor hour × labor hourly cost. The running cost included cultivation materials input (e.g. seed price, nutrient solution), greenhouse and instruments maintenance according to local surveys and previous production data from MKV Dream Co.

Means (n = 4) with different letters within each row are significantly different by Tukey's HSD test at P < 0.05.
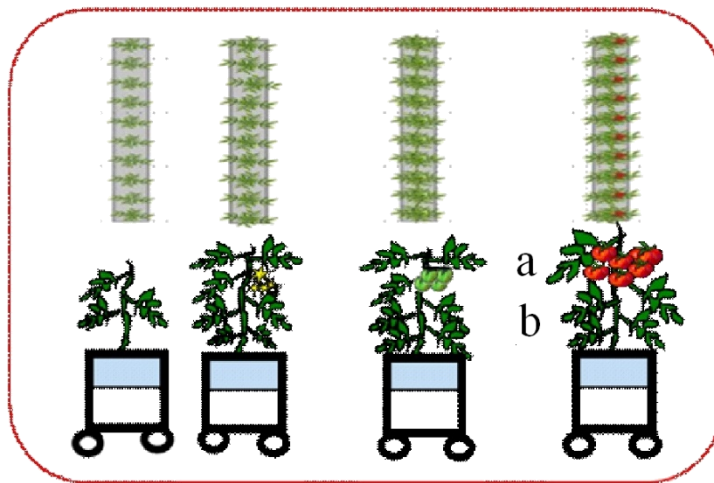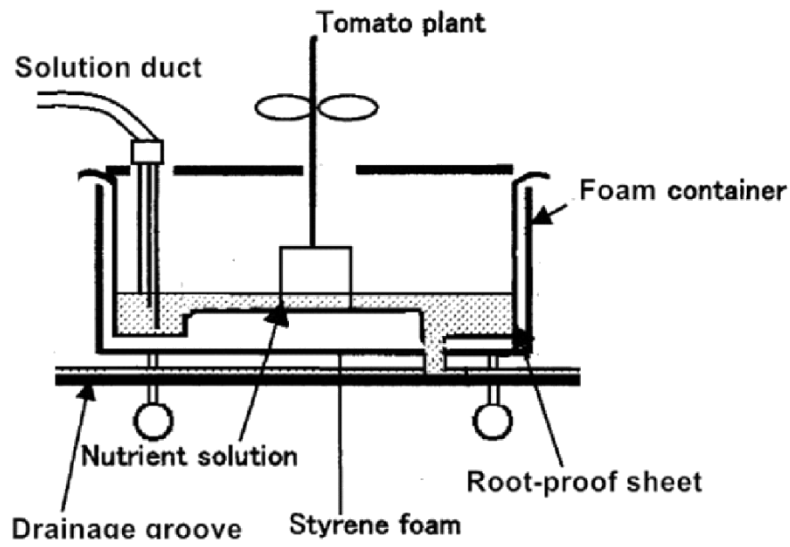
Figure 1 │Schematic diagram of movable bench system. Bench shading index = (1-b/a) × 100%, both side light irradiation checks were carried out every day at 10:00, 12:00, 14:00 and 16:00.
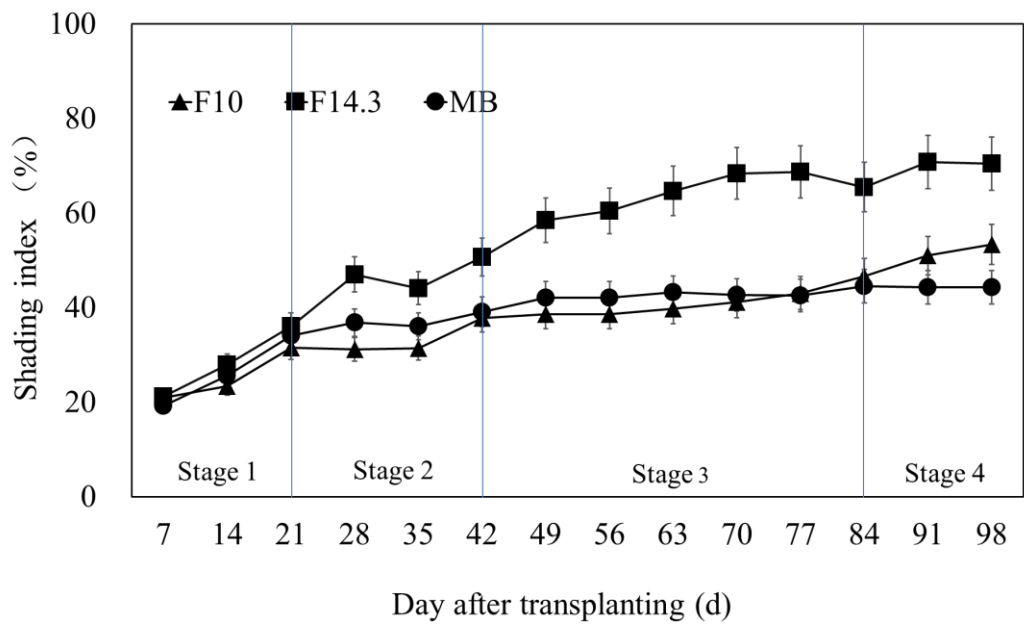
Figure 2 │ Changes of the shading index with fixed and movable bench treatments in single-truss tomato production system. Vertical bars represent standard errors of the means (*n* = 12).
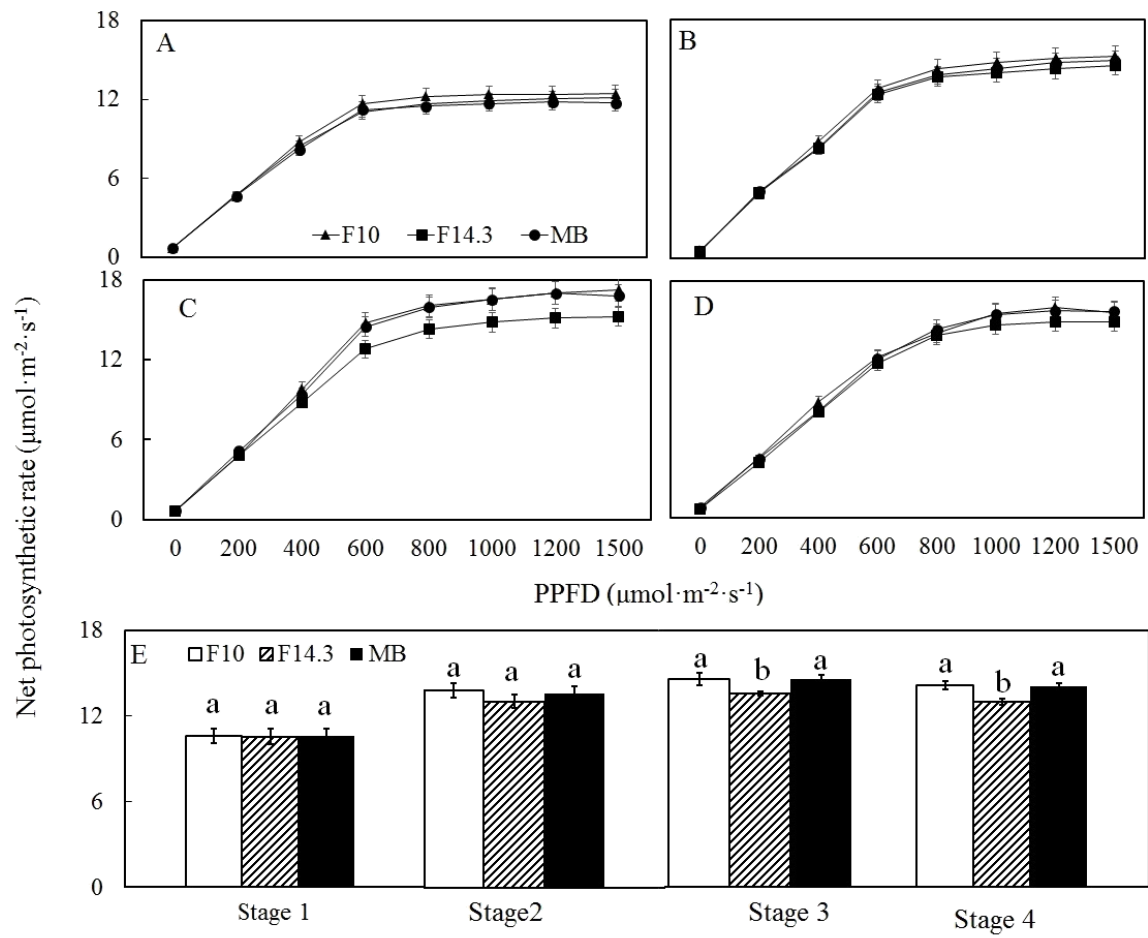
Figure 3 │ Effects of different plant density (PD) treatments on photosynthesis. Light response curve was measured at photosynthetic photon flux density of 0, 200, 400, 600, 800, 1000, 1200, and 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in leaves of tomato plants at different PDs at Stage 1(A), Stage 2 (B), Stage 3 (C) and Stage 4 (D) and actual net photosynthetic rate of each stage was determined under growth condition (E). Vertical bars represent standard errors of the means (n = 8–16). Means with an asterisk (*) within each panel are significantly different by Tukey's HSD at $P < 0.05$.
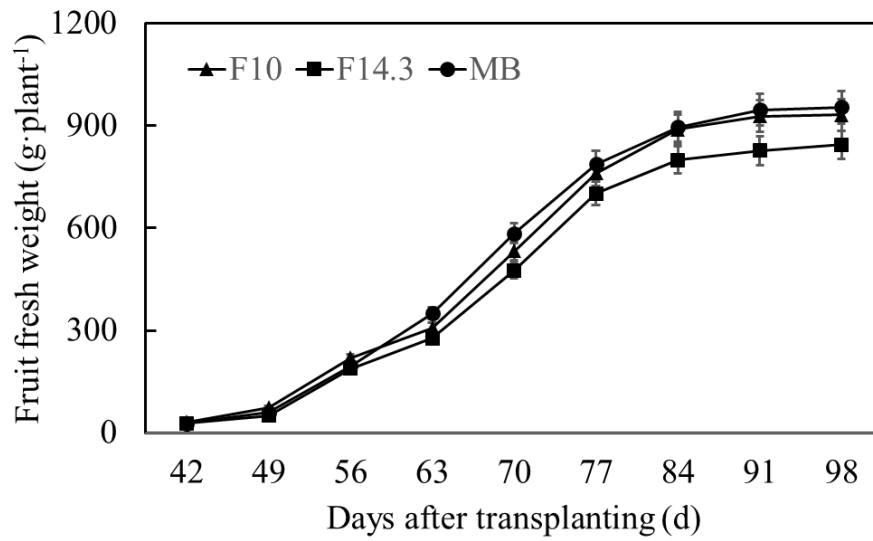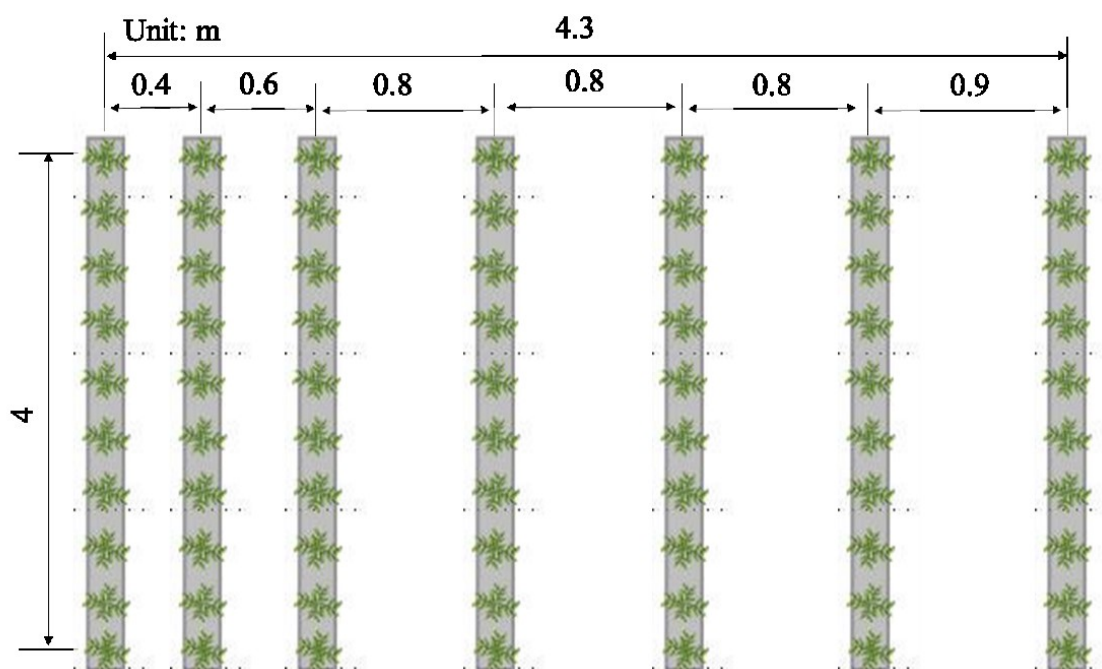
Figure 4 │ The variation of cumulative fruit growth of tomato plants in different treatments. Vertical bars represent standard errors of the means ($n = 12$).

Supplemental Figure 1 │Schematic diagram of the winter actual cultivation area used to calculate the actual calculated plant density (ACPD) in the movable bench system. Plants were transplanted every 14 days and the bench distance was adjusted according to the physiological stages described previously. There were seven benches and each bench contained 40 plants in the winter production period. Thus, the ACPD in MB for the total yield calculation was 16.27 plants·m^{-2}.

Chapter 3

Periodic altered plant density enhances tomato leaf photosynthesis, plant growth and fruit productivity in the summer by optimizing the inter-plant light environment

3-1 Introduction

Light not only actuates photosynthesis to provide energy but also triggers numerous physiological processes, such as seed germination (De Villers et al., 1994; Zohar et al., 1975), leaf development (Evans et al., 2001; Fu et al., 2011), flowering (Cerdán et al., 2003; Goto et al., 1991), stomatal regulation (O'Carrigan et al., 2014; Shimazaki et al., 2007) and the membrane transport of cells (Mullineaux et al., 2002) to extensively regulate the growth and development of plants, which largely determines greenhouse crop productivity. In greenhouse-based winter crop production, insufficient amounts of light reaching the canopy levels appears to be a consequence of intensive cultivation schedules. However, the situation is usually reversed in the summer cultivation period. In Japan, the average photosynthetic photon flux density (PPFD) at the top canopy exceeds 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the midday period on sunny days. Excessive light irradiation leads to changes, ranging from those at the macroscopic whole-plant level to those at the microscopic ion investment level (Evans et al., 2001). The biomass balance of plants can be disturbed by surplus irradiation (Björkman 1981), causing a decreased allocation to leaves and stems, while increasing the fraction to roots (Brouwer 1962; Poorter et al., 2000) to maintain a constant transpiration rate per unit root mass (Sims et al., 1994). Oxidative damages also occur when plants are treated with excessive

light, which impairs the photosynthetic apparatus and induces the bleaching, chlorosis and bronzing of leaves (Karpinski et al., 1999; Mullineaux et al., 2002). To fight such oxidative damage, plants have developed a series of protective strategies, such as chloroplast avoidance (Kagawa et al., 2001), decreases in photosynthetic reaction centers (Walters et al., 1999), altered stomatal behaviors (Willmer et al., 1996), leaf curling (Neuner et al., 1999) and even increases in leaf epidermal wax deposits (Horton et al., 1996), which, however, weaken electron transport rates, obstruct photosynthetic quantum yields and generate plant growth delays, resulting in death in extreme cases (Jiang et al., 2006).

In general, extra sunshade can decrease the solar irradiation entering a greenhouse, but this method also decreases the light intensity in the lower canopy, leading to reductions in leaf photosynthesis and potential yields (Hovi, et al., 2004; Lu, 2012). In addition, as plants grow, mutual shading will spontaneously occur, which can obstruct excessive solar light along the plant profile. Thus, mutual shading may solve the conflictive situation caused by extra sunshade. Meanwhile, plant density (PD), similar to mutual shading, can affect plant architecture, and alter growth and developmental patterns (Bleasdale et al., 1960; Casal et al., 1985; Sangoi, 2001). Although previous studies on PD have investigated organ development and crop productivity in different species (Dong et al., 2005; Gardner et al., 1985; Sangoi, 2001; Verheul, 2012), the research has mainly focused on fixed PD conditions through the production process, and reports investigating suitable PDs at different growth stages are limited.

Tomato (*Solanum lycopersicum* L.) is an important horticultural crop worldwide. During the midday period, in the hot summers of the Tokyo Region of Japan, the PPFD of the plant's

top canopy often exceeds 2000 μmol·m^{-2}·s^{-1} and the inter-plant temperature often exceeds 30°C, which strongly suppresses the tomato growth process and reduces production. Tomato seedlings become significantly more spindly when continuously irradiated at PPFDs of over 800 μmol·m^{-2}·s^{-1} (Fan et al., 2013), and the fruit size and development are significantly affected when the PPFD is over 1200 μmol·m^{-2}·s^{-1} and the temperatures are over 30°C (Charles et al., 1972; Dumas et al., 2003; Kläring et al., 2013). Since the inter-plant temperature is closely correlated with light irradiation (Bristow et al., 1984), it is possible to improve the growth and productivity of tomato plants in the summer season by optimizing the inter-plant light conditions. Information on the relationship between periodic altered PDs and inter-plant environments, and the cultivation effects of this technique under high-light irradiation conditions, are limited in the present literature. In this study, we assumed that a periodic altered PD, which was determined based on the plant developmental stage, could create suitable mutual shading to remit excessive light stress with less influence on plant productivity under high-light irradiation conditions. We compared the whole-plant morphologies, leaf gas exchange rates, and fruit yields and qualities of tomatoes cultivated under fixed and periodic altered PD conditions to investigate the effects of the latter on the optimization of inter-plant irradiation and to better understand the physiological mechanisms involved in tomato growth, development and productivity under stressful conditions.

3-2 Materials and methods

Plant material and growth conditions

Tomato ('Momotaro York'; Takii Seed Co., Ltd., Kyoto, Japan) was cultivated

hydroponically, adopting the single-truss tomato production system in a Venlo-type greenhouse in Kashiwa-no-ha, Kashiwa, Chiba, Japan (34°53'29.46''N, 139°65'14.1''E) from June to September 2015. Seeds were sown into plug trays filled with a commercial substrate (Best Mix; Nippon Rockwool Co., Tokyo, Japan) and germinated in darkness for 3 days, then grown in a temperature-controlled chamber equipped with fluorescent tubes (Nae Terrace; MKV Dream Co., Ltd., Tsukubamirai, Japan) for 21days before transplanting. The chamber was operated at a PPFD of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a 16 h photoperiod, 23/18°C day/night temperatures and 800 $\mu mol \cdot mol^{-1}$ CO_2 concentration. The trays were sub-irrigated every other day with a commercial nutrient solution at an electrical conductivity (EC) of 1.5 $dS \cdot m^{-1}$. After transplanting, seedlings were cultivated in the greenhouse at an average air temperature of 33.2°C/25.6°C (day/night) and an average relative humidity above 60%. A drip irrigation nutrient solution adjusted to 1.8 $dS \cdot m^{-1}$ EC was automatically supplied, with a feeding rate of 100–120 mL per irrigation event per plant from 6:00 until 18:00 h. The EC of the nutrient solution was gradually increased from 1.8 to 6.5 $dS \cdot m^{-1}$, depending on plant growth and development. Plant leaves were pruned to have three leaves left under the fruit truss after anthesis, and the first top pinching occurred on the 28th day after transplanting, leaving three leaves above the fruit. Other maintenance, including pruning lower leaves and removing side shoots, took place on a weekly basis. A 4-chlorophenoxy acetate-containing solution (Tomato Tone, ISK Biosciences K.K., Tokyo, Japan) was sprayed on completely blooming flowers, and then five most productive fruits were selected for future fruit production.

PD treatment

Seedlings were transplanted into foam containers on cultivation benches. In each row,

the plant distance was fixed at 0.1 m owing to the commercial production system. Two fixed bench distances were settle as controls, resulting in row distances of 0.7 and 1.0 m, causing PDs of 14.3 plant·m^{-2} (F14.3; Lu, 2012) and 10 plant·m^{-2} (F10), respectively (Table 1) (Kobayashi, 1997; 1999). Movable benches (MB) were used for periodic altered PD group cultivation (Figure 1).

To identify the light irradiation loss caused by branch and leaf shading between two rows of plants at a certain bench distance, we defined the bench shading index and calculated it as [1− (light received by the second leaf under the fruit)/ (light received by the first leaf above the fruit)] × 100% to indirectly measure distance changes that were required during different physiological periods. Light irradiation accumulation was measured using Quantum Sensors (SQ/MQ-200; Apogee Instruments, Utah, USA) on the surface of the leaves. The inter-plant temperature was measure by Thermal Sensors (RS-13H; Espec Inc., Aichi, Japan) in the middle canopy.

Based on previous research, we divide the plant developmental period into four stages (Lu et al., 2012a; McAvoy et al., 1989): Stage 1 occurred from after transplanting to anthesis, 14 d; Stage 2 occurred from anthesis to initial fruit set, 14 d; Stage 3 occurred from fruit set to mature green, 42 d; and Stage 4 occurred from mature green to red-ripe, 14 d. In this experiment, we used a bench distance that maintained a bench-shading index of 40 ± 3% for each stage to maintain nearly 60% of light could reach lower canopy (calculated according to Lu et al., 2012a), which meant row distances were 0.3 m (Stage 1), 0.5 m (Stage 2), 0.7 m (Stage 3) and 0.8 m (Stage 4), resulting in corresponding PDs of 33.3, 20, 14.3 and 12.5 plant·m^{-2} (Table 1).

Leaf gas-exchange measurements

Leaf gas-exchange measurements were carried out on the third fully matured leaflets of leaves on both trusses above and below the fruit truss (Matsuda et al., 2014; Tewolde et al., 2016), using a portable gas-exchange measurement system (LI-6400; LI-COR Inc., Lincoln, NE, USA) between 9:00 and 14:00 on the 14th, 28th, 70th and 84th day after transplanting. The net photosynthesis rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r) were measured at the PPFD of 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a leaf temperature of 28 ± 1°C, CO_2 concentration of 400 ± 2 $\mu mol \cdot m^{-2}$, and relative humidity of 63 ± 2%. The light photosynthesis curve was constructed through step-wise increases in the irradiance from darkness to 1,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at a CO_2 concentration of 400 ± 2 $\mu mol \cdot m^{-2}$.

Plant growth and development analyses

The plant growth and development analyses were carried out essentially as described by Matsuda et al. (2014). The stem lengths and leaf chlorophyll contents were measured every 7 days after transplanting with a ruler and a chlorophyll meter (SPAD–502; Minolta, Osaka, Japan), respectively. The stem elongation rate (SER) of each plant was calculated as the slope of the first-order regression equation of the time course of stem length. On the 14th, 28th, 70th and 84th day after transplanting, samples were divided into leaves and stem, the fresh weights and total leaf areas were measured, and the materials were oven dried at 100°C for 1 h, followed by 80°C for 3 days to measure the dry weights (DWs). Leaf area was measured using an area meter (LI-3000C; LI-COR Inc., Lincoln, NE, USA). Roots were not subjected to measurements because of the difficulty of retrieving them from the substrate. The growth

analysis of shoots (leaves and stem) was carried out using the dry weight of each organ and the total leaf area. The net assimilation rate (NAR), leaf area ratio (LAR) and leaf area index (LAI) of shoots were calculated according to the standard time-averaged equations (Radford,1967; Poorter et al., 1996; Peterson, et al. 2004), except that shoot DW, instead of whole-plant DW (which included the root), was used for the calculations.

Fruit yield and quality analyses

The fruit were harvest from September 14th to 21st, and fruit diameters as well as fresh and dry weights were measured. The relationship between fruit diameter and fresh weight was determined by a regression equation as follows: $y = 0.061x^2 - 1.25x - 15.144$, with $R^2 = 0.993$, where y = fruit fresh weight and x = fruit diameter. The fruit diameter of each group was measured every 7 days from the fruit sizing stage to harvest, and the fruit fresh weight of each stage was estimated based on the equation listed above. The fruit quality parameters included dry weight, hardness, juice pH, juice EC, soluble solid content and ascorbic acid content. The fruit hardness was determined using a hardness tester (FR-5120; Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan), and both juice pH and EC were determined using a conductivity meter (ES-51; Horiba Co., Ltd., Kyoto, Japan). The fruit soluble solid content was determine using a refractometer (PAL-1; Atago Co., Ltd., Tokyo, Japan), and the ascorbic acid content was determined using an RQ Flex plus (Merck Co., Ltd., Darnstadt, Germany).

Economic performance analyses

The net profit of each treatments was calculated to analyze to economic performance. The gross profit was calculated as total yield × wholesale price. The labor cost was calculated

as total labor hour × labor hourly cost, as labor hour was calculated as total working hour/cultivation area. The tomato wholesale price was collected from data published by Tokyo Metropolitan Central Wholesale Market and labor hourly cost was based on local survey. Since the moving bench system used in this study was primitive and the bench cost was almost the same as commonly used fixed bench. Therefore, we evaluated the economic performance based on the running costs. The running cost included cultivation materials input (e.g. seed price, nutrient solution), greenhouse and instruments maintenance was general estimated according to local surveys and previous production data from MKV Dream Co.

Statistical analyses

Statistical software (SPSS 11.0, SPSS Inc., Chicago, IL, USA) was used for all analyses. Mean separations were conducted using a Tukey's honest significant difference test predicted by an analysis of variance at $P < 0.05$.

3-3 Results

Light conditions and thermal environments of plants under different treatments

During the cultivation process in Stages 1 and 2 the shading index within the plant canopy remained steady and was lower than 40% at every PD before fruit developed (Figure 2). As fruit developed, the shading index increased in F10 and F14.3, becoming greater than 55%, and this trend continued until the final harvest. Within the fixed bench treatments, the variation of shading in F14.3 was more acute than in F10. The thermal environment within the canopy was relevant to the inter-plant light condition. Generally, the daily high temperature in the MB was lower than that of the F10 and F14.3, except for at Stage 4, during which a high

shading effect occurred in the fixed bench treatments and the increase was less than 0.5°C.

This indicated that plants in the MB had greater thermal dissipation capabilities.

Leaf photosynthesis characters

The light response curves of the leaves (Figure 3 Ai, Aii, Aiii and Aiv) showed that at a

low light irradiation (PPFD less than 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$), the P_n of each treatment was almost

the same. However, as the light intensity increased, a difference in the P_n occurred and,

generally, the MB showed their highest saturated P_n, while F10 and F14.3 presented a similar

capacity, excepted in Stages 2 and 3. The decrements between the MB and fixed bench groups

gradually widened as the plants grew and were greatest in Stage 4, during which the Pn (1,500)

in the MB was 24.2% and 26.4% higher than those of the F14.3 and F10, respectively. In

Stages 1 and 2, the leaf P_n, G_s, C_i and T_r showed the same trend as the MB, followed by F14.3

and then F10, and the data from the MB were significantly higher than those of the other

groups (Figure 3Bi, Bii, Ci, Cii, Di, Dii, Ei and Eii). However, in Stages 3 and 4, the P_n and

G_s followed the same trend (Figure 3Biii, Biv, Ciii and Civ), while the C_i's trend was the

reverse (Figure 3Ciii and Civ). No significant differences in the T_r were observed among the

bench systems and plant densities (Figure 3Eiii and Eiv).

Whole plant growth and development

Except for the stem diameter, the data on final stem height, total leaf area and shoot DW

were significantly higher in the MB (Table 1). Generally, there were significant differences

among different treatments, but the stem heights of the F10 and F14.3, and the stem diameters

of the F14.3 and MB, presented at similar levels. Table 2 shows the results of the growth

analysis. There was a peak of plant morphological development at Stage 1 and the rate declined after entering the reproductive stages, as demonstrated by the variation in the SER and NAR. The MB plants had the highest SER, NAR and chlorophyll content, but the lowest LAR among the treatments. Generally, there was a negative relationship between the NAR and LAR.

Tomato fruit development and yield

The fruit weight increased the most during Stage 3, and the cumulative fresh weights of fruit were higher in the MB and F14.3 than in the F10, especially during Stage 4 (Figure 4 A). Total fruit fresh weight per plant in the MB increased 10.1% and 5.3%, compared with F10 and F14.3 respectively. The yield per cultivated area at each PD treatment was calculated as the fruit fresh weight per plant × plant density. For the annual production in MB, plants were transplanted every 14 days. Thus, we calculated the actual calculated plant density (ACPD) of the MB. In this study, values for 84 d in total 7 benches were calculated (Figure 5). The final distance from the 1st to 7th bench was 3.7 m. In total, 40 plants were cultivated in each bench, with a distance of 4 m. Based on the calculation (40 plants × 7 benches) / (3.7 m × 4 m), the ACPD was determined to be 18.9 plant·m^{-2}. Therefore, the total yield in the MB was significantly high, followed by F14.3 and F10 (Figure 4 B).

The fruit quality indexes were affected by the PD treatments to varying degrees (Table 3). Except for fruit hardness and juice PH, the data of other indexes were significantly different among the three PD treatments. The fruit DW of the MB was 11.4% and 26.4% higher than F14.3 and F10, respectively. The juice EC of F14.3 was the highest, while that of the MB was the lowest. The soluble solid content of the MB was 28.3% and 11.5% higher than those of

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the F14.3 and F10, respectively, and the ascorbic acid content of the MB was 18.1% and 10.6% higher than those of the F14.3 and F10, respectively.

Economic value of different treatments

The Table 4 shows that labor cost was significantly different among treatments, and the number in MB was significantly highest followed by F14.3 and F10. The other cost was significantly lower in the F10 while there was no significant difference between the F14.3 and MB. However, the net profit of MB was still significantly highest, followed by F14.3 and F10.

3-4 Discussion

In addition to genetics, environmental factors greatly affect plant morphogenesis and development (Aldesuquy et al., 2000; Hogewoning et al 2010). Compared with other factors, light irradiation conditions not only influence plant growth as a separate abiotic factor that provides an energy source for physiological reactions and regulatory signals (Franklin et al., 2005; Hoenecke et al., 1992), but they also regulate the thermal environment that synergistically and indirectly affects plant development (Bristow et al., 1984; Greffet et al., 2002). In the high-light irradiation season, surplus irradiation can disrupt the biomass allocation balance (Poorter et al., 2000), causing oxidative damage (Karpinski et al., 1999; Mullineaux et al., 2002), and impairing the photosynthetic apparatus (Jiang et al., 2006; Willmer et al., 1996), as well as increasing the surrounding temperatures, which induces thermal stress on plants, ultimately restricting the productive capacity and quality of greenhouse crop production. Previous studies mainly focused on avoiding shading in cultivation practices because it leads to decreasing yields and benefits (Cockshull et al., 1992;

Xu et al., 1997). However, in the high-light irradiation season, shading is necessary, because the failure to dissipate or avoid excessive light induces physiological damage to plants (Karpinski et al., 1999; Mullineaux et al., 2002). In this study, the plant's vegetative growth was concentrated in Stages 1 and 2 according to the SER, NAR and LAR data (Table 2), indicating that leaves underwent the greatest development during these periods and were also more sensitive to excessive light stress. The inter-plant shading in fixed PD treatments was lower than in the MB, even at the end of Stage 2, when the shading indexes in the F10 and F14.3 were 21.7% and 7.1%, respectively, lower than in the MB (Figure 2). During the experimental period, the average PPFD at the plant's top canopy exceeded 2,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the midday period on sunny days. This suggested that possible excessive irradiation damage might occur to the leaf morphology and photosynthetic apparatus, compared with in Jiang et al. (2006), in which photo damage occurred in leaves after being continuously irradiated at greater than 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Additionally, the temperature in the MB was lower than those in the F10 and F14.3, providing a more suitable micro-climate for plant development. At Stages 3 and 4, although the shading in the F10 and F14.3 was higher than in the MB, the inter-plant temperature was not theoretically higher in the MB (Bristow et al., 1984; Greffet et al., 2002), indicating that plant morphology changes, such as leaf curling and senescence, had occurred in the fixed PD treatment.

Plant photosynthesis is extremely sensitive to supra-optimal light conditions. The P_n– PPFD curves (Figure 3 Ai, Aii, Aiii and Aiv) of tomato plants illustrated the differences in the photosynthetic capabilities in leaves among different PD treatments. Compared with F10 and F14.3, the MB showed a relatively steady saturated P_n that was the highest for the whole

process. The leaf chlorophyll content (Table 2) in the MB was significantly higher, indicating that the integrity of the photosynthetic apparatus and the light harvest efficiency were not affected in the MB. Generally, it demonstrated that the light condition in the MB treatment guaranteed a photosynthetic capacity of leaves greater than inhibition. Takahashi et al. (2008) reported that photosystem I could be readily photo-inhibited by high-light stress, and the repair system of photosystem II would be inhibited simultaneously. In our study, leaves of different PD treatments presented the same trends for P_n, G_s, C_i and T_r, with those of MB being highest during Stages 1 and 2 (Figure 3 Bi, Bii, Ci, Cii, Di, Dii, Ei and Eii), indicating that the increased P_n in the MB was probably due to the improvement of stomatal conductance, which enabled sufficient CO_2 for photosynthesis. As plants enter the reproductive stage, leaves start aging and undergo senescence (Steinger, 2003; von Caemmerer, 2000), but in Stages 3 and 4, the data of the index showed no large declined in the P_n of the MB, the G_s was still significantly higher in the MB, while the C_i was the lowest and the Tr showed no significant difference among treatments (Figure 3 Biii, Biv, Ciii, Civ, Diii, Div, Eiii and Eiv), indicating that the increased P_n in the MB was caused by an improved CO_2 supplying and assimilation. According to the Farquhar, von Caemmerer and Berry model (Farquhar et al., 1980; von Caemmerer, 2000), at a normal CO2 atmosphere, photosynthesis under higher PPFD is limited by the ribulose-1,5-bisphosphate carboxylation capacity of Rubisco, as well as by the CO_2 diffusivity from the intracellular atmosphere to the chloroplast stroma. Therefore, our results suggested that the MB could maintain a higher ribulose-1,5-bisphosphate carboxylation capacity and/or CO_2 diffusion from the intercellular space to stroma, especially during the reproductive stage. As a result of the enhanced

photosynthesis behavior, the significantly longest stem, heaviest shoot DW, and largest leaf area were all observed in the MB treatment (Table 1), confirming that the inter-plant shading could relieve the influence caused by the direct exposure to high-light irradiation. The impact was tracked using the NAR and LAI, which were also higher in the MB, of different physiological stages (Table 2). According to Heuvelink (2005), the average amount of light reaching the lower canopy at 3 $m^2 \cdot m^{-2}$ LAI was 54% less than that at 1 $m^2 \cdot m^{-2}$ LAI. Thus, at the peak period of plant morphology, Stages 1 and 2, the higher LAI in the MB could prevent more than 50% of the excessive light from reaching the inter-canopy. This would prevent approximately 40% of the photochemical-induced damage to fragile and immature leaves (Jiang et al., 2006) and decrease the photosynthetic productivity loss, which was confirmed by the variation of the P_n data among treatments during different stages (Figure 3 Bi, Bii, Biii and Biv). Combining the P_n variation with G_s, C_i and T_r, as well as shoot DW (Table 1), SER, NAR and LAI (Table 2), indicated that the plants in the MB were characterized by increased ion transportation capabilities and carbon transmissions.

An optimized light environment can improve crop yields (Hovi et al., 2004; McAvoy et al., 1989; Pettersen et al., 2010). In our experiment, the MB treatment presented a significantly higher fruit development rate (Figure 4 A) and product yield compared with F10 and F14.3 (Figure 4 B). In Stage 3, tomato fruit assimilate import and net carbon fixation rates are highest (Scholberg et al., 2000; Heuvelink, 2005). Fruit at this stage are the first priority of plant assimilate partitioning and this fractional partition increases until a steady state occurs before harvesting (Heuvelink, 2005; McAvoy et al., 1989). Thus, the photosynthetic difference during this sensitive stage and along the inter-canopy level should

reflect most of the fruit production. In our experiment, the P_n in the MB was significantly higher (Figure 3 Bi, Bii, Biii and Biv) and correspondingly promoted significantly higher fruit fresh weights (Figure 4 B).

Light stress decreases tomato fruit quality and further affects market consumption (Dumas et al., 2003; Mc Collum, 1944; Yanagi et al., 1995). Although most fruit development occurs in Stage 3, and PD was the same in the MB and F14.3 and higher than in the F10. The difference in the fruit quality index indicated that after two stages of development, the ability of plants to reproduce varied. The total soluble solids content determines fruit taste, and is an important index of the tomato's commercial quality. The total soluble solids content of plants in the MB was significantly higher than those of the F10 and F14.3 (Table 3). There is a correlation between plant photosynthetic assimilation and the fruit sugar content in strawberry (Anttonen et al., 2006), grape (Reynolds et al., 1994) and apple (Robinson et al., 1983). Thus, the higher soluble solids content level could be regarded as a result of greater net photosynthetic assimilation in plants. Similarly, the fruit ascorbic acid content can increase 35% when tomato fruit is exposed to higher light irradiation (Mc Collum, 1944; Gautier et al., 2008; Hovi et al., 2004). Thus, the enhanced ascorbic acid content in the MB as a result of fruit being exposed higher levels of direct light could be caused by the lower level of shading from inter-plantings at this stage (Figure 2). The juice PH and EC values are regarded as measures of the cell-free mineral content, and they readily fluctuate with changes in the thermal environment because the minerals are transported from roots to aerial organs through water transpiration, which is affected by air temperature (Riga et al., 2008). Although the long-distance transport and accumulation of minerals in leaves and fruit is not proportional to

plant transpiration (Schulze et al., 1984; Tanner et al., 2001), leaf transpiration affects fruit mineral accumulation in berries (Boselli et al., 1998). The low values of leaf T_r in Stages 3 and 4 (Figure 3 Diii and Div) can be explained by the lowest EC level (Table 3) in the MB because the ion accumulation in tomatoes is dependent not only on leaf transpiration, but also on undetermined physiological processes affected by temperature (Riga et al., 2008). However, environmental factors do not affect all of the fruit quality indexes. Fruit hardness is usually determined by the amount of pectin and cellulose in fruit cells, which is mainly controlled by expressed genetic traits rather than environmental factors (Hadfield et al., 1998; Rose, 1997). This explains why no variability in fruit hardness was found among the treatments.

Similar to winter production results, labor cost is still consider as highest expense in greenhouse production (Frantz et al., 2010), and the treatment of periodic alteration of PD added significantly more labor hour compared with fixed PD cultivation, resulting in significantly higher labor input (Table 4). This suggests the treatment of periodic alteration of PD in high irradiation season would cause extra labor cost compared with regular intensive cultivation with high PD, which is different with the results in winter production. Moreover, though without significant difference between the F14.3 and MB, the other cost was significantly higher than F10. However, due to the significantly higher total yield, the net profit of MB was still highest, re-demonstrating the highest economic efficiency of the treatment of periodic alteration of PD in STTPS.

3-5 Conclusion

Periodic altering PD treatment could optimize the inter-plant light condition and thermal

environment in high irradiation season. Tomato plant accumulated most vegetative growth at Stage 1 and fruit developed most at Stage 3. Leaf photosynthesis ability was guaranteed by periodic altering PD treatment via declining curling and senescence and enhancing of CO_2 assimilation. The optimized inter-plant environment also improved fruit carbon assimilation which promoted yield and quality. Although the treatment of periodic alteration of PD in high irradiation season would cause extra labor cost compared with regular intensive cultivation with high PD. However, due to the significantly higher total yield, the net profit of MB was still highest. Therefore, the periodic altering PD treatment was effective in dissipating excessive light stress and maintaining high economic efficient for greenhouse tomato production.

Table 1 Final stem heights, diameters, total leaf areas and shoot dry weights (DWs) of tomato plants growing in fixed and movable bench systems in a single tomato production scheme.

Treatment	Stem height (cm)	Stem diameter (mm)	Total leaf area (m^2)	Shoot DW (g)
F10	125.2 b	14.8 a	0.47 c	51.2 c
F14.3	124.8 b	14.2 b	0.51 b	53.8 b
MB	138.1 a	14.1 b	0.61 a	59.1 a

Means (n = 12) with different letters within each row are significantly different by Tukey's HSD test at $P < 0.05$.

Table 2 Stem elongation rate (SER), net assimilation rate (NAR), leaf area ratio (LAR), leaf area index (LAI) and leaf chlorophyll content of tomato plants.

Growth stage	Treatment	SER (cm·d^{-1})	NAR (g·m^{-2}·d^{-1})	LAR (cm^2·g^{-1})	LAI (m^2·m^{-2})	SPAD
	F10	3.01 a	5.42 a	402 a	2.4 b	40 b
Stage 1	F14.3	3.03 a	5.46 a	395 b	3.6 b	42 b
	MB	3.06 a	5.64 a	390 c	9.3 a	45 a
	F10	1.23 b	3.37 b	301 b	3.6 c	36 b
Stage 2	F14.3	1.25 b	3.40 b	338 a	4.7 b	41 ab
	MB	1.31 a	3.81 a	307 b	7.5 a	46 a
	F10	0.20 b	2.05 b	281 ab	4.3 c	41 b
Stage 3	F14.3	0.27 a	2.02 b	291 a	5.6 b	40 b
	MB	0.26 a	2.12 a	276 b	6.9 a	49 a
	F10	0.04 b	1.53 a	252 a	4.7 b	40 b
Stage 4	F14.3	0.07 a	1.50 a	277 a	7.3 a	46 ab
	MB	0.07 a	1.59 a	223 b	7.6 a	52 a

Means (n = 12), within the same stage, with different letters within each row are significantly different by Tukey's HSD test at $P < 0.05$.

Table 3 Fruit hardness, dry weight, juice PH and EC, and contents of soluble solids and ascorbic acid for tomatoes grown at different plant densities in fixed and movable bench systems.

Treatment	Fruit hardness ($kg \cdot (LB \cdot Newton)^{-1}$)	Dry weight ($g \cdot plant^{-1}$)	PH	EC ($dS \cdot m^{-1}$)	Soluble solid content (Bix%)	Ascorbic acid content ($mg \cdot kgFW^{-1}$)
F10	4.31 a	31.8 c	4.5 a	5.4 b	6.1 b	142 b
F14.3	4.25 a	36.1 b	4.4 a	5.7 a	5.3 c	133 c
MB	4.26 a	40.2 a	4.5 a	5.1 c	6.8 a	157 a

Means (n = 12) with different letters within each row are significantly different by Tukey's HSD test at $P < 0.05$.

Table 4 Economical calculations of different treatments.

Treatment	Gross profit (JPY·m^{-2})	Labor hour (h·m^{-2})	Labor cost (JPY·m^{-2})	Running costs (JPY·m^{-2})	Net profit (JPY·m^{-2})
F10	4270.0 c	1.67 c	1586.5 c	2071.6 b	611.9 c
F14.3	6425.2 b	2.01 b	1909.5 b	2486.1 a	2029.6 b
MB	8962.0 a	2.43 a	2308.5 a	2519.7 a	4133.8 a

Based on data published by Tokyo Metropolitan Central Wholesale Market, the wholesale price of tomato was 449 JPY· kg^{-1} (September, 2015, when fruit harvest) and the labor hourly cost was 950 JPY· h^{-1} based on local survey. The gross profit was calculated as total yield × wholsale price. The labor hour was calculated as total working hour/cultivation area. The labor cost was calculated as total labor hour × labor hourly cost. The running cost included cultivation materials input (e.g. seed price, nutrient solution), greenhouse and instruments maintenance according to local surveys and previous production data from MKV Dream Co.

Means (n = 3) with different letters within each row are significantly different by Tukey's HSD test at $P < 0.05$.
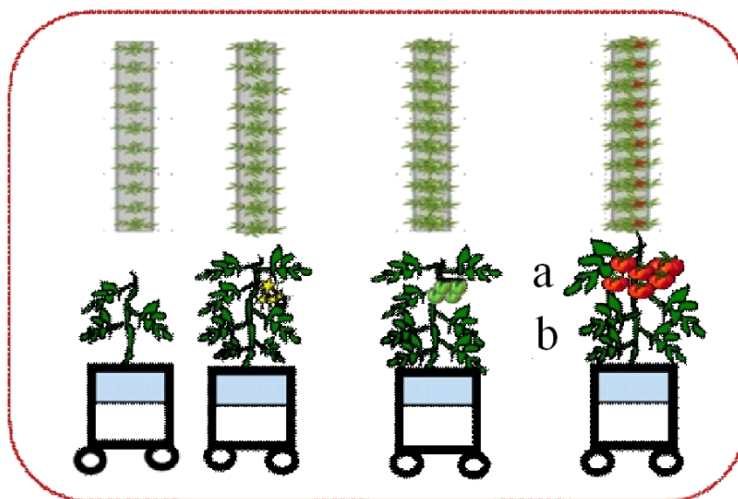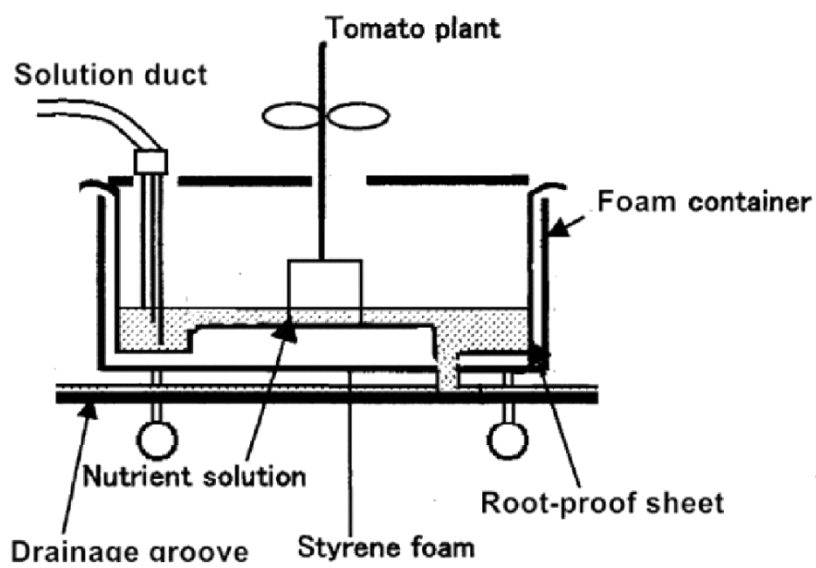
Figure 1 │Schematic diagram of movable bench system. Bench shading index = $(1-b/a) \times 100\%$, both side light irradiation checks were carried out every day at 10:00, 12:00, 14:00 and 16:00.
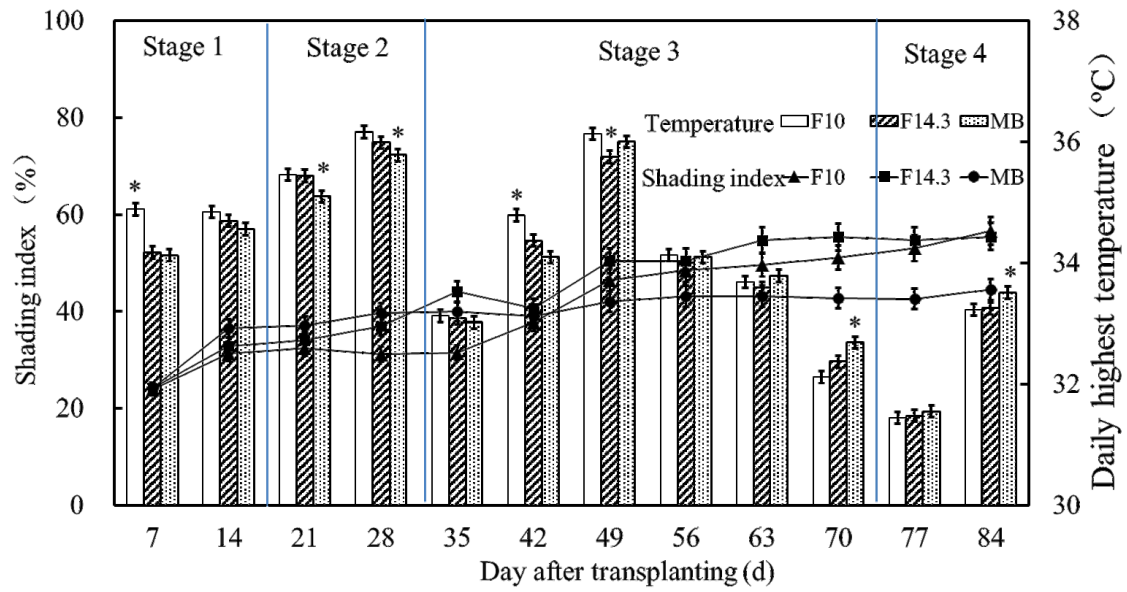
Figure 2 │ Change of shading index and daily highest temperature within canopy of plants cultivated with fixed and movable bench systems. Vertical bars represent standard errors of the means (n = 3), and * indicate significant differences at P < 0.05 according to Tukey's HSD test.
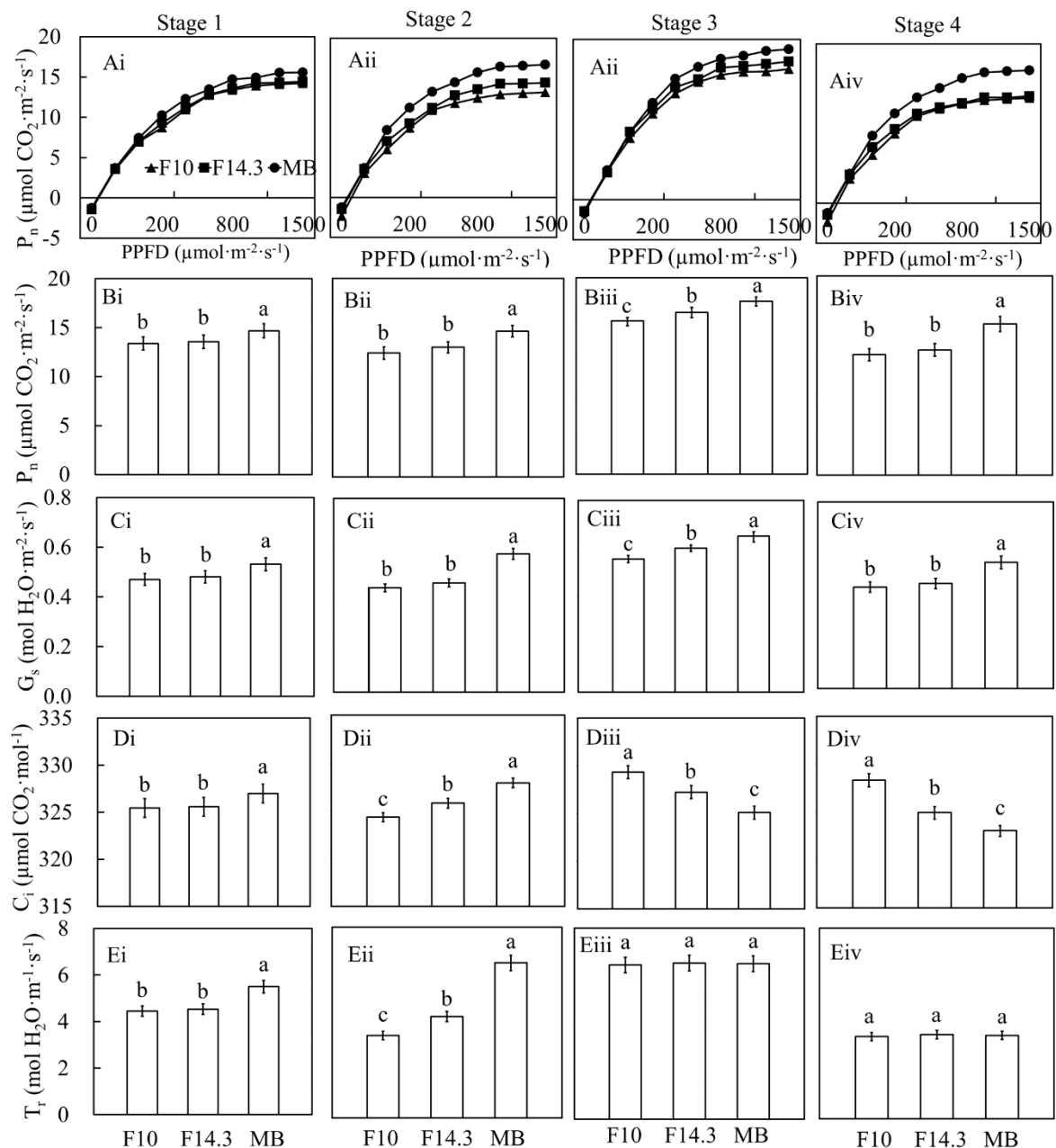
Figure 3 │ Effects of different plant density treatments on photosynthesis parameters. Light curve (A), PN (B), G_s (C), C_i (D), T_r (E) were measured in leaves of tomato plants in altered PD at Stage 1(i), Stage 2 (ii), Stage 3 (iii) and Stage 4 (iv). Measurements were made at 9:00 to 14:00 with leaf temperature of 28 ± 1°C, CO_2 concentration of 400 ± 2 $\mu mol \cdot mol^{-1}$ and relative humidity (RH) of 63 ± 2%. Vertical bars represent standard errors of the means (n = 8–16). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.
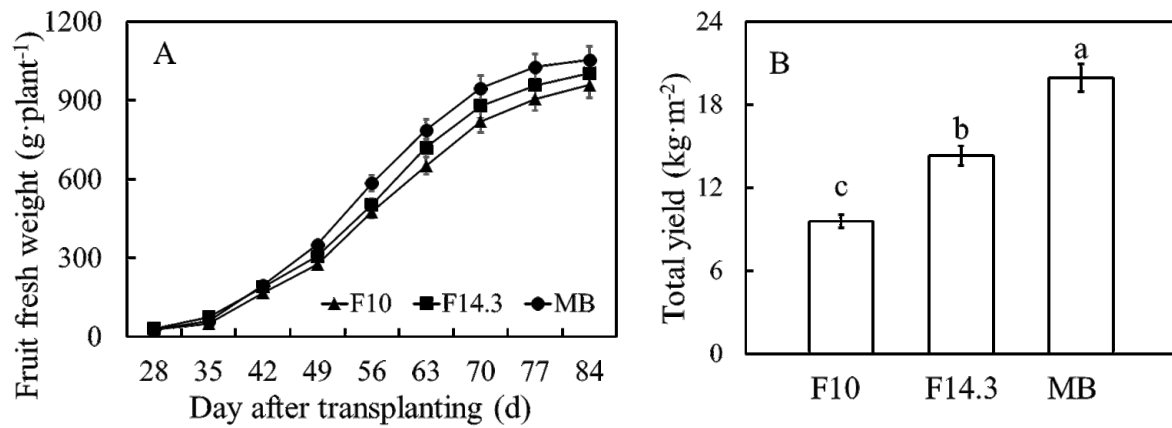
Figure 4 │ The variation of cumulative fruit growth (A) and total yield (B) of tomato plants in different treatment. The fruit weight was estimated based on the equation $y = 0.061x^2 - 1.25x - 15.144$, with $R^2 = 0.993$ (y = fruit fresh weight and x = fruit diameter), with fruit diameter of each group measured every 7d since fruit sizing stage. Total yield was calculated as fruit fresh weight per plant × plant density of each group (MB using actual calculated plant density, Supplemental Figure 1). Vertical bars represent standard errors of the means ($n = 12$). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.
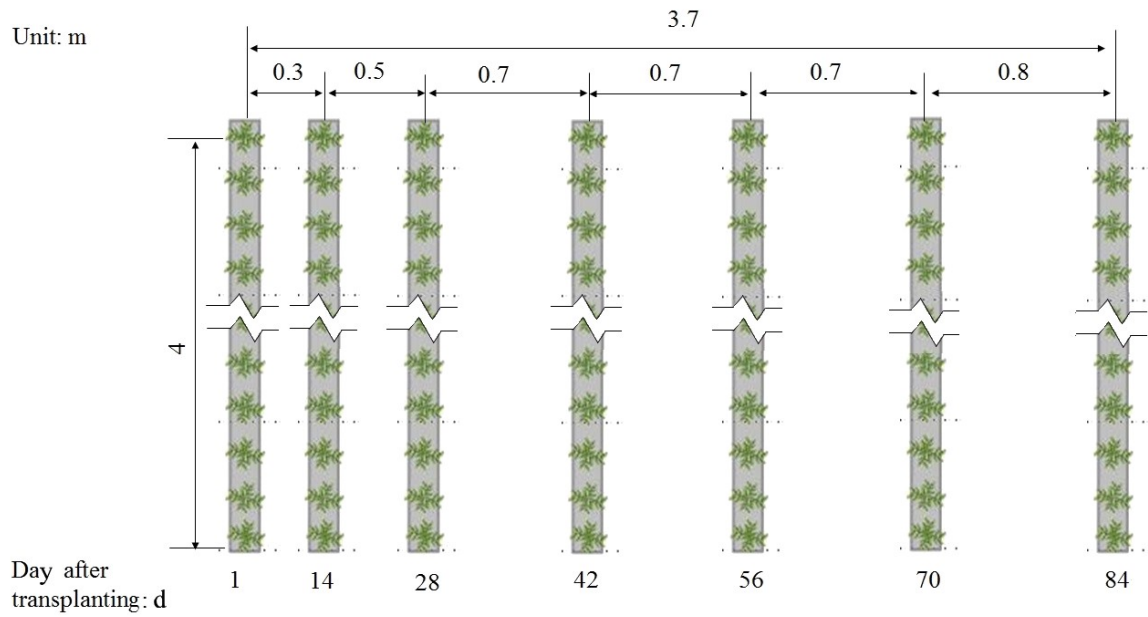
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Figure 5 │Schematic diagram of summer actual cultivation area to calculate the actual calculated plant density (ACPD) in movable bench system. Plants transplanted every 14d and bench distance arranged according to physiology stages described before. Each bench contained 40 plants and 7 benches were considered into summer production period. Thus the ACPD in MB for total yield calculation was 18.9 plant·m^{-2}.

Chapter 4

Supplemental lighting applied to inner or underneath canopy enhanced tomato growth under limiting light condition by promoting leaf photosynthesis and stomatal regulation

4-1 Introduction

Light is one of the most important factors that limits greenhouse crop productivity. Intensive cultivation schedules are adopted worldwide to achieve high yields of greenhouse products, and result in insufficient light to canopies and altered plant morphogenesis and photosynthesis, with variable effects among species (Hogewoning et al., 2010; Terfa et al., 2013). In greenhouse tomato cultivation, light interception of each canopy layer decreases sharply down the plant profile and mutual shading also occurs (Lu et al., 2012a; Tewolde et al., 2016)—less than 35% of total intercepted solar light can be received in leaves under fruit trusses (Lu et al., 2012a). In leaves that are even lower, extremely low net photosynthetic rate is observed as a result of low incident light and premature leaf senescence (Acock et al., 1978; Xu et al., 1997).

Foliar supplemental lighting is considered an efficient method to relieve low-light stress on plants. Numerous studies of responses of plant growth and photosynthesis to different supplemental lighting have been conducted in various species, concentrating on light source categories, canopy layer, light wavelength, light intensity, and light period (Frantz et al., 2000; Hovi et al., 2004, 2008; Okamoto et al., 1996; Pettersen et al., 2010; Lu et al., 2012a, 2012b; Tewolde et al., 2016). The results show that light-emitting diodes (LEDs) are considered a

suitable supplemental lighting source for less heat emitting, better wavelength specificity and longer operating lifetime compared with other lamps (Okamoto et al., 1996; Tewolde et al., 2016). And supplemental lighting to the lower canopy of plants could be effective in promoting tomato plant productivity (Lu et al., 2012a, 2012b; Tewolde et al., 2016), improve cucumber fruit quality (Hovi et al., 2004, 2008; Pettersen et al., 2010) and delay senescence of the cowpea interior leaves (Frantz et al., 2000). However, previous researches also demonstrate that the leaf internal maximum photosynthesis rates were not near the leaf surface where light intensity was highest, but occurred in the middle and lower palisade layers (Nishio et al., 1993; Evans, 1995; Evans et al., 2003; Sun et al., 1998, 2001). These deeper layers have higher electron transport activities and greater amounts of photosynthetic proteins (Terashima et al., 1985, 1988; Sun et al., 2001). In addition, when lettuce exposed to upward lighting from underneath, the senescence of outer leaves was retarded and photosynthetic rate was increased (Zhang et al., 2015). All these results indicated that in tomato cultivation, supplemental lighting from underneath the canopy (USL, with supplemental lighting orientated to abaxial epidermis) might function better in improving leaf and plant development than using inner canopy supplemental lighting (ISL, with supplemental lighting orientated to adaxial epidermis).

Photosynthesis is essential for plant growth and development, and improved leaf photosynthesis capacity would enhance crop development (Tewolde et al., 2016; Trouwborst et al., 2010). Stomatal regulation, highly correlated with leaf photosynthesis, governs overall carbon dioxide (CO_2) assimilation and water loss from plants (Casson et al., 2010; Araújo et al., 2011), and can be affected by numerous biotic and abiotic factors, including hormones,

humidity, and CO_2 concentration (Fan et al., 2004; Mott et al., 2008; Chen et al., 2012). Among those, light wavelength and intensity can regulate stomatal behavior through energy conversion, membrane ion transport, and metabolic activity in guard cells (Shimazaki et al., 2007; Araújo et al., 2011; O'Carrigan et al., 2014). However, the information regarding the effects of different supplemental lighting orientations on photosynthetic characteristics of tomato plants and stomatal behavior is still literally insufficient. The objective of this study is to investigate the effects of USL and ISL to the lower canopy on intensive cultivated tomato growth, leaf photosynthesis, and stomatal regulation.

4-2 Materials and methods

Plant material and growth conditions

The experiment was conducted in an ethylene-tetra fluoroethylene film greenhouse (Venlo-type with double spans, north–south oriented) in Kashiwa-no-ha, Kashiwa, Chiba, Japan (34°53′29.46″N, 139°65′14.1″E) from January to April, 2015. Tomato (*Solanum lycopersicum* 'Momotaro York'; Takii Seed Co. Ltd., Kyoto, Japan) seeds were sown into plug trays filled with a commercial substrate (Best Mix No. 3, Nippon Rockwool Co., Tokyo, Japan), germinated and grown in a closed nursery production system with fluorescent light (Nae Terrace, Mitsubishi Plastics Agri Dream Co. Ltd., Tsukubamirai, Japan) for 24 days. The room was maintained at photosynthetic photon flux density (PPFD) of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 16 h photoperiod, 23/18°C day/night temperatures, and 800 $\mu mol \cdot mol^{-1}$ CO_2 concentration, according to Matsuda et al. (2011a, 2014). The trays were sub-irrigated every other day with a commercial nutrient solution at an electrical conductivity (EC) of 1.5 $dS \cdot m^{-1}$.

At 24 days after seeding, each seedling was transplanted to foam blocks on a cultivation bench and irrigated with nutrient solution (Nakano et al., 2010) with EC of 1.8 dS·m^{-1} and pH 7.0 ± 0.5, and automatically supplied with a feeding rate of 100–120 mL per irrigation event per plant during 6:00–18:00. The distance between plants within a bench was 10 cm and the distance between rows was 0.6 cm, making a plant density of 16.6 plant·m^{-2}. The basic environmental data during the experiment period was shown in Supplemental Table 1.

Supplemental lighting treatment

Three treatments were imposed after transplanting: without supplemental lighting as the control (CK), ISL, and USL. Each treatment consisted of three bench rows of plants, with each row containing 20 plants with a 16-h photo cycle each day (during 6:00–22:00). LEDs (provided by Philips Co. Ltd., Netherlands) were used as the light source and were fixed to movable girders that ensured lighting distance from the abaxial epidermis of the lowest leaf (USL) or the adaxial epidermis of inner canopy leaves (ISL) was 10 cm (Figure 1). The measured PPFD was 100 µmol·m^{-2}·s^{-1} at 10 cm from the LED module.

Light distribution along the plant profile

The canopies were divided into the following three levels: top (leaves above the flower truss), middle (three leaf trusses around the flower truss), and low (bottom leaf trusses). A quantum sensor (LI-190SA; Li-Cor Inc., Lincoln, NE, USA) was used to measure the distribution of light at each canopy level. The sensor was positioned at an angle consistent with the representative canopy leaves. Light distribution was measured for USL- and ISL-treated plants on sunny and cloudy days.

Photosynthetic activity measurements

Leaf photosynthetic activity measurements were conducted with a portable photosynthesis system (Li-6400XT; Li-Cor Inc., Lincoln, NE, USA) under cultivation condition during 9:00–14:00 at 28 days after transplanting with measurement light source provided from red and blue LEDs (6400-02B; Li-Cor Inc. used in CK and ISL, while in USL the bottom of chamber was changed to clear bottom in keeping with growth condition).Measurements were conducted on the second terminal leaflets of leaves on the fifth youngest node (Matsuda et al., 2014), which were around the middle and low canopy. Photosynthetic capacity was determined by measuring light and CO_2 response curve, respectively. Light response curve measurement was conducted with a leaf temperature setting of 25°C and PPFD settings of 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 25, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The CO_2 response curve measurement was conducted with a leaf temperature of 25°C, CO_2 concentrations of 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 25, and 0 $\mu mol \cdot mol^{-1}$ and light intensity of 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The P_n–PPFD and P_n–C_i curves were plotted using P_N data and the corresponding light intensity or intercellular CO_2 concentration, respectively. Light-saturated maximum photosynthetic rate (P_{nmax}), apparent quantum yield (AQY), CO_2-saturated maximum photosynthetic rate (A_{max}), and carboxylation efficiency (CE) was calculated from P_n–PPFD and P_n–C_i curves. Index definitions were was as follows: P_{nmax}, the maximum net photosynthetic rate at saturation light intensity; AQY, the initial slope of the P_N–PPFD curves (Lambers et al., 2008; Skillman, 2008); A_{max}, the maximum net photosynthetic rate at a saturated CO_2 concentration; and CE, the initial slope of the P_n–Ci curve (Farquhar et al., 1980; Sharkey et al., 2007; Sun et al, 2016). Actual

photosynthetic rate were measured including net photosynthetic rate (P_N), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) with PPFD, leaf temperature, CO_2 concentration, and relative humidity at 350 ± 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, $25 \pm 1°C$, 400 ± 2 $\mu mol \cdot m^{-2}$, and $62 \pm 2\%$, respectively.

Leaf optical properties measurements

After gas exchange measurement, leaves were collected for reflectance and transmittance measurements. The measured light spectrum was 400-800 nm. Five measurements were made on both sides of each leaf, parallel to the middle vein (Soares et al., 2008). Reflectance and transmittance were measured using integration hemispheres constructed in our laboratory, connected to a USB-2000 spectroradiometer (SR9910; Irradian Ltd., Tranent, Scotland, UK). The measuring light was provided by a stabilized LED (Philips Co., Ltd.). Leaf absorption was calculated for each wavelength, as 1 – (reflectance + transmittance). Data represent the average values of measurements from the adaxial and the abaxial side.

Stomatal assays

Stomatal assays were carried out essentially as described in work of O'Carrigan et al.(2014) and conducted on abaxial epidermal strips of the leaves in the same position for gas exchange measurement at 29 days after transplanting during 9:00-15:00. The samples were peeled, immersed in a transparent nail polish buffer, and mounted on glass slides before micro-imaging. Images of each epidermal strip were taken under a Leica microscope (Leica Microsystems AG, Solms, Germany) fitted with a Nikon NIS-F1 CCD camera and a Nikon DS-U3 controller (Nikon, Tokyo, Japan), and analyzed with Nikon NIS Element software.

Stomatal density was the number of stomata per mm^2 and stomatal index was calculated as {([number of stomata] / [number of epidermal cells + number of stomata]) × 100} (Kubínová, 1994). Stomatal pore area was calculated by assuming an oval pore shape according to Chen et al. (2012) and Meckel et al. (2007).

Plant growth analyses

At 30 days after transplanting, plants were destructively harvested for the determination of fresh and dry weights of shoots and roots, height and diameter of stems, leaf areas, and leaf chlorophyll contents. Plants were washed with distilled water and weighed after wiping the water off. Leaf area per plant was measured using a leaf area meter (LI-3000C; Li-Cor Inc.). Leaves in the same position of gas exchange measurement were chosen for chlorophyll content was determined using a chlorophyll meter (SPAD-502; Minolta, Osaka, Japan). Samples were oven dried at 80°C until a constant weight was attained, and the dry weight subsequently recorded.

Statistical analyses

Data are presented as mean ± standard error (SE). All data were statistically analyzed with SPSS 11.0 software (SPSS Inc., Chicago, IL, USA) using the Tukey's HSD test at $P <$ 0.05.

4-3 Results

Light and thermal environment among plants

The distribution of light decreased dramatically from the middle to low canopies, and the

application of supplemental lighting improved the situation (Figure 2). On sunny day (Figure 2A), only 16.9% of solar light could reach middle canopy and 6.5% could irradiated to low canopy. With ISL and USL treatment, the light intensity on middle canopy could significantly increase 86.2% and 52.3%, respectively. On low canopy, compared with CK, USL increased the light irradiation level by nearly 273.8%, and enhancement in ISL was 106.7%. On cloudy day (Figure 2B), the effects of SL were more obvious. The light irradiation levels in middle canopy were nearly identical to those measured on sunny days. USL could maintain a steady light intensity even in low canopy, while the light intensity in ISL decreased obviously from middle to low canopy. The CK level deteriorated seriously on cloudy days. The differences between USL and ISL treatments in low canopy were significant both on sunny day and cloudy day. However, the application of supplemental lighting did not significantly altered the inter-plant thermal environment on both sunny and cloudy day (Supplemental Table 2).

Measurements of leaf optical properties

Leaf absorbance, transmittance, and reflectance profiles were similar of plants across the light spectrum of 400-800 nm for ISL, USL, and CK treatments (Figure 3). The transmittance and reflectance showed similar trends and were at maxima after wavelength of 700 nm while absorbance showed an opposite trend.

Photosynthesis parameters

P_n increased with the increase of PPFD but stabilized after light intensity reached 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating that light saturation for tomato plants was approximately 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figure 4A). The P_n also increased with intercellular CO_2 concentration and

stabilized around 800 $\mu mol \cdot mol^{-1}$, indicating CO_2 saturation for tomato was approximately 800 $\mu mol \cdot mol^{-1}$ (Figure 4B). Compared with CK, ISL significantly increased P_{nmax} (+18.7%) and A_{max} (+26.1%) (Figure 4 C, D), but had no significant effect on AQY or CE (Figure 4 E, F), while USL significantly increased P_{nmax} (+33.2%), A_{max} (+46.7%), AQY (+22.3%), and CE (+22.5%). Compared with CK, ISL significantly increased P_n (+20.3%), G_s (+18.9%), T_r (+31.3%), and C_i (+15.1%) (Figure 5A-D); and USL significantly increased P_n (+36.6%), but decreased C_i (-10.6%), and had no significant effects on G_s and T_r.

Stomatal characteristics

Compared with CK, ISL significantly decreased stomatal aperture length (-11.2%), and increased aperture width (+20.6%), stomatal pore area (+7.1%), the stomatal index and stomatal density also increased 13.1% and 18.7%, respectively (Figure 6). USL significantly increased stomatal index and stomatal density by 13.3 and 37.8%, respectively, with no significant difference in aperture length, aperture width, and stomatal pore area compared with CK.

Plant growth

ISL and USL had positive effects on plant growth. Compared with CK, ISL significantly increased plant shoot fresh weight (+8.2%), shoot dry weight (+26.5%), root fresh weight (+14.9%), root dry weight (+13.8%), stem diameter (+22.8%), total leaf area (+36.8%), specific leaf area (+9.3%), and flower number (+29.8%), while stem height decreased by 7.7% (Table 1). USL significantly increased shoot fresh weight (+15.7%), shoot dry weight (+35.1%), root fresh weight (+40.5%), root dry weight (+16.3%), stem diameter (+23.6%), total leaf area (+47.2%), specific leaf area (+10.5%), and flower number (+53.9%), while

stem height decreased by 9.1%, compared with CK plants (Table 1). However, chlorophyll content (SPAD) was not significantly affected by either ISL or USL.

4-4 Discussion

Besides genetics control, environmental factors, such as light quality, intensity, and period, could largely regulate plant morphogenesis and development (Pearcy, 1988, 1990; Aldesuquy et al., 2000; Naumburg et al., 2002; Hogewoning et al., 2010; Wahidin et al., 2013). In intensive greenhouse crop cultivation, leaf architecture and multi shading will prevent light penetrating to lower canopy, cause inter-plant light insufficiency. This low irradiation condition suppress photosynthesis activity and consequently reduces plant growth and restricts productive capacity (Steinger, 2003; Shimazaki et al., 2007). Therefore, optimizing light environment to lower canopy can be viable for promoting plant development in greenhouse production (Demers et al., 1998, 2002; Frantz et al., 2000).

Our study reconfirmed the serious unbalance of light distribution along the middle and low canopy leaves. Without supplemental lighting, the PPFD at the leaf was only 225 and 97 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the middle and low canopies, respectively, even on sunny days (Figure 2). These values are much lower than necessary for normal plant biomass accumulation (Fan et al., 2013; McCree, K.J. 1972; O'Carrigan et al., 2014). PPFDs below 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ will attenuate the development of mesophyll tissue, palisade cells, and spongy cells of tomato leaves, and decrease the specific leaf area and dry weight of plant (Fan et al., 2013; Fu et al., 2011). In this study, supplemental lighting maintained the light intensity at 300-350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the middle canopy. And PPFD under USL was stable at above 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the low canopy, which was barely exposed to solar light. This light intensity

level escapes low light stress on plant biomass accumulation and development. Additionally, given the relative steady irradiation level, the application of USL improved the distribution of light more than the use of ISL. This observation indicates that light originating from underneath the canopy is transmitted to the leaves more efficiently and provides more light directly to the low canopy than light provided within the inner canopy.

Plant photosynthesis is extremely sensitive to supra-optimal light condition and photosynthesis decrease dramatically under irradiation stress by damaging photosynthetic apparatus (Anderson et al., 1995; Terashima et al., 1988; Tikkanen et al., 2012), degrading photosynthetic pigments (Aldesuquy et al., 2000; Hogewoning et al., 2010; Wahidin et al., 2013), inducing stomatal closure which prevents CO_2 entering the mesophyll cells and suppresses carbon assimilation (Araújo et al., 2011; Mott et al., 2008; O'Carrigan et al., 2014). In this study, the improved light distribution directly led to a significant promotion of photosynthetic capacity in plant treated with supplemental lighting (Figure 4). This was also supported by data that leaf actual photosynthetic rate under growth condition enhanced in supplemental lighting treatment, compared with the CK (Figure 5 A). This results was in accordance with Tewolde et al., (2016), who found LED inter-lighting enhanced diurnal photosynthesis rate compared to control leaves that were grown under only soar light. However, the investigation of data differences between USL and ISL clearly showed that enhancement in photosynthetic rate (Figure 4 A-D, Figure 5 A) from USL was significantly higher than ISL. Given supplemental lighting did not affect the leaf optical property (Figure 3) and chlorophyll content (SPAD, Table 1), the variation of increased P_n from supplemental lighting treatment was probably owing to different improvements in CO_2 supply and/or

assimilation. P_{nmax}, A_{max}, AQY, and CE (Figure 4C-F), calculated from P_n–PPFD and P_n–Ci (Figure 4A and B) curves, showed the response of photosynthesis capacity in tomato plants to the treatments. P_{nmax} represents the maximum net photosynthetic rate at saturation light intensity; while A_{max} represents the maximum net photosynthetic rate at a saturated CO_2 concentration and is related to the activities of photosynthetic electron transport and phosphorylation (Coste et al., 2005; Farquhar et al., 1980). AQY represents CO_2 assimilation or oxygen release when one photon is absorbed by the plant, and CE represents carboxylation efficiency, which positively correlates to Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activity, which could regulated CO_2 assimilation (Farquhar et al., 1982; Reng et al., 2003). Compared with the CK, the P_{nmax} and A_{max} (Figure 4 C, D) were significantly increased by ISL, whereas AQY and CE (Figure 4 E, F) were unaffected, suggesting that P_n improvement by ISL was related to the promotion of photosynthetic electron transport activity and phosphorylation, rather than enhanced CO_2 assimilation efficiency. Under USL treatment, P_{nmax}, A_{max}, AQY, and CE all increased, suggesting that P_n improvement by USL was not only related to promotion of photosynthetic electron transport activity, but also to enhanced CO_2 assimilation efficiency.

This hypothesis was confirmed in data of actual photosynthetic parameters, that is both ISL and USL increased P_n, but G_s, T_r, and C_i were not presented a similar variation (Figure 5A-D). Under ISL condition, P_n, G_s, T_r, and C_i all increased, suggest that the improved stomatal conductance, which enabled sufficient CO_2 for photosynthesis, led to increase in P_n (according to Farquhar et al., 1980, 1982). This result was in accordance with results of Hao et al. (1999), who found that when cucumber was treated with supplemental lighting, the P_n,

G_s, T_r were increased, consequently improved leaf biomass partitioning and altered inner water transportation that in turn increased shoot water potential and directly enhanced stomatal aperture. However, though P_n of tomato plants was significantly increased by USL, C_i was decreased, while G_s and T_r were unaffected, compared with CK. These results combined with the lack of changes in stomatal aperture morphology and stomatal pore area (Figure 6A-C), suggest that the increase of P_n by USL was related to improved CO_2 assimilation, rather than to CO_2 supply being enhanced by stomatal conductance. Studies on the effects of abaxial lighting treatment on plant photosynthesis in *Paspalum dilatatum* (Soares et al., 2008) and *Helianthus annuus* (Wang et al., 2008) also showed photosynthesis improvements related to CE.

Change in stomatal numbers in response to light can influence photosynthesis and internal CO_2 concentration (Lake et al., 2001). In this study, stomatal density and stomatal index (Figure 6 D, E) under USL and ISL conditions were significantly higher than CK. This was consistent with higher stomatal densities in various plant species exposed to full sunlight or at high light intensities compared with plants grown in shade (Gay et al., 1975; Schoch et al., 1980; Lake et al., 2001; Thomas et al., 2003). However, compared with the CK, ISL significantly decreased stomatal aperture length but increased aperture width, consequently led to larger stomatal pore area (Figure 6 A-C), indicating that ISL could stimulate stomatal opening. This reconfirmed the increased P_n form ISL was resulted from improvement of CO_2 supply, which reflected on increase in G_s. The stomatal aperture width and length was unaffected by USL, suggesting that abaxial lighting had no significant effect on the morphology of stomata. Meanwhile, there was no significant difference in stomatal index

between the two supplemental lighting treatments, while stomatal density was higher for USL. These results indicate that USL induced greater stomatal numbers with no effect on stomatal area, which explained the relatively decreased G_s and T_r and reconfirmed the increased P_n was due to higher CO_2 assimilation efficiency, compared with ISL.

As results of significantly improved light distribution along plant profile and photosynthetic activity, in this study, supplemental lighting notably influenced tomato morphological features (Table 1), reconfirming the feasibility of cultivating tomato intensively through applying SL to the lower canopy (Lu et al., 2012 a, 2012 b; Tewolde et al., 2016). Compared with ISL, the USL remarkably improved fresh weight of both stems and roots, stem diameter, and flower number notably, indicating that tomato development was effectively promoted by application of USL. This provides a new way to improve tomato growth during low irradiation conditions.

4-5 Conclusion

Both ISL and USL significantly improved light distribution of lower canopy and effectively increased tomato photosynthetic efficiency and plant growth. The application of USL maintained relatively steady light irradiation level in the lower canopy of tomato plants. The USL performed greater photosynthetic enhancement as results of different photosynthesis improvement mechanisms. The USL improved photosynthesis of tomato plants through promoting photosynthetic electron transport activity as well as enhancing CO_2 assimilation efficiency, rather than stomatal morphology regulation. The ISL improved photosynthesis by increasing stomatal behavior and stomatal conductance to enhance CO_2 supply, thereby promoting photosynthetic electron transport activity.

TABLE 1 │ Effects of different treatments on tomato growth and leaf chlorophyll content (SPAD).

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Stem height (cm)	Stem diameter (mm)	Total leaf area (m²)	Specific leaf area (cm²·g⁻¹)	Flower number per plant	SPAD
CK	341.73 c	16.85 b	20.17 c	1.96 b	79.5 a	12.3 a	0.20 b	107.34 b	2.15 c	43.63 a
ISL	369.86 b	21.32 a	23.17 b	2.23 a	73.4 b	15.1 b	0.28 a	117.28 a	2.79 b	44.23 a
USL	395.44 a	22.77 a	28.33 a	2.28 a	72.3 b	15.2 b	0.30 a	118.60 a	3.31 a	44.45 a

CK= the control; ISL= supplemental lighting from the inner canopy; USL= supplemental lighting from the underneath canopy

Mean ± SE (n = 9) with different letters within each row indicating significant difference by Tukey's HSD test at P < 0.05.

Supplemental Table 1 │ Basic environmental data during experiment period.

Integral amount of solar radiation (MJ·m^{-2}·day^{-1})	Sunlight duration* (h·day^{-1})	Mean air temperature (°C)		Relative humidity (%)	
		Daytime	Night time	Daytime	Night time
9.72	5.8	26.7	19.3	65.7	74.7

Solar radiation sensors were positioned outside at height of 2 m from the ground without any shelters. Temperature and relative humidity sensors were positioned at center of the greenhouse with a height of 1.7 m from the ground without any shelter of plants.

* calculated as total time of direct solar radiation exceeds 120 w·m^{-2} according to World Meteorological organization, 2003: Manual on the Global Observing System. WMO-No. 544,

Supplemental Table 2 ｜ Effects of different treatments on daily tomato inter-plant temperature and humidity in both sunny and cloudy days.

Treatment	Temperature (°C)		Relative humidity (%)	
	Sunny day	Cloudy day	Sunny day	Cloudy day
CK	21.2 a	19.9 a	60.5 a	65.5 a
ISL	21.7 a	20.1 a	59.3 a	64.3 a
USL	21.4 a	20.3 a	59.7 a	64.7 a

CK= the control; ISL= supplemental lighting from the inner canopy; USL= supplemental lighting from the underneath canopy

Sensors were positioned within mid-canopy leaves at an average distance of 20 cm from LED modules. Data represent mean ± SE (n = 5) with different letters within each row indicating significant difference by Tukey's HSD test at $P < 0.05$.
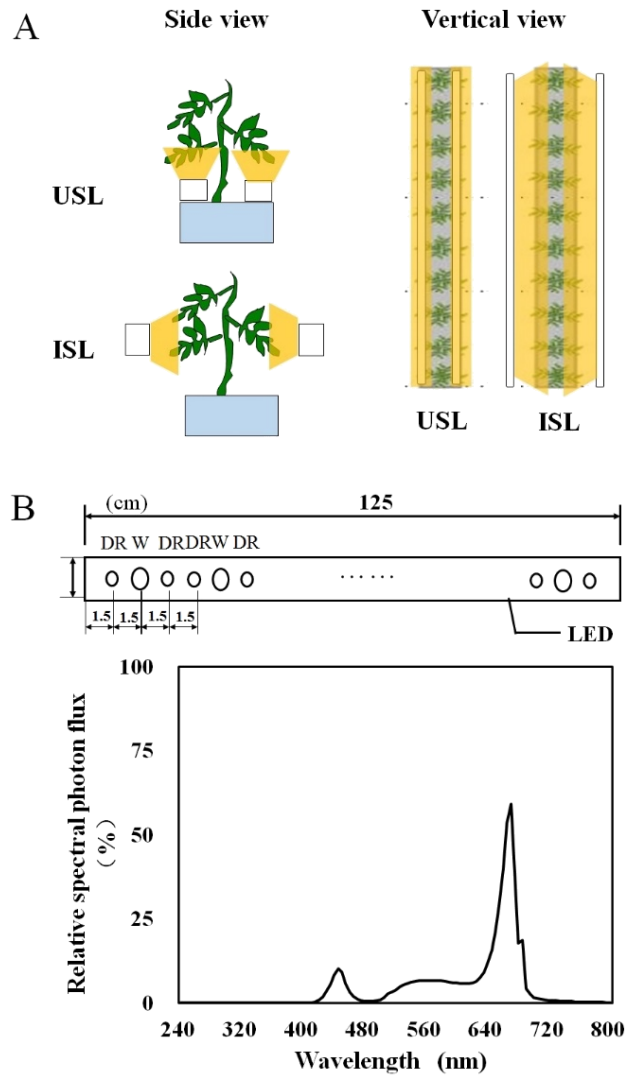
Figure 1 │ Schematic diagram of the supplemental lighting treatment (A) and LED module characterestic (B) in this experiment. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants since transplanting with no supplemental lighting as control (CK). Each supplemental lighting kept fixed 10cm distance to abaxial or adaxial side of leaf, respectively with a PPFD of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A digital timer, dimmer, and transformer were used to maintain the light period (16h, 6:00 to 22:00.) and light intensity. Each LED module unit was in 1.25 m length and contained deep red and white bulb with distance 1.5 cm. The wavelength was measured between 240-800 nm with a spectroradiometer.
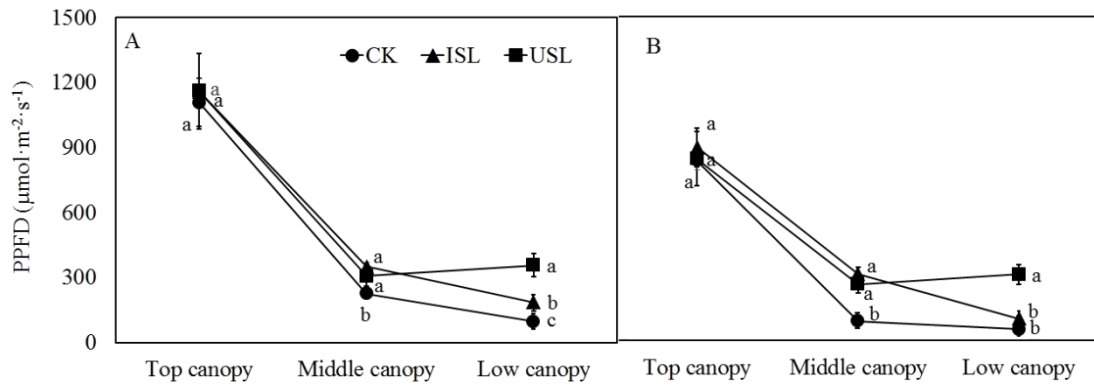
Figure 2 │ Light condition of top, middle and low canopy of tomato plants with different supplemental lighting treatments in sunny day (A) and cloudy day (B) in this experiment. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants since transplanting with no supplemental lighting as control (CK). Photosynthetic photon flux density was measured by using a quantum sensor positioned at the inclination angle of representative canopy. Data represent mean ± SE (n=9). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.
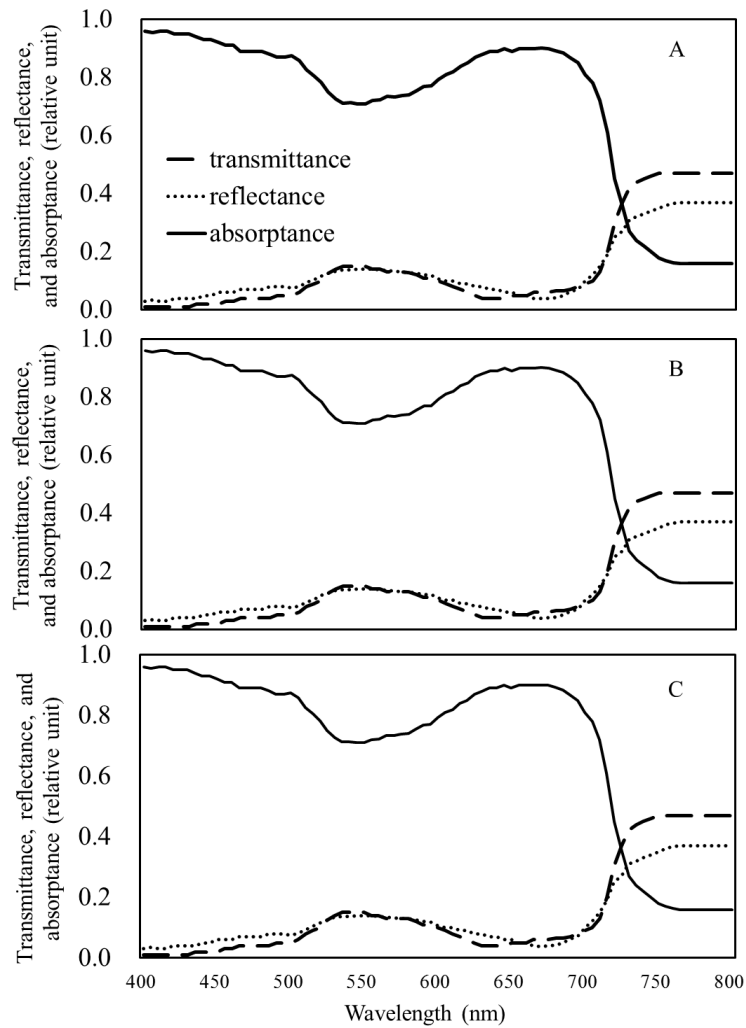
Figure 3 │ Leaf optical properties of tomato plants with different supplemental lighting treatments. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants since transplanting with no supplemental lighting as control (CK). Effects of ISL (A), USL (B) and CK (C) on the transmittance (dashed line), reflectance (dotted line) and absorption (solid line) spectra of tomato leaves. Data average values of results from both adaxial and abaxial side of leaves in each treatment.
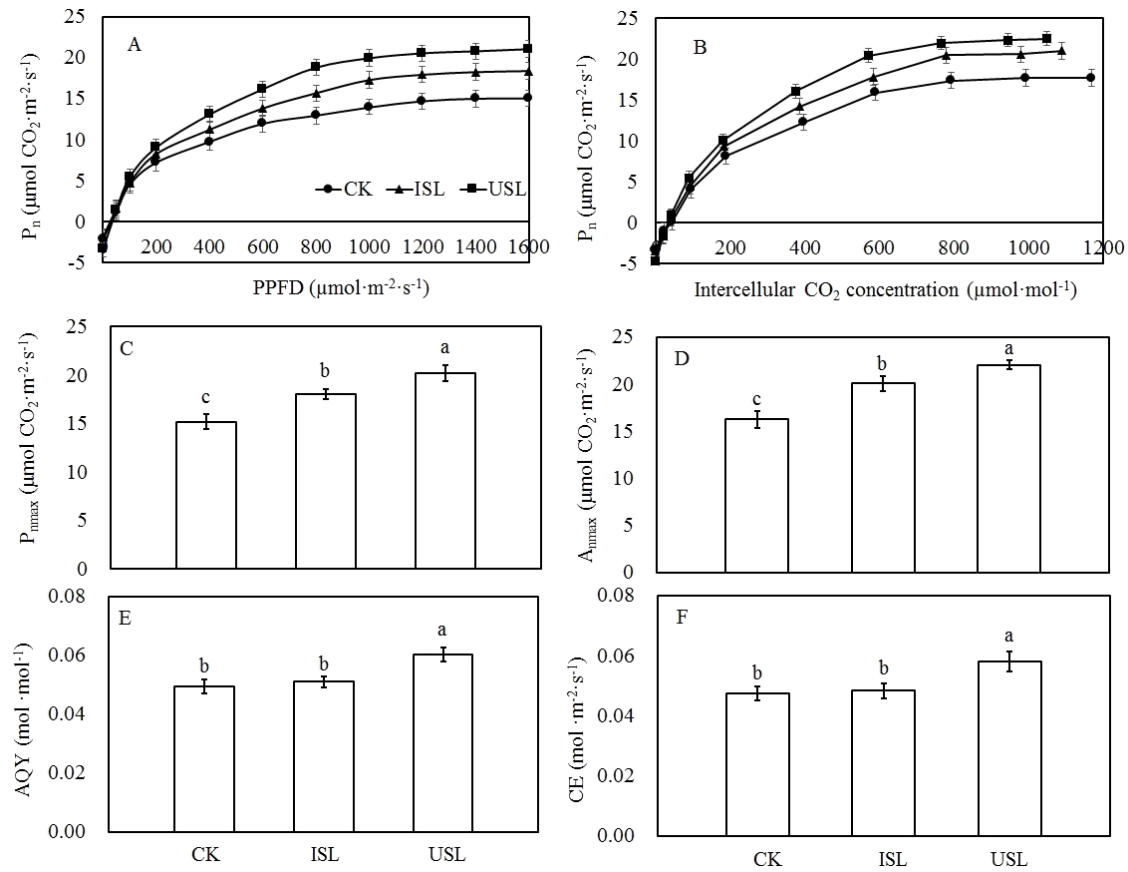
Figure 4 │ Photosynthesis capacity of tomato plants with different supplemental lighting treatments. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants since transplanting with no supplemental lighting as control (CK). Effects on (A) light response curve (P_n–PPFD); (B) CO_2 response curve (P_n–C_i); (C) P_{nmax}; (D) A_{max}; (E) apparent quantum yield (AQY), and (F) carboxylic efficiency (CE) in tomato leaves. Data represent mean ± SE (n=9). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.
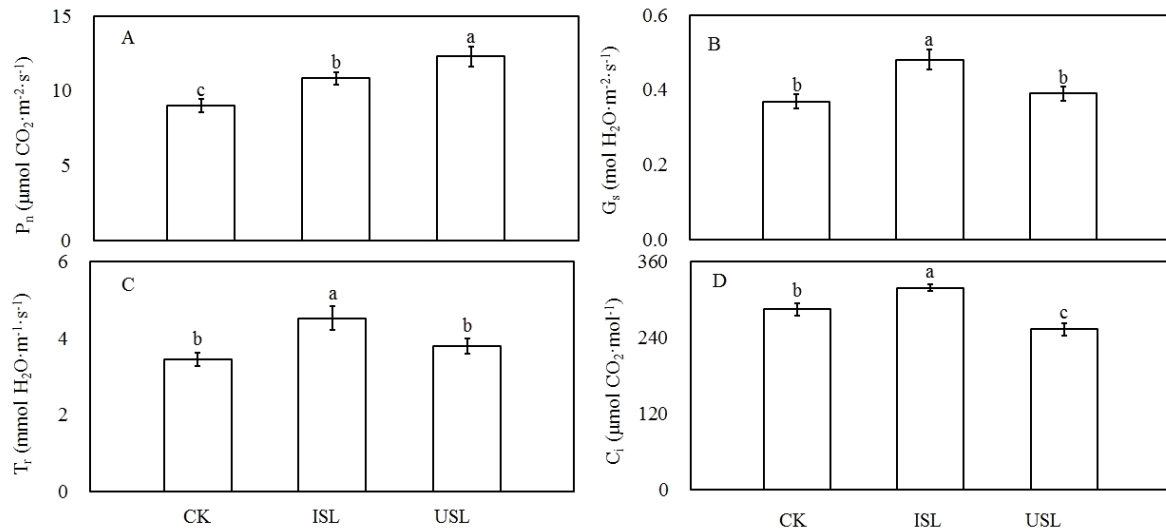
Figure 5│Actual photosynthesis parameters of tomato plants with different supplemental lighting treatments. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants since transplanting with no supplemental lighting as control (CK). Effects on (A) net photosynthetic rate (P_n), (B) stomatal conductance (G_s), (C) transpiration rate (T_r), and (D) intercellular CO_2 concentration (C_i) in leaves of tomato plants. Photosynthetic photon flux density, Leaf temperature, CO_2 concentration, and relative humidity were 350 ± 3 µmol·m^{-2}·s^{-1}, $25 \pm 1°C$, 400 ± 2 µmol·m^{-2}, and $62 \pm 2\%$, respectively. Data represent mean $\pm$ SE (n=9). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.
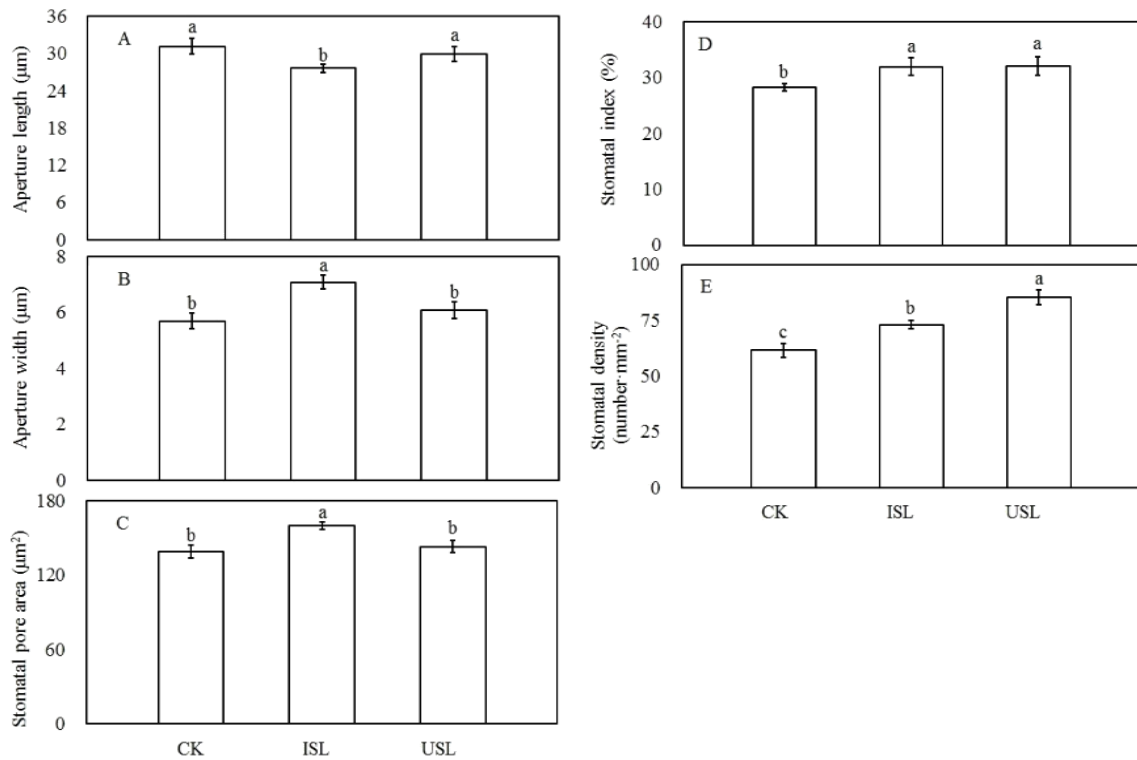
Figure 6 │ Stomatal regulation of tomato plants with different supplemental lighting treatments under growth conditions. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants since transplanting with no supplemental lighting as control (CK). Effects on (A) aperture length, (B) aperture width, (C) stomatal pore area, (D) stomatal index, and (E) stomatal density in tomato leaves. Data represent mean ± SE (n=18). Different letters indicate significant differences at P < 0.05 according to Tukey's HSD test.

Chapter 5

Photosynthesis, Plant Growth, and Tomato Fruit Production Improves with Supplemental Lighting Provided from Underneath or Within the Inner Canopy

5-1 Introduction

Light conditions considerably affect plant physiological activities at all growth stages. In addition to serving as the energy source for photosynthesis, light functions as a signal that regulates several processes such as seed germination, leaf development, flowering, and fruit development (e.g., expansion and coloring) (Assmann et al., 1985; Fan et al., 2004; Kubínová, 1991; Lee et al., 2007; Shimazaki et al., 2007; Talbott et al., 2006; Talbott and Zeiger, 1993; Walters et al., 2003). Crop production in greenhouses often involves intensive cultivation systems to achieve high annual yields. The resulting high plant densities usually lead to light insufficiency for the growing plants. A lack of light has adverse consequences for plant morphogenesis and photosynthesis, with variable effects among species (Hogewoning et al., 2010; Terfa et al., 2013). It also induces many light stress responses via photoreceptors, including phytochromes, cryptochromes, and phototropins, which alter the expression of several genes (Barnes et al., 1997; Whitelam and Halliday, 2007).

Light insufficiency during greenhouse intensive crop production is often caused by decreased vertical distribution of light along the plant profile as well as mutual shading (Talbott and Zeiger, 1993; Walters et al., 2003; Zhang et al., 2015). The irradiation of leaves rapidly decreases with increasing canopy depths. Data in tall trees (e.g., oak) show that the

light intensity at the canopy layer may be 10% lower than that at the emergent layer (Kull et al., 1999). And in a single-truss tomato production system, leaves growing under a fruit truss receive less than 35% of the total intercepted solar light (Lu et al., 2012a). With low incident light, the understory leaves exhibit an extremely low net photosynthetic rate and premature senescence (Acock et al., 1978; Xu et al., 1997). This results in decreased plant growth and limited productivity (Frantz et al., 2000; Shimazaki et al., 2007; Steinger, 2003). Generally, a decrease in cumulative daily light of 1% leads to a yield loss of 1% for greenhouse-grown crops (Cockshull et al., 1992).

The application of supplemental lighting (SL) within the lower canopy (i.e., inter-lighting) may represent a better way to mitigate light insufficiency than traditional top-mounted SL. Previous studies have revealed that inter-lighting improves net photosynthesis, leading to a 50% increase in the yield of various crops (Adams et al., 2002; Hovi et al., 2004; 2008; Pettersen et al., 2010; Lu et al., 2012a; 2012b). SL also delays senescence of the interior leaves in both cowpea (Frantz et al., 2000) and lettuce (Zhang et al., 2015). These results confirm that SL applied to the lower canopy is beneficial for crop production. However, investigations of the structure-function relationships concluded that the highest internal photosynthesis rates do not occur near the adaxial side leaf surface where the light intensity is highest, but are observed in the middle and lower palisade layers (Nishio et al., 1993; Evans, 1995; 2003; Sun et al., 1998; 2001). These layers have higher electron transport activities and greater abundance of photosynthetic proteins (Terashima et al., 1985; 1988; Sun et al., 2001). Additionally, applying SL from underneath the canopy (USL) in lettuce increases the marketable leaf fresh weight by delaying the senescence of the outer

leaves (Zhang et al., 2015). These results suggest the use of USL during tomato production may enhance leaf photosynthesis and increase yields better than the application of SL from within the inner canopy (ISL).

Compared with other commercially-available light sources, light-emitting diodes (LEDs) may be the most suitable for SL. Their advantages include greater wavelength specificity, longer operating lifetimes, and less heat production, which limits any effects on the thermal environment surrounding leaves. Numerous studies involving various species have investigated effective LED wavelengths (Goins et al., 1997; Kim et al., 2006; Massa et al., 2008; McCree, 1972; Okamoto et al., 1996; Lu et al., 2012b), suitable light intensities (Marschner et al., 1989; Fan et al., 2013), and appropriate lighting periods (Tewolde et al., 2016). However, to the best of our knowledge, no study has attempted to optimize the SL orientation in the lower canopy of tomato plants. In this study, we used LEDs for USL and ISL during intensive tomato cultivation to study the effect of foliar SL orientation on tomato leaf photosynthesis and plant development, and to analyze the resulting economic benefits.

5-2 Materials and methods

Plant material and growth conditions

Tomato (*Solanum lycopersicon* L., 'Momotaro York', Takii Seed Co., Ltd., Kyoto, Japan) was cultivated hydroponically with a single-truss production system in a greenhouse in Kashiwa-no-ha, Kashiwa, Chiba, Japan (34°53′29.46″N, 139°65′14.1″E) from November 2014 to March 2015. Seeds were sown into 128-cell plug trays filled with the Best Mix commercial substrate (Nippon Rockwool, Tokyo, Japan), and germinated in darkness for 3

days, and then grown in a temperature-controlled chamber equipped with fluorescent tubes (Nae Terrace; MKV Dream, Tsukubamirai, Japan) for 21 days. Seedlings were exposed to a photosynthetic photon flux density (PPFD) of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$, under a 16-h light (23°C)/8-h dark (18°C) photoperiod and 800 $\mu mol \cdot mol^{-1}$ CO_2 concentration. The trays were sub-irrigated every second day with a commercial nutrient solution at an electrical conductivity (EC) of 1.5 $dS \cdot m^{-1}$.

On 24th day after seeding, seedlings were transplanted to foam blocks cultivation bench in the greenhouse, at a plant density of 10 $plant \cdot m^{-2}$ (Kobayashi, 1997; 1999). A drip irrigation nutrient solution (Nakano et al., 2010) adjusted to 1.8 $dS \cdot m^{-1}$ EC was automatically supplied at a feeding rate of 100-120mL per irrigation event per plant from 6:00 to 18:00. The EC of the nutrient solution was gradually increased from 1.8 to 6.5 $dS \cdot m^{-1}$ depending on plant growth and development. Leaves were pruned to ensure only three leaf trusses present under fruit truss at anthesis. The growth point at the top of stems was pinched under the second truss 35 days after transplanting, with three leaves remaining above fruit trusses. Plants were maintained (e.g., pruning of the lower leaves and removal of side shoots) on a weekly basis. A 4-chlorophenoxy acetate-containing solution (Tomato Tone, ISK Biosciences K.K., Tokyo, Japan) was sprayed on flowers at the full bloom stage, and the five most productive fruits were selected.

Plants were grown in a Venlo-type greenhouse with double spans, oriented north and south, and covered with an ethylene tetrafluoroethylene film. The greenhouse was equipped with an air-conditioner to provide heat during the winter. This was supplemented by natural ventilation from the roof and side windows, which were automatically adjusted based on

internal air temperatures. The daytime and nighttime mean air temperatures were 24-30 °C and 17-22 °C, respectively. The daily mean relative humidity was maintained at about 60%. Although the CO_2 concentration was not measured, it was assumed to be close to the outside level, based on measurements during the same period in a previous year (data not shown).

SL treatment

SL was applied to seedlings from 42th to 84th day after transplanting. This period corresponded to the initial fruit set to mature green fruit stage (Lu et al., 2012; McAvoy et al., 1989), which was also the most effective period for the application of SL (Lu et al., 2012). The understory leaves were illuminated with LEDs (Philips Green Power LED inter-lighting module DR/W; Philips, Eindhoven, Netherlands). The applied spectrum consisted of deep red and white light (Figure 1A) at a PPFD (10 cm from the LED module) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The USL LED modules were positioned 10 cm from the abaxial epidermis of the lowest leaf truss, and 30 cm from the stem (on both sides). The ISL LED modules were positioned on both sides of the plant at a distance of 10 cm from the inner canopy leaves under fruit trusses, and 30 cm from stems (on average) (Figure 1B). Tomato plants were treated daily with SL for 16 h (6:00–22:00), with untreated plants serving as controls (CK).

Light distribution along the plant profile

The canopies were divided into the following three levels: top (two leaf trusses above the fruit truss), middle (two leaf trusses around the fruit truss), and low (two leaf trusses below the fruit truss). A quantum sensor (LI-190SA; Li-Cor Inc., Lincoln, NE, USA) was used to measure the distribution of light at each canopy level. The sensor was positioned at an angle

consistent with the canopy leaves to enable analysis of the adaxial epidermis. Light distribution was measured for USL- and ISL-treated plants on sunny and cloudy days.

Gas-exchange parameter measurements

Gas-exchange was assessed at the low canopy level, which contributed more to fruit production than other levels (Lu, 2012). Fully expanded leaves (Hogewoning et al., 2010; Kim et al., 2006; Matsuda et al., 2014) were analyzed with a portable photosynthesis system (Li-6400XT, Li-Cor Inc.) between 9:00 and 14:00 every week during the SL treatment period. The net photosynthetic rate (P_N), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) were measured. Light was provided from red and blue LED (6400-02B, Li-Cor Inc.). The PPFD was measured at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the leaf temperature, CO_2 concentration, and relative humidity (RH) were 28 ± 1ºC, 400 ± 2 $\mu mol \cdot m^{-2}$, and $63 \pm 2\%$, respectively. Light response curve measurement of each canopy level was conducted with leaf temperature setting at 25 ºC and PPFD settings from 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 25 and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The P_N–PPFD and P_N–C_i curve was plotted using P_N data and the corresponding light intensity and intercellular CO_2 concentration, respectively. The curves were fitted using least squares (Bassman et al., 1991) to calculate light-saturated maximum photosynthetic rate (P_{Nmax}) and apparent quantum yield (AQY), the initial slope of the P_N–PPFD curves.

Plant growth analyses

Plants were harvested 98 days after transplanting to measure shoot and root fresh and dry weights, stem height and diameter, leaf area, and leaf chlorophyll content. Plants were washed

with distilled water and weighed after drying. Leaf area per plant was measured using a leaf area meter (LI-3000C; Li-Cor Inc.). Leaf chlorophyll content was determined using a chlorophyll meter (SPAD-502; Minolta, Osaka, Japan). Samples were oven-dried at 80 °C until the weight stabilized (i.e., dry weight). The specific leaf area and health index were calculated according to the method described by Fan et al. (2013).

Fruit yield and quality analyses

Fruits were harvested to measure fruit diameter and fresh and dry weights. The tomato fruit soluble solid contents were determined using a refractometer (PAL-1; Atago, Tokyo, Japan), while ascorbic acid content was determined by RQ Flex Plus (Merck, Darnstadt, Germany). Fruit hardness was analyzed using a fruit hardness tester (FR-5120; Lutron Electronic Enterprise, Taipei, Taiwan). The relationship between fruit diameter and fresh weight was determined according to the following regression equation: $y = 0.0003x^3 + 0.0215x^2 - 0.6325x + 3.444$, with $R^2 = 0.9908$ [y = fruit fresh weight (FFW), g; x = fruit diameter, mm]. Fruit diameter was measured every 7 days from the fruit sizing stage to harvest, and the FFW at each stage was estimated based on the equation provided above.

Use of electricity and cost performance

Electricity consumption for the LED modules was measured with a multimeter and clamp ammeter (Hioki 3169-01; Hioki, Nagano, Japan) as previously described by Zhang et al. (2015). Electricity efficiency ($kg \cdot kWh^{-1}$) and cost performance (return / cost) were calculated as below, while tomato price and electricity unit price were collected from market investigation and Tokyo Electricity Power Company.

Electric energy efficiency = [Yield enhancement with SL treatment (kg·m^{-2})] / [Electricity consumption (kWh·m^{-2})].

Cost performance = [Price of tomato (Yen· kg^{-1}) × Yield enhancement (kg·m^{-2})] / [Price of electricity (Yen·kWh^{-1}) ×Electricity consumption (kWh·m^{-2})]

Statistical analyses

Data were analyzed using SPSS 11.0 (SPSS, Chicago, USA), with mean separations determined using Tukey's HSD test at $P < 0.05$.

5-3 Results

Light condition and inter-canopy thermal environment of plant with different treatments

The distribution of light decreased dramatically from the middle to low canopies, and the application of SL improved the situation (Figure 2). On sunny day (Figure 2A), only 15% of solar light could reach middle canopy and less than 7% light could irradiated to low canopy. With USL and ISL treatment, the light intensity on middle canopy could significantly increase 167% and 188%, respectively, to reach a PPFD of 550-600 μmol·m^{-2}·s^{-1}. On low canopy, compared to CK, USL increased the light irradiation level by nearly 455%, and maintained the PPFD at 400 μmol·m^{-2}·s^{-1}. The ISL increased the irradiation level by 325%, while the PPFD was nearly 320 μmol·m^{-2}·s^{-1}. On cloudy day (Figure 2B), the effects of SL were more obvious. The light irradiation levels in middle and low canopy were nearly identical to those measured on sunny days. In contrast, the CK level deteriorated seriously on cloudy days. The differences between USL and ISL treatments in low canopy were significant on sunny day and

cloudy day. However, the application of SL did not caused significant changes in temperature and relative humidity within canopy, compared with the CK (Supplemental table 1).

Leaf photosynthesis capacity

As observed in a previous study on the relationship between carbon assimilation and leaf position, low canopy leaf trusses used most of the assimilated carbon to produce fruits (Lu, 2012) and the photosynthetic characteristics of the low canopy largely influenced tomato productivity. Compared with the CK, the ISL-treated plants exhibited significantly increased P_N (Figure 3A), G_s (Figure 3B), T_r (Figure 3C), and C_i (Figure 3D). Plants treated with USL also had significantly increased P_N, G_s, and C_i but T_r was unchanged. There were no significant differences in C_i between the USL- and ISL-treated plants.

Increases in PPFD resulted in increased P_N in the middle and low canopy leaves, until P_N eventually stabilized at a light intensity of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This result implied that the light saturation point for tomato plants is approximately 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Figure 4 A, B). Compared with the CK plants, the SL-treated plants exhibited improved leaf light responses. Leaves treated with USL generated a similar light response curve regardless of leaf position. In contrast, leaves in the low canopy of ISL-treated plants had weaker responses to light compared with leaves from the middle canopy (Figure 4 A, B).The application of SL significantly elevated P_{Nmax} (Figure 4 E, F) and AQY (Figure 4 G, H) in the middle and low canopies. The P_{Nmax} of ISL-treated plants increased by 17.5% and 31.3% in the middle and low canopies, respectively, while the increases in USL-treated plants were 15.7% and 39.1%, respectively. The increases in the AQY of the middle canopy were the same for both SL treatments. The enhancement of the AQY following USL treatment was highest in the low

canopy, reaching 25%, while the corresponding increase in ISL-treated plants was 12.3%.

Whole plant growth and development

Plant growth and development were positively affected by exposure to ISL and USL. The ISL treatment increased shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, stem diameter, specific leaf area, and health index by 4.1, 4.6, 7.1, 13.1, 7.6, 5.7, and 16.3%, respectively. In contrast, stem height decreased by 1.9% (Table 1). Exposure to USL increased shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, stem diameter, specific leaf area, and health index by 5.3, 8.4, 12.3, 16.3, 6.2, 7.1, and 18.3%, respectively. Stem height decreased by 1.6% (Table 1). However, total leaf area and chlorophyll content (SPAD) were not significantly affected by ISL or USL.

Tomato fruit development and yield

The harvested fresh and dry tomato yields significantly increased with the use of SL (Table 2). Fresh yields of ISL- and USL-treated plants increased by 13.6% and 19.7%, respectively, while the dry yield increased by 11.8% and 17.6%, respectively. There were significant differences in the effects of ISL and USL treatments. The fruit soluble solid contents were 11.4% higher in ISL-treated plants than in CK plants. Meanwhile in plants exposed to USL, the enhancement reached 15.7%. The ascorbic acid content was enhanced by application of SL, while no significant differences were observed between ISL and USL. However, the fruit hardness were not significantly different among the various treatments.

The slope of the weekly changes in FFW (Figure 5) reflected the fruit growth rate, which was highest in USL-treated plants, followed by ISL-treated plants. The significant increase in

FFW in plants exposed to SL occurred starting 63 days after transplanting. Furthermore, the increases in FFW were maintained even after the SL was removed 84 days after transplanting. The FFW in CK plants remained steady during this period.

Economic value of SL

Because the daily photoperiod (16 h) was kept constant, the total electricity consumed was similar (40.32 kWh·m^{-2}) for 42-day SL treatments. Using fresh yield data from Table 2, we calculated the yield enhancement, and analyzed the economic performance of the SL treatments (Table 3). The USL-treated plants were 46.6% more energy efficient than the plants exposed to ISL. Both SL treatments provided an economic benefit. Additionally, the cost performance for USL was 45.0% higher than that of ISL.

5-4 Discussion

Plant morphogenesis and development are regulated by genetics and environmental factors (Aldesuquy et al., 2000; Hogewoning et al., 2010). Light provides energy source for physiological activities, and also acts as an abiotic signal influnces various plant developmental processes (Franklin et al., 2005; Hoenecke et al., 1992; Wahidin et al., 2013). Light insufficiency in intensive cultivation is an outstanding problem, especially for the lower canopy, which ultimately inhibits plant growth and restricts productivity (Demers et al., 1998; 2002; Frantz et al., 2000; Shimazaki et al., 2007; Steinger, 2003).

The distribution of light along the middle and low canopy leaves was very low. Fan et al. (2013) revealed that PPFDs below 300 μmol·m^{-2}·s^{-1} attenuate the development of mesophyll tissue, palisade cells, and spongy cells of tomato leaves, and decrease the plant health index.

In our study, without SL, the PPFD at the leaf epidermis was only 208 and 92 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the middle and low canopies, respectively, even on sunny days (Figure 2). These values are much lower than necessary for normal plant biomass accumulation (Fan et al., 2013; McCree, K.J. 1972; O'Carrigan et al., 2014). On cloudy days, the light insufficiency in the lower canopy worsened. However, excessive light irradiation induced the photosynthetic apparatus stress response resulting in dissipated energy. Takahashi et al. (2008) reported that high intensity light stress inhibits photosystem I and prevents the repair of photosystem II. Similarly, exposure to PPFDs above 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for long periods results in changes to leaf structures and stomatal traits, and damages to the photosynthetic apparatus (Fan et al., 2013; Karpinski et al., 1997; 1999; O'Carrigan et al., 2014). In our study, the light intensity in the top canopy was approximately1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating that extra shading might be necessary for this canopy level. SL maintained the light intensity at 379-600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the middle canopy. The PPFD was stable at approximately 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the low canopy, which was barely exposed to solar light. This light intensity level provides a suitable light environment for plant biomass accumulation and development. Additionally, the application of USL improved the distribution of light more than the use of ISL. This observation indicates that light originating from underneath the canopy is transmitted to the leaves more efficiently and provides more light directly to the low canopy than light provided within the inner canopy.

Photosynthetic activities are extremely sensitive to supra-optimal light conditions and low light stress would damage photosynthetic apparatus (Petterson et al., 2010), degrade photosynthetic pigments (Aldesuquy et al., 2000; Hogewoning et al 2010), and induce

stomatal closure (Mott et al., 2008; O'Carrigan et al., 2014), all consequently decreased

photosynthesis rates. Similar to previous research on other species (Hovi et al., 2004; 2008;

Massa et al., 2008), our results presented that SL improved tomato P_N (Figure 3, 4)

accompanied with increased light irradiation (Figure 2). However, the leaf chlorophyll content

(Table 1) was not affected by SL, indicating the integrity of the photosynthetic apparatus and

light harvest efficiency were unaffected. Therefore, the increased P_N due to SL was likely the

results of increased CO_2 supply or/and assimilation. The application of ISL and USL

increased the P_N (Figure 3A), G_s (Figure 3B) and C_i (Figure3D) of tomato plants, suggesting

that increased P_N was caused by improved stomatal conductance, which provided sufficient

CO_2 for photosynthesis. Our results are consistent with those of Hovi et al. (2004), who

observed that G_s increased in cucumber irradiated with SL. Previous studies on *Paspalum*

dilatatum (Soares et al., 2008) and *Helianthus annuus* (Wang et al., 2008) confirmed that

abaxial lighting treatment improved photosynthesis correlated to carboxylic efficiency.

Therefore, the significant difference in P_N, G_s and T_r between plants treated with USL and ISL

suggest increases in P_N caused by USL are correlated with improved CO_2 assimilation as well

as stomatal conductance.

The P_N–PPFD (Figure 4 A, B), P_N–C_i curves(Figure 4 C, D), P_{Nmax} (Figure 4 E, F), AQY

(Figure 4 G, H) of tomato plants were analyzed to understand the differences between the ISL

and USL treatments regarding leaf photosynthetic ability in different canopy levels. The P_N–

PPFD and P_N–C_i curves (Figure 4 A-D) indicated that leaf photosynthetic ability under ISL

treatment decreased from the middle to low canopies, while photosynthetic activities were

stable among canopies of USL-treated plants. P_{Nmax} represents the maximum net

photosynthetic rate at saturation light intensity, while AQY represents CO_2 assimilation or O_2 release when one photon absorbed by plant. Compared to CK, SL significantly increased P_{Nmax} (Figure 4E, F) and AQY (Figure 4G, H), suggesting SL promoted photosynthetic electron transport activity. Except for P_{Nmax} and AQY in the middle canopy (Figure 4E, G), the data for USL-treated plants were significantly higher than those of ISL-treated plants, further revealing the distinct effects of USL.

According to the C_3 photosynthesis model (i.e., FvCB model) (Farquhar et al., 1980; von Caemmerer, 2000), at normal atmosphere CO_2 levels, photosynthesis at low PPFDs is limited by light harvesting and electron transport capacities, while at high PPFDs is limited by the ribulose-1,5-bisphosphate (RuBP) carboxylation capacity of Rubisco, as well as by CO_2 diffusivity from the intracellular atmosphere to chloroplast stroma. Therefore, our results suggested that ISL promoted RuBP carboxylation capacity and/or CO_2 diffusion in middle canopy (Figure 2). In low canopy (Figure 2) leaves photosynthesis was more limited by light-harvesting and/or electron-transport capacities under ISL treatment than under USL treatment.

Increased light intensity improves plant growth (Frantz et al., 2000; Shimazaki et al., 2007; Steinger, 2003). In this study, tomato morphological features were considerably affected by the use of SL (Table 1), proving the feasibility of intensive tomato cultivation through the application of SL to the lower canopy. Further comparisons of the effects of ISL and USL revealed that only USL results in increased shoot and root fresh weights. This observation suggests USL-treated plants have higher water contents. Additionally, the differences in leaf photosynthetic ability affected carbon transport for fruit assimilation more

than whole plant morphology.

Previous researches has demonstrated that application of SL in low light condition could improve fruit assimilation rate and enhanced crop yield (McAvoy et al., 1988; Hovi et al., 2004; Pettersen et al., 2010). Our study revealed that SL significantly increases the fruit growth rate (Figure 5) and the fresh and dry yields (Table 2). Lu (2012) used ^{13}C-tracer method to identify most effective leaves on fruit carbon assimilation supply, and determined that the three leaf trusses under fruit truss contributed the most to fruit production. This indicates that differences in photosynthetic activities in this canopy level should be reflected most by fruit production. We observed that P_N was highest in USL-treated plants, which consequently produced the highest FFWs. Meanwhile, Wang et al. (2008) studied the sunflower leaf response to direct illuminated light as well as naturally transmitted light. They reported that naturally transmitted light (e.g., through leaves) resulted in more efficient photosynthesis than direct illumination. In our study, light from USL sources were transmitted through the leaves themselves (i.e., from the abaxial epidermis to spongy cells, then palisade cells, and finally the chloroplast) or through other leaves (i.e., leaves in the top canopy received scattered or refracted light from the low canopy, which was directly irradiated). Thus, leaves treated with USL behaved similarly to the leaves described by Wang et al. (2008). They exhibited a more efficient use of light energy and a better fruit carbon assimilation rate (Figure 5) than the ISL-treated leaves.

Low-light stress affects tomato fruit quality (McCollum, 1944; Yanagi et al., 1995). Total soluble solids content decrease drastically with decreasing light levels (McCollum, 1944; Yanagi et al., 1995), and ascorbic acid content could increase 35% when tomato fruit exposed

to relatively higher light (Mc Collum, 1944; Hovi et al., 2004). In our study, both ISL and USL enhanced the total soluble solids content and ascorbic acid content of single-truss tomato fruit (Table 2). The total soluble solid contents in ISL-treated plants were significantly higher than those of USL-treated plants. This finding may have been because fruits on ISL-treated plants were exposed to more scattered light than the fruits of USL-treated plants. This exposure to higher light intensity likely led to increased sugar contents in fruits (Gautier et al., 2008). Fruit hardness is usually determined by the degradation of pectin and cellulose in fruits, which is primarily regulated by genes rather than environmental factors (Hadfield et al., 1998; Rose, 1997). This may explain the lack of variability in fruit hardness among treatments.

Cost is an important consideration when evaluating a crop production system. The cost of the energy consumed by SL may prevent this strategy from being financially viable. Therefore, energy-efficient lighting options are needed for modern greenhouse crop production systems. In our study, although USL and ISL treatments enhanced tomato fruit yield (Table 3), the use of USL was more energy efficient, with a better cost performance.

5-5 Conclusion

Both ISL and USL effectively increased single-truss tomato leaf photosynthetic efficiency, plant growth and fruit productivity while USL could achieve higher economic benefit. USL maintain relatively steady light irradiation condition in tomato lower canopy and promoted leaf photosynthesis ability through improvement of CO_2 assimilation in addition to stomatal conductance, which contributed more on fruit carbon assimilation compare to ISL. However, ISL could introduced more soluble solid content on fruit because of more scattered light illuminated on fruit.

Table 1 Fresh and dry weight of shoot and root, height and diameter of stem, total leaf area and leaf chlorophyll content (SPAD) of tomato plants from different treatments.

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Stem height (cm)	Stem diameter (mm)	Total leaf area (m^{-2})	Specific leaf area ($cm^{-2}·g^{-1}$)	Health index	SPAD
CK	441.73 c	38.53 b	23.17 c	1.96 b	117.1 a	14.3 b	0.58 a	118.15 b	0.49 b	46.03 a
ISL	459.86 b	40.32 a	24.81 b	2.23 a	114.9 b	15.4 a	0.55 a	124.26 a	0.57 a	46.23 a
USL	465.44 a	41.77 a	26.03 s	2.28 a	115.2 b	15.2 a	0.57 a	126.58 a	0.58 a	47.55 a

CK= the control; ISL= supplemental lighting from the inner canopy; USL= supplemental lighting from the underneath canopy

Means (n=12) with different letters within each column are significantly different according to Tukey's HSD test ($P < 0.05$).

Table 2 Fresh and dry yields and tomato fruit quality resulting from different treatments.

Treatment	Fresh yield (kg·m^{-2})	Dry yield (kg·m^{-2})	Soluble solid content (Bix%)	Ascorbic acid content (mg·kgFW^{-1})	Fruit hardness (kg·(LB·Newton)$^{-1}$)
CK	9.54 c	0.34 c	7.0 c	130 b	4.26 a
ISL	10.84 b	0.38 b	8.1 a	141 a	4.23 a
USL	11.42 a	0.40 a	7.8 b	142 a	4.25 a

CK= the control; ISL= supplemental lighting from the inner canopy; USL= supplemental lighting from the underneath canopy

Means (n=12) with different letters within each column are significantly different according to Tukey's HSD test ($P < 0.05$).

Table 3 Energy efficiencies of supplemental lighting treatments.

Treatment	Electricity consumption (kWh·m^{-2})	Yield enhancement (kg·m^{-2})	Electric energy efficiency (g·kWh^{-1})	Cost performance (return/cost)
ISL	40.32 a	1.30 b	32.24 b	1.11 b
USL	40.32 a	1.88 a	46.62 a	1.61 a

ISL= supplemental lighting from the inner canopy; USL= supplemental lighting from the underneath canopy

Electric energy efficiency = [Yield enhancement with SL treatment (kg·m^{-2})] / [Electricity consumption (kWh·m^{-2})]. Cost performance = [Price of tomato (Yen· kg^{-1}) × Yield enhancement (kg·m^{-2})] / [Price of electricity (Yen·kWh^{-1}) ×Electricity consumption (kWh·m^{-2})]. Price of tomato was 514 JPY· kg^{-1} (March 2015, Tokyo Metropolitan Central Wholesale Market) and price of electricity was 14.87 JPY· kWh^{-1} (Tokyo Electricity Power Company).

Means (n=12) with different letters within each column are significantly different according to Tukey's HSD test ($P < 0.05$).

Supplemental Table 1. Effects of different treatments on daily tomato inter-plant temperature and humidity in both sunny and cloudy days.

Treatment	Temperature (°C)		Relative humidity (%)	
	Sunny day	Cloudy day	Sunny day	Cloudy day
CK	21.0 a	19.6 a	60.5 a	65.9 a
ISL	21.7 a	20.0 a	60.3 a	65.4 a
USL	21.4 a	19.9 a	59.9 a	65.7 a

CK= the control; ISL= supplemental lighting from the inner canopy; USL= supplemental lighting from the underneath canopy

Sensors were positioned within mid-canopy leaves at an average distance of 20 cm from LED modules. Data represent mean ± SE (n = 5) with different letters within each row indicating significant difference by Tukey's HSD test ($P < 0.05$).

A　　LED unit and light spectrum
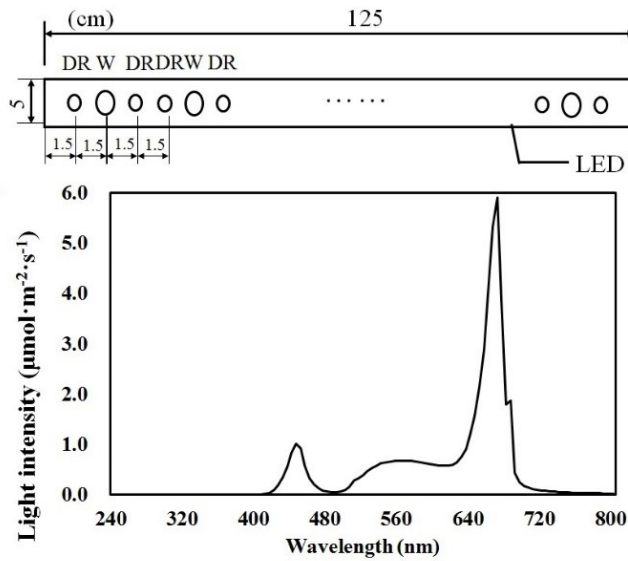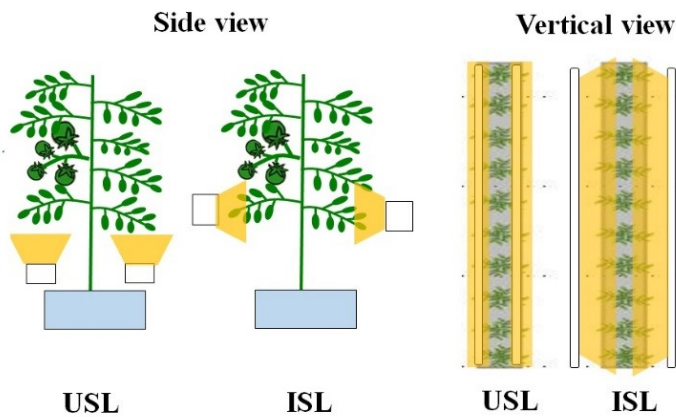

B　　SL system arrangement

Figure 1 │Schematic diagram of light emitting diode characters (A) and the supplemental lighting arrangement (B). Supplemental lighting from underneath (USL) or within the inner canopy (ISL) was applied to plants from the 42th to 84th day after transplanting. The supplemental lighting (SL) was provided 10 cm from the abaxial or adaxial epidermis of leaves, with a photosynthetic photon flux density (PPFD) of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A digital timer, dimmer, and transformer were used to maintain a consistent photoperiod (16h, 6:00 to 22:00) and light intensity.
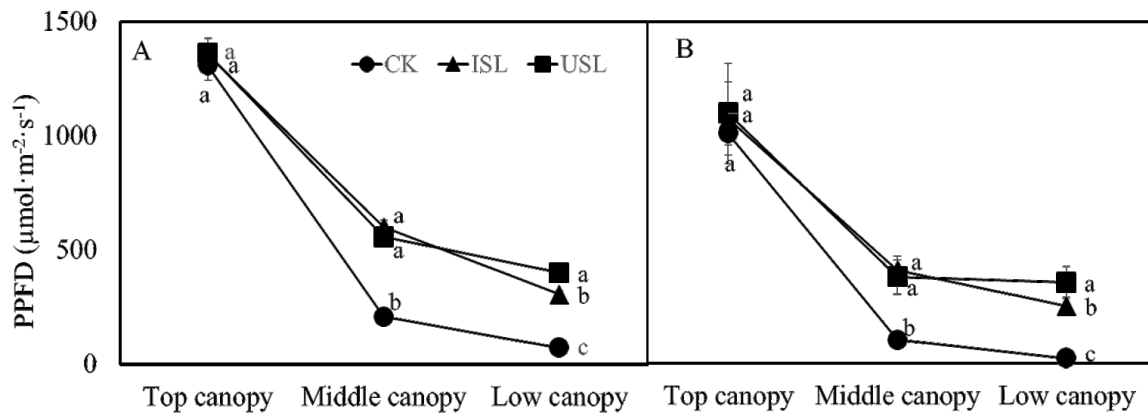
Figure 2 │ Light conditions in the top, middle, and low canopies of tomato plants exposed to different supplemental lighting treatments on sunny (A) and cloudy (B) days. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants from the 42th to 84th day after transplanting, while no supplemental lighting as control (CK). Photosynthetic photon flux density was measured by using a quantum sensor positioned at the inclination angle of representative canopy. Data represent mean ± SE (n=9). Different letters indicate significant differences at $P < 0.05$ according to Tukey's HSD test.
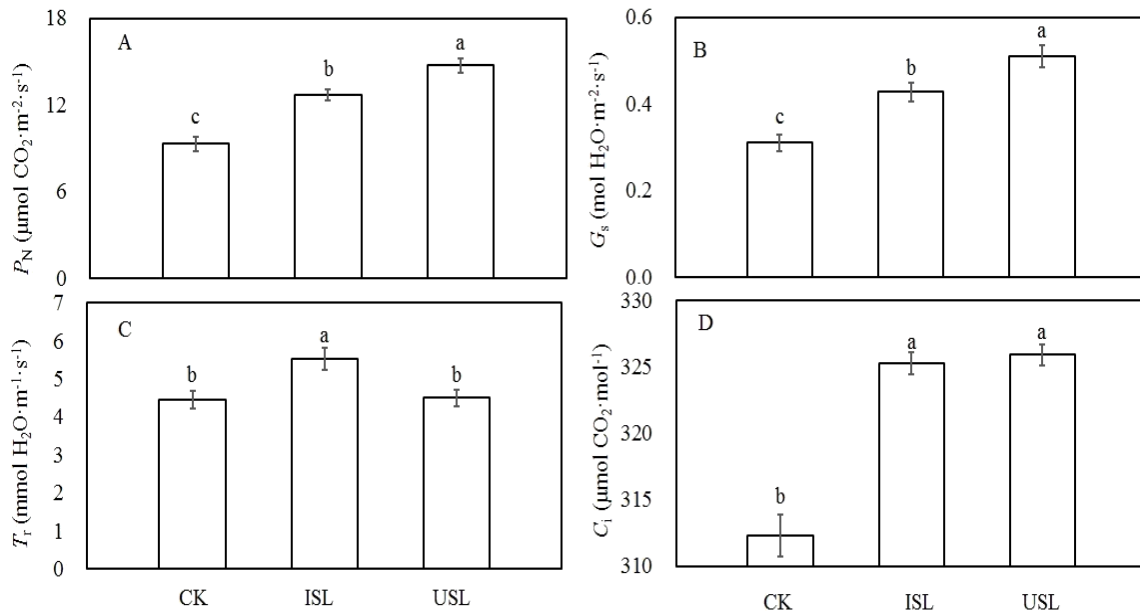
Figure 3 │Effects of different treatments on (A) net photosynthetic rate (P_N), (B) stomatal conductance (G_s), (C) transpiration rate (T_r), and (D) intercellular CO_2 concentration (C_i) of tomato plants from the low canopy. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants from the 42th to 84th day after transplanting, while no supplemental lighting as control (CK).Fully expanded leaves were used for measurements. Means ± SE (n=9) with different letters indicate significant differences ($P < 0.05$) according to Tukey's HSD test.

Figure 4 │ **Effects of different treatments on light response curve (P_N–PPFD) (A, B); intercellular CO_2 response curve (P_N–C_i) (C, D); light-saturated maximum photosynthetic rate (P_{Nmax}) (E, F), and apparent quantum yield (AQY) (G, H) of tomato leaves from the middle (A, C, E, G) and low (B, D, F, H) canopies.** Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants from the 42th to 84th day after transplanting, while no supplemental lighting as control (CK).Fully expanded leaves were used for measurements. Means± SE (n=9) with different letters indicate significant differences ($P < 0.05$) according to Tukey's HSD test.
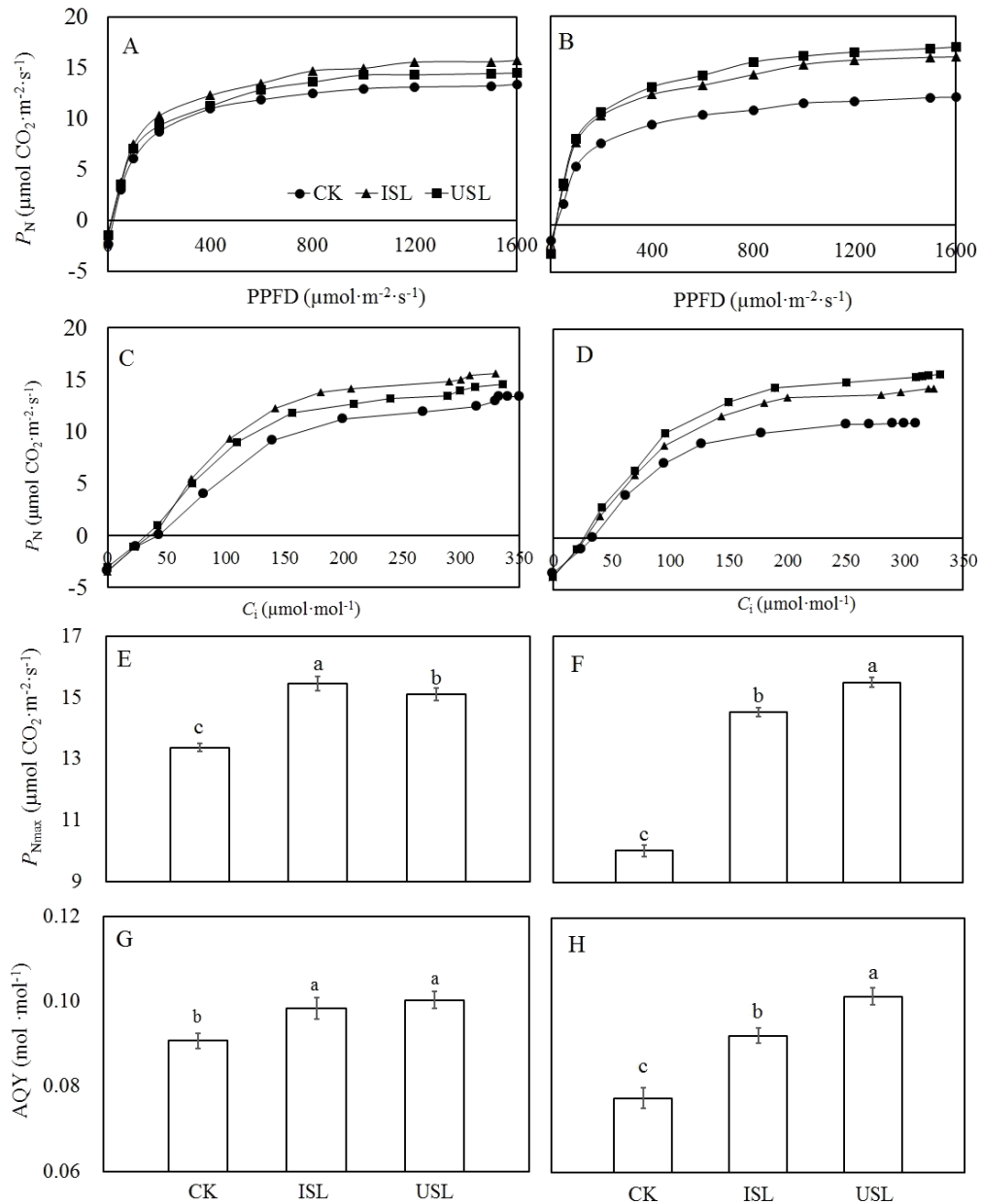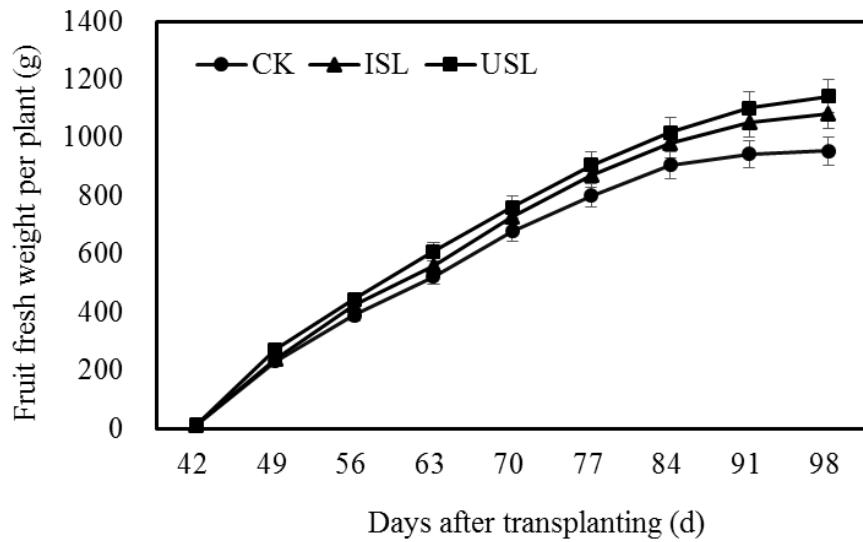
Figure 5 │ Variations in tomato fruit growth resulting from different treatments. Supplemental lighting from underneath (USL) or inner canopy (ISL) was applied to plants from the 42th to 84th day after transplanting, while no supplemental lighting as control (CK).Vertical bars represent standard errors of the means ($n = 12$). Fresh fruit weight (FFW) was calculated as follows: $y = 0.0003x^3 + 0.0215x^2 - 0.6325x + 3.444$, with $R^2 = 0.9908$ [y = fruit fresh weight (FFW), g; x = fruit diameter, mm]

Chapter 6

Polychromatic supplemental lighting from underneath canopy is more effective to enhance tomato plant development by improving leaf photosynthesis and stomatal regulation

6-1 Introduction

In greenhouse tomato cultivation, the light interception of each canopy layer decreases sharply down the plant profile, and mutual shading also occurs (Acock et al., 1978; Lu et al., 2012a; Tewold et al., 2016; Xu et al., 1997). No more than 35% of the total intercepted solar light reaches the leaves under the tomato fruit trusses (Cockshull et al., 1992; Lu et al., 2012a), and such a shortage of light triggers an extremely low net photosynthetic rate and premature leaf senescence (Acock et al., 1978; Xu et al., 1997). Supplemental lighting, using artificial light resources and employed in lower canopies, is considered to be an efficient method for relieving low-light stress on plants. Numerous studies the effects of application of SL have been conducted on various species via aspects of the canopy layer (Hovi et al., 2004; 2008; Pettersen et al., 2010), light source (Lu et al., 2012a; 2012b; Piringer et al., 1960), light intensity (Dorais, 2003; Demers et al., 1998), and light period (Piringer et al., 1960; Tewolde et al., 2016). Among those, the selection of optimized light wavelength is more complex and is often reported with mixed results (Lu et al., 2012b; Ni et al., 2009; Li et al., 2009; Okamoto et al., 1996). For example, blue light suppresses hypocotyl elongation in wheat (Goins et al., 1997) and tomato (Massa et al., 2008), but improves the dry matter production and the photosynthetic capacity in pepper (Brown et al., 1995), wheat (Goins et al., 1997), and

spinach (Matsuda et al., 2007). In contrast, red light seems to be most effective in the biomass assimilation of lettuce (Yanagi et al., 1996; Kim et al., 2006), but not spinach and radish (Okamoto et al., 1996; Yorio et al., 2001). Similarly, different red/far-red ratios show contrary results in phytochemical content (Alokam et al., 2002) and plant photomorphogenesis (Brown et al., 1995; Kirdmanee et al., 1993; Runkle et al., 2001). These results have shown the viability of optimizing the light quality in promoting plant morphology and productivity to eventually improve the greenhouse economic benefits. However, previous studies have mainly examined only a few selected sole light qualities or the compound spectrum with only the combination of red/blue or red/far-red at one time, and there are no reports examining the effects of polychromatic light quality (the combination of white, blue, red and far-red) affecting plant growth and development. Therefore, it is necessary to investigate the polychromatic light quality effects when provided as supplemental lighting resource applied for horticultural crop production.

Photosynthesis is sensitive to light condition and essential for plant growth. Improved leaf photosynthesis would enhance plant development (Hovi et al., 2004, 2008; Pettersen et al., 2010). The investigation of leaf structure–function relationships in photosynthesis shows that internal maximum photosynthesis rates were not close to the leaf surface of the upper epidermis, where light intensity was highest, but instead occurred in the middle and lower palisade layers (Nishio et al., 1993; Evans, 1995, 2003; Sun et al., 1998, 2001). These deeper layers have higher electron transport activities and greater amounts of photosynthetic proteins (Terashima et al., 1985, 1988; Sun et al., 2001). This indicated that the supplemental lighting from the underneath canopy (with light orientation to the abaxial epidermis) might function

better in improving leaf and plant development than using the supplemental lighting from the inner canopy (with light orientation to the adaxial epidermis).

Stomatal regulation, which is highly correlated with leaf photosynthesis, governs the overall CO_2 assimilation and water loss from plants (Casson et al., 2010; Araújo et al., 2011). Stomatal behaviour can be affected by the light wavelength through energy conversion (Chen et al., 2012; Shimazaki et al., 2007), membrane ion transport (Araújo et al., 2011; Fan et al., 2004), and metabolic activity in guard cells (Mott et al., 2008; O'Carrigan et al., 2014). Tomato (*Lycopersicon esculentum* Mill.) is regarded as one of the most important horticultural crops in the world and stomata are mostly distributed on the abaxial epidermis of tomato leaves. We thus hypothesized that supplemental lighting from the underneath canopy have more obvious improvement on the stomatal regulation and photosynthesis capacity, which might consequently better stimulate plant growth and development, compared with supplemental lighting from the inner canopy. White light has a higher penetration rate through the tomato canopy than other colours (Lu et al., 2012b), blue light could contribute a larger stomata size (Loreto et al., 2009; Sharkey et al., 1981) in leaves and a higher health index of tomato plants (Chang et al., 2010; Chen et al., 2014), and red/blue and red/far-red light are the most commonly used in horticulture cultivation. Thus, we added white light to the spectrum in this study to investigate the effects of polychromatic supplemental lighting. Plants undergo the most vegetative growth before anthesis and the fluctuation of environment factors would cause largely morphological changes at this stage (Masuda et al., 2014). Therefore, in this study, to understand how plants respond to the interaction of light quality and light orientation, we treated young tomato plants with/without supplemental lighting with different

polychromatic light quality levels orientated from underneath or inner canopy for four weeks, and investigated the resulting fluctuations in leaf photosynthesis and stomatal behavior, as well as the consequent response of plant morphologic development.

6-2 Materials and methods

Plant material and growth conditions

The experiment was conducted in a glass greenhouse (Venlo-type, with double spans and a north–south orientation) in Urumqi, China (43°46′12″N, 87°40′48″E) from September, 2015 to April, 2016. Tomato ('NS3389', Agricultural Science and Technology Co. Ltd., Guangzhou, China) seeds were sown into trays with commercial substrate (Peilei No. 2, Peile Organic Fertilizer Co., Zhenjiang, China) and germinated in an environmentally controlled box (RTOP-1000D, Top Yun Co. Ltd., Hanzhou, China) for 24 days. Other environment factors were fixed, including the photosynthetic photon flux density (PPFD), photocycle, day/night temperatures and CO_2 concentration of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 16 h, 23/18°C, and 800 $\mu mol \cdot mol^{-1}$, according to Matsuda et al. (2011b, 2014). The trays were sub-irrigated every other day, with a commercial nutrient solution at an electrical conductivity of 1.5 $dS \cdot m^{-1}$.

At 24 days after sowing, each seedling was transplanted into the greenhouse at a plant density of 16.6 $plant \cdot m^{-2}$ with an automatically irrigated nutrient solution (Nakano et al., 2010). The greenhouse environment was maintained, with daytime mean air temperature of 27 ± 2°C, a night-time mean air temperature of 20 ± 2°C, and a daily mean relative humidity above 60%. Although the CO_2 concentration was not measured, it was assumed to be close to the outside level, based on measurements in the same season in another year (data not shown).

Supplemental lighting treatment

Light-emitting diodes (LEDs) (Philips Co. Ltd., Netherlands) were used as the light source. Four polychromatic light, red+ blue (R/B, R: B=3:1), white + red+ blue (W/R/B, W: R: B=3:2:1), white + red + far-red (W/R/FR, W: R: FR=3:2:1) and white + blue (W/B, W: B=2:1), were applied from two light orientations: supplemental lighting from the underneath and inner canopy (Figure 1). LEDs were fixed to movable girders that ensured the lighting distance from the adaxial epidermis of inner canopy leaves or the abaxial epidermis of the lowest leaf truss was maintained at 10 cm. The measured PPFD was 200 μmol·m^{-2}·s^{-1} at 10 cm from the LED module. Plants without supplemental lighting were considered to be the control plants. Each treatment consisted of three bench rows of plants, with each row containing 20 plants, with a 16 h photo cycle each day (during 8:00-24:00 at GMT +8, which is 6:00-22:00 local time).

Gas-exchange parameter measurements

Gas-exchange measurements were conducted on the second terminal leaflets of leaves on the fifth youngest node (Matsuda et al., 2014) with a portable photosynthesis system (Li-6400XT; Li-Cor Inc., Lincoln, NE, USA) during 11:00-16:00, GMT +8 (9:00-14:00, local time) on the 28th day after transplanting. The net photosynthetic rate (P_N), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) were measured. Measurements were conducted with PPFD, leaf temperature, CO_2 concentration, and relative humidity at 800 ± 5 μmol·m^{-2}·s^{-1}, 28 ± 1°C, 400 ± 2 μmol·m^{-2}, and 63 ± 2%, respectively.

The light and CO$_2$ response curve measurement was conducted to calculate the light-saturated maximum photosynthetic rate (P_{Nmax}), apparent quantum yield (AQY), CO$_2$-saturated maximum photosynthetic rate (A$_{max}$), and carboxylation efficiency (CE). The leaf temperature was set at 25°C, and the PPFD and CO$_2$ concentrations ranged from 1600 to 0 μmol·m^{-2}·s^{-1}and 1200 to 0 μmol·mol^{-1}, respectively. The P_N–PPFD and P_N–C_i curves were plotted using a non-linear curving-fitting routine with the P$_N$ data and the corresponding light intensity or intercellular CO$_2$ concentration, respectively. Indexes were identified as P_{Nmax} and A_{max}, the maximum net photosynthetic rate at the saturation light intensity and CO$_2$ concentration, respectively. AQY were the initial slope of the P_N–PPFD curves (Lambers et al., 2008; Skillman, 2008), and CE also known as V_{cmax}, the maximum velocity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) for carboxylation, which can be calculated from P_N–C_i curve according to equation from FvCB model (Farquhar et al., 1980; Sharkey et al., 2007):

$$P_N = V_{cmax}\left[\frac{C_c - \Gamma^*}{C_c + K_c(1 + O/K_o)}\right] - R_d \quad (1)$$

where V_{cmax} is the maximum velocity of Rubisco for carboxylation, C_c is the CO$_2$ partial pressure at Rubisco, K_C is the Michaelis constant of Rubisco for carbon dioxide, O is the partial pressure of oxygen at Rubisco and K_O is the inhibition constant (usually taken to be the Michaelis constant) of Rubisco for oxygen. The symbol Γ* is the photorespiratory compensation point and R_d is day respiration. This equation lends itself to a linear regression approach to estimating V$_{cmax}$ as the slope and -R_d as the intercept.

Chlorophyll fluorescence parameter measurements

Chlorophyll fluorescence parameters were measured to evaluate the light absorption,

electron transfer, thermal dissipation, and excitation distribution in the photosystem of tomato plants treated with or without supplemental lighting from the underneath or inner canopy with polychromatic LEDs after the adaption of the leaves to stable light or dark states. Leaf chlorophyll fluorescences levels were measured simultaneously using a portable photosynthesis system (Li-6400XT, Li-Cor Inc.) with an integrated fluorescence fluorometer (Li 6400-40 leaf chamber fluorometer, Li-Cor Inc.). The gas supply (ambient CO_2 concentrations and 21% O_2), actinic light (LED with 90% red light, 630nm; 10% blue light, 470nm) and measurement light (630nm, 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were all setting in accordance with Sun et al. (2016). The steady state chlorophyll fluorescence level (F_s), minimum chlorophyll fluorescence at the open PSII center (F_o, dark treated; F'_o, light adapted), maximum chlorophyll fluorescence at the closed PSII center (F_m, dark treated; F'_m, light adapted) were all determined in accordance with the work of Kramer et al. (2004). The maximum quantum yield of the PSII primary photochemistry [F_v/F_m; $(F_m-F_o)/F_m$], efficiency of excitation energy capture by open PSII reaction centers [$F'_v/F'_m = (F'_m - F'_o)/F'_m$], quantum yield of the PSII electron transport [$\Phi PSII$; $(F'_m - F_s)/=F'_m$], and non-photochemical quenching [NPQ= $(F_m - F'_m)/ F'_m$] were calculated from the measured parameters (Maxwell et al., 2000).

Stomatal assays

Stomatal assays were carried out essentially as described in work of O'Carrigan et al.(2014) and conducted on abaxial epidermal strips of the leaves at the same position of photosynthesis measurement on the 29th day after transplanting during 11:00-16:00, GMT +8 (9:00-14:00, local time). The samples were peeled, immersed in a transparent nail polish buffer, and mounted on glass slides before micro-imaging. Images of each epidermal strip

were taken under a Leica microscope (Leica Microsystems AG, Solms, Germany) fitted with a Nikon NIS-F1 CCD camera and a Nikon DS-U3 controller (Nikon, Tokyo, Japan), and analyzed with a Nikon NIS Element software. Stomatal density was defined as the number of stomata per mm^2 and stomatal index was calculated as ([number of stomata]/[number of epidermal cells + number of stomata]) × 100 (Kubínová, 1994). The stomatal aperture width and length was defined in Figure 5, and stomatal pore area was calculated by assuming an oval pore shape according to Chen et al. (2012).

Plant growth analyses

On the 30th day after transplantation, plants were destructively harvested for the determination of the dry weights of the shoots and roots, the height and diameter of stems, health index, leaf areas, specific leaf area (leaf area per unit leaf mass), leaf chlorophyll contents, flower number, and carbohydrate determination. Plants were washed with distilled water and weighed after wiping the water off. The leaf area per plant was measured using a leaf area metre (LI-3000C; Li-Cor Inc.). The leaf chlorophyll content was determined using a chlorophyll metre (SPAD-502; Minolta, Osaka, Japan). Samples were oven dried at 80°C until a constant weight was attained, and the dry weight subsequently recorded. The health index, widely used as general evaluation of the young plant growth quality (Chen et al., 2014; Fujii, 1952; 1953; Huang et al., 2012; Song, 1999; Yang et al, 2010; Zhang et al., 1992), was calculated as (stem diameter/stem height) × total dry weight, according to Fan et al. (2013). The carbohydrates, including the soluble-sugar and starch, were measured in samples of the milled leaf material. Soluble sugars were extracted with 80% (v/v) ethanol at 80°C and their contents were determined enzymatically, and starch in the 80% ethanol-insoluble fraction was

extracted and digested, and the resultant glucose content was assayed by Nelson–Somogyi's

method (Matsuda et al., 2014).

Statistical analyses

Duncan's multiple range test was performed at P < 0.05 for among all treatments and

two-way analysis of variance (ANOVA) was performed at P < 0.05 with supplemental light

quality and light orientation as sources of variation. SPSS 11.0 software (SPSS Inc., Chicago,

IL, USA) was used for the statistical analyses.

6-3 Results

Gas-exchange parameter

Compared with the control, regardless of the light quality, the supplemental lighting

from the inner canopy significantly increased the P_N, G_s, C_i, and T_r (Figure 2A-D). In contrast,

while the supplemental lighting from the underneath canopy significantly increased the P_N,

G_s, and T_r, it had no significant effects on C_i. Among the supplemental lighting treatments, as

regard of light quality, data in the W/R/B and W/B was significantly higher than those in the

R/B and W/R/FR. The leaf photosynthesis capacity was significantly promoted by

supplemental lighting. Compared with the control, supplemental lighting significantly

increased the P_{Nmax}, A_{max}, AQY, and CE (Figure 3). Among the supplemental lighting

treatments, the data in the supplemental lighting from the underneath canopy were generally

higher than those in the supplemental lighting from the inner canopy and data in the W/R/B

and W/B were significantly higher than those in the R/B and W/R/FR. The P_{Nmax}, A_{max} and

AQY were highest in the W/R/B from treatments of supplemental lighting from the

underneath canopy and increased by 86.5, 70.0, 53.6%, respectively, compared with the control (Figure 3 C-E). The CE was highest in the W/B from treatment of supplemental lighting from the underneath canopy and increased 57.5% compared with the control (Figure 3F).

Chlorophyll fluorescence parameter

Compared with the control, the supplemental lighting from the underneath canopy significantly increased the efficiency of the excitation energy captured by the open PSII reaction centers (F'_v/F'_m; Figure 4B), the quantum yield of the PSII electron transport (ΦPSII; Figure 4C), and the non-photochemical quenching (NPQ; Figure 4 D;), but had no effect on the maximum quantum yield of the PSII primary photochemistry (F_v/F_m; Figure 4A). Under treatment of supplemental lighting from the inner canopy, the W/R/B and W/B significantly increased ΦPSII and NPQ but had no effect on F_v/F_m, F'_v/F'_m, while R/B and W/R/FR had no effect on any of the indexes.

Stomatal characteristics

Compared with the control, the supplemental lighting significantly increased stomatal density but not influenced stomatal index (Figure 6). The data on the stomatal density in the treatments of supplemental lighting from the underneath canopy were significantly higher than those in the treatments of supplemental lighting from the inner canopy, while no significant difference among the light quality was observed. The stomatal aperture size was significantly affected by the supplemental lighting (Figure 5; Figure 7). The aperture length was significantly decreased, while the aperture width was significantly increased when the leaf was exposed to supplemental lighting (Figure 7 A; B), resulting in a significantly higher

width/length ratio and a larger stomatal pore area (Figure 7 C; D). The data in the W/R/B and W/B were generally higher than those in the R/B and W/R/FR, and the data in the treatments of supplemental lighting from the underneath canopy were generally higher than those in the treatments of supplemental lighting from the inner canopy.

Plant growth and carbohydrate accumulation

Supplemental lighting had positive effects on the plant growth and carbohydrate accumulation (Table 1). With the exception of the stem height, the data in supplemental lighting were obviously higher than the data in the control. Generally, the data in the W/R/B and W/B were higher than those in other light quality and the data in the treatments of supplemental lighting from the underneath canopy were higher than the inner canopy treatments. With the excepted of the leaf area, the light quality, light orientation or quality × orientation had a significant influence on the measured index.

Correlation analysis of growth, photosynthesis and stomatal parameters

The aperture width/length, stomatal pore area and stomatal index, F_v/F_m, ΦPSII, starch content, stem dry weight, specific leaf area, flower number, and health index were highly significantly correlated to photosynthetic performance and growth development of tomato plants ($P < 0.05$; Figure 8; Table 2). Furthermore, two-way ANOVA analysis showed that there were highly significant effects of light quality, light orientation, and interaction factors between the quality×orientation on the growth, stomatal and gas exchange parameters (Table 1; 3). However, there were no significant quality effects on the stem diameter, leaf area (Table 1), F'_v/F'_m, stomatal density and aperture length (Table 3). The orientation had no effects on

the leaf area, specific leaf area, chlorophyll content, soluble sugar content (Table 1), F_v/F_m, stomatal index, aperture length, aperture width/aperture length (Table 3).There was also no significant quality×orientation effects on the shoot dry weight, root dry weight, leaf area (Table 1), F_v/F_m, stomatal index, and aperture length (Table 3).

6-4 Discussion

Plant photosynthesis is extremely sensitive to supra-optimal light conditions. Low light stress can damage the photosynthetic apparatus (Naumburg et al., 2002), degrade the photosynthetic pigments (Aldesuquy et al., 2000), and suppress the carbon assimilation (Nawrocki et al., 2015). In our study, supplemental lighting treatment was found to improve the photosynthesis ability of leaves (Figure 2, Figure 3; Figure 4), which was in accordance with the previous work done to other species (Hovi et al., 2004, 2008; Massa et al., 2008). Chlorophyll captures light and soaks up the energy from it. The chlorophyll content closely related to the photosynthesis ability of leaf (Tewolde et al., 2016), and lack of the light-harvesting complex will affect chloroplasts structure and decrease chlorophyll content (Kovács et al., 2006). Powles (1984) had found that when plant suffered from photoinhibition induced by visible light, the photosynthetic apparatus was injured and chlorophyll content decreased dramatically, while Sokawa et al. (1967) declared that in low light condition, enhanced light illumination would trigger chlorophyll formation and accompanied with increased light harvesting and modified photoreceptor. In this study, the chlorophyll content was enhanced in plants treated with supplemental lighting (SPAD, Table 1), which indicated the improvement of photosynthetic apparatus integrity and light harvesting efficiency. However, there was no significant difference in the chlorophyll content among the

supplemental lighting treatments, indicating that the variation in the increased P_N among the supplemental lighting treatments was probably due to variations in the CO_2 supply (the quantity that entered leaf through stomatal aperture, not the ambient CO_2 concentration) and/or assimilation efficiency. We observed significantly higher P_N and G_s (Figure 2 A; B) in the W/R/B and W/B treatment conditions, indicating that plants treated with these types of polychromatic supplemental lighting had performed better CO_2 utilization efficiency. Considering the relative spectral distribution, there are larger proportions of blue light in W/R/B and W/B. Tough pure blue light has negative effects on photosynthesis, especially on tree species (McCree, 1972; Sarala et al., 2009; Pallozzi et al., 2013), adding blue light to the other spectrum could stimulate photosynthesis in wheat (Goins et al., 1997) and tomato (Arena et al., 2016). Sharkey et al. (1981) found that blue light could induce stomatal opening, thus increasing the stomatal pore area (Figure 7 D) allowing for a higher availability of CO_2 in the mesophyll. The data of P_{Nmax}, A_{max}, AQY, and CE (Figure 3 C-F) are also significantly higher in W/R/B and W/B. P_{Nmax} and A_{max} are related to the activities of photosynthetic electron transport and phosphorylation. AQY represents CO_2 assimilation or oxygen release when one photon is absorbed by the plant, and CE represents the carboxylation efficiency (Reng et al., 2003; Farquhar et al., 1980). These improved photosynthetic parameters confirmed the hypothesis that the enhanced blue light fraction in polychromatic illumination could promote photosynthetic electron transport activity and enhance the CO_2 assimilation efficiency. This result was in consistent with the findings of Hogewoning et al. (2010b), who determined that the photosynthetic capacity of cucumber leaves increased as the blue light fraction increased. Chlorophyll fluorescence parameter variations provided the further

explanation of optimized photosynthetic regulation under SL treatment. We observed significantly higher ΦPSII (Figure 4 C) and NPQ (Figure 4 D) under the W/R/B and W/B treatments. ΦPSII represents the electron supply for photosynthesis, highly correlated to P_N (Table 2), while NPQ suggests excessive energy dissipation ability, a most common form of photo protector against stress (Maxwell et al., 2000). Thus W/R/B and W/B improved the actual quantum yield of PSII electron transport and relieved light insufficiency stress in tomato leaves.

On the other side, compared with the control, plants under the treatments of supplemental lighting from the inner canopy generally presented with increased P_N, G_s, C_i, and T_r (Figure 2 A-D), which indicated that, in addition to the enhancement of chlorophyll content (Table 1), the increase in P_N was mostly caused by improved stomatal conductance, which provide sufficient CO_2 for photosynthesis (Farquhar et al., 1982). This result was in accordance with the research on cucumber (Hao et al., 1999), which demonstrates that after treatment of supplemental lighting, the P_N was increased with G_s and expanded stomatal aperture. However, in the treatments of supplemental lighting from the underneath canopy, accompanied with an increase in the P_N, G_s and T_r of tomato plants, C_i was unaffected compared with the control. Combining the increased in P_{Nmax}, A_{max}, AQY, and CE (Figure 3 C-F), these results suggest that in addition to the influence from chlorophyll content, the increase in the P_N by supplemental lighting from the underneath canopy was mostly related to the highly improved CO_2 assimilation efficiency, rather than to the simply enhanced CO_2 supply. Studies on the effects of abaxial lighting treatment on plant photosynthesis in *Paspalum dilatatum* (Soares et al., 2008) and *Helianthus annuus* (Wang et al., 2008) also

showed photosynthesis improvements closely related to CE. Given that T_r is similar to that in treatments of supplemental lighting from the inner canopy, the CO_2 assimilation efficiency should be the determining factor of P_N variation between the treatments of supplemental lighting from the underneath and inner canopy conditions. F_v/F_m (Figure 4 A) was not statistically changed, reconfirming that the variation of increased P_N among supplemental lighting treatments was due to variation in CO_2 utilization and independent of light-harvesting. However, the increased F'_v/F'_m, ΦPSII and NPQ (Figure 4 B-D) suggested that supplemental lighting from the underneath canopy improved the quantum yield of PSII electron transport and the excessive energy dissipation ability of tomato leaves. The increased ΦPSII meant that the majority of the photons absorbed by PSII and used in photochemistry were promoted to increase the level of the photorespiration rate. Therefore, the supplemental lighting from the underneath canopy could promote quantum yields of both PSII electron transport and carboxylation rates of tomato plants, leading to an increase in the photosynthetic efficiency, which is in accordance with the observed photosynthesis improvements by the application of abaxial lighting treatment on sunflower plants (Wang et al., 2008). In this study, the supplemental lighting from the inner canopy did not affect F'_v/F'_m (Figure 4 B), and only partly increased ΦPSII and NPQ (in W/R/B and W/B, Figures 4 C-D). Data of the above parameters were significantly lower than those in the treatments of supplemental lighting from the underneath canopy. This results reconfirmed the lower carboxylation efficiency in plants treated with supplemental lighting from inner canopy, and this induced a relatively lower P_N, compared with the other kind of light orientation treatment (Figure 2).

Stomatal morphogenesis and behaviour are controlled by genetic as well as

environmental factors, such as in light (Meckel et al., 2007; Mott et al., 2008; O'Carrigan et al., 2014). In this study, stomatal density was not affected by the light quality of supplemental lighting, and the stomatal index was not affected by the supplemental lighting orientation and quality×orientation (Figure 6; Table 3). However, the stomatal form and aperture size was significantly affected by the supplemental lighting conditions (Figure 5, Figure 7). The aperture width was significantly increased in supplemental lighting treatment, accompanied by an increased aperture width/length ratio and stomatal pore area (Figure 7), suggesting that supplemental lighting could remit stomatal closure (Figure 8) to promote enter-cell CO_2 supply other than enhancing the stomatal number, which was in accordance with previous research on the cowpea (Schoch et al., 1980) and other tomato species (Gay et al., 1975; Lee et al., 2007). The stomatal closure, usually induced by environmental stress, prevents CO_2 from entering the mesophyll cells (Mott et al., 2008; Araújo et al., 2011) and decreases the internal CO_2 concentration (Lake et al., 2001). Additionally, the stomatal morphology and density are correlated with leaf photosynthesis and plant development (Figure 8, Table 2). The aperture width/length and stomatal pore area are highly positively correlated to the P_N, G_s, specific leaf area, flower number, and health index, but are negatively linked to the stem height (Table 2). This was in accordance with the work of O'Carrigan et al. (2014), who found that a decrease in the aperture area could reduce the P_N of tomato leave and induce excessive plant growth and a decrease in the flower number. These results indicated that the enlargement of the aperture could increase the CO_2 supply and that stomatal morphology should be an important determinant of photosynthesis and growth of greenhouse cultivated tomato.

Enhanced leaf photosynthesis capacity and optimized stomatal regulation can enhance plant development (Hovi et al., 2004, 2008; O'Carrigan et al., 2014; Pettersen et al., 2010). In this study, tomato morphological features were notably influenced by the application of supplemental lighting (Table 1), reconfirming that plant morphology could be improved by increasing the light intensity (Seibert et al., 1975; Marschner et al., 1989) and also demonstrating the feasibility of cultivating tomato intensively through the application of supplemental lighting to the lower canopy. The dry weight of both stems and roots, specific leaf area, health index, and flower number were remarkably improved in W/R/B and W/B (Table 1), reconfirming that the enlarging blue light fraction in polychromatic illumination has better performance. Meanwhile, the indoleacetic acid (IAA) oxidase activity can be promoted by enhancing blue light proportion in illumination, which decreases the IAA level, consequently preventing excessive growth and guaranteeing reproductive development in various species, such as broad bean (Assmann et al., 1985), pepper (Brown et al., 1995) and lettuce (Li et al., 2009). Additionally, under red light conditions, adding blue light irradiation could trigger epidermal cell elongation of the abaxial side and inhibit leaf epinasty in the geranium (Fukuda et al., 2008), results in more direct irradiation interception. Although R/B consisted of blue light, the green light spectrum was added to the W/R/B and W/B, and the addition of green light could enhance the photochemical content (Li et al., 2009) and drive leaf photosynthesis more efficiently than red light (Terashima et al., 2009). A large proportional increase in the far-red light could significantly limit the biomass accumulation (Wang et al., 2007), which explained the inhibition of tomato plant growth in W/R/FR condition compared with other supplemental lighting treatment conditions.

6-5 Conclusion

Supplemental lighting with polychromatic light applied from either inner canopy or underneath canopy effectively increased tomato photosynthetic efficiency, reduced stomatal closure and improved plant development. W/R/B and W/B from underneath canopy promoted plants with higher health index and faster development. CO_2 utilization efficiency determined the variation of photosynthetic performance among the supplemental lighting treatments. An enhanced blue light fraction in W/R/B and W/B could better stimulate stomatal opening and promote photosynthetic electron transport activity, thus better improving photosynthetic rate. The mechanisms of photosynthesis improvement differed for the two light orientation treatments. The supplemental lighting from the inner canopy improved the photosynthesis of tomato plants by increasing the stomatal conductance to enhance the CO_2 supply for leaf, thereby promoting photosynthetic electron transport activity. The supplemental lighting from the underneath canopy improved photosynthesis by enhancing the CO_2 supply as well as increasing the CO_2 assimilation efficiency and excessive energy dissipation, of which the enhancement contributed to a higher photosynthetic rate compared with the treatment of supplemental lighting from the inner canopy. Stomatal morphology was highly positively associated with leaf photosynthesis and plant development, and is therefore believed to be an important determinant for photosynthesis and growth of greenhouse cultivated tomato.

TABLE 1 │ Effects of polychromatic supplemental lighting quality and light orientation on tomato plant morphological characteristics and carbohydrate accumulation.

Treatment — Light quality	Treatment — Light orientation	Shoot dry weight (g)	Root dry weight (g)	Stem height (cm)	Stem diameter (mm)	Leaf area (m^2)	Specific leaf area ($cm^{-2}\cdot g^{-1}$)	Health index	SPAD	Flower number	Soluble sugar content ($mg.g^{-1}$)	Starch content ($mg.g^{-1}$)
CK		19.03 ± 0.86 e	2.06 ± 0.06 e	85.55 ± 0.77 a	13.0 ± 0.34 e	0.22 ± 0.04 a	104.65 ± 1.04 e	0.32 ± 0.03 f	40.03 ± 1.21 b	1.1 ± 0.11 d	78.3 ± 9.41 d	35.3 ± 1.41 d
R/B	ISL	25.32 ± 0.43 c	2.43 ± 0.03 d	80.63 ± 0.67 b	13.2 ± 0.12 de	0.23 ± 0.03 a	120.21 ± 0.81 c	0.45 ± 0.02 de	45.23 ± 0.64 a	1.7 ± 0.13 b	126.3 ± 4.54 c	43.6 ± 0.91 c
	USL	29.01 ± 0.51 b	3.18 ± 0.04 b	72.25 ± 0.41 de	14.3 ± 0.22 bc	0.24 ± 0.03 a	120.28 ± 0.62 c	0.63 ± 0.02 b	45.14 ± 0.61 a	1.8 ± 0.22 b	125.7 ± 4.61 c	44.1 ± 0.64 c
W/R/B	ISL	28.97 ± 0.54 b	3.15 ± 0.03 b	76.88 ± 0.25 cd	14.0 ± 0.14 c	0.23 ± 0.04 a	127.98 ± 0.44 b	0.58 ± 0.02 bc	45.55 ± 0.33 a	2.3 ± 0.23 a	160.8 ± 5.43 a	47.9 ± 0.73 b
	USL	32.98 ± 0.31 a	3.41 ± 0.02 a	70.24 ± 0.42 e	15.0 ± 0.42 a	0.25 ± 0.02 a	128.23 ± 0.62 b	0.78 ± 0.02 a	45.68 ± 0.24 a	2.4 ± 0.27 a	150.9 ± 4.34 ab	48.1 ± 0.74 b
W/R/FR	ISL	22.53 ± 0.12 d	2.48 ± 0.02 d	79.51 ± 0.53 bc	13.4 ± 0.43 d	0.22 ± 0.02 a	114.78 ± 0.82 d	0.42 ± 0.01 e	45.32 ± 0.51 a	1.4 ± 0.07 c	161.5 ± 6.51 a	43.7 ± 1.11 c
	USL	25.07 ± 0.31 c	2.83 ± 0.03 c	77.42 ± 0.12 cd	14.1 ± 0.12 bc	0.23 ± 0.02 a	115.03 ± 0.71 d	0.50 ± 0.02 d	45.17 ± 0.34 a	1.6 ± 0.10 bc	129.4 ± 4.34 c	43.8 ± 1.08 c
W/B	ISL	25.28 ± 0.32 c	2.80 ± 0.03 c	75.28 ± 0.68 d	14.5 ± 0.28 b	0.25 ± 0.03 a	128.14 ± 0.93 b	0.54 ± 0.01 cd	45.61 ± 0.42 a	2.4 ± 0.33 a	140.9 ± 2.42 b	49.6 ± 0.52 ab
	USL	33.01 ± 0.31 a	3.41 ± 0.01 a	71.01 ± 0.23 e	15.2 ± 0.34 a	0.27 ± 0.01 a	133.32 ± 0.83 a	0.78 ± 0.03 a	45.51 ± 0.63 a	2.5 ± 0.26 a	144.4 ± 2.33 b	50.5 ± 0.93 a
Quality		*	*	*	N.S.	N.S.	*	*.	*	*	*	*	*
Orientation		*	*	*	*	N.S.	N.S.	*	N.S.	*	N.S.	*
Quality × Orientation		N.S.	N.S	*	*	N.S.	*	*	N.S.	*	*	*

Supplemental lighting from the underneath canopy (USL) or from the inner canopy (ISL) was applied to plants from the time of transplantation while a no supplemental lighting condition was considered to be the control (CK).

Means ± SE (n= 8) with different letters within each row indicating significant difference by Duncan's multiple range test at $P < 0.05$.

*, significant by two-way ANOVA at $P < 0.05$; N.S., nonsignificant.

TABLE 2 │ Correlation analysis of selected stomatal parameters, photosynthetic characteristics and plant development of tomato plants under different treatments.

Parameter [a]	AW/AL	SPA	SI	P_N	G_s	F_v/F_m	ΦPSII	SSC	SC	SH	SDW	RDW	SLA	FN	HI
AW/WL	1														
SPA	0.966*[b]	1													
SI	0.884	0.930*	1												
P_N	0.964*	0.957*	0.940*	1											
G_s	0.960*	0.969*	0.942*	0.900	1										
F_v/F_m	0.882	0.889	0.848	0.946*	0.848	1									
ΦPSII	0.783	0.862	0.865	0.934*	0.875	0.816	1								
SSC	0.778	0.681	0.590	0.583	0.611	0.853	0.442	1							
SC	0.963*	0.944*	0.821	0.954 *	0.905*	0.878	0.705	0.798	1						
SH	-0.752	-0.840	-0.897	-0.836	-0.856	-0.713	-0.901*	-0.364	-0.692	1					
SDW	0.847	0.904*	0.922*	0.901*	0.883	0.831	0.883	0.578	0.752	-0.903*	1				
RDW	0.905*	0.918*	0.932*	0.852	0.963*	0.891	0.935*	0.615	0.843	-0.867	0.865	1			
SLA	0.919*	0.950*	0.859	0.908*	0.926*	0.816	0.729	0.634	0.944*	-0.777	0.818	0.834	1		
FN	0.915*	0.965*	0.902*	0.958*	0.929*	0.815	0.753	0.616	0.936*	-0.771	0.809	0.846	0.967*	1	
HI	0.916*	0.921*	0.900*	0.936 *	0.885	0.774	0.899	0.461	0.757	-0.965*	0.965*	0.860	0.853	0.828	1

a Aperture wide/aperture length (AW/AL), stomatal pore area (SPA), stomatal index (SI), net photosynthesis rate (P_N), stomatal conductance (G_s), maximum quantum yield of the PSII primary photochemistry (F_v/F_m), quantum yield of PSII electron transport (ΦPSII), soluble sugar content (SSC), starch content (SC), stem height (SH), stem dry weight (SDW), root dry weight (RDW), specific leaf area (SLA), flower number (FN), health index (HI)

b *, significant by t-test at $P < 0.05$.

TABLE 3 │ Two-way ANOVA analysis of the effects of supplemental light quality, light orientation, and quality × orientation interaction on photosynthetic characteristics and stomatal parameters of tomato plants under different treatment conditions.

Parameter [a]	P_N	G_s	T_r	C_i	P_{Nmax}	AQY	A_{max}	CE	F_v/F_m	F'_v/F'_m	ΦPSII	qN	SD	SI	AL	AW	AW/AL	SPA
Quality	*[b]	*	*	*	*	*	*	*	*	N.S	*	*	N.S	*	N.S	*	*	*
Orientation	*	*	*	*	*	*	*	*	N.S	*	*	*	*	N.S	N.S	*	N.S	*
Quality × Orientation	*	*	*	*	*	*	*	*	N.S	*	*	*	*	N.S	N.S	*	*	*

[a] Net photosynthesis rate (P_N), stomatal conductance (G_s), transpiration rate (T_r), intercellular CO_2 concentration (C_i), light-saturated maximum photosynthetic rate (P_{Nmax}), apparent quantum yield (AQY), CO_2-saturated maximum photosynthetic rate (A_{max}), carboxylation efficiency (CE), maximum quantum yield of the PSII primary photochemistry (F_v/F_m), efficiency of excitation energy capture by open PSII reaction centers (F'_v/F'_m), quantum yield of PSII electron transport (ΦPSII), non-photochemical quenching (qN), stomatal density (SD), stomatal index (SI), aperture length (AL), aperture width (AW), aperture wide/aperture length (AW/AL), stomatal pore area (SPA)

[b] *, significant by two-way ANOVA at $P < 0.05$; N.S., nonsignificant.
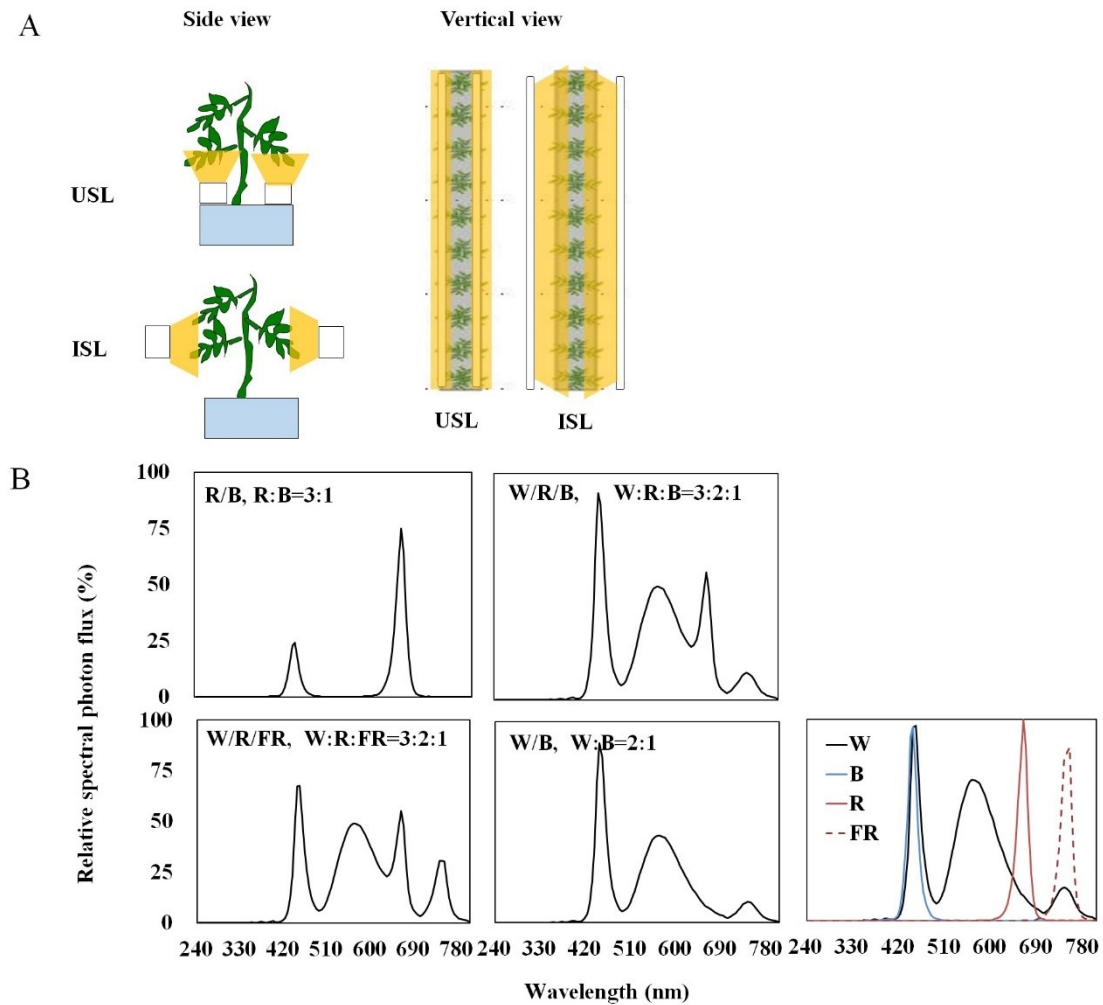
Figure 1 │**Schematic diagram (A) and relative spectral photon flux of polychromatic LEDs (B) of the supplemental lighting treatment in this experiment.** Supplemental lighting from the underneath canopy (USL) or from the inner canopy (ISL) was applied to plants from the time of transplantation while a no supplemental lighting condition was considered to be the control. Each supplemental lighting module was kept fixed at a 10cm distance to the abaxial or adaxial epidermis of the leaf, respectively, with a PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The light quality contains red + blue (R/B, R: B=3:1), white + red + blue (W/R/B, W: R: B=3:2:1), white + red + far-red (W/R/FR, W: R: FR=3:2:1) and white + blue (W/B, W: B=2:1). The spectral property of each LED module used for polychromatic LEDs combination also shown in (B). The wavelengths of the light sources were recorded at 240-800 nm with a spectrometer (SR9910-v7, Irradiant Ltd., Tranent, UK).A digital timer, dimmer, and transformer were used to maintain the light period (16h, GMT+8 8:00-24:00) and light intensity.
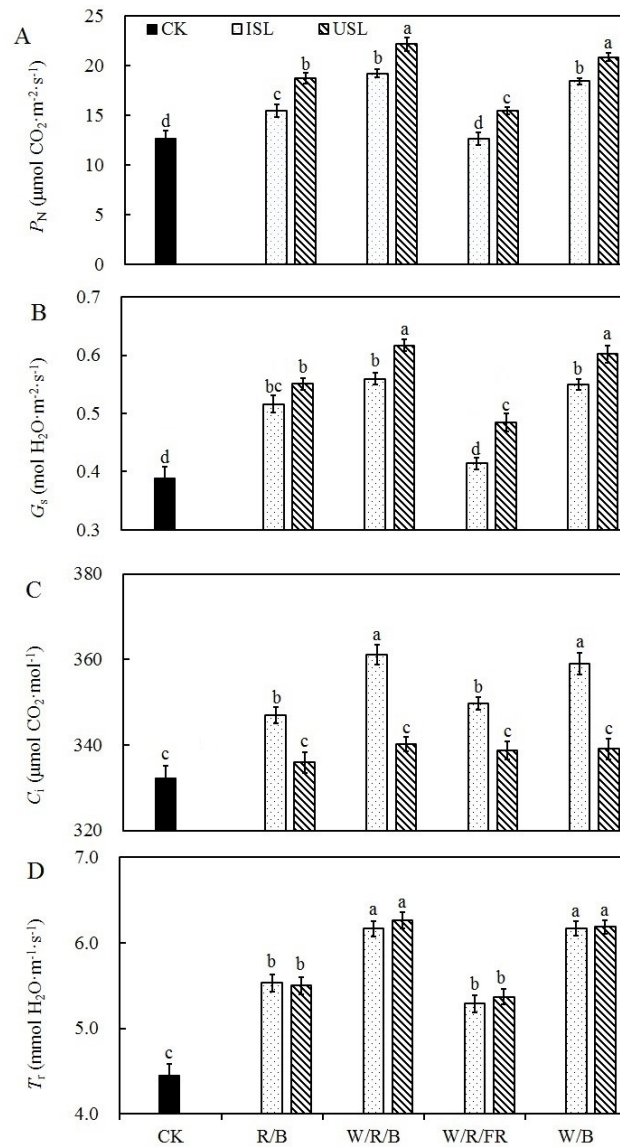
Figure 2 │The effects of different treatments on the net photosynthetic rate (P_N; A), stomatal conductance (G_s; B), intercellular CO_2 concentration (C_i; C), and transpiration rate (T_r; D) in the leaves of tomato plants. Supplemental lighting from the underneath canopy (USL) or from the inner canopy (ISL) was applied to plants from the time of transplantation while a no supplemental lighting condition was considered to be the control (CK). Parameters were measured on the second terminal leaflets of leaves on the fifth youngest node for each treatment. Measurements were conducted with PPFD, leaf temperature, CO_2 concentration, and relative humidity at 800 ± 5 μmol·m^{-2}·s^{-1}, 28 ± 1°C, 400 ± 2 μmol·m^{-2}, and 63 ± 2%, respectively. Means $\pm$ SE (n= 8) different letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.
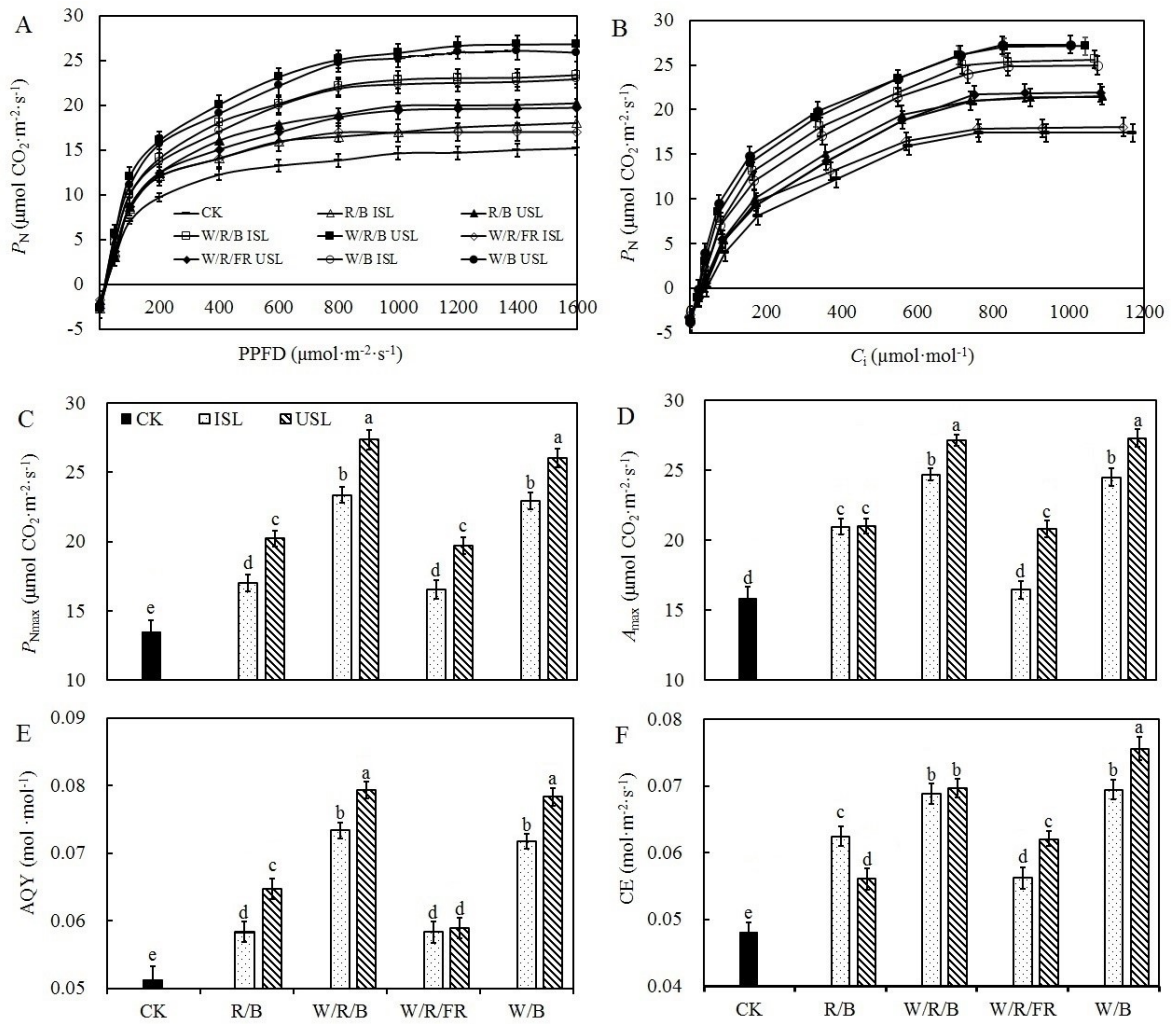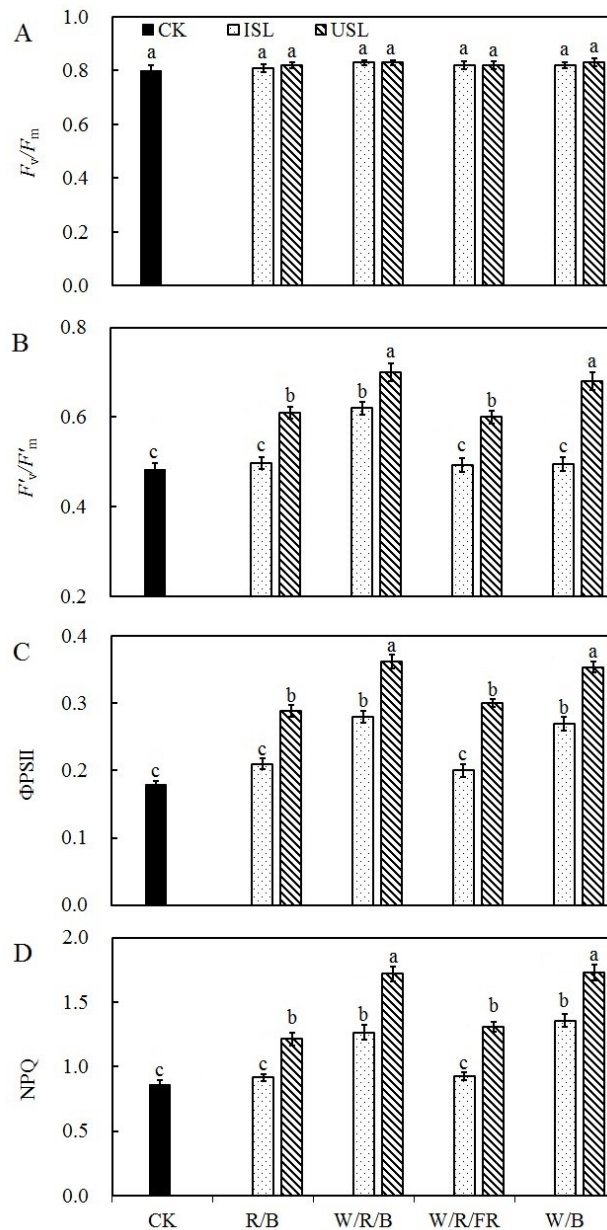
Figure 3 │ **The effects of different treatments on the light response curve (A), CO₂ response curve (B), light-saturated maximum photosynthetic rate (P_{Nmax}; C), CO₂-saturated maximum photosynthetic rate (A_{max}; D), apparent quantum yield (AQY; E), and carboxylic efficiency (CE; F) in the leaves of tomato plants.** Supplemental lighting from the underneath canopy (USL) or from the inner canopy (ISL) was applied to plants from the time of transplantation while a no supplemental lighting condition was considered to be the control (CK). Parameters were measured on the second terminal leaflets of leaves from the fifth youngest node for each treatment. Means ± SE (n= 8) different letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

Figure 4 │ The effects of different treatments on maximum quantum yield of the PSII primary photochemistry (F_v/F_m; A), the efficiency of excitation energy capture by PSII (F'_v/F'_m; B), the quantum yield of PSII electron transport (ΦPSII; C), and non-photochemical quenching (NPQ; D) in leaves of tomato plants. Supplemental lighting from the underneath canopy (USL) or from the inner canopy (ISL) was applied to plants from the time of transplantation while a no supplemental lighting condition was considered to be the control (CK). Parameters were measured on the second terminal leaflets of leaves on the fifth youngest node for each treatment. Means ± SE (n= 8) different letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.
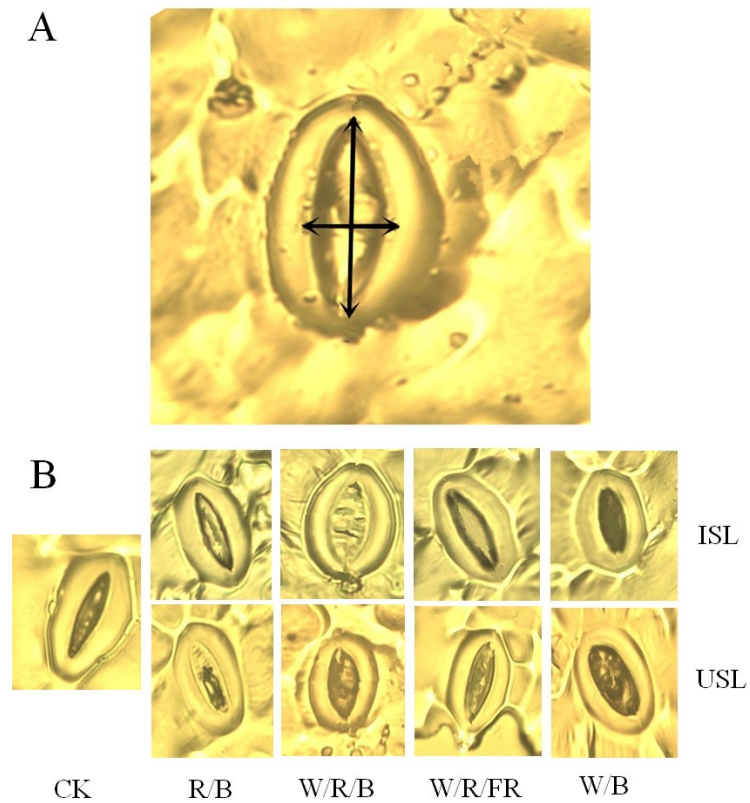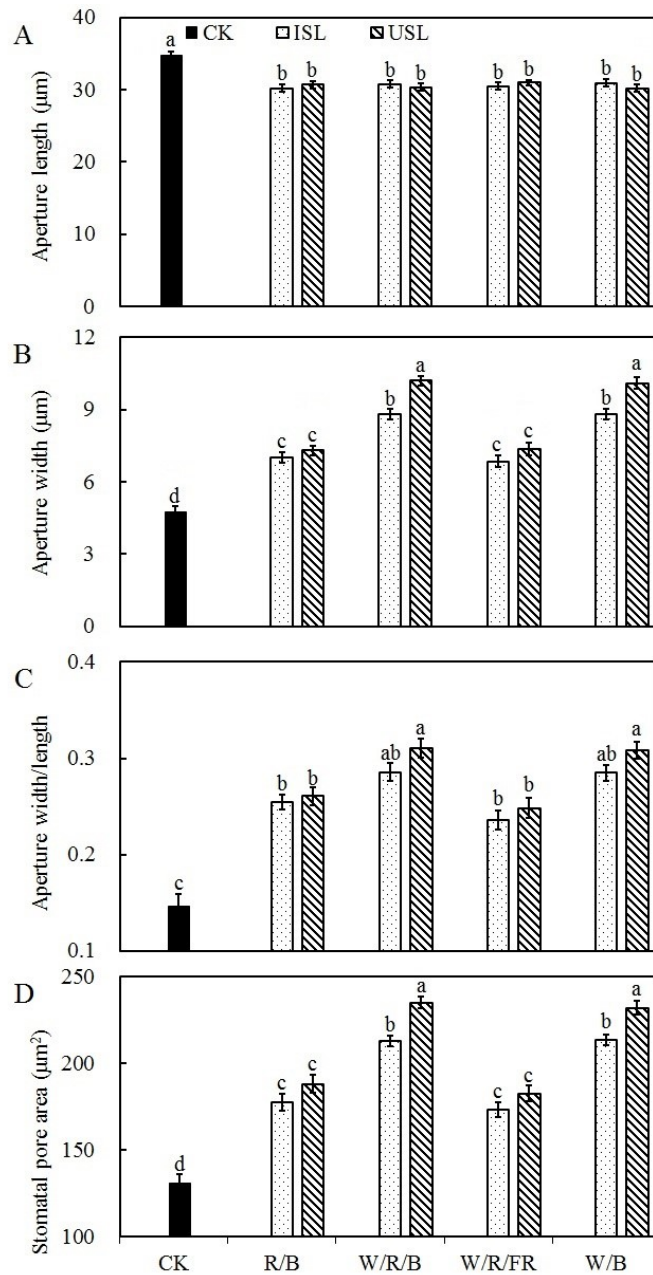
Figure 5 │Representative stomatal images for the measurements of stomatal parameters. Supplemental lighting from the underneath canopy (USL) or from the inner canopy (ISL) was applied to plants from the time of transplantation while a no supplemental lighting condition was considered to be the control (CK). (A) A light micrograph of stomata aperture width (horizontal arrow) and aperture length (vertical arrow) are indicated by double arrows. (B) Typical stomata aperture closure responses to different treatments in this experiment are shown.
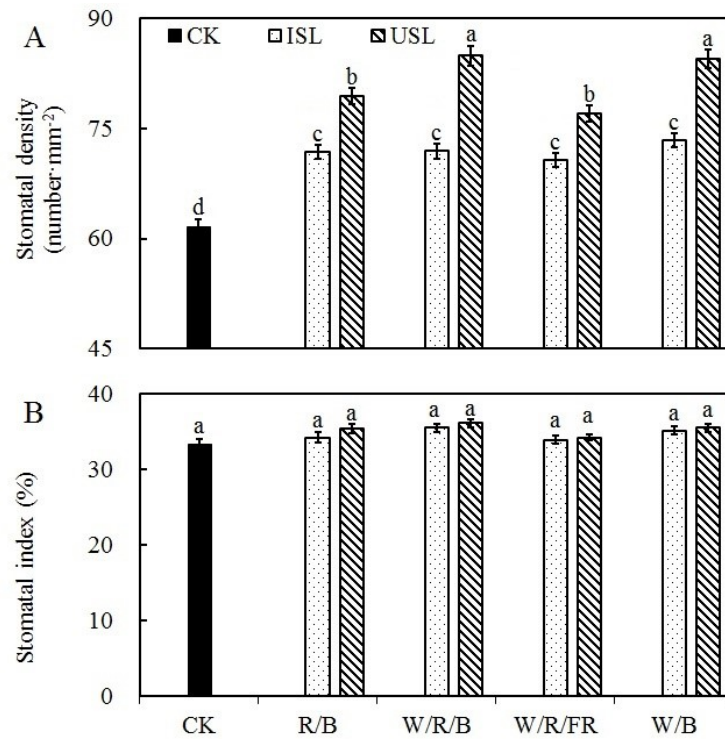
Figure 6 │**The effects of different treatments on the stomatal density (A) and stomatal index (B) in the leaves of tomato plants.** Supplemental lighting from the underneath canopy (USL) or from the inner canopy (ISL) was applied to plants from the time of transplantation while a no supplemental lighting condition was considered to be the control (CK). Parameters were measured on the second terminal leaflets of leaves on the fifth youngest node for each treatment. Means ± SE (n= 16) different letters indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

Figure 7 │ The effects of different treatments on the aperture length (A), aperture width (B), aperture width/length (C), and stomatal pore area (D) in the leaves of tomato plants. Supplemental lighting from the underneath canopy (USL) or from the inner canopy (ISL) was applied to plants from the time of transplantation while a no supplemental lighting condition was considered to be the control (CK). Parameters were measured on the second terminal leaflets of leaves on the fifth youngest node for each treatment. Means ± SE (n= 16) different letters indicate significant differences at *P* < 0.05 according to Duncan's multiple range test.

Chapter 7

Concluding Remarks

7-1 Summary of this thesis

The inter-plant light environment of single-truss tomato production system (STTPS) is complex and fluctuated. In winter production, intensive cultivation often induce light insufficient stress on plant and the understory leaves usually present an extremely low net photosynthetic rate and premature senescence. However, in summer cultivation period, the situation of light insufficient to canopies usually reverses and excessive light irradiation leads to changes ranging from macroscopic whole-plant level to microscopic ion investment. To further enhance the vegetative accumulation and fruit productivity of STTPS, application of periodic alteration of plant density (PD) and supplemental lighting from different orientations were employed in this study. The inter-light condition fluctuation, the plant physiological response mechanism, the optimized PD schedule and economic benefit maximized SL technique were investigated.

In winter production period, seedlings were transplanted to either movable or fixed cultivation benches for treatments with PD fixed PDs of 14.3 plants·m^{-2} (F14.3), and 10 plants·m^{-2} (F10), and unfixed PDs in a movable bench (MB; 25 plants·m^{-2} after transplanting to anthesis/Stage 1, 16.6 plants·m^{-2} at anthesis to initial fruit set/Stage 2, 12.5 plants·m^{-2} at fruit set to mature green/Stage 3 and 11.1 plants·m^{-2} at mature green to red-ripe/Stage 4). The fixed high PD (F14.3) would cause excessive morphologic growth, presenting highest stems but lowest leaf area and shoot dry weight. MB could obviously remit the low light stress in

lower canopies especially in reproductive stage. The relative high PD in vegetative stage would not cause a significant decrease in both leaf photosynthetic capacity and plant morphological development, and it did not affect the plant reproductive potential. However, the PD at least needs to be adjusted to 12.5 plant·m^{-2} at the fruit development stage. Both fruit yield per plant and total yield were highest in MB treatment, and the soluble solids content was also increased in MB and F10 compared with F14.3, while no significant differences in ascorbic acid content or fruit hardness were observed among treatments. In addition, the periodic altering PD treatment would not cause extra labor cost compared with regular intensive cultivation with high PD (F14.3), resulting in highest net profit in MB treatment. Therefore, a high PD in the vegetative stage but relatively lower PD in the fruit development stage was highly economically efficient.

In summer production period, the periodic alteration of PD setting was 33.3 plants·m^{-2} (Stage 1), 20.0 plants·m^{-2} (Stage 2), 14.3 plants·m^{-2} (Stage 3) and 12.5 plants·m^{-2} (Stage 4). The periodic altering PD treatment could not only save cultivation space but also optimize the inter-plant light condition and thermal environment in high irradiation season. Tomato leaf photosynthesis ability was guaranteed by periodic altering PD treatment via declining curling and senescence and enhancing of CO_2 assimilation. The optimized inter-plant environment also improved fruit carbon assimilation which promoted yield and quality. Although different from the results of winter production, the treatment of periodic alteration of PD in high irradiation season would cause extra labor cost compared with regular intensive cultivation with high PD (F14.3). However, due to the significantly higher total yield, the net profit of MB was still highest, re-demonstrating the highest economic efficiency of the treatment of

periodic alteration of PD in STTPS. Thus, periodic altering PD treatment was effective in dissipating excessive light stress and maintaining high economic efficient for greenhouse tomato production.

Application of supplemental lighting from underneath the canopy (USL) with light orientation to the abaxial epidermis and from the inner canopy (ISL) with light orientation to the adaxial epidermis to young tomato plants showed the results that both ISL and USL effectively increased tomato plant growth and photosynthetic efficiency, while USL promoted fast developed plants. The mechanisms of photosynthesis improvement differed for the two treatments. The former improved photosynthesis of tomato plants through increasing stomatal behavior and stomatal conductance to enhance CO_2 supply, thereby promoting photosynthetic electron transport activity and phosphorylation, rather than enhancing CO_2 assimilation efficiency. The latter improved photosynthesis by enhancing CO_2 assimilation efficiency rather than stomatal regulation.

Further investigation of actual economic benefit of ISL and USL on STTPS, showed that both ISL and USL effectively increased tomato leaf photosynthetic efficiency, plant growth and fruit productivity while USL could achieve higher economic benefit. USL maintain relatively steady light irradiation condition in tomato lower canopy and promoted leaf photosynthesis ability through improvement of CO_2 assimilation in addition to stomatal conductance, which contributed more on fruit carbon assimilation compare to ISL. However, ISL could introduced more soluble solid content on fruit because of more scattered light illuminated on fruit.

7-2 Future research aspects

The plant density not only makes the shading index, also affects other plant biological traits. However, in this study, we concentrated on using different morphological shapes and space demanding of plants at different stages, to remit the light insufficiency stress in winter cultivation and excessive irradiation harm in summer cultivation. To directly evaluate the effect of periodic alteration plant density on light condition around lower canopy, we chose shading index to describe the extent of light insufficiency among the treatments. The changes in light condition shall cause plant physiological response. Thus, we chose both photosynthetic ability, growth index (e.g. RGR, NAR) and fruit quality, which were important for evaluation of cultivation economic benefit, to describe the effect on such plant biological trait. However, plant biological traits cover more than what we list above. Due to the limitation of research aspect and experiment condition, we could not discuss further about plant's organ or tissue. Therefore, further study on plant biological traits is considered necessary for clearer understanding of plant response to periodic alteration plant density.

On the other aspect, supplemental lighting applied to underneath or inner canopy of tomato is efficient to enhance plant growth and fruit development and USL promotes more leaf photosynthesis and higher CO_2 assimilation efficiency compared with ISL. However, light quality is also an important factor affecting supplemental lighting economic benefit. In this study, due to the experiment limitation, we did not take the light quality in to consideration. Therefore, in future research, the combination of light quality and light orientation should be considered for optimal supplemental lighting technique. Meanwhile, although the USL could maintain a relative steady light condition along the plant profile, the

fruit soluble solid content of this USL-treated plant was lower than that of ISL-treated plant. This fact that relatively lack of scattered light illuminated on fruit in USL treatment indicates the location of USL might need adjustment (e.g. distance between leaf surface and LED lamps), and further research on this aspect is also necessary.

Reference

Adams, S.R., Woodward, G.C., and Valdés, V.M. (2002). The effects of leaf removal and of modifying temperature set-points with solar radiation on tomato yields. *Journal of Horticultural Science and Biotechnology*. 77: 733-738.

Acock, B., Charles-Edwards, D.A., Fitter, D.J., Hand, D.W., Ludwig, L.J., Warren Wilson, J. (1978). The contribution of leaves from different levels within a tomato crop to canopy net photosynthesis: an experimental examination of two canopy models. *Journal of Experimental Botany*. 111: 815-827.

Aldesuquy, H.S., Abdel-Fattah, G.M., Baka, Z.A. (2000). Changes in chlorophyll, polyamines and chloroplast ultrastructure of puccinia striiformis induced 'greenislands' on detached leaves of *Triticum aestivum*. *Plant Physiology and Biochemistry*. 38: 613–620.

Allen, M.T., Pearcy, R.W. (2000). Stomatal versus biochemical limitations to dynamic photosynthetic performance in four tropical rainforest shrub species. *Oecologi*a. 122:479-486.

Alokam, S., Chinnappa, C.C., Reid, D.M. (2002). Red/far-red light mediated stem elongation and anthocyanin accumulation in *Stellaria longipes*: differential response of alpine and prairie ecotypes. *Canadian Journal of Botany*. 80:72-81.

Alonso, A., Pérez, P., Morcuende, R., Martinez-Carrasco, R. (2008). Future CO_2 concentrations, though not warmer temperatures, enhance wheat photosynthesis temperature responses. *Physiologia Plantarum*, 132: 102-112.

Anttonen, M.J., Hoppula, K.I., Nestby, R., Verheul, M.J., Karjalainen, R.O. (2006).Influence of fertilization, mulch color, early forcing, fruit order, planting date, shading, growing

environment, and genotype on the contents of selected phenolics in strawberry (*Fragaria × ananassa Duch*.) fruits. *Journal of Agricultural and Food Chemistry*. 54:2614-2620.

Araújo, W.L., Fernie, A.R., Nunes-Nesi, A. (2011). Control of stomatal aperture. A renaissance of the old guard. *Plant Signaling & Behavior*. 6:1305-1311.

Arena, C., Tsonev, T., Donevac, D., De Micco, V., Michelozzi, M., Brunetti, C., Centritto, M., Fineschi, S., Velikova, V., Loreto, F. (2016).The effect of light quality on growth, photosynthesis, leaf anatomy and volatile isoprenoids of a monoterpene-emitting herbaceous species (*Solanum lycopersicum* L.) and an isoprene-emitting tree (*Platanus orientalis* L.). *Environmental and Experimental Botany*.130:122-132.

Assmann, S.M., Simoncini, L., Schroeder, J.I. (1985). Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*. *Nature*. 318: 285-287.

Bai, K.D., Liao, D.B., Jiang, D.B., Cao, K.F. (2008). Photosynthetic induction in leaves of co-occurring *Fagus lucida* and *Castanopsis lamontii* saplings grown in contrasting light environments. *Trees*. 22:449-462.

Baroli, I., Price, G.D., Badger, M.R., von Caemmerer, S. (2008). The contribution of photosynthesis to the red light response of stomatal conductance. *Plant Physiology*. 146: 737-747.

Bassman, J.B., Zwier, J.G. (1991).Gas exchange characteristics of *Populus trichocarpa*, *Populus deltoides* and *Populus trichocarpa × P. deltoides* clones. *Tree Physiology*. 11:145-149.

Berger, D., Altmann, T. (2000). A subtilisin-like serine protease involved in the regulation of stomatal density and distribution in *Arabidopsis thaliana*. *Genes & Development*.

14:1119-1131.

Bernacchi, C. J., Singsaas, E. L., Pimentel, C., Portis Jr, A. R., Long, S. P. (2001). Improved temperature response functions for models of Rubisco‐limited photosynthesis. *Plant, Cell & Environment*. 24: 253-259.

Bernacchi, C.J., Bagley, J.E., Serbin, S.P., Ruiz-Vera, U., Rosenthal, D.M., Vanloocke, A. (2013). Modelling C_3 photosynthesis from the chloroplast to the ecosystem. *Plant, Cell & Environment*. 36:1641-1657.

Björkman O. (1981). Responses to different quantum flux densities. In Physiological Plant Ecology I. Responses to the Physical Environment (eds O.L. Lange, P.S. Nobel, C.B. Osmond & H. Ziegler). *Encyclopedia of Plant Physiology*, New Series, Vol. 12A, pp. 57-107. Springer-Verlag, Berlin.

Bleasdale, J.K.A., Nelde, J.A. (1960). Plant population and crop yield. *Nature*. 188: 342.

Boselli, M., DiVaio, C., Pica, B. (1998). Effect of soil moisture and transpiration on mineral content in leaves and berries of Cabernet sauvignon grapevine. *Journal of Plant Nutrition*. 21:1163-1178.

Bristow, K.L., Campbell, G.S. (1984). On the relationship between incoming solar radiation and daily maximum and minimum temperature. *Agricultural and Forest Meteorology*. 31: 159-166.

Brouwer, R. (1962). Distribution of dry matter in the plant. *Netherlands Journal of Agricultural Sciences*. 10: 361-376.

Brown, C.S., Schuerger, A.C., Sager, J.C. (1995). Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. *Journal*

of the American Society for Horticultural Science. 120: 808-813.

Bunce, J.A. (1997). Does transpiration control stomatal responses to water vapour pressure deficit? *Plant, Cell & Environment*. 20:131-135.

Casal, J.J., Deregibus, V.A., Sánchez, R.A. (1985). Variations in tiller dynamics and morphology in *Lolium multiflorum Lam*. vegetative and reproductive plants as affected by differences in red/far-red irradiation. *Annals of Botany*.56:533-559.

Casson, S.A., Hetherington, A.M. (2010). Environmental regulation of stomatal development. *Current Opinion in Plant Biology*. 13: 90-95.

Cerdán, P. D., Chory, J. (2003). Regulation of flowering time by light quality. *Nature*. 423: 881-885.

Charles, W.B., Harris, R.E. (1972). Tomato fruit-set at high and low temperatures. *Canadian Journal of Plant Science*. 52: 497-506.

Chazdon, R.L., Pearcy, R.W. (1986) Photosynthetic responses to light variation in rainforest species. II. Carbon gain and photosynthetic efficiency during light flecks. *Oecologia*. 69:524-531.

Chen, Z.H., Hills, A., Batz, U., Amtmann, A., Lew, V.L., Blatt, M.R. (2012). Systems dynamic modeling of the stomatal guard cell predicts emergent behaviors in transport, signaling, and volume control. *Plant Physiology*. 159:1235-1251.

Chen, J.W., Zhang, Q., Li, X.S., Cao, K.F. (2011). Steady and dynamic photosynthetic responses of seedlings from contrasting successional groups under low-light growth conditions. *Physiologia Plantarum*.141:84-95.

Chen, C., Huang, M., Kuan-Hung Lin, K., Wong, S., Huang, W., Yang, D. (2014). Effects of

light quality on the growth, development and metabolism of rice seedlings (*Oryza sativa* L.). *Research Journal of Biotechnology*. 9:15-24.

Cockshull, K.E., Graves, C.J., and Cave, C.R.J. (1992). The influence of shading on yield of greenhouse tomatoes. *Journal of Horticultural Science and Biotechnology*. 67:11-24.

Coupe, S.A., Palmer, B.G., Lake, J.A., Overy, S.A., Oxborough, K., Woodward, F.I., Gray, J.E., Quick, W.P. (2006). Systemic signalling of environmental cues in Arabidopsis leaves. *Journal of Experimental Botany*. 57: 329-341.

Demers, D.A., Dorais, M., Wien, C.H., Gosselin, A. (1998). Effects of supplemental light duration on greenhouse tomato (*Lycopersicon esculentum Mill.*) plants and fruit yields. *Scientia Horticulturae*. 74: 295-306.

Demers, D.A., Gosselin, A. (2002). Growing greenhouse tomato and sweet pepper under supplemental lighting: optimal photoperiod, negative effects of long photoperiod and their causes. *Acta Horticulturae*. 580: 83-88.

De Villers, A. J., Van Rooyen, M. W., Theron, G. K., Van de Venter, H. A. (1994). Germination of three Namaqualand pioneer species, as influenced by salinity, temperature and light. *Seed Science and Technology*. 22: 427-433.

Dong, H., Zhang, D., Tang, W., Li, W., Li Z. (2005). Effects of planting system, plant density and flower removal on yield and quality of hybrid seed in cotton. *Field Crops Research*. 93:74-84.

Dougher, T.A.O., Bugbee, B. (2001). Differences in the response of wheat, soybean and lettuce to reduced blue radiation. *Photochemistry and Photobiology*. 73:199-207.

Dumas, Y., Dadomo, M., Lucca, G.D., Grolier, P. (2003). Effects of environmental factors and

agricultural techniques on antioxidant content of tomatoes. *Journal of the Science of Food and Agriculture*. 83: 369-382.

Enochs, I. C., Manzello, D.P., Carlton, R., Schopmeyer, S., van Hooidonk, R., Lirman, D. (2014). Effects of light and elevated p CO_2 on the growth and photochemical efficiency of *Acropora cervicornis*. *Coral Reefs*. 33: 477-485.

Evans, J.R. (1995). Carbon fixation profiles do reflect light absorption profiles in leaves. *Australian Journal of Plant Physiology*. 22: 865-873.

Evans, J.R., Poorter, H. (2001). Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen in maximizing carbon gain. *Plant Cell and Environment*. 24:755-767.

Evans J.R, Vogelmann TC. (2003). Profiles of ^{14}C fixation through spinach leaves in relation to light absorption and photosynthetic capacity. *Plant, Cell & Environment*. 26: 547-560.

Fan, L.M., Zhao, Z., Assmann, S.M. (2004). Guard cells: a dynamic signaling model. *Current Opinion in Plant Biology*. 7: 537-546.

Fan, X., Xu, Z., Liu, X., Tang, C., Wang, L., Han, X. (2013). Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. Scientia Horticulturae. 153: 50-55.

Farquhar, G.D., Sharkey, T.D. (1982a).Stomatal conductance and photosynthesis. *Annual Review of Plant Physiol*. 33: 317-345.

Farquhar, G.D., von Caemmerer, S. (1982b). Modelling of photosynthetic response to environmental conditions. In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (Eds.), Physiological Plant Ecology II: Water Relations and Carbon Assimilation.

Springer-Verlag, Berlin, pp. 549-587.

Farquhar, G.D., von Caemmerer, S., Berry, J.A. (1980). A biochemical model of photo-synthetic CO_2assimilation in leaves of C_3 species. *Planta*. 149: 78-90.

Fisher, D.F., Giacomelli, G.A., Janes, H.W. (1990). A system of intensive tomato production using ebb-flood benches. *Professional Horticulture*. 4: 99-106.

Franklin, K.A., Larner, V.S., Whitelam, G.C. (2005). The signal transducing photoreceptors of plants. *International Journal of Developmental Biology*. 49:653-664.

Frantz, J.M., Joly, R.J., and Mitchell, C.A. (2000). Intra-canopy lighting influences radiation capture, productivity, and leaf senescence in cowpea canopies. *Journal of American Society for Horticulture Science*. 125: 694-701.

Fu, Q., Zhao, B., Wang, X., Wang, Y., Ren, S., Guo, Y. (2011). The responses of morphological trait, leaf ultrastructure, photosynthetic and biochemical performance of tomato to differential light availabilities. *Agricultural Sciences in China*. 10: 1887-1897.

Fujii, T. (1952). Cultivation Techniques of Tomato. Kyoto: Takii Shubyo Shuppanbu Press (in Japanese).

Fujii, T. (1953). Vegetables Horticulture, 8*th* E*dn*. Tokyo: Yokendo Press (in Japanese).

Gardner, F.P., Pearce, R.B., Mitchell, R.L. (1985). *Physiology of crop plants*. Iowa State University Press. p327.

Gautier, H., Diakou-Verdin, V., Benard, C., Reich, M., Buret, M., Bourgaud, F., Poëssel, J.L., Caris-Veyrat, C., Génard, M. (2008). How does tomato quality (sugar, acid and nutritional quality) vary with ripening stage, temperature and irradiation. *Journal of Agricultural and Food Chemistry*. 56:1241-1250.

Gautire, H., Massot, C., Stevens, R., Sérino, S., Génard, M. (2009). Regulation of tomato fruit ascorbate content is more highly dependent on fruit irradiance than leaf irradiance. *Annals of Botany*.103:495-504.

Gay, A.P., Hurd, R.G., 1975. The influence of light on stomatal density in the tomato. *New Phytologist*. 75: 37-46.

Giniger, M.S., McAvoy, R.J., Giacomelli, G.A., Janes, H.W. (1988). Computer simulation of a single truss tomato cropping system. *Transactions of the ASAE*. 31:1176-1179.

Goins, G.D., Yorio, N.C., Sanwo, M.M., Brown, C.S. (1997). Photo morphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. *Journal of Horticultural Science and Biotechnology*. 48: 1407–1413.

Goto, N., Kumagai, T., Koornneef, M. (1991) Flowering responses to light-breaks in photomorphogenic mutants of Arabidopsis thaliana, a long-day plant. *Physiologia Plantarum*. 83:209-215.

Govindasamy. R. (1996). A re-examination of supply response in the Northeastern fresh tomato market: Evidence from cointegration and error correction analysis. *New Jersey Agr. Expt. Sta. Res. Rpt*. pp96.

Greffet, J.J., Carminat, R., Joulain, K., Mulet J. P. Mainguy, S., Chen, Y. (2002).Coherent emission of light by thermal sources. *Nature*. 416:61-64.

Groenendijk, M., Dolman, A.J., van der Molen, M.K., Leuning, R., Arneth, A., Delpierre, N., Gash, J.H.C., Lindroth, A., Richardson, A.D., Verbeeck, H., Wohlfahrt, G. (2011). Assessing parameter variability in a photosynthesis model within and between plant

functional types using global Fluxnet eddy covariance data. *Agricultural and Forest Meteorology*. 151:22-38.

Gunnlaugsson, E., Adalsteinsson, S. (2006). Inter light and PD in year-round production of tomato at northern latitudes. *Acta Horticulturae*. 711: 71-75.

Hadfield, K. A., Rose, J.K.C., Yaver, D.S., Berka, R.M., Bennett, A.B. (1998). Polygalacturonase gene expression in ripe melon fruit supports a role for Polygalacturonase in pipening-associated pectin disassembly. *Plant Physiology*. 117: 363-373.

Han Q, Yamaguchi E, Odaka N, Kakubari Y (1999) Photosynthetic induction responses to variable light under field conditions in three species grown in the gap and understory of a *Fagus crenata* forest. *Tree Physiology*. 19:625-634.

Hao, X., Papadopoulos, A.P. (1999) Effects of supplemental lighting and cover materials on growth, photosynthesis, biomass partitioning, early yield and quality of greenhouse cucumber. *Scientia Horticulturae*. 80:1-18.

Heuvelink, E., Dorais, M., (2005). Crop growth and yield. In: Heuvelink, E. (Ed.), Tomatoes. CABI Publishing, Wallingford, pp. 81-144.

Higashide, T., Heuvelink, E., (2009). Physiological and morphological changes over the past 50 years in yield components in tomato. *Journal of the American Society for Horticultural Science*. 134:460-465.

Hills, A., Chen, Z.H., Amtmann, A., Blatt, M.R., Lew, V.L. (2012). OnGuard, a computational platform for quantitative kinetic modelling of guard cell physiology. *Plant Physiology*. 159:1026-1042.

Hoenecke, M., Bula, R.J., Tibbitts, T.W. (1992). Importance of 'blue' photon levels for lettuce seedings grown under red light-emitting diodes. *Hort Science*. 27:427-430.

Hogewoning, S.W., Douwstra, P., Trouwborst, G., van Ieperen, W., Harbinson, J. (2010a). An artificial solar spectrum substantially alters plant development compared with usual climate room irradiance spectra. *Journal of Experimental Botany*. 61, 1267-1276.

Hogewoning, S.W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W., Harbinson, J. (2010b).Blue light dose–responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. J*ournal of Experimental Botany*. 61: 3107-3117.

Horton, P., Ruban, A.V., Walters, R.G. (1996). Regulation of light harvesting in green plants. *Annual Review of Plant Physiology and Plant Molecular Biology*. 47:655-684.

Hovi, T., Nakkila, J., and Tahvonen, R. (2004). Intra-canopy lighting improves production of year-round cucumber. *Scientia Horticulturae*. 102: 283-294.

Hovi, T., Tahvonen, R. (2008). Effect of inter-lighting on yield and external fruit quality in year-round cultivated cucumber. *Scientia Horticulturae*. 116: 152-161.

Huang, S., Xu, F., Wang, W., Du, J., Ru, M., Wang, J., Cao X. (2012). Seedling index of Salvia miltiorrhiza and its simulation model. *Chinese Journal of Applied Ecology.* 23: 2779-2785 (in Chinese with English abstract).

Janes, H.W., McAvoy, R.J. (1989). Alternative greenhouse tomato production. The Rutgers single-cluster system. *American Vegetable Grower*. 37: 14-16.

Janes, H.W., McAvoy, R.J. (1991). Environmental control of a single-cluster greenhouse tomato crop. *Hort Technology*. 1:110-114.

Jiang, C., Gao, H., Zou, Q., Jiang, G., Li L. (2006). Leaf orientation, photorespiration and xanthophyll cycle protect young soybean leaves against high irradiance in field. *Environmental and Experimental Botany*. 55:87-96.

Kagawa, T., Sakai, T., Suetsugo, N., Oikawa, K., Ishiguro, S., Kato, T., Tabata, S., Okada, K., Wada, M. (2001). Arabidopsis NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response. *Science*. 291:2138-2141.

Karpinski, S., Escobar, C., Karpinska, B., Creissen, G., Mullineaux, P.M. (1997). Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in Arabidopsis during excess light stress. *Plant Cell*. 9: 627-640.

Karpinski, S., Reynolds, H., Karpinska, B., Wingsle, G., Creissen, G., Mullineaux, P.M. (1999). Systemic signalling and acclimation in response to excess excitation energy in Arabidopsis. *Science*. 284: 654-657.

Khazaei, H., Monneveux, P., Hongbo, S., Mohammady, S. (2010). Variation for stomatal characteristics and water use efficiency among diploid, tetraploid and hexaploid Iranian wheat landraces. *Genetic Resources & Crop Evolution*. 57, 307-314.

Kim, H.H., Wheeler, R.M., Sager, J.C., Goins, G.D., Norikane, J.H. (2006). Evaluation of lettuce growth using supplemental green light with red and blue light-emitting diodes in a controlled environment-a review of research at Kennedy Space Center. *Acta Horticulturae*. 711:111-119.

Kirdmanee, C., Kitaya, Y., Kozai, T. (1993). Effect of supplemental far-red lighting and photosynthetic photon flux density on stem elongation and dry weight increase of Eucalyptus camaldulensis in vitro plantlets. *XVth International Botanical Congress*,

Yokohama, Japan. 537.

Kläring, H.P., Krumbein, A. (2013). The effect of constraining the intensity of solar radiation on the photosynthesis, growth, yield and product quality of tomato. *Journal of Agronomy and Crop Science*. 199: 351-359.

Kobayashi, S. (1997). A study on single-truss tomato production by hydroponics. Ⅰ.Plant growth and fruit yield by different sowing dates over the year. Journal of the Society of Agricultural Structures, Japan. 27:199-206. (in Japanese, with English abstract).

Kobayashi, S. (1999). A study on single-truss tomato production by hydroponics. Ⅲ. Effects of plant density and number of leaf above the truss on plant growth and fruit yield. Journal of the Society of Agricultural Structures, Japan. 30:53-60. (in Japanese, with English abstract).

Kobayashi, S. (1997). A study on single-truss tomato production by hydroponics. Ⅰ.Plant growth and fruit yield by different sowing dates over the year. *Journal of the Society of Agricultural Structures, Japan*. 27:199-206. (in Japanese, with English abstract).

Kobayashi, S. (1999). A study on single-truss tomato production by hydroponics. Ⅲ. Effects of plant density and number of leaf above the tr uss on plant growth and fruit yield. *Journal of the Society of Agricultural Structures, Japan*. 30:53-60. (in Japanese, with English abstract).

Kozai, T., Kubota, C., Fujiwara, K., Ibaraki, Y., Sase, S. (1996). Proceedings of the international symposium on plant production in closed ecosystems, *International Society for Horticultural Science*, pp 674.

Kozai, T. (2005). Closed systems for high quality transplants using minimum resources. In:

Plant Tissue Culture Engineering (ed. by Gupta, S. and Y. Ibaraki, 480 pp.), Springer, Berlin. 275-312.

Kozai, T., Niu, G. (2016). "Plant factory as a resource-efficient closed plant production system", In *Plant factory: an indoor vertical farming system for efficient quality food production*, eds. Kozai, T., Niu, G., and Takagaki, M (Elsevier), 69-90.

Kubínová, L. (1991). Stomata and mesophyll characteristics of barley leaf as affected by light: stereological analysis. *Journal of Experimental Botany*. 42; 995-1001.

Kull, O., Broadmeadow, M., Kruijt, B., Meir, P. (1999). Light distribution and foliage structure in an oak canopy. *Trees*. 14: 55-64.

Lake, J.A., Quick, W.P., Beerling, D.J., Woodward, F.I., (2001). Plant development: signals from mature to new leaves. *Nature*. 411:154.

Lambers, H., Chapin III, F. S., Pons, T,L. (2008).Plant Physiological Ecology. Springer Science & Business Media. pp. 27.

Leakey, A.D.B., Scholes, J.D., Press, M.C. (2004). Physiological and ecological significance of sunflecks for dipterocarp seedlings. *Journal of Experimental Botany*. 56:469-482.

Lee, S.H., Tewari, R.K., Hahn, E.J., Park, K.Y. (2007). Photon flux density and light quality induce changes in growth, stomatal development, photosynthesis and transpiration of *Withania somnifera L.* Dunal. plantlets. *Plant Cell, Tissue and Organ Culture*. 90:141-151.

Li, Q., Kubota, C. (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany*. 67: 59-64.

Logendra, L.S., Janes, H.W. (1999). Hydroponics tomato production: Growing media

requirements. *Acta Horticulturae*. 481:483-486.

Long, S.P., Zhu, X.G., Naidu, S.L., Ort, D.R. (2006). Can improvement in photosynthesis increase crop yields? *Plant, Cell & Environment*. 29:315-330.

Loreto, F., Tsonev, T., Centritto, M. (2009).The impact of blue light on leaf mesophyll conductance. *Journal of Experimental Botany*. 60: 2283-2290.

Lu, N. (2012). Study on application of supplemental lighting within the canopy to enhance yield and quality of single-truss tomato. Doctor thesis. Chiba University.

Lu, N., Maruo, T., Johkan, M., Hohjo, M., Tsukagoshi, S., Ito, Y., et al. (2012a). Effects of supplemental lighting within the canopy at different developing stages on tomato yield and quality of single-truss tomato plants grown at high density. *Environmental Control in Biology*. 500: 1-11.

Lu, N., Maruo, T., Johkan, M., Hohjo, M., Tsukagoshi, S., Ito, Y., et al. (2012b). Effects of supplemental lighting with light-emitting diodes (LEDs) on tomato yield and quality of single-truss tomato plants grown at high planting density. Environmental Control in Biology. 50: 63-74.

Marschner, H., Cakmak, I. (1989). High light intensity enhances chlorosis and necrosis in leaves of Zinc, Potassium, and Magnesium deficient bean (*Phaseolus vulgaris*) Plants. *Journal of Plant Physiology*. 134: 308-315.

Massa, G.D., Kim, H.H., Wheeler, R.M., Mitchell, C.A. (2008).Plant productivity in response to LED lighting. *Hort Science*. 43: 1951-1956.

Matsuda, R., Ohashi-Kaneko, K., Fujiwara, K., Kurata, K. (2007). Analysis of the relationship between blue-light photon flux density and the photosynthetic properties of spinach

(*Spinaciaoleracea*, L.) leaves with regard to the acclimation of photosynthesis to growth irradiance. *Soil Science and Plant Nutrition*. 53: 459-465.

Matsuda, R., Nakano, A., Ahn, D.H., Suzuki, K., Yasuba, K.I., Takaichi, M. (2011a). Growth characteristic and sink strength of fruit at different CO_2 concentrations in a Japanese and a Dutch tomato cultivar. *Scientia Horticulturae*. 127: 528-534.

Matsuda, R., Suzuki, K., Nakano, A., Higashide, T., Takaichi, M. (2011b).Responses of leaf photosynthesis and plant growth to altered source–sink balance in a Japanese and a Dutch tomato cultivar. *Scientia Horticulturae*.127: 520-527.

Matsuda, R., Ozawa, N., Fujiwara, K. (2014). Leaf photosynthesis, plant growth, and carbohydrate accumulation of tomato under different photoperiods and diurnal temperature differences. *Scientia Horticulturae*.170: 150-158.

Marschner, H., Cakmak, I. (1989). High Light Intensity Enhances Chlorosis and Necrosis in Leaves of Zinc, Potassium, and Magnesium Deficient Bean (Phaseolus vulgaris) Plants. Journal of Plant Physiology. 134: 308-315.

Mc Avoy, R.J., Janes, H.W. (1988). Alternative production strategies for greenhouse tomato using supplemental lighting. *Scientia Horticulturae*. 35: 161-166.

Mc Avoy, R.J., Janes, H.W., Godfriaux, B.L., Secks, M., Duchai, D., Wittman. W.K. (1989). The effect of total available photosynthetic photon flux on single truss tomato growth and production. *Journal of Horticultural Science*. 64:331-338.

Mc Collum, J.P. (1944). Some factors affecting the ascorbic acid content of tomatoes. *American Society of Horticulture Science*. 45: 382-386.

Mc Cree, K.J. (1972). The action spectrum, absorptance and quantum yield of photosynthesis

in crop plants. *Agricultural Meteorology*. 9: 191-216.

Meckel, T., Gall, L., Semrau, S., Homann, U., Thiel, G., (2007). Guard cells elongate: relationship of volume and surface area during stomatal movement. *Biophysical Journal*. 92:1072-1080.

Mitchell, C.A., Both, A.J., Bourget, C.M., Burr, J.F., Kubota, C., Lopez, R.G. (2012). LEDs: The future of greenhouse lighting! *Chronica Horticulture*. 52: 6-11.

Mott, K.A., Sibbernsen, E.D., Shope, J.C. (2008). The role of the mesophyll in stomatal responses to light and CO_2. *Plant Cell and Environment*. 31: 1299-1306.

Mullineaux, P., Karpinski, S., (2002). Signal transduction in response to excess light getting out of the chloroplast. *Current Opinion in Plant Biology*. 5: 43-48.

Naumburg, E., Ellsworth, D.S. (2002) Short-term light and leaf photosynthetic dynamics affect estimates of daily understory photosynthesis in four tree species. *Tree Physiology*. 22:393-401.

Nawrocki, W.J., Tourasse, N.J., Taly, A., Rappaport, F., Wollman, F.A. (2015) The plastid terminal oxidase: its elusive function points to multiple contributions to plastid physiology. *Annual Review Plant Biology*. 66:49-74.

Neuner, G., Braun, V., Buchner, O., Taschler, D. (1999). Leaf rosette closure in the alpine rock species Saxifraga paniculata Mill.: significance for survival of drought and heat under high irradiation. *Plant, Cell and Environment*. 22:1539-1548.

Ni, J., Chen X., Chen C., Xu Q. (2009).Effects of supplemental different light qualities on growth, photosynthesis, biomass partition and early yield of greenhouse cucumber. *Scientia Agricultura Sinica*. 42:2615-2623.

Nishio, J.N., Sun, J., Vogelmann, T.C. (1993). Carbon fixation gradients across spinach leaves do not follow internal light gradients. Plant Cell 5: 953–961.

Nishio, J.N., Sun, J., Vogelmann, T.C. (1993). Carbon fixation gradients across spinach leaves do not follow internal light gradients. *Plant Cell*. 5: 953-961.

O'Carrigan, A., Hinde, E., Lu, N., Xu X, Duanc, H., Huang, G., Mak, M., Bellotti, B., a, Chen, Z. (2014). Effects of light irradiance on stomatal regulation and growth of tomato. *Environmental and Experimental Botany*. 98: 65-73.

Ögren, E., Evans, J.R. (1993). Photosynthetic light response curves. 1. The influence of CO2 partial pressure and leaf inversion. *Planta*. 189:182-190.

Okamoto, K., Yanagi, T., Takita, S., Tanaka, M., Higuchi, T., Ushida, Y. (1996). Development of plant growth apparatus using blue and red LED as artificial light source. *Acta Horticulturae*. 440: 111-116.

Okano, K., Nakano, Y., Watanabe, S. (2001). Single truss tomato system-a labor saving management system for tomato production. *Japan Agricultural Research Quarterly*.35:177-184.

Pallozzi, E., Tsonev, T., Marino, G., Copolovici, L., Niinemets, Ü., Loreto, F., Centritto, M. (2013). Isoprenoid emissions, photosynthesis and mesophyll diffusion conductance in response to blue light. *Environmental and Experimental Botany*.95: 50-58.

Pearcy RW. (1988). Photosynthetic utilization of light flecks by understory plants. *Australian Journal of Plant Physiology*. 15:223-238.

Pearcy RW. (1990). Sunflecks and photosynthesis in plant canopies. *Annual review of plant physiology and plant molecular biology*. 41:421-453.

Pearcy, R.W., Way, D.A. (2012). Two decades of sunfleck research: looking back to move forward. *Tree Physiology*. 32:1059-1061.

Peterson, A.G., Neofotis, P.G. (2004). A hierarchical analysis of the interactive effects of elevated CO2 and water availability on the nitrogen transpiration productivities of velvet mesquite seedlings. *Oecologia*. 141: 629–640.

Pettersen, R.I., Torre, S., and Gislerod, H.R. (2010). Effects of intera-canopy lighting on photosynthesis characteristics in cucumber. *Scientia Horticulturae*. 125: 77-81.

Piringer, A.A., Cathey, H.M. (1960). Effect of photoperiod, kind of supplemental light and temperature on the growth and flowering of petunia plants. Proceedings. *American Society for Horticultural Science*. 76: 649-60.

Poorter, H., Garnier, E. (1996). Plant growth analysis: an evaluation of experimental design and computational methods. *Journal of Experimental Botany*. 47:1343-1351.

Poorter, H., Nagel, O.W. (2000). The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Australian Journal of Plant Physiology. 27: 595-607.

Radford, P.J. (1967). Growth analysis formulae - their use and abuse. *Crop Science*. 7:171-175.

Rawson, H.M., Begg, J.E., Woodward, R.G. (1977). The effect of atmospheric humidity on photosynthesis, transpiration and water use efficiency of leaves of several plant species. *Planta*. 134:5-10.

Reynolds, A.G., Price, S.F., Wardle, D.A., Watson, B.T. (1994). Fruit Environment and Crop Level Effects on Pinot noir. I. Vine Performance and Fruit Composition in British

Columbia. *American Journal of Enology and Viticulture*. 45: 452-459.

Rijkers, T., de Vries, P.J., Pons, T.L., Bongers, F. (2000). Photosynthetic induction in saplings of three shade-tolerant tree species: comparing understory and gap habitats in a French Guiana rain forest. *Oecologia*. 125:331-340.

Robinson, T.L., Seeley, E.J., Barritt, B.H. (1983). Effect of light environment and spur age on "Delicious" apple fruit size and quality. Journal of the American Society for Horticultural Science. 108:855-861.

Rose, J.K.C., Lee, H.H., Bennett, A.B. (1997). Expression of a divergent expansin gene is fruit-specific and ripening-regulated. *Proceedings of the National Academy of Sciences*. 94: 5955–5960.

Rousseaux, M.C., Hall, A.J., Sánchez, R.A. (1996). Far-red enrichment and photosynthetically active radiation level influence leaf senescence in field-grown sunflower. *Physiologia Plantarum*. 96:217-224.

Runkle, E.S., Heins, R.D. (2001). Specific functions of red, far red, and blue light in flowering and stem extension of long-day plants. *Journal of the American society for horticultural science*. 126: 275-282.

Sangoi, L. (2001). Understanding plant density effects on maize growth and development: an important issue to maximize grain yield. *Ciência Rural*. 31:159-168.

Sarala, M., Taulavuori, E., Karhu, J., Savonen, E.-M., Laine, K., Kubin, E., Taulavuori, K. (2009). Improved elongation of Scots pine seedlings under blue light depletion isnot dependent on resource acquisition. *Functional Plant Biology*. 36:742–751.

Schoch, P.G., Zinsou, C., Sibi, M., (1980). Dependence of the stomatal index on

environmental factors during stomatal differentiation in leaves of *Vigna sinensis L.*:1. Effect of light intensity. *Journal of Experimental Botany*. 31: 1211-1216.

Scholberg, J., McNeal, B.L., Jones, J.W., Boote, K.J., Stanley, C.D., Obreza, T. A. (2000). Growth and canopy characteristics of field-grown tomato. *Agronomy Journal*. 92:152-159.

Schulze, E.D., Bloom, A.J. (1984).Relationship between mineral nitrogen influx and transpiration in radish and tomato. *Plant Physiology*. 76:827-828.

Seibert, M., Wetherbee, P.J., Job, D.D. (1975) The Effects of Light Intensity and Spectral Quality on Growth and Shoot Initiation in Tobacco Callus. *American Society of Plant Biologists*. 56: 130-139.

Sharkey, T.D. (1985). Photosynthesis in intact leaves of C_3 plants: physics, physiology and rate limitations. *The Botanical Review*. 51: 53-105.

Sharkey, T.D., Raschke, K. (1981). Effect of light quality on stomatal opening in leaves of *Xanthium strumarium* L. *Plant Physiology*. 68:1170–1174.

Shimazaki, K., Doi, M., Assmann, S.M., Kinoshita, T. (2007). Light regulation of stomatal movement. *Annual Review of Plant Biology*. 58: 219-247.

Sims, D.A., Pearcy, R.W. (1993) Sunfleck frequency and duration affect growth-rate of the understorey plant, *Alocasia macrorrhiza. Functional Ecology*. 7:683-689.

Sims, D.A., Pearcy, R.W. (1994). Scaling sun and shade photosynthetic acclimation of *Alocasia macrorrhiza* to whole plant performance–I. Carbon balance and allocation at different daily photon flux densities. *Plant, Cell and Environment*. 17: 881-887.

Skillman, J.B. (2008). Quantum yield variation across the three pathways of photosynthesis:

not yet out of the dark. *Journal of Experimental Botany*. 59: 1647-1661.

Soares, A.S., Driscoll, S.P., Olmos, E., Harbinson, J., Arrabaça, M.C., Foyer, C.H. (2008). Adaxial/abaxial specification in the regulation of photosynthesis and stomatal opening with respect to light orientation and growth with CO_2 enrichment in the C_4 species Paspalum dilatatum. *New Phytologist*. 177: 186-198.

Sokawa, Y., Hase, E. (1967). Effect of light on the chlorophyll formation in the "glucose-bleached" cells of *Chlorella protothecoides*. *Plant and Cell Physiology*. 8: 495-508.

Song, S. (1999). Study on the index of the healthy seedling of cucumber. *Journal of Hebei Normal University of Science & Technology*. 13: 58-63 (in Chinese with English abstract).

Steinger, T., Roy, B.A., Stanton, M.L. (2003). Evolution in stressful environments II: adaptive value and costs of plasticity in response to low light in *Sinapis arvensis*. *Journal of Evolutionary Biology*. 16:313-323.

Sun J, Nishio, J.N. (2001). Why abaxial illumination limits photosynthetic carbon fixation in spinach leaves? *Plant and Cell Physiology*. 42: 1-8.

Sun J, Nishio, J.N., Vogelmann, T.C. (1998). Green light drives CO_2 fixation deep within leaves. *Plant and Cell Physiology*. 39: 1020-1026.

Takahashi, S., Murata, N. (2008). How do environmental stresses accelerate photo inhibition? *Trends Plant*. 13:178-182.

Talbott, L.D., Hammad, J.W., Harn, L.C., Nguyen, V.H., Patel, J., Zeiger, E. (2006). Reversal by green light of blue light-stimulated stomatal opening in intact, attached leaves of

Arabidopsis operates only in the potassium dependent, morning phase of movement. *Plant and Cell Physiology*. 47: 332-339.

Talbott, L.D., Zeiger, E. (1993). Sugar and organic acid accumulation in guard cells of Vicia faba in response to red and blue light. *Plant Physiology*. 102: 1163-1169.

Tanner, W., Beevers, H. (2001).Transpiration, a prerequisite for long distance transport of minerals in plants. *Proceedings of the National Academy of Sciences*. 98:9443-9447.

Terfa, M.T., Solhaug, K.A., Gislerød, H.R., Olsen, J.E., Torre, S. (2013). A high proportion of blue light increases the photosynthesis capacity and leaf formation rate of Rosa × hybrida but does not affect time to flower opening. *Physiologia Plantarum*. 148:146-159.

Terashima I., Evans J.R. (1988). Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. *Plant and Cell Physiology*. 29: 143-155.

Terashima I, Inoue Y. (1985). Palisade tissue chloroplasts and spongy tissue chloroplasts in spinach: biochemical and ultrastructural differences. *Plant and Cell Physiology*. 26: 63-75.

Tewolde, F.T., Lu, N., Shiina, K., Maruo, T., Takagaki, M., Kozai, T., Yamori, W. (2016). Nighttime supplemental LED inter-lighting improves growth and yield of single-truss tomatoes by enhancing photosynthesis in both winter and summer. *Frontiers in Plant Science*. 7:448.

Thomas, W.P., Woodward, F.I., Quick, W.P., (2003). Systemic irradiance signalling in tobacco. *New Phytologist*. 161: 193-198.

Tibbitts, T.W., Morgan, D.C., Warrington, I.J. (1983).Growth of lettuce, spinach, mustard, and wheat plants under four combinations of high-pressure sodium, metal halide, and

tungsten halogen lamps at equal PPFD (Photosynthetic photon flux density). *Journal of the American Society for Horticultural Science*. 108:622-630.

Ting, K.C., Giacomelli, G.A., Fang, W. (1993). Decision support system for single truss tomato production. XXV CIOSTA-CIGR V Congress, Wageningen, Netherlands.

Torres, C.A., Andrews, P.K., and Davies, N.M. (2006). Physiological and biological response of fruit exocarp of tomato (Lycopersicon esculentum Mill.) mutants to natural photo-oxidative conditions. Journal of Experimental Botany. 57: 1933-1947.

Trouwborst, G., Oosterkamp, J., Hogewoning, S.W., Harbinson, J., Ieperen, W.V. (2010). The response of light interception, photosynthesis and fruit yield of cucumber to LED-lighting within the canopy. *Physiologia Plantrum*. 138: 289-300.

Valladares, F., Allen, M.T., Pearcy, R.W. (1997). Photosynthetic responses to dynamic light under field conditions in six tropical rainforest shrubs occurring along a light gradient. *Oecologia*. 111:505-514.

van der Ploeg, A., van der Meer, M., Heuvelink, E. (2007). Breeding for a more energy efficient greenhouse tomato: past and future perspectives. *Euphytica*.158: 129-138.

Verheul, M.J. (2012). Effects of plant density, leaf removal and light intensity on tomato quality and yield. *Acta Horticulturae*. 956:365-372.

von Caemmerer, S. (2000). Biochemical Models of Leaf Photosynthesis. CSIRO Pub-lishing, Victoria.

von Caemmerer, S., Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*. 153: 376-387.

Wada, T., Ikeda, H., Matsushita, K., Kambara, A., Hirai H., Abe, K. (2006). Effects of shading

in summer on yield and quality of tomatoes grown on a single-truss system. *Journal of the Japanese Society for Horticultural Science*. 75: 51-58.

Wahidin, S. Idris, A., Shaleh, S.R.M. (2013) The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. *Bioresource technology*. 129:7-11.

Walters, R.G., Rogers, J.J.M., Shephard, F., Horton, P. (1999). Acclimation of Arabidopsis thaliana to the light environment: the role of photoreceptors. *Planta*. 209:517-527.

Walters, R.G., Shephard, F., Rogers, J.J.M., Rolfe, S.A., Horton, P. (2003). Identification of mutants of Arabidopsis defective in acclimation of photosynthesis to the light environment. *Plant Physiology*. 131: 472-481.

Watling, J.R., Ball, M.C., Woodrow, I.E. (1997). The utilization of light flecks for growth in four Australian rain-forest species. *Functional Ecology*. 11:231-239.

Wang, H., Gu, M., Cui, J., Shi, K., Zhou, Y., Yu, J. (2009). Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. *Journal of Photochemistry and Photobiology B: Biology*. 96: 30-37

Wang, Y., Noguchi, K., Terashima, I. (2008). Distinct light responses of the adaxial and abaxial stomata in intact leaves of *Helianthus annuus L. Plant, Cell and Environment*. 31: 1307-1316.

Wheeler, R.M., Mackeowiak, C.L., Sager, J.C. (1991). Soybean stem growth under high pressure sodium with supplemental blue lighting. *Agronomy journal*. 83:903-906.

Wingler, A., Marès, M., Pourtau, N. (2004). Spatial patterns and metabolic regulation of

photosynthetic parameters during leaf senescence. *New Phytologist*. 161: 781–789.

Willmer, C., Fricker, M. (1996). *Stomata*. Chapman & Hall Publisher, London.

Xu, H.L., Gauthier, L., Desjardins, Y., and Gosselin, A. (1997). Photosynthesis in leaves, fruits, stem and petioles of greenhouse-grown tomato plants. *Photosynthetica*. 33: 113-123.

Yanagi, T., Ueda, E., Sato, H., Hirai, H., Oda, Y. (1995). Effects of shading and fruit set order on fruit quality in single-truss tomato (in Japanese with English summary). *Journal of the Japanese Society for Horticultural Science*. 64:291-297.

Yanagi, T., Okamoto, K., Takita, S. (1996). Effects of blue, red, and blue/red lights of two different PPF levels on growth and morphogenesis of lettuce plants. *Acta Horticulturae*. 440:117-122.

Yasuba, K. I., Suzuki, K., Sasaki, H., Higashide, T., Takaichi, M. (2011). Fruit yield and environmental condition under integrative environmental condition for high yielding production at long-term culture of tomato. *Bull. Natl. Inst. Veg. Tea Sci.* pp 10 (in Japanese, with English abstract).

Yin, Z.H., Johnson, G.N. (2000). Photosynthetic acclimation of higher plants to growth in fluctuating light environments. *Photosynthesis Research*. 63:97-107.

Yorio, N.C., Goins, G.D., Kagie, H.R., Wheeler, R.M., Sager, J.C. (2001). Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *Hort Science*. 36:380-383.

Zhang, G., Shen, S., Takagaki, M., Kozai, T., Yamori, W. (2015). Supplemental upward lighting from underneath to obtain higher marketable lettuce (Lactuca sativa) leaf fresh

weight by retarding senescence of outer leaves. *Frontiers in Plant Science*. 6, 1110.

Zhu, X.G., Portis, A.R., Long, S.P. (2004). Would transformation of C_3 crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. *Plant, Cell and Environment*. 27:155-165.

Zhu, X.G., Long, S.P., Ort, D.R. (2010). Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology*. 61:235-261.

Zohar, Y., Waisel, Y., Karschon, R. (1975). Effects of light, temperature and osmotic stress on seed germination of *Eucalyptus occidentalis Endl*. *Australian Journal of Botany*. 23: 391-397.

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