Study on Strawberry Photosynthesis and Growth under Optimized Plant Factory Conditions

最適化された植物工場条件下におけるイチゴの光合成と成長に関する研究

Doctoral Thesis

学位論文

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Abstract

Strawberry is a high economic fruit, rich in nutrients, and is especially paid attention to due to the medicinal potential for human health. Consequently, strawberries are produced worldwide; the production, however, is restricted by geographic factors as they require a suitable climate for growth and productivity. Currently, farming environments are constantly fluctuating and unstable because disasters such as droughts, floods, and typhoons frequently occur due to global climate change. Plant factory is an advanced method with strictly controlled cultivation environments, which may be completely independent of natural conditions. Crop production using plant factories is a sustainable solution and could be applied anywhere without concerning to geographical limitation. This study focused on evaluating and determining the optimal environments to grow strawberries in a plant factory for application in tropical and subtropical areas. The optimizations of cultivation environments were assessed through photosynthetic responses, plant growth traits, and fruit sugar accumulation.

Two photosynthetic measurement methods (single-leaf method [SL] and whole-plant method [Wp]) used on strawberry plants were compared. Besides, diurnal photosynthetic patterns of strawberry under different light intensity (200 and 1000 μmol m^{-2} s^{-1}) and CO_2 (400 and 1000 μmol mol^{-1}) conditions were also investigated (Chapter 2). The photosynthetic values between the two methods were only significantly different under low CO_2 and PPFD, which mainly resulted from the different numbers of leaves used for the measurements and the different light intensities depending on the leaf positions. The magnitude and tendency of diurnal photosynthesis of strawberries were stable at a low level under low CO_2 and PPFD. Meanwhile, it was at a high level but gradually decreased during the photoperiod under elevated CO_2 and PPFD.

The effects of different light conditions on photosynthetic responses and reproductive ability of strawberry grown in a closed-type plant factory using sunlight (solar plant factory, SPF) were investigated (Chapter 3). The strawberries were grown under two different light conditions of full sunlight (S) or 10% sunlight + fluorescent light (SA). The fluctuation of light intensity following day length was a driving factor to reduce or maintain the photosynthetic capacity. Besides, under SPF conditions, strawberries could maintain regular reproductive potentials such as flowering and fruiting.

The impacts of different phosphorus concentrations (2, 6, and 12 mM) and light spectra (purple LED light [PL], white LED light [WL], and white fluorescence light [WF]) on the

reproductive growth and its relationship with fruit sugar accumulation of strawberry were examined (Chapter 4). Phosphorus dominantly affected the magnitude and tendency of net assimilation rate, which led to a major variation in the relative growth rate at the reproduction stage of strawberry. The light spectra only enhanced vegetative growth during the reproduction period. Besides, the combination of 6 mM phosphorus and WL enhanced the relative growth rate of reproduction and activity of sucrose-phosphate synthase which led to the increases of fruit yield and fruit sugar accumulation.

In conclusion, SPF could be used for strawberry production and its limitation could be overcome by applying supplemental lighting. The optimization of environmental factors, including light quality and phosphorus concentration, could drive plant growth and fruit sugar accumulation of strawberries grown in the plant factory.

要旨

イチゴは経済的価値が高く，また栄養素が豊富で，特に人体への薬効が高いために注目されている果物である．そのため，イチゴは世界的に広く生産されているが，栄養成長および生殖成長は気候の影響を強く受けることから，その生産は地理的な要因によって制限されている．現在，地球規模の気候変動により干ばつや洪水，台風などの災害が度々生じることから，農業環境は常に変動し，不安定である．植物工場は自然条件からは完全に独立し精密に管理された環境をつくりだせる高度な栽培システムである．植物工場を用いた作物生産は地理的な制限を伴わないため，あらゆる土地において適用可能かつ持続可能な技術である．本研究では，熱帯および亜熱帯地域におけるイチゴ生産への植物工場の適用を目的として，光合成反応，成長特性および果実への糖蓄積に基づき最適な栽培環境の評価および決定を行った．

まず，イチゴに用いられる 2 通りの光合成測定法（単葉および全個体）の比較を行った．加えて，異なる光強度（200，1000 μmol m^{-2} s^{-1}）および CO_2 濃度（400，1000 μmol mol^{-1}）下での光合成の日変化パターンも調査した（第 2 章）．光合成測定値は低光強度および低 CO_2 条件下でのみ方法間で有意差が確認されたが，この差には主に測定対象となる葉の枚数や葉位による光強度の違いが関与していると考えられた．日中の光合成は低光強度および低 CO_2 条件下において低い値で安定していたのに対し，高光強度および高 CO_2 条件下では高い値から徐々に減少する様子が観察された．

次に，太陽光を利用した閉鎖型植物工場において，光環境の違いが光合成応答および生殖能力に与える影響について調査するため，太陽光のみまたは 10% 太陽光 + 蛍光灯の 2 つの条件下でイチゴの栽培を行った（第 3 章）．日長に伴い変化する光強度は光合成能の低下または維持に働く要因であると考えられた．加えて，太陽光利用型植物工場下においてもイチゴは花芽や果実の形成など正常な生殖能力を維持することがわかった．

栽培養液中のリン濃度（2，6，12 mM）および光源（紫色 LED 光，白色 LED 光，白色蛍光灯）の違いが生殖成長および果実の糖蓄積に与える影響について調査した（第 4 章）．リンは主に純同化率に影響を与え，イチゴの生殖成長期の相対成長速度が大きくばらつく要因となった．光源は生殖期における栄養成長のみを促進した．また，6 mM リンと白色 LED 光の組み合わせにより生殖期の相対成長速度および SPS 活性が促進された結果、果実収量と糖度が増加したと考えられた．

結論として，太陽光利用型植物工場はイチゴ生産に利用可能であるが、補光の実施が必要だと考えられた。また、光質やリン濃度などの環境要因を最適化することで，植物工場で栽培されたイチゴの成育および果実の糖蓄積を向上できることが本研究から明らかになった.

List of Figures

List of Tables

Contents

Chapter 1.

General Introduction

Current status of strawberry production and plant factory application potential

Strawberry and current production status

Strawberries are perennial rosette plants belonging to the *Fragaria* genus, Rosacea family. The commercial species are the *Fragaria* × *ananassa* Duch., which were discovered from the spontaneous hybrids between wild octoploid species (Whitaker et al., 2020). Because of a microclimatic crop, strawberry requires special environmental conditions for growth and reproduction; specifically, strawberries demand a temperature range of 15–25°C for flowering and fruit growth (Sønsteby and Heide, 2009; Hidaka et al., 2017; Menzel, 2019). Consequently, strawberry production is somewhat restricted by geographic areas, namely, in tropical and subtropical areas characterized by year-round high temperatures.

Strawberry, a kind of berry fruit, is prized for the human diet in three main aspects. Firstly, its high nutritional value (rich in K, Mg, Mn, Fe, vitamin C, folate). Secondly, it is the potential for preventions of oxidative stress, cardiovascular disease, certain types of cancers, type II diabetes, and neurodegeneration. Finally, it is attractive by characteristic flavors (sweetness and scents of volatile compounds such as linalool, mesifuranes) (Giampieri et al., 2012; Whitaker et al., 2020). Cultivated in 76 countries around the world, the economic value of strawberries is quite high, up to 10,208 USD/tone (data of producer price from Japan in 2017, FAOSTAT, 2020). During the past fifteen years, from 2003 to 2018, the total world production area increased by 55,270 hectares, accounting for about 17.4%; meanwhile, the production reached 8.3 million tons (2018), nearly 65.3% increased (FAOSTAT, 2020). This information is an indication of the importance and potential value of the strawberry production industry in the future.

Plant factory and application potential in the Subtropics

A plant factory is a facility whose inward environmental factors are controlled strictly for crop production. The environment is controlled automatically according to a pre-established procedure, and it may completely separate from the external conditions (Kozai, 2013). These are the remarkable advantages of plant factory compared with traditional farming methods. Plant factories are divided into three main categories based on the light source applied, including Type I - plant factory with artificial light (PFAL, 100% artificial light), Type II - using 100% sunlight, and Type III - a mixed type using both artificial and sunlight (Anpo et al., 2019). Plant factory Type I is also known as closed plant factory, vertical farming system, or indoor cultivation system. Type II is usually a greenhouse that can control the inner environments (controlled greenhouse). Type III is a variant of Type II with the supplemental lighting systems. Also, in 2017, a new type of plant factory was introduced by Ueno and Kawamitsu as Okinawa type plant factory or Solar plant factory (SPF) (Ueno and Kawamitsu, 2017). It is a variation of Type I (fully enclosed space) but combining sunlight and artificial light.

Because in plant factories, a separate cultural environment could be generated; hence, the application of plant factories for strawberry production in tropical or subtropical areas is quite feasible. The only challenge is energy consumption for the plant factory operation. However, the development of current technology, namely, light-emitting diodes (LED), and renewable energy applications (Ueno and Kawamitsu, 2017) may facilitate the issues solving.

Strawberry cultivation using plant factory Type I

Because of small size, strawberry plants are suitable for production in the plant factory Type I. Currently, studies on strawberry using the plant factory Type II and III are very common (Itani et al., 1998; Hidaka et al., 2016; Díaz-Galián et al., 2020). In contrast, studies using Type I are modest but gaining attention. These studies focused on the role of

monochromatic light (Yoshida et al., 2016) and photoperiod (Yoshida et al., 2013) in growth and flower induction in strawberries. Interestingly, an artificial pollination method for strawberry flowers by ultrasonic waves is also underway (Shimizu, 2019). Simultaneously, the plant factory Type I is also used to study the growth and accumulation of secondary compounds in transgenic strawberries under high-temperature conditions (Hikosaka et al., 2019). Indispensably, the role of mineral nutritional factors in strawberry growth responses under plant factory conditions will be the suitable direction for upcoming studies.

The role of environmental factors in strawberry photosynthesis and growth response

Light qualities

Light spectrum and light intensity are two indispensable components of light quality that played important role in plant photosynthesis. Monochrome blue and red light have been studied much on strawberry, and reported that red light was more effective in photosynthesis than blue light, and photosynthetic efficiency under red light was twice as high as under blue light conditions (Yanagi et al., 1996; Yoshida et al., 2012). Additionally, blue light (405–470 nm) promoted more flowering in strawberries, while red light with a long wavelength (680 nm) stimulated early flowering (Yoshida et al., 2016). However, the role of the light spectra on strawberry fruit qualities is currently not much research information. Light intensity is one of the critical driven factors of plant physiology. Low light intensity was considered as a cause of photosynthesis decrease, increasing chlorophyll fluorescence in strawberry leaves, thus leading to a reduction in the number of flowers and fruits (Awang and Atherton, 1995; Demirsoy et al., 2007; Choi et al., 2016). Nevertheless, the impacts of constant or fluctuation of light intensity during the photoperiod on strawberries remain limited information.

Phosphorus nutrient

Together with participating in ATP formation and reducing CO_2 to the form of Triose-phosphate in the photosynthesis process, phosphorous also play the role of sucrose phosphate synthase (SPS) regulator in the sucrose synthesis pathway. Most of the studies on strawberry, however, have been focused on improving the amount of phosphorus fertilizer to increase yield and fruit quality. Specifically, May and Pritt (1993) reported that the strawberry yield responded to the phosphorus concentration in the form of a quadratic function; also, Estrada-Ortiz et al., (2012) and Cao et al., (2015) found that supplemental phosphorus increased sugar, the soluble solids content in the fruits. The role of phosphorus in the early growth stage, reproduction features, or its correlation with SPS activity has not been studied much in strawberries.

Growth evaluation parameters: P_n, RGR, GRC

Net photosynthetic rate (P_n) or CO_2 assimilation rate (A) is one of the essential parameters for plant growth and yield evaluation. Therefore, the most accurate results of photosynthetic measurement methods should be considered, especially under the condition of more and more measurement methods are proposed. Currently, there are two popular photosynthetic measurement methods, namely, the single-leaf measurement method (using a portable photosynthesis system, Li-COR) and the whole-plant measurement method (using the measuring chambers). The single-leaf measurement method is widely used, while the whole-plant measurement method is less usual. However, the compatibility of the P_n values of strawberry plants measured between the two methods has not been studied in detail.

RGR is a widely used parameter to estimate the growth of plants from crops to the ecosystem, from grass to woody plants (Gent, 1986; Pommerening and Muszta, 2015). RGR is analysed based on its two components, including NAR and LAR, which play a major role in RGR changes (Medek et al., 2007; Hoang et al., 2017; Poorter, 1989). Determining whether

NAR or LAR is the main factor directing the change in RGR is the key to understanding how the environmental conditions impacted plant growth. In order to identify the clearest and accurate correlation between NAR, LAR and RGR, GRC parameters were proposed (Poorter and Werf, 1998). Strawberry plants fulfil both the vegetative and reproductive growth simultaneously during the reproduction period. Therefore, developing indicators to identify vegetative and reproductive growth traits during this period may provide useful knowledge for studying strawberry production. This may be considered as an additional aspect of strawberry research.

Research purposes and objectives

This study aims to optimize the cultivation conditions of strawberry in a plant factory for application in the tropics and subtropics. These areas maintain all year round with strong radiation and high temperatures that restrict strawberry production on the open field or normal greenhouse. Moreover, the climate of the tropics and subtropics is favorable for insects and plant diseases to grow, so that the excessive and uncontrolled usage of plant protection chemicals leads to a reduction in strawberry quality and also adversely affecting the surrounding environment and human health. The application of a plant factory may overcome the mentioned limitations to strawberry production in the tropics and subtropics. This study evaluates the optimization of environmental factors basing on plant photosynthetic responses, plant growth traits and sugar accumulation in the fruits. Light qualities (spectrum, intensity, and light sources) and phosphorus nutrients were the main focus. Additionally, the impacts of different photosynthetic measurement methods on the determination of the photosynthetic values were also investigated. To achieve the above purpose, this study was established with three objectives related to the chapters as follows.

Chapter 2 - Whole-plant and single-leaf photosynthesis of strawberry under various environmental conditions. The objective of this study is to (1) determine the suitable

measurement manner of the photosynthesis applied to the strawberry plant, (2) to determine the diurnal photosynthetic response pattern of strawberry plants to different light intensities and CO_2 concentrations under plant factory conditions.

Chapter 3 - Photosynthetic responses and reproductive ability of strawberry following sunlight application in a plant factory closed system in subtropical Okinawa. The goal of this study is to (1) evaluate the photosynthetic response and reproductive ability of strawberry under the different light conditions, (2) evaluate the feasibility of the solar plant factory model in growing strawberries under subtropical climatic conditions.

Chapter 4 - Improvement of growth and fruit sugar accumulation in strawberry under plant factory conditions through manipulation of phosphorus and light spectrum applications. This study inherits the results of using low and constant light intensity from Chapter 3 to investigate the impacts of light spectra and phosphorus concentration on strawberry. The study planned to answer three questions. The first question is: during the reproductive growth period, what is the distribution rate of RGR to vegetative and reproductive growth? Second, what are the impacts of the light spectrum and phosphorus concentration on the RGR distribution? Finally, how are the light spectrum and phosphorus concentration related to fruit sugar accumulation in strawberry?

Chapter 2.

Whole-Plant and Single-Leaf Photosynthesis of Strawberry Under Various Environmental Conditions

Introduction

Photosynthesis is a crucial process for the existence, development, and productivity of crops, which directly impacts the world food security (Amthor, 2000; Simkin, 2019). Therefore, the photosynthesis of plants has been meticulously studied. One of the concerned research directions is to build plant growth models based on the photosynthesis process (Fourcaud et al., 2008; Wu et al., 2019; Amitrano et al., 2020). To build accurate models, the photosynthetic measurement methods should be suitable and engender the most accurate results possible.

Currently, two popular photosynthesis measurement methods are available which are, namely, the single-leaf measurement method and the whole-plant measurement method (for short, single-leaf, and whole-plant method, respectively). The application of the whole-plant method will overcome deviations in photosynthesis of plants introduced by differences in canopy structure, leaf ages, mutual shading, and leaf orientation (Lanoue et al., 2017; Nomura et al., 2020). The single-leaf method may not overcome those deviations without applying a proper practice. On the contrary, the advantage of the single-leaf method is its convenience and ease of measurement, and it can be flexibly moved in the measurement site. In this study, I compared measurements of the whole-plant and single-leaf method on strawberry plants (*Fragaria* × *ananassa* Duch.) grown under plant factory conditions to provide an overview of measurement methods for this plant species. Additionally, the diurnal photosynthetic response pattern of strawberry plants to different light intensities and CO_2 concentrations under plant factory conditions were also investigated.

Materials and Methods

Plant materials

'Sachinoka' variety of strawberry (*Fragaria* × *ananassa* Duch.) was used as materials. The plants were grown on a vertical-shelf system using the circulation hydroponics method in a plant factory with artificial light (PFAL) at the University of the Ryukyus, Okinawa, Japan (26°15′N, 127°45′E).

Cultivation conditions

The environmental conditions in the PFAL were established as a CO_2 concentration of 1000 µmol mol^{-1}, PPFD of 150 µmol m^{-2} s^{-1}, photoperiod of 10h day^{-1}, a temperature of 23°C, and humidity 70 ± 10%. The light source was white light-emitting diode (LED) (LED FL40 AX-120AD, 19W, Ryukyu Kougaku Kenkyuu Unit, Japan). The nutrient solution was modified Hoagland and Arnon's solution (1950), and renewed every seven days. Nutrient solution composition (macronutrients in mM, micronutrients in µM): 12 N-NO_3^-, 2 P, 10 K, 4 Ca, 2 Mg, 46 B, 15 Mn, 2 Zn, 5 Cu, 0.5 Mo and 100 Fe-EDTA with an EC of 0.21 S m^{-1}, and pH of 6.0–6.2.

The strawberries were used for two investigations, namely, (1) evaluating leaf area, SPAD values, and the maximum photosynthetic potential (A_{max}) at different leaf ages, (2) measuring whole-plant diurnal photosynthesis, light response curves and single leaf photosynthesis.

Under this cultivation conditions, after the 12 day of age, leaves expressed small superficial necrotic lesions. However, the individuals still exhibited normal growth appearance. The necrotic lesions strongly reduced on the leaves when growing under higher PPFD (from 250 µmol m^{-2} s^{-1} upward, data not show).

Data collection

The leaf age was determined in terms of days (1, 3, 6, 9, 12, 15, 18, and 21 days) after the three leaflets of each trifoliate leaf (hereafter called leaf) were fully opened. Leaf areas were calculated using the formula "y = 0.6271x + 1.0696" obtained by the correlation between length × width and area of leaflets (Fig. 2.1). The leaf area was the sum of the three leaflet areas, and SPAD values measured by a SPAD meter (SPAD-502; Minolta, Japan) were the mean values of the three leaflet values.

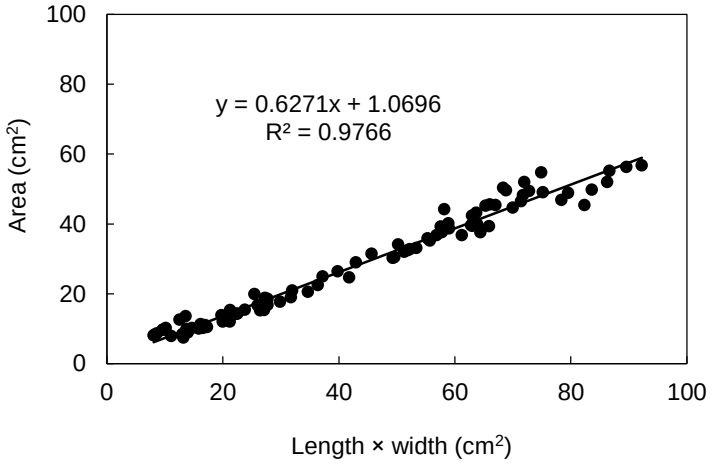

Fig. 2.1 Correlation between length × width and area of strawberry leaflets (n = 100). The formula used for leaf area calculation is y = 0.6271x + 1.0696.

Gas exchange measurement

The photosynthesis of strawberries was measured using the single-leaf method and whole-plant methods. In the single-leaf method, the leaflet was measured using a portable photosynthesis system (Li-6400; Li-COR, Lincoln, Nebraska, USA). In the whole-plant method (Wp method), an open-chamber measurement system described in Fig. 2.2 was applied. The system consists of two assimilation chambers (A and B) made of transparent acryl resin (ø

30 cm, H 14.5 cm, thickness 3 mm, Fig. 2.3A), which were set up in the same conditions to measured simultaneously. The assimilation chambers were placed in a container chamber; the temperature of the container chamber was controlled at 23°C by a radiator connected to a constant water temperature circulator (MTC-1500, As One, Japan). Firstly, an air compressor pumped atmospheric air passed through a low-range pressure regulator (PR) and then through a mass flow controller to a pipe column (ø 10 cm, length 100 cm) of Soda-lime (No. 2, Wako Co., Ltd, Japan) to remove CO_2. This airflow (zero CO_2) continued went through a dew point control system connected to a constant water temperature circulator (CTW802, Komatsu-Yamato, Japan). The airflow was then mixed with 10% CO_2 (N_2 balance) in an air mixing box to make 400, 1000, or 1500 µmol mol^{-1} CO_2, and this airflow was fed into the assimilation chambers at a flow rate of 14.0 L min^{-1}. The assimilation chambers and leaf temperature were measured with 0.07 mm copper-constantan thermocouples (T-type). The CO_2 concentration in the reference airflow, inlet, and outlet airflow of the assimilation chamber A and B were monitored by an IRGA (LI-840A, Shimadzu, Japan) and three solenoid valves. Signals from IRGA and thermal sensors were converted by analog to digital converter (DA100, Yokogawa) and linked to the computer by a LAN system. The system automatically recorded data with an interval of 3 minutes 20 seconds for each assimilation chamber. The light source was a 600W LED system (model PFQ-600DT, NK system limited, Japan). The maximum PPFD of this system was 1500 µmol m^{-2} s^{-1}. The whole plant of strawberries with seven leaves were placed into the assimilation chamber as Fig. 2.3B to conduct the measurement.

A_{max} measurement conditions: applying the single-leaf method, chamber temperature of 23°C, vapour pressure deficit (VPD) value of 1.2±2 kPa, and airflow rate of 400 µmol s^{-1}, CO_2 concentration of 1500 µmol mol^{-1}, and PPFD of 2000 µmol m^{-2} s^{-1}. Data were recorded after 5 minutes when the photosynthesis was stable.

Whole-plant diurnal photosynthesis measurement conditions: using the whole-plant method, relative humidity of 85±5%, airflow rate of 14 L min^{-1}, photoperiod of 12h (6:00 – 18:00 light on, 18:00 – 6:00 light off), PPFD combined with CO_2 concentration as 200 µmol m^{-2} s^{-1} with 400 µmol mol^{-1} (chamber temperature day/night time of 23/23°C), and 1000 µmol m^{-2} s^{-1} with 1000 µmol mol^{-1} (chamber temperature day/night time of 27/23°C). The high temperature of the chamber in the day time (27°C) was the result of high PPFD established (1000 µmol m^{-2} s^{-1}).

Whole-plant light response curves measurement conditions: using the whole-plant method; chamber temperature of 23°C; relative humidity of 80±10%; airflow rate of 14 L min^{-1}; PPFD of 200, 400, 600, 800, 1000, 1200, 1500 µmol m^{-2} s^{-1}. Each PPFD level was changed after 30 minutes; data were manually recorded after 30 minutes respectively. The measurement was fulfilled under three CO_2 levels of 400, 1000, and 1500 µmol mol^{-1}.

Single-leaf photosynthesis measurement conditions: using the single-leaf method, chamber temperature of 23°C, VPD value of 1.2±2 kPa, and airflow rate of 400 µmol s^{-1}. The CO_2 and PPFD conditions were same as in Whole-plant diurnal photosynthesis measurement conditions. This measurement was used to compare with the photosynthesis values obtained from the whole-plant values. The Single-leaf photosynthesis was measured in two manners, specifically, (1) measuring all the leaves, then calculating the mean value (averaged single-leaf method, ASL method), and (2) measuring only the 4th leaf (the 4th full-open leaf from the top of the plant, L4 method). The same plants used in the whole-plant diurnal photosynthesis measurements were used for this measurement to make the comparison between single-leaf and whole-plant method.

The water use efficiency (WUE) was calculated following the formula reported by Dinh et al. (2017)

For the single-leaf method: WUE = Photosynthetic rate (A)/transpiration rate (E)

For the whole-plant method WUE = CO_2 exchange rate (CER)/transpiration rate (E)

Statistical analyses

All data were prepared using Microsoft Excel 2016 software and statistically analyzed using STAGRAPHIC software. The mean values were compared using the least significant difference (LSD) test with a significance level of $P < 0.01$, or $P < 0.05$.

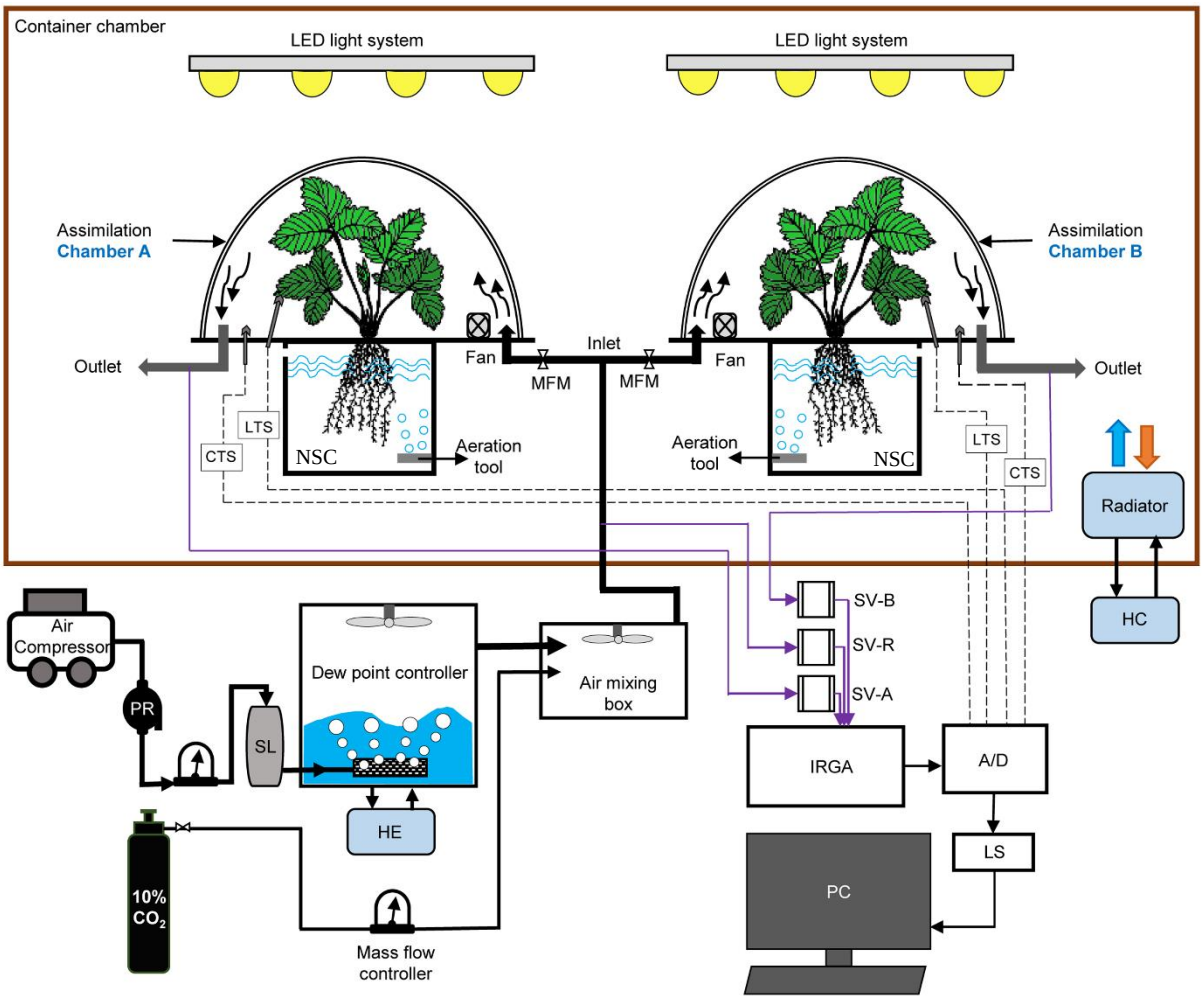

Fig. 2.2 Schematic diagram of photosynthetic close-chamber measurement system used for strawberry plant.

A/D: Analog to digital converter

CTS: Chamber temperature sensor

HE: Heat exchange (CTW802, Yamato)

HC: Heat controller (MTC-1500, As One)

IRGA: Infra-red gas analyzer (LI-840A, Li-COR)

LTS: Leaf temperature sensor

LS: LAN system

MFM: Mass flow meter

PC: Personal computer

SV-A: Solenoid valve for chamber A

SV-B: Solenoid valve for chamber B

SV-R: Solenoid valve for Reference check

Plant nutrient solution container (NSC) specification: diameter, 20 cm; high, 16.5 cm

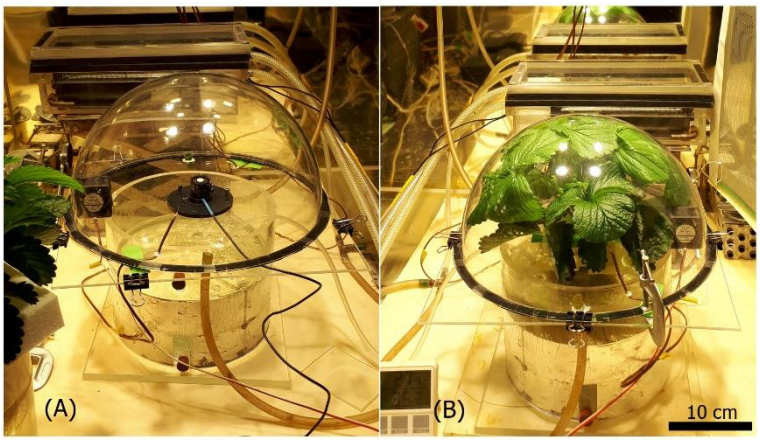

Fig. 2.3 Assimilation chamber without plant (A) and with plant (B) used for the whole-plant method (Wp method) to measure the whole-plant diurnal photosynthesis and whole-plant light response curve.

Results

Photosynthetic potential for single leaves at different ages

The strawberry leaf area rapidly and continuously increased from the leaf opening point on day 1 to day 9 (Fig. 2.4A). The increasing rate gradually decreased from 19.1 to 12.6 cm^2 $leaf^{-1}$ day^{-1} for the first nine days and reduced to 0.8 cm^2 $leaf^{-1}$ day^{-1} until day 12. From day 12, the leaf area remained constant; the mean leaf area (included three leaflets) at the steady stage reached 208.2 cm^2 $leaf^{-1}$.

In growing leaves, after the leaf opening point, SPAD values continuously and simultaneously increased with the increase of leaf area (Fig. 2.4B). However, a rapid rise only occurred in the first nine days with an increasing rate of 1.2–2.3 $leaf^{-1}$ day^{-1}. Afterward, the increment was slowed down from 0.7 to 0.3 $leaf^{-1}$ day^{-1} and tended to stabilize until the 21st day. The mean SPAD values of leaves from day 12 to day 21 were around 48.4.

The stability of leaf area and SPAD values are one of the criteria to evaluate leaf maturity in terms of its structure and function. However, a determination of leaf photosynthetic potential using specific ages can provide more accurate clues for the complete leaf function.

Fig. 2.4C shows the variation in maximum photosynthetic potential (A_{max}) of strawberry leaves at different ages (ranged with a six-day interval for each measurement time). A_{max} values (25.4 µmol m^{-2} s^{-1}) increased from leaves at three days of age and peaked (29.7 µmol m^{-2} s^{-1}) at day 15, then sharply decreased (24.3 µmol m^{-2} s^{-1}) at day 21.

The stomatal conductance (gs) and transpiration rate (E) indicated the same trend as in A_{max} (Figs. 2.4D and 2.4E). However, both gs and E values were not different in leaves between 9 and 15 days of age.

Water use efficiency (WUE) of leaves tended to be stable in magnitude between different leaf ages despite a slight increase in leaves of 21 days of age (Fig. 2.4F).

From these results, for strawberry plants grown under plant factory conditions, we recommend that the leaves at 12–15 days of age (equivalent to the 4th full-open leaf from the top of the plant) are the best options to measure photosynthesis in case of only one leaf measurement.

Diurnal changes in CO_2 exchange rate (CER), E, gs, and WUE of the whole plant

Under the low condition of CO_2 and PPFD (400 µmol mol^{-1} and 200 µmol m^{-2} s^{-1}, respectively), the whole plant CER remained stable at 5.0 µmol m^{-2} s^{-1} during the photoperiod (Fig. 2.5A). Whereas, under elevated CO_2 and PPFD conditions (1000 µmol mol^{-1} and 1000 µmol m^{-2} s^{-1}, respectively), the whole plant CER increased approximately 18.5% (equivalent to a 5.4-fold increase) compared with that under low CO_2 and PPFD conditions. On the other hand, under high CO_2 and PPFD conditions, the whole plant CER slightly decreased during the photoperiod. Specifically, the whole plant CER increased rapidly and reached a maximum (27.4 µmol m^{-2} s^{-1}) about an hour after the light was on, then slightly decreased (from 27.4 to 25.4 µmol m^{-2} s^{-1}) in the next hours until the light was off again. Regardless of CO_2 and PPFD conditions, the whole plant CER pattern was the same as in the dark period; suggesting that the

differences in CO_2 and PPFD in this study did not affect the respiration pattern in our model plant.

The whole-plant transpiration rates were significantly higher (approximately 40%) in photoperiod and the dark period when CO_2 and PPFD increased to 1000 µmol mol^{-1} and 1000 µmol m^{-2} s^{-1}, respectively (Fig. 2.5B). A higher-level transpiration rate during the night-time suggested that elevated CO_2 and PPFD conditions control the respiration intensity of the plants.

Fig. 2.5C shows the same diurnal pattern and magnitude of the whole-plant gs regardless of CO_2 and PPFD conditions. Interestingly, during the dark period, the stomata of the strawberry plants were not completely closed. Therefore, both E and gs values were nonzero (Figs. 2.5B and 2.5C).

Elevated CO_2 and PPFD conditions were factors driving the increase in WUE of the whole-plant diurnal photosynthesis (Fig. 2.5D). WUE increased by approximately 61% compared with that under low CO_2 and PPFD. In this measurement, the stomata activity did not play a dominant role in E and CER values of the whole plant (Fig. 2.5C).

The results of the whole-plant methods show an overview of the trends and the magnitude of CER, E, gs, and WUE of the whole-plant diurnal pattern, which is difficult to observe using the single-leaf method.

Whole-plant light response curve patterns

The whole-plant light response curve proportionally increased with CO_2 and PPFD (Fig. 2.6A). One important result was that the fully-functioning of CO_2 concentration only occurred apparently when PPFDs were at high levels. Specifically, under a PPFD of 200 µmol m^{-2} s^{-1}, the photosynthesis rate slightly differed at three levels of CO_2. When the PPFD increased from 400 to 600 µmol m^{-2} s^{-1}, the photosynthesis rate was divided into two distinctive groups (i.e., high and low). The high group was under 1000 – 1500 µmol mol^{-1} of CO_2, and the low group was under 400 µmol mol^{-1} of CO_2. Finally, when the PPFD was at a higher level (from 800 to

1500 µmol m^{-2} s^{-1}), the photosynthetic rate difference between three levels of CO_2 became apparent.

Our results confirm the dependence of CO_2 concentration on PPFD in controlling the trend and magnitude of the photosynthesis rate. Moreover, these results may also be a basis to assess the CO_2 use efficiency in a plant factory operation.

In contrast with the photosynthetic rate, the transpiration rate (Fig. 2.6B) between CO_2 levels had no difference and tended to slightly increase when PPFD increases.

The whole plant stomata activities widely varied under low PPFD under three CO_2 levels (Fig. 2.6C). However, when the PPFD was close to the highest PPFD (1500 µmol m^{-2} s^{-1}), the gs did not differ between the CO_2 levels.

WUE of the whole plant proportionally increased with the rise of PPFD (Fig. 2.6D). Interestingly, under high PPFD, A and WUE distinctly increased (Figs. 2.6A and 2.6D) across CO_2 levels, while the E and gs (Figs. 2.6B and 2.6C) were not different. These results proved the role of high CO_2 concentration in the water use efficiency to fasten the photosynthetic rate.

In Fig. 2.6, the A, E, gs, and WUE of the single leaves were added (identified by the black arrows) to compare trends of these parameters between the whole plant and single-leaf measurement methods. All parameters indicated the same trend except for gs. An evaluation of the magnitude of these parameters is presented in Table 2.1.

Comparison between whole plant and single-leaf photosynthesis measurement methods

Differences in A and gs of the two measurement methods only appeared under low CO_2 and PPFD (Table 2.1). However, these differences were eliminated under elevated CO_2 and PPFD. Under low CO_2 and PPFD, there were no significant differences in A and E of ASL and L4 methods, but both were significantly higher than those in the Wp method (Table 2.1). Specifically, the A and E increased by 62% and 190%, respectively, when comparing L4 with Wp methods. In contrast, the Wp measurement shows a higher WUE of those in the remaining

measurements. Besides, gs was also higher in the Wp measurement compared with the other methods. Under elevated CO_2 and PPFD, E and WUE indicated the same trend but increased in magnitude.

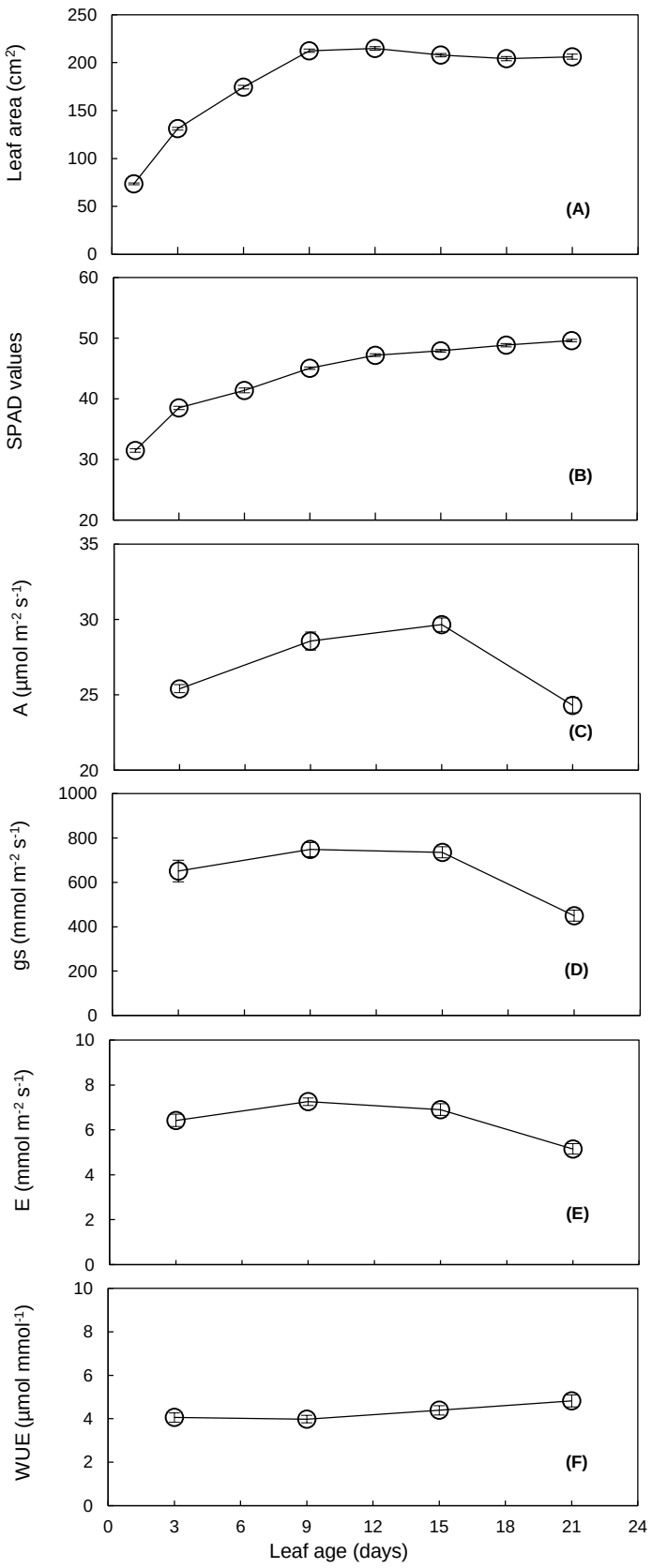

Fig. 2.4 Changes in area (A), SPAD values (B), maximize photosynthetic rate (C), stomatal conductance (D), transpiration rate (E) and water use efficiency (F) of strawberry leaves at different ages. Values are means; vertical bars represent SE (n = 50 for leaf area and SPAD values; n = 12 for A, gs, E and WUE).

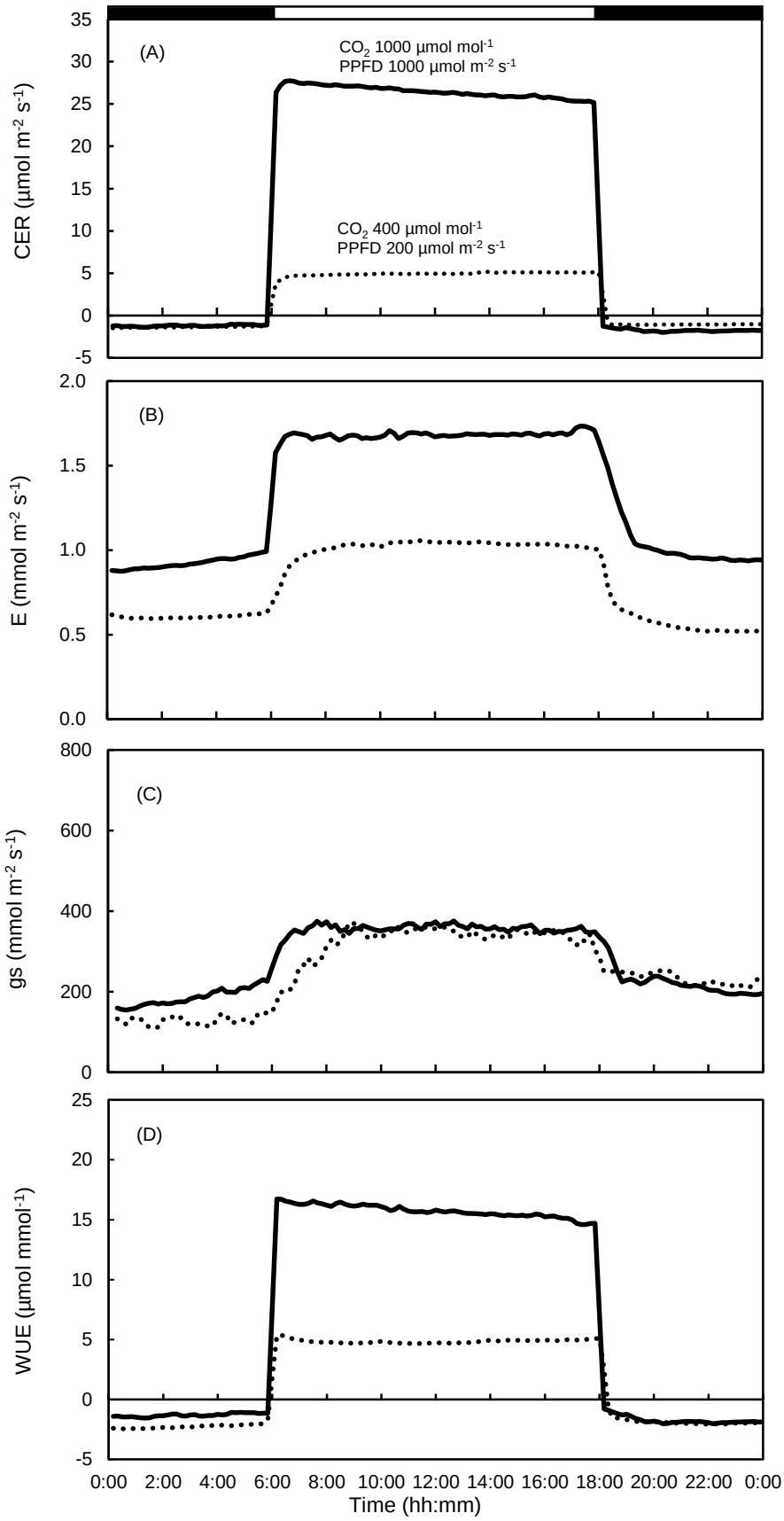

Fig. 2.5 Diurnal CO_2 exchange rate (A), transpiration rate (B), stomatal conductance (C), and water use efficiency (D) of whole plant of strawberry measured by closed chamber methods. Values are means (n = 6).

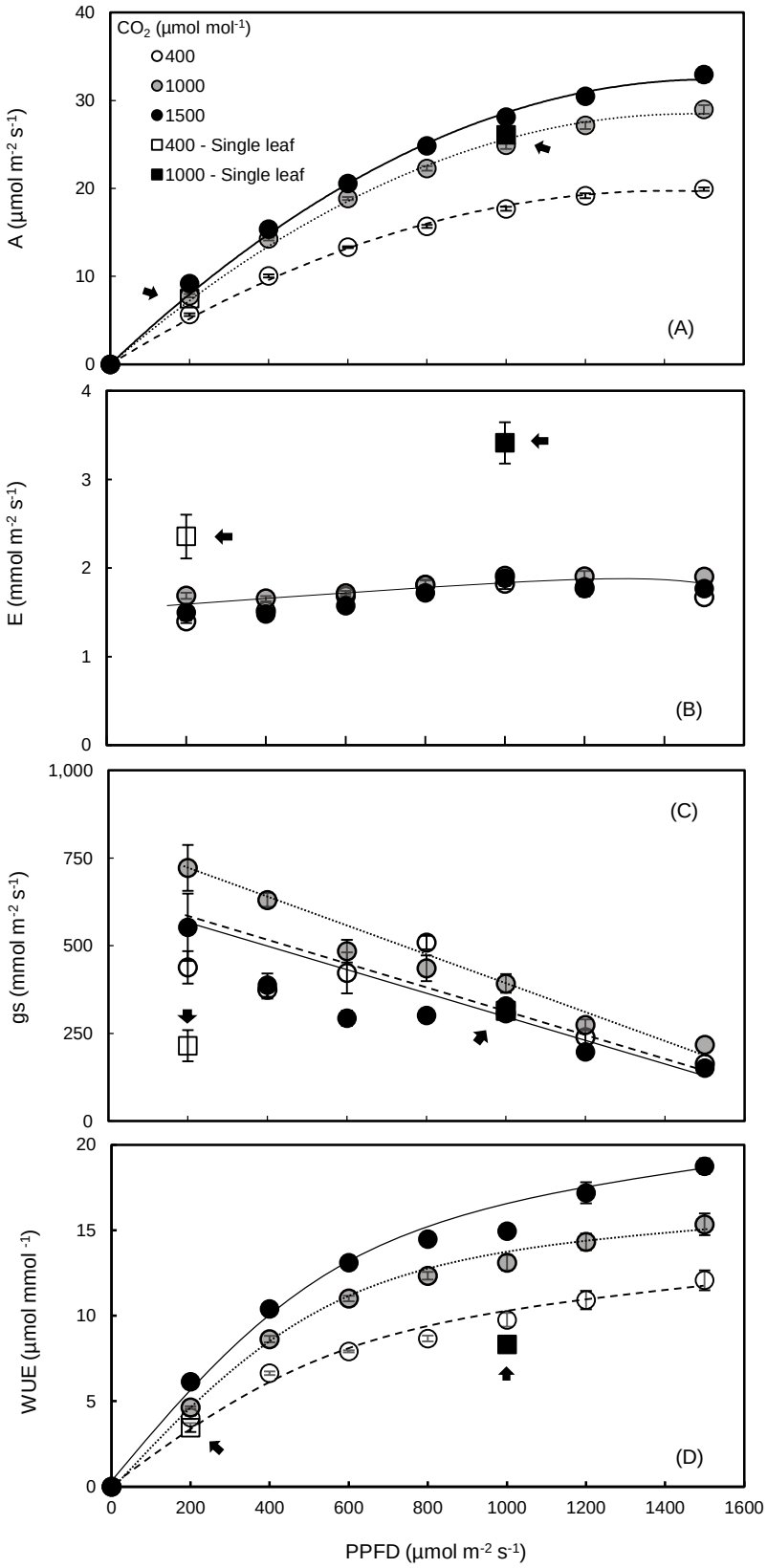

Fig. 2.6 Light response curves of photosynthesis (A), transpiration (B), stomatal conductance (C), and water use efficiency (D) of the whole plant and single leaf of strawberry under different CO_2 concentrations. The arrows indicate positions of the values measured from single leaves. Values are means; vertical bars represent SE (n = 6).

Table 2.1. The different in photosynthetic rate (A, μmol m^{-2} s^{-1}), stomatal conductance (gs, mmol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), and water use efficiency (WUE, μmol $mmol^{-1}$) measured from the averaged single leaves (ASL), 4th leaf (L4) and the whole plant (Wp) of strawberries under different CO_2 and PPFD conditions.

Treatment code	CO_2 of 400 μmol mol^{-1} PPFD of 200 μmol m^{-2} s^{-1}				CO_2 of 1000 μmol mol^{-1} PPFD of 1000 μmol m^{-2} s^{-1}			
	A	gs	E	WUE	A	gs	E	WUE
ASL	7.5 a[z]	215 b	2.4 a	3.5 b	26.1	314	3.4 a	8.3 b
L4	8.1 a	280 ab	2.9 a	3.0 b	28.5	421	4.4 a	6.9 b
Wp	5.0 b	347 a	1.0 b	6.2 a	26.4	351	1.7 b	15.9 a
ANOVA[y]	**	*	**	*	NS	NS	**	**

[z] Values within a column followed by the same letter are not significant different by LSD test.

[y] **, * or NS denote the significant different at $P < 0.01$, $P < 0.05$ or non-significant.

Discussion

Photosynthetic potential for single leaves at different ages

The photosynthetic capacity of leaves noticeably depended on their growth features (Fig. 2.4). However, the selection of suitable leaves to measure photosynthesis has not been consistent in previous studies (Paul and Pellny, 2003; Hidaka et al., 2013; Choi and Kang, 2019). I supposed that the photosynthesis measured from a single leaf at a particular age to represent the whole plant should be carefully considered to its accuracy because different-aged leaves can photosynthesize and contribute differently to the total capacity of the whole plant (Fig. 2.4, Table 2.1). The single-leaf method remains undeniably valid when the whole-plant method is impossible to fulfil. Therefore, the number of leaves as well as their age or position and the measurement conditions needs to be considered when measuring a plant using the single-leaf method. On the other hand, the photosynthesis capacity of leaves can also be the cornerstone for reverse predictions of the physiological state and development of leaves (Fig. 2.4).

Diurnal changes in CER, E, gs, and WUE of the whole plant

CO_2 and PPFD were determinants of the equilibrium or a slight decrease in the photosynthesis rate (Fig. 2.5A). The photosynthetic reduction might be due to an imbalance in the synthesis and translocation of photoassimilates. Specifically, under elevated CO_2 and PPFD, the synthetic rate of photoassimilates was faster than their translocation rate. Consequently, it caused feedback inhibitions, which led to a photosynthetic rate decrease (Upmeyer and Koller, 1973; Paul and Pellny, 2003; Figueroa et al., 2016). On the contrary, under low CO_2 and PPFD, the balance between the two processes were maintained, resulting in a constant rate of photosynthesis throughout the photoperiod (Fig. 2.5A). I recommend that the time-period to measure photosynthesis of strawberries under plant factory conditions should not be fixed during the photoperiod because the light intensity is always kept steady at a low level (typically below 300 μmol m^{-2} s^{-1}) in a plant factory. In case the PPFD reaches 1500 μmol m^{-2} s^{-1}, a slight change of photosynthetic rate may not lead to a large error in the photosynthetic capacity of leaves.

The increase of A, E, and WUE (Figs. 2.5A, B, and D) shows that these trends were typical responses of plants under elevated CO_2 and PPFD. Similar results were observed in previous studies (Chandra et al., 2008; Baligar et al., 2012).

During the dark periods, gs values were nonzero (Fig. 2.5C), and the dark transpiration rates were approximately 40% higher in the plants measured under elevated CO_2 and PPFD (Fig. 2.5B). These results can be explained by three factors. Firstly, the stomata are not completely closed at night due to the need for the plant respiration (Caird et al., 2007). Secondly, plants grown under elevated CO_2 increase their respiration (Wang et al., 2001), and leaf transpiration linearly correlates with the respiratory process (Stoyanova, 1996). Finally, the leaf transpiration drives the mineral absorption, responding to the growth during night-time (Tanner and Beevers, 2001).

Whole-plant light response curve patterns

The light response pattern of A, E, and WUE did not differ when the whole-plant and single-leaf light response curves were compared (Fig. 2.6). Mochizuki et al. (2019) also reported similar patterns when using the single-leaf method.

The gs patterns of the whole-plant and single-leaf measurements were dissimilar (Fig. 2.6C). This difference is because, in our Wp measurement system, high power LEDs caused a rise of temperature (from 23 to 27°C) in the assimilation chambers when the PPFD was in the range of 1000–1500 μmol m^{-2} s^{-1}. Consequently, the relative humidity in the chambers was reduced leading the VPD to increase from ~4 kPa to ~10 kPa (data not shown). Therefore, the response of the plants to the increase of VPD was that the gs decreased (Merilo et al., 2017).

Comparison between whole-plant and single-leaf photosynthesis measurement methods

The A and E from the two measurement methods only differed under low CO_2 and PPFD (Table 2.1). The A and E reductions of the whole plant may emerge from (1) mutual shading and (2) different leaf age. The effect of mutual shading on plants is reducing the PPFD received by the leaves at the lower position within a plant canopy. As a result, the low PPFD directly reduces photosynthetic rate (A) of lower leaves. Kitano et al. (2007) and Chandra et al. (2008) reported that the changes in leaf transpiration rate were PPDF-dependent and were coordinated with the changes of photosynthetic rate. Whereas, in the single-leaf method, a small leaf area was measured with adequate light intensity provided; hence the mutual shading effect was removed. Consequently, the A and E values of ASL and L4 were significantly higher than that of Wp.

In contrast, under 1000 μmol mol^{-1} CO_2 combined with a PPFD of 1000 μmol m^{-2} s^{-1}, the interleaf mutual shading may be noticeably reduced. Moreover, elevated CO_2 can enhance the leaves reaching close to the A_{max} values. Therefore, the variation in A caused by PPFD and

leaf ages may also be reduced. As a result, the difference in photosynthetic rate values of the two methods becomes negligible (Table 2.1).

The Wp may be the most suitable measure, however, if there are appropriate manners, the single-leaf method is still valid. Based on the results presented in Fig. 2.4, Fig. 2.6, and Table 2.1, the ASL method can be used instead of the Wp under high CO_2 and PPFD conditions; Also, measuring only the 4^{th} leaf (L4 method) to represent the whole plant seem to be acceptable. Otherwise, under low PPFD (regardless of CO_2 concentrations), the ASL and L4 methods still need to be further considered to achieve better results in terms of accuracy.

Chapter 3.

Photosynthetic Responses and Reproductive Ability of Strawberry Following Sunlight Application in a Plant Factory Closed System in Subtropical Okinawa

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a fruit with high potential to promote human health (Giampieri et al., 2015), having high economic values, and commercially produced worldwide (Simpson, 2018). However, strawberry is a temperate plant species whose cultivation is restricted by regional climates, especially in the tropical and subtropical regions. Specifically, in Vietnam, the country in wholly located in the tropical climate zone; strawberry production in the central and southern regions is almost impossible. In Vietnam, strawberries are mainly produced in the highland, the tropical monsoon climate area (Lam Dong province); nonetheless, most of field production areas are strongly damaged by pests and diseases that lead to increase utilization of plant protection chemicals and reduce fruit qualities (Lam Dong crop production and plant protection Sub Department, Vietnam, 2013, data not show). Together with the regional climate limitation, pests and disease are also a further disadvantage in the tropics and subtropics against strawberry production. Therefore, to expand strawberry cultivation in the tropical and subtropical regions and to eradicate the utilization of plant protection chemicals, an effective production system is required.

In this situation, application of plant factory systems could be a potential cultivation method for strawberry production. A plant factory, known as plant factory using artificial light (PFAL), is a system used to grow plants under strictly controlled environments (Kozai, 2013), allowing the production of high-quality and pesticide-free crops (Nakamura and Shimizu, 2019). Moreover, to use sunlight advantageously in subtropics and to reduce energy

consumption in PFAL, an “Okinawa-type plant factory” (in this study, it was mentioned as solar plant factory, SPF) was developed basely on modification of a PFAL to apply sunlight as a lighting source. It was reported that introducing sunlight, utilization of LED light, and renewable energy in the SPF could reduce up to 30% of total energy consumption (Kawasaki et al., 2015; Ueno and Kawamitsu, 2017). This advantage could facilitate the application of SPF in tropical and subtropical areas. Hence, strawberry production in the SPF system could bring more advantages to its adoption to the tropical and subtropical climate conditions. However, there is a limitation that sunlight introduced into SPF is unstable on cloudy and rainy days and it fluctuates season by season.

Light factor is the driving force of plant growth through direct effects on photosynthesis process. Under different lighting conditions, plants exhibit different levels of photosynthesis. Photosynthetic rate (P_n) of leaves reflects the physiological and biochemical conditions of plants in responding to environmental conditions (Upmeyer and Koller, 1972; Carlen et al., 2009). The P_n closely relates to the fruit productivity of strawberry (Choi et al., 2016); Goto et al. (2018), Hidaka et al. (2016), and Smeet (1980) demonstrated that reproductive growth characteristics, including earlier flowering, flower numbers, the percentage of fruit set, and fruit numbers of strawberry were increased by the enhanced P_n from the supplemental lighting conditions. Therefore, photosynthetic responses and reproductive traits of plants could be used as the parameters to evaluate the light effectiveness for strawberry cultivation in a plant factory.

With the target of applying SPF in Vietnam for strawberry production in the future, I continue to study to overcome the above-mentioned disadvantage of the SPF developed in Okinawa. In this study, the limitation of sunlight introduced into SPF was investigated based on photosynthetic responses and reproductive ability of strawberries grown under different sunlight application conditions.

Materials and Methods

Plant materials and growth conditions

The June-bearing strawberry cultivar ‘Sachinoka’ was used in this study. Strawberry plantlets sourced from tissue culture were first acclimatized to the plant factory conditions for 30 days and were then grown hydroponically in a SPF located in Nakagusuku, Okinawa, Japan (26°15′N, 127°47′E). The advantage of micro-propagated plantlets is genetically homogeneous and disease-free; in this study, to minimize differences in growth responses between plants caused by genetic factors, the plantlets were propagated from a mother plant. The shoots were cultured on MS (Murashige and Skoog) medium without plant growth regulators for root formation for 15 days before transferring to the acclimatization stage. The plantlets preparation protocol was briefly described in the Appendix 1. The acclimation was conducted in a PFAL from June 27 to July 27, 2018, and environmental conditions were set up at a photosynthetic photon flux density (PPFD) of 150 µmol m^{-2} s^{-1}, a photoperiod of 12h, a CO_2 concentration of 1200 µmol mol^{-1}, a relative humidity of 50% ± 10%, and a temperature of 23°C. The plantlets were placed in a sowing tray (30 cm wide, 60 cm long, 4 cm deep) that contained 1.5 L Hoagland and Arnon’s (1950) nutrient solution modified the same as mentioned below. The solution was adjusted to electrical conductivity of 0.12 S m^{-1} with pH 6.0 for the young plantlets.

In SPF, the strawberry plantlets were grown on vertical shelves, and nutrient solution was distributed by a circulation system. A pump (45 L min^{-1}, 70 W; Iwaki Co., Ltd.) was used to supply the nutrient solution continuously from a container to the cultivation shelves; these shelves were connected to each other and to the container by supply pipes. The nutrient solution was a modification of Hoagland and Arnon’s (1950) solution containing 3 mM $Ca(NO_3)_2$, 2 mM KNO_3, 2 mM KH_2PO_4, 2 mM $MgSO_4$, 25 µM H_3BO_3, 6 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM Na_2MoO_4, and 0.1 mM Fe-EDTA. This solution had an electrical

conductivity of 0.18 S m^{-1} with pH 6.0, and was renewed every 2 weeks. The plants were grown under 1200 μmol mol^{-1} CO_2 at a relative humidity of 50% ± 10% and temperature established at 23°C. The flowering induction of June-bearing strawberries can be achieved under short-day conditions with temperatures ranging from 15°C to less than 25°C (Ruan et al., 2011). Besides, in this study, I first focused on examining the effect of light conditions on plant growths at an appropriate temperature. Therefore, the plants were grown under a temperature of 23°C without chilling treatments.

In Okinawa, the periods July to October and November to January represent long-day and short-day conditions, respectively (Jiro and Hiroshi, 1987). Therefore, since flower induction in this June-bearing cultivar occurs under short-day conditions, the experiment was conducted from July 28, 2018 to January 10, 2019 to investigate the growth responses of the strawberries in both vegetative and reproductive stages. For more details, the timing of strawberry growth stages was described in Fig. 3.1.

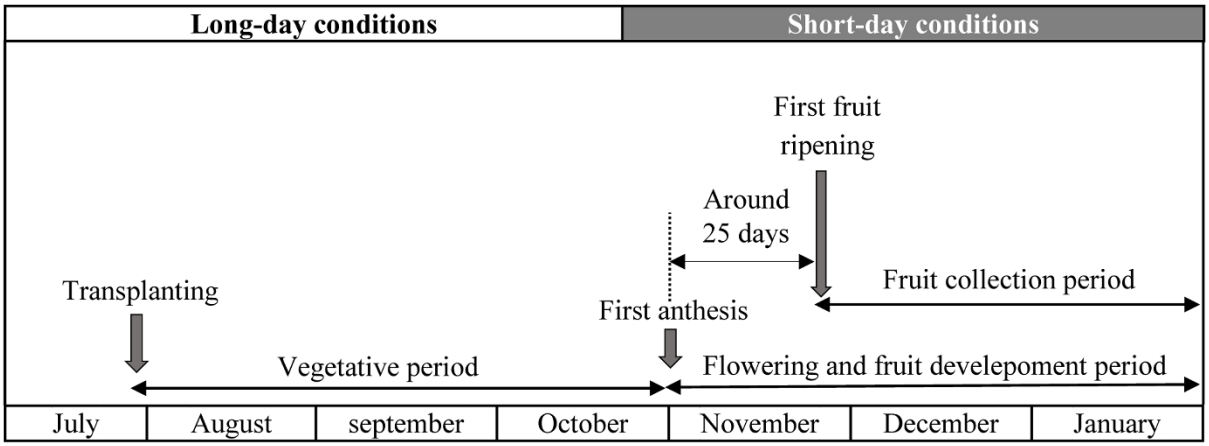

Fig. 3.1. Descriptions of the timing of strawberry growth stages in the solar plant factory under long-day and short-day conditions.

SPF specifications

The SPF used in this study was described in detail by Ueno and Kawamitsu (2017). Briefly, a rooftop was modified with a pair of transparent, heat-insulating glass panes (90 × 180 cm, 3 mm thick, 70% solar transmittance rate; Asahi Glass Co. Ltd., Japan) with an air layer of 2 mm between them. The SPF with a modified rooftop and vertical cultivation system is shown in Fig. 3.2. Cultivation trays (28 cm wide, 120 cm long, 7 cm deep) were placed on the tiers in the SPF to grow plants (Figs. 3.2C, D). The trays on the top tiers were 100 cm from the heat-insulating glass and exposed to sunlight (S treatment). Those on the lower tiers partly received sunlight intensity (approximately 10% on a sunny day) and were supplemented with fluorescent light using a PPFD of 65 ± 5 μmol m^{-2} s^{-1} (SA treatment). The photoperiod in the SA treatment was controlled to 13 h (5:00 am to 7:00 pm) during long-day periods and 10 h (7:00 am to 5:00 pm) during short-day periods, following the day length in the S treatment. The PPFD in the SA treatment was recorded at the beginning of the experiment on a sunny day (including the supplemented fluorescent light, maximum = 180 μmol m^{-2} s^{-1}) using a LI-250A light meter (LI-COR, Inc., USA).

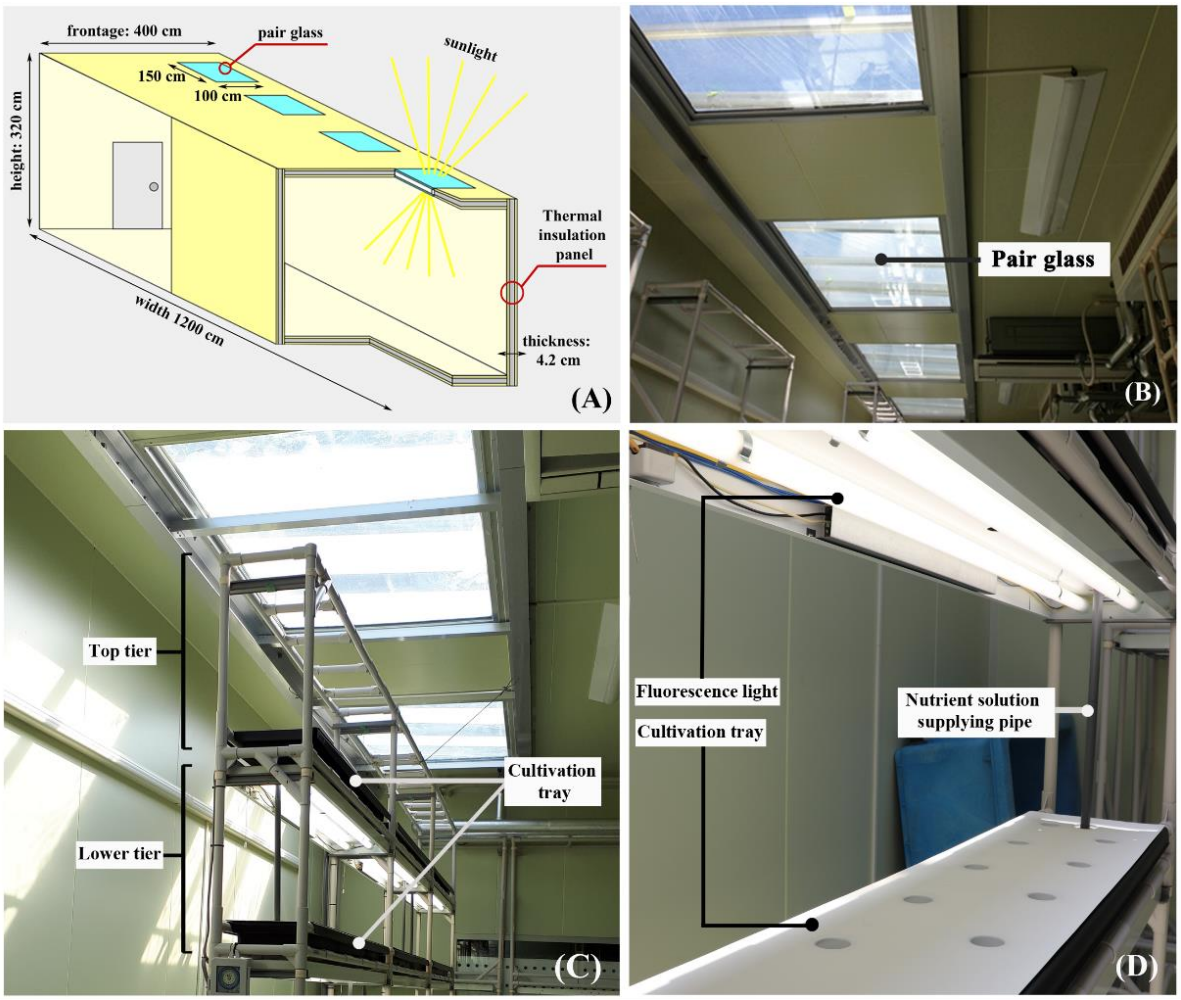

Fig. 3.2. Solar plant factory diagram (A), revised rooftop with pair glasses (B), vertical cultivation shelves with the top tier (S, sunlight) and the lower tier (SA, low sunlight and artificial light) (C), and cultivation tray (D).

Experimental design

The strawberry plants were exposed to two different light treatments: sunlight only (S) and low sunlight + fluorescent light (SA). In the S treatment, light intensity completely relies on the sunlight introduced in the SPF. The SA treatment was designed in order to simulate the low light conditions (Figs. 3.2C, D). The supplemented fluorescent light is to maintain a stable low light intensity of this treatment at a base of 65 ± 5 µmol m^{-2} s^{-1} against sunlight fluctuation at

a low level in short-day conditions. The experiment was performed in triplicates with four plants per treatment in each replicate.

Gas exchange measurement

Photosynthesis was measured using the third fully expanded leaves from the tops of the plants. The net photosynthetic rate (P_n) was measured using a portable photosynthesis system (Li-6400; Li-COR, Lincoln, Nebraska, USA), with a flow rate of 300 μmol s^{-1}, an atmospheric CO_2 concentration of 1200 μmol mol^{-1}, and a block temperature of 25°C. The plants were first exposed to the highest PPFD of 2000 μmol m^{-2} s^{-1}, followed by progressively lower intensities of 1600, 1200, 800, 400, 150, 50, and 0 μmol m^{-2} s^{-1}. Photosynthetic measurements were performed twice in the vegetative [49 days after transplanting (DAT) under long-day conditions] and reproductive (154 DAT under short-day conditions) stages.

Data collection

Experimental data collection was divided into the vegetative and reproductive growth stages. Vegetative response was investigated only during the long-day period. Vegetative growth parameters such as the leaf number, shoot number, and SPAD values were collected every 4 weeks. The SPAD values, of the 4^{th} wide-open leaves from the top of the plants, were measured using a SPAD meter (SPAD-502; Minolta, Japan). The reproductive growth parameters were collected during the short-day period (December to January) and included the flower number, inflorescence number, and fruit developmental parameters (weight, length, and diameter). The harvested fruits were sorted into three categories by weight (type I: 0–5 g; type II: 5–10 g; and type III: >10 g), and the percentage of each fruit type was determined by calculating the ratio of the fruit number of a particular type to the total number of fruits harvested in each treatment.

The light intensity and temperature in the SPF were recorded every 10 min during the experimental period using a TR-74Ui illuminance UV recorder (T&D Corporation, Japan) that

was placed on the top or lower tier of the cultivation shelf, and light intensities during the photosynthetic measurement processes were automatically recorded by the Li-6400 portable photosynthesis system (Fig. 3.4).

Flower pollination

Manual pollination was applied to strawberry flowers instead of the use of bees because bees can pose some risks (namely, fungus and bacteria that are carried by bees or develop on bee dead bodies) to the sanitary environments inside the plant factory (Hiroshi, 2019). The flowers were pollinated manually using a small paintbrush. The pollens from the stamens were brushed over the stigmas of the fully open flowers.

Statistical analysis

The mean and standard error of each parameter were calculated for each treatment and they were compared between treatments using Student's t-test. All analyses were performed in Excel 2016 using a significance level of $P < 0.05$.

Results

Light conditions in the SPF

Variation in sunlight intensity in the SPF differed among the long and short-day periods (Fig. 3.3A). Under long-day conditions, the light intensity varied over a wide range of 0–1400 μmol m^{-2} s^{-1} with the S treatment and over a narrower range of 0–180 μmol m^{-2} s^{-1} with the SA treatment. In contrast, under short-day conditions, the light intensity varied over a smaller range for both S (0–200 μmol m^{-2} s^{-1}) and SA (0–80 μmol m^{-2} s^{-1}) treatments. The results in Figs. 3.4C, D showed that fluctuations of sunlight intensity in photoperiod at S treatment could occur at very low levels (20–60 μmol m^{-2} s^{-1}) when cloudy. Meanwhile, the supplementary light revealed a positive effect on the light intensity in SA treatment. It was more stable and maintained at a higher level (55–80 μmol m^{-2} s^{-1}) in the short-day conditions.

In this experiment, the temperature variation was not focused; however, there might be queries about the changes in temperature when introducing sunlight in a PFAL. The thermal information is mentioned in this section to confirm that there were no harmful effects from the temperature. Under long-day conditions, the air temperature inside the SPF fluctuated from 22°C during the night-time to 29°C during the daytime. However, under short-day conditions, the air temperature remained more stable, fluctuating from 22°C during the night-time to 24°C during the daytime. The air conditioner in the SPF was set to 23°C to prevent high temperatures during high solar radiation outside the plant factory. Notably, from 11:00 am to 3:00 pm in September, the average temperature in the SPF was 24.6°C, which was approximately 6°C lower than the average ambient temperature outside the plant factory (30.7°C) (Figs. 3.3B, C). Thus, while the temperature inside the SPF was not perfectly controlled to 23°C during this time, it was maintained within an acceptable range (22–29°C) to prevent any negative effects on the photosynthesis and growth of the strawberry plants. Kadir and Sidhu (2006), and Carlen et al., (2009) previously demonstrated that the photosynthesis of strawberry was inhibited at a temperature of 40°C, with 30°C being optimal for P_n and vegetative growth.

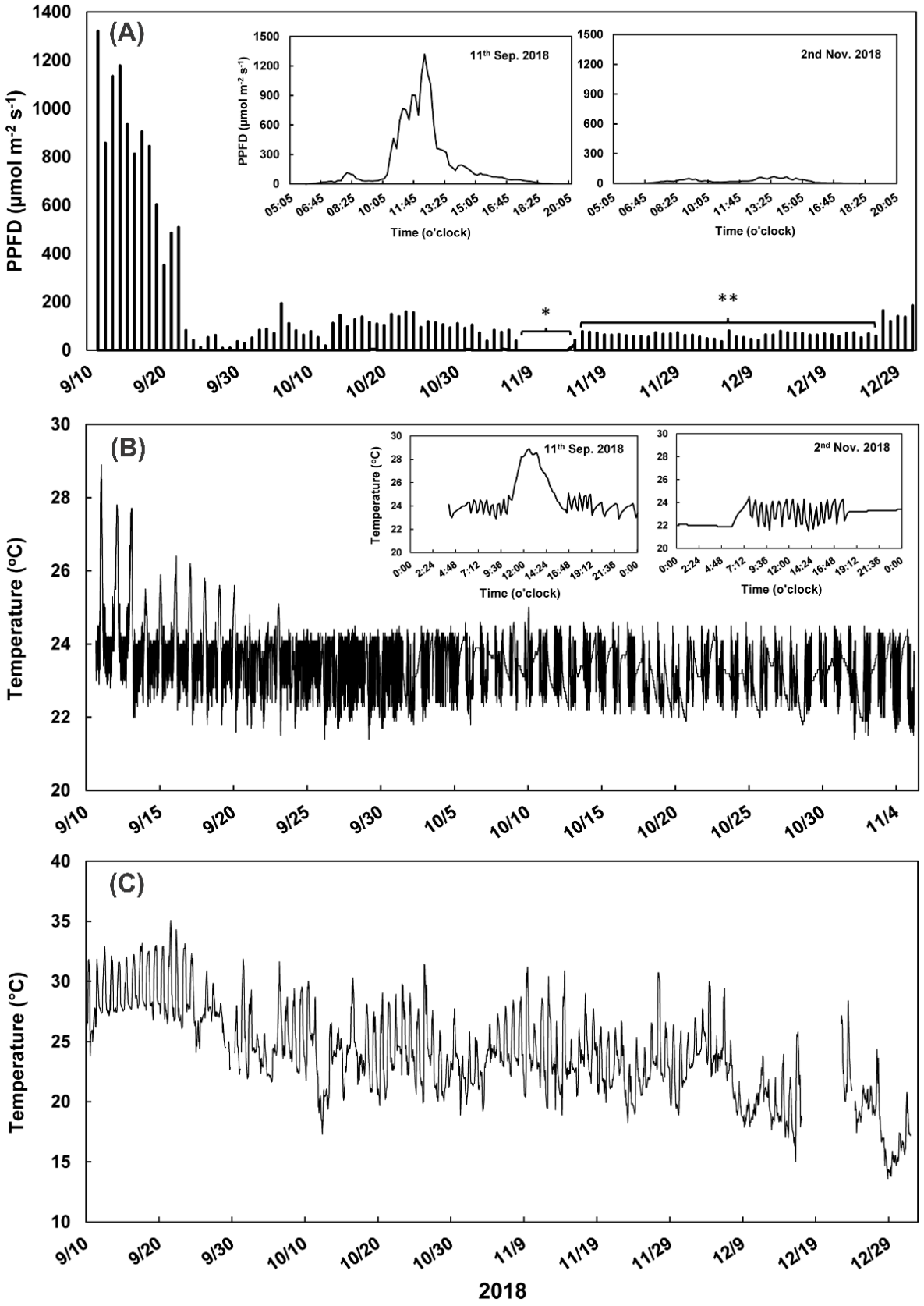

Fig. 3.3. Sunlight intensity in S treatment (A) and air temperature (B) in solar plant factory, air temperature outside the plant factory (C); lost data (∗), data collected on SA treatment (∗∗).

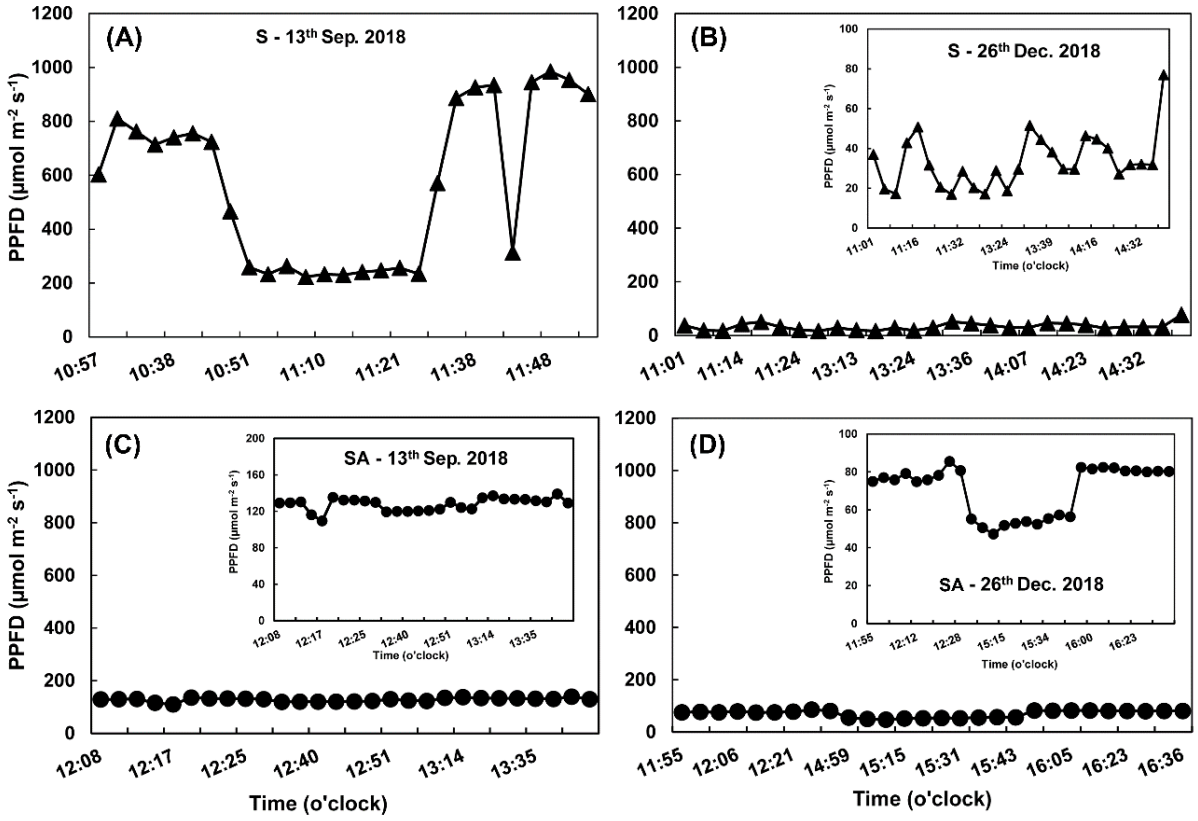

Fig. 3.4. Ambient light intensity during photosynthetic measurement process in S treatment in long-day (A) and short-day conditions (B), and in SA treatments in long-day (C) and short-day conditions (D).

Leaf traits and photosynthetic profile

The strawberry plants increased leaf number during the cultivation period from August to October, reaching a maximum in October irrespective of the light conditions (Table 3.1). The leaf number was significantly lower under the SA treatment than with the S treatment at all times. The crown number of the strawberry plants increased with the S treatment (1.9–3.1 crown plant^{-1}) but remained at a low level with the SA treatment (1.4–1.8 crown plant^{-1}) during the cultivation period (Table 3.1).

The results in Figs. 3.5A and C showed the photosynthetic capacity of the strawberry plants changed depending on the day length regardless of S or SA treatments. Considering PPFD from 300 to 2000 µmol m^{-2} s^{-1}, P_n of the leaves in S and SA treatments (23 and 26 µmol

m^{-2} s^{-1}, respectively) were lower than those (30 and 32 μmol m^{-2} s^{-1}, respectively) under short-day compared with long-day conditions. Additionally, under long-day conditions, P_n and light use efficiency (presented by initial slopes) did not significantly differ between strawberry leaves in S and SA treatments (Figs. 3.5A, B). In contrast, P_n and the light use efficiency were significantly higher in SA compared with those in S treatment under short-day conditions (Figs. 3.5C, D).

Reproductive growth traits

The flower numbers of the plants in S treatment (3.7 inflorescence $plant^{-1}$) were significantly higher than that in treatment SA (2.2 inflorescence $plant^{-1}$) (Table 3.2). Consequently, flower numbers and fruit numbers were also significantly higher in S compared to SA treatment. The fruit Type I was not recorded in SA treatment, while fruit Type I was accounted for 45% in S treatment. Fruit types II and III tended to be higher in the plants of SA treatment compared to plants in S treatment (Table 3.2).

Fruit development characteristics (including fruit weight, length, and fruit diameter) were not statistically different between the two treatments, even though fruit weights tended to be larger with treatment SA (Table 3.3).

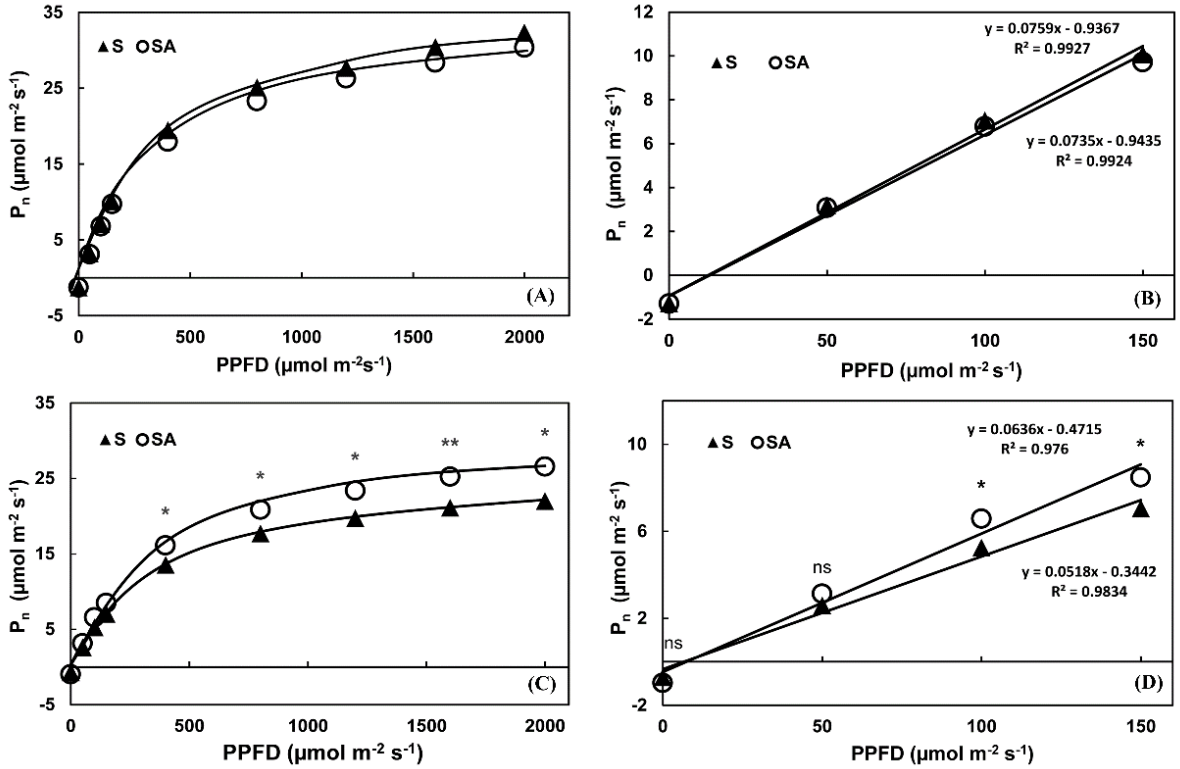

Fig. 3.5. Net photosynthetic rate (A) and initial slope (B) in long-day conditions, net photosynthetic rate (C) and initial slope (D) in short-day conditions.

ns, *, ** denote non-significant, significant at P< 0.05 or 0.01, respectively (means data compared by t-test).

Table 3.1. Leaf number, crown number and SPAD value of strawberry under SPF conditions (means ± standard error).

Treatment	Leaf number plant^{-1}			Crown number plant^{-1}			Leaf SPAD value		
	Aug.	Sep.	Oct.	Aug.	Sep.	Oct.	Aug.	Sep.	Oct.
S	9.3 ± 1.2*	21.4 ± 1.6*	28 ± 1.8*	1.9 ± 0.9^{ns}	2.3 ± 0.7^{ns}	3.1 ± 0.8*	37.7 ± 1.6^{ns}	39.9 ± 1.3^{ns}	38.2 ± 1.6^{ns}
SA	6.6 ± 0.9	14.6 ± 1.9	18 ± 1.7	1.4 ± 0.7	1.9 ± 0.9	1.8 ± 0.7	38.8 ± 1.6	41.2 ± 1.3	38.6 ± 1.5

S or SA denotes plant grown under full sunlight or under low sunlight + fluorescence light

*, ^{ns} indicates significant difference, non-significant difference at P < 0.05 by t-test, respectively.

Table 3.2. Number of flower (No. Flower), inflorescence (No. inflorescence), fruit (No. fruit) per plant, and percentage of fruit type of strawberry grown in SPF (means ± standard error).

Treatment	No. Flower plant^{-1}	No. inflorescence plant^{-1}	No. fruit plant^{-1}	Percentage of fruit type (%)		
				Type I (<5g)	Type II (>5g)	Type III (>10g)
S	21.5 ± 0.8*	3.7 ± 0.2*	6.8 ± 0.3*	45.0	45.0	10.0
SA	17.8 ± 0.8	2.2 ± 0.1	3.1 ± 0.2	-	54.2	45.8

S or SA denotes plant grown under full sunlight of under low sunlight + fluorescence light

* indicates significant difference at P < 0.05 by t-test, respectively.

Table 3.3. Fruit weight and fruit size of strawberry under different light conditions in SPF (means ± SE).

Treatment	Fruit type I (<5g)			Fruit type I (>5g)			Fruit type I (>10g)		
	Weight (g)	Length (cm)	Diameter (cm)	Weight (g)	Length (cm)	Diameter (cm)	Weight (g)	Length (cm)	Diameter (cm)
S	3.7 ± 0.2	2.9 ± 0.6	1.9 ± 0.7	6.0 ± 0.4^{ns}	3.4 ± 0.6^{ns}	2.6 ± 0.6^{ns}	10.9 ± 0.8^{ns}	3.6 ± 0.8^{ns}	2.9 ± 0.7^{ns}
SA	-	-	-	7.2 ± 0.4	3.3 ± 0.1	2.4 ± 0.2	12.4 ± 0.4	3.8 ± 0.1	3.1 ± 0.1

S or SA denotes plant grown under full sunlight of under low sunlight + fluorescence light

^{ns} indicates non-significant difference at P < 0.05 by t-test, respectively.

Discussion

Light conditions in the SPF

The light intensity varied following the day length, and its fluctuation occurred in both long-day and short-day conditions (Figs. 3.3A, 3.4). The findings showed that introducing sunlight to PFAL has two main issues that need attention. First was the seasonal fluctuation in which the light intensity tended to decrease sharply in the short-day conditions or winter (often cloudy and rainy). The second was the fluctuation range of light intensity could be very low (even downward to 20 μmol m^{-2} s^{-1}) in the short-day conditions. Therefore, the use of artificial light was the manner to maintain a stable level of light intensity against the low range fluctuation.

Leaf traits and photosynthetic profile

The increment of leaf and crown numbers proved that the vegetative growth response of strawberries in SPF was as normal as other plants; most plants have an increase in the leaf numbers, shoot numbers and their biomass when grown under high light intensity (Yao et al., 2017; Feng et al., 2019; Aspinall and Paleg, 1964). Moreover, this result also revealed clearly the potential of leveraging sunlight into the PFAL to promote the growth of strawberry plants.

Under long-day conditions, there was no significant difference between the S and SA treatments with respect to the light response curves of the strawberry leaves measured at PPFD values of 0–2000 μmol m^{-2} s^{-1} (Fig. 3.5A, B). This result indicated that light variation in the range of 60–1400 μmol m^{-2} s^{-1} did not limit the photosynthetic ability of the strawberries grown in SPF. The strawberry plants grown under a low-light intensity in the SA treatment had a similar photosynthetic ability to plants grown under full sunlight in the S treatment indicated that low-light intensity (60–180 μmol m^{-2} s^{-1}) conditions had no adverse effect on the physiology of the strawberry leaves. Besides, the normal physiological condition of leaves is

also expressed through SPAD value, which is one of the criteria to evaluate chlorophyll content in leaves and to reflect the biochemical status of leaves (Guer and Ozcelik, 2007). In our results, SPAD values were relatively constant with both treatments regardless of low or high light conditions (Table 3.1). Furthermore, at PPFDs of 2000 μmol m^{-2} s^{-1}, the maximum P_n values in both S and SA treatments were approximately 32 μmol m^{-2} s^{-1}, which are not inferior to the usual P_n values of strawberry plants growing in a CO_2-enriched greenhouse (Hidaka et al., 2016).

Under short-day conditions, the P_n values of the strawberry leaves were lower than those measured under long-day conditions in both S and SA treatments (Figs. 3.5A, C). However, the photosynthetic ability and the initial slope of the light response curve of the strawberry leaves were significantly higher with the SA treatment than with the S treatment (0.0636, r = 0.988; 0.0518, r = 0.992) (Figs. 3.5C, D). In combination with data presented in Figs. 3.5B and D, these results made it clear that in short-day conditions, the light fluctuation combined with low intensity in the SPF were the main reasons that led to reducing the photosynthetic ability of the strawberries. Vialet-Chabrand et al., (2017) demonstrated that plants reduced their photosynthetic ability when grown under fluctuating light intensity compared to those grown under stable light conditions. Therefore, the maintenance of light stability even at low intensity (55–80 μmol m^{-2} s^{-1}), such as in the SA treatment, showed a positive effect on improving the photosynthesis ability of the strawberries (Figs. 3.5C, D). Thus, the supplemented lighting to maintain the stability of light intensity could overcome the disadvantage of fluctuation and low light intensity in short-day conditions in SPF.

Reproductive growth traits

The strawberry plants in the SPF bloomed and set fruits regardless of the light conditions. Hidaka et al., (2016) reported that strawberry plants grown in a greenhouse under elevated CO_2 produced 14.2 and 21.8 flowers per plant under non-supplemental and supplemental lighting,

respectively. However, the use of full sunlight or a low sunlight intensity supplemented with fluorescent light resulted in high flower numbers ranging from 17.8 to 21.5 flowers per plant (Table 3.2); thus, indicating that plants that were grown in the SPF had similar reproductive abilities to those under controlled environmental greenhouse conditions. A higher photosynthesis activity combined with the strong growth in leaf and shoot numbers under the effect of high light intensity enhanced reproductive traits (Smeet, 1980; Goto et al., 2018), which induced the production of significantly higher numbers of inflorescences, flowers, and fruit in S treatment than those in the SA treatment (Tables 3.1, 3.2).

The size and weight of the harvested fruits were similar for the two light conditions. However, plants in the SA treatment did not produce any type I fruit, and produced a higher proportion of type II (54.2%) than type III (45.8%) fruit. Plants in the S treatment produced equal proportions of type I and II fruits (45% each) and a much lower proportion of type III fruits (10%). The number of fruits per strawberry plant was low (3.1–6.8 fruit plant^{-1}) despite the high flower numbers (Table 3.2). This low fruit set may have resulted from the low efficiency of manual pollination, which may have led to the significant difference in fruit numbers between the two treatments. It is also possible that a plant with a large number of fruits would experience less fruit size enlargement than a plant with a smaller number of fruits. This is reflected by the fact that plants in the S treatment produced twice as many fruits as those in the SA treatment group but had a lower proportion of type III fruits (Table 3.2).

Interestingly, the plants grown in the S treatment showed superiorities in vegetative and reproductive growths compared with that in the SA treatment. It indicates the efficiency of using sunlight in SPF. Wang and Wang (2014) agreed that higher light intensity from full sunlight conditions enhances photosynthesis and vegetative growth, specifically in leaf area, which results in better reproductive growth. Similarly, previous studies reported negative effects of shading on strawberry growth under greenhouse and field conditions. The lower light

intensity due to shading leads to the reductions in leaf number, crown number, and the subsequent reduction in the flower number, fruit number, and smaller fruit sizes compared with those grown under full sunlight (Awang and Atherton, 1995; Demirsoy et al., 2007; Wang and Wang, 2014). In this study, high light intensity along with long photoperiod could give advantages on vegetative growth with better leaf number, crown number, and SPAD values, which potential for higher reproductive growth in flower number, fruit number in S treatment. In the opposite way, more stable and higher light intensity during short-day conditions since supplemental lighting in SA treatment could give advance in the higher percentage of the fruit type of bigger size (Table 3.2). In order to ensure better plant growth, continuous and year-round production, the supplemental lighting during the short-day conditions, and increasing the light intensity in the lower tiers should be required.

In conclusion, when sunlight is applied in a closed-type plant factory, it is important that both the light intensity and its fluctuation have to be considered, as each of these could affect crop growth responses and variate markedly between seasons. Under long-day conditions, the photosynthetic ability of the strawberry plants was not affected significantly by the light conditions in SPF; however, the growth parameters measured were greater under the full sunlight condition, which contributed to an increased fruit productivity in terms of the numbers of inflorescences, flowers, and fruits. In contrast, the low and fluctuating light intensity that occurred under short-day conditions reduced the photosynthetic ability of the strawberry plants. The strawberry plants in SPF showed a high potential to produce flowers and fruits, which were not inferior to those under other controlled environment productions. To address the issue of fluctuation and low-light intensity under short-day conditions in the SPF and allow the year-round cultivation of strawberry in the tropics or subtropics, the supplemental lighting to maintain the stability of light intensity is recommended. Therefore, strawberry production using SPF is practicable in the subtropics. Some treatments for flowering induction may be

considered in the long-day condition to advantageously use sunlight to promote the growth of flowers and fruits. The effects of other factors on the productivity of the strawberry plants in the SPF, such as mineral nutrition or pollination methods, were not considered in the present study and should be investigated in the future.

Chapter 4.

Improvement of Growth and Fruit Sugar Accumulation in Strawberry under Plant Factory Conditions through Manipulation of Phosphorus and Light Spectrum Applications

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a microclimatic crop typically in the temperate region (López-Aranda et al., 2011). Its cultivation is limited in tropical and subtropical countries. Strawberry is the fruit of high economic value, expected for sweetness, attractive color, and medical values in cardiovascular diseases and cancer prevention (Giampieri et al., 2012). However, strawberries are highly perishable after harvest, requiring producers to apply plant protection chemicals during cultivation until pre-harvest (Feliziani et al., 2016) and use post-harvest techniques to maintain fruit qualities (Hikawa-Endo, 2020). Therefore, in tropical or subtropical countries, plant factory technology is the solution for strawberry production to maintain the fruit qualities without depending on the external environments and plant protection chemicals (Le et al., 2020). Additionally, producing strawberry in the consumption area can reduce transportation and storage times of fruits. In this study, the plant growth responses and fruit sugar accumulation of strawberry grown in plant factory conditions were focused.

Phosphorus, an important macronutrient for both the vegetative and reproductive growth of plants, participates directly in the pathway that converts solar energy into the chemical energy of adenosine triphosphate (ATP) in the light phase of photosynthesis and regulates sucrose biosynthesis in the leaves (Rychter et al., 2016). Phosphorus has been reported to be involved in controlling reproduction and increasing crop productivity and quality (Sandaña and Pinochet, 2014; Taliman et al., 2019). However, there is limited information about the role of

phosphorus in the division ratio between reproductive and vegetative growth rates of plants during the reproduction period and its role in the relationship between reproduction and fruit sugar accumulation.

The light spectrum also governs plant growth by affecting photosynthesis and the signals received by light receptors, with various light spectra having been reported to have pronounced effects on the growth, quality, and metabolic processes of cultivated plants (Muneer et al., 2014; Zhang et al., 2017). The use of light-emitting diodes (LEDs) as artificial light sources have the advantages of providing a well-controlled light spectrum, saving energy, and improving the efficiency of plant production in a plant factory (Yoshikoshi and Yamamoto, 2014; Kawasaki et al., 2015; Ueno and Kawamitsu, 2017). However, the interaction between the light spectrum and phosphorus has not been well studied on plant growth responses. Therefore, in this study, I was interested in examining the impacts of different phosphorus concentrations in combination with light spectra from LED and fluorescent light sources on the growth and fruit sugar accumulation in strawberry under plant factory conditions.

Materials and Methods

Growth conditions and plant materials

This experiment was conducted in a plant factory with artificial lighting (PFAL) at the University of the Ryukyus, Okinawa, Japan (26°15′N, 127°45′E) under the following conditions: light intensity, 150 μmol m^{-2} s^{-1}; photoperiod, 10 h; CO_2 concentration, 1200 μmol mol^{-1}; relative humidity, 70 ± 10 %; and temperature, 23°C. The strawberry cultivar ‘Sachinoka’ was used as the plant material in this study. To minimize any experimental errors due to genetic differences among the plants, the plantlets that were used originated from a single mother plant and were prepared using the tissue culture method. The plantlets were adapted to the environmental conditions in the PFAL for 30 days prior to the experiment.

There were three cultivation systems; each cultivation system consisted of a vertical shelf stratified system (or block) with three tiers, each of which was 260 cm long, 60 cm wide, and 10 cm high. Light tubes were located 25 cm above the surface of each tier, where the light intensity was determined for each treatment. A circulating hydroponic method was used to provide nutrients to the plants in three blocks, whereby one tier in each block corresponded to one treatment. The nutrient solutions were kept in three containers (70 L) that were separate from the three blocks, each of which contained a pump (RSD-50A, 70 W, Iwaki Co., Ltd., Japan) that supplied nutrient solution simultaneously to all three tiers. The water flows were kept continuously with a flow rate of 45 L min^{-1}. There was a drainage pipe system at the end of each tier that returned the solution to the container.

Flower pollination

On each inflorescence about three flowers were retained (one primary and two secondary flowers) and pollinated manually using a small paintbrush.

Experimental design

The experiment was designed as a randomized complete block design that included two factors, namely phosphorus concentrations (2, 6, and 12 mM with EC of 0.21, 0.25 and 0.31 S m^{-1}) in combination with three different light spectra [purple LED light (PL; PT3RB120V24, Showa Denko Group), white LED light (WL; LED FL40 AX-120AD, 19W, Ryukyu Kougaku Kenkyuu Unit), and white fluorescence light (WF; model FHF32EX-D-HX-S, 32 W, NEC Writing Co., Ltd.)]. The spectral photon distribution of the LED and fluorescence light sources were measured using a field portable spectroradiometer (Field Spec Pro, ASD Inc., UK) (Fig. 4.1). The experiment included nine treatments; each treatment consisted of 12 plants divided into three replicates, which were conducted simultaneously. Each cultural block was exposed to the three different light spectra on different tiers but the same concentration of phosphorus

across all three tiers. The nutrient solution used in this experiment was a modification of Hoagland and Arnon's solution (1950) (Appendix 2) and was renewed every 7 days.

When plants entered the reproductive stage (after day 42), symptoms of tip burn were very likely to occur. Therefore, 1 mM $CaCl_2$ was added to all treatments and the pH of the nutrient solution was maintained at 6.2 ± 1 using KOH to control this.

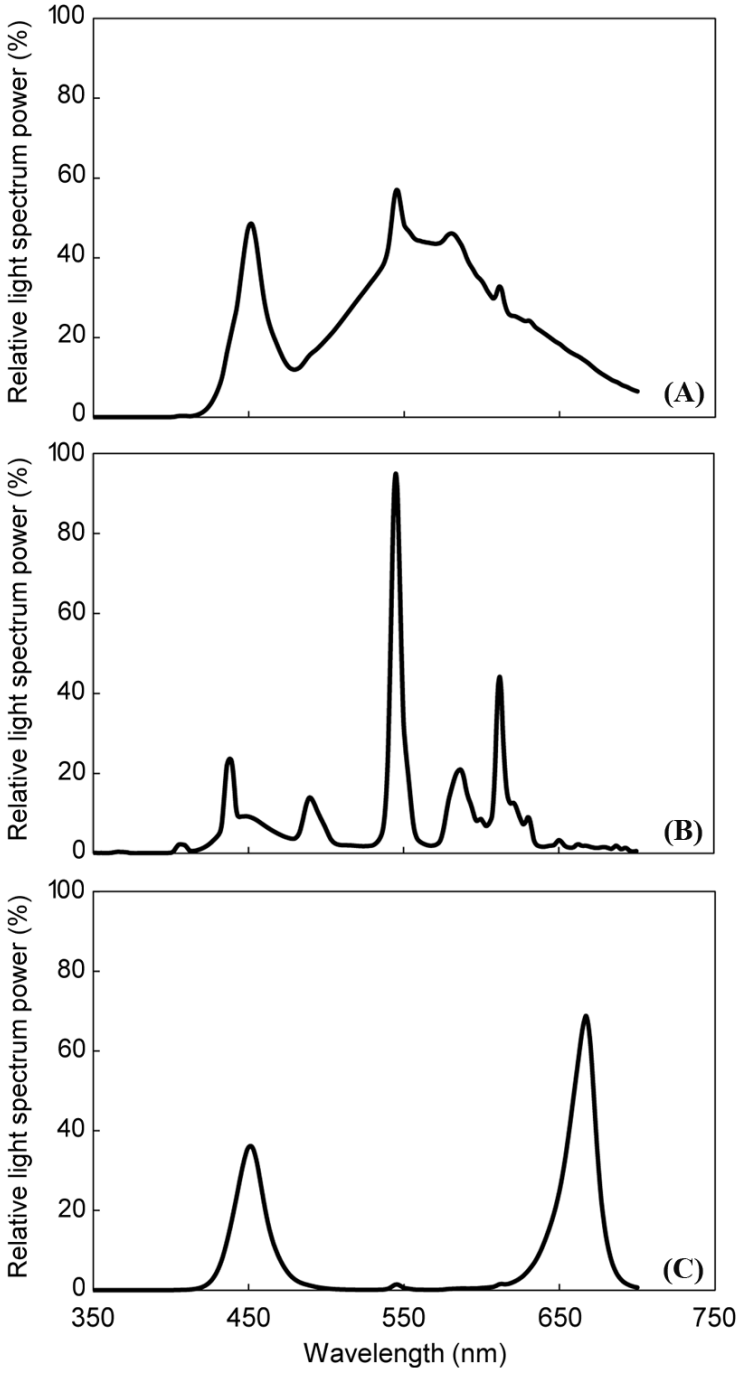

Fig. 4.1. The spectral photon distribution of (A) white LED light (WL), (B) white fluorescence light (WF), and (C) purple LED light (PL).

Growth analysis

Growth parameters (biomass data and leaf area) were collected twice during the experiment: on day 42 in the end of vegetative stage (when bracts appear) and on day 119 in the reproductive stage (marking the end of the experiment). Flower and ripe fruit data (flower number, fruit number, fruit length, fruit diameter, and fruit weight) were also collected during the reproductive period.

The relative growth rate (RGR) is widely used to analyze the growth of plants. RGR has two components, the net assimilation rate (NAR) and leaf area ratio (LAR), which are crucial to its direction and magnitude (Poorter, 1989). In order to indicate the proportional difference in NAR or LAR scaled to the proportional difference in RGR, growth response coefficient (GRC) parameters should be applied (Poorter and Werf, 1998).

The values of strawberry (sugar content, fruit weight) are mainly assessed based on the fresh fruit. Therefore, I considered evaluating the reproductive growth rate of the whole plant also by measuring the fresh biomass of both the flowers and fruit combined with the biomass data. However, RGR is usually calculated using dry biomass data. Therefore, I first checked the difference between RGR values calculated with fresh and dry biomass data (excluding flower and fruit data) to ensure that the fresh biomass data could be used. The results showed that there were negligible differences in the trend and magnitude of RGR calculated with fresh and dry biomass data (Appendix 3). Therefore, the various growth parameters (RGR, NAR, and LAR) were calculated with fresh biomass data using the following formulas of Hoffmann and Poorter (2002), Poorter (1989), and Williams (1946):

$$\mathrm{RGR} = \frac{\overline{\ln W_2} - \overline{\ln W_1}}{t_2 - t_1}$$

$$\mathrm{NAR} = \frac{\ln L_{A2} - \ln L_{A1}}{t_2 - t_1} \times \frac{W_2 - W_1}{L_{A2} - L_{A1}}$$

$$LAR = \frac{\overline{\ln W_2} - \overline{\ln W_1}}{W_2 - W_1} \times \frac{L_{A2} - L_{A1}}{\ln L_{A2} - \ln L_{A1}}$$

where W is the plant fresh weight (including flower and fruit weight), L_A is the leaf area, and 1 and 2 indicate the sample collection times (on days 42 and 119, respectively).

In addition, GRC values were calculated using the following formula proposed by Poorter and Werf (1998):

$$GRC_X = \frac{\ln X_A - \ln X_B}{\ln RGR_A - \ln RGR_B}$$

where X is the growth parameters (i.e., NAR and LAR) and A and B denote the two treatments in the comparison. The unit of the RGRs used in this formula was g g^{-1} d^{-1}.

Two new parameters of RGR that characterized the relative vegetative and reproductive growth of strawberry plants were also calculated using the following formulas:

$$RGR_V = \frac{\overline{\ln W_{V2}} - \overline{\ln W_{V1}}}{t_2 - t_1}$$

$$RGR_{RP} = RGR - RGR_V$$

where RGR_V and RGR_{RP} represent the relative growth rate of vegetative growth and reproductive growth, respectively. W_V is the fresh weight of vegetative organs (crow, root, and leaf fresh weight; excluding flower and fruit weight), and 1 and 2 indicate the sample collection times (on days 42 and 119, respectively).

These parameters were used to calculate the percentage of vegetative and reproductive growth rate that comprised RGR:

$$\%RGR_V = (RGR_V/RGR) * 100$$

$$\%RGR_{RP} = (RGR_{RP}/RGR) * 100$$

The increased leaf areas (ILA) and increased plant fresh weight (IPFW) were calculated as the formula below:

$$ILA = LA_1 - LA_2$$

$$\mathrm{IPFW} = \mathrm{PFW}_1 - \mathrm{PFW}_2$$

where LA and PFW are leaf area and plant fresh weight of the plants, and 1 and 2 indicate the sample collection times on days 42 and 119, respectively.

Extraction and analysis of sucrose phosphate synthase (SPS)

During the reproduction period (when the plans get flowers and harvested fruits), approximately 8 cm^2 of the fourth wide-open leaf from the top of each plant was collected between 12:00 and 13:00 and immediately placed in liquid nitrogen and stored at −80°C for later analysis. Enzyme extraction and analysis were based on the method of Du et al. (1998). Briefly, each leaf sample was ground in 4 mL extraction buffer with 40 mg polyvinylpolypyrrolidone and 0.2 g sea sand in a cold mortar. The extraction buffer consisted of 10 mM $MgCl_2$, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-NaOH (pH 7.5), 1 mM ethylenediaminetetraacetic acid (EDTA)-NaOH (pH 7.0), 5 mM dithiothreitol (DTT), 1% (w/v) bovine serum albumin (BSA), and 0.02% (w/v) Triton X-100. The grinding fluid was then filtered through a single layer of Miracloth (Calbiochem-Novabiochem, La Jolla, USA) and the filtrate was centrifuged at 18000 rpm at 4°C for 10 min. The supernatant was desalted by passing through a 5-mL Sephadex G-25 column (HiTrap Desalting, Pharmacia Biotech, Sweden), and the filtrates were used immediately for SPS assay.

SPS was assayed based on the reaction by which uridine diphosphate (UDP) glucose and fructose-6-phosphate (F6P) are converted into sucrose-6-phosphate. The assay mixtures (70 µL) contained 50 mM HEPES-NaOH (pH 7.5), 15 mM $MgCl_2$, 25 mg UDP glucose, 10 mM F6P, and an aliquot of leaf extract, and were incubated at 30°C for 10 min, following which 70 µL of 1 N NaOH was added to stop the reaction. The resulting mixtures were kept in a water bath at 100°C for 10 min to destroy any unreacted fructose and F6P. During cooling, 0.25 mL of 0.1% (w/v) resorcinol in 95% ethanol and 0.75 mL of 30% HCl were added, and the mixtures were incubated at 80°C for 8 min. The absorbance of each mixture at 520 nm was then

measured with a spectrophotometer (V500, Jasco, Japan). SPS activity was measured against a blank obtained by the same procedure as outlined above except that 70 µL of 1 N NaOH was added to the reaction mixture before adding leaf extract. Sucrose concentrations were estimated from a standard curve that was produced using sucrose standards.

Sugar analysis

During the reproductive period, the fruit were harvested from plants in each treatment and stored at −80°C. Three samples of fruit (30 g each) were randomly selected from the mixing of fruits from three replications in each treatment, defrosted at room temperature, and then pressed to obtain the juices. The juices were diluted 20 times and then filtered through filter paper (Advantec quantitative ashless No. 6, Toyo Roshi Kaisha, Ltd., Japan). The filtered juices were then re-filtered through a membrane of cellulose acetate (0.45 µm; Toyo Roshi Kaisha, Ltd., Japan) and used directly for sucrose, glucose, and fructose analysis by high-performance liquid chromatography (HPLC) (LC-20A, Shimadzu).

Statistical analysis

All of the data obtained in this experiment were prepared using the Excel 2016 software and statistically analyzed using the STAGRAPHIC software. The mean values of the treatments were compared using Duncan's multiple range test with a significance level of $P < 0.05$.

Results

Plant growth in the early growth stage

After 42 days of cultivation, there was a significant difference in strawberry growth among treatments. In terms of the phosphorus concentration, the total leaf area and fresh weight were significantly higher in plants that were grown with 6 mM phosphorus (0.16 m^2 and 110.7 g, respectively) than the other concentrations (Table 4.1, single factor). In terms of the different

light spectra, there was no significant difference in the leaf area or plant fresh weight between the PL and WL conditions. However, both parameters were significantly lower under WF (0.14 m^2 and 90.7 g, respectively) (Table 4.1, single factor). There was no significant interaction between phosphorus concentration and light spectrum, but plants in the 6WL treatment group showed superior growth compared with all other treatments.

Plant growth in the reproductive period

In the reproductive period (from day 42 to day 119), there was no significant difference in leaf area between any of the treatments (Table 4.1). However, the plant fresh weight differed among treatments, becoming significantly lower when the phosphorus concentration increased from 2 to 12 mM and the light spectrum remained constant (from 332.2 to 229.6 g, from 302.8 to 249.4 g, and from 264.4 to 225.3 g under PL, WL, and WF conditions, respectively) (Table 4.1).

The phosphorus concentration significantly affected both the increased leaf area (ILA) and the increased plant fresh weight (IPFW), while the light spectrum only affected IPFW (Table 4.1, single factor). Plants that were grown in 2 mM phosphorus had significantly higher ILA (0.17 m^2) and IPFW (208.2 g) than those grown in 6 or 12 mM phosphorus (Table 4.1, single factor). In addition, the IPFW was significantly higher under PL (186.5 g) than under white light conditions, with WF giving the lowest value (153.3 g) (Table 4.1, single factor).

Table 4.1. Leaf area and plant fresh weight on day 42 (vegetative stage), leaf area and plant fresh weight on day 119 (reproductive stage), increased leaf area (ILA, compared between day 42 and day 119) and increased plant fresh weight (IPFW, compared between day 42 and day 119) of strawberries grown under different phosphorus concentrations and light spectra

Treatment[y]	Leaf area (m^2)			Plant fresh weight (g)		
	Day 42	Day 119	ILA	Day 42	Day 119	IPFW
2PL	0.14	0.33 a[x]	0.19 a	94.2	332.2 a	238.1 a
6PL	0.16	0.29 abc	0.13 bc	110.1	301.7 b	191.6 c
12PL	0.15	0.24 c	0.09 c	99.8	229.6 f	129.7 f
2WL	0.14	0.28 abc	0.14 abc	99.3	302.8 b	203.5 b
6WL	0.17	0.27 bc	0.09 c	125.6	287.7 c	162.1 d
12WL	0.14	0.31 ab	0.16 ab	98.6	249.4 e	150.9 e
2WF	0.13	0.30 ab	0.17 ab	81.3	264.4 d	183.1 c
6WF	0.15	0.29 abc	0.14 abc	96.5	242.3 e	145.8 e
12WF	0.14	0.28 abc	0.14 abc	94.2	225.3 f	131.1 f
Separating into single factor						
Phosphorus[y]	Day 42	Day 119	ILA	Day 42	Day 119	IPFW
2	0.14 b[x]	0.30	0.17 a	91.6 b	299.8 a	208.2 a
6	0.16 a	0.28	0.12 b	110.7 a	277.2 b	166.5 b
12	0.14 b	0.28	0.13 b	97.5 b	234.8 c	137.2 c
Spectrum[y]	Day 42	Day 119	ILA	Day 42	Day 119	IPFW
PL	0.15 a[x]	0.29	0.14	101.4 a	287.8 a	186.5 a
WL	0.15 a	0.28	0.13	107.8 a	280.0 b	172.2 b
WF	0.14 b	0.29	0.15	90.7 b	244.0 c	153.3 c
Spectrum (S)	*	NS	NS	*	*	*
Phosphorus (P)	*	NS	*	*	*	*
S x P	NS	*	*	NS	*	*

[y] Treatment codes with number and letters, the numbers denote phosphorus concentration (2, 6 or 12 mM), the letters denote kind of spectrum (PL – purple, WL – white LED, WF – white fluoresence).

[x] Means with the different letters in a column are significantly different by Duncan test. NS, * indicate non-significant, significant different at $P<0.05$.

Changes in RGR and its components in the reproductive period

RGR varied from 10.6 to 16.3 mg g^{-1} d^{-1} among the treatments (Fig. 4.2A). RGR decreased with increasing concentrations of phosphorus under all light spectrum conditions. Furthermore, for concentration of 2 or 6 mM phosphorus, plants that were grown under PL had higher RGRs (16.3 and 13.0 mg g^{-1} d^{-1}, respectively) than those grown under WL or WF (Fig. 4.2A). Similarly, NAR varied over a fairly wide range among treatments (from 8.4 to 14.0 g m^{-2} day^{-1}) and depended on the phosphorus concentration, decreasing as the concentration increased from 2 to 12 mM (Fig. 4.2B). By contrast, LAR varied over a narrow range (from 1.1 to 1.33 m^{-2} kg^{-1}) (Fig. 4.2C) and was not as strongly correlated with RGR as NAR. Furthermore, the difference in LAR was controlled more by the light spectrum than the phosphorus concentration, with higher values being obtained under WF (Fig. 4.2C).

Analysis of the GRC values further showed that NAR was the main factor that governed the changes in the trend and magnitude of RGR, while LAR made a negligible contribution (Fig. 4.3). The contribution ratios of NAR ranged from 0.93 to 1.89, while those of LAR ranged from −0.89 to 0.07 (Fig. 4.3), whereby a higher ratio indicates a higher proportional difference with the proportional difference in RGR. An interesting observation was made in the comparison between 6WL and 12WL (the plants grown in 6 or 12 mM phosphorus, respectively, under WL conditions), where NAR became a limiting factor (ratio = −0.64), resulting in treatment 6WL having a significantly lower RGR than treatment 12WL (Fig. 4.3) and LAR making a very positive contribution to the change in RGR (ratio = 1.64). This result revealed that WL combined with a high phosphorus concentration (12 mM) caused a positive dominant shift from NAR to LAR in RGR. In 12WL treatment, the LAR increased led its RGR increase (Fig. 4.2A & C), which reduced the difference in magnitude with RGR of 2WL treatment. Therefore, the denominator of the comparison (2-12) _WL in the GRC formula was always lower than that of the other comparisons. Consequently, the ratio of GRCs (GRC_{NAR}

and GRC_{LAR}) in the (2-12) _WL was always higher than the other (Fig. 4.3) and still ensured the sum of the GRCs was 1.0 (Poorter and Werf, 1998).

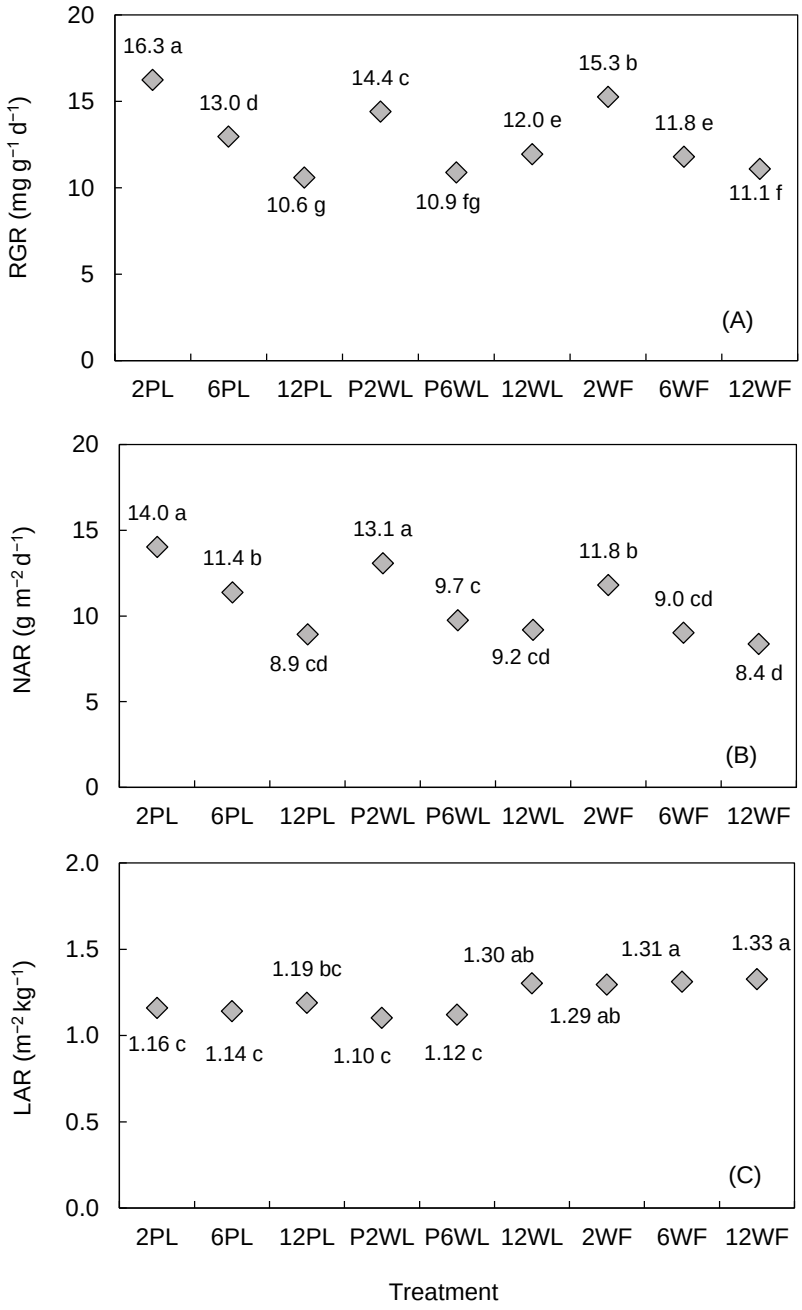

Fig. 4.2. Trend of relative growth rate (RGR), NAR (net assimilation rate) and LAR (leaf area ratio) of strawberry plants impacted by different phosphorus concentrations and light spectra.

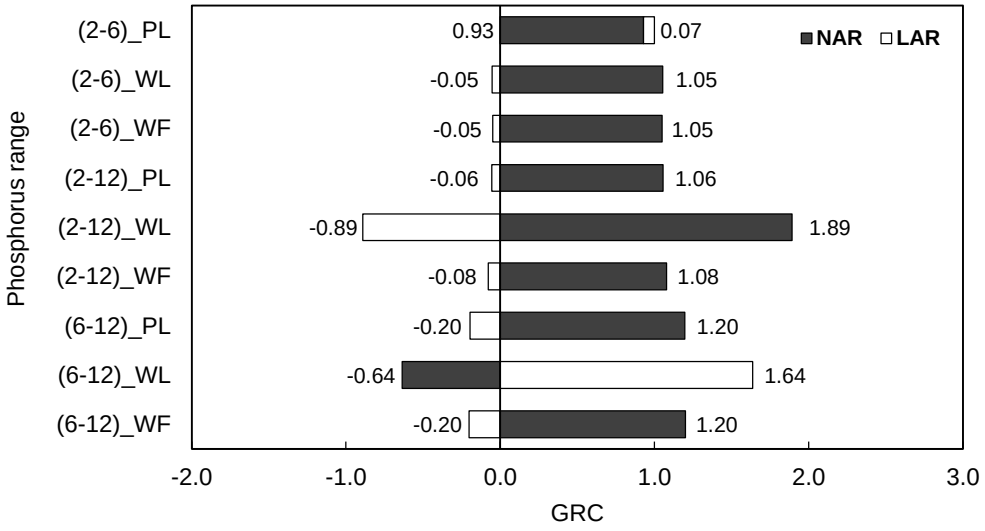

Fig. 4.3. Growth response coefficient (GRC) of NAR and LAR. The ratios were obtained when comparing between two treatments with different phosphorus concentration. For example, the comparison code (2-6)_PL means $GRC_{NAR\ or\ LAR}$ were calculated for treatment 2PL and 6PL.

Vegetative and reproductive growth rates

There was a significant difference in RGR_V but not RGR_{RP} among treatments (Table 4.2). A decrease in phosphorus concentration from 12 to 2 mM significantly increased the value of RGR_V (Table 4.2, single factor), leading to a decline in $\%RGR_{RP}$ and an increase in $\%RGR_V$. Different light spectra caused significant differences in RGR_V without influencing the percentage allocation of RGR to RGR_V and RGR_{RP}, with the highest value of RGR_V being observed under PL (11.3 mg g^{-1} day^{-1}) and the lowest under WL (10.2 mg g^{-1} day^{-1}) (Table 4.2, single factor).

The strawberry plants invested more than 80% of RGR in vegetative growth and less than 20% in reproductive growth during its reproduction period, with an inverse relationship between the percentage of RGR allocated to vegetative and reproductive growth. Thus, plants with the highest $\%RGR_V$ (87.7%, treatment 2PL) had the lowest $\%RGR_{RP}$ (12.3%), and conversely, plants with the highest $\%RGR_{RP}$ (19.6%, treatment 6WL) had the lowest $\%RGR_V$

(80.4%) (Table 4.2, single factor). In this analysis, phosphorus concentration was the main factor contributing to the difference in the percentage allocation of RGR to $\%RGR_V$ and $\%RGR_{RP}$, while the light spectrum did not play a significant role.

Flower and fruit development

After 119 days of cultivation, the number of flowers tended to be highest in plants grown under WL and lowest in those grown under WF (Table 4.3). However, the number of harvested fruit tended to be higher under both WL and WF than under PL across all phosphorus concentrations, with the highest numbers of fruit being recorded for treatments 2WL (11.9 fruit) and 6WL (11.1 fruit) (Table 4.3). There was no clear difference in the fruit weights among treatments, but these tended to be higher in treatments that had a lower number of harvested fruit (Table 4.3). There was no significant difference in the various fruit size parameters, including fruit length and fruit diameter, among the treatments.

Table 4.2. Relative growth rate (RGR), RGR of vegetative growth (RGR_V), RGR of reproductive growth (RGR_{RP}), percentage of RGR_V and RGR_{RP} of strawberry during the reproduction stage

Treatment[y]	RGR (mg g^{-1} d^{-1})	RGR_V (mg g^{-1} d^{-1})	RGR_{RP} (mg g^{-1} d^{-1})	$\%RGR_V$	$\%RGR_{RP}$
2PL	16.2 a[x]	14.2 a	2.0	87.7	12.3
6PL	13.0 d	11.0 d	2.0	84.8	15.2
12PL	10.6 g	8.7 g	1.9	82.1	17.9
2WL	14.4 c	12.2 c	2.1	85.1	14.9
6WL	10.9 fg	8.7 g	2.2	80.4	19.6
12WL	11.9 e	9.7 ef	2.3	81.1	18.9
2WF	15.3 b	13.1 b	2.2	85.6	14.4
6WF	11.8 e	10.0 e	1.8	84.8	15.2
12WF	11.1 f	9.3 fg	1.8	83.5	16.5
Separating into single factor					
Phosphorus	RGR (mg g^{-1} d^{-1})	RGR_V (mg g^{-1} d^{-1})	RGR_{RP} (mg g^{-1} d^{-1})	$\%RGR_V$	$\%RGR_{RP}$
2	15.3 a	13.2 a	2.1	86.1 a	13.9 b
6	11.9 b	9.9 b	2.0	83.3 b	16.7 a
12	11.2 c	9.2 c	2.0	82.2 b	17.8 a
Spectrum	RGR (mg g^{-1} d^{-1})	RGR_V (mg g^{-1} d^{-1})	RGR_{RP} (mg g^{-1} d^{-1})	$\%RGR_V$	$\%RGR_{RP}$
PL	13.3 a	11.3 a	2.0	84.9	15.1
WL	12.4 c	10.2 c	2.2	82.2	17.8
WF	12.7 b	10.8 b	1.9	84.6	15.4
Spectrum (S)	*	*	NS	NS	NS
Phosphorus (P)	*	*	NS	*	*
S x P	*	*	NS	NS	NS

[y] Treatment codes with number and letters, the numbers denote phosphorus concentration (2, 6 or 12 mM), the letters denote kind of spectrum (PL – purple LED, WL – white LED, WF – white fluoresence).

[x] Means with the different letters in a column are significantly different by Duncan test. NS, * indicate non-significant, significant different at $P<0.05$.

Table 4.3. Flower numbers (flower plant^{-1}), harvested Fruit numbers (fruit plant^{-1}) and fruit characteristics of strawberry grown under different spectrum and phosphorus conditions

Treatment[y]	Flower No.[x]	Harvested Fruit No.	One Fruit		
			Weight (g)	Length (mm)	Diameter (mm)
2PL	69.3 ± 3.3	3.7 ± 0.6	10.3 ± 1.0	32.7 ± 3.3	27.6 ± 2.5
6PL	72.9 ± 1.1	4.3 ± 0.8	11.5 ± 2.0	35.7 ± 6.3	28.1 ± 4.6
12PL	52.6 ± 2.8	4.4 ± 0.7	9.1 ± 0.8	34.0 ± 0.9	25.9 ± 0.7
2WL	74.1 ± 2.7	11.9 ± 1.2	9.8 ± 0.5	34.0 ± 0.6	26.5 ± 0.6
6WL	80.8 ± 6.2	11.1 ± 0.7	9.7 ± 0.5	33.3 ± 0.8	27.0 ± 0.8
12WL	72.3 ± 4.1	7.8 ± 0.6	10.1 ± 0.6	33.9 ± 0.9	27.5 ± 0.7
2WF	57.9 ± 2.5	9.0 ± 0.8	9.9 ± 0.6	34.8 ± 0.7	28.5 ± 1.4
6WF	61.4 ± 2.7	8.0 ± 0.8	10.0 ± 0.5	34.6 ± 0.9	27.5 ± 0.8
12WF	56.8 ± 3.5	8.0 ± 0.8	9.1 ± 0.4	34.4 ± 0.7	26.4 ± 0.4

[y] Treatment codes with number and letters, the numbers denote phosphorus concentration (2, 6 or 12 mM), the letters denote kind of spectrum (PL – purple LED, WL – white LED, WF – white fluoresence).

[x] Means ± SE

SPS activity in the leaves

SPS activity tended to be largely controlled by the light spectrum, with greater activity occurring under WL and WF than under PL (Fig. 4.4). SPS activity was also positively affected by the phosphorus concentration (6 or 12 mM) when combined with WL.

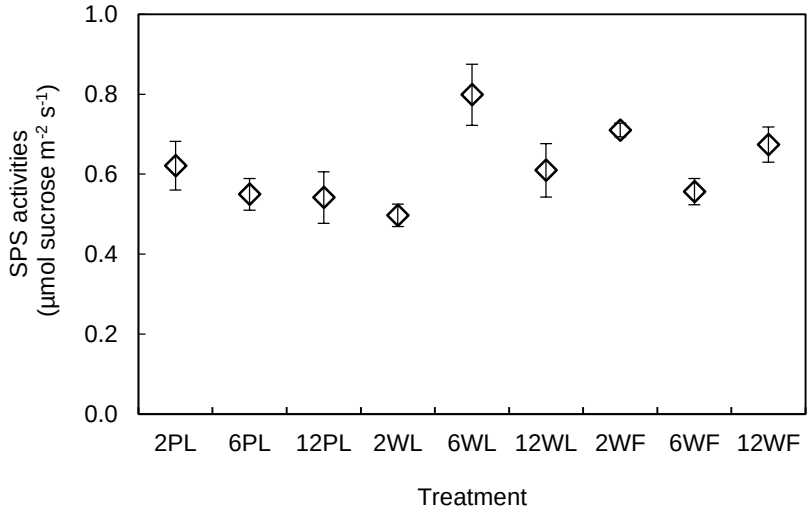

Fig. 4.4. SPS activity in strawberry leaves under different light spectra and phosphorus concentrations. Samples were taken within 12:00-13:00, n=4.

Fruit sugar content

The total sugar content of the fruit increased when the phosphorus concentration increased from 2 to 6 mM but decreased when it rose to 12 mM under the same light conditions (Table 4.4). Glucose and fructose contents showed the same trend, whereas the sucrose content tended to decrease as the phosphorus concentration increased from 2 to 12 mM. Comparison of the different light spectra with phosphorus concentrations of 6 or 12 mM showed that the total sugar, glucose, and fructose contents of the fruit tended to be highest under WL, while the sucrose content was higher under WF (Table 4.4). By contrast, with 2 mM phosphorus, the

total sugar, glucose, and fructose contents were highest under PL, whereas the sucrose content was highest under WL.

Table 4.4. The change of sugar content and its components (g per 100 g fruit) in strawberry fruit affected by light spectra and phosphorus concentrations.

Treatment[y]	Sucrose[x]	Glucose	Fructose	Total Sugar
2PL	1.08 ± 0.14	2.66 ± 0.04	2.90 ± 0.12	6.66 ± 0.26
6PL	0.78 ± 0.08	3.06 ± 0.25	3.26 ± 0.22	7.11 ± 0.5
12PL	0.21 ± 0.01	2.74 ± 0.08	3.04 ± 0.09	5.99 ± 0.17
2WL	1.25 ± 0.07	2.37 ± 0.18	2.50 ± 0.15	6.12 ± 0.29
6WL	0.59 ± 0.02	3.59 ± 0.41	3.86 ± 0.49	8.03 ± 0.75
12WL	0.34 ± 0.02	2.84 ± 0.16	3.08 ± 0.16	6.27 ± 0.29
2WF	0.93 ± 0.02	2.51 ± 0.06	2.63 ± 0.09	6.07 ± 0.14
6WF	0.84 ± 0.07	2.99 ± 0.16	3.26 ± 0.14	7.09 ± 0.32
12WF	0.41 ± 0.05	2.56 ± 0.17	2.81 ± 0.21	5.79 ± 0.38

[y] Treatment codes with number and letters, the numbers denote phosphorus concentration (2, 6 or 12 mM), the letters denote kind of spectrum (PL – purple LED, WL – white LED, WF – white fluoresence).

[x] Means ± SE

Discussion

Early growth stage

Although the total amount of N in the solutions remained constant, the NH_4^+ concentrations in P2, P6, and P12 solutions (0, 0.5, and 3 mM, respectively) could not be adjusted equally. However, according to Cárdenas-Navarro et al. (2006), the NH_4+ concentration in a solution up to 4 mM did not affect strawberry growth. Hence, the effects on plant growths (Table 4.1, Day 42) caused by differential NH_4^+ concentrations could be eliminated. On the other hand, strawberry growth was optimal in a solution EC of 0.25 S m^{-1}, exceeding this level caused growth inhibition (D'Anna et al., 2003). Similar results were found

when strawberry growths increased from the P2 (EC = 0.21 S m^{-1}) and reached the highest in the P6 (EC = 0.25 S m^{-1}) then decreased in the P12 solution (EC = 0.31 S m^{-1}). Therefore, phosphorus concentration in the solution may affect the EC, which induces a difference in plant growth. Currently, 1-2 mM phosphorus is widely used to grow the strawberry in greenhouses (D'Anna et al., 2003; Tafarnia et al., 2010); in the plant factory, however, 6 mM appeared to be optimal for the plant growth while 2 mM and 12 mM did not. This finding could be the new information on strawberry production in a plant factory.

While many studies have demonstrated the positive effects of monochromatic light on plant growth, use of the white light spectrum has many advantages, such as increasing plant qualities, increasing biomass, and maintaining the stability of the quantum yield of photosystem II (PSII) (Kim et al., 2004; Lin et al., 2013; Arena et al., 2016). In the present study, it was found that WL in combination with 6 mM phosphorus provided the best conditions for vegetative growth in the early growth stage of strawberry, although growth did not significantly differ between the PL and WL conditions (Table 4.1, day 42). By contrast, WF was less effective for plant growth than WL (Table 4.1), possibly due to the difference in the specifications of the light spectrum components between WL and WF (Fig. 4.1) (Hidaka et al., 2013; Arena et al., 2016).

Plant growth in reproductive period

During the reproductive period, the phosphorus concentration affected both the ILA and IPFW of the entire plant. In the early growth stage, the leaf area and plant fresh weight were significantly lower with 2 mM phosphorus than with 6 mM phosphorus (Table 4.1, data day 42, single factor). However, there were negligible differences between these concentrations at day 119 and the direction of this effect then reversed, so that both calculated parameters ILA and IPFW of the plants showed significantly higher under 2 mM phosphorus (Table 4.1, single factor). The observed changes in RGR followed the changes in leaf area and plant fresh weight

(Table 4.1, Fig. 4.2), indicating that analysis of the characteristics of the RGR components (NAR and LAR) could contribute to our understanding of the causes of plant growth responses in the preproduction stage.

Changes in RGR and its components in the reproductive period

A low phosphorus concentration (2 mM) stimulated a significant increase in RGR regardless of the light spectrum (Fig. 4.2B). The changes in RGR were primarily governed by variation in NAR (Fig. 4.1, 4.2), supporting the findings of Poorter and Werf (1998), who showed that NAR plays a key role in the observed changes in RGR within a species. The high demand of strawberry plants for essential macronutrients, such as nitrogen, phosphorus, and potassium, is undeniable (Wu et al., 2020), so an increase in NAR could reflect a specific response to a lack of these essential mineral elements. Deng and Woodward (1998) previously reported that the NAR values of strawberry plants increased from 50% to more than 90% when grown under severe nitrogen deficiency compared with full nitrogen conditions, while Chi et al. (1991) reported elevated NAR values under low nutrient conditions in tomato (*Solanum lycopersicum*) plants. In the present study, nitrogen was maintained at a high level across all treatments (12 mM; Appendix 1). However, phosphorus is known to be an essential and irreplaceable factor for reproduction in strawberry (Li et al., 2010), so a phosphorus concentration below the required level could stimulate an increase in NAR in the same way as nitrogen-limited conditions. This interpretation is particularly appropriate when considering the NAR of plants treated with 6 mM phosphorus, which showed optimal growth in the early stage but had lower NAR values than plants exposed to 2 mM phosphorus when transitioning to the reproductive stage (Table 4.1, Fig. 4.2). These results suggest that NAR could be a useful indicator for evaluating the response of strawberry to different phosphorus concentrations in the reproductive growth stage.

Relationship between RGR_V and RGR_{RP}

In this study, different light spectra and phosphorus concentrations did not make a difference in the reproductive growth rate values. The low phosphorus (2 mM) and PL conditions, however, encouraged the significant increase of RGR_V, meanwhile only the high phosphorus concentrations (6 or 12 mM) drove the increase of $\%RGR_{RP}$ in the RGR of the whole plants (Table 4.2).

The dramatic increase in RGR_V under low phosphorus (2 mM) was evident from the observed increase in leaf area and plant fresh weight from day 42 to day 119 (Table 4.1). Previous studies have also demonstrated that when the phosphorus concentration is insufficient to meet a plant's demands, reproduction is delayed (Fujita et al., 2014) to enhance the uptake and utilization of phosphorus for vegetative growth of the whole plant (Nord et al., 2011). Therefore, RGR_V and its percentage in RGR was significantly increased under phosphorus concentration of 2 mM. By contrast, RGR_{RP} was favored under higher levels of phosphorus (6 or 12 mM), so that its percentage in RGR was significantly increased. A high $\%RGR_{RP}$ increased the numbers of flowers and harvested fruit (treatment 6WL, Table 4.3), while a high $\%RGR_V$ drove a reduction in these reproductive characteristics (treatment 2PL). Estrada-Ortiz et al. (2013) also showed that the reproductive traits of strawberry were significantly improved when the supply of phosphorus to the plants was increased.

Changing the light spectrum only affected RGR_V, especially with PL and WF, whose RGR_V increment stimulated by low growth status in the early growth stage (Tables 1, 2, single factor). However, strawberry plants grown under WL tended to have a higher $\%RGR_{RP}$, although there was no statistical difference between the WL and PL conditions for this parameter (Table 4.3, single factor). The numbers of flowers and harvested fruit also tended to be higher in plants grown under WL rather than PL, even at a phosphorus concentration of 2 mM (Table 4.3).

Sugar content of the fruit

I supposed that source–sink interactions would drive sugar accumulation in strawberry fruit. In the present study, the response of the source and sink to changes in the phosphorus concentration and light spectrum were represented by $\%RGR_V$ and $\%RGR_{RP}$, respectively. The optimal phosphorus concentration (6 mM) stimulated plants to increase flower and fruit production, which means increasing the sink size or $\%RGR_{RP}$. Thus, a reduction in $\%RGR_V$ and an increase in $\%RGR_{RP}$ (Table 4.2) indicated that plant growth prioritized responding to sink demand. An increase in sink size promotes growth of the whole plant (Reekie et al., 1998) and also regulates metabolic processes in plants (Smith et al., 2018). Thus, sugar accumulation in the fruit could be one of the metabolic processes that is governed by $\%RGR_{RP}$. It has been shown that there were connections between the increasing the amount of phosphorus available to strawberry plants and the sugar content in the fruit (Estrada-Ortiz et al., 2012; Wang et al., 2017; Zhang et al., 2017) and that the enhancement of SPS activity also promotes sugar accumulation in plants (Lingle and Dunlap, 1987; Anur et al., 2020). Therefore, the increase in SPS activity and high $\%RGR_{RP}$ that was observed in plants grown under 6 mM phosphorus and WL could explain the high fruit sugar content in the 6WL treatment (Tables 2, 4, Fig. 4.4).

In conclusion, a phosphorus concentration of 6 mM combined with WL (i.e., treatment 6WL) resulted in optimal growth of the strawberry plants in the early growth stage, which affected the magnitude of the growth rate in the subsequent reproductive stage. During reproductive growth, the phosphorus concentration was the main factor that controlled RGR by increasing or decreasing NAR. In addition, high phosphorus concentrations (6 or 12 mM) promoted the allocation of RGR to RGR_{RP} (i.e., $\%RGR_{RP}$), the magnitude of which could be determined through the numbers of flowers and harvested fruit, which are considered indicators of the sink size, while low phosphorus concentrations (2 mM) enhanced RGR_V and its percentage ($\%RGR_V$). The light spectrum primarily played a role in maintaining vegetative

growth and was less important for reproductive growth. Finally, an increased SPS activity in combination with a high $\%RGR_{RP}$ directed an increase in sugar accumulation in strawberry fruit.

Chapter 5.

General Discussion

This study demonstrated that optimized strawberry cultivation environments in the plant factory oriented for production in the tropics or subtropics is feasible. Moreover, the results from this study also contributed to the strawberry research field some useful pieces of information, such as the information of photosynthetic response patterns, growth rate distribution, and fruit sugar accumulation of strawberry under controlled conditions. Additionally, the study also identified the disadvantages of the current photosynthesis measurement methods for strawberry plants.

Under controlled environments, the diurnal photosynthesis pattern was either stable (under low light intensity with low CO_2 concentration) or decreased gradually during the photoperiod (under high light intensity with elevated CO_2) (Chapter 2). In a plant factory using artificial light, whose intensity is constantly maintained from the light turned on until the light turned off. Therefore, the photosynthesis rate of plants was mainly influenced by the established light intensity and CO_2 concentration. From this result, a question of how does the variation of light intensity affect the photosynthesis capacity of the strawberry plant emerged? The Answer of this question will contribute to providing a suitable solution for the application of sunlight to the plant factory. The impact of light intensity variation on photosynthesis capacity was clearly reported in Chapter 3.

Effects of light intensity variation were considered according to the long-day and short-day conditions. During the long-day conditions, the light intensity fluctuation took place under a high average light intensity in a day. In contrast, during short-day conditions, the fluctuation also occurred but under the low level of average light intensity in a day. Therefore, the photosynthesis capacity was significantly reduced under short-day conditions when the light intensity fluctuated (Chapter 3). Supplemental lighting to maintain light intensity stability

resulting in effectively maintained photosynthesis capacity at a high level. Furthermore, under low and stable light intensity, the strawberry plants still ensured their normal flowering and fruit development. The results of this study could provide two solutions for using a plant factory in energy-saving manners. Firstly, using full sunlight and only combining with artificial light when the light intensity fluctuating in the low range. Secondly, using only artificial light with low and stable light intensity. Based on the second solution, a follow-up study was conducted under low light intensity conditions to show plant growth and fruit sugar accumulation of strawberry under varying spectra and phosphorus concentration in a closed-type plant factory (Chapter 4).

Relative growth rate of vegetative and reproductive growths of strawberry during the reproduction period and its correlation with fruit sugar accumulation were reported (Chapter 4). During the reproduction period, strawberry plants spent about 80% of the whole-plant RGR to vegetative growth and about 20% to reproductive growth. Phosphorus concentration was the main factor responsible for the distribution of reproductive growth rates to promote flowering and fruit development. Additionally, the appropriate phosphorus level promoted significant plant growth in the early stages. The light spectrum maintained the vegetative growth of plants during the reproductive stage. Besides, the white LED light spectrum combined with 6 mM phosphorus concentration enhanced SPS activity in the leaves and lead to an increase of sugar accumulation in the fruit. These findings confirm controlling phosphorus concentration and light spectrum could manage the plant growth tendency and fruit sugar accumulation even under low light intensity conditions.

In conclusion, the optimization of environmental factors, including light quality and phosphorus concentration, based on photosynthetic and growth responses, could drive plant growth and fruit sugar accumulation of strawberries grown in a plant factory. Strawberry production using plant factory in the tropics or subtropics is completely feasible. Not only

strawberries but also other temperate plants, this study is vivid evidence to grow plants under the optimum controlled environments without concerning the geographic factors.

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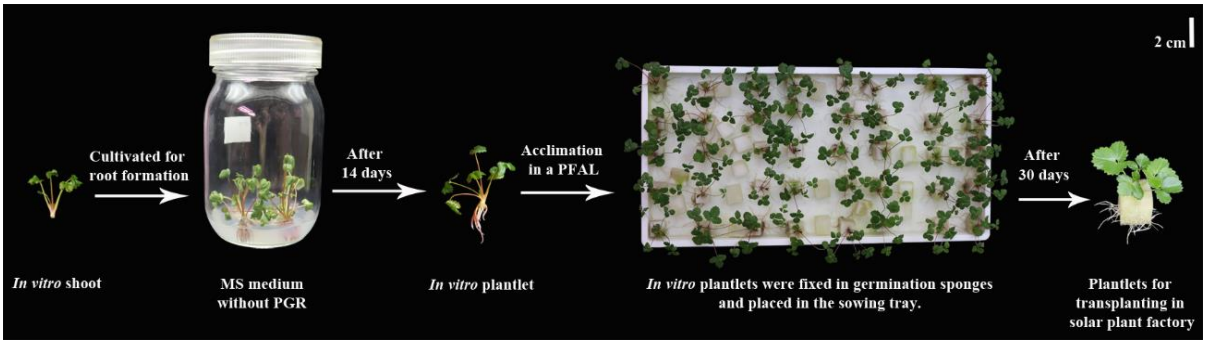
Appendix 1.



Strawberry plantlets preparation protocol.

Conditions of cultivation for root formation: MS medium without PGR, PPFD of 65 µmol m^{-2} s^{-1}, photoperiod of 12h, temperature of 23°C.

The acclimation conditions: PPFD of 150 µmol m^{-2} s^{-1}, photoperiod of 12h, CO_2 concentration of 1200 µmol mol^{-1}, relative humidity of 50% ± 10%, temperature of 23°C, and nutrient solution of Hoagland and Arnon's (1950) (modified) with electrical conductivity of 0.12 S m^{-1}.

MS medium: Murashige and Skoog's medium used for plant tissue culture, PGR: plant growth regulator, sowing tray size: 30 cm wide, 60 cm long, 4 cm deep (in the figure, the 2 cm bar could not use for estimating the real dimension of the sowing tray).

Appendix 2.

Nutrient solution formulas of different phosphorus concentration (2, 6 and 12 mM) used in this study.

Chemicals	Utilized amount (mM)				
	Ca	N	P	K	Mg
Phosphorus 2 mM					
$Ca(NO_3)_2.4H_2O$	3	6	-	-	-
KNO_3	-	3	-	3	-
$MgSO_4.7H_2O$	-	-	-	-	2
$NaNO_3$	-	3	-	-	-
KH_2PO_4	-	-	2	2	-
K_2SO_4	-	-	-	5	-
$CaCl_2$	1	-	-	-	-
Total	*4*	*12*	***2***	*10*	*2*
Phosphorus 6 mM					
$Ca(NO_3)_2.4H_2O$	3	6	-	-	-
KNO_3	-	1	-	1	-
$MgSO_4.7H_2O$	-	-	-	-	2
NH_4NO_3	-	1	-	-	-
NaH_2PO_4	-	-	2	-	-
KH_2PO_4	-	-	4	4	-
$NaNO_3$	-	4	-	-	-
K_2SO_4	-	-	-	5	-
$CaCl_2$	1	-	-	-	-
Total	*4*	*12*	***6***	*10*	*2*
Phosphorus 12 mM					
$Ca(NO_3)_2.4H_2O$	3	6	-	-	-
KH_2PO_4	-	-	5	5	-
$MgSO_4.7H_2O$	-	-	-	-	2
NH_4NO_3	-	6	-	-	-
$NaH_2PO_4.2H_2O$	-	-	7	-	-
K_2SO_4	-	-	-	5	-
$CaCl_2$	1	-	-	-	-
Total	*4*	*12*	***12***	*10*	*2*

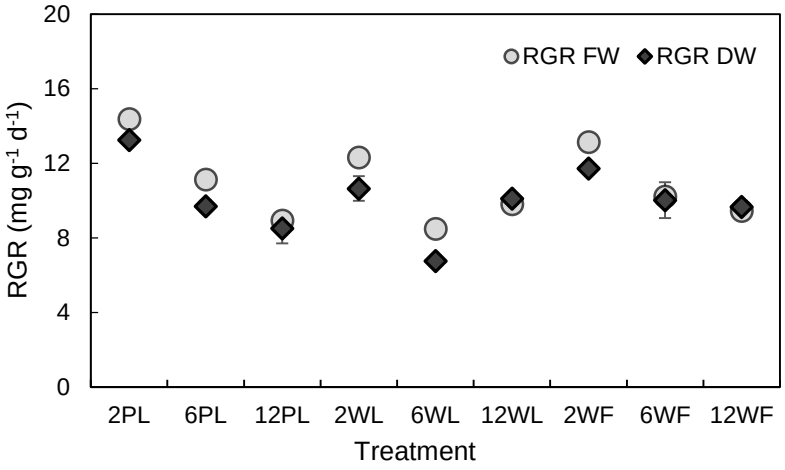
These amount used for 1 litre

$CaCl_2$ only added after day 42 to remove the tip burn effects.

* Micronutrient followed Hoagland and Arnon (1950)

FeEDTA concentration of 0.1 mM

Appendix 3.



Comparison between RGR calculated by dry weight (RGR DW) and RGR calculated by fresh weight (RGR FW)
Data were plant dry weight and fresh weight in the reproduction stage (not including flower and fruit data)
* The amount of water in the plant hardly created any difference in the trend and magnitude of the RGR was calculated in each treatment.