國立臺灣大學生物資源暨農學院農藝學系

博士論文

Department of Agronomy
College of Bioresources and Agriculture
National Taiwan University

Doctoral Dissertation

光質對水稻幼苗形態與光合生理之影響

The Effect of Light Quality on Morphology and Photosynthetic Physiology in Rice Seedling

陳昶璋

Chang-Chang Chen

指導教授：黃文達 博士

楊棋明 博士

Advisor: Dr. Wen-Dar Huang

Dr. Chi-Ming Yang

中華民國 103 年 6 月

June 2014

國立臺灣大學博士學位論文
口試委員會審定書

光質對水稻幼苗形態與光合生理之影響
The Effect of Light Quality on Morphology and Photosynthetic Physiology in Rice Seedling

本論文係陳昶璋君（D99621103）在國立臺灣大學農藝學系完成之博士學位論文，於民國一百零三年六月三十日承下列考試委員審查通過及口試及格，特此證明

口試委員：

國立台灣大學農藝系助理教授
黃　文　達　博士（本論文指導教授）　黃文達

中央研究院生物多樣性研究中心副研究員
楊　棋　明　博士（本論文指導教授）　楊棋明

農業委員會特有生物研究保育中心助理研究員
翁　韶　良　博士　翁韶良

台灣中油股份有限公司煉製研究所研究員
許　明　晃　博士　許明晃

農業委員會桃園區農業改良場助理研究員
楊　志　維　博士　楊志維

誌　謝

本論文完成，首要感謝兩位指導教授　黃文達老師以及　楊棋明多年來的指導、愛護與包容。論文修改過程，感謝翁韶良博士、許明晃博士與楊志維博士提供寶貴建議。求學期間感謝　葉學文老師多年來耐心指導、訓練與提攜。並感謝國立台灣大學農藝學系與中央研究院生物多樣性研究中心提供良好研究環境與資源，使研究得以順利進行。

在台大農藝完成博士學位，乃因緣際會。彭雲明老師與盧虎生老師的推薦函成為博士班的契子。回顧博士班生涯，與盧老師雖然師生緣分不長，但在那三個學期，受到朱鈞老師、盧虎生老師與張孟基老師在邏輯思維上的訓練，奠定良好研究基礎。在提交論文此刻，對於當時老師的諄諄教誨仍深深烙印在心裏，在未來亦時時自我警惕。感謝作物生理實驗室的夥伴在實驗上提供協助，特別是李佳諭博士及楊蓁瑋博士。

2005 年還是大學生的我開始踏入 308 實驗室，轉眼就是 2014 年，今日能完成學業，猶如站在巨人的肩膀上，看得更廣，卻也更顯渺小。張新軒教授、蔡養正教授、蔡文福教授與曾美倉教授平時的指導與薰陶，讓我明白學海無涯。感謝黃秀鳳博士，從試驗的方法學乃至生活細節，都照顧得無微不至。感謝黃盟元博士多年的關懷及照顧。

感謝實驗室歷年助理、研究生與大學部的研究夥伴：彭懷慈、許毓鈞、林柏齡、彭元慶、陳傑君、郭宇翔、張瀞文、李雅蓁、羅啟元、陳怡夙、林奎庭、藍秋月、陳相全、周庭聿、陳煥文、徐芷陶、楊敬屏，你們的協助、討論、陪伴與關懷都是論文完成的推手。台大農藝全職博士生王群山、林亞平、楊琇淳、華德揚同學，感謝你們，文字敘述在這裡是累贅卻又不足。

I have to thank Dr. Karyne Rogers, Andy Phillips and all SIL people. I am grateful for your patient during the training in National Isotope Centre, GNS science. And thank Simon Stewart for sample running and calibration of rough isotopic data. Meanwhile, I am thankful for all of your kinds and let me feel welcome and at home in New Zealand.

最後，感謝我的祖母與父母親，你們的愛就如同無盡的海洋，包容了我的一切。謝謝我生命中的所有人，成就今天的我。感謝上天。

陳昶璋　謹誌

July, 2014

摘要

本研究主要評估光質對於水稻幼苗之形態與光合生理之影響。先行探討幼苗生長、發育與碳、氮代謝方面對於不同光質之反應。水耕幼苗栽培於紅(R)、綠(G)、藍(B)與紅藍(RB)發光二極體(LED)照射之生長箱。紅光誘導地上部伸長。藍光抑制伸長並促進壯苗指數。相較於紅藍混和，葉片總蛋白在藍光照射下含量較高。另外光合生理方面，藍光可提高幼苗葉片之光系統 II 有效光量子產量(Φ_{PSII})與光化學消散(q_P)，同時降低非光化學消散(NPQ)。水稻幼苗對紅光與綠光的反應相當類似。幼苗葉片花青素含量以 RB 最高，R、B 卻低於 G。葉片中葉綠素 *a/b* 比例則受不同光質波段所調控。

另外結果顯示，不同光質對於光合作用與氮素代謝具不同效果，而此類生理反應與水分利用效率 (WUE)、氮素吸收具相關性。進一步探討水稻在不同光質處理下，WUE、穩定性碳同位素分辨率($\Delta^{13}C$)與氮素吸收反應，另以螢光燈(FL)為對照。發現 R 之幼苗 WUE 最高，而後依序為 G、RB、B。除了 FL 處理之外，WUE 與 $\Delta^{13}C$ 具顯著正相關($P<0.01$)。另利用氮含量與氮同位素值($\delta^{15}N$)評估不同光質對於氮肥吸收之結果顯示，幼苗中化學肥貢獻之氮素(N_f)以 B 最高而 R 最低。因此，推測藍光可促進氣孔導度與蒸散作用，造成 WUE 降低而促進根部氮素吸收。

在此水稻幼苗葉片之葉綠素(Chl)與其生合成中間產物(protoporphyrin IX, PPIX; magnesium protoporphyrin IX, MGPP; protochlorophyllide, Pchlide)、降解代謝產物 (chlorophyllide, Chlide; pheophytin, Phe; pheophorbide, Pho)以及胡蘿蔔素(Car)。葉片中 Chl 與 Car 在綠光下較低。光質並未影響生合成途徑中卟啉(porphyrins) 莫爾百分比。Phe/Chlide 在 G 與 FL 照明下數值較低，顯示綠光較高之環境會促進葉片中 Chlilde 分解途徑。

為了釐清綠光在 Chl 分解途徑之效應，將幼苗在固定紅、藍光強度 40 μmol m^{-2} s^{-1})下生長，分別以 4 個綠光等級 (0, 20, 40 and 60 μmol m^{-2} s^{-1}) 處理。同時調查部分形態與光合生理。結果顯示，在固定紅、藍光下隨著綠光強度增加，水稻幼苗

具較長之葉鞘且葉片角度較為直立，具避蔭效應(shade avoidance symptoms, SAS)。且增強綠光亦會造成葉綠素、Φ_{PSII} 降低與 NPQ 提高。另外增強綠光下，也發現較高之 Chlide 與較低之 Phe/Chlide 比例。以上結果顯示綠光可誘導水稻幼苗之 SAS 產生且調節 Chl 分解途徑。

關鍵字：水稻幼苗、光質、光形態發生、降解代謝、穩定性同位素、水分利用效率、氮素吸收、葉綠素分解途徑、避蔭效應

Abstract

Our objective in this study was to evaluate the effect of light quality on the morphology and photosynthetic physiology of rice seedlings. We examined the growth, development, and metabolic responses of rice seedlings to varying light quality firstly. Seedlings were hydroponically cultured under red (R) light-emitting diodes (LED), green LED (G), blue LEDs (B), and red + blue LED (RB) inside growth chambers. Red light induced shoot elongation. B light inhibited shoot elongation and promoted health index values. B light also resulted in higher total protein content in tested leaves compared to RB. Blue light enhanced the effective quantum yield of PSII photochemistry (Φ_{PSII}) and photochemical quenching (q_P) while reducing non-photochemical quenching (NPQ) in seedling leaves. The responses of rice seedlings to green and red light were quite similar. The anthocyanin content of seedling leaves was observed to be highest in RB but less so in R and B, the latter two being even lower than in G. Different wavelengths mediated the chlorophyll (Chl) *a/b* ratio of the leaves.

Light quality influenced photosynthetic potential and nitrogen metabolism, which are related to water-use efficiency (WUE) and nitrogen uptake. We further investigated the response of time-integrated WUE, ^{13}C discrimination ($\Delta^{13}C$), and nitrogen uptake in hydroponic seedlings of rice grown under different light treatments with fluorescent light (FL) as the control. The WUE response was highest for seedlings

grown under R light, then (in decreasing order) seedlings grown under G, RB, and B light. WUE had a significantly positive correlation with $\Delta^{13}C$ except under FL light (P<0.01). Nitrogen content (%N) and $\delta^{15}N$ values were used to estimate the effects of fertilizer uptake under different lighting conditions. The amount of N in seedlings derived from fertilizer (N_f) was highest under B light and lowest under R light. Therefore, we conclude that blue light may increase stomatal conductance and transpiration, decrease WUE, and promote root N uptake.

The dynamics of Chl, biosynthetic intermediates (protoporphyrin IX, PPIX; magnesium protoporphyrin IX, MGPP; protochlorophyllide, Pchlide), degradation intermediates (chlorophyllide, Chlide; pheophytin, Phe; pheophorbide, Pho), and carotenoids (Car) in leaves of rice seedlings were also investigated. Lower levels of Chl and Car in leaves were observed under G lighting. Light quality did not mediate the mole percent of porphyrins in biosynthetic pathways. Lower Phe/Chlide ratios were observed under G and FL lighting conditions, indicating that green-enriched environments may up-regulate the Chlide degradation route in leaves.

In order to clarify the effect of green light on the Chl degradation pathway, seedlings were grown under equal intensity (40 μmol m^{-2} s^{-1}) of red and blue light with four levels of green light intensity (0, 20, 40, and 60 μmol m^{-2} s^{-1}). Some morphological traits and photosynthetic physiology were also investigated at the same time. Sheaths of

rice seedling leaves became elongated and leaves grew more erectly under red and blue light with increasing green light intensity. These morphological traits are known as shade avoidance symptoms (SAS). Lower Chl, decreasing Φ_{PSII}, and increasing NPQ were also observed under increasing green light intensity. Higher Chlide levels and lower Phe/Chlide ratios were observed under increases in green light intensity. These results indicated that green light induced SAS and mediated Chl degradation routes in rice seedlings.

Key words: Rice seedling, Light quality, Photomorphogensis, Metabolism, Stable isotope, Water-use efficiency, Nitrogen uptake, Chlorophyll degradation pathway, Shade avoidance symptoms

Organization

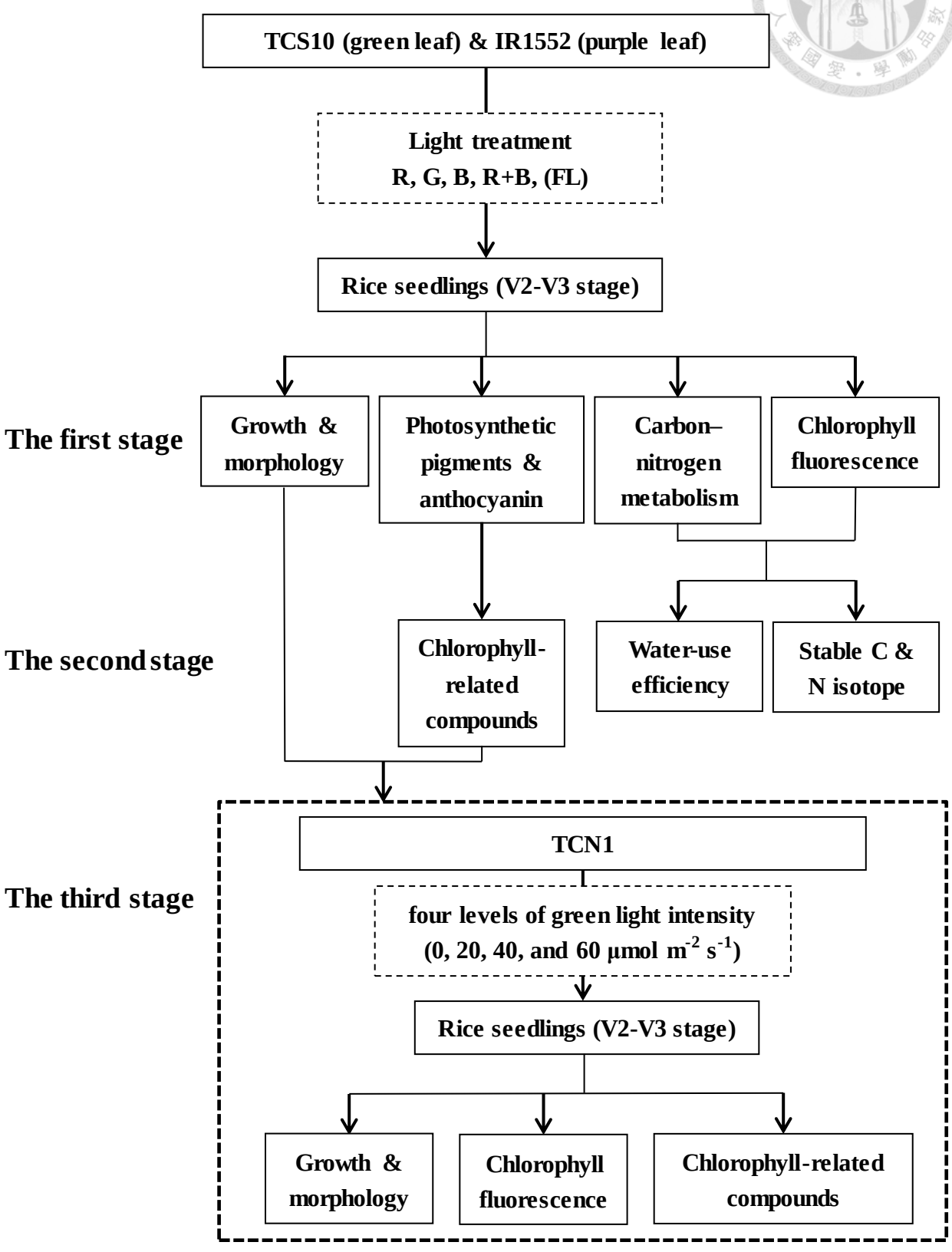


Table of Contents

List of Figures

List of Tables

I. Effects of Light Quality on the Growth, Development and Metabolism of Rice Seedlings (*Oryza sativa* L.)

Abstract

The V3 seedlings of two rice cultivars, IR1552 (purple leaf) and Taichung sen 10 (TCS10, green leaf) were hydroponically cultured under 12 h photoperiod at 30/25℃ (day/night), 70% relative humidity and 160 μmol m^{-2} s^{-1} photon flux density under red light-emitting diodes (LEDs) (R), green LEDs (G), blue LEDs (B) and red + blue LEDs (RB) inside growth chambers for 14 days (starting 2 days after sowing). The results showed that shoot elongation was induced under the exposure of R and G. The maximum health index [(stem diameter/plant height) × biomass)] occurred under B because blue light inhibited shoot elongation. The root length under RB was the shortest. Different wavelengths mediated the chlorophyll (Chl) *a/b* ratio of the leaves.

The content of anthocyanin (Ant) in seedling leaves was observed to be highest in RB but less in R and B, the latter pair being even lower than in G. B light LEDs enhanced effective quantum yield of PSII photochemistry (Φ_{PSII}) and photochemical quenching (q_P), but reduced non-photochemical quenching (NPQ) of seedling leaves. B LEDs also showed higher total protein content in the tested leaves compared to B plus R. In summary, precise management of irradiance and wavelength may hold promise in maximizing the economic efficiency of plant growth, development and metabolic

potential of rice seedlings grown in controlled environments.

Keywords: Light-emitting diode, Light quality, Rice, Photomorphogensis, Metabolism

Introduction

Light is the main energy source for plant photosynthesis and is an environmental signal used to trigger growth and structural differentiation in plants. Light quality, quantity and photoperiod control the morphogenesis, growth and differentiation of plant cells, tissue and organ cultures (Abidi et al., 2013). Plant development is strongly influenced by light quality which refers to the colors or wavelengths reaching a plant's surface (Johkan et al., 2010). Red (R) and blue (B) lights have the greatest impact on plant growth because they are the major energy sources for photosynthetic CO_2 assimilation in plants. It is well known that spectra have action maxima in the B and R ranges (Kasajima et al., 2008). The integration, quality, duration and intensity of red light/far red light, blue light, mixed red and blue lights (RB), UV-A (320–500 nm) or UV-B (280–320 nm) and hormone signaling pathways have a profound influence on plants by triggering or halting physiological reactions and controlling the growth and development of plants (Clouse, 2001; Shin et al., 2008). Recent studies reported that green (G) light also affects the morphology, metabolism and photosynthesis of plants (Johkan et al., 2012; Zhang et al., 2011).

Light sources such as fluorescent, metal-halide, high-pressure sodium and incandescent lamps are generally used for plant cultivation. These sources are applied to increase photosynthetic photon flux levels but contain unnecessary wavelengths that are

located outside the photosynthetically active radiation spectrum and are of low quality for promoting growth (Kim et al., 2004b). Compared to those conventional light sources, gallium-aluminum-arsenide light-emitting diode (LED) lighting systems have several unique advantages, including the ability to control spectral composition, small size, durability, long operating lifetime, wavelength specificity, relatively cool emitting surfaces and photon output that is linear with electrical input current. These solid-state light sources are therefore ideal for use in plant lighting designs and allow wavelengths to be matched to plant photoreceptors for providing more optimal production and influencing plant morphology and metabolism (Bourget, 2008; Massa et al., 2008; Morrow, 2008).

The LED light spectra in many reported experiments were inconsistent with light intensity being non-uniform because the investigators were unable to precisely modulate and quantify spectral energy parameters (Liu et al., 2011). Furthermore, experimental results may have been influenced in part by differences in light intensity and this often presents a problem when comparing results from experiments conducted under inconsistent lighting parameters. While it is widely understood that light intensity can positively affect photochemical accumulation (Fu et al., 2012; Li and Kubota, 2009), the effects of light quality are more complex and mixed results have often been reported.

Spectral light changes evoke different morphogenetic and photosynthetic responses that can vary among different plant species. Such photo responses are of practical importance in recent plant cultivation technologies since the feasibility of tailoring illumination spectra enables one to control plant growth, development and nutritional quality. The effects of LED light sources on several plants such as maize (Felker et al., 1995), cotton (Li et al., 2010) and peas (Wu et al., 2007) have been reported and indicate that LED lights are more suitable for plant growth than fluorescent lights.

Rice (*Oryza sativa* L.) is a staple food in Asia. During the vegetative growth stage, rice plants grow better under RB lights than under R alone (Matsuda et al., 2004; Ohashi-Kaneko et al., 2006b). The quality of V3 seedlings during growth is therefore an important factor in rice production, especially when mechanically transplanting seedlings to the field. The seedlings incubated under RB LEDs were more robust than when incubated under other LED spectra in terms of root number, stem diameter, health index and soluble sugars (Guo et al., 2011b). Therefore, in order to apply the findings to rice seedling quality and production, we considered it important to investigate the effects of light quality when provided by R, B, G and RB LED systems to meet different purposes. Hence, in this study, the growth, development and quality of rice hydroponically grown under various LEDs at the same light intensity were investigated

to determine the efficacy of this promising radiation source.

In order to clarify the different response of green and purple leaf rice, rice seedlings of two indica rice varieties, IR1552 (purple leaf) and Taichung sen 10 (TCS10, green leaf), were cultivated under different light environments at the same light density. Fourteen day old seedlings were collected to investigate the effects of light quality on growth and metabolism of rice seedlings. Controlled climates and LEDs may be practical issue for rice seedling stages before transplanting to field conditions. An optimal strategy of light quality regulation will help in designing growth chambers or greenhouse light environments to obtain maximum economic benefit for rice growers.

Material and Methods

Plant materials and growth conditions

Seeds of *indica* rice (*Oryza sativa* L.) cultivar, IR1552, were donated by Dr. Su-Jein Chang, Miaoli District Agricultural Research and Extension Station, Taiwan. IR1552 is famous for its purple leaf. In addition, Taichung shen 10 (TCS10, green leaf), one of the most widely grown rice cultivars in Taiwan, was also used in this study. Seeds were sterilized with 2% sodium hypochlorite for 20 min, washed extensively with distilled water and then germinated in Petri dishes with wetted filter paper at 37℃ in the dark. After 48 h of incubation, uniformly germinated seeds were selected and cultivated

in a 250 ml beaker containing a half-strength Kimura B nutrient solution with the following macro and microelements: 182.3 μM $(NH_4)_2SO_4$, 91.6 μM KNO_3, 273.9 μM $MgSO_4 \cdot 7H_2O$, 91.1 μM KH_2PO_4, 182.5 μM $Ca(NO_3)_2$, 30.6 μM Fe-citrate, 0.25 μM H_3BO_3, 0.2 μM $MnSO_4 \cdot H_2O$, 0.2 μM $ZnSO_4 \cdot 7H_2O$, 0.05 μM $CuSO_4 \cdot 5H_2O$ and 0.07 μM H_2MoO_4.

Nutrient solutions (pH 4.7) were replaced every 3 d. Hydroponically cultivated rice seedlings were raised in growth chambers with the LED lighting system set at 30℃ and 25℃ for day and night respectively and 70% relative humidity under a 12 h photoperiod.

Light treatments

LED lighting systems designed by GRE Technology Co. (Taipei, Taiwan) were used to control light quality. The spectral distribution of the relative energy of the blue (peak at 460 nm), red (peak at 630 nm) and green (peak at 530 nm) regions were measured using a spectroradiometer (LI-COR1800, Lincoln, NE, USA) in the 300-800 nm range. These peak emissions of LEDs closely coincide with the absorption peaks of chlorophylls a and b and the reported wavelengths are at their respective maximum photosynthetic efficiencies (McCree, 1972). Light treatments for rice seedlings, proliferation and differentiation included red LEDs (R), blue LEDs (B), green LEDs (G)

and red + blue LEDs (R:B = 4:1 by photon flux density; RB) (Figure I-1), with photon flux density (PPFD) being set at 160 μmol $m^{-2}s^{-1}$. The experiment was independently performed three times for a randomized design of growth conditions and measurements representing the means of 15 plants (three reps consisting of five plants each) were taken.

Plant growth parameters

Rice seedlings were sampled after 14 d of growth after reaching the V3 stage according to Counce et al. (2000). Three seedlings for each beaker and 3 beakers for each light treatment were randomly selected for growth analysis. Plant height and root length were measured from the base of the seedling to the top of the third leaf and from the root base to the seed root tip respectively. Column diameter was measured in the seedling base with a Vernier caliper. Fresh weights (FW) and dry weights (DW) of seedlings were measured with an electronic balance. To determine DW, seedlings were dried at 80℃ until constant weights were achieved. Moisture content (%) was calculated as [1- (DW/FW)] × 100%. The health index was calculated as (stem diameter / plant height) × biomass according to Guo et al. (2011b).

Chlorophyll fluorescence measurements

Seedlings were kept in the dark for approximately 20 min before measurement. Chlorophyll fluorescence was measured at the middle portion of the second leaf of the seedlings taken at ambient temperatures with a Portable Chlorophyll Fluorometer PAM-2100 (Walz, Effeltrich, Germany). Actinic light and saturating light intensities were set at 280 μmol $m^{-2}s^{-1}$ and 2500 μmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR) respectively. The maximal photochemical efficiency of PSII (F_v/F_m), relative quantum efficiency of PSII photochemistry (Φ_{PSII}), photochemical quenching (q_P) and non-photochemical quenching (NPQ) were measured and calculated according to the method described previously (Kooten and Snel, 1990).

Chlorophyll (Chl), carotenoid (Car) and anthocyanin (Ant) contents

Chl and Car contents were eluted from the second leaf DW samples (0.01 g) with 5 ml of 80% acetone at 4℃ overnight and determined using the methods by Porra et al. (1989) and Holm (1954) respectively. Samples were then centrifuged at 13,000 g for 5 min. Supernatants were tested to determine the absorbances of Chl *a*, Chl *b* and Car in acetone as measured with a spectrophotometer (U-2000, Hitachi, Tokyo, Japan) at wavelengths of 663.6, 646.6 and 440.5 nm respectively. Concentrations (μg g^{-1} DW) of Chl a, Chl b and Car were determined using the following equations:

Chl *a* = (12.25 × $OD_{663.6}$ – 2.55 × $A_{646.6}$) × volume of supernatant (ml) / sample weight (g)

Chl *b* = (20.31 × $A_{646.6}$ – 4.91 × $A_{663.6}$) × volume of supernatant (ml) / sample weight (g)

Car = [(4.69 × $A_{440.5}$ × volume of supernatant (ml) / sample weight (g)) – 0.267 × (Chl *a* + Chl *b*).

Ant content was measured according to the protocol of Mancinelli et al. (1975). A mixture of 80% methanol containing 1% HCl of solvent was used to extract the powder samples. The mixture was then centrifuged at 4℃ and 3,000 rpm for 5 min and the supernatant was used to measure the absorbance at 530 nm and 657 nm. Ant content (μg g^{-1} DW) was calculated as (A_{530} - 0.33 × A_{657} / 31.6) × volume of supernatant (ml) / sample weight (g).

Free amino acid, soluble sugar and starch contents

DW samples of the second leaf (0.05 g) were placed into 15 ml tubes and then 5 ml of distilled water was added and mixed in. The supernatant was collected after 30 min in a water bath at 85℃. This step was repeated once and then distilled water was added to obtain 10 ml of the extract for use in determining soluble sugar and free amino

acid contents (mg g^{-1} DW). The soluble sugar content was determined using the sulfuric acid anthrone method at a wavelength of 630 nm (Morris, 1948). Free amino acid content was determined using the ninhydrin method at a wavelength of 570 nm (Moore and Stein, 1948). Starch was extracted according to the procedures from Takahashi et al. (1995).

The residue obtained after distilled water extraction was dried and then 1 ml of distilled water was added. The mixture was placed in a water bath for 30 min at 100℃. The gelatinized starch was digested after cooling with 1 ml 9.2 N perchloric acid for 10 min. Two ml of distilled water was added and the mixture centrifuged at 8,000 *g* for 6 min. After the extract was transferred to a 15 ml tube, 1 ml of 4.6 N perchloric acid was added and stirred for 10 min. Three ml of distilled water were added to the final volume after centrifugation. Starch contents (mg g^{-1} DW) were determined using the same method for soluble sugar.

Total protein content

Total proteins were measured using the method of Bradford (1976). Samples (0.05 g FW) were ground in a mortar with liquid nitrogen to which 3 ml of a phosphate buffered solution (pH 7.0) was added. The extract was centrifuged at 13,000 *g* for 15 min at 4℃ and 0.1 ml of the supernatant was combined with 5 ml of Coomassie

brilliant blue G-250 solution (0.1 g l^{-1}). The soluble protein content (mg g^{-1} FW) was determined after 2 min at a wavelength of 595 nm.

Statistical analysis

All measurements were evaluated for significance using analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the $P < 0.05$ level. All statistical analyses were conducted using SAS 9.2 (SAS Institute; Cary, NC, USA).

Results

Plant growth and morphology

The effects of light quality treatments (T) on the two rice varieties (V) were monitored by measuring changes in plant height, root length, stem diameter, shoot and root biomasses, moisture content and health index at 14 d seedling. In this experiment, a factorial experiment design with a completely randomized arrangement was used. Table 1 presents that all the measured components of growth parameters were significant at the 5% level for the main effects, except for plant height and shoot moisture content in V and shoot biomass and root moisture content in both V and T which showed negligible differences. Moreover, when the V × T interaction was examined for significance, all parameters significantly differed except the plant height, shoot biomass,

moisture content of shoot and root and health index.

Plant heights of both varieties were significantly shorter (12.9 and 13.1 cm) and stem diameters were larger (0.19 and 0.16 cm) under B than other lighting treatments (Table I-1). Root lengths of both varieties were significantly shorter (12.2 and 9.1 cm) under RB than under other lighting conditions. However, shoot biomass and root moisture contents were not significantly different among all lighting environments. Different light quality treatments affected the growth of rice seedlings and blue light likely inhibited the elongation of rice seedlings. The shoot moisture content of both varieties was lower (83.3 ~84.5%) under blue light than without (85.0~ 86.2%) indicating that blue light could increase water transport. Root biomass of TCS10 was significantly higher (0.019 g) under B compared to other lighting environments. Lighting environments not only affected shoot growth but also mediated root elongation and root biomass accumulation.

The shoot/root dry weight ratios of TCS10 under B (1.91) and RB (2.16) were significantly lower than without blue light, but there was no significant difference in the S/R DW ratios of IR1552 among all treatments. A normal appearance and compact morphology with vigorous roots in TCS10 seedlings treated with B LED light were observed. However, seedlings grown under B light looked small or even severely dwarfed (photos not shown). The health index was used to describe the morphological

quality of rice seedlings and a higher index number contributed to shorter shoot height and larger stem diameter. Under B LED light, values were significantly higher (0.525 and 0.431) than under other lighting colors.

Chlorophyll (Chl), carotenoid (Car) and anthocyanin (Ant) contents

ANOVA was used to uncover the main effects of variety (V) and light quality treatment (T) and their interaction effects (V × T) for different pigments as summarized in Table I-2. All pigments displayed significant differences ($P< 0.05$) for the main effects, with the exception of Car levels. Only total Chl and Ant contents constituted significant differences for the interaction effect.

Pigment content in leaves was influenced by different lighting environments. Total Chl content in leaves of TCS10 was not significantly different among all treatments but in IR1552 it was highest (18.25 mg g^{-1} DW) under RB and lowest (13.24 mg g^{-1} DW) under R condition (Table I-2). The Chl *a/b* ratio of both varieties was higher (2.81 and 2.47 mg g^{-1} DW) under B than other lighting treatments.

The Car content in leaves of both varieties was not significantly different among all lighting environments. Changes in the Car/Chl ratio were therefore attributed to the level of total Chl content in the leaves. The level of Ant in the purple leaves of IR1552 was sensitive to lighting. Ant content in IR1552 was significantly higher (150 μg g^{-1}

DW) under RB as compared to other conditions, indicating that light quality affected the synthesis of pigments (Chl and Ant) in rice seedling leaves.

Chlorophyll (Chl) fluorescence

Chl fluorescence components were used to indirectly measure the different functional levels of photosynthesis. Figure I-2 shows the effects of light quality on Chl fluorescence in 14 d rice leaves. The Fv/Fm ratios of both varieties were not significantly different among all lighting conditions. In healthy leaves, the F_v/F_m ratio is close to 0.8, a value typical for uninhibited plants. A lower value indicates that a portion of the PSII reaction center is damaged (Jung et al., 1998; Somersalo and Krause, 1989). The Φ_{PSII} and q_P of the two varieties under B were highest (0.85~0.87) among all lighting treatments and those under R and G were at similar levels. The exception was that the q_P of IR1552 under R (0.82) was significantly higher than under G (0.71).

Therefore, blue light might promote the photosynthetic potential of rice seedlings. The seedlings of TS10 under R (1.4) and G (1.3) exhibited higher NPQ than those grown in the blue light environment (1.0). This indicated thermal energy dissipation in the antennae. In IR1552, there was no significant difference in the NPQ among the R (1.0), G (0.8) and B (0.8) lighting but NPQ under RB (1.2) was slightly higher than under other lighting qualities. In general, cultivars responded differently to

light quality due to a different photosynthetic apparatus and the Chl fluorescence of two varieties varied in response to RB LED conditions.

Free amino acid, soluble sugar, starch and total protein contents: ANOVAs for variety (V), light quality treatment (T) and their interaction (V × T) for carbon–nitrogen metabolism in 14 d seedlings are tabulated in Table I-3. There were significant differences in soluble sugar, free amino acid and total protein content between the two varieties. Moreover, total protein levels were significantly affected by T and soluble sugar appeared to significantly differ in V × T.

The soluble sugar content of IR1552 was significantly greater in seedling leaves under R and G (47~48.6 mg g^{-1} DW) than under B and RB (37~38.7 mg g^{-1} DW) (Table I-3). A similar trend was observed in starch levels where R and G (23~25.4 mg g^{-1} DW) were greater than B and RB (14.0~15.4 mg g^{-1} DW), suggesting that R and G lights might stimulate carbohydrate accumulation. The soluble sugar content in TS10 seedling leaves was not significantly different among all treatments but the starch content was greatest (54.3 mg g^{-1} DW) when exposed to G. The only significant difference in free amino acid content was the value from TS10 seedling leaves under RB which showed the lowest level (15.3 mg g^{-1} DW) among all lighting qualities. The total protein of leaves was greatest (43.7 mg g^{-1} DW) in IR1552 under B and lowest (33.1 mg g^{-1} DW) in TS10 under R. Furthermore, total proteins of IR1552 under R (35.6 mg g^{-1} DW) were

significantly lower in comparison to other LED conditions (41.1~43.7 mg g^{-1} DW) indicating that blue light might promote protein synthesis in seedling leaves.

Discussion

Growth and morphological quality

Rice is widely grown in Taiwan and its production is very important economically and commercially. The spectral quality of lighting is defined as the relative intensity and quantity of different wavelengths emitted by a light source and perceived by photoreceptors within a plant. Plant yields and quality are the result of interactions of various environmental factors under which plants are grown. The present study examined the effects of different spectral lighting conditions on growth parameters, pigments, chlorophyll fluorescence and carbon–nitrogen metabolism of two genotypes of rice seedling plants grown under identical environmental conditions. Plants showed distinct growth responses to different light-quality treatments. Results from table 1 demonstrated that light quality influenced the growth and morphology of rice seedlings and blue light inhibited shoot elongation. Seedling height was shortest and stem diameter greatest under B LED conditions.

Similar results were observed in rice seedlings (Guo et al., 2011b), strawberry plantlets (Nhut et al., 2003a), sprouting broccoli (Kopsell and Sams, 2013), grapes

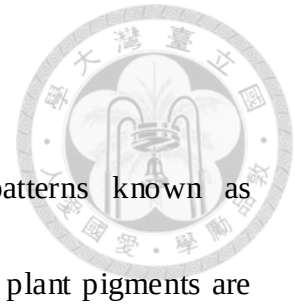
(Poudel et al., 2008), roses (Abidi et al., 2013) and *Cymbidium plantlets* (Tanaka et al., 1998b). In addition, several studies (Johkan et al., 2010; Kim et al., 2004b; Lee et al., 2007; Li et al., 2010; Nhut et al., 2003b; Ohashi-Kaneko et al., 2006b; Tanaka et al., 1998b) showed that blue and red mixed LEDs increased biomass accumulation. In our study, however, shoot biomass was unaffected by light quality. It is unlikely that red and blue mixed LEDs could promote shoot biomass. Root length was the shortest and root biomass the lowest in seedlings of the two varieties under RB LED conditions. This agrees with the reports by Guo et al. (2011b) and Nhut et al. (2003b) but differs from previous studies (Johkan et al., 2010; Lee et al., 2007; Tanaka et al., 1998b) in which red and blue mixed LEDs were shown to induce root elongation.

Liu et al. (2011) reported that a different red to blue ratio affected root morphology and an increase in blue radiation caused a longer root length. The B:R (3:1) LED light was suitable for rapeseed plantlet growth and can be used as a priority light source in the rapeseed culture system (Li et al., 2013). In our study, the energy distribution of RB LEDs was 80% red and 20% blue in PPFD (data not shown). B LED light is important for leaf expansion and enhances biomass production (Hogewoning et al., 2010; Johkan et al., 2012; Li et al., 2010). Yorio et al. (2001) also reported that there was higher dry weight accumulation in lettuce grown under R light supplemented with B light than in lettuce grown under R light alone. These results indicate that plant

responses to light quality are species- or cultivar- dependent.

The morphological quality of rice seedlings can be described by the S/R DW ratio and the health index. TCS10 seedlings under B exhibited the lowest S/R ratio (1.91) which contributed to an increase in root biomass. A higher seedling root biomass supports shoot growth by fully supplying the plant with water and mineral nutrition and may increase successful transplantation into the field. Poor roots cannot supply sufficient water for large shoots so plants with high S/R ratios are unsuitable for active growth (Johkan et al., 2010). In our study, the S/R DW in TS10 was not optimal under G (2.80) compared to other light colors. This observation is indicative of the poor growth of roots under G light and also indicates that root induction is probably also dependent on the spectral quality of lighting.

In addition, a growth-retarding effect might have been caused by an insufficient quality of light. The seedling health index was greatest under B which is in agreement with Guo et al. (2011b). The higher health index under the blue light environment contributed to the shorter shoot height and larger stem diameter which can provide a higher lodging resistance potential. Consequently, B LED light was an effective light source for plant growth and development and light spectra, intensities and durations can easily be controlled by growers in artificial growing environments.



Photosynthetic pigments and chlorophyll fluorescence

Plant pigments have specific wavelength absorption patterns known as absorption spectra. Biosynthetic wavelengths for the production of plant pigments are referred to as action spectra (Wang et al., 2009). Chl and Car have high light absorptions at 400–500 and at 630–680 nm respectively and low light absorption at 530–610 nm. Previous studies (Guo et al., 2011b; Johkan et al., 2010; Lee et al., 2007; Lin et al., 2011; Liu et al., 2011; Nhut et al., 2003a; Tanaka et al., 1998b) demonstrated that blue light induces the synthesis of Chl and Car. In our study, light quality also affected photosynthetic pigments in rice seedling leaves (Table I-2). Total Chl in IR1552 seedling leaves under RB was higher than other light conditions but Car in seedling leaves was not responsive to different light qualities. Although different quality lighting for all treatments were applied at the same PPFD level, plants showed similar absorption spectra of photosynthetic pigments, total Chl and Car (Table I-2).

Perhaps the applied PPFD level (160 μmol m^{-2} s^{-1}) had reached a certain minimum that is necessary for sufficient synthesis and activity of photosynthetic pigments and electron carriers. The Chl *a/b* ratio was mediated by lighting treatments in seedling leaves of two varieties and was higher under B compared to other lighting environments. This result is consistent with those of previous studies (Johkan et al., 2010; Lee et al., 2007; Lin et al., 2011; Wang et al., 2009). An increase in Chl *a/b* is

usually observed in higher irradiation environments (Evans and Poorter, 2001) suggesting it as an indicator for estimating relative photosystem stoichiometry (Pfannschmidt et al., 1999). Plants grown under all treatments appeared to synthesize more Chl *a* because it has a wider spectrum compared to that of Chl *b* and Chl *a* is the molecule that makes photosynthesis possible (Calatayud and Barreno, 2004).

Furthermore, a change in the Chl *a/b* ratio is usually correlated with variation in PSII light-harvesting antenna size and PSII:PSI content (Leong and Anderson, 1984). This inference is strengthened by our findings on Chl fluorescence (Figure I-2). The q_P and Φ_{PSII} of the tested samples under B were higher than those of under R and G which may indicate non-radiative (thermal) energy dissipation. The thermal dissipation process is called non-photochemical quenching (NPQ), referring to the fact that the thermal dissipation of Chl's excited states competes with fluorescence emission as well as with photochemistry (i.e. photosynthesis). The decreases in NPQ are associated with decreases in non-photochemical quenching. PSII activity may regulate the response of photosynthesis to light quality changes. Blue light promoted the Φ_{PSII} and q_P of seedling leaves and is in agreement with the findings by Wang et al. (2009) who indicated that the decrease in Φ_{PSII} was due to the lower q_P. This might be caused by rate-limiting processes including the PSI and cytochrome *b*6/*f* complex processes (Wang et al., 2009). Yu and Ong (2003) found a reduction of Φ_{PSII} and q_P in leaves under red or yellow light

compared with blue light.

In addition, blue light is essential for high light acclimation and photoprotection in the diatom *Phaeodactylum tricornutum* (Costa et al., 2013). These results imply that the Chl fluorescence parameters were genotype- and light quality-specific and were not expressed solely in response to an increasing excess of photon energy. Chloroplast development in TCS10 may be particularly sensitive to blue lights. Electron transport would be inhibited under conditions without blue light and NPQ would increase in TS10 seedling leaves. Both genotypes behaved similarly when their leaves were developed at 30/25℃ and 160 μmol $m^{-2}s^{-1}$ PPFD inside growth chambers for 14 d and hence the genotypic differences might be related to adaptation mechanisms induced by light quality.

Carbon–nitrogen metabolism

The selected LED lights differentially affected the metabolic system of the investigated rice varieties. Seedlings under B were observed to have higher total protein content in leaves than under other monochromatic lights (Table I-3) which is in agreement with the findings by Lin et al. (2011), Guo et al. (2011b), Wang et al. (2009) and (Eskins et al., 1991). Blue light influences nitrate reductase activity for mediating the rate of nitrogen assimilation in radish plants (Maevskaya and Bukhov, 2005).

Ohashi-Kaneko et al. (2006b) and (Matsuda et al., 2004) reported that a red light environment with a supplemental blue light caused an increase in the total N content of rice leaves. This included Rubisco, cytochrome *f* and light-harvesting complex II and was positively correlated with photosynthetic rate and stomatal conductance. These results were consistent with our findings on Chl *a/b* (Table I-2) and Chl fluorescence (Figure I-2). Previous studies found that the density, length and width of stomata were enhanced in blue light-enriched environments (Kim et al., 2004b; Li et al., 2010; Poudel et al., 2008; Wang et al., 2009).

In contrast, leaves in the blue light-enriched environments of our study exhibited stronger water transport, contributing to lower moisture content. The accumulation of carbohydrates in IR1552 leaves was promoted significantly under R and G LED conditions (Table I-3). This outcome was similar to those published for rice seedlings (Guo et al., 2011b), *Oncidium* (Liu et al., 2011) and upland cotton plantlets (Li et al., 2010) but was opposite to the findings of (Wang et al., 2009) which indicated blue light-induced carbohydrates were accumulated in leaves. Red light induces the accumulation of carbohydrates which is attributed to inhibiting the translocation of photosynthetic products from leaves (Sæbø et al., 1995).

IR1552 had the higher soluble sugar and starch contents under R and G LEDs, so these light sources might be beneficial for the accumulation of soluble sugars and

starches in plants. However, the amount of free amino acids in all plant leaves showed no significant differences among all treatments except for RB in TS10 leaves. This suggests that the light spectrum might not be advantageous for free amino acids synthesis.

Function of green light

Anthocyanins are one group of polyphenols that are thought to protect plants against unsuitable environments (Winkel-Shirley, 2001). A study of red leaf lettuce discovered that blue light induced the synthesis of Ant in seedling leaves (Johkan et al., 2010). Our results showed that RB lighting also induced Ant synthesis in purple leaf IR1552; however, the efficiency of green light was higher than other monochromatic lights (Table I-2). Johkan et al. (2012) tested the effects of green light wavelengths on red leaf lettuce and found that green LEDs (peak wavelength 510 nm) induced Ant synthesis in baby lettuce leaves. In our study, G LEDs had a peak wavelength of 525 nm and induced more Ant synthesis than red or blue light (Figure I-1).

Furthermore, the morphology, photosynthetic pigments, Chl fluorescence and metabolites under G performed similarly to those under R (Table I-1, Table I-2 and Table I-3; Figure I-2), which is in agreement with the trend that was observed in cucumbers (Wang et al., 2009). Green light acts as a signal source affecting the

development of wheat (Kasajima et al., 2008) and the rosette architecture of *Arabidopsis* (Zhang et al., 2011). However, the function of green light is not clear (Wang and Folta, 2013); hence the effect of green light on plants is worthy of further evaluation. In addition, it will be interesting to test more rice varieties and lines for seedling growth when illuminated by various monochromatic light spectra and combinations under a wide range of light intensities.

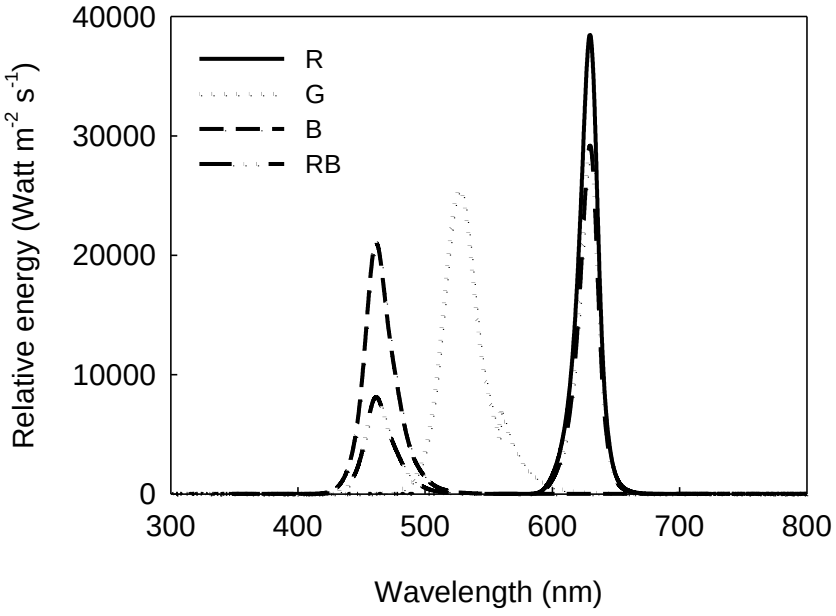


Figure I-1. The spectral distributions of different light treatments. Spectral scans were recorded at the top of the plant canopy with a spectroradiometer.

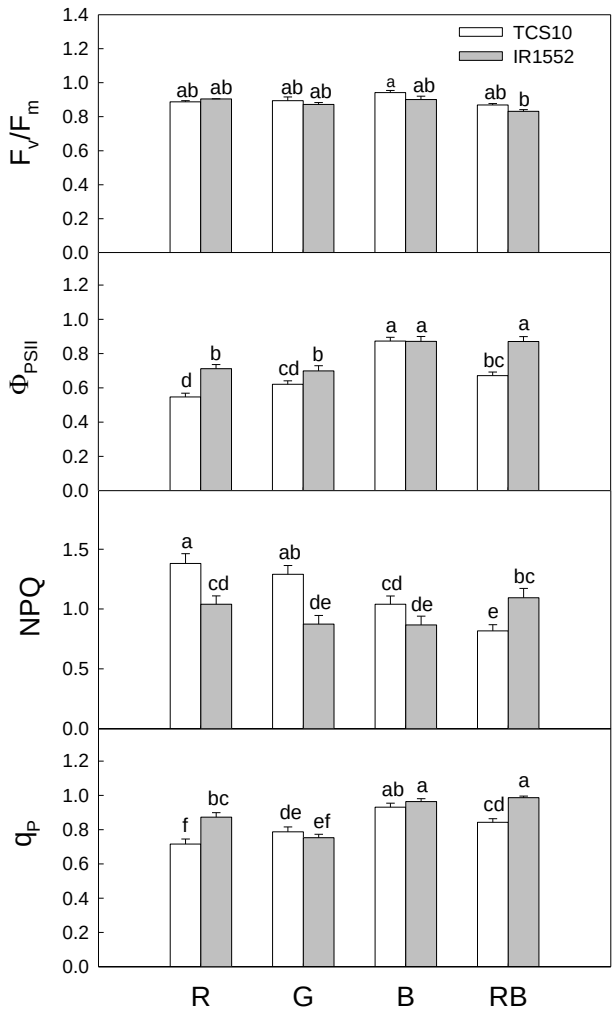


Figure I-2. Effects of light quality on the relative value of chlorophyll fluorescence. Leaves were analyzed from 14 d seedlings under different light environments. Values are the mean of ten plants from two replicates consisting of five plants each. The values followed by the different letter show statistically significant differences at $P < 0.05$.

Table I-1. The growth parameters of 14 d seedlings cultivated under different light environments.

Variety	Treatment	Plant height (cm)	Root length (cm)	Stem diameter (cm)	Shoot biomass (g)	Root biomass (g)	Shoot/Root Ratio (w/w)	Shoot moisture content (%)	Root moisture content (%)	Health index
TCS10	R	19.5a	13.7 ab	0.16 b	0.038a	0.015b	2.49 b	85.0 ab	92.3a	0.310c
	G	20.1a	14.4 a	0.17 ab	0.036a	0.013c	2.80 a	86.1 a	93.2a	0.310c
	B	12.9c	14.1 ab	0.19 a	0.035a	0.019a	1.91 c	83.3 d	93.1a	0.525a
	RB	19.1a	12.2 c	0.16 b	0.036a	0.017b	2.16 c	84.2 cd	93.0a	0.307c
IR1552	R	19.6a	13.0 bc	0.13 c	0.034a	0.013c	2.66 ab	85.9 ab	92.4a	0.218d
	G	19.5a	12.1 c	0.12 c	0.034a	0.013c	2.57 ab	86.2 a	93.3a	0.221d
	B	13.1c	11.9 c	0.16 b	0.036a	0.013c	2.79 a	84.5 bcd	92.9a	0.431b
	RB	16.8b	9.1 d	0.15 b	0.036a	0.013c	2.69 ab	83.8 cd	92.8a	0.340c
ANOVA *F* tests										
Variety (V)		ns	<0.0001	<0.0001	ns	<0.0001	<0.0001	ns	ns	0.0045
Treatment (T)		<0.0001	<0.0001	0.0003	ns	0.0016	0.0041	0.0007	ns	<0.0001
V × T		ns	0.0489	0.0160	ns	0.0007	<0.0001	ns	ns	ns

Biomass is the total weight of 3 seedlings. Values for ANOVA *F* tests are type I observed significance levels. Within columns, means followed by the same letter are not significantly different according to LSD (0.05). ns, non-significant at $P < 0.05$.

Table I-2. The effect of light quality on pigments in 14 d seedling leaves.

Variety	Treatment	Total Chl (mg g^{-1} DW)	Chl *a/b*	Car (mg g^{-1} DW)	Car/Chl	Ant (μg g^{-1} DW)
TCS10	R	14.49 bc	2.55 bc	3.52 ab	0.24 ab	nd
	G	14.02 bc	2.77 ab	3.70 ab	0.26 a	nd
	B	13.45 c	2.81 a	3.52 ab	0.26 a	nd
	RB	13.94 bc	2.53 bc	3.20 ab	0.23 ab	nd
IR1552	R	13.24 c	2.19 d	2.84 b	0.22 bc	15 c
	G	15.48 b	2.20 d	3.35 ab	0.22 b	33 b
	B	15.19 b	2.47 c	3.52 ab	0.23 ab	17 c
	RB	18.25 a	2.06 d	3.24 ab	0.18 c	150 a
				ANOVA *F* tests		
Variety (V)		0.0015	<0.0001	ns	0.0006	<.0001
Treatment (T)		0.0083	0.0060	ns	0.0239	<.0001
V × T		0.0020	ns	ns	ns	<.0001

nd, non-detectable. Values for ANOVA *F* tests are type I observed significance levels. Within columns, means followed by the same letter are not significantly different according to LSD (0.05); ns, non-significant at $P < 0.05$.

Table I-3. Effects of light quality on the carbon–nitrogen metabolism of seedling leaves collected from 14 d seedlings under different light environments.

Variety	Treatment	Soluble sugar (mg g^{-1} DW)	Starch (mg g^{-1} DW)	Free amino acid (mg g^{-1} DW)	Total protein (mg g^{-1} FW)
TCS10	R	50.3 ab	23.1b	21.4 a	33.1 d
	G	53.5 ab	54.3a	19.2 ab	34.7 cd
	B	52.3 ab	21.5b	19.1 ab	41.0 ab
	RB	57.6 a	21.5b	15.3 b	38.0 bc
IR1552	R	48.6 b	25.4b	19.6 a	35.6 cd
	G	47.0 b	23.0b	21.6 a	41.1 ab
	B	37.0 c	14.0c	21.4 a	43.7 a
	RB	38.7 c	15.4c	21.2 a	41.7 ab
ANOVA *F* tests					
Variety (V)		<0.0001	ns	0.0362	0.0034
Treatment (T)		ns	ns	ns	0.0010
V × T		0.0162	ns	ns	ns

Values for ANOVA *F* tests are type I observed significance levels. Within columns, means followed by the same letter are not significantly different according to LSD (0.05); ns, non-significant at $P < 0.05$.

II. Water-use efficiency and nitrogen uptake in rice seedlings grown under different light treatments

Abstract

In this study, our objective was to investigate the response of time-integrated water-use efficiency (WUE), ^{13}C discrimination ($\Delta^{13}C$) and nitrogen uptake in hydroponic seedlings (V2-V3) of rice (*Oryza sativa* L.) cultivars, Taichung shen 10 (TCS10) and IR1552, grown under different light treatments. Light emitting diode (LED) lighting systems were used to control light quality. Light treatments for rice seedlings included red (R), blue (B), green (G) and red + blue (RB), with fluorescent light (FL) as control, photon flux density (PPFD) set at 105 μmol m^{-2} s^{-1}. The WUE response was highest for seedlings grown under R light, then (in decreasing order) seedlings grown under G, RB, and B light. WUE had a high positive correlation with $\Delta^{13}C$, except under FL light.

Nitrogen content (%N) and $\delta^{15}N$ values were used to estimate the effect of fertilizer uptake under different lighting conditions. The results showed that the amount of N in seedlings derived from fertilizer (N_f) was highest under B light, and was lowest under R light. Therefore, we concluded that blue light may increase stomatal conductance and transpiration, decrease WUE, and promote root N uptake. In this study, we also demonstrated the application of stable C and N isotope techniques for crop

physiology.

Key words: Light quality, Rice seedling, Water-use efficiency, ^{13}C discrimination, Nitrogen uptake, ^{15}N isotope, ^{13}C isotope

Introduction

Light is the main energy source for plant photosynthesis and is used as an environmental signal to trigger growth and structural differentiation in plants. Light quality, quantity, and photoperiod controls the morphogenesis, growth, and differentiation of plant cells, tissues, and organ cultures (Abidi et al., 2013). Plant development is strongly influenced by light quality, which refers to the color or wavelengths reaching a plant's surfaces (Johkan et al., 2010). Red (R) and blue (B) lights have the greatest impact on plant growth because they are the major energy sources for photosynthetic CO_2 assimilation in plants. It is well known that spectra have action maxima in the B and R ranges (Kasajima et al., 2008). The integration, quality, duration, and intensity of red light/far red light, blue light, mixed red and blue lights (RB), UV-A (320–500 nm) or UV-B (280–320 nm), and hormone signaling pathways have a profound influence on plants by triggering or halting physiological reactions and controlling the growth and development of plants (Clouse, 2001; Shin et al., 2008). Recent research and review articles report that green light also affects the morphology, metabolism, and photosynthesis of plants (Johkan et al., 2012; Wang and Folta, 2013; Zhang et al., 2011).

Light sources such as fluorescent, metal-halide, high-pressure sodium and incandescent lamps are generally used for crop cultivation. These sources are applied to

increase photosynthetic photon flux levels but contain unnecessary wavelengths that are located outside the photosynthetically active radiation spectrum and are of low quality for promoting growth (Kim et al., 2004b). Compared to artificial light sources, light-emitting diode (LED) lighting systems are ideal for use in plant light studies and allow wavelengths to be matched to plant photoreceptors for providing more optimal production and influencing plant morphology and metabolism (Bourget, 2008; Massa et al., 2008). Spectral light changes induce different morphogenetic and photosynthetic responses that can vary among different plant species. Such photo responses are of practical importance in recent plant cultivation technologies since the feasibility of tailoring illumination spectra enables one to control plant growth, development and nutritional quality.

Water-use efficiency (WUE), which is defined as dry matter produced per unit of water transpired, is perceived as an important attribute for growth and influenced by genetic (Chen et al., 2012; Monclus et al., 2012; Takai et al., 2009) and environmental factors, including water (Grant et al., 2012; Liu et al., 2012; Wang et al., 2013b; Yasir et al., 2013), fertilizer (Guo et al., 2011a; Wang et al., 2013b), and tillage level (Dalal et al., 2013). At the agronomic scale (crop level), WUE is considered as the accumulated dry matter divided by water consumed by the crop during the whole growth cycle (Condon et al., 2004; Tambussi et al., 2007). Carbon isotope discrimination ($\Delta^{13}C$) is an

alternative screening technique for water use efficiency which is highly correlated with transpiration efficiency, and has been demonstrated to be a simple but reliable measure of WUE (Farquhar and Richards, 1984; Farquhar et al., 1982). The advantages of $\Delta^{13}C$ expressed on a dry matter basis are that it gives integrated data over a whole period of growth and is suitable for high-throughput screening, because samples can be stored for further measurement (Condon et al., 1987). Several studies (Cabrera-Bosquet et al., 2007; Chen et al., 2012; Glenn, 2014; Moghaddam et al., 2013; Monclus et al., 2012; This et al., 2010) have utilized $\Delta^{13}C$ as a tool for screening WUE of different C_3 plant species. Similarly, intrinsic differences in the nitrogen isotope value ($\delta^{15}N$) of different N sources (such as soil and fertilizer) can be used to estimate their relative contribution to the crop N uptake (Shearer and Kohl 1993). If differences in natural abundance of $\delta^{15}N$ values of different N sources occur, then this approach can be successfully used. Dalal et al. (2013) successful evaluated the N uptake and NUE of wheat using $\delta^{15}N$ values.

Our previous studies (Chen et al., 2014a) found that blue light LEDs enhanced relative quantum efficiency of PSII photochemistry and photochemical quenching, but reduced non-photochemical quenching of seedling leaves, and also showed higher total protein content in the tested leaves compared to B plus RB. Light quality might mediate the photosynthetic potential and nitrogen uptake/metabolism in rice seedling.

Furthermore, WUE is also mediated under different light spectral compositions. However, there are fewer studies describing the effects of LED lighting on WUE and nitrogen uptake. Our objective is to investigate the effect of light quality on WUE and N uptake of rice seedling using a stable isotope approach.

Materials and Methods

Plant species and growth conditions

In this study, we used rice (*Oryza sativa* L.) cultivar IR1552, which is famous for its purple leaves, and rice cultivar Taichung shen 10 (TCS10, green leaf), one of the most widely grown rice cultivars in Taiwan. Seeds were sterilized with 2% sodium hypochlorite for 20 min, washed extensively with distilled water, and germinated in Petri dishes on wet filter paper at 37 ℃ in the dark. After 48 h of incubation, uniformly germinated seeds were selected and cultivated in a 150 ml beaker containing a half-strength Kimura B nutrient solution with the following macro and microelements: 182.3 μM $(NH_4)_2SO_4$, 91.6 μM KNO_3, 273.9 μM $MgSO_4 \cdot 7H_2O$, 91.1 μM KH_2PO_4, 182.5 μM $Ca(NO_3)_2$, 30.6 μM Fe-citrate, 0.25 μM H_3BO_3, 0.2 μM $MnSO_4 \cdot H_2O$, 0.2 μM $ZnSO_4 \cdot 7H_2O$, 0.05 μM $CuSO_4 \cdot 5H_2O$, and 0.07 μM H_2MoO_4. Nutrient solutions (pH 4.7) were replaced every 3 d. Hydroponically cultivated rice seedlings were raised in growth chambers under LED lights at 30 ℃ and 25 ℃, held day and night, respectively, over a

12 h photoperiod. All hydroponic seedlings were collected on day 14 after reaching stage V2 or V3 according to Counce et al. (2000). The shoots and roots of the seedlings were frozen and freeze-dried before analysis.

Light treatments

LED lighting systems designed by GRE Technology (Taipei, Taiwan) were used to control light quality. Spectral distributions of blue (peak at 460 nm), red (peak at 630 nm), and green (peak at 530 nm) were measured using a spectro-radiometer (LI-COR1800, Lincoln, NE, USA) in the 300-800 nm range. These LED emission peaks closely coincide with the absorption peaks of chlorophyll *a* and *b*, and the reported wavelengths are at their respective maximum photosynthetic efficiencies (McCree, 1972). Light treatments for rice seedlings, proliferation, and differentiation consisted of red LEDs (R), blue LEDs (B), green LEDs (G), a mixture of red plus blue LEDs (R:B = 4:1 by photon flux density; RB), and fluorescent lighting (FL). Photosynthetic photon flux density (PPFD) was uniformly set at 105 μmol m^{-2} s^{-1}. The experiment was independently performed three times under randomized growth conditions

Sample collection and analysis

WUE was defined as the ratio of dry biomass produced to total water transpired during the experimental period. It was calculated as

$$WUE\ (mg\ DW\ g^{-1}) = BM/W$$

where BM was the biomass of shoot and root, and W was the total amount of water transpired during the period. The amount of water transpired was calculated by the difference in volume between beakers with and without rice seedlings. The loss of water in beakers without seedlings should be only evaporation.

The freeze-dried shoots and seeds of both cultivars were well ground to a fine, homogeneous powder by automatic homogenizer. Subsamples of powdered inorganic fertilizers were taken from three types of nitrogen fertilizer in Kimura solution. Appropriate weights of powdered samples were transferred into 6×4 mm tin capsules. Carbon and nitrogen contents and isotopic composition from shoots and seeds of both cultivars were analyzed in duplicate at the Stable Isotope Laboratory, GNS Science, New Zealand, using a VG Isoprime (Isoprime Ltd. U.K.) isotope ratio mass spectrometer, interfaced to a EuroVector elemental analyser (EuroVector Ltd. Italy.) in continuous flow mode (EA-IRMS). The carbon dioxide gas was resolved from nitrogen

gas using gas chromatographic separation on a column at 65 ℃ and analyzed simultaneously for isotopic abundance as well as total organic carbon and nitrogen. International and working reference standards (NISTN1, IAEA-CH6, leucine, EDTA, and cane sugar) and blanks were included during each run for calibration. Isotopic ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) are expressed as isotopic deviations δ defined as

$$\delta(‰) = \frac{R_S - R_{Ref}}{R_{Ref}} \times 1000$$

where R_s is the isotopic ratio measured for the sample and R_{Ref} that of the international standards. The $\delta^{13}C$ value is relative to the international Vienna Pee Dee Belemnite (VPDB) standard, and the $\delta^{15}N$ value is relative to atmospheric air. Results are expressed in δ (‰) versus the specific reference. The analytical precision of the measurements is 0.2 ‰, and reproducibility of the results is within ±0.2 ‰ for carbon and ±0.3 ‰ for nitrogen (1σn).

Carbon isotope discrimination ($\Delta^{13}C$) was calculated as

$$\Delta^{13}C\ (‰) = \frac{\delta_a - \delta_p}{1 + \delta_p} \times 1000$$

where δ_p is the $\delta^{13}C$ of the sample and δ_a is the $\delta^{13}C$ of atmospheric CO_2. On the PDB scale, atmospheric CO_2 has a current deviation of approximately −8‰ (Farquhar et al.,

1989).

The total %N of shoot (N_{shoot}) was determined from %N of seedling and shoot biomass. The amount of N in plant derived from fertilizer (N_f) was estimated from $\delta^{15}N$ values using a mixed model as follows

$$N_f = \frac{\delta^{15}N_{seedling} - \delta^{15}N_s}{\delta^{15}N_F - \delta^{15}N_s} \times N_{shoot}$$

where $\delta^{15}N_s$, $\delta^{15}N_{seedling}$, $\delta^{15}N_F$ are the $\delta^{15}N$ of seeds, seedlings and nitrogen of Kimura fertilizer (nutrient) solution (Table II-1), respectively.

Statistical analysis

All measurements were evaluated for significance using an analysis of variance (ANOVA) followed by a least significant difference (LSD) test at the $P < 0.05$ level. All statistical analyses were conducted using SAS 9.3 (SAS Institute; Cary, NC, USA). A regression analysis was conducted to relate the WUE and $\Delta^{13}C$.

Results

Biomass, transpired water, and water-use efficiency

The changes of biomass, total amount of water transpired, and WUE of both cultivars under different light quality are listed in Table II-2. In both cultivars, the

highest accumulation of biomass was observed under FL light. The biomass of TCS10 and IR1552 were 304 and 227 mgDW pot^{-1} respectively. Seedlings from cultivar IR1552 grown under B light had the highest biomass out of all the other LED light types, but there were no significant differences with TCS10 cultivar seedlings. A similar trend was observed in the total amount of water transpired. WUE is defined as dry matter produced per unit of water transpired. The WUE response was the highest for seedlings grown under R light. The WUE of TCS10 and IR1552 were 5.0 and 7.1 mg g^{-1} respectively. The next highest WUE (in decreasing order) were from seedlings grown under G, RB, B, and FL lighting.

Carbon isotope discrimination and water-use efficiency

The $\Delta^{13}C$, calculated from the $\delta^{13}C$ of the seedling shoot and well-defined atmospheric CO_2, also responded to different light conditions and showed a similar trend as WUE with the exception of cultivars grown under FL light (Table II-2 and Table II-3). By excluding data from seedlings grown under FL light, WUE was highly correlated to $\Delta^{13}C$ (Figure II-1).

Percentage of carbon and nitrogen, and C/N ratio

%C of both cultivars was significantly higher under B than other light treatments

(44.0 and 43.3% for TCS10 and IR1552, respectively). %N of IR1552 was lower under FL (5.2%) and higher under B (6.1%) and RB (6.0%) than other light treatments, and the trend was also observed in results of TCS10 with exception of seedling grown under B. The C/N ratio of IR1552 seedlings was significantly higher in seedlings grown under FL (8.1), than under R and G light (7.6), and the lowest C/N ratio was under B and RB light. The C/N ratio of TCS10 seedlings was also moderately influenced by light quality (Table II-3).

Nitrogen in plant derived from fertilizer

The total amount of plant nitrogen derived from fertilizer (N_f), (or uptake efficiency) was estimated from the nitrogen content, and $\delta^{15}N$ values of seedlings, seed, and fertilizer (Figure II-2). N_f of TCS10 and IR1552 cultivars was highest under B (7.26 and 6.40 mg pot^{-1}) and lowest under R (6.71 and 3.69 mg pot^{-1}) light treatments. The N_f response to light quality showed different patterns between TSC10 and IR1552 cultivars. The N_f reduced significantly under R, G, and RB in IR1552 cultivars, but not in TCS10 cultivars.

Discussion

WUE is controlled by two factors, CO_2 assimilation and transpiration. Red light

with supplemental blue light can induce higher net photosynthetic rate and stomatal conductance (Hogewoning et al., 2010; Wang et al., 2009). However, some studies demonstrated that blue light reduced net assimilation (Evans and Vogelmann, 2003; Loreto et al., 2009). The change in stomatal conductance was caused by different stomatal aperture and density, and the morphogenesis of stomata was highly influenced by light quality (Savvides et al., 2012). Blue light induced higher numbers of stomata in leaves (Kim et al., 2004c; Poudel et al., 2008). According to our results, seedlings grown under B and RB light showed lower WUE among all the LED light treatments (Table II-2). The amount of water transpired by seedlings grown under LED light treatment was responsive to different light quality, but the biomass was not. The effect of LED lighting may influence the development of stomata and stomatal conductance rather than net assimilation. Lee et al. (2007) reported higher stomatal conductance, transpiration, net photosynthetic rate and water-use efficiency under fluorescent light and red plus blue light. Dong et al. (2014) also demonstrated that leaves of wheat grown under red plus white light had higher photosynthetic rates and stomatal conductance. We found the highest accumulation of biomass was under FL light, but there was a lower WUE, as most of the water was transpired by seedlings grown under FL light (Table II-2).

Under blue light, we observed an increase photosynthetic processing of proteins

(which included Rubisco, light-harvesting chlorophyll-binding proteins of photosystem II and Cyt *f*) (Matsuda et al., 2004; Matsuda et al., 2007; Murakami et al., 2014; Ohashi-Kaneko et al., 2006a; Ohashi-Kaneko et al., 2006b), higher N content (Matsuda et al., 2004; Matsuda et al., 2007; Ohashi-Kaneko et al., 2006b), and higher accumulation of carbohydrates (Sivakumar et al., 2006; Wang et al., 2009) in leaves were also observed. Chen et al. (2014a) also found an increase in total protein from leaves of rice seedlings grown under B light than other mono-spectrum LED light. On average, higher %C and N, and lower C/N ratio were found in seedlings grown under B and RB light than other light treatments in our study (Table II-3). These results suggested a higher carbohydrate accumulation and increased amino acid/protein biosynthesis in leaves of rice seedlings grown under blue enriched light conditions. In addition, fertilizer uptake efficiency (N_f) of seedlings was higher under B light than other light conditions (Figure II-2).This result suggested that seedlings grown under B light showed an increased N uptake ability from roots and N translocation from root to shoot, while red light might inhibit the N absorption/translocation from root.

$\Delta^{13}C$ in C_3 plants is highly associated with transpiration efficiency, the ratio of intercellular to ambient CO_2 concentration (Ci/Ca) (Farquhar et al., 1982) and stomatal conductance (Motzo et al., 2013). Furthermore, Yasir et al. (2013) investigated the relationship between $\Delta^{13}C$, yield, harvest index, and biomass in wheat, and Dalal et al.

(2013) showed the relationship between $\Delta^{13}C$ and nitrogen-use efficiency of wheat grain. In this study, $\Delta^{13}C$ of seedlings was responsive to different light quality (Table II-3), and the seedlings grown under LED light showed a positive correlation between $\Delta^{13}C$ and WUE (Figure II-1). However, the data from seedlings grown under FL light were not as well correlated compared to the LED light treatments, because seedlings grown under FL light were grown in different growth chambers than those grown under LED lighting. The LED growth chamber was almost entirely enclosed, while the FL growth chamber was well ventilated. The exchange rate of air in FL lit chamber most likely influenced the CO_2 concentration of the chamber and subsequent transpiration of leaves. Furthermore, $\Delta^{13}C$ was calculated from the $\delta^{13}C$ value of well-defined atmosphere CO_2 (-8 ‰), which was not the real-time value of either growth chambers in this study. Nevertheless, this study confirms that the WUE could be estimated from $\Delta^{13}C$ of rice seedlings grown in well-controlled environments under LED light.

In conclusion, our study shows lower WUE and higher N absorption from rice seedling roots under B lighting. This suggests that by supplementing growing conditions with blue light, we can improve and promote net photosynthetic rates, transpiration and N uptake/translocation through improved stomatal conductance in rice plants. In this study, we also demonstrated the application of stable C and N isotope techniques to determine uptake efficiency of fertilizer and CO_2 for crop physiology. $\Delta^{13}C$ values are

positively correlated with increasing WUE and $\delta^{15}N$ values reflect the efficiency of fertilizer nutrient uptake.

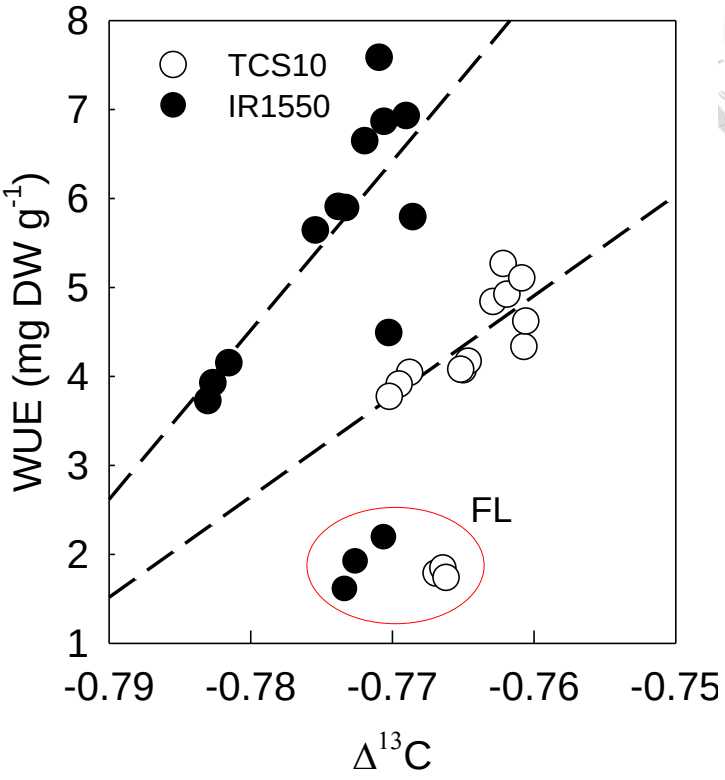


Figure II-1. The relationship between $\Delta^{13}C$ and WUE. Dashed lines correlate various light treatments with the exception of FL light conditions. R^2_{TCS10}=0.6166, P<0.01; R^2_{IR1552} = 0.6112, P<0.01.

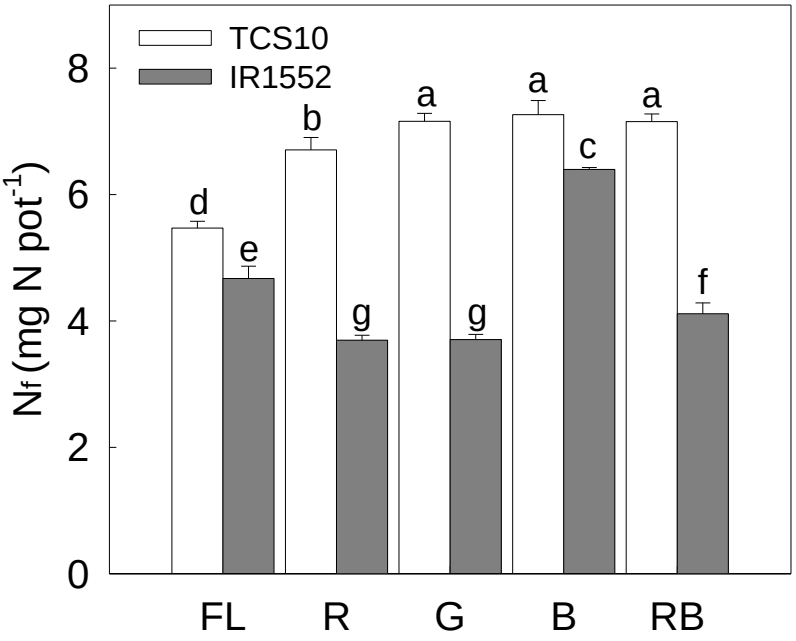


Figure II-2. Nutrient dynamics (N_f) of TCS10 and IR1552 cultivars under different light conditions. Values are a mean of three replicate samples. The values followed by the different letters show statistically significant differences at $P < 0.05$.

Table II-1. $\delta^{15}N$ values of seeds of two cultivars and three fertilizers in Kimura solution, and the estimated value of bulk Kimura solution.

Fertilizer	Mean $\delta^{15}N$(‰) ± 0.3‰ (*n*=2)	Mean %N (*n*=2)
Seeds of TCS10	4.7	1.4
Seeds of IR1552	8.9	2.2
KNO_3	3.1	13.8
$(NH_4)_2SO_4$	-9.4	21.5
$Ca(NO_3)_2$	-1.0	11.9
Bulk Kimura	-4.3*	--

*estimated value

Table II-2. The response of biomass (BM), total amount of water transpired (W), and WUE of TCS10 and IR1552 under different light conditions.

Cultivar	Treatment	BM (mg pot^{-1})	W (g pot^{-1})	WUE (mgDW g^{-1})
TCS10	FL	304a	210 a	1.78 g
	R	257b	66 d	5.00 cd
	G	262b	72 cd	4.68 de
	B	261b	87 c	3.90 f
	RB	254b	80 cd	4.10 ef
IR1552	FL	227c	154 b	1.90 g
	R	188e	35 e	7.12 a
	G	188e	45 e	5.64 bc
	B	209d	70 d	3.93 f
	RB	177e	42 e	5.81 b

Within each column, the mean followed by the same letter is not significantly different according to LSD (0.05).

Table II-3. %C, %N, C/N ratio, $\delta^{13}C$, $\delta^{15}N$, and $\Delta^{13}C$ of rice seedlings.

Cultivar	Treatment	%C	%N	C/N	$\delta^{13}C$	$\delta^{15}N$	$\Delta^{13}C$
TCS10	FL	43.0 bc	3.6 g	11.8 a	-31.0b	0.2 d	-0.766c
	R	43.0 bc	4.4 ef	9.8 bc	-30.4a	-0.6 e	-0.762 b
	G	42.6 cd	4.4 f	9.8 c	-30.2a	-0.9 ef	-0.761 a
	B	44.0 a	4.4 ef	10.0 b	-31.4c	-1.0 f	-0.769 d
	RB	43.2 bc	4.5 e	9.6 c	-30.8b	-0.9 ef	-0.765 c
IR1552	FL	41.8 e	5.2 d	8.1 d	-31.7d	3.6 b	-0.772 e
	R	41.9 e	5.5 c	7.6 e	-31.5c	4.2 a	-0.770 d
	G	41.9 e	5.5 c	7.6 e	-31.5c	4.2 a	-0.770 d
	B	43.3 b	6.1 a	7.1 f	-33.2f	2.3 c	-0.782 g
	RB	42.2 de	6.0 b	7.1 f	-32.0e	3.8 b	-0.774 f

Within each column, the mean followed by the same letter is not significantly different according to LSD (0.05).

III. Light Quality Influences the Chlorophyll Degradation Pathway in Rice Seedling Leaves

Abstract

The objective of this study was to investigate the dynamics of chlorophyll (Chl), biosynthetic intermediates (protoporphyrin IX, PPIX; magnesium protoporphyrin IX, MGPP; protochlorophyllide, Pchlide), degradation intermediates (chlorophyllide, Chlide; pheophytin, Phe; pheophorbide, Pho), and carotenoids (Car) in leaves of rice (*Oryza sativa* L.) seedlings. Seedlings of two rice varieties, Taichung shen 10 (TCS10) and IR1552, were grown under different light quality conditions. Quality conditions controlled by light emitting diodes (LED). Lighting treatments for rice seedlings included red (R), blue (B), green (G), and red + blue (RB), with fluorescent lighting (FL) as the control and photosynthetic photon flux density (PPFD) being set at 105 μmol m^{-2} s^{-1}. Lower levels of Chl and Car in leaves were observed under G lighting. Light quality did not mediate the mole percent of porphyrins in biosynthetic pathways. Rice seedling leaves took Chl→Phe→Pho and Chl→Chlide→Pho as the major and minor degradation routes, respectively. Furthermore, lower Phe/Chlide ratios were observed under G and FL lighting conditions, indicating that green-enriched environments may up-regulate the minor degradation route in leaves.

Keywords: Chlorophyll, Biosynthesis and degradation pathway, Light quality, Rice seedling

Introduction

Light is the main energy source for plant photosynthesis and is used as an environmental signal to trigger growth and structural differentiation in plants. Light quality, quantity, and photoperiod control the morphogenesis, growth, and differentiation of plant cells, tissues, and organ cultures (Abidi et al., 2013). Plant development is strongly influenced by light quality, which refers to the colors or wavelengths reaching a plant's surfaces (Johkan et al., 2010). Red (R) and blue (B) lights have the greatest impact on plant growth because they are the major energy sources for photosynthetic CO_2 assimilation in plants. It is well known that spectra have action maxima in the B and R ranges (Kasajima et al., 2008). The integration, quality, duration, and intensity of red light/far red light, blue light, mixed red and blue lights (RB), UV-A (320–500 nm) or UV-B (280–320 nm), and hormone signaling pathways have a profound influence on plants by triggering or halting physiological reactions and controlling the growth and development of plants (Clouse, 2001; Shin et al., 2008). Recent studies report that green (G) light also affects the morphology, metabolism, and photosynthesis of plants (Johkan et al., 2012; Zhang et al., 2011).

Chlorophylls (Chl) and carotenoids are the main and accessory photosynthetic pigments, respectively. Chl accumulation is a combined effect of the Chl biosynthesis pathway and the Chl degradation pathway. The Chl biosynthesis pathway requires light

(Hoober and Eggink, 1999; Jilani et al., 1996). Different spectrums also influence the formation of photosynthetic pigments. Blue light induces higher Chl *a/b* ratios (Chen et al., 2014a; Demarsac and Houmard, 1993; Rivkin, 1989) and greater accumulations of Chl (Kurilčik et al., 2008; Poudel et al., 2008). Red light inhibits Chl synthesis at lower concentrations of Chl and precursors like 5-aminolevulinic acid (ALA) (Sood et al., 2005; Tanaka et al., 1998a), PPIX, MGPP, and Pchlide (Fan et al., 2013). Furthermore, the mole percentages of PPIX, MGPP, and Pchlide also respond to various physiological conditions and varieties of genotype (Hsu et al., 2003; Hsu et al., 2011; Huang et al., 2014; Yang et al., 2012) .

Chlorophyllase and Mg-dechelatase actions, which are responsible for the first steps in the Chl degradation pathway, are elicited by *Rhopalosiphum padi* and *Diuraphis noxia* (Ni et al., 2002; Wang et al., 2004). Chlorophyllase 1 of *Arabidopsis thaliana*, encoded by *AtCLH1*, is indicated to be involved in plant damage control and can modulate the balance between different plant defense pathways (Kariola et al., 2005). The leaves of sweet potato (Hsu et al., 2003), rice (Yang et al., 2012), and *Machilus thunbergii* (Yang et al., 2003) might use Chl→Phe→Pho as the major route for chlorophyll degradation, whereas the leaves of banana (Hsu et al., 2011) might use Chl→Chlide→Pho as the major route. Furthermore, some biotic/abiotic factors affect the degradation pathway (Hsu et al., 2003; Hsu et al., 2011; Huang et al., 2014; Yang et

al., 2003; Yang et al., 2012). However, there are no reports describing the effects of LED lighting on the Chl degradation pathway. Our objective was to investigate Chl biosynthetic and degradation pathways in leaves of rice seedlings grown under different lighting spectra.

Materials and Methods

Plant materials and growth conditions

In this study, we used rice (*Oryza sativa* L.) cultivar IR1552, which is famous for its purple leaves, and cultivar Taichung shen 10 (TCS10, green leaf), one of the most widely grown rice cultivars in Taiwan. Seeds were sterilized with 2% sodium hypochlorite for 20 min, washed extensively with distilled water, and germinated in Petri dishes on wetted filter paper at 37 ℃ in the dark. After 48 h of incubation, uniformly germinated seeds were selected and cultivated in a 150 ml beaker containing a half-strength Kimura B nutrient solution with the following macro and microelements: 182.3 μM $(NH_4)_2SO_4$, 91.6 μM KNO_3, 273.9 μM $MgSO_4 \cdot 7H_2O$, 91.1 μM KH_2PO_4, 182.5 μM $Ca(NO_3)_2$, 30.6 μM Fe-citrate, 0.25 μM H_3BO_3, 0.2 μM $MnSO_4 \cdot H_2O$, 0.2 μM $ZnSO_4 \cdot 7H_2O$, 0.05 μM $CuSO_4 \cdot 5H_2O$, and 0.07 μM H_2MoO_4. Nutrient solutions (pH 4.7) were replaced every 3 d. Hydroponically cultivated rice seedlings were raised in growth chambers with the LED lighting at 30 ℃ and 25 ℃ for day and night, respectively,

under a 12 h photoperiod.

Light treatments

LED lighting systems designed by GRE Technology (Taipei, Taiwan) were used to control light quality. Spectral distributions of blue (peak at 460 nm), red (peak at 630 nm), and green (peak at 530 nm) were measured using a spectroradiometer (LI-COR1800, Lincoln, NE, USA) in the 300-800 nm range. These peak LED emissions closely coincide with the absorption peaks of chlorophyll *a* and *b*, and the reported wavelengths are at their respective maximum photosynthetic efficiencies (McCree, 1972). Light treatments for rice seedlings, proliferation, and differentiation consisted of red LEDs (R), blue LEDs (B), green LEDs (G), a mixture of red plus blue LEDs (R:B = 4:1 by photon flux density; RB), and fluorescent lighting (FL). Photosynthetic photon flux density (PPFD) was uniformly set at 105 μmol m^{-2} s^{-1}. The experiment was independently performed three times under randomized growth conditions, and measurements representing the means of nine plants (three replications consisting of three plants each) were taken.

Pigment analysis

All hydroponic seedlings were collected on day 14 after reaching stage V2 or V3

according to Counce et al. (2000). The second fully expanded leaves of the seedlings were detached, frozen with liquid nitrogen, and extracted with 80% acetone. The concentrations of Car, less polar (LP) Car, more polar (MP) Car, and Chl-related compounds (*i.e.*, PPIX, MGPP, Pchlide, Chl, Chlide, and Phe) were determined according to a combined procedure described by Yang et al. (1998) with a spectrophotometer (Hitachi U3010, Tokyo, Japan). The mole percent of individual porphyrin is defined as [(PPIX, MGPP or Pchlide) / (PPIX + MGPP + Pchlide)] x 100%. The values of phytylated and/or dephytylated pigments in samples were read directly at absorbances of 661 and 666 nm (A_{661} and A_{666} g^{-1} DW), respectively.

Statistical analysis

All measurements were evaluated for significance using an analysis of variance (ANOVA) followed by a least significant difference (LSD) test at the $P < 0.05$ level. All statistical analyses were conducted using SAS 9.3 (SAS Institute; Cary, NC, USA).

Results

Chl and Car

The dynamics of photosynthetic pigments in leaves of both cultivars under different lighting quality are listed in Table III-1. On average, concentrations of Chl and

Car in leaves of IR1552 were higher than TCS10. In both cultivars, the concentration of Chl was lower under G. The differences in Chl among B, BR, and FL were not significant. A similar trend was observed in Car in the leaves of TCS10, but there were no significant differences with IR1552.

Concentrations of LP Car were dramatically reduced in leaves of both cultivars under G. A lower accumulation of LP Car was also observed in leaves of TCS10 under FL (369 μg g^{-1}DW). Significantly lower concentrations of MP Car were observed in leaves of TCS10 under G and R lighting. However, the effects of LED lighting were insignificant for MP Car in IR1552. Light quality influenced LP Car/MP Car ratios in TCS10 strongly, but not in IR1552.

Porphyrins and their mole percentages

Porphyrins are the Chl biosynthesis intermediates. The accumulation of porphyrins in leaves of IR1552 was higher than in TCS10 on average (Table III-2). The concentration of porphyrins in TCS10 was not significantly different among all treatments (2840~3126 nmol g^{-1} DW). IR1552 showed a similar response, but the levels of porphyrins under B were lower than in other treatments. The percentages of PPIX and MGPP were insignificant among all lighting conditions for both cultivars. The percentage of Phchlide in TCS10 was higher than in other treatments, and it was

irresponsive to lighting quality in IR1552.

Chl degradation intermediates

The concentration of Phe was higher than Chlide for both cultivars on average. Leaves of rice seedlings take Chl→Phe→Pho as the major degradation route and Chl→ Chlide→Pho as the minor degradation route (Table III-3). In both cultivars, the concentration of Phe under G was lower than in other treatments, and the highest concentration of Phe was under RB. The concentration of Chlide under FL was greater than in other treatments. The Phe/Chlide ratios of IR1552 under G and FL were lower than other treatments on average (insignificant at P <0.05). A similar trend was observed in TCS10, with the exception of G. The results of phytylated and dephytylated pigments and their ratios also showed a similar trend in the Phe/Chlide ratio (Table III-4).

Discussion

Chl and Car

Plant pigments have specific wavelength absorption patterns known as absorption spectra. Biosynthetic wavelengths for the production of plant pigments are referred to as action spectra (Wang et al., 2009). Chl and Car have high light absorptions

at 400–500 and 630–680 nm, respectively, and low light absorption at 530–610 nm. Previous studies (Fan et al., 2013; Guo et al., 2011b; Johkan et al., 2010; Lee et al., 2007; Lin et al., 2011; Liu et al., 2011; Nhut et al., 2003a) demonstrated that blue light induces the synthesis of Chl and Car. In this study, the concentrations of Chl and Car were greater under FL, B, and RB, with the exception of IR1552 under B, and were lower under G (Table 1). However, our previous study showed that Car levels were not responsive to light quality (Chen et al., 2014a). The differing results under the same experimental conditions, including light quality and rice variety, might be due to the change in light irradiance, as higher light irradiance (160 μmole m^{-2} s^{-1}) results in insignificant differences in Car levels.

MP and LP Car levels respond to different aging and senescent conditions (Hsu et al., 2003). MP Car levels decrease as LP Car levels increase during the maturation/ageing process. This study shows that MP and LP Car levels are also mediated by light quality, including the possibility that green light might inhibit LP Car synthesis (Table III-1).

Chl biosynthetic pathway

Light is an important environmental signal and induces chlorophyll biosynthesis (Jilani et al., 1996). Chl reduction was observed under red light as a result of a decrease

in ALA (Sood et al., 2005; Tanaka et al., 1998a), PPIX, MGPP, and Pchlide (Fan et al., 2013). In this study, porphyrin levels were not reduced by red light (Table III-2).

The mole percentages of PPIX, MGPP, and Pchlide are responsive to maturing/aging in sweet potato leaves (Hsu et al., 2003), the vegetative/reproductive stage of rice (Yang et al., 2012), and tissues infected by disease/insects (Hsu et al., 2011; Huang et al., 2014). Light quality also influences the mole percentages of these three precursors. According to Fan *et al.* (2013), green light increases the mole percentage of PPIX but decreases the mole percentage of MGPP and Pchlide. In our study, the mole percentages of the three precursors were insignificant among all treatments except TCS10 under B (Table III-2). Our resulting Chl biosynthetic intermediates suggest that light quality did not affect the Chl biosynthetic pathway.

Chl degradation pathway

The removal of Mg or the phytol chain, catalyzed by Mg-dechelatase and chlorophyllase, respectively, are the two possible routes in the initial period of Chl degradation. Their products are Chlide and Phe, respectively, which are further converted into Pho and continue to degrade into even smaller molecules (Matile et al., 1996). The four products in the initial period of Chl degradation can be divided into two categories according to their chemical structures. The first category contains phytylated

pigments, such as Chl and Phe, all of which contain a phytol chain in their structure, but the first example contains Mg while the second does not. The other category contains dephytylated pigments, including Chlide and Pho, but the first contains Mg while the second does not (Matile et al., 1996).

Sweet potato, rice, and *Machilus thunbergii* use Chl→Phe→Pho and Chl→Chlide→Pho as the major and minor routes for chlorophyll degradation, respectively (Hsu et al., 2003; Huang et al., 2014; Yang et al., 2012). Some physiological conditions, such as aging (Hsu et al., 2003), growth stage (Yang et al., 2012), disease (Hsu et al., 2011), and infestation by insects (Huang et al., 2014), are important factors for mediating the ratio between these two routes and phytylated and dephytylated pigments.

In this study, rice seedlings also took Chl→Phe→Pho as the major route (Table III-3). A lower Phe/Chlide ratio under G was observed among three mono-spectrum lighting conditions, and the ratio under FL was also lower between the two poly-spectrum lighting conditions. Furthermore, higher levels of Chlide were generally apparent under G and FL in our study. Phytylated/dephytylated ratios showed similar trends (Table III-4). These results suggest that a green light-enriched environment might promote the minor route for Chl degradation. This phenomenon warrants further investigation. The different response between TCS10 and IR1552 under G lighting might due to the accumulation of anthocyanin in leaves of IR1552.

We conclude that light quality not only influences the accumulation of photosynthetic pigments, but also mediates the Chl degradation pathway in rice seedling leaves, possibly promoting the minor route of Chl degradation in rice seedling leaves.

Acknowledgments

Seeds of IR1552, were donated by Dr. Su-Jein Chang, Miaoli District Agricultural Research and Extension Station, Taiwan.

Table III-1. Effects of light quality on the levels of Chl, Car, LP Car, MP Car, and their ratios in seedling leaves collected from 14 d seedlings under different lighting environments.

Variety	Light treatment	Chl (mg g^{-1} DW)	Car (μg g^{-1}DW)	LP Car (μg g^{-1}DW)	MP Car (μg g^{-1}DW)	LP/MP (w/w)
TCS10	FL	12.08 c	4.55 cd	369 de	575 a	0.64 c
	R	10.6 d	4.2 de	400 cde	377b	1.06 a
	G	9.67 d	3.88 e	342 e	348b	0.98 ab
	B	12.96 bc	4.88 bc	394 de	544a	0.79 bc
	RB	12.75 c	4.63 c	446 cd	518a	0.90 abc
IR1552	FL	15.01 a	5.49 a	593 a	604a	0.99 ab
	R	14.16 ab	5.23 ab	554 ab	541a	1.02 ab
	G	12.63 c	4.93 bc	486 bc	500a	0.97 ab
	B	14.27 a	4.95 bc	543 ab	511a	1.06 a
	RB	15.35 a	5.20 ab	613 a	584a	1.05 ab

Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table III-2. Effects of light quality on the levels and mole percentages of porphyrins in leaves collected from 14 d seedlings under different lighting environments.

Variety	Light treatment	Prophyrins (μmol g^{-1} DW)	Mole percent of porphyrin (%) PPIX	MGPP	Pchlide
TCS10	FL	3.13 d	72.5 cde	26.6 abc	0.9b
	R	3.07 d	72.6 cde	26.9 ab	0.5b
	G	2.84 d	72.3 de	26.9 ab	0.8b
	B	3.11 d	69.4 e	27.3 a	3.2a
	RB	2.92 d	72.7 bcde	26.2 abcd	1.1b
IR1552	FL	4.47 a	76.1 ab	23.9 cd	0.0b
	R	4.38 ab	76.2 a	23.8 d	0.0b
	G	4.00 bc	73.9 abcd	25.6 abcd	0.5b
	B	3.66 c	75.9 abc	23.9 cd	0.1b
	RB	4.24 ab	75.6 abcd	24.4 bcd	0.0b

Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table III-3. Effects of light quality on Chlide, Phe, and their ratios in 14 d seedling leaves.

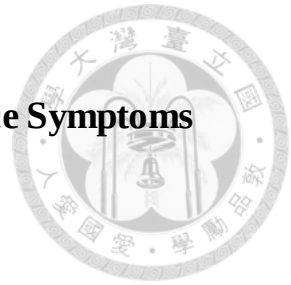
Variety	Light treatment	Chlide (mmole g^{-1} DW)	Phe (mg g^{-1} DW)	Phe/Chlide (g/mol)
TCS10	FL	1.24 b	3.20 ef	2.58 d
	R	1.02 d	3.29 ef	3.23 abcd
	G	0.95 d	2.67 f	2.82 cd
	B	1.23 bc	3.53 de	2.89 bcd
	RB	1.08 cd	3.84 cde	3.56 ab
IR1552	FL	1.44 a	4.73 a	3.30 abc
	R	1.22 bc	4.51 abc	3.70 a
	G	1.35 ab	3.99 bcd	2.97 bcd
	B	1.28 b	4.53 ab	3.57 ab
	RB	1.31 ab	5.04 a	3.85 a

Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table III-4. Effects of light quality on phytylated and dephytylated pigments and their ratios in 14 d seedling leaves.

Variety	Light treatment	Phytylated (A661 g^{-1} DW)		Dephytylated (A666 g^{-1} DW)		Phytylated/ Dephytylated	
TCS10	FL	598	d	25	ab	23.60	c
	R	609	cd	15	d	39.67	a
	G	554	d	14	d	38.82	ab
	B	707	bc	23	abc	31.88	abc
	RB	715	b	19	cd	38.17	ab
IR1552	FL	842	a	29	a	29.67	bc
	R	776	ab	22	bc	37.01	ab
	G	704	bc	28	a	25.30	c
	B	798	ab	28	a	29.68	bc
	RB	839	a	26	ab	32.42	abc

Within columns, means followed by the same letter are not significantly different according to LSD (0.05).



IV. Effects of Green Light Intensity on Shade Avoidance Symptoms and Chlorophyll Degradation in Rice Seedlings

Abstract

Our objectives in this study were to investigate morphological traits and dynamics of chlorophyll (Chl) degradation intermediates (chlorophyllide, Chlide; pheophytin, Phe; pheophorbide, Pho) in leaves of rice (*Oryza sativa* L.) seedlings under increasing green light intensity. Seedlings of Taichung Native 1 (TCN1) were grown under equal intensities (40 μmol m^{-2} s^{-1}) of red and blue light with four levels of green light intensity (0, 20, 40, and 60 μmol m^{-2} s^{-1}). Light emitting diodes (LED) were used to control lighting treatments. Sheaths of rice seedling leaves elongated and leaves grew erectly under red and blue light with increasing green light intensity. These morphological traits are known as shade avoidance symptoms (SAS). Increasing green light intensity resulted in decreases in total chlorophyll, the effective quantum yield of photosystem II (Φ_{PSII}), and Phe/Chlide ratios, and increases in non-photochemical quenching (NPQ) and Chlide levels. These results indicated that green light induced SAS and mediated Chl degradation routes in rice seedlings.

Key words: Green light, Rice seedling, Shade avoidance symptoms, Chlorophyll degradation pathway

Introduction

Light quality, quantity, and duration control morphogenesis, growth, and differentiation in plants (Chen et al., 2004; Kami et al., 2010; Spalding and Folta, 2005). Red and blue light are the major energy sources for CO_2 assimilation and impact plant growth greatly because chlorophyll (Chl) *a* and *b* and different types of carotenoids (Car) in green tissue/organs capture light for photosynthesis (Nishio, 2000). Red/infrared, blue, and mixed red and blue light, UV-A and –B, and hormone signaling pathways have profound influences on plants by triggering or halting physiological reactions and controlling their growth and development (Clouse, 2001; Shin et al., 2008).

Green light, which is absorbed less than red and blue by photosynthetic pigments in green tissue, can penetrate into a canopy better than red or blue light (Klein, 1992). Therefore, plant growth is promoted in lettuce under blue and red light with supplemental green light (Kim et al., 2004a; Kim et al., 2004b). Furthermore, different spectra of green light induce different responses in morphogenesis and photosynthesis (Johkan et al., 2012). The rate of green light, which could be a signal source, enhanced the development of wheat (Kasajima et al., 2008). Some studies indicate that the partial responses of plants to green, red, and blue light are quite similar (Chen et al., 2014a; Wang et al., 2009), because phytochromes (phy), cryptochromes (cry), and phototropin 1 might be green-light receptors (Macedo et al., 2011; Wang et al., 2013a). Green light

also plays a role in the physiology of plant growth and development, including responses to shading (Wang and Folta, 2013).

Shade avoidance symptoms (SAS), which are morphological adjustments in response to shading, include elongated internodes and petioles, leaf hyponasty, and early-flowering behavior in *Arabidopsis thaliana*. Low red/infrared ratios and low levels of blue light are known to induce SAS in shade-avoiding plants grown under high lighting conditions, and phyB and cry are the major photoreceptors mediating SAS (Keuskamp et al., 2011; Stamm and Kumar, 2010). The lower canopy, which is enriched in green light, is one such kind of shaded environment. Zhang et al. (2011) proved that green light induces a shade morphology, which is cry- and phyB-independent in *Arabidopsis thaliana*. However, there are no reports describing the shade response of higher plants, such as rice, to green light signals.

The accumulation of Chl, which is the main photosynthetic pigment, is a combined effect of the Chl biosynthesis and degradation pathway. The Chl biosynthetic pathway requires light (Hoober and Eggink, 1999; Jilani et al., 1996). Blue light induces higher Chl *a/b* ratios (Chen et al., 2014a; Demarsac and Houmard, 1993; Rivkin, 1989) and greater accumulations of Chl (Kurilčik et al., 2008; Poudel et al., 2008). Red light inhibits Chl synthesis at lower concentrations of Chl and its precursors like PPIX, MGPP, and Pchlide (Fan et al., 2013). Chlorophyllase and Mg-dechelatase actions,

which are responsible for the first steps in the Chl degradation pathway, are elicited by the aphids *Rhopalosiphum padi* and *Diuraphis noxia* (Ni et al., 2002; Wang et al., 2004). Chlorophyllase 1 of *Arabidopsis thaliana,* encoded by *AtCLH1,* is indicated to be involved in plant damage control and can modulate the balance between different plant defense pathways (Kariola et al., 2005). Other biotic/abiotic factors also affect the degradation pathway (Hsu et al., 2003; Hsu et al., 2011; Huang et al., 2014; Yang et al., 2003; Yang et al., 2012).

Our previous study investigating the effect of light quality on the Chl biosynthetic and degradation pathway suggested that green light enrichment might mediate the degradation pathway (Chen et al., 2014b). We therefore conducted two experiments in the present study, the first to cultivate rice seedlings under four levels of green light intensity (0, 20, 40, and 60 μmol m^{-2} s^{-1}) with standardized background red and blue light levels, and the second to confirm that green light induces SAS in rice seedlings and mediates the Chl degradation pathway in rice seedling leaves under uniform intensity.

Materials and Methods

Plant materials and growth conditions

Seeds of rice (*Oryza sativa* L.) cultivar Taichung Native 1 (TCN1) were

sterilized with 2% sodium hypochlorite for 20 min, washed extensively with distilled water, and germinated in Petri dishes on wetted filter paper at 37 ℃ in the dark. After 48 h of incubation, uniformly germinated seeds were selected and cultivated in a 150 ml beaker containing a half-strength Kimura B nutrient solution with the following macro- and microelements: 182.3 μM $(NH_4)_2SO_4$, 91.6 μM KNO_3, 273.9 μM $MgSO_4 \cdot 7H_2O$, 91.1 μM KH_2PO_4, 182.5 μM $Ca(NO_3)_2$, 30.6 μM Fe-citrate, 0.25 μM H_3BO_3, 0.2 μM $MnSO_4 \cdot H_2O$, 0.2 μM $ZnSO_4 \cdot 7H_2O$, 0.05 μM $CuSO_4 \cdot 5H_2O$, and 0.07 μM H_2MoO_4. Nutrient solutions (pH 4.7) were replaced every 3 d. Hydroponically cultivated rice seedlings were raised under a 12 h photoperiod in growth chambers with LED lighting at 25 and 20 ℃ for day and night, respectively.

Light treatments

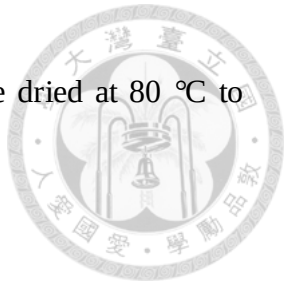
LED lighting systems designed by GRE Technology (Taipei, Taiwan) were used to control light quality. Spectral distributions of blue (peak at 460 nm), red (peak at 630 nm), and green (peak at 530 nm) were measured with a spectroradiometer (LI-COR1800, Lincoln, NE, USA) in the 300-800 nm range. These peak LED emissions closely coincide with the absorption peaks of chlorophyll *a* and *b*, and the reported wavelengths are at their respective maximum photosynthetic efficiencies (McCree, 1972). The composition and intensity of all light treatments in both

experiments are listed in Table IV-1. Rice seedlings in experiment 1 were given equal intensities (40 μmol m^{-2} s^{-1}) of red and blue light plus four levels of green light intensity, 0, 20, 40, and 60 μmol m^{-2} s^{-1} (G0, G20, G40 and G60). Experiment 2, which was designed to confirm the contributions of green light, were given red, green, and blue light of uniform intensity (140 μmol m^{-2} s^{-1}). For each light treatment, only one color light was set at 60 μmol m^{-2} s^{-1} (B60, G60 and R60) and the others at 40 μmol m^{-2} s^{-1}. The two experiments were performed independently three times under randomized growth conditions, and measurements represent the means of nine plants (three replications consisting of three plants each).

Plant growth and morphological parameters

Rice seedlings were sampled during the V2-V3 stage according to Counce et al. (2000) Three seedlings for each beaker and three beakers for each light treatment were randomly selected for growth analysis. Plant height and root length were measured from the base of the seedling to the top of the leaf and from the root base to the seed root tip, respectively. Blade and sheath lengths of the second leaf were measured from blade tip to blade ring and from blade ring to seedling base, respectively. Column diameter was measured in the seedling base with a Vernier caliper. Leaf angle was measured in the angle between the second leaf and stem with a protractor. Dry weights (DW) of

seedlings were measured with an electronic balance. Seedlings were dried at 80 ℃ to constant weights to determine DW.



Chlorophyll fluorescence measurements

Seedlings were kept in the dark for approximately 20 min before being measured. Chlorophyll fluorescence was measured at the middle of the second leaf at ambient temperature with a Portable Chlorophyll Fluorometer PAM-2100 (Walz, Effeltrich, Germany). Actinic light and saturating light intensities were set at 250 μmol m^{-2} s^{-1} and 2500 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR), respectively. The maximal photochemical efficiency of PSII (F_v/F_m), relative quantum efficiency of PSII photochemistry (Φ_{PSII}), photochemical quenching (q_P), and non-photochemical quenching (NPQ) were measured and calculated according to the methods of Kooten and Snel (1990).

Pigment analysis

The second fully expanded leaves were detached, frozen with liquid nitrogen, and extracted with 80% acetone. The concentrations of carotenoids (Car), Chl-related compounds including Chl *a*, *b*, and Chl biosynthetic intermediates (protoporphyrin IX, PPIX; magnesium protoporphyrin IX, MGPP; protochlorophyllide, Pchlide), and

degradation intermediates (chlorophyllide, Chlide; pheophytin, Phe) were determined according to a combined procedure described by Yang et al. (1998) with a spectrophotometer (Hitachi U3010, Tokyo, Japan). The mole percent of individual porphyrin is defined as [(PPIX, MGPP, or Pchlide) / (PPIX + MGPP + Pchlide)] x 100%. The values of phytylated and/or dephytylated pigments in samples were read directly at absorbances of 661 and 666 nm (A_{661} and A_{666} g^{-1} DW), respectively.

Statistical analysis

Experiments 1 and 2 were analyzed independently. All measurements were evaluated for significance using an analysis of variance (ANOVA) followed by a least significant difference (LSD) test at the $P < 0.05$ level. All statistical analyses were conducted using SAS 9.3 (SAS Institute; Cary, NC, USA).

Results

Growth and morphology

The responses of growth and morphological traits to different light treatments in both experiments are listed in Table IV-2. The shoots and roots lengthened more and a higher biomass was observed with supplemental green light in experiment 1. Plant height increased by 15.2 - 16.11 cm and root length extended slightly under increasing

green light intensity. Shoot and root biomass increased 26.6 and 38.9%, respectively, under G60 as compared with G0. The shortest plant heights were under B60 and the lowest root biomass was under R60 in experiment 2. There were no significant differences in shoot biomass and root length. Stem diameter did not respond to different light treatments in either experiment.

Second-leaf blade and sheath lengths responded to light treatments in both experiments (data not shown). The increase in sheath length was positively correlated to increases in green light. The ratio of blade length to sheath length (blade/sheath ratio), which is more sensitive to supplemental green light, decreased under green light enrichment. The most erect leaves were under G40 (11.1°) in experiment 1 and G60 (14.5°) in experiment 2.

Chlorophyll fluorescence

Chlorophyll fluorescence components were used to indirectly measure the different functional levels of photosynthesis. Figure IV-1 shows the effects of light treatments in both experiments on chlorophyll fluorescence in rice seedling leaves. The F_v/F_m ratios of both varieties were not significantly different among all lighting conditions. In healthy leaves, the F_v/F_m ratio is close to 0.8, a value typical for uninhibited plants. A lower value indicates that a portion of the PSII reaction center is

damaged (Somersalo and Krause, 1989).

Under G20, q_P and Φ_{PSII} increased slightly and then decreased under higher supplemental green light treatments in experiment 1, but were lower under G60 than B60 and R60 in experiment 2. Therefore, the photosynthetic potential of rice seedlings would be suppressed when supplemental green light is higher than 40 μmol m^{-2} s^{-1}. However, the dynamics of NPQ exhibited an opposite trend, with NPQ decreasing initially and then increasing under increasing green light intensity in experiment 1 and was highest under G60.

Chl and Car

The responses of Chl and Car in rice seedling leaves to different light treatments in both experiments are listed in Table IV-3. The Chl level in leaves decreased from 17.07 to 15.70 mg g^{-1} DW under increasing green light intensity in experiment 1. Lower Chl levels occurred under G60 than B60 and R60. The highest Chl *a/b* ratio (2.55) was under G60 in experiment 1; however, the differences in Chl *a/b* between G60 and either R60 or B60 were insignificant in experiment 2. The change in Car levels was not significant among all treatments in both experiments. The Car/Chl ratio only responded to changes in total Chl.

Chl biosynthetic and degradation intermediates

Porphyrin levels were not significantly different among all treatments in both experiments (Table IV-4). Furthermore, the mole percentages of PPIX and MGPP were also insignificant among all lighting conditions. Chl biosynthetic intermediates were irresponsive to light treatments.

The dynamics of Phe, Chlide, and phytylated and dephytylated pigments and their ratios are listed in Table IV-5. The Phe level decreased slightly while the mean of Chlilde levels increased with an increase in green light intensity in experiment 1. Meanwhile, lower Phe levels occurred under G60 relative to R60 and B60 in experiment 2. Changes in Phe/Chlide ratios were more responsive to increases in green light intensity. The Phe/Chlide ratio decreased significantly with increases in additional green light in experiment 1. Mean Phe/Chlide ratios were also lower, but insignificant, under G60. The results of phytylated and dephytylated pigments and their ratios showed a similar Phe/Chlide ratio trend.

Discussions

Plant growth

Our previous study showed that similar growth and morphology occurs in rice seedlings grown under red and green light (Chen et al., 2014a). Other studies indicate

that plants produced more biomass and have higher leaf areas under supplemental green light (Kim et al., 2004a; Kim et al., 2004b). Green light intensity greatly influences the development and growth of wheat, including panicle and leaf morphologies, development rate, and culm elongation (Kasajima et al., 2008). Lettuce grown under different green light wavelengths and intensities exhibits varied responses in photosynthesis and development, and shoot growth and net CO_2 assimilation of plants grown under 510 nm light were greater than under 520 and 530 nm lighting (Johkan et al., 2012).

In this study, increases in plant height, root length, and biomass with supplemental green light (peak at 530 nm) were observed in experiment 1 (Table IV-2). However, differences in growth were insignificant except for greater plant heights under G60 and shorter root lengths under R60 in experiment 2. The contribution of supplemental green light to growth should be enhanced by such irradiance, but not be due to any specific function of green light.

Shade avoidance

Plants grown under low red/infrared ratio lighting or blue light environments display SAS that alter plant morphology to suit the spectral shift induced by shade (Keuskamp et al., 2011; Stamm and Kumar, 2010). Zhang et al. (2011) indicate that

green light could be an environmental signal for inducing erect leaves and longer petioles in *Arabidopsis thaliana*. In this study, rice seedlings exhibited more erect leaf angles, longer sheaths (data not shown), and lower blade/sheath ratios with supplemental green light in experiment 1 and a similar response was observed under G60 in experiment 2 (Table IV-2). Our results are consistent with Zhang et al. (2011) and support the hypothesis that green light induces SAS.

Photosynthetic pigments and chlorophyll fluorescence

Chl and Car have high absorptions at 400–500 and 630–680 nm, respectively, and low absorption at 530–610 nm. Wang et al. (2009) showed that Chl levels in leaves are lower under green than red or blue light. The differences in Chl and Car levels in leaves are insignificant whether fluorescent lighting is supplemented with green light or not (Li and Kubota, 2009). Our previous study showed that Chl and Car levels and the Chl *a/b* ratio of plants grown under green light are similar to those under red or blue lighting (Chen et al., 2014a). In the present study, a decrease in total Chl and an increase in Chl *a/b* were observed under supplemental green light in experiment 1, and lower Chl levels occurred under G60 in experiment 2 (Table IV-3).

An increase in Chl *a/b*, which is usually correlated with variations in PSII light-harvesting antenna size and PSII:PSI content (Leong and Anderson, 1984), is

usually observed under higher irradiation (Evans and Poorter, 2001), suggesting that it may be an indicator for estimating relative photosystem stoichiometry (Pfannschmidt et al., 1999). Chlorophyll fluorescence experiments could strengthen this inference (Figure IV-1). Reductions of Φ_{PSII} and q_P occurred with higher supplemental green light in both experiments, while NPQ was enhanced. NPQ is the thermal dissipation process. Our chlorophyll fluorescence results suggested that the energy emitted by thermal dissipation was more enriched under a green light environment.

Chl biosynthetic pathway

Light is an important environmental signal and induces chlorophyll biosynthesis (Jilani et al., 1996). Chl reduction was observed under red light as a result of a decrease in ALA (Sood et al., 2005; Tanaka et al., 1998a), PPIX, MGPP, and Pchlide (Fan et al., 2013). The mole percentages of PPIX, MGPP, and Pchlide are responsive to maturing/aging in sweet potato leaves (Hsu et al., 2003), vegetative/reproductive stages in rice (Yang et al., 2012), and tissues infected by disease/insects (Hsu et al., 2011; Huang et al., 2014). Light quality also influences the mole percentages of these three precursors. According to Fan *et al.* (2013), green light increases the mole percentage of PPIX but decreases the mole percentage of MGPP and Pchlide. Our previous study showed that light quality did not affect the Chl biosynthetic pathway (Chen et al.,

2014b). In this study, porphyrin levels and mole percentages of PPIX, MGPP, and Pchlide did not respond to an increase in supplemental green light (Table IV-4).

Chl degradation pathway

The removal of Mg and the phytol chain, catalyzed by Mg-dechelatase and chlorophyllase, respectively, are the two possible routes in the initial degradation of Chl. Their products are Chlide and Phe, respectively, which are further converted into Pho and continue to degrade into even smaller molecules (Matile et al., 1996). The four products in the initial period of Chl degradation can be divided into two categories according to their chemical structures. The first category contains phytylated pigments, such as Chl and Phe, all of which contain a phytol chain in their structure, but the first example contains Mg while the second does not. The other category contains dephytylated pigments, including Chlide and Pho, and again the first contains Mg while the second does not (Matile et al., 1996).

Our previous study (Chen et al., 2014b) indicated that a green-enriched environment may up-regulate the degradation route of Chlide in leaves. In this study, a higher level Chlide and lower Phe/Chlide ratio in leaves exposed to red and blue light with an increase of supplemental green light were observed in experiment 1, and a lower Phe/Chlide ratio occurred under G60 in experiment 2 (Table IV-5). According our

findings on the changes in Chlide levels and Phe/Chlide ratios, green light intensity can mediate the Chl degradation pathway.

In conclusion, Green light intensity induces morphological and metabolic changes in rice seedlings. Erect leaves and elongated sheaths, referred to as shade avoidance symptoms, and higher Chlide levels and lower Phe/Chlide ratios were observed in rice seedlings grown under red and blue light with an increase of supplemental green light intensity.

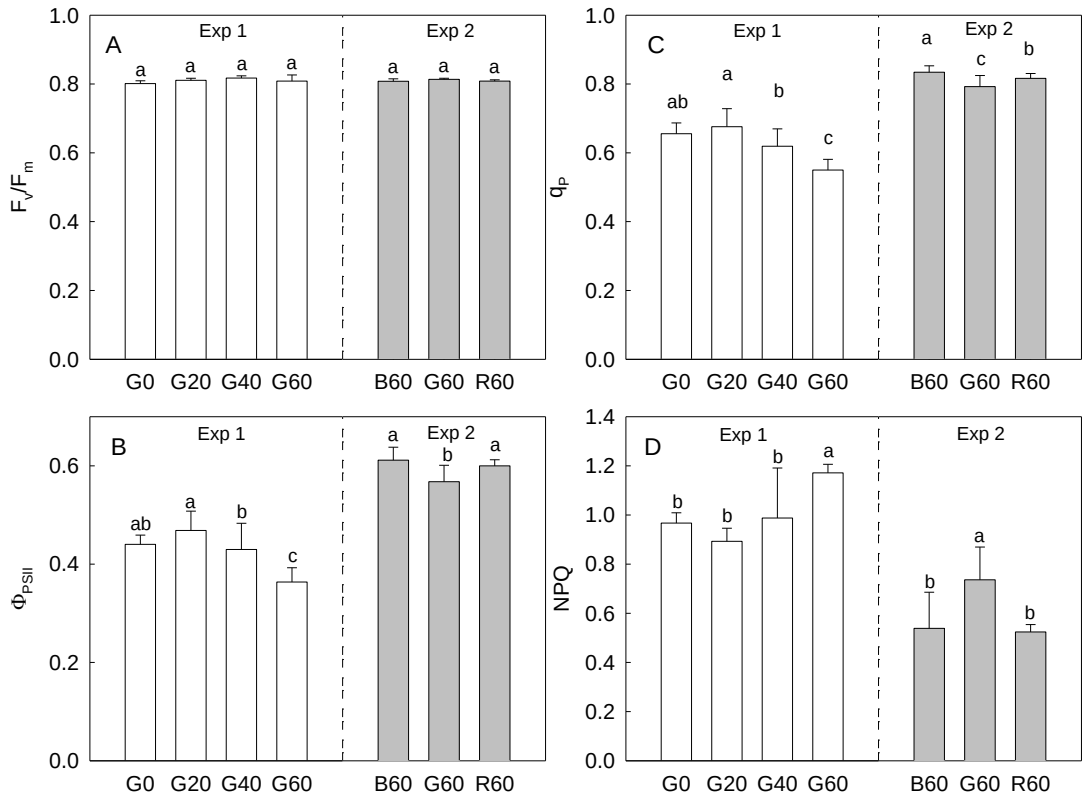


Figure IV-1. Effects of different light treatments on the maximal photochemical efficiency of PSII (F_v/F_m, A), relative quantum efficiency of PSII photochemistry (Φ_{PSII}, B), photochemical quenching (q_P, C), and non-photochemical quenching (NPQ, D). Values are the means of three replicates with standard errors shown by vertical bars. Experiments 1 and 2 were analyzed separately. Different letters represent statistically different means (P <0.05).

Table IV-1. Photon flux densities in each treatment.

Light treatment	Red	Green	Blue	Total
		(μmol m^{-2} s^{-1})		
Exp. 1				
G0	40	0	40	80
G20	40	20	40	100
G40	40	40	40	120
G60	40	60	40	140
Exp. 2				
B60	40	40	60	140
G60	40	60	40	140
R60	60	40	40	140

Table IV-2. Growth and morphological traits of rice seedlings grown under different light treatments.

Light treatment	Plant height (cm)	Stem diameter (cm)	Shoot biomass (g)	Root length (cm)	Root biomass (g)	Blade/sheath ratio (cm/cm)	Leaf angle (degree)
Exp. 1							
G0	15.0b	0.19a	0.029b	8.2 b	0.013b	1.26 a	18.2a
G20	14.7b	0.18a	0.028b	7.9 b	0.013b	1.25 a	17.6a
G40	16.0a	0.18a	0.033a	9.0 a	0.015b	1.14 b	11.1b
G60	16.1a	0.18a	0.036a	8.7 ab	0.018a	1.19 ab	18.7a
Exp. 2							
B60	15.74b	0.16a	0.035a	7.7 a	0.019a	1.19 b	22.0b
G60	17.22a	0.18a	0.036a	7.6 a	0.019a	1.13 c	14.5c
R60	16.61a	0.16a	0.034a	7.9 a	0.017b	1.24 a	29.0a

Biomass is the total weight of three seedlings. Experiments 1 and 2 were analyzed separately. Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table IV-3. Effects of different light treatments on Chl, Car, and their ratios in seedling leaves.

Light treatment	Total Chls (mg g^{-1}DW)	Chl *a/b*	Car (mg g^{-1}DW)	Car/Chl
Exp. 1				
G0	17.07 a	2.31 b	6.45a	0.38b
G20	17.13 a	2.33 b	6.50a	0.38b
G40	16.06 b	2.45 ab	6.36a	0.40a
G60	15.70 b	2.55 a	6.35a	0.41a
Exp. 2				
B60	15.48 ab	2.63 a	6.28a	0.41a
G60	15.26 b	2.50 ab	6.14a	0.40a
R60	16.47 a	2.41 b	6.42a	0.39b

Experiments 1 and 2 were analyzed separately. Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table IV-4. Effects of different light treatments on the levels and mole percentages of porphyrins in seedling leaves.

Light treatment	Porphyrin (μmol g^{-1} DW)	Mole percent of porphyrin (%) PPIX	MGPP	Pchlide
Exp. 1				
G0	1.62 a	71.9a	28.2a	n.d.
G20	1.24 a	66.3a	31.7a	2.0
G40	1.54 a	69.0a	31.0a	n.d.
G60	1.63 a	70.4a	29.4a	0.2
Exp. 2				
B60	1.09 a	71.4a	28.6a	n.d.
G60	1.20 a	78.0a	22.0a	n.d.
R60	1.22 a	67.9a	25.4a	6.6

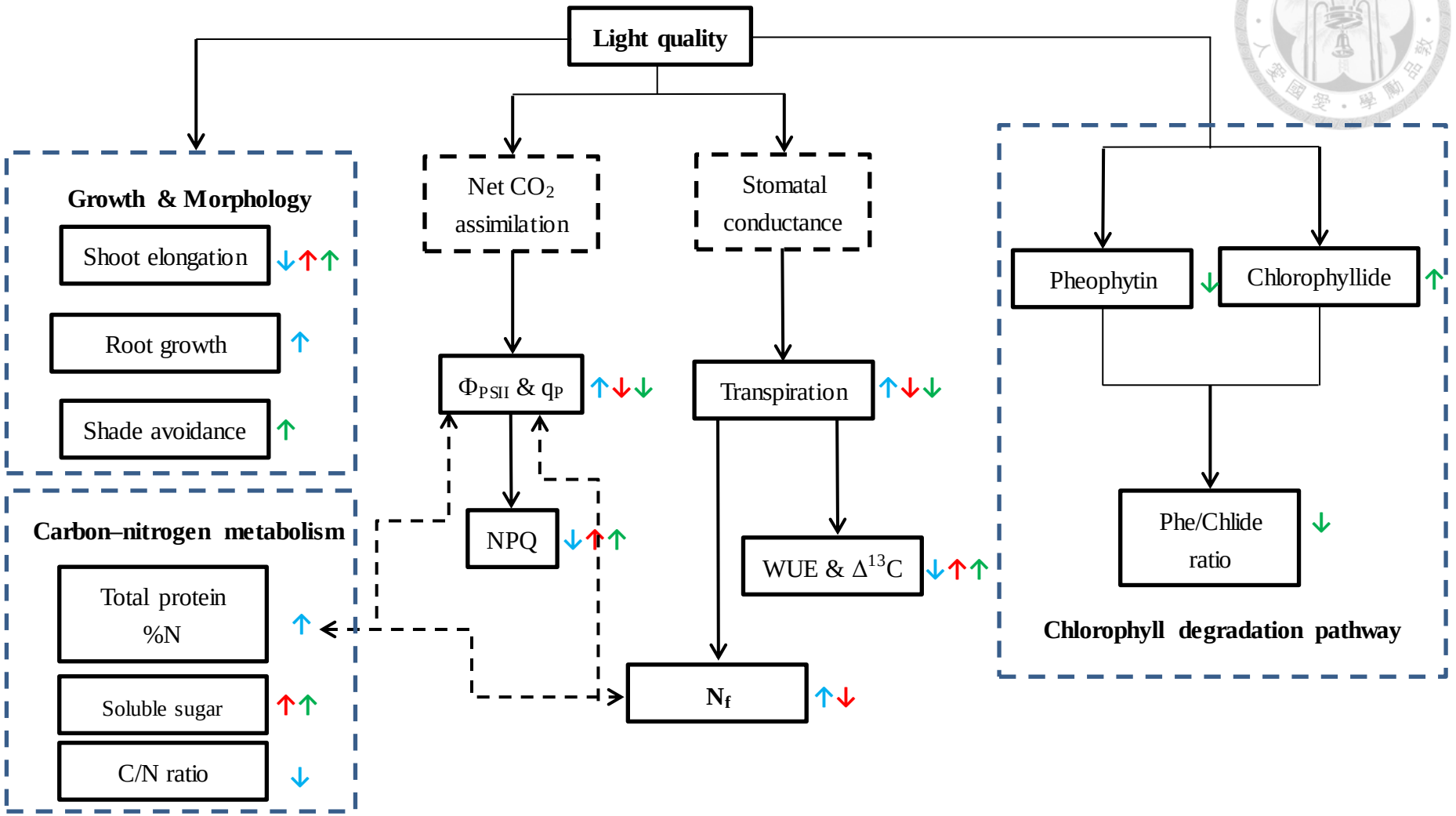
Experiments 1 and 2 were analyzed separately. Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table IV-5. Effects of different light treatments on Chlide, Phe, phytylated, and dephytylated pigments, and their ratios in seedling leaves.

Light treatment	Chlide (mmole g^{-1} DW)	Phe (mg g^{-1} DW)	Phe/Chlide (g/mol)	Phytylated (A661 g^{-1}DW)	Dephytylated (A666^{-1}DW)	Phytylated/ Dephytylated
Exp. 1						
G0	0.69 a	5.34 a	7.71 a	926a	24a	38.55 ab
G20	0.75 a	5.41 a	7.56 a	945a	24a	40.16 a
G40	0.80 a	5.05 a	6.33 ab	887a	24a	37.66 ab
G60	1.07 a	5.07 a	5.23 b	904a	37a	28.64 b
Exp. 2						
B60	0.38 a	4.82 ab	13.13 a	794a	13a	63.15 a
G60	0.43 a	4.57 b	11.34 a	770a	15a	54.13 a
R60	0.43 a	5.17 a	12.53 a	822a	14a	61.49 a

Experiments 1 and 2 were analyzed separately. Within columns, means followed by the same letter are not significantly different according to LSD (0.05)

Graphical Summary



References

Abidi F., Girault T., Douillet O., Guillemain G., Sintes G., Laffaire M., Ahmed H.B., Smiti S., Huché-Thélier L., Leduc N. (2013) Blue light effects on rose photosynthesis and photomorphogenesis. Plant Biology 15:67-74.

Bourget C.M. (2008) An introduction to light-emitting diodes. Hortscience 43:1944-1946.

Bradford M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry 72:248-254.

Cabrera-Bosquet L., Molero G., Bort J., Nogués S., Araus J. (2007) The combined effect of constant water deficit and nitrogen supply on WUE, NUE and $\Delta^{13}C$ in durum wheat potted plants. Annals of Applied Biology 151:277-289.

Calatayud A., Barreno E. (2004) Response to ozone in two lettuce varieties on chlorophyll *a* fluorescence, photosynthetic pigments and lipid peroxidation. Plant Physiology and Biochemistry 42:549-555.

Chen C.C., Huang M.Y., Lin K.H., Wong S.L., Huang W.D. (2014a) Effects of light quality on the growth, development and metabolism of rice seedlings (*Oryza sativa* L.). Research Journal of Biotechnology 9:15-24.

Chen C.C., Huang W.D., Yang C.M. (2014b) Light quality influences the chlorophyll degradation pathway in rice seedling leaves. (submitted).

Chen J., Chang S.X., Anyia A.O. (2012) Quantitative trait loci for water-use efficiency in barley (*Hordeum vulgare* L.) measured by carbon isotope discrimination under rain-fed conditions on the Canadian Prairies. Theoretical and Applied Genetics 125:71-90. DOI: 10.1007/s00122-012-1817-7.

Chen M., Chory J., Fankhauser C. (2004) Light signal transduction in higher plants. Annual Review of Genetics 38:87-117.

Clouse S.D. (2001) Integration of light and brassinosteroid signals in etiolated seedling growth. Trends in plant science 6:443-445.

Condon A., Richards R., Farquhar G. (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. Crop Science 27:996-1001.

Condon A., Richards R., Rebetzke G., Farquhar G. (2004) Breeding for high water-use efficiency. Journal of Experimental Botany 55:2447-2460.

Costa B.S., Jungandreas A., Jakob T., Weisheit W., Mittag M., Wilhelm C. (2013) Blue light is essential for high light acclimation and photoprotection in the diatom *Phaeodactylum tricornutum*. Journal of Experimental Botany 64:483-493.

Counce P.A., Keisling T.C., Mitchell A.J. (2000) A uniform, objective, and adaptive

system for expressing rice development. Crop Science 40:436-443.

Dalal R.C., Strong W.M., Cooper J.E., King A.J. (2013) Relationship between water use and nitrogen use efficiency discerned by ^{13}C discrimination and ^{15}N isotope ratio in bread wheat grown under no-till. Soil and Tillage Research 128:110-118. DOI: 10.1016/j.still.2012.07.019.

Demarsac N.T., Houmard J. (1993) Adaptation of Cyanobacteria to Environmental Stimuli - New Steps Towards Molecular Mechanisms. Fems Microbiology Letters 104:119-189.

Dong C., Fu Y., Liu G., Liu H. (2014) Growth, photosynthetic characteristics, antioxidant capacity and biomass yield and quality of wheat (*Triticum aestivum* L.) exposed to LED light sources with different spectra combinations. Journal of Agronomy and Crop Science 200:219-230. DOI: 10.1111/jac.12059.

Eskins K., Jiang C.Z., Shibles R. (1991) Light-quality and irradiance effects on pigments, light-harvesting proteins and Rubisco activity in a chlorophyll-and light-harvesting-deficient soybean mutant. Physiologia Plantarum 83:47-53.

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

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

Fan X., Zang J., Xu Z., Guo S., Jiao X., Liu X., Gao Y. (2013) Effects of different light quality on growth, chlorophyll concentration and chlorophyll biosynthesis precursors of non-heading Chinese cabbage (*Brassica campestris* L.). Acta Physiologiae Plantarum 35:2721-2726. DOI: 10.1007/s11738-013-1304-z.

Farquhar G., Richards R. (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Functional Plant Biology 11:539-552.

Farquhar G.D., Ehleringer J.R., Hubick K.T. (1989) Carbon isotope discrimination and photosynthesis. Annual review of plant biology 40:503-537.

Farquhar G.D., O'leary M., Berry J. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Functional Plant Biology 9:121-137.

Felker F.C., Doehlert D.C., Eskins K. (1995) Effects of red and blue light on the composition and morphology of maize kernels grown *in vitro*. Plant Cell, Tissue and Organ Culture 42:147-152.

Fu W., Li P., Wu Y. (2012) Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. Scientia Horticulturae 135:45-51.

Glenn D.M. (2014) An analysis of ash and isotopic carbon discrimination ($\Delta^{13}C$)

methods to evaluate water use efficiency in apple. Scientia Horticulturae 171:32-36.

Grant O.M., Davies M.J., Johnson A.W., Simpson D.W. (2012) Physiological and growth responses to water deficits in cultivated strawberry (*Fragaria×ananassa*) and in one of its progenitors, *Fragaria chiloensis*. Environmental and Experimental Botany 83:23-32. DOI: 10.1016/j.envexpbot.2012.04.004.

Guo R.Q., Ruan H., Yang W.J., Liu B., Sun S.C. (2011a) Differential responses of leaf water-use efficiency and photosynthetic nitrogen-use efficiency to fertilization in Bt-introduced and conventional rice lines. Photosynthetica 49:507-514. DOI: 10.1007/s11099-011-0060-9.

Guo Y.S., Gu A.S., Cui J. (2011b) Effects of light quality on rice seedlings grow th and physiological characteristics. Chinese Journal of Applied Ecology 22:1485-1492.

Hogewoning S.W., Trouwborst G., Maljaars H., Poorter H., van Ieperen W., Harbinson J. (2010) Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of Cucumis sativus grown under different combinations of red and blue light. Journal of Experimental Botany 61:3107-17. DOI: 10.1093/jxb/erq132.

Holm G. (1954) Chlorophyll mutations in barley. Acta Agriculturae Scandinavica 4:457-471.

Hoober J.K., Eggink L.L. (1999) Assembly of light-harvesting complex II and biogenesis of thylakoid membranes in chloroplasts. Photosynthesis Research 61:197-215.

Hsu M.H., Huang W.D., Yang Z.W., Tsai Y.Z., Yang C.M., Chang S.-S. (2003) Study on the chlorophyyll biosynthetic and degradative pathway in the leaves of three sweet potatoes. Chinese Agronomy Journal 3:87-98.

Hsu M.H., Yang Z.W., Huang W.D., Yang C.M. (2011) Study on the Chlorophyll Biosynthetic and Degradative Pathway in the Leaves of Banana Infected with Fusarium Wilt. Scientific Agriculture, Taiwan 59:21-28.

Huang M.Y., Huang W.D., Chou H.M., Chen C.C., Chang Y.T., Yang C.M. (2014) Herbivorous insects alter the chlorophyll biosynthetic and degradation pathway of galls on host plant. Journal of Asia-Pacific Entomology 17:431-434.

Jilani A., Kar S., Bose S., Tripathy B.C. (1996) Regulation of the carotenoid content and chloroplast development by levulinic acid. Physiologia Plantarum 96:139-145.

Johkan M., Shoji K., Goto F., Hahida S., Yoshihara T. (2012) Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. Environmental and Experimental Botany 75:128-133. DOI: 10.1016/j.envexpbot.2011.08.010.

Johkan M., Shoji K., Goto F., Hashida S.-n., Yoshihara T. (2010) Blue light-emitting

diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. Hortscience 45:1809-1814.

Jung S., Steffen K.L., Jae Lee H. (1998) Comparative photoinhibition of a high and a low altitude ecotype of tomato (*Lycopersicon hirsutum*) to chilling stress under high and low light conditions. Plant Science 134:69-77.

Kami C., Lorrain S., Hornitschek P., Fankhauser C. (2010) Light-Regulated Plant Growth and Development. Current topics in developmental biology 91:29-66.

Kariola T., Brader G., Li J., Palva E.T. (2005) Chlorophyllase 1, a damage control enzyme, affects the balance between defense pathways in plants. Plant Cell 17:282-294. DOI: 10.1105/tpc.104.025817.

Kasajima S.-y., Inoue N., Mahmud R., Kato M. (2008) Developmental responses of wheat cv. Norin 61 to fluence rate of green light. Plant production science 11:76-81.

Keuskamp D.H., Sasidharan R., Vos I., Peeters A.J., Voesenek L.A., Pierik R. (2011) Blue-light-mediated shade avoidance requires combined auxin and brassinosteroid action in *Arabidopsis* seedlings. The Plant Journal 67:208-217.

Kim H.H., Goins G.D., Wheeler R.M., Sager J.C. (2004a) Green-light supplementation for enhanced lettuce growth under red-and blue-light-emitting diodes. Hortscience 39:1617-1622.

Kim H.H., Goins G.D., Wheeler R.M., Sager J.C. (2004b) Stomatal conductance of lettuce grown under or exposed to different light qualities. Annals of Botany 94:691-697.

Kim S.J., Hahn E.J., Heo J.W., Paek K.Y. (2004c) Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets in vitro. Scientia Horticulturae 101:143-151. DOI: 10.1016/j.scienta.2003.10.003.

Klein R.M. (1992) Effects of green light on biological systems. Biological Reviews 67:199-284.

Kooten O., Snel J.F. (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynthesis Research 25:147-150.

Kopsell D.A., Sams C.E. (2013) Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. Journal of the American Society for Horticultural Science 138:31-37.

Kurilčik A., Miklušytė-Čanova R., Dapkūnienė S., Žilinskaitė S., Kurilčik G., Tamulaitis G., Duchovskis P., Žukauskas A. (2008) *In vitro* culture of *Chrysanthemum* plantlets using light-emitting diodes. Central European Journal of Biology 3:161-167. DOI: 10.2478/s11535-008-0006-9.

Lee S.H., Tewari R.K., Hahn E.J., Paek K.Y. (2007) Photon flux density and light quality induce changes in growth, stomatal development, photosynthesis and

transpiration of *Withania Somnifera* (L.) Dunal. plantlets. Plant Cell, Tissue and Organ Culture 90:141-151. DOI: 10.1007/s11240-006-9191-2.

Leong T.Y., Anderson J.M. (1984) Adaptation of the thylakoid membranes of pea chloroplasts to light intensities. I. Study on the distribution of chlorophyll-protein complexes. Photosynthesis Research 5:105-115.

Li H., Tang C., Xu Z. (2013) The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis *in vitro*. Scientia Horticulturae 150:117-124.

Li H., Xu Z., Tang C. (2010) Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets in vitro. Plant Cell, Tissue and Organ Culture 103:155-163.

Li Q., Kubota C. (2009) Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environmental and Experimental Botany 67:59-64. DOI: 10.1016/j.envexpbot.2009.06.011.

Lin Y., Li J., Li B., He T., Chun Z. (2011) Effects of light quality on growth and development of protocorm-like bodies of *Dendrobium officinale in vitro*. Plant Cell, Tissue and Organ Culture 105:329-335. DOI: 10.1007/s11240-010-9871-9.

Liu B.H., Cheng L., Liang D., Zou Y.J., Ma F.W. (2012) Growth, gas exchange, water-use efficiency, and carbon isotope composition of 'Gale Gala' apple trees grafted onto 9 wild Chinese rootstocks in response to drought stress. Photosynthetica 50:401-410. DOI: 10.1007/s11099-012-0048-0.

Liu M.X., Xu Z.G., Yang Y., Feng Y.J. (2011) Effects of different spectral lights on *Oncidium* PLBs induction, proliferation, and plant regeneration. Plant Cell, Tissue and Organ Culture 106:1-10. DOI: 10.1007/s11240-010-9887-1.

Loreto F., Tsonev T., Centritto M. (2009) The impact of blue light on leaf mesophyll conductance. Journal of Experimental Botany 60:2283-2290. DOI: 10.1093/jxb/erp112.

Macedo A.F., Leal-Costa M.V., Tavares E.S., Lage C.L.S., Esquibel M.A. (2011) The effect of light quality on leaf production and development of in vitro-cultured plants of *Alternanthera brasiliana* Kuntze. Environmental and Experimental Botany 70:43-50. DOI: 10.1016/j.envexpbot.2010.05.012.

Maevskaya S., Bukhov N. (2005) Effect of light quality on nitrogen metabolism of radish plants. Russian Journal of Plant Physiology 52:304-310.

Mancinelli A., Yang C.-P.H., Lindquist P., Anderson O., Rabino I. (1975) Photocontrol of anthocyanin synthesis III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant Physiology 55:251-257.

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

Matile P., Hortensteiner S., Thomas H., Krautler B. (1996) Chlorophyll Breakdown in

Senescent Leaves. Plant Physiology 112:1403-1409.

Matsuda R., Ohashi-Kaneko K., Fujiwara K., Goto E., Kurata K. (2004) Photosynthetic characteristics of rice leaves grown under red light with or without supplemental blue light. Plant and cell physiology 45:1870-1874.

Matsuda R., Ohashi-Kaneko K., Fujiwara K., Kurata K. (2007) Analysis of the relationship between blue-light photon flux density and the photosynthetic properties of spinach (*Spinacia oleraceaL*.) leaves with regard to the acclimation of photosynthesis to growth irradiance. Soil Science and Plant Nutrition 53:459-465. DOI: 10.1111/j.1747-0765.2007.00150.x.

McCree K. (1972) Test of current definitions of photosynthetically active radiation against leaf photosynthesis data. Agricultural Meteorology 10:443-453.

Moghaddam A., Raza A., Vollmann J., Ardakani M., Wanek W., Gollner G., Friedel J. (2013) Carbon isotope discrimination and water use efficiency relationships of alfalfa genotypes under irrigated and rain-fed organic farming. European Journal of Agronomy 50:82-89.

Monclus R., Leple J.C., Bastien C., Bert P.F., Villar M., Marron N., Brignolas F., Jorge V. (2012) Integrating genome annotation and QTL position to identify candidate genes for productivity, architecture and water-use efficiency in *Populus* spp. BMC Plant Biology 12:173-187. DOI: 10.1186/1471-2229-12-173.

Moore S., Stein W.H. (1948) Photometric ninhydrin method for use in the chromatography of amino acids. Journal of biological chemistry 176:367-388.

Morris D.L. (1948) Quantitative determination of carbohydrates with Dreywood's anthrone reagent. Science 107:254-255.

Morrow R.C. (2008) LED lighting in horticulture. Hortscience 43:1947-1950.

Motzo R., Pruneddu G., Giunta F. (2013) The role of stomatal conductance for water and radiation use efficiency of durum wheat and triticale in a Mediterranean environment. European Journal of Agronomy 44:87-97. DOI: 10.1016/j.eja.2012.09.002.

Murakami K., Matsuda R., Fujiwara K. (2014) Light-induced systemic regulation of photosynthesis in primary and trifoliate leaves of *Phaseolus vulgaris*: effects of photosynthetic photon flux density (PPFD) *versus* spectrum. Plant Biology. DOI: 10.1111/plb.12055.

Nhut D.T., Takamura T., Watanabe H., Okamoto K., Tanaka M. (2003a) Responses of strawberry plantlets cultured in vitro under superbright red and blue light-emitting diodes (LEDs). Plant Cell, Tissue and Organ Culture 73:43-52. DOI: 10.1023/A:1022638508007.

Nhut D.T., Takamura T., Watanabe H., Tanaka M. (2003b) Efficiency of a novel culture system by using light-emitting diode (LED) on in vitro and subsequent growth of micropropagated banana plantlets. Acta Horticulturae 161:121-127.

Ni X.Z., Quisenberry S.S., Heng-Moss T., Markwell J., Higley L., Baxendale F., Sarath G., Klucas R. (2002) Dynamic change in photosynthetic pigments and chlorophyll degradation elicited by cereal aphid feeding. Entomologia Experimentalis Et Applicata 105:43-53. DOI: 10.1046/j.1570-7458.2002.01031.x.

Nishio J. (2000) Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. Plant, Cell and Environment 23:539-548.

Ohashi-Kaneko K., Fujiwara K., Kimura Y., Kurata K. (2006a) Effect of blue-light PPFD percentage in red and blue LED low-light irradiation during storage on the contents of chlorophyll and Rubisco in grafted tomato plug seedlings. Environment control in biology 44:309-314.

Ohashi-Kaneko K., Matsuda R., Goto E., Fujiwara K., Kurata K. (2006b) Growth of rice plants under red light with or without supplemental blue light. Soil Science and Plant Nutrition 52:444-452. DOI: 10.1111/j.1747-0765.2006.00063.x.

Pfannschmidt T., Nilsson A., Allen J.F. (1999) Photosynthetic control of chloroplast gene expression. Nature 397:625-628.

Porra R., Thompson W., Kriedemann P. (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta - Bioenergetics 975:384-394.

Poudel P.R., Kataoka I., Mochioka R. (2008) Effect of red- and blue-light-emitting diodes on growth and morphogenesis of grapes. Plant Cell, Tissue and Organ Culture 92:147-153. DOI: 10.1007/s11240-007-9317-1.

Rivkin R.B. (1989) Influence of Irradiance and Spectral Quality on the Carbon Metabolism of Phytoplankton .1. Photosynthesis, Chemical-Composition and Growth. Marine Ecology Progress Series 55:291-304. DOI: 10.3354/Meps055291.

Sæbø A., Krekling T., Appelgren M. (1995) Light quality affects photosynthesis and leaf anatomy of birch plantlets in vitro. Plant Cell, Tissue and Organ Culture 41:177-185.

Savvides A., Fanourakis D., van Ieperen W. (2012) Co-ordination of hydraulic and stomatal conductances across light qualities in cucumber leaves. Journal of Experimental Botany 63:1135-1143. DOI: 10.1093/jxb/err348.

Shin K.S., Murthy H.N., Heo J.W., Hahn E.J., Paek K.Y. (2008) The effect of light quality on the growth and development of in vitro cultured *Doritaenopsis* plants. Acta Physiologiae Plantarum 30:339-343.

Sivakumar G., Heo J., Kozai T., Paek K. (2006) Effect of continuous or intermittent radiation on sweet potato plantlets in vitro. Journal of Horticultural Science and

Biotechnology 81:546-548.

Somersalo S., Krause G. (1989) Photoinhibition at chilling temperature. Planta 177:409-416.

Sood S., Gupta V., Tripathy B.C. (2005) Photoregulation of the greening process of wheat seedlings grown in red light. Plant Molecular Biology 59:269-287. DOI: 10.1007/s11103-005-8880-2.

Spalding E., Folta K. (2005) Illuminating topics in plant photobiology. Plant, Cell and Environment 28:39-53.

Stamm P., Kumar P.P. (2010) The phytohormone signal network regulating elongation growth during shade avoidance. Journal of Experimental Botany 61:2889-2903.

Takahashi K., Fujino K., Kikuta Y., Koda Y. (1995) Involvement of the accumulation of sucrose and the synthesis of cell wall polysaccharides in the expansion of potato cells in response to jasmonic acid. Plant Science 111:11-18.

Takai T., Ohsumi A., San-oh Y., Laza M.R., Kondo M., Yamamoto T., Yano M. (2009) Detection of a quantitative trait locus controlling carbon isotope discrimination and its contribution to stomatal conductance in *japonica* rice. Theoretical and Applied Genetics 118:1401-1410. DOI: 10.1007/s00122-009-0990-9.

Tambussi E., Bort J., Araus J. (2007) Water use efficiency in C_3 cereals under Mediterranean conditions: a review of physiological aspects. Annals of Applied Biology 150:307-321.

Tanaka A., Ito H., Tanaka R., Tanaka N.K., Yoshida K., Okada K. (1998a) Chlorophyll a oxygenase (CAO) is involved in chlorophyll b formation from chlorophyll a. Proceedings of the National Academy of Sciences of the United States of America 95:12719-12723. DOI: 10.1073/pnas.95.21.12719.

Tanaka M., Takamura T., Watanabe H., Endo M., Yanagi T., Okamoto K. (1998b) In vitro growth of *Cymbidium* plantlets cultured under superbright red and blue light-emitting diodes (LEDs). Journal of Horticultural Science and Biotechnology 73:39-44 .

This D., Comstock J., Courtois B., Xu Y., Ahmadi N., Vonhof W.M., Fleet C., Setter T., McCouch S. (2010) Genetic analysis of water use efficiency in rice (*Oryza sativa* L.) at the leaf level. Rice 3:72-86.

Wang H., Gu M., Cui J.X., Shi K., Zhou Y.H., Yu J.Q. (2009) Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. Journal of Photochemistry and Photobiology B: Biology 96:30-37. DOI: 10.1016/j.jphotobiol.2009.03.010.

Wang T., Quisenberry S.S., Ni X.Z., Tolmay V. (2004) Enzymatic chlorophyll degradation in wheat near-isogenic lines elicited by cereal aphid (Homoptera : Aphididae) feeding. Journal of Economic Entomology 97:661-667. DOI:

10.1603/0022-0493-97.2.661.

Wang Y., Folta K.M. (2013) Contributions of green light to plant growth and development. American Journal of Botany 100:70-78. DOI: 10.3732/ajb.1200354.

Wang Y., Maruhnich S.A., Mageroy M.H., Justice J.R., Folta K.M. (2013a) Phototropin 1 and cryptochrome action in response to green light in combination with other wavelengths. Planta 237:225-237. DOI: 10.1007/s00425-012-1767-y.

Wang Y., Zhang X., Liu X., Zhang X., Shao L., Sun H., Chen S. (2013b) The effects of nitrogen supply and water regime on instantaneous WUE, time-integrated WUE and carbon isotope discrimination in winter wheat. Field Crops Research 144:236-244. DOI: 10.1016/j.fcr.2013.01.021.

Winkel-Shirley B. (2001) Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiology 126:485-493.

Wu M.C., Hou C.Y., Jiang C.M., Wang Y.T., Wang C.Y., Chen H.H., Chang H.M. (2007) A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. Food Chemistry 101:1753-1758.

Yang C.M., Chang K.W., Yin M.H., Huang H.M. (1998) Methods for the determination of chlorophylls and their derivatives. Taiwania 43:116-122.

Yang C.M., Yang M.M., Hsu J.M., Jane W.N. (2003) Herbivorous insect causes deficiency of pigment-protein complexes in an oval-pointed cecidomyiid gall of *Machilus thunbergii* leaf. Botanical Bulletin of Academia Sinica 44:315-321.

Yang Z.W., Huang W.D., Yang C.M. (2012) Study on the Chlorophyll Biosynthetic and Degradative Pathways in the Leaves of Paddy Rice. Bulletin of the Taoyuan District Agricultural Research and Extension Station,COA 71:17-34.

Yasir T.A., Min D., Chen X., Condon A.G., Hu Y.-G. (2013) The association of carbon isotope discrimination (Δ) with gas exchange parameters and yield traits in Chinese bread wheat cultivars under two water regimes. Agricultural Water Management 119:111-120. DOI: 10.1016/j.agwat.2012.11.020.

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

Yu H., Ong B.-L. (2003) Effect of radiation quality on growth and photosynthesis of *Acacia mangium* seedlings. Photosynthetica 41:349-355.

Zhang T., Maruhnich S.A., Folta K.M. (2011) Green light induces shade avoidance symptoms. Plant Physiology 157:1528-1536. DOI: 10.1104/pp.111.180661.