Plant Cell Physiol. 45(12): 1870–1874 (2004)

Short Communication

Photosynthetic Characteristics of Rice Leaves Grown under Red Light with or without Supplemental Blue Light

Ryo Matsuda [1], Keiko Ohashi-Kaneko, Kazuhiro Fujiwara, Eiji Goto [2] and Kenji Kurata

Department of Biological and Environmental Engineering, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, 113-8657 Japan

In rice plants grown under red light supplemented with blue light (red/blue-light PPFD ratio was 4/1), photosynthetic rates per unit leaf area measured under white light at 1,600 and 250 μmol m^{-2} s^{-1} were higher than those in the plants grown under red light alone. The higher photosynthetic rates were associated with higher total N content of leaves, which was accompanied by larger amounts of key components of photosynthesis-limiting processes, including Rubisco, Cyt *f*, Chl and LHCII. These results suggested that the increase in total N content of leaves induced by supplemental blue light enhanced both light-saturated and light-limited photosynthesis.

Keywords: Blue light — Gas exchange (photosynthesis) — Light-emitting diode (LED) — Light quality — Nitrogen (leaf) — Rice (*Oryza sativa* L.).

Abbreviations: LED, light-emitting diode; LHCII, light-harvesting Chl *a*/*b*-binding protein of PSII; pCa, atmospheric CO_2 partial pressure; PPFD, photosynthetic photon flux density; R, red light; RB, red light supplemented with blue light; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; SPD, spectral photon-number distribution.

Light quality (spectral distribution of light) affects various characteristics of plants. Blue light is vital for the growth and development of higher plants, because blue-light photoreceptors participate in many events of photomorphogenesis (for reviews, see Briggs and Huala 1999, Christie and Briggs 2001). Furthermore, supplementing red light from light-emitting diodes (LEDs) with blue light promotes dry matter production in several plant species, including pepper (Brown et al. 1995), wheat (Goins et al. 1997), spinach, radish and lettuce (Yorio et al. 2001). These results suggest that blue light plays important roles in both photomorphogenesis and dry matter production. Blue light generally promotes stomatal opening more than other light wavelengths (Sharkey and Raschke 1981, Karlsson 1986), and this stimulation of stomatal opening may contribute to the enhancement of dry matter production. In fact, Goins et al. (1997) observed in wheat that photosynthetic rates were higher along with the increase in stomatal conductance in leaves under red-LED light supplemented with blue light. They suggested that the increase in photosynthetic rate by increased stomatal conductance may be related to enhancement of dry matter accumulation under red-LED light by supplemental blue light. However, Yorio et al. (2001) reported that stomatal opening was stimulated but photosynthesis was not enhanced in leaves of lettuce under red-LED light supplemented with blue light. Thus, the effects of blue light on dry matter productivity and leaf photosynthesis remain unclear, and there may be variation with plant species.

On the other hand, several studies have shown that blue light influences the biochemical properties of photosynthesis in leaves. Plants grown under blue fluorescent lamps had higher Chl *a*/*b* ratios (for a review, see Senger and Bauer 1987), smaller amounts of light-harvesting Chl *a*/*b*-binding protein of PSII (LHCII) per unit Chl content (Leong and Anderson 1984) or per unit total protein content (Eskins et al. 1991), higher photosynthetic electron-transport activities per unit Chl content (Leong and Anderson 1984) and higher ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) activities per unit leaf area (Eskins et al. 1991) than plants grown under red fluorescent lamps. However, it is not known whether such changes in the biochemical properties of photosynthesis are responsible for the differences in gas exchange between the leaves of plants grown under red light with or without supplemental blue light.

In the present study we investigated how supplementing red light with blue light during growth affects the photosynthetic characteristics of leaves. We grew rice (*Oryza sativa* L.) plants hydroponically under red light alone (R) or supplemented with blue light (RB) in nutrient solutions with 0.5, 2 or 8 mM N and examined the photosynthetic characteristics of young, fully expanded leaf blades. Because leaf photosynthesis is strongly dependent on total leaf N content and N partitioning among photosynthetic components (for reviews, see Evans 1989, Terashima and Hikosaka 1995), we evaluated gas-

[1] Corresponding author: E-mail, aa47070@mail.ecc.u-tokyo.ac.jp; Fax, +81-3-5841-8172.
[2] Present address: Department of Bioproduction Science, Faculty of Horticulture, Chiba University, 648 Matsudo, Matsudo, Chiba, 271-8510 Japan.

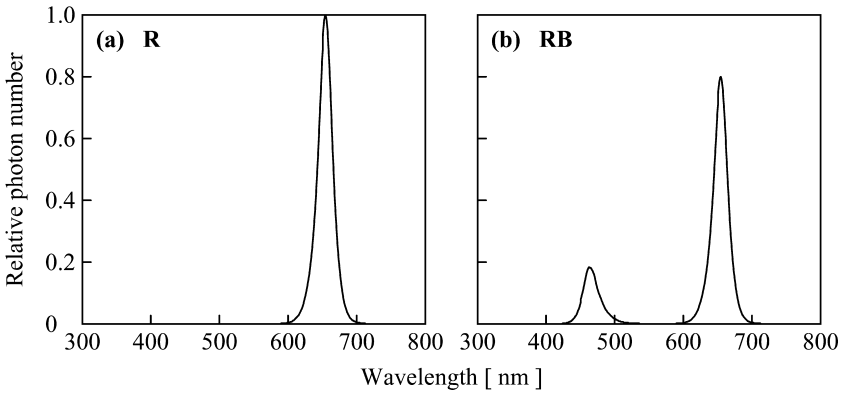

Fig. 1 Relative SPDs of R (a) and RB (b). Light was provided from red and blue LEDs. The number of photons was counted for every 1 nm.

exchange characteristics in relation to total leaf N content and N partitioning among photosynthetic components. Rice was used as the plant material because the response of photosynthesis to leaf N content has been characterized in detail in this plant (for a review, see Makino 2003).

As a light source, red and blue LEDs, which characteristically have narrow bandwidths, were used to avoid contamination with undesired wave bands. Fig. 1 shows the spectral photon-number distributions (SPDs) of R and RB. For RB, blue light was mixed with red light at 20% of the total photosynthetic photon flux density (PPFD). This ratio was determined based on the suggestion that 1–10% blue light was effective in enhancing dry matter production and leaf photosynthesis (Brown et al. 1995, Goins et al. 1997, Yorio et al. 2001). PPFD of both R and RB was adjusted to 240 μmol m^{-2} s^{-1} at the tops of the plants. The photon flux densities absorbed by the leaf blade of rice, calculated from Fig. 1 and the spectral absorptance from 400 to 700 nm in the leaf blade, was about 210 μmol m^{-2} s^{-1} for either R or RB. This means that the number of photons available for photochemical reactions was nearly the same in the plants under R and RB. In addition, phytochrome photoequilibria, the ratios of active phytochrome (P_{FR}) to total phytochrome (P_{FR} + P_R), under R and RB were nearly the same. The calculated values of phytochrome photoequilibria were 0.89 and 0.88 under R and RB, respectively. Therefore, differences in the reversible action of phytochrome between the plants under R and RB were negligible.

We first measured light-saturated and light-limited rates of photosynthesis per unit leaf area at PPFDs of 1,600 and 250 μmol m^{-2} s^{-1}, respectively, and an atmospheric CO_2 partial pressure (pCa) of 36 Pa. A white halogen lamp was used as the measurement light source. The light-saturated rate of photosynthesis in the RB-grown plants was 88% higher than that in the R-grown plants in the nutrient solution with 2 mM N (Table 1). The light-limited rate of photosynthesis was also 53% higher in the RB-grown plants than in the R-grown plants (Table 1). The RB-grown plants had a significantly higher total leaf N content in addition to higher rates of photosynthesis than the R-grown plants (Table 1). Similar differences in the photosynthetic rates and total leaf N content between the plants grown under R and RB were observed when the plants were grown in a nutrient solution containing 0.5 or 8 mM N (data not shown).

The light-saturated photosynthetic rate in the plants grown in the nutrient solutions with three N concentrations was linearly correlated with total leaf N content in each light treatment (Fig. 2a). Although the *y*-intercept of the regression line for the RB-grown plants was significantly different from that for the R-grown plants, the higher total leaf N content in the RB-grown plants greatly contributed to the higher photosynthetic rate. The light-limited photosynthetic rates plotted against total leaf N content regressed to the same line in both R- and RB-grown plants (Fig. 2b). The slope of the regression line under light-limiting conditions (250 μmol m^{-2} s^{-1}) tended to be gentler than that under light-saturating conditions (1,600 μmol m^{-2} s^{-1}).

Table 1 Net photosynthetic rates and total N content per unit leaf area in rice plants grown under R or RB in a nutrient solution with 2 mM N

Light treatment	Net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) [a, b]		Total leaf N content (mmol m^{-2}) [a]
	1,600 μmol m^{-2} s^{-1} [c]	250 μmol m^{-2} s^{-1} [c]	
R	5.8 ± 1.08*	4.04 ± 0.442*	49.9 ± 0.98*
RB	10.9 ± 1.33*	6.18 ± 0.646*	57.1 ± 2.38*

[a] Mean ± standard errors (n = 4–6). Means in each column with an asterisk (*) are significantly different at the 5% level by t test.

[b] Measurements were made at a pCa of 36 Pa, a leaf temperature of 27°C, and a leaf-to-air vapor pressure difference of 1.1 ± 0.1 kPa.

[c] Measurement PPFD. Light was provided by a white halogen lamp.

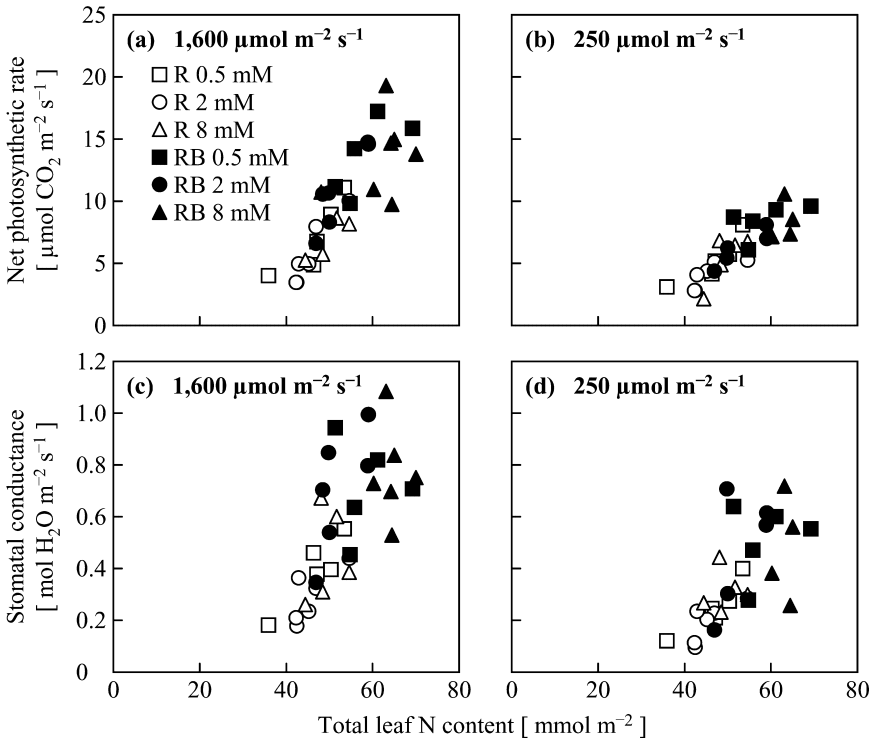

Fig. 2 Net photosynthetic rates (a, b) and stomatal conductances (c, d) per unit leaf area versus total N content per unit leaf area in rice plants grown under R (open circle) or RB (closed triangle) in a nutrient solution with 0.5 (square), 2 (circle) or 8 (triangle) mM N. Measurements were made at a PPFD of 1,600 (a, c) or 250 (b, d) µmol m^{-2} s^{-1}, a pCa of 36 Pa, a leaf temperature of 27°C, and a leaf-to-air vapor pressure difference of 1.1 ± 0.1 kPa. Light for measurements was provided from a white halogen lamp. For (a), $y = 0.414x - 12.7$, $r^2 = 0.66$ (R); $y = 0.307x - 5.62$, $r^2 = 0.33$ (RB). For (b), $y = 0.238x - 6.27$, $r^2 = 0.74$ (R and RB). Separate regression equations were given for R and RB when the slopes or *y*-intercepts were significantly different at the 5% level by *F* test in the analysis of covariance; otherwise a single regression equation was given.

Moreover, although the mean value of stomatal conductance in the RB-grown plants was higher than that in the R-grown plants under either measurement PPFD (data not shown), higher stomatal conductance in the RB-grown plants was associated with a higher total leaf N content (Fig. 2c, d). These results suggest that enhancement of both light-saturated and light-limited photosynthesis by supplemental blue light primarily results from the increase in total leaf N content. Hirose and Werger (1987) found that the positive correlation between photosynthetic rate and leaf N content was gradually lost as the PPFD lowered. This trend may reflect the fact that light-limited photosynthesis is not necessarily limited by leaf N content (Hirose and Werger 1987, Makino et al. 1997b). In rice, however, a positive correlation between photosynthetic rate and leaf N content was observed at 350 µmol m^{-2} s^{-1} PPFD, particularly in leaves with a low N content (Makino et al. 1997a, Makino et al. 1997b). In this study, both R- and RB-grown plants had a relatively low total leaf N content, and therefore the photosynthetic rate measured at 250 µmol m^{-2} s^{-1} PPFD may be positively correlated with total leaf N content.

We next compared N allocations to several key components of photosynthesis-limiting processes in the RB-grown plants with those in the R-grown plants. We determined the amounts of Rubisco, a key enzyme of RuBP carboxylation, Cyt *f*, a rate-limiting factor for electron transport, and Chl and LHCII, light-harvesting components (Makino et al. 1994). The mean amounts of all measured photosynthetic components were significantly larger in the RB-grown plants than in the R-grown plants under all N-treatment conditions (data not shown). The larger amounts of photosynthetic components in the RB-grown plants than in the R-grown plants were mainly caused by the higher total leaf N content (Fig. 3), as was the case for the higher photosynthetic rate caused by RB irradiation. For Rubisco, Chl and LHCII, there were significant differences in the *y*-intercepts of the regression lines between the plants grown under R and RB conditions. However, the ranges in which data points overlapped between the plants grown under R and RB conditions were small. Therefore, it is difficult to conclude whether the increase in the amounts of photosynthetic components in the RB-grown plants can be explained solely by the increase in total leaf N content, or whether slight changes in N partitioning among photosynthetic components are additionally involved.

In summary, rice plants grown under RB conditions showed higher light-saturated and light-limited photosynthetic rates than those grown under R conditions, and this was associated with a higher total leaf N content. The increased total leaf N content was accompanied by increases in the amounts of key components of photosynthesis-limiting processes. We considered that this was a major cause of the enhancement of both light-saturated and light-limited photosynthesis by supplementing red light with blue light in rice plants.

Anderson et al. (1995) proposed that specific blue-light perception might be involved in adjustments of photosynthetic apparatus stoichiometry in response to different light environments. Blue light is more effective in the formation of 'sun-type' chloroplasts than red light in pea (López-Juez and Hughes 1995) and *Arabidopsis thaliana* (Walters and Horton

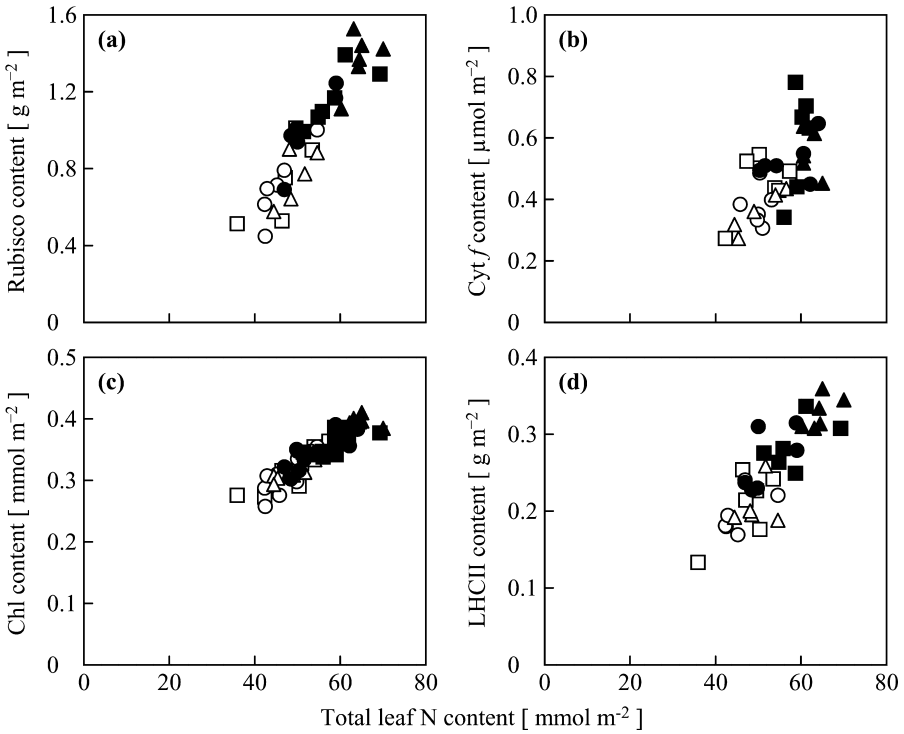

Fig. 3 Rubisco (a), Cyt *f* (b), Chl (c) and LHCII (d) contents per unit leaf area versus total N content per unit leaf area. For (a), $y = 0.0269x - 0.535$, $r^2 = 0.57$ (R); $y = 0.0271x - 0.406$, $r^2 = 0.79$ (RB). For (b), $y = 0.0136x - 0.269$, $r^2 = 0.45$ (R and RB). For (c), $y = 0.00458x + 0.0916$, $r^2 = 0.75$ (R); $y = 0.00385x + 0.140$, $r^2 = 0.69$ (RB). For (d), $y = 0.00396x - 0.0161$, $r^2 = 0.34$ (R); $y = 0.00442x - 0.0349$, $r^2 = 0.60$ (RB). Separate regression equations were given for R and RB when the slopes or *y*-intercepts were significantly different at the 5% level by *F* test in the analysis of covariance; otherwise a single regression equation was given. Symbols are as described for Fig. 2.

1995). According to Makino et al. (1997a), when rice plants were grown under white light, leaves developing under higher irradiance had a lower Chl content, a higher Chl *a*/*b* ratio and slightly larger amounts of electron-transport components at a given leaf N content than those developing under lower irradiance. N allocation to Rubisco did not differ between sun- and shade-type leaves of rice (Makino et al. 1997a). In this study, the Chl *a*/*b* ratio in the plants grown under RB was slightly higher than that in the plants grown under R. The mean values of Chl *a*/*b* ratio obtained under all N-treatment conditions were 3.20 ± 0.017 and 3.27 ± 0.014 in the R- and RB-grown plants, respectively. However, no apparent difference due to light treatment in N partitioning between the light-harvesting and electron-transport components was found under the conditions of this study. Thus, it is difficult to determine whether rice leaves developing under RB have sun-type characteristics when compared with those developing under R. It has been reported that dicotyledonous species tend to be more sensitive to blue light than monocotyledonous species, at least with regard to morphogenesis at the whole-plant level (Britz and Sager 1990, Dougher and Bugbee 2001). It is possible that the response of the photosynthetic properties at the single leaf level to blue light also differs between monocots and dicots. Further studies focusing on the differences between species and quantitative analysis of responses to blue light are required.

Rice (cv. Sasanishiki) plants were grown hydroponically in environmentally controlled chambers equipped with a LED panel (LHP0364–040; Iwasaki Electric Co., Tokyo, Japan), which was composed of 1,296 red and 648 blue LEDs. Seeds were germinated on a plastic net floating on tap water and grown for 21 d under white fluorescent lamps in an environmentally controlled room (320 ± 20 μmol m^{-2} s^{-1} PPFD at the tops of the plants during a 16-h photoperiod, 25 ± 2/20 ± 2°C day/night temperatures). Two seedlings were then transplanted to each of 18 plastic bottles (500 ml) containing a nutrient solution and were grown under R or RB in the environmentally controlled chamber. A total of 18 seedlings were subjected to each set of light treatment conditions. Growth conditions were as follows: 240 μmol m^{-2} s^{-1} PPFD at the tops of the plants during the 12-h photoperiod, 27 ± 1/20 ± 1°C day/night temperatures and 75 ± 10% relative humidity. The basal nutrient solution used was prepared according to Mae and Ohira (1981) and was renewed once a week. The strength of the nutrient solution was varied depending on plant growth as follows (days after germination): 1/3, on 21 and 28 d; 1/2, on 35 d; 2/3, on 42 d; and full, from 49 d. From 42 d after germination, six plants under R and RB were supplied with a nutrient solution with 1/4-, 1- or 4-fold N concentration relative to the standard N concentration. The N concentrations in nutrient solutions from 49 d were 0.5, 2 and 8 mM. All measurements were made on young, fully expanded leaf blades of 54- to 87-day-old plants.

PPFD was measured using a quantum sensor (LI-190SA; Li-Cor Inc., Lincoln, NE, U.S.A.). SPD was calculated from the spectral energy distributions of the red and blue LEDs, which were measured using a spectroradiometer (MSR-7000; Opto Research Co., Tokyo, Japan). Spectral absorptance was measured using the spectroradiometer with an integrating

sphere. SPD and spectral absorptance were counted for every 1 nm. Phytochrome photoequilibrium was calculated according to Sager et al. (1988).

Gas-exchange rates were measured using a portable gas-exchange measurement system (LI-6400; Li-Cor Inc.). Light was provided from a 35-W white halogen lamp (JR12V35WKW/3GZ4; Phoenix Electric Co., Hyogo, Japan), and PPFD was adjusted by shading with cloths. All measurements were made at a leaf temperature of 27°C and a leaf-to-air vapor pressure difference of 1.1 ± 0.1 kPa.

A single leaf blade was homogenized with a chilled pestle and mortar in 50 mM Na-phosphate buffer (pH 7.0) containing 2 mM Na-iodoacetate, 0.8% (v/v) 2-mercaptoethanol and 5% (v/v) glycerol. Chl, Rubisco and total leaf N content was determined from a part of this homogenate according to the methods of Makino et al. (1994). A calibration curve for Rubisco determination was made with Rubisco purified from tomato leaves. For determination of LHCII, the insoluble fraction was prepared according to the method of Makino et al. (2003) and was subjected to SDS–PAGE. The LHCII content was determined spectrophotometrically by formamide extraction of the Coomassie-Brilliant-Blue-R-250-stained bands (Makino et al. 1986) corresponding to approximately 27 and 25 kDa from the gel. A calibration curve was made with bovine serum albumin. The 27- and 25-kDa polypeptides were identified as LHCII apoproteins by immunoblotting using a polyclonal anti-LHCII antiserum. Cyt *f* content was estimated according to the method of Ohashi et al. (1998). The millimolar extinction coefficient used was 20 mM^{-1} cm^{-1} (Bendall et al. 1971).

Acknowledgments

We wish to thank Dr. Hiroyuki Ishida (Tohoku University) for his technical assistance in Rubisco purification and immunoblotting as well as for kindly providing antibodies against LHCII. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 14760168 to K. O.-K.).

References

Anderson, J.M., Chow, W.S. and Park, Y.-I. (1995) The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. *Photosynth. Res.* 46: 129–139.

Bendall, D.S., Davenport, H.E. and Hill, R. (1971) Cytochrome components in chloroplasts of the higher plants. *Methods Enzymol.* 33: 327–344.

Briggs, W.R. and Huala, E. (1999) Blue-light photoreceptors in higher plants. *Annu. Rev. Cell Dev. Biol.* 15: 33–62.

Britz, S.J. and Sager, J.C. (1990) Photomorphogenesis and photoassimilation in soybean and sorghum grown under broad spectrum or blue-deficient light sources. *Plant Physiol.* 94: 448–454.

Brown, C.S., Schuerger, A.C. and Sager, J.C. (1995) Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. *J. Amer. Soc. Hort. Sci.* 120: 808–813.

Christie, J.M. and Briggs, W.R. (2001) Blue light sensing in higher plants. *J. Biol. Chem.* 276: 11457–11460.

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

Eskins, K., Jiang, C.Z. and 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. *Physiol. Plant.* 83: 47–53.

Evans, J.R. (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78: 9–19.

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

Hirose, T. and Werger, M.J.A. (1987) Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiol. Plant.* 70: 215–222.

Karlsson, P.E. (1986) Blue light regulation of stomata in wheat seedlings. II. Action spectrum and search for action dichroism. *Physiol. Plant.* 66: 207–210.

Leong, T.-Y. and Anderson, J.M. (1984) Effect of light quality on the composition and function of thylakoid membranes in *Atriplex triangularis*. *Biochim. Biophys. Acta* 766: 533–541.

López-Juez, E. and Hughes, M.J.G. (1995) Effect of blue light and red light on the control of chloroplast acclimation of light-grown pea leaves to increased fluence rates. *Photochem. Photobiol.* 61: 106–111.

Mae, T. and Ohira, K. (1981) The remobilization of nitrogen related to leaf growth and senescence in rice plants (*Oryza sativa* L.). *Plant Cell Physiol.* 22: 1067–1074.

Makino, A. (2003) Rubisco and nitrogen relationships in rice: leaf photosynthesis and plant growth. *Soil Sci. Plant Nutr.* 49: 319–327.

Makino, A., Mae, T. and Ohira, K. (1986) Colorimetric measurement of protein stained with Coomassie Brilliant Blue R on sodium dodecyl sulfate-polyacrylamide gel electrophoresis by eluting with formamide. *Agric. Biol. Chem.* 50: 1911–1912.

Makino, A., Nakano, H. and Mae, T. (1994) Responses of ribulose-1,5-bisphosphate carboxylase, cytochrome *f*, and sucrose synthesis enzymes in rice leaves to leaf nitrogen and their relationships to photosynthesis. *Plant Physiol.* 105: 173–179.

Makino, A., Sakuma, H., Sudo, E. and Mae, T. (2003) Differences between maize and rice in N-use efficiency for photosynthesis and protein allocation. *Plant Cell Physiol.* 44: 952–956.

Makino, A., Sato, T., Nakano, H. and Mae, T. (1997a) Leaf photosynthesis, plant growth and nitrogen allocation in rice under different irradiances. *Planta* 203: 390–398.

Makino, A., Shimada, T., Takumi, S., Kaneko, K., Matsuoka, M., Shimamoto, K., Nakano, H., Miyao-Tokutomi, M., Mae, T. and Yamamoto, N. (1997b) Does decrease in ribulose-1,5-bisphosphate carboxylase by antisense *rbc*S lead to a higher N-use efficiency of photosynthesis under conditions of saturating CO_2 and light in rice plants? *Plant Physiol.* 114: 483–491.

Ohashi, K., Makino, A. and Mae, T. (1998) Gas exchange characteristics in rice leaves grown under the conditions of physiologically low temperature and irradiance. *Plant Cell Physiol.* 39: 1384–1387.

Sager, J.C., Smith, W.O., Edwards, J.L. and Cyr, K.L. (1988) Photosynthetic efficiency and phytochrome photoequilibria determination using spectral data. *Trans. ASAE* 31: 1882–1889.

Senger, H. and Bauer, B. (1987) The influence of light quality on adaptation and function of the photosynthetic apparatus. *Photochem. Photobiol.* 45: 939–946.

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

Terashima, I. and Hikosaka, K. (1995) Comparative ecophysiology of leaf and canopy photosynthesis. *Plant Cell Environ.* 18: 1111–1128.

Walters, R.G. and Horton, P. (1995) Acclimation of *Arabidopsis thaliana* to the light environment: regulation of chloroplast composition. *Planta* 197: 475–481.

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

(Received July 28, 2004; Accepted September 16, 2004)