Effects of Blue Light Deficiency on Acclimation of Light Energy Partitioning in PSII and CO₂ Assimilation Capacity to High Irradiance in Spinach Leaves

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Blue light effects on the acclimation of energy partitioning characteristics in PSII and CO₂ assimilation capacity in spinach to high growth irradiance were investigated. Plants were grown hydroponically in different light treatments that were a combination of two light qualities and two irradiances, i.e. white light and blue-deficient light at photosynthetic photon flux densities (PPFDs) of 100 and 500 μmol m⁻² s⁻¹. The CO₂ assimilation rate, the quantum efficiency of PSII (ΦPSII) and thermal dissipation activity \( \frac{F_v}{F_m} \) in young, fully expanded leaves were measured under 1,600 μmol m⁻² s⁻¹ white light. The CO₂ assimilation rate and ΦPSII were higher, while \( \frac{F_v}{F_m} \) was lower in plants grown under high irradiance than in plants grown under low irradiance. These responses were observed irrespective of the presence or absence of blue light during growth. The extent of the increase in the CO₂ assimilation rate and ΦPSII and the decrease in \( \frac{F_v}{F_m} \) by high growth irradiance was smaller under blue light-deficient conditions. These results indicate that blue light helps to boost the acclimation responses of energy partitioning in PSII and CO₂ assimilation to high irradiance. Similarly, leaf N, Cyt f and Chl contents per unit leaf area increased by high growth irradiance, and the extent of the increment in leaf N, Cyt f and Chl was smaller under blue light-deficient conditions. Regression analysis showed that the differences in energy partitioning in PSII and CO₂ assimilation between plants grown under high white light and high blue-deficient light were closely related to the difference in leaf N.

**Keywords:** Chlorophyll fluorescence — Gas exchange (photosynthesis) — Light acclimation — Light quality — Nitrogen (leaf) — Spinach (*Spinacia oleracea* L.).

Abbreviations: CRY, cryptochrome; LHCII, light-harvesting Chl-binding protein of PSII; PFD, photon flux density; PHOT, phototropin; PPFD, photosynthetic photon flux density; ΦPSII, quantum efficiency of PSII; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; SPD, spectral photon flux density distribution.

**Introduction**

Blue light strongly influences growth and development of higher plants. In addition to well-known photomorphogenic responses mediated by blue light photoreceptors (Briggs and Huala 1999, Lin 2000), blue light is thought to participate in the acclimation of leaf photosynthesis to irradiance during growth (Senger and Bauer 1987, Anderson et al. 1995, Walters 2005). Plants grown under blue light exhibit photosynthetic characteristics more similar to those of plants acclimating to high irradiance than to those grown under red light, such as a higher Chl \( a/b \) ratio (Buschmann et al. 1978, Lichtenhaler et al. 1980, Leong and Anderson 1984, López-Juez and Hughes 1995), a greater Cyt f content (Leong and Anderson 1984, López-Juez and Hughes 1995) and a greater ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content (Eskins et al. 1991, López-Juez and Hughes 1995). We recently confirmed that the ratio of Cyt f content to light-harvesting Chl-binding protein of PSII (LHCII) content in spinach leaves decreased, as it did ‘shade-type’ leaves, with lowering of the blue light photon flux density (PFD) from 100 to 0 μmol m⁻² s⁻¹ at a constant total photosynthetic photon flux density (PPFD) of 300 μmol m⁻² s⁻¹ (Matsuda et al. 2007). This suggests that blue light is involved in acclimation at least at the chloroplast level in spinach.

The mechanism of acclimation of photosynthesis to irradiance has been frequently discussed based on monitoring the changes in photosynthetic rates and the amounts of photosynthetic components. However, when acclimating to irradiance, plants alter not only photosynthetic rates and the amounts of photosynthetic components, but also several other physiological characteristics. Light energy partitioning in PSII is one such characteristic that plants alter depending on irradiance. The main energy-consuming processes at PSII are photochemistry, heat dissipation and Chl fluorescence (Kräuse and Weis 1991). According to the data of Kato et al. (2003), *Chenopodium album* leaves grown
under high irradiance utilize more energy in photochemistry, whereas they dissipate less energy as heat than those grown under low irradiance when compared under saturating light conditions. Such changes in energy partitioning in PSII depending on irradiance can contribute to efficient use of light energy for photosynthesis while avoiding photoinactivation due to excess energy under high irradiance. However, there are no available data on how long-term blue light irradiation is related to the change in energy partitioning characteristics in PSII depending on growth irradiance. In addition, some authors suggested that acclimation responses to irradiance at the chloroplast level and at the leaf morphological level could be regulated by different mechanisms (Murchie and Horton 1998, Yano and Terashima 2001). It is possible that, even within chloroplasts, different mechanisms participate in the regulation of various acclimation responses. However, it is uncertain whether blue light also affects the responses of energy partitioning characteristics in PSII to growth irradiance as well as that of the amounts of photosynthetic components.

In this study, we grew spinach plants under white light and blue-deficient light at low and high irradiances and measured the CO₂ assimilation rate and Chl fluorescence parameters of leaves under saturating light conditions. We then evaluated the effects of blue light on acclimation responses of photosynthetic capacity and light energy partitioning in PSII to high irradiance. We also determined leaf N content, which affects the capacities for photosynthesis and heat dissipation in PSII (Verhoeven et al. 1997, Ushio et al. 2003).

**Results and Discussion**

We first grew spinach plants in different light treatments that were a combination of two light qualities and two irradiances, i.e. white light (W) and blue-deficient light (BD) at PPFDs of 100 (L) and 500 (H) μmol m⁻² s⁻¹, with an N concentration of 12 mM in the nutrient solution (experiment 1). The four light treatments were referred to as W-L, W-H, BD-L and BD-H. Fig. 1A shows the CO₂ assimilation rate measured at a PPFD of 1,600 μmol m⁻² s⁻¹ and an ambient CO₂ partial pressure of 36 Pa using a white halogen lamp. Plants grown under W-L and BD-L showed almost the same CO₂ assimilation rate. The CO₂ assimilation rate of plants grown under BD-H was significantly lower than that of plants grown under W-H. The ratios of the CO₂ assimilation rate of the H-grown plants to that of the L-grown plants were 1.77 for W treatment and 1.60 for BD treatment. Thus, although photosynthetic capacity was significantly enhanced by high irradiance during growth irrespective of the presence or absence of blue light, the extent of enhancement was slightly smaller under conditions of absence of blue light. Stomatal conductance was

![Graph A: CO₂ assimilation rate](image1)

**Fig. 1** CO₂ assimilation rate (A), stomatal conductance (B) and intercellular CO₂ partial pressure (C) in spinach leaves grown under white light (W) or blue-deficient light (BD) at a PPFD of 100 (L) or 500 (H) μmol m⁻² s⁻¹. Measurements were made at a PPFD of 1,600 μmol m⁻² s⁻¹, an atmospheric CO₂ partial pressure of 36 Pa, a leaf temperature of 25°C and a leaf-to-air vapor pressure deficit of 1.1 ± 0.1 kPa. Vertical bars represent SEMs (n = 4). Means with different letters are significantly different by Tukey’s HSD test (P < 0.05).
significantly higher in W-H and BD-H plants than in W-L and BD-L plants (Fig. 1B), but intercellular CO₂ partial pressure did not differ among the treatments (Fig. 1C). This means that stomatal responses played no part in the observed differences in the CO₂ assimilation rate. \( F_v/F_m \) ranged between 0.80 and 0.82 for all light treatments (data not shown), indicating that none of the light treatments led to photoinhibition.

The quantum efficiency of PSII (ΦPSII) under saturating light conditions showed a similar trend to the CO₂ assimilation rate (Fig. 2A). Although ΦPSII significantly increased with increasing growth irradiance irrespective of light quality, the ΦPSII increment was smaller in the BD treatment than in the W treatment. \( F_v/F_m - F'_v/F'_m \) significantly decreased as growth irradiance increased, and the difference in \( F_v/F_m - F'_v/F'_m \) between the L- and H-grown plants was smaller in the BD treatment than in the W treatment (Fig. 2B). These results indicate that blue light is not essential for spinach to change its photosynthetic capacity and energy partitioning in PSII to acclimate to high irradiance. However, blue light helps to boost increases in photosynthetic capacity and ΦPSII, and a decrease in thermal dissipation capacity to acclimate to high irradiance.

Significant differences in CO₂ assimilation capacity and light energy partitioning in PSII between W- and BD-grown plants were observed only in the H treatment, not in the L treatment. Which factors are related to such differences between W-H and BD-H plants? We assumed that the N content of leaves is involved. Plants grown with a limiting N supply exhibit decreased photosynthetic capacity and ΦPSII (Demmig-Adams et al. 1995, Verhoeven et al. 1997, Logan et al. 1999). The decreases in photosynthetic capacity and ΦPSII due to N deficiency are associated with an increased level of xanthophyll cycle-dependent heat dissipation (Ushio et al. 2003). On the other hand, we previously found that plants grown under red light alone have lower leaf N content per unit leaf area than those grown under a mixture of red and blue light, both for rice (Matsuda et al. 2004, Ohashi-Kaneko et al. 2006) and for spinach (Matsuda et al. 2007). Leaf N content per unit leaf area of plants grown under BD-H was significantly smaller than for those grown under W-H (Table 1). There was no significant difference in leaf N content per unit leaf area between plants grown under W-L and BD-L. The amounts of photosynthetic components such as Cyt f, one of the rate-limiting factors for electron transport, and Chl were associated with the amount of leaf N content (Table 1). The Chl a/b ratio was higher in H-grown plants than in L-grown plants under both light quality conditions (Table 1). In order to confirm whether the differences in photosynthetic capacity, ΦPSII and \( F_v/F_m - F'_v/F'_m \) between W-H and BD-H were quantitatively related to leaf N content per unit leaf area, we grew plants under W-H or BD-H with 1.0, 4.0 or 12.0 mM N in the nutrient solution (experiment 2). Fig. 3 shows the CO₂ assimilation rate, ΦPSII and \( F_v/F_m - F'_v/F'_m \) at 1,600 μmol m⁻² s⁻¹ PPFD in W-H and BD-H plants plotted against leaf N content per unit leaf area for each light quality treatment, while \( F_v/F_m - F'_v/F'_m \) (Fig. 3C) was negatively correlated. Analysis of covariance indicated that, in all relationships, neither slopes nor intercepts were significantly different between regression equations for W-H and BD-H. Thus, the differences in the CO₂
assimilation rate, \( \Phi_{\text{PSII}} \) and \( F_v/F_m - F'_v/F'_m \) between plants grown under W-H and BD-H were considered to be closely related to the difference in leaf N content.

To summarize the results, blue light is not essential for spinach to change photosynthetic capacity and energy partitioning characteristics in PSII to high irradiance during growth, but blue light has a role in boosting the acclimation responses. The differences in acclimation responses to high irradiance caused by the presence or absence of the blue light fraction seemed to be related to the difference in leaf N content.

Walters and Horton (1995) reported that neither the light-saturated rate of photosynthesis nor the Chl \( a/b \) ratio in Arabidopsis thaliana were greatly affected by irradiance of red light which was completely deficient in blue light. Their results suggest that blue light is an indispensable waveband for triggering the acclimation to irradiance. This is inconsistent with our conclusion. The reason for the difference between their results and ours is not known, but it might be attributed to the differences in species and/or spectrum of the blue-deficient light. The blue-deficient light in Walters and Horton (1995) was rich in red (600–700 nm) and far-red (700–750 nm) light and deficient in green light (500–600 nm), which is quite different from ours (Fig. 4B, C). Interactions of blue light with green and far-red light on acclimation to irradiance should be studied in future studies. In addition, the spectrum of white light tested in the present study was considerably different from that of natural sunlight. The effect of blue light on acclimation to irradiance in natural sunlight should also be addressed. A light-emitting diode-artificial sunlight source system that is able to control relative spectral power distribution (Fujiwara and Sawada 2006) could be used for such experiments.

It is known that higher plants have blue light photoreceptors capable of specifically detecting blue light irradiance. For example, the transcriptional level of \( psbD \) that encodes the D2 protein in PSII increases with increasing blue and white light irradiance from 0 to 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), but the extent of the increase in the transcriptional level is higher under blue light than under white light (Christopher and Mullet 1994). Increasing red light irradiance has little effect on the \( psbD \) transcriptional level (Christopher and Mullet 1994). This blue light irradiance-specific activation of \( psbD \) transcription is regulated by cryptochrome (CRY) 1 and CRY2 as well as phytochrome A in \( A. \text{thaliana} \) (Thum et al. 2001). This indicates that CRY1 and CRY2 play an important role in perception of blue light irradiance to regulate photosynthetic properties toward changing irradiance. Similar blue light irradiance-specific activation of transcription has also been observed in \( rbcS \), which encodes the small subunit of Rubisco (Sawbridge et al. 1994). Furthermore, it has been recently shown that FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) protein in \( A. \text{thaliana} \) works as a blue light photoreceptor in photoperiodic-specific light signaling in flowering and can sense blue light irradiance at least up to 66 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) (Imaizumi et al. 2003, Sawa et al. 2007). It is possible that these blue light photoreceptors are involved in the mechanism underlying the ‘boosting’ effect of blue light on acclimation to high irradiance. On the other hand, Weston et al. (2000) reported that involvement of CRY1, CRY2 and phototropin (PHOT) 1 in changing leaf thickness in response to growth irradiance was unlikely in \( A. \text{thaliana} \). Involvement of blue light photoreceptors in acclimation responses to growth irradiance, including those that have not been studied yet such as PHOT2 and FKF1, is to be tested further in future studies.

Under high irradiance, the Chl \( a/b \) ratio was not affected by the blue light deficiency, although other parameters such as the photosynthetic rate, energy partitioning in PSII and the amounts of leaf N, Chl and Cyt \( f \) were affected (Table 1). Different mechanisms might participate between changes in the Chl \( a/b \) ratio and in the other parameters tested in this study. The redox state of the photosynthetic electron transport chain may be one of the mechanisms changing the Chl \( a/b \) ratio, since the LHCII content is influenced by the redox state (Yang et al. 2001).

The lower leaf N content per unit leaf area in BD-H plants than in W-H plants (Table 1) might be related to a decrease in uptake and/or reduction of N caused by blue light deficiency, as we previously discussed (Ohashi-Kaneko et al. 2006). In general, blue light promotes stomatal opening (Sharkey and Raschke 1981, Karlsson 1986) and nitrate

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>Leaf N (mmol m(^{-2}))</th>
<th>Cyt ( f ) (( \mu \text{mol m}^{-2} ))</th>
<th>Chl ( f ) (mmol m(^{-2}))</th>
<th>Chl ( a/b ) (mol mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>W-L</td>
<td>69 ± 2.9bc</td>
<td>0.86 ± 0.035c</td>
<td>424 ± 19.4c</td>
<td>2.96 ± 0.063b</td>
</tr>
<tr>
<td>W-H</td>
<td>119 ± 6.0a</td>
<td>1.73 ± 0.053a</td>
<td>651 ± 19.5a</td>
<td>3.22 ± 0.036a</td>
</tr>
<tr>
<td>BD-L</td>
<td>65 ± 3.2c</td>
<td>0.73 ± 0.021c</td>
<td>378 ± 12.9c</td>
<td>2.93 ± 0.023b</td>
</tr>
<tr>
<td>BD-H</td>
<td>87 ± 5.0b</td>
<td>1.39 ± 0.073b</td>
<td>538 ± 22.8b</td>
<td>3.26 ± 0.031a</td>
</tr>
</tbody>
</table>

\(^{2}\)Mean ± SE (n = 4). Means with different letters are significantly different by Tukey’s HSD test (\( P < 0.05 \)).
Spinach (Spinacia oleracea L. cv. Megaton) plants were grown hydroponically in environmentally controlled chambers equipped with white fluorescent lamps (FPL55EX-L; Iwaki Electric Co., Tokyo, Japan). The chambers were maintained with 300 μmol m⁻² s⁻¹ PPFD at the tops of plants during a 12 h photoperiod and 20 ± 1°C/15 ± 1°C day/night temperatures. Seeds were sown in vermiculite, and each 17-day-old seedling was transplanted to a 0.51 plastic bottle containing a nutrient solution.

For experiment 1, seedlings were then grown under white light (W) or blue-deficient light (BD) at a PPFD of 100 (L) or 500 (H) μmol m⁻² s⁻¹. W and BD were obtained from light provided by white fluorescent lamps passing through long wavelength-pass glass filters with a transition wavelength of 420 ± 5 nm (L-42; HOYA CANDEO OPTRONICS Co., Saitama, Japan) and 500 ± 5 nm (Y-50; HOYA CANDEO OPTRONICS Co.), respectively. The basal nutrient solution used was prepared according to Makino et al. (1988) except that 12.0 mM N (9.0 mM NaNO₃ plus 1.5 mM NH₄NO₃) was used instead of 2.0 mM N. The nutrient solution was renewed every 5 d and was continuously aerated. The strength of the nutrient solution was varied depending on plant growth (days after germination): half strength between 7 and 11 d and full strength after 12 d.

For experiment 2, seedlings were grown under W-H or BD-H from 17 d after germination and supplied with either 1.0 mM N (0.75 mM NaNO₃ plus 0.125 mM NH₄NO₃), 4.0 mM N (3.0 mM NaNO₃ plus 0.5 mM NH₄NO₃) or 12.0 mM N (9.0 mM NaNO₃ plus 1.5 mM NH₄NO₃) for each light quality treatment. PPFD was measured using a quantum sensor (LI-190SA with LI-250; LI-COR, Inc., Lincoln, NE, USA) and adjusted with shade cloths. The spectral PFD distributions (SPDs) were calculated from spectral energy distributions determined every 0.4–0.5 nm by a spectroradiometer (HR2000; Ocean Optics, Inc., Dunedin, FL, USA). Fig. 4 shows the SPDs for the four light treatments. The ratios of PFD between 400 and 500 nm to PPFD (400–700 nm) were 11 and 0% for W and BD, respectively. Phytochrome photoequilibria were calculated from the SPDs of W and BD with the data of photoconversion of the cross-section of phytochrome and equation 5 in Sager et al. (1988). Phytochrome photoequilibria
were 0.86 and 0.87 for W and BD treatments, respectively, indicating that the difference in the reversible action of phytochrome caused by the treatment was negligible.

All measurements were carried out on young, fully expanded second leaves of 27- to 32-day-old plants. Chl fluorescence and gas exchange were simultaneously measured with a pulse amplitude-modulated Chl fluorometer (Mini-PAM; Heinz Walz GmbH, Effeltrich, Germany) and a portable gas exchange measurement system (LI-6400: LI-COR, Inc.). Actinic light was provided by an external 100 W halogen cold light source through an optical fiber. Measurements were made at an ambient CO2 partial pressure of 36 Pa, a leaf temperature of 25°C and a leaf-to-air vapor pressure deficit of 1.1±0.1 kPa. The leaves were first kept in the dark for 30 min, and the initial and the maximal Chl fluorescence yields (Fo and Fm) were determined. Then the actinic light was turned on, and, after gas exchange had reached the steady-state rate at a PPFD of 1,600 µmol m⁻² s⁻¹, the steady-state and the maximal Chl fluorescence yields (F and Fm) and gas exchange rates were determined. The maximal quantum efficiency of PSII was determined as

\[
\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m}
\]

where Fv and Fm are the maximal and the steady-state Chl fluorescence yields, respectively, and F0 is the initial Chl fluorescence yield. The level of thermal dissipation was estimated from

\[
\frac{F_v}{F_m} = \frac{F_m - F_0}{F_0}
\]

which is frequently used as an indicator for the fraction of light-dependent thermal dissipation (Demmig-Adams et al. 1996, Kato et al. 2003, Hirotsu et al. 2005). Fv was calculated without measuring F0, according to Oxborough and Baker (1997).

Leaf N and Chl contents were determined by the methods of Makino et al. (1994). The Cyt f content was estimated from the difference between the hydroquinone-reduced and ferricyanide-oxidized spectra of the thylakoid membrane according to Ohashi et al. (1998). The millimolar extinction coefficient used was 20 mM⁻¹ cm⁻¹ (Bendall et al. 1971).

Fig. 4 Spectral photon flux density (PFD) distributions of white light (W, upper panels) and blue-deficient light (BD, lower panels) at PPFDs of 100 (L, left panels) and 500 (H, right panels) µmol m⁻² s⁻¹. PFD was measured every 0.4–0.5 nm.

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**References**


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