Biochimica et Biophysica Acta 850 (1986) 57–63
Elsevier

BBA 42012

Light-quality and irradiance adaptation of the composition and function of pea-thylakoid membranes

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(Received October 23rd, 1985)

Key words: Photoregulation; Light quality; Irradiance adaptation; Membrane composition; (Pea thylakoid)

The effect of light quality and light quantity during growth on the composition and function of pea thylakoids was investigated. Pea plants were grown at seven irradiance levels in four light-quality regimes with different red to far-red ratios (control, far-red-enriched, far-red-deficient and red). Both the chlorophyll *a*/chlorophyll *b* (Chl *a*/Chl *b*) ratios and the coupling-factor activities increased with increasing levels of irradiance in a curvilinear response in each light regime. This results in a linear relationship between Chl *a*/Chl *b* ratios, and hence modulations of chlorophyll-proteins and coupling-factor activities in each case, regardless of the red/far-red ratio of the four light-quality sources. Under conditions of long-term irradiance in peas, the modulations of the amounts of core Photosystem II (PS II) complex, core Photosystem I complex, cytochrome *b*/*f* complex and ATP synthetase on the one hand vs. the antenna chlorophyll *a*/*b*-proteins of PS II and PS I on the other do not appear to be influenced by the phytochrome photoequilibrium. We suggest that the levels of NADPH and ATP formed during photosynthesis are the crucial factor in promoting gene expression and regulating the composition, structure and function of thylakoid membranes.

Introduction

Light is essential for the development and growth of plants, since it controls both photosynthesis and morphogenesis. In higher plants, the photomorphogenetic responses are mediated by the red to far-red response of phytochrome [1,2] and the blue-light receptors [3,4]. Chlorophyll synthesis also requires light for the conversion of protochlorophyllide to chlorophyllide [5]. Hence, there is a complex interaction between the photoregulation of the expression of the plant genomes and photosynthesis. The synthesis and assembly of the intrinsic protein complexes and the mobile electron carriers of thylakoid membranes require a strict coordination of regulation, since each intrinsic membrane-spanning complex is composed of both nuclear- and chloroplast-encoded subunits [6]. Althouth the transcription of mRNA for some nuclear- and chloroplast-encoded thylakoid proteins has been shown to be light-stimulated, it is already clear that transcriptional changes are only part of the regulatory process [6,7].

It is established that plants grown in high irradiance or sunlight have high photosynthetic capacities which saturate at high light intensities,

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Abbreviations: Chl, chlorophyll, PS II, Photosystem II; PS I, Photosystem I; Cyt, cytochrome; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethylethyl]glycine; CF_1, coupling factor one; C, control; −FR, far-red-deficient; +FR, far-red-enriched; R, red, LHC, light-harvesting chlorophyll; P_i inorganic phosphate; Phyt, phytochrome.

while those grown at low irradiance have low photosynthetic capacities which saturate at low intensites [8–10]. Photosynthetic capacity is regulated partly by the relative proportions of light-harvesting protein complexes, electron-transport complexes and ATP synthetase in pea leaves [11–13]. In turn, the concentrations of both chlorophyll-proteins and electron carriers are strictly dependent on the irradiance available during plant growth. The other aspect of light control on chloroplast development involves the quality of illumination received during plant growth. Several plant species grown in blue light have chloroplasts which have similar composition, function and structure to sun plant chloroplasts, and those grown in red light have chloroplasts which resemble shade plant chloroplasts [10,14]. Shade plants receive intermittent sun flecks of high intensity (40–70% of the total quantum flux density) and diffuse irradiation enriched in far-red and green and depleted in red and blue [15]. Thus, a shade habitat combines low intensity with a very low red/far-red ratio, which is thought to influence the activation state of phytochrome [16].

Many of the light-quality studies [10,17–19] on thylakoid adaptation have focussed on comparison of varying light qualities at only one irradiance level. Alternatively, other studies have focussed on the very early events in chloroplast development from dark-grown plants [14]. In order to test the interactions of light quality and light quantity on the composition and function of pea thylakoid membranes during long-term irradiance, we have grown pea seedlings at seven different light intensities in each of four light quality regimes which have very different red/far-red ratios. We have asked the following questions. (i) How does the chlorophyll composition and coupling-factor activity adapt in plants grown at increasing levels of irradiance in the four light-quality regimes? (ii) Does light quality influence the coordination of regulation of thylakoid-intrinsic complexes? (iii) Does phytochrome equilibrium influence the relative proportion of thylakoid complexes under conditions of long-term light growth?

Materials and Methods

Pea seedlings (*Pisum sativum* L.) were grown in vermiculite watered with Hoagland's nutrient solution in growth cabinets at 20°C for approx. 14 days in four different light quality climates on a 16 h light/8 h dark regime. In each of the four growth cabinets, peas were grown at seven different levels of irradiance which were obtained by adjusting the distance between the seedlings and the light source as well as by using neutral density screens.

Light-quality climates:

C Control; fluorescent and tungsten lamps
−FR Far-red-deficient; fluorescent lamps
+FR Far-red-enriched; tungsten lamps
R Red; red plexiglass filter placed over fluorescent and tungsten lamps

The fluoresence tubes were Philips 65/80 W 33 RS and incandescent tungsten lamps were 100 W. The emission spectra of the light sources were recorded with a Techtum Spectroradiometer, QSM 2500. The 660 nm/730 nm ratios of each light-quality regime were determined by measuring the photon fluence rate at 660 and 730 nm with a monochromatic instrument (IL 700 Research Radiometer) at 1 nm band width. The irradiance levels were measured with a Li-Cor Quantum Photometer (Model Li-185A).

Thylakoid membranes were isolated from whole seedlings to eliminate physiological differences in leaves as described previously [12] in 50 mM Tricine buffer (pH 8.0)/0.4 M Sucrose/100 mM NaCl/5 mM $MgCl_2$. Thylakoids were subsequently washed with 50 mM Tricine buffer (pH 8.0) and resuspended in 50 mM Tricine buffer (pH 8.0) (1–4 mg Chl/ml) prior to storage in liquid N_2. Total Chl and Chl *a*/Chl *b* ratios were determined in 80% acetone [20].

Non-denaturing green gels were run as previously described following the solubilization of thylakoids at an SDS/Chl ratio of 7.5 [12]. The Mg^{2+}-specific ATPase activity of chloroplast coupling factor, CF_1 was assayed in the presence of octylglucoside by the method of Pick and Bassilian [21]. Atrazine-binding analysis was performed according to the method of Tischer and Strotmann [22] as described previously [13]. Atrazine (16 mCi/mmol) was purchased from Amersham International Plastoquinone was extracted from ethanol-treated thylakoids (400 μg Chl) with methanol/chloroform/heptane (1 : 1 : 1, v/v) and assayed as described [23]. Cytochrome *f*

was estimated from (hydroquinone-reduced)-minus-(ferricyanide-oxidized) difference spectra [24] and P-700 from (ferricyanide-oxidized)-minus-(ascorbate-reduced) difference spectra [25].

Results

Light-quality regimes

To probe the interactions of light quality and quantity, pea seedlings were grown at seven different intensities under each of four light quality regimes of varying spectral quality in a 16 h light/8 h dark regime. The spectroradiometric scans from 400 to 740 nm of regimes (C, +FR, −FR, and R) are shown in Fig. 1. The 660 nm/730 nm ratios of these light-quality climates (Table I) are very different with the far-red-deficient light source having a 5–6-fold greater red/far-red ratio than that of the far-red-enriched light source.

Chlorophyll content

Within each of the four light-quality regimes, there was an increase in the Chl *a*/Chl *b* ratio of pea thylakoids with increasing levels of irradiance. When the Chl *a*/Chl *b* ratios were plotted against intensity, there was a curvilinear response to irradiance (Fig. 2), as was described previously for the control climate [12]. For all four light climates the rate of increase of the Chl *a*/Chl *b* ratio was greater in the lower-intensity range when compared to that in the higher-intensity range. Note, however, that the transition point of the bilinear response occurred at a different intensity for each light-quality regime.

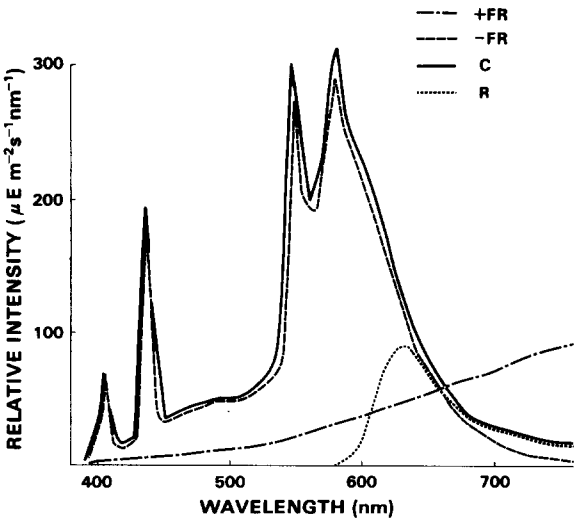

Fig. 1. The spectral irradiance of light quality regimes. C, Control (fluorescent and tungsten lamps); −FR, far-red-deficient (fluorescent lamps); +FR, far-red-enriched (tungsten lamps); R, red (red plexiglass under fluorescent and tungsten lamps).

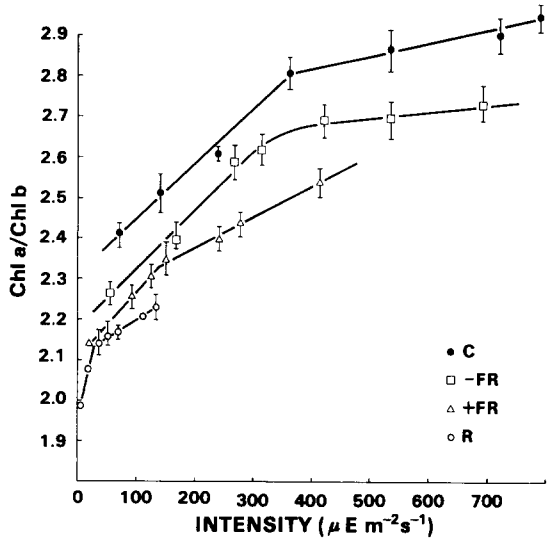

Fig. 2. Comparison of the Chl *a*/Chl *b* ratios of thylakoid membranes isolated from pea plants grown at seven different irradiance levels in four light quality regimes (see Fig. 1).

The Chl *a*/Chl *b* ratios of pea thylakoids have been shown to be an index of the relative amounts of chlorophyll-protein complexes [12]. PS II and PS I each contain inner-core Chl-*a*-proteins (CPa and CP1, respectively) and antenna light-harvesting Chl *a*/*b*-proteins (LHC II and LHC I, respectively). Hence, lower Chl *a*/Chl *b* ratios reflect

TABLE I

COMPARISON OF THE 660 nm/730 nm RATIOS OF THE FOUR LIGHT-QUALITY REGIMES USED FOR GROWING PEA PLANTS

Light-quality regime	660/730 nm ratio
C (Control)	2.88
−FR (far-red-deficient)	4.24
+FR (far-red-enriched)	0.75
R (red)	3.40

increased amounts of LHC II and LHC I, and, concomitantly, decreased amounts of the inner-core Chl *a*-proteins of PS II and PS I.

Coupling-factor activity

The octylglucoside-stimulated ATPase activity of the coupling factor was also determined for the light-adapted pea thylakoid membranes. When the coupling-factor activity on a chlorophyll basis was plotted against photon-fluence rate, the response to light intensity was also non-linear. In each light-quality climate there was a greater rate of increase in CF_1 activity at the lower-irradiance levels when compared to that at the higher-irradiance levels used for the growth of pea seedlings (Fig. 3). Further, the transition point observed for each light-quality climate is roughly similar to that observed for the Chl *a*/Chl *b* ratios (Fig. 2). As the response of coupling-factor activity to irradiance parallels that of the Chl *a*/Chl *b* ratios, there is a linear relationship between CF_1 activity and Chl *a*/Chl *b* ratios for each light climate

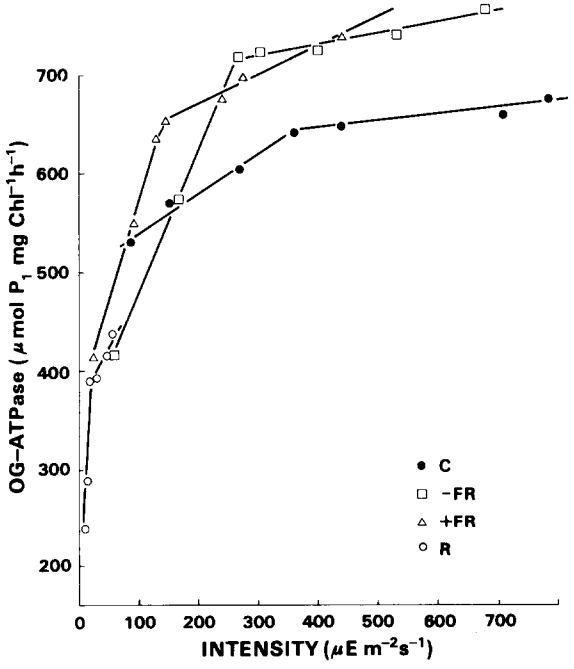

Fig. 3. Comparison of the coupling-factor activity of thylakoid membranes isolated from pea plants grown at seven different irradiance levels in four light-quality regimes described in Fig. 1. Coupling-factor activity was measured as the octylglucoside (OG) activated Mg^{2+}-ATPase activity [21].

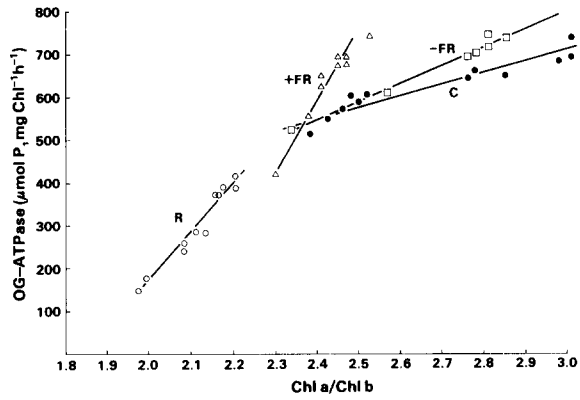

Fig. 4. Relationship between the coupling-factor activity (see Fig. 3) and Chl *a*/Chl *b* ratios (see Fig. 2) of thylakoid membranes isolated from pea plants grown at seven different light intensities in four light-quality regimes, C, control; −FR, far-red-deficient; +FR, far-red-enriched; and R, red light. OG, octylglucoside.

(Fig. 4). These results suggest a marked co-ordination between the relative amounts of the core Chl *a*-proteins and the Chl *a*/*b*-proteins, vs. the ATP synthetase activity.

Photoadaptation of electron-transport carriers

Modulations in the proportions of several electron-transport carriers per unit chlorophyll were compared for pea thylakoids isolated from plants grown in the control light-quality regime. These include P-700, Cyt *f*, plastoquinone and Q, the primary electron acceptor of PS II. The amount of

TABLE II

THE RELATIVE ADAPTATION OF THYLAKOID COMPLEXES TO LIGHT INTENSITY FOR PEAS GROWN UNDER THE CONTROL WHITE LIGHT REGIME C

The amounts of Q, cytochrome *f*, P-700 and coupling-factor activity of peas grown at 42 $\mu E \cdot m^{-2} \cdot s^{-1}$ were each given a value of 1 (see text).

Intensity of growth ($\mu E \cdot m^{-2} \cdot s^{-1}$)	PS II (Q) (relative amounts per unit chlorophyll)	Cyt *f*	PS I (P-700)	CF_1 activity
42	1.0	1.0	1.0	1.0
93	1.5	1.3	1.02	1.6
215	2.2	1.6	1.04	2.1
440	2.4	2.1	1.12	2.5
840	2.8	2.9	1.15	2.8

Q was measured as [^{14}C]atrazine binding to the Q_B protein of PS II (22,26). When Chl/Cyt *f*, Chl/PQ and Chl/Q ratios were plotted against light intensity, similarly shaped curves were obtained to those of Figs. 2 and 3. However, there was much less change in the Chl/P-700 ratios with irradiance (data not shown).

In order to compare the adaptation of these components to light irradiance the relative increase in each component was compared to the level present in thylakoids from peas grown at an irradiance of 42 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Table II). It must be stressed that the actual stoichiometry of Q (atrazine binding) to Cyt *f* to P-700 was not 1 : 1 : 1. At 42 $\mu E \cdot m^{-2} \cdot s^{-1}$, the amounts in μmol per mmol^{-1} Chl were as follows: Q (atrazine binding), 2.36; Cyt *f*, 1.63; and P-700, 2.24. The octylglucoside coupling-factor activity was 310 μmol P_i per mg Chl per h. The relative increases in Q (atrazine binding), Cyt *f* and ATP synthetase activity are fairly similar. By contrast, the adaptation of P-700 per unit Chl is much less marked (Table II).

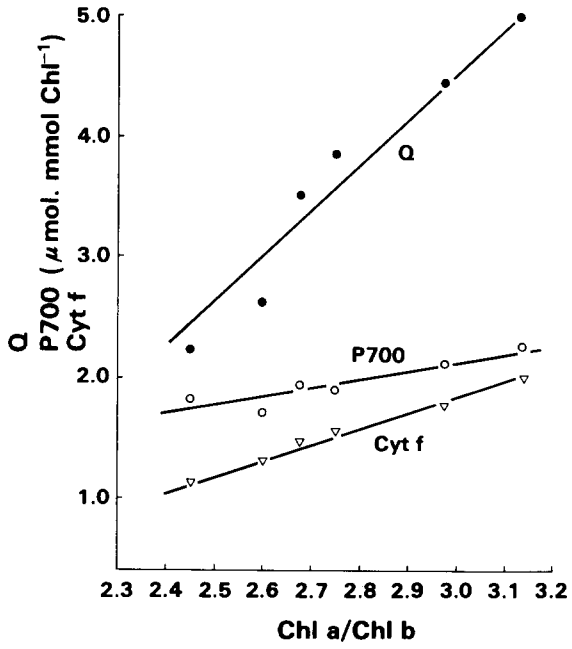

Fig. 5. Relationship between some electron-transport carriers and Chl *a*/Chl *b* ratios of thylakoids isolated from pea plants grown at seven different light intensities in the control light regime C. Concentrations of the electron-transport carriers are given in μmol per mmol Chl and were determined as follows: Q, the primary electron acceptor of PS II by [^{14}C]atrazine binding [22], cytochrome *f* [24] and P-700 [25].

The amounts of atrazine-binding sites, plastoquinone and Cyt *f* all show linear relationships with respect to Chl *a*/Chl *b* ratios (Fig. 5), as is the case for coupling-factor activity (Fig. 4). Since the coupling-factor activity was also linear with respect to Chl *a*/Chl *b* ratios in the light quality regimes, C, +FR, −FR and R, we assume that the amounts of electron-transport carriers in these three light regimes would show similar responses.

Discussion

Pea seedlings grown in four different light-quality regimes each showed a curvilinear response of the Chl *a*/Chl *b* ratio with respect to irradiance levels with a faster rate of increase at the lower irradiance level and a lower rate of increase at the higher irradiance levels (Fig. 2). Previously, we have shown that the increase in Chl *a*/Chl *b* ratios with irradiance in the control light climate is due to modulations in the relative amounts of chlorophyll-protein complexes [12]. Low Chl *a*/Chl *b* ratios were due to relatively more Chl *a*/*b*-proteins of PS II and PS I, LHC II and LHC I, and, concomitantly, less of the core Chl *a*-proteins, CP*a* and CP1. Hence, modulations in the amounts of LHC II and LHC I vs. CPa and CP1 were found also in thylakoids isolated from peas grown in the far-red enriched, far-red deficient and red-light sources as well as for the control-light source. The coupling-factor activity also showed a curvilinear response to irradiance in all four light climates (Fig. 3) again with greater rates of increase occurring at the lower irradiance levels in all cases, regardless of the red/far-red ratios of the light sources.

In peas there is a linear relationship between the Chl *a*/Chl *b* ratios, and hence between the relative Chl-protein composition and the CF_1 activity for each light regime (Fig. 4). Previous investigations on two cultivars of sunflowers grown at different temperatures also show that the levels of CF_1 activity follow closely the variations in Chl *a*/Chl *b* ratios [13,27]. Light intensity during growth clearly influences not only the relative proportions of chlorophyll-protein complexes as reflected by the Chl *a*/Chl *b* ratios, but also the

electron-transport capacities of both PS II and PS I, concentrations of electron carriers and reaction centres, and ATP synthetase activity (Fig. 5). At the lower irradiance levels in all four light sources, plants compensate for the limited light by increasing the apparent antenna unit size of PS II, and possibly PS I, whereas at higher irradiance they have less Chl *a/b*-proteins and more Chl *a*-proteins present. Since the stoichiometry of the two Chl *a* 47 and 43 kDa apoproteins of PS II remains constant, more CPa and less LHC II implies more P-680 per unit Chl in PS II, and this will partly account for the increased electron-transport capacity. The amounts of electron carriers per unit Chl such as Q, plastoquinone and cytochrome *f* also increase with increasing irradiance. In line with the increased electron-transport capacity, the amounts of ATP synthetase need also to be increased.

Phytochrome is known to increase the levels of mRNA of some thylakoid proteins in the light development of etioplasts [7]. Phytochrome also influences chlorophyll synthesis [28]. The effect of phytochrome is thought to depend on the amount of red light-absorbing ($Phyt_R$) and far-red light absorbing forms ($Phyt_{FR}$) which are in photoequilibrium, due to the $Phyt_{FR}$-destroying enzyme being activated above a threshold level of $Phyt_{FR}$ [16,29]. The relationship between phytochrome photoequilibrium ($Phyt_{FR}/Phyt_{total}$) and the R/FR ratio was shown by Smith and Holmes [30] to approximate a rectangular hyperbole; hence at low values of the R/FR ratio, small changes in this parameter would greatly affect phytochrome. However, in green tissue the photoequilibrium of phytochrome would be suppressed due to the specific absorption of red light by chlorophyll. Moreover, varying R/FR ratios might result in imbalances between the excitation of PS II and PS I, and plants may have the ability to compensate for this during long-term irradiance. It is significant therefore that our results show no direct correlation between the red/far-red ratio and the adaptive responses seen in Chl *a*/Chl *b* ratios or coupling-factor activities of the light-adapted pea thylakoids.

Although the various light conditions influenced the leaf and stem size in our experiments (data not shown), it appears that the overall chlorophyll-protein composition and coupling-factor activity – and hence, by extrapolation, the modulations of core PS II complex, PS I complex, cytochrome *b/f* complex and ATP synthetase on the one hand vs. the modulations of antenna Chl *a/b*-proteins on the other – are not greatly influenced by the red/far-red ratio during growth under long-term irradiance. We did not calculate the $Phyt_{FR}/Phyt_{total}$ ratios of the four light-quality regimes by the method of Smith and Holmes [30], but clearly the $Phyt_{FR}/Phyt_{total}$ ratios would be very different in each of the light regimes, and these should have elicited great changes in phytochrome effectiveness. The varying effects of phytochrome were clearly seen in alterations to stem length. We suggest, therefore, that phytochrome is not the dominant factor in determining the overall amounts of thylakoid complexes under long-term irradiance, although it may be involved initially in some accelerated gene expression of various thylakoid components [31]. Recently, Eskins et al. [32] showed that the adaptive effects of development of corn seedlings under far-red light could be duplicated by exceedingly low red light. Significantly, phytochrome did not appear to influence thylakoid pigments or chlorophyll-protein composition in either bundle sheath or mesophyll chloroplasts under long-term irradiance [32]. Our studies and those of Eskins et al. [32] suggest that phytochrome is not the dominant factor in determining the composition and function of thylakoid membranes under long-term irradiance.

Our data point also to the possible effect of a blue-light response. The transition points in Figs. 2 and 3, and the plots in Fig. 4 are similar for the control and far-red-deficient light regimes, but different for the red- and far-red-enhanced regimes. The light regimes used for the control and far-red-deficient treatments have a greater blue/(far-red + red) ratio compared to the red- and far-red-enhanced light.

The interacting effects of light quality and quantity both in promoting gene expression and in regulating photosynthesis itself are bound to be very complex. It is evident already, that transcriptional changes which are often influenced by phytochrome are only part of the light-regulatory process [6,7,31]. It may be also that the levels of intermediate metabolites, NADPH and ATP, will

influence gene expression. In particular, the levels of NADPH and ATP formed during photosynthesis will be influenced by any imbalances in the rate of excitation of PS II and PS I; such imbalances would certainly occur in our light regimes which have varying amounts of far-red light.

Acknowledgements

We are grateful for discussions with Drs. W.S. Chow, J.R. Evans, and R.W. King. We thank Dr. C.S. Wong for the red- and far-red-light-intensity measurements, and the Phytotron staff for maintaining the plants.

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