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Special Issue – Mini Review

Green Light Drives Leaf Photosynthesis More Efficiently than Red Light in Strong White Light: Revisiting the Enigmatic Question of Why Leaves are Green

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The literature and our present examinations indicate that the intra-leaf light absorption profile is in most cases steeper than the photosynthetic capacity profile. In strong white light, therefore, the quantum yield of photosynthesis would be lower in the upper chloroplasts, located near the illuminated surface, than that in the lower chloroplasts. Because green light can penetrate further into the leaf than red or blue light, in strong white light, any additional green light absorbed by the lower chloroplasts would increase leaf photosynthesis to a greater extent than would additional red or blue light. Based on the assessment of effects of the additional monochromatic light on leaf photosynthesis, we developed the differential quantum yield method that quantifies efficiency of any monochromatic light in white light. Application of this method to sunflower leaves clearly showed that, in moderate to strong white light, green light drove photosynthesis more effectively than red light. The green leaf should have a considerable volume of chloroplasts to accommodate the inefficient carboxylation enzyme, Rubisco, and deliver appropriate light to all the chloroplasts. By using chlorophylls that absorb green light weakly, modifying mesophyll structure and adjusting the Rubisco/chlorophyll ratio, the leaf appears to satisfy two somewhat conflicting requirements: to increase the absorptance of photosynthetically active radiation, and to drive photosynthesis efficiently in all the chloroplasts. We also discuss some serious problems that are caused by neglecting these intra-leaf profiles when estimating whole leaf electron transport rates and assessing photoinhibition by fluorescence techniques.

Keywords: Chlorophyll • Fluorescence • Palisade tissue • Photoinhibition • Quantum yield • Spongy tissue.

Abbreviations: A, absorbance; A_n, net photosynthetic rate; E, excess energy; F_m (F_m'), maximum fluorescence in the fully relaxed state (in the light); F_s', steady-state fluorescence in the light; F_v (F_v'), variable fluorescence in the fully relaxed state (in the light), $F_v = F_m - F_0$ ($F_v' = F_m' - F_0'$); F_0 (F_0'), minimum fluorescence in the fully relaxed state (in the light); Φ, mean quantum yield of monochromatic light in white light; ϕ, differential quantum yield of monochromatic light in white light; PAM, pulse amplitude modulated; PPFD, photosynthetically active photon flux density; R, reflectance; RuBP, ribulose-1,5-bisphosphate; T, transmittance.

Introduction

Absorbance spectra of chlorophylls or pigments extracted from green leaves show that green light is absorbed only weakly. Action spectra of photosynthesis for thin algal solutions, transparent thalli of ordinary green algae, and leaves of aquatic angiosperms also show that green light is less effective than red light. As has been pointed out by Nishio (2000), these facts are often confused, and it is frequently argued that green light is inefficient for photosynthesis in green leaves. However, many spectra of absorptance (the absolute value of light absorption) measured with integrating spheres have shown clearly that ordinary, green leaves of land plants absorb a substantial fraction of green light (McCree 1972, Inada 1976, Gates 1980). It is also known that green light, once absorbed by the leaves, drives photosynthesis with high

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efficiency (Björkmann 1968, Balegh and Biddulph 1970, McCree 1972, Inada 1976). On an absorbed quantum basis, the efficiency or photosynthetic quantum yield of green light is comparable with that of red light, and greater than that of blue light. The difference between the quantum yields of green and blue light is particularly large in woody plants grown outdoors in high light. The question of how much green light is absorbed and used in photosynthesis by the green leaves of land plants has therefore been solved. In this mini-review, however, we aim at further clarifying another important role of green light in photosynthesis, by considering the intra-leaf profiles of light absorption and photosynthetic capacity of chloroplasts. First, we briefly explain light absorption by the leaf. Secondly, we examine the light environment within the leaf. Thirdly, we compare the vertical, intra-leaf profile of photosynthetic capacity with that of light absorption. We also discuss some serious problems with the use of pulse amplitude modulated (PAM) fluorometry in assessing leaf electron transport rate and photoinhibition. Fourthly, we propose a new method to measure the quantum yield of any monochromatic light in white light, and demonstrate the effectiveness of green light in strong white light. Based on these arguments, we finally revisit the enigmatic question of why leaves are green.

Absorption of light by the leaf

Lambert–Beer's law defines absorbance, A, as,

$$A = -\log_{10}\frac{I_T}{I_0} = \varepsilon c l \qquad (1)$$

where I_0 is the intensity of the monochromatic light incident on the surface of the optical cuvette, and I_T is that of the transmitted light. A is equal to εcl, where ε is the absorption coefficient of the pigment in the solution ($m^2\ mol^{-1}$), c is the concentration of the pigment (mol m^{-3}) and l is length of the light path (m). Transmittance, T, is defined as I_T/I_0. The absorbance spectrum is the spectrum of A (or ε) plotted against the wavelength of monochromatic light. From Equation 1, it is obvious that the absorption spectrum does not express absorptance straightforwardly. As we will see below, it is dangerous to infer the absorptance of the leaf from the apparent impression of the absorbance spectrum. Although A is often called absorption, the common noun of the verb 'absorb', we use the term 'absorbance' in this article to avoid confusion between absorption and absorptance.

As an optical system, the leaf differs from a pigment solution in two aspects: the concentration of pigments into chloroplasts and the diffusive nature of plant tissues. The first factor decreases the opportunity for light to encounter pigments and generally decreases light absorption, and has been called the sieve or flattening effect.

Once light that is strongly absorbed by chlorophylls, such as blue or red, encounters a chloroplast, most of the light is absorbed. Let us make the drastic assumption that the chloroplast is a sac containing a solution of chlorophylls at a concentration of 100 mol m^{-3}. This value is chosen because (i) ordinary green leaves are a few hundred micrometers thick; (ii) 50–80% of their volume comprises cells; and (iii) chloroplasts occupy 5–10% of the cell volume. Given that the values of ε for the mixture of chlorophylls at blue and red wavelengths are $> 1.0\times10^4\ m^2\ mol^{-1}$, and the chloroplast thickness is 2 µm, then A of the chloroplast calculated using Equation 1 is > 2. In other words, < 1% of the red or blue light is transmitted through the chloroplast. On the other hand, for wavelengths that are weakly absorbed, such as green light, T is considerable. When ε for green light is assumed to be $500\ m^2\ mol^{-1}$, A and T would be 0.05 and 79.4%, respectively.

Using a simple model shown in **Fig. 1**, let us consider how the sieve effect is influenced by wavelength. In the left-hand cuvette, photosynthetic pigments are uniformly distributed, whereas the right-hand model comprises one half-cuvette with the pigments concentrated 2-fold and another half-cuvette containing only the solvent. At wavelengths with strong absorption, the loss of absorptance by the sieve effect is large. On the other hand, at wavelengths of weak absorption such as green, the loss is marginal. The sieve effect, therefore, strongly decreases absorptance at wavelengths of strong absorption such as red and blue light. Because of this, absorption spectra with strong sieve effects show flattened absorption peaks; hence the alternative term 'flattening effect'.

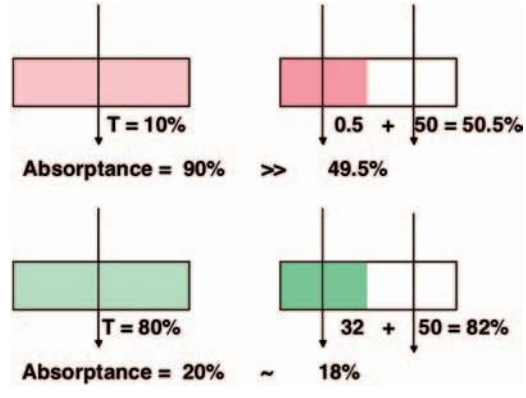

Fig. 1 Model explaining the sieve effect on absorptance. Left: a cuvette containing a pigment solution. Right: the pigment is concentrated in a half-cuvette, while another half-cuvette contains only the solvent. When the cuvette is uniformly irradiated with a strongly absorbed monochromatic light, the decrease in absorptance by the sieve effect was large (above), while in the case of weakly absorbed monochromatic light the decrease in absorptance is small (below).

The second point that distinguishes leaves from a simple pigment solution is that leaf tissues are diffusive. This is due to the fact that the leaf consists of cells and intercellular air spaces. The refractive index, which depends on both the material and wavelength of the light, of the bulk plant cells is around 1.48, compared with 1.33 for water and 1.0 for air. The diffusive nature of leaf tissues increases the light path length (détour effect) and thereby the opportunity for light to encounter chloroplasts, leading to the increase in absorptance (Vogelmann 1993). On the other hand, the diffusive nature of the leaf tissues inevitably increases the reflectance, *R*, of the leaf to some extent. Leaves appear to minimize *R* of the adaxial side by having a greater contact area between the adaxial epidermis and palisade tissue cells per unit leaf surface area than that between the abaxial epidermis and spongy tissue cells. In some species, palisade tissue cells are funnel-shaped, which further increases the contact area with the epidermis (Haberlandt 1914). By reducing the chances of refraction at the interfaces between cells and air, *R* decreases to a considerable extent (compare the differences in *R* between the adaxial and abaxial sides).

The increase in absorptance due to light diffusion (détour effect) is significant in the spongy tissues in bifacial leaves whose abaxial surfaces are paler than their adaxial surfaces (Terashima and Saeki 1983, Vogelmann 1993). In such leaves, spongy tissues have cell surfaces facing various directions and fewer chloroplasts (or chlorophyll) per unit mesophyll volume. In leaves of *Camellia japonica*, a typical example, lengthening of the optical path is more marked in the spongy tissue than in the palisade tissue (Terashima and Saeki 1983). On the other hand, in spinach, where the difference in the chlorophyll content per unit mesophyll volume between the palisade and spongy tissues use is small, the optical path length does not differ much between the tissues (Vogelmann and Evans 2002).

The consequence of lengthening the optical path can be shown using the same model (**Fig. 2**). In this model, the path length increases by 3-fold (see Vogelmann 1993). At strongly absorbed wavelengths, the increase in absorptance achieved by lengthening the light path is 11% (while the increase in *A* is, of course, 3-fold). In contrast, for weakly absorbed wavelengths such as green light, the increase in absorptance is much greater.

In summary, for strongly absorbed light such as red or blue, the sieve effect decreases absorptance considerably, whereas the détour effect increases absorptance marginally. On the other hand, for green light, loss in the efficiency of absorptance by the sieve effect is small, while gain in absorptance by the détour effect is large. Consequently, green leaves absorb much green light. Typical values of absorptance at 550 nm range from 50% in *Lactuca sativa* (lettuce) to 90% in evergreen broad-leaved trees (Inada 1976). The corresponding absorptance values for blue and red lights

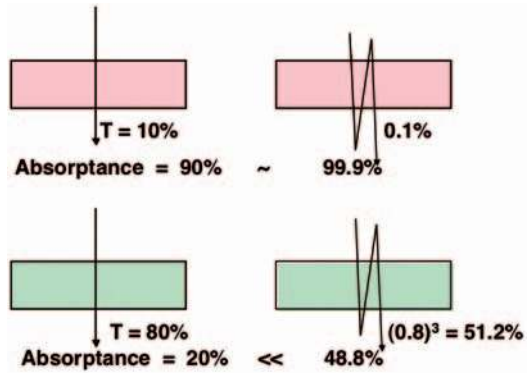

Fig. 2 Model explaining the détour effect on absorptance. Left: no détour effect. Right: the light path is lengthened 3-fold by the détour effect. For strongly absorbed monochromatic light, the increase in absorptance by the détour effect is small (above), while for weakly absorbed light the increase is marked (below).

range from 80 to 95%. Moreover, as already mentioned above, it has been clearly shown that the quantum yield of photosynthesis based on absorbed photosynthetically active photon flux density (PPFD), measured at low PPFDs, was comparable between green and red light. When measured in leaves grown under natural conditions, particularly for those of trees, the quantum yield of green light is considerably greater than that of blue light (Inada 1976), because some fraction of blue light is absorbed by flavonoids in vacuoles and/or carotenoids in chloroplast envelopes. Moreover, some carotenoids in thylakoid membranes do not transfer energy to reaction centers, or transfer with an efficiency significantly less than 1.0 (Akimoto and Mimuro 2005). For example, one of the most abundant carotenoids in thylakoids, lutein, transfers its energy to chlorophyll with an efficiency of 0.7 (Akimoto et al. 2005). The efficiency for neoxanthin is even less, at most 0.09 (Akimoto et al. 2005). Accumulation of flavonoids and carotenoids is well known to increase in response to ultraviolet and/or strong light (Lambers et al. 2008). This probably explains to a considerable extent why the quantum yield of blue light is low.

Evans and Anderson (1987) reconstructed the absorbance spectrum of thylakoid membranes from those of the chlorophyll–protein complexes and estimated the relative excitation of PSII and PSI. Evans (1987) argued that imbalance of PSII/PSI excitation would occur at wavelengths where light is absorbed by Chl *b* because energy is preferentially transferred to PSII. This might also explain why the quantum yield of blue light on an absorbed quantum basis is low. If this effect is large, a decrease in the PSII quantum yield (Genty's parameter, see below) might be expected at wavelengths strongly absorbed by Chl *b*. In a preliminary study with rice leaf discs illuminated with monochoromatic lights at a low PPFD of 5–12 μmol m^{-2} s^{-1},

we observed small reduction of the PSII reaction center [decreased $(F_m' - F_s')/F_m'$ mainly due to the decrease in photochemical quenching] in two wavelength regions with peaks at 470 and 650 nm, respectively, implying overexcitation of PSII at these wavelengths. However, the decreases observed were not enough to account for the large decrease in the quantum yield of blue light.

Light environment within the leaf

Although there were some classical works, the light environment within the leaf was first intensively studied in the early 1980s. The micro fiberoptic method is the most efficient in measuring the flux of light within a leaf (Vogelmann et al. 1991, Vogelmann 1993). Because the viewing angle of the optical fiber is narrow, the angular distribution of the light flux, including backward scattering, is measured by inserting the fiber into the leaf from various directions. On the other hand, it is not possible to measure the absorption profile using this method alone. Paradermal sectioning, i.e. sectioning of the leaves parallel to the leaf epidermis, is suitable for examining the optical properties of leaf tissues (Terashima and Saeki 1983). This sectioning method is also used to measure the profiles of photosynthetic properties within the leaf (Terashima and Hikosaka 1995). Sectioning after exposure of the leaf to $^{14}CO_2$ has been used to reveal the photosynthetic profile in vivo across the leaf (for a review, see Nishio 2000). Fukshansky and his colleagues applied the Kubelka–Munk theory to predict the light environment within the leaf and to characterize the optical properties of leaf tissues (Richter and Fukshansky 1996a, Richter and Fukshansky 1996b). Fluorescence techniques have also been used. Takahashi et al. (1994) devised a method to illuminate a leaf segment normal to its epidermis and measure fluorescence from the transversely cut surface of the segment in order to analyze the light absorption gradient.

To analyze the light environment within the leaf in relation to photosynthesis, it is necessary to know the light absorption profile, not the light fluxes per se. This is because only those photons absorbed by pigments can work photochemically (the law of photochemistry, see Clayton 1970). There are no straightforward methods to measure the light absorption profile, but the method of Takahashi et al. (1994) originally developed for rice leaves has been successfully used for estimation of the light absorption profiles for leaves of *Rhizophora mucronata* and *C. japonica* (Koizumi et al. 1998), *Spinacia oleracea* (Vogelmann and Evans 2002, Vogelmann and Evans 2003) and *Eucalyptus pauciflora* (Evans and Vogelmann 2006).

Here, applying the Kubelka–Munk theory to the transmittance and reflectance data from paradermal leaf sections (Terashima and Saeki 1983), we have reconstructed the light environment and absorption profile of a *C. japonica* L. leaf. The method of Allen and Richardson (1968) was used to fit the data (Gates 1980). For a leaf containing Chl $a+b$ at C mol m^{-2}, the downward flux (I) and the upward flux (J) are considered at the plane parallel to the irradiated leaf surface. Let the cumulative Chl $a+b$ from the surface to the plane be c mol m^{-2}. Then, introducing the absorption parameter, k, and the scattering parameter, s, we obtain the following set of differential equations:

$$\begin{aligned} dI &= -(k+s)\cdot I(c)\cdot dc + sJ(c)\cdot dc \\ dJ &= -s\cdot I(c)\cdot dc + (s+k)\cdot J(c)\cdot dc \end{aligned} \quad (2)$$

Boundary conditions are

$$I(0)=1,\ J(0)=R_L,\ I(C)=T_L,\ \text{and}\ J(C)=R_0 I(C) \quad (3)$$

where R_L is the reflectance of the leaf, T_L is the transmittance of the leaf and R_0 is the reflectance of the lower surface of the leaf when light is applied from inside the leaf (Gates 1980). For the fitting, we used data from paradermal sections of *C. japonica* leaves with upper epidermes, and reflectance data from paradermal sections with lower epidermes (Terashima and Saeki 1983). Through fitting the data, k and s for the palisade tissue and spongy tissues for 680 and 550 nm were obtained, respectively; 680 nm is the red absorption peak of chlorophyll *a* in vivo, while 550 nm is green light at which leaves show maximal T_L and R_L.

Fig. 3 shows fitting of the Kubelka–Munk model to the sections. The adopted k and s sufficiently describe the optical properties of these leaf tissues. **Fig. 4** shows the downward flux, I, the ratio of the upward flux to the downward flux, J/I, the sum of both fluxes, $I+J$, and absorption, $k(I+J)$, calculated for a model *C. japonica* leaf. The calculated values showed abrupt changes at the interface between the palisade and spongy tissues. This is because k and s for these tissues differed. Both k and s for the spongy tissue were much greater than those for the palisade tissue (see legend of **Fig. 3**), reflecting the enhancement of absorption by the détour effect and diffusive nature of the spongy tissue. If s were zero or there were no scattering, the differential equations would degenerate into the Lambert–Beer's law and $k/2.3$ would be equal to ε(cf. $\log_{10}e = 2.3$). The value of k for the palisade tissue at 680 nm, 10^4, corresponds to ε of 4,300 m^2 mol^{-1}, which is about half the value for the red peak of chlorophyll in the organic solvent. On the other hand, for 550 nm, $k/2.3$ was 1,500, much greater than ε in the green region (<500 m^2 mol^{-1} for the solution of chlorophylls) even for the palisade tissue. These absolute values are somewhat greater than those obtained for blue light (2,600–2,900 m^2 mol^{-1}) and green light (1,000–1,300 m^2 mol^{-1}) in spinach leaves (Vogelmann and Evans 2002).

Our absorptance data agree well with a previous calculation of the light absorption gradient, which was based purely on the experimental data (Terashima and Saeki 1985). When compared

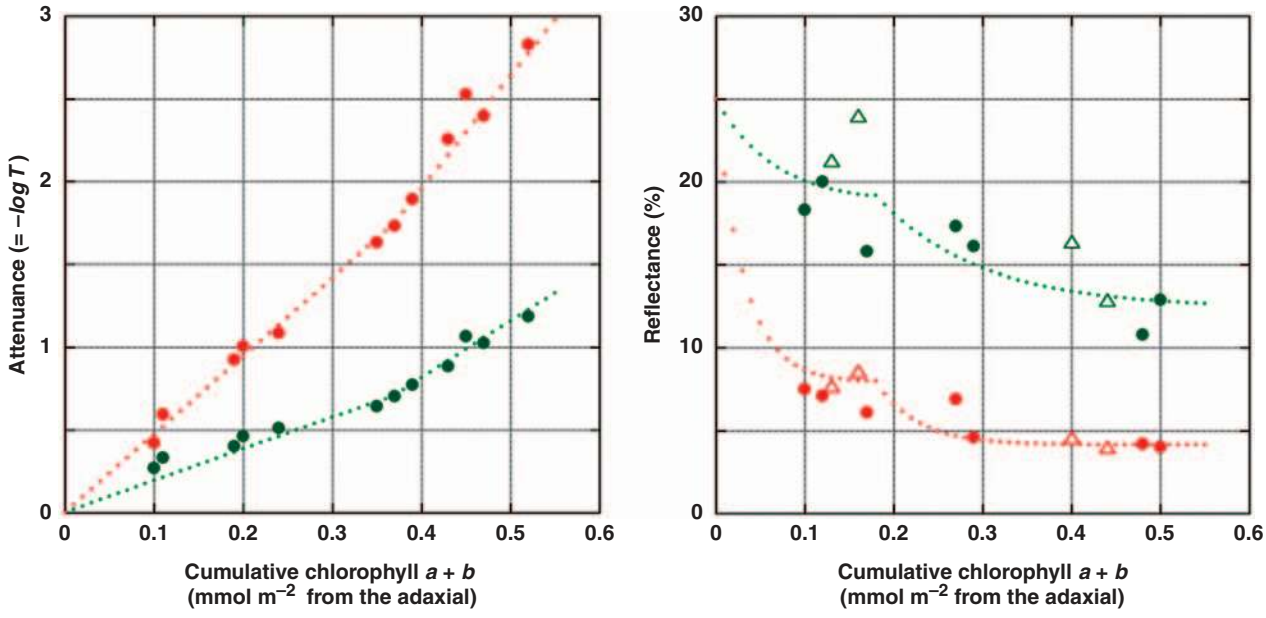

Fig. 3 Fitting of the Kubelka–Munk theory to the transmittance and reflectance data of the paradermal sections of leaves of *Camellia japonica*. Left: attenuance = $-\log_{10}T$ of paradermal sections with adaxial epidermes. Although attenuance can be defined by the same mathematical equation that defines absorbance (A), attenuance is used when the decrease in T due to R is substantial. The monochromatic light at 680 or 550 nm was applied to the adaxial epidermis. Right: reflectance of the paradermal sections having the abaxial epidermes. Monochromatic light was irradiated from the cut surface. Reflectance was measured with an integrating sphere. Different symbols indicate different leaves. The reflectance of the abaxial epidermis was assumed to be 0.25, for both 680 and 550 nm. The boundary between the palisade and spongy tissues was assumed to be at 0.37 mmol chlorophyll m^{-2} (note the inflections of the curves). k_{680} and s_{680} for the palisade tissue were 10,000 and 900, and k_{680} and s_{680} for the spongy tissue were 13,400 and 2,500, respectively. k_{550} and s_{550} for the palisade tissue were 3,400 and 1,100, and k_{550} and s_{550} for the spongy tissue were 5,300 and 3,500, respectively. For the unit of these numbers, see the text. The data of transmittance and reflectance were adopted from Terashima and Saeki (1983).

on a unit chlorophyll basis, the chloroplasts in the lowermost part of the leaf absorb <10% of those in the uppermost part, even at a wavelength of 550 nm at which the absorption gradient is most moderate. For spinach, various estimations have been published. Using the method of Takahashi et al. (1994), Vogelmann and Evans (2002) and Evans and Vogelmann (2003) indicated that, on a unit chlorophyll basis, the chloroplasts in the lowermost part absorb about 10 and <20%, respectively, of the green light of those in the uppermost part. For wavelengths with strong absorption, such as red and blue, the fractions are much smaller. In *C. japonica*, the absorption of 680 nm (red) light by the lowermost chloroplasts is <2% of the absorption by the uppermost chloroplasts on a unit chlorophyll basis. For blue light in spinach, the estimated absorption by the lowermost chloroplasts was <5% of that of the uppermost (Vogelmann and Evans 2002, Evans and Vogelmann 2003).

Comparison of the profiles of light absorption and photosynthetic capacity

The profiles of photosynthetic capacity along the gradient of light absorption have been reported for *Spinacia oleracea* (Terashima and Hikosaka 1995, Nishio 2000, Evans and Vogelmann 2003) and *E. pauciflora* (Evans and Vogelmann 2006). The differences in photosynthetic properties found between the chloroplasts in the upper and lower parts of the leaf are essentially identical to those found between sun and shade leaves, or between sun and shade plants (Terashima and Hikosaka, 1995). Thus, the formation of an intra-leaf profile of photosynthetic capacity can be regarded as an acclimation process (Terashima et al. 2005). It is also worth mentioning that we verified acclimation of light sensitivity of stomatal opening to the intra-leaf light environment with *Helianthus annuus* leaves: stomata in the abaxial epidermis, which are located in a light environment enriched in green light, open in response to monochromatic green light, whereas those in the adaxial epidermis do not (Wang et al. 2008).

Based on observations of the differences in the shape of light response curves depending on the direction of irradiation, Oja and Laisk (1976) predicted the existence of an intra-leaf gradient in photosynthetic capacity. The profile in photosynthetic capacity and the differentiation of optical properties between palisade and spongy tissues are adaptive,

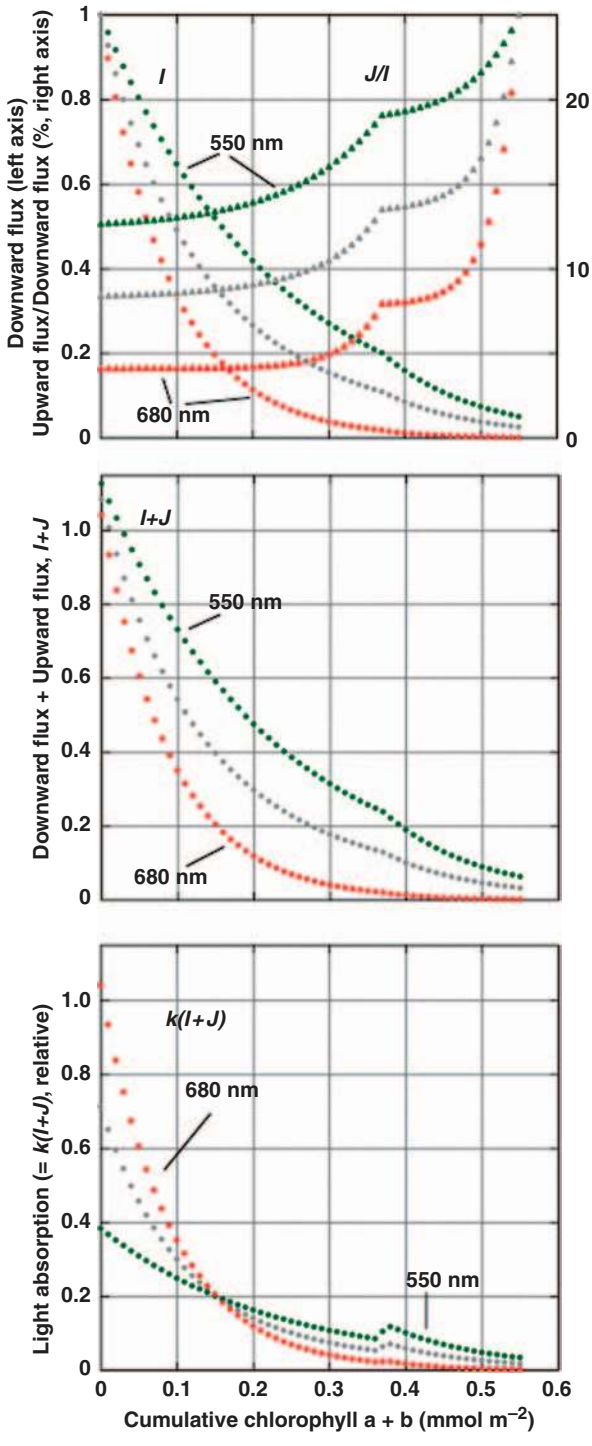

Fig. 4 Light environment and light absorption profile within a *Camellia japonica* leaf predicted by the Kubelka–Munk theory. The *k* and *s* values fitted to the data (**Fig. 3**) were used. *I*, downward flux; *J*, upward flux; and $k\cdot(I+J)$, absorptance per unit chlorophyll (relative value). The calculated results for 680 and 550 nm are shown together with the mean values of 680 and 550 nm values (in gray). Unpublished results of I. Terashima.

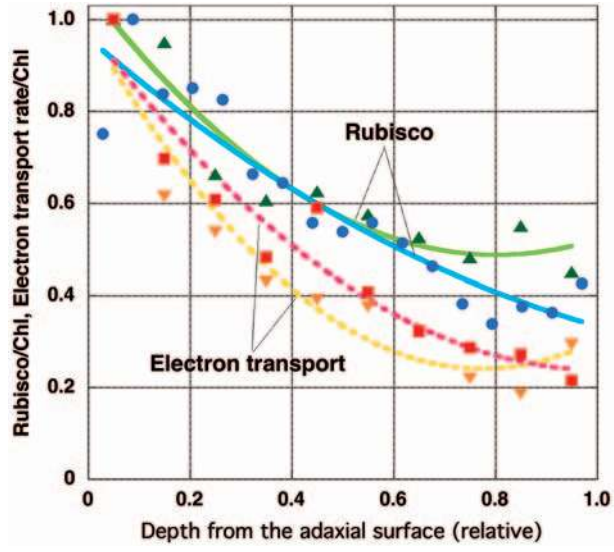

Fig. 5 Intra-leaf profiles of photosynthetic capacities in leaves of *Spinacia oleracea*. Green triangles, Rubisco content/Chl (Terashima and Inoue 1985); blue circles, Rubisco content/Chl (Nishio et al. 1993); red squares, dichloroindophenol reduction rate/Chl (Terashima and Inoue 1985); and orange triangles, dichloroindophenol reduction rate/Chl (Terashima 1989). Quadratic equations were fitted to the data.

because these features improve the efficiencies of both light use and nitrogen use in photosynthesis (Terashima and Saeki 1985, Farquhar 1989, Terashima and Hikosaka 1995). The most efficient situation is realized when the profile of light absorption and the profile of photosynthetic capacity are perfectly matched, and all the chloroplasts in the leaf behave synchronously with respect to photosynthetic light saturation (Farquhar 1989, Terashima and Hikosaka 1995, Richter and Fukshansky 1998). **Fig. 5** shows the gradients of Rubisco content per Chl, and electron transport rate from water to dichlorophenol indophenol per Chl. The gradients for electron transport rate were steeper than those for the Rubisco. There appears to be a gradient in the balance between ribulose-1,5-bisphosphate (RuBP) carboxylation and RuBP regeneration capacities. Although the influence of light level on this balance has not been studied intensively (see Evans and Vogelmann 2003), it may be an important subject, particularly with respect to photoinhibition (see below). The gradients show that even chloroplasts in the lowermost part of the leaf exhibit 20–40% of the maximal photosynthetic capacity of the uppermost chloroplasts. Given that the profile of photosynthetic capacity may be similar to that of light absorption when bifacial spinach leaves are irradiated from the adaxial side with green monochromatic light (Evans and Vogelmann 2003), and that T_L values of ordinary green leaves were at most 10–15% for PPFD (McCree 1972, Inada 1973), the gradients of photosynthetic capacity are in most cases more gradual than those of light absorption in situ.

The underlying reason(s) for the more gradual gradient of photosynthetic capacity have not been clarified. It is likely, however, that it is too costly for a given plant to be prepared to acclimate to a very wide range of light environment. In other words, in any given species, there is a limit to the dynamic range of acclimational adjustment of chloroplast properties to the light environment.

Detection of discrepancy of the profiles of light absorption and photosynthetic capacity

When leaves are irradiated from the upper side, therefore, there will be a situation in which the upper chloroplasts are light saturated while the chloroplasts in the lower parts still need additional light to reach saturation. In other words, the quantum yield of photosynthesis differs within the leaf, being less in the uppermost part. This discrepancy has been detected by comparing the electron transport rates estimated from the gas-exchange technique and from Genty's parameter (Tsuyama et al. 2003; for Genty's parameter, see below). For upright or pendulous leaves, it has long been known that the sharpest light response curves are obtained when these leaves are irradiated equally from both sides (Moss 1964, Tanaka and Matsushima 1970, Evans et al. 1993). Thus, unilateral illumination should cause a considerable discrepancy between the profile of light absorption and the profile of photosynthetic capacity.

A more straightforward method to detect such a difference in light saturation would be to monitor fluorescence from both sides of the leaf, in order to assess the PSII quantum yields or Genty's parameters for each side (Genty et al. 1989).

After formulation by Genty et al. (1989), the linear electron transport rate from water to $NADP^+$ for the whole leaf has been frequently estimated as:

$$J = I \cdot \alpha \cdot \varphi_{PSII} \cdot \frac{F_m' - F_s'}{F_m'} \quad (4)$$

where α is the absorptance of the leaf, φ_{PSII} is the fraction of excitation energy allocated to PSII, F_m' is the maximal fluorescence in the light, and F_s' is the fluorescence level in the presence of actinic light. The last term, $(F_m' - F_s')/F_m'$, expresses the quantum yield of PSII in actinic light and is often called Genty's parameter.

Marked differences in Genty's parameter have already been shown, for example for thick (~300 µm) horizontal leaves of *Eucalyptus maculata* (Evans et al. 1993). When the adaxial surface was irradiated with strong white light, $(F_m' - F_s')/F_m'$ estimated from fluorescence signals from the abaxial side was markedly greater than that estimated for the adaxial side. **Fig. 6** shows an example for a relatively thin leaf from a shade-grown *Alocasia odora* plant. At PPFDs > 600 µmol m^{-2} s^{-1}, $(F_m' - F_s')/F_m'$ obtained from the upper side was lower than that obtained from the lower side. These differences are clear proofs of the discrepancies between the light absorption profile and that of the photosynthetic capacity.

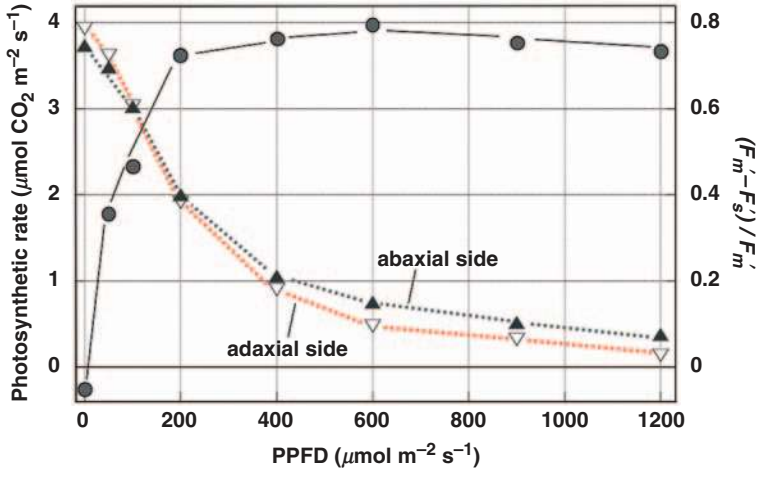

Fig. 6 Light response curves of the rate of photosynthesis (A_n) and quantum yield of PSII in the light in a leaf of a shade-grown *Alocasia odora* plant. A_n was measured with a portable gas-exchange system (LI-6400, Li-Cor, Lincoln, NE, USA) at a cuvette temperature of 25°C in air containing 380 µl l^{-1} CO_2. The leaf was sandwiched with two half-chambers that had transparent windows. White light from a metal-halide lamp was provided via an optical fiber with a rectangular light emitter. The fluorescence signals were measured from the adaxial and abaxial sides with two PAM fluorometers (PAM 101/102/103, Walz, Effltliche, Germany). The optical fiber connected to one of the fluorometers was placed in the central space of the rectangular emitter for the actinic light. The other probe was placed below the lower half-chamber. Unpublished results of T. Inoue.

Because $(F_m' - F_s')/F_m'$ would not be uniform within the leaf, indiscriminate use of the adaxial fluorescence signals to represent the whole leaf is very problematical.

Use of the adaxial fluorescence signals in photoinhibition studies

The fluorescence method has been used in studies of photoinhibition for the past three decades. When PSII is damaged by strong light, $(F_m - F_0)/F_m$ decreases (Powles 1984). There are two main hypotheses for the mechanism of photoinhibition: the excess energy hypothesis (Weis and Lechtenberg 1989, Osmond 1994, Demmig-Adams et al. 1996, Kato et al. 2003) and the two-step hypothesis (Hakala et al. 2005, Ohnishi et al. 2005, Nishiyama et al. 2006). The energy of photons absorbed by PSII pigments is dissipated as either heat or fluorescence in PSII antennae, $F_0'/F_m' = 1 - F_v'/F_m'$, drives photosynthesis, $(F_m' - F_s')/F_m'$ (Genty's parameter), or migrates to closed PSII reaction centers and is dissipated non-photochemically, $(F_s' - F_0')/F_m'$ (excess):

$$(F_m' - F_s')/F_m' + (F_s' - F_0')/F_m' + F_0'/F_m' = 1 \quad (5)$$

(Stefanov and Terashima 2008). Excess energy, E, can be calculated as:

$$E = I \cdot \alpha \cdot \varphi_{PSII} \cdot \frac{F_s' - F_0'}{F_m'} \quad (6)$$

The excess hypothesis claims that the excess energy, the energy migrated to closed PSII centers, is responsible for photoinhibition. According to this hypothesis, photoinhibition would not occur when PSII reaction centers are open. On the other hand, the two-step hypothesis claims that the manganese cluster in the oxygen-evolving complexes is the primary site of damage. The action spectrum for manganese damage indicates that the effect is strongest in the UV region and is progressively weaker in blue, green then red light (Hakala et al. 2005, Ohnishi et al. 2005). Once the oxygen-evolving complex is damaged, damage to the PSII reaction center occurs subsequently. These two hypotheses are not necessarily mutually exclusive, and it is probable that both mechanisms are important in nature (R. Oguchi and W. S. Chow in preparation).

In the context of the topic of this review, it is important to note that photoinhibitory damage does not occur uniformly throughout the leaf, irrespective of the *mechanisms*. In the paradigm of the excess hypothesis, if $(F_s' - F_0')/F_m'$ does not differ much across the leaf, the extent of E should depend on the depth within the leaf and the wavelength of the light, as is apparent from Equation 6. For the two-step hypothesis, if the first step is irreversible, the damage to the manganese cluster should depend on the absolute number of photons (UV and blue) absorbed by chloroplasts. Therefore, the damage to the manganese cluster should also differ depending both on the depth within the leaf and the wavelength. In reality, $(F_s' - F_0')/F_m'$ would differ considerably across the leaf [as has been argued above for $(F_m' - F_s')/F_m'$; see also **Fig. 6**], and the damage to the manganese cluster appears to be reversible. These imply that photoinhibitory damage should be greater in the chloroplasts near the irradiated surfaces.

In the PAM system from Walz (Effeltrich, Germany), the measuring beam is red light with an intensity peak around 650 nm. When the beam is irradiated from one side of the leaf, the beam is absorbed mainly in the shallow part of the mesophyll, and thereby fluorescence emitted from chloroplasts near the irradiated surface is preferentially detected. As expected from the argument above, large differences in F_v/F_m between the leaf surfaces have been reported for leaves of *H. annuus* that were unilaterally irradiated with strong light (Evans et al. 1993). When leaves of *Capsicum annuum* were photoinhibited by irradiation to the upper side, the fluorescence signals from the upper side indicated considerable decreases in F_v/F_m, whereas F_v/F_m calculated from the fluorescence signals obtained from the abaxial side hardly decreased (R. Oguchi and W. S. Chow in preparation).

To analyze photoinhibition within the leaf, the pulse-modulated fiberoptic fluorometer can be used. Schreiber et al. (1996) devised this system (Microfiber PAM) and demonstrated several applications including the measurement of the profiles of F_v/F_m within leaves of *Syringa vulgaris*. When the leaf was irradiated from the adaxial side with strong white light, the authors observed the lowest F_v/F_m in the uppermost part of the mesophyll, and the value increased with depth. Using *C. annuum* leaves, we also confirmed the same trend (**Fig. 7**, left). We further analyzed effects on the photoinhibition profile of treatments with broad-band red, green and blue lights at the same PPFD (**Fig. 7**, right). Because the manganese cluster would show strong absorption in UV and blue regions, and the absorption by the manganese cluster should decrease markedly with the increase in wavelength, we might expect that the strongest photoinhibition would occur with blue light, followed by green and then red light. On the other hand, absorption of photons by the chloroplasts near the irradiated surface should be greatest when the leaf is irradiated by blue light, followed by red light, and it should be lowest in green light. The results shown in **Fig. 7B** may be explained by these two effects. The greatest decrease in F_v/F_m in the uppermost part of the leaf was observed with blue light, and F_v/F_m approached high levels at depth. The second greatest damage to the surface chloroplasts was observed with red light, but the damage was confined to the irradiated half of the leaf. On the other hand, damage to the surface chloroplasts was least with green light, but continued deep into the leaf, probably because

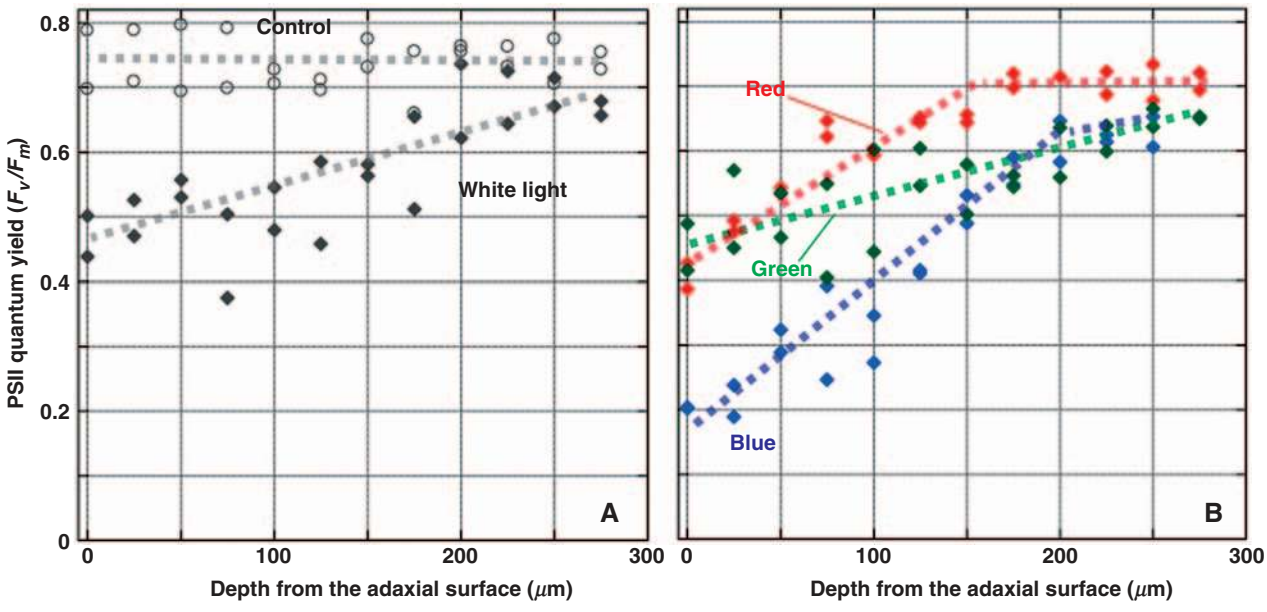

Fig. 7 Intra-leal profiles of F_v/F_m in leaves of *Capsicum annuum*. Leaf discs of *C. annuum* were treated with lincomycin, an inhibitor of protein synthesis, and subsequently photoinhibited at 2,000 µmol photon m^{-2} s^{-1} at room temperature for 60 min by white light, broad-band blue (400–500 nm), green (500–600 nm) or red (600–700 nm, right). A (left), results with white light; B (right), results with broad-band monochromatic lights. A Microfiber PAM (Walz, Effelt, Germany) having an optical microfiber of 30 µm diameter was used to measure intra-leaf profiles of F_v/F_m. The micro optical fiber was inserted into the leaf tissues with the aid of a three-dimensional water-pressure micromanipulator (WR-60, Narishige, Tokyo, Japan). Unpublished data of R. Oguchi.

sufficient green light penetrated and was absorbed by the chloroplasts in the abaxial side.

The Microfiber PAM system uses photomultipliers to detect fluorescence. Although the system is operated in the pulse amplitude modulation mode, strong background light interferes with the measurements. It could be possible to follow the recovery of fluorescence yield after rapidly turning off the actinic light *by extrapolation*. We would then be able to estimate PSII quantum yield in the light and thereby the profile of photosynthetic capacity. However, we are still awaiting suitable modification of the system to allow such an experiment.

As has been explained above, it is dangerous to assume that the fluorescence signals obtained from the irradiated side of a leaf represent the quantum yield of the chloroplasts within the whole leaf. In particular, when the chloroplasts near the irradiated surface are photoinhibited, the misleading effect would be very large. It also causes some artifact in estimating mesophyll conductance (or internal conductance), the conductance from the intercellular spaces to chloroplast stroma for CO_2 diffusion, because the combined gas-exchange and fluorometry method for estimating this conductance assumes that the quantum yield of PSII is uniform in the chloroplasts in the whole leaf. Because this problem is beyond the scope of this review, readers should refer to appropriate papers (see Evans 2009).

In contrast to chlorophyll fluorescence, radiation at 810 nm is hardly absorbed by chlorophyll, so measurements based on an absorbance change at 810 nm associated with the oxidation of P700 (the special chlorophyll pair in PSI) monitor all P700 rather equally at all depths of a leaf. Using the 810 nm signal to determine the relative content of PSII after photoinhibition, Losciale et al. (2008) found a single relationship that applied to leaves of diverse anatomy.

In situ quantum yield of monochromatic light in white light

As Nishio (2000) clearly postulated, and as we have detailed so far, red or blue light is preferentially absorbed by the chloroplasts in the upper part of the leaf. Then, when PPFD is high, the energy of these wavelengths tends to be dissipated as heat by the upper chloroplasts, while green light drives photosynthesis in the lower chloroplasts that are not light saturated (Sun et al. 1998, Nishio 2000). However, there has been no quantitative evaluation of this possibility. Here, we propose a new method to quantify the quantum yield of monochromatic light contained in white light.

Theory

We can measure the differential quantum yield of the monochromatic light in any background white light at a PPFD of I, $\phi_\lambda(I)$, as in **Fig. 8**. Initially, a leaf is illuminated with the

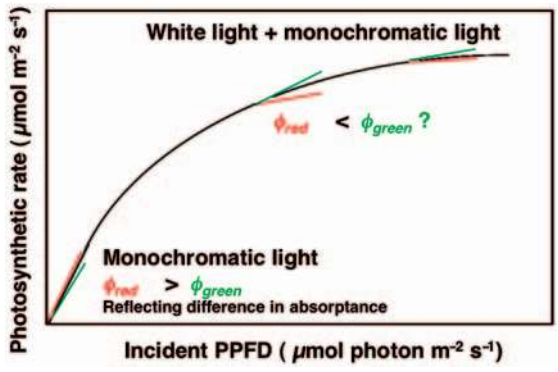

Fig. 8 The differential quantum yield measurement. In the presence of white light at PPFD of I, weak monochromatic light (dI) is given. The ratio of increment of the photosynthetic rate (dA_n) to PPFD of the monochromatic light (dI), dA_n/dI, gives the differential quantum yield $\phi(I)$. When $I = 0$, the differential quantum yield is the same as the ordinary quantum yield on an incident PPFD basis.

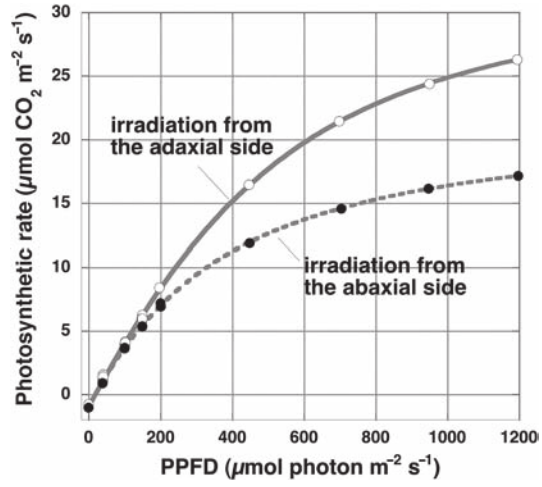

Fig. 9 Light response curves of the rate of net photosynthesis (A_n) in a leaf of *Helianthus annuus* obtained with irradiation to the adaxial or abaxial side. The rate of photosynthesis was measured with a portable gas-exchange system (LI-6400, Li-Cor), at a leaf temperature of 25°C in air containing 390 μl l^{-1} CO_2. Vapor pressure deficit was <0.7 kPa. The light from a halogen lamp in a slide projector was delivered by a tri-furcated optical fiber. *Helianthus annuus* plants were grown at 350 μmol m^{-2} s^{-1} at canopy height at 23°C with a photoperiod of 12 h. Unpublished data of T. Fujita.

white light at I. Then, weak monochromatic light at the wavelength of λ (dI) is added to the background white light. The increment of the photosynthetic rate (A_n), dA_n, divided by dI, dA_n/dI, is defined as $\phi_\lambda(I)$.

Let us then define the mean quantum yield of monochromatic light at the wavelength of λ contained in the white light at I as $\Phi_\lambda(I)$. When the fraction of the PPFD of the monochromatic light at λ to that of the whole white light is r_λ, then $r_\lambda \cdot I \cdot \Phi_\lambda(I)$ is the photosynthesis driven by the monochromatic light at λ when the PPFD of the white light is I. At a PPFD of $I + \Delta I$, the photosynthesis driven by the monochromatic light at λ is $r_\lambda \cdot (I + \Delta I) \cdot \Phi_\lambda(I + \Delta I)$, which can be written as:

$$r_\lambda (I+\Delta I)\cdot \Phi_\lambda (I+\Delta I) = r_\lambda \cdot I \cdot \Phi_\lambda (I) + r_\lambda \cdot \Delta I \cdot \phi_\lambda (I) \quad (7)$$

Rearrangement of this equation leads to:

$$\lim_{\Delta I \to 0} \frac{(I+\Delta I)\cdot \Phi_\lambda (I+\Delta I) - I \cdot \Phi_\lambda (I)}{\Delta I} = \phi_\lambda (I) \quad (8)$$

Then, integrating $\phi_\lambda(I)$ with respect to I, one obtains:

$$I \cdot \Phi_\lambda (I) = \int_0^I \phi_\lambda (I) dI \quad (9)$$

Thus, it is possible to estimate $\Phi_\lambda(I)$, the mean quantum yield of any monochromatic light in the white light.

Because the Chl *a*/*b* ratio and the carotenoids/chlorophyll ratio are usually higher in sun-type chloroplasts, the quantum yield of photosynthesis driven by blue light would differ between sun and shade chloroplasts (Lichtenthaler and Balari 2004). Thus, in this study, we compared the effects of green light at 550 nm and red light at 668 nm. Judging from the action spectra of green leaves (McCree 1972, Inada 1976), the red light at 668 nm used in this study would not cause the marked red-drop effect. Moreover, the measurements were conducted in the presence of the background white light.

We obtained ϕ_{red} and ϕ_{green} in sunflower leaves irradi-ated from the adaxial side and abaxial side, respectively. Typical light response curves obtained by irradiating from the adaxial and abaxial sides of the same sunflower leaf are shown in **Fig. 9**. The different curves depending on the direction of irradiation were reported for several species (Moss 1964, Oja and Laisk 1976, Terashima 1986, Ögren and Evans 1993), and the difference between the curves can be explained by the profile of photosynthetic capacity and the difference in optical properties between the palisade and spongy tissues. When the leaf is irradiated from the lower side, light is preferentially absorbed by the spongy tissue. Then, PPFDs have to be increased to very high levels to deliver sufficient light energy to the upper chloroplasts for their light saturation (Oja and Laisk 1976, Terashima 1986, Ögren and Evans 1993, Terashima and Hikosaka 1995, Sun and Nishio 2001).

When a sunflower leaf was irradiated from the adaxial side, ϕ_{red} was at first greater than ϕ_{green} (**Fig. 10**). This can be attributed to the difference in absorptance between these two wavelengths. With the increase in PPFD of the white light, both ϕ decreased but, as expected, the decrease was more marked in ϕ_{red} than in ϕ_{green}. When the leaf was irradiated from the abaxial side, ϕ_{red} was greater only at the lowest PPFD, and green light was more effective in higher

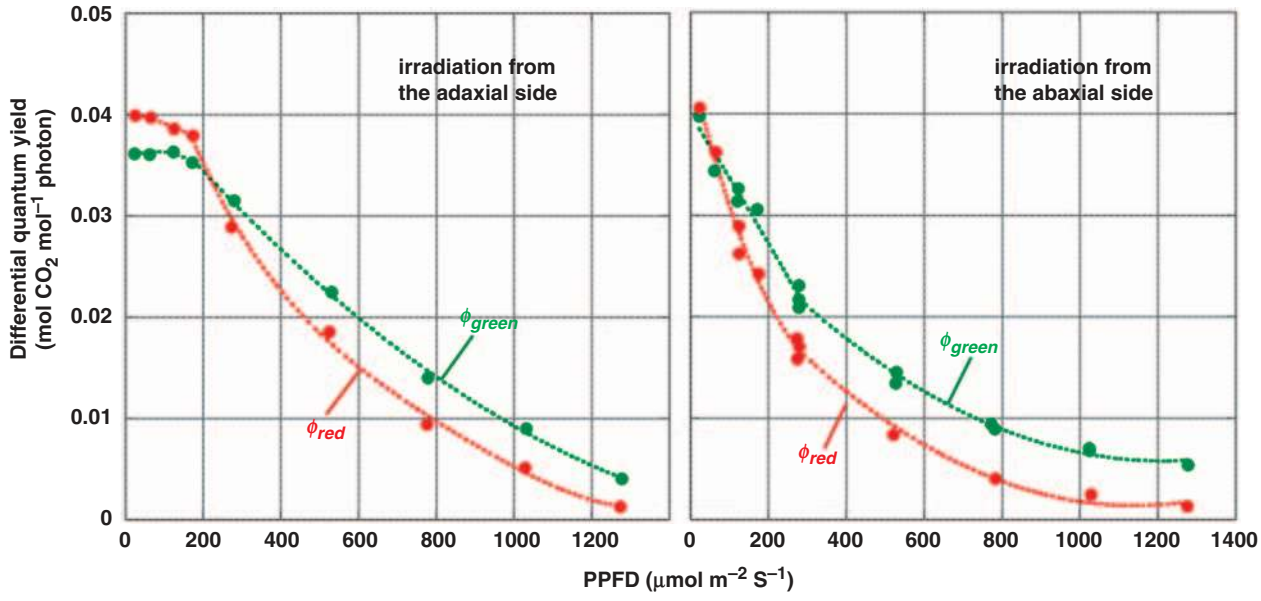

Fig. 10 Differential quantum yields for red and green monochromatic light in a leaf of *Helianthus annuus*. Left: data obtained by irradiation to the adaxial side. Right: data obtained by irradiation to the abaxial side. The white light source was a halogen lamp delivered through a tri-furcated optical fiber. Red or green monochromatic light was obtained by passing the light from a halogen lamp through optical filters and delivered through another tri-furcated optical fiber. A hexagonal holder made of Plexiglas was used to hold six optical fibers to secure uniform and constant irradiation. The peak of the green monochromatic light was 550 nm with the half-band width of ±30 nm. The red light had a maximum at 668 nm. Half the maximum transmittance occurred at 641 and 690 nm. The PPFDs of the white light were 0, 40, 100, 150, 200, 450, 700, 950 and 1,200 μmol photons m^{-2} s^{-1}. The light response curves obtained by irradiation with the white light are shown in **Fig. 9**. The PPFD of the monochromatic light was either 50 μmol m^{-2} s^{-1} (for the white light at 0, 40, 100 and 150 μmol m^{-2} s^{-1}) or 150 μmol m^{-2} s^{-1} (for the white light at 200, 450, 700, 950 and 1,200 μmol m^{-2} s^{-1}). The differential quantum yield is plotted against the PPFD (PPFD of the white light + half the PPFD of the monochromatic light). The data were obtained with the same leaf of *H. annuus* that was used for **Fig. 9**. Once A_n attained a stable value in the white light at the ambient CO_2 concentration (C_a) at 390 nm, then monochromatic light was added, keeping the intercellular CO_2 concentration (C_i) constant by manipulating C_a. Therefore, C_i was constant for three measurements, in the white light, the white light + red monochromatic light and the white light + green monochromatic light. Quadratic or quadruple equations were fitted to the data. $\phi_{green,adax}$ ($I \leq 180$ μmol m^{-2} s^{-1}) $= -1.0938 \cdot 10^{-7} I^2 + 1.7422 \cdot 10^{-5} I + 3.5598 \cdot 10^{-2}$ ($r^2 = 0.803$); $\phi_{green,adax}$ ($I \geq 180$ μmol m^{-2} s^{-1}) $= 1.1978 \cdot 10^{-8} I^2 - 4.5847 \cdot 10^{-5} I + 4.3115 \cdot 10^{-2}$ ($r^2 = 0.999$); $\phi_{red,adax}$ ($I \leq 180$ μmol m^{-2} s^{-1}) $= -3.8099 \cdot 10^{-8} I^2 - 6.3947 \cdot 10^{-6} I + 4.0115 \cdot 10^{-2}$ ($r^2 = 0.980$); $\phi_{red,adax}$ ($I \geq 180$ μmol m^{-2} s^{-1}) $= 4.1802 \cdot 10^{-14} I^4 - 1.4411 \cdot 10^{-10} I^3 + 1.9467 \cdot 10^{-7} I^2 + 1.264 \cdot 10^{-10} I + 5.7508 \cdot 10^{-2}$ ($r^2 = 0.999$); $\phi_{green,abax}$ ($I \leq 280$ μmol m^{-2} s^{-1}) $= -1.0914 \cdot 10^{-8} I^2 - 6.1311 \cdot 10^{-5} I + 3.9941 \cdot 10^{-2}$ ($r^2 = 0.968$); $\phi_{green,abax}$ ($I \geq 280$ μmol m^{-2} s^{-1}) $= 1.8263 \cdot 10^{-8} I^2 - 4.4363 \cdot 10^{-5} I + 3.2675 \cdot 10^{-2}$ ($r^2 = 0.990$); $\phi_{red,abax}$ ($I \leq 280$ μmol m^{-2} s^{-1}) $= 2.0084 \cdot 10^{-7} I^2 - 1.556 \cdot 10^{-4} I + 4.4579 \cdot 10^{-2}$ ($r^2 = 0.985$), and $\phi_{red,abax}$ ($I \geq 280$ μmol m^{-2} s^{-1}) $= 2.0539 \cdot 10^{-8} I^2 - 4.6917 \cdot 10^{-5} I + 2.8126 \cdot 10^{-2}$ ($r^2 = 0.988$) Unpublished data of T. Fujita and I. Terashima.

PPFDs. We also noted that both ϕ_{red} and ϕ_{green} decreased more abruptly than when irradiated from the adaxial side. When the mean quantum yields, Φ, were compared in the experiment in which the adaxial side was irradiated, Φ_{red} was greater at low PPFDs, but at PPFDs above approximately 450 μmol m^{-2} s^{-1}, Φ_{green} became greater than Φ_{red} (**Fig. 11**). When irradiated from the abaxial side, Φ_{red} was greater than Φ_{green} only at very low PPFDs. We initially expected that the ϕ and Φ at very low PPFDs would be greater when the leaf was irradiated from the adaxial side compared with the abaxial side because, in ordinary bifacial leaves, R is smaller and leaf absorptance is greater when irradiated from the adaxial side. However, ϕ and Φ values at very low PPFD were greater when the leaf was irradiated from the abaxial side. In these measurements, we maintained the CO_2 concentration in the leaf cuvette at 390 μmol mol^{-1}, and, as reported by Wang et al. (2008), the stomatal conductance at low PPFDs was greater when the leaf was irradiated from the abaxial side than when irradiated from the adaxial side (data not shown). The difference in stomatal conductance caused considerable differences in CO_2 concentration in the intercellular spaces, which explains the present results. The data shown in **Figs. 10** and **11** clearly demonstrate that green light more effectively drove photosynthesis than red light in the white light at high PPFDs.

If we analyze the changes in the PPFD and spectrum of daylight with time of day in the natural environment, it would be possible to compare the efficiencies of green and

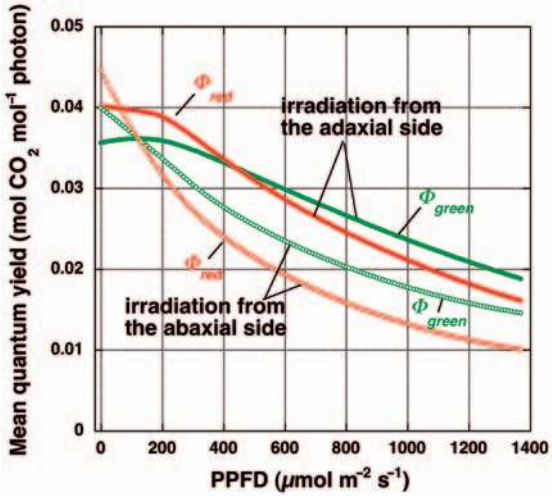

Fig. 11 Calculated mean quantum yield of monochromatic light in white light, $\Phi(I)$. The values were obtained by integration of each of the curves shown in **Fig. 10** with respect to I from 0 to I. Unpublished results of T. Fujita and I. Terashima.

other monochromatic lights in an ecological context. The method enables us to measure in situ quantum yield and opens the way to obtaining ecologically meaningful action spectra. Further studies are, of course, awaited.

Although the light absorption profiles calculated by Nishio (2000) are spurious (Vogelmann and Evans 2002), his argument has nevertheless been proven experimentally to be correct using our differential quantum yield method. Namely, red light is more effective than green light in white light at low PPFDs, but as PPFD increases, light energy absorbed by the uppermost chloroplasts tends to be dissipated as heat, while penetrating green light increases photosynthesis by exciting chloroplasts located deep in the mesophyll. Thus, for leaves, it could be adaptive to use chlorophylls as photosynthetic pigments, because, by having chlorophyll with a 'green window' the leaves are able to maintain high quantum yields for the whole leaf in both weak and strong light conditions.

Some green algae such as *Codium fragile* and *Ulva pertusa*, inhabiting the deepest part of the green algae zonation, appear very black, because they contain a keto-carotenoid, siphonaxanthin, which absorbs green light with a peak at 535 nm and transfers energy to chlorophylls with an efficiency of 1.0 (Kageyama et al. 1977, Akimoto et al. 2004, Akimoto et al. 2007). Because the peak of available PPFD shifts toward blue wavelengths with depth of sea-water, it has been argued that siphonaxanthin is a useful carotenoid to absorb green light. If leaves of land plants had black chloroplasts with siphonaxanthin, the leaves could close the so-called 'green window' and increase their absorptance. If the carboxylation enzyme, Rubisco, were very efficient, land plants would indeed be able to have thin black leaves. However, having the inefficient Rubisco as their primary carboxylation enzyme, leaves receiving high light need considerable chloroplast volumes to contain it (Terashima et al. 2005, Terashima et al. 2006). Moreover, to supply CO_2 efficiently to the chloroplasts, the leaf also needs a large cumulative cell surface area per leaf area, so the chloroplasts must be distributed throughout the leaf (Terashima et al. 2001, Terashima et al. 2005, Terashima et al. 2006). Given these constraints, it would be ideal to have chlorophyll that enables considerable light absorptance, due to the high absorptivity of blue and red light, but also penetration of green light to the lower chloroplasts. As Nishio (2000) argued, this may explain why land plants adopted Chl *a* and *b* from green algae but did not develop other pigment systems.

If a gradient in the ratio of Rubisco to photosynthetic pigments freely changes in response to PPFD, leaves could exist with black chloroplasts containing both chlorophylls and siphonaxanthin. When light absorption is plotted against the cumulative black pigment content for such leaves, the gradient would be very steep, because absorption coefficients would now be high for green as well as blue and red light. In the upper chloroplasts, the ratio of Rubisco to black pigments would then need to be very large but to decrease drastically with depth. Noting that the dynamic range of acclimational modification of chloroplast properties is limited within a given species, it would be impossible to counterbalance the profile of light absorption by drastically changing the Rubisco/black pigment ratio. It is, therefore, worth mentioning again that, by having chlorophylls with a 'green window' to the most abundant photosynthetically active wavelengths of solar radiation, green leaves have succeeded in moderating the intra-leaf light gradient to a considerable extent.

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References

Akimoto, S., Mimuro, M., (2005) Excitation relaxation dynamics of carotenoids probed by ultrafast fluorescence spectroscopy. *In* Recent Progress of Bio/Chemiluminescence and Fluorescence Analysis in Photosynthesis. Edited by Wada, N. and Mimuro, M. pp. 213–214. Research Signpost, Kerala, India.

Akimoto, S., Tomo, T., Naitoh, Y., Otomo, A., Murakami, A. and Mimuro, M. (2007) Identification of a new excited state responsible for the in vivo unique absorption band of siphonaxanthin in the green alga *Codium fragile*. *J. Phys. Chem. B.* 111: 9179–9181.

Akimoto, S., Yamazaki, I., Murakami, A., Takaichi, S. and Mimuro, M. (2004) Ultrafast excitation relaxation dynamics and energy transfer in the siphonaxanthin-containing green alga *Codium fragile*. *Chem. Phys. Lett.* 390: 45–49.

Akimoto, S., Yokono, M., Ohmae, M., Yamazaki, I., Tanaka, A., Higuchi, M., et al. (2005) Ultrafast excitation–relaxation dynamics of lutein in solution and in the light-harvesting complex II isolated from *Arabidopsis thaliana*. *J. Phys. Chem. B.* 109: 12612–12619.

Allen, W.A. and Richardson, A.J. (1968) Interaction of light with a plant canopy. *J. Opt. Soc. Amer.* 58: 1023–1031.

Balegh, S.E. and Biddulph, O. (1970) The photosynthetic action spectrum of the bean plant. *Plant Physiol.* 46: 1–5.

Björkman, O. (1968) Further studies on differentiation of photosynthetic properties in sun and shade ecotypes of *Solidago virgaurea*. *Physiol. Plant.* Vol. 21: 84–99.

Clayton, R.K., (1970) Light and Living Matter, Vol. 1: The Physical Part. McGraw-Hill, New York.

Demmig-Adams, B., Adams, W.W. III, Barker, D.H., Logan, B.A., Dowling, D.R. and Verhoeven, A.S. (1996) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiol. Plant.* 98: 253–264.

Evans, J.R. (1987) The dependence of quantum yield on wavelength and growth irradiance. *Aust. J. Plant Physiol.* 14: 69–79.

Evans, J.R. (2009) Exploring chlorophyll fluorescence with a multilayer leaf model. *Plant Cell Physiol.* 50: in press.

Evans, J.R. and Anderson, J.M. (1987) Absolute absorption spectra for the five major chlorophyll–protein complexes and their 77K fluorescence excitation spectra. *Biochim. Biophys. Acta* 892: 75–82.

Evans, J.R., Jakobsen, I. and Ögren, E. (1993) Photosynthetic light-response curves. 2. Gradients of light absorption and photosynthetic capacity. *Planta* 189: 191–200.

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

Evans, J.R. and Vogelmann, T.C. (2006) Photosynthesis within isobilateral *Eucalyptus pauciflora* leaves. *New Phytol.* 171: 771–782.

Farquhar, G.D. (1989) Models of integrated photosynthesis of cells and leaves. *Philos. Trans. R. Soc. B: Biol. Sci.* 323: 357–367.

Gates, D.M., (1980) *Biophysical Ecology*. Springer Verlag, New York.

Genty, B., Briantais, J.-M. and Baker, N.R. (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990: 87–92.

Haberlandt, G., (1914) *Physiological Plant Anatomy*. Macmillan, London.

Hakala, M., Tuominen, I., Keränen, M., Tyystjärvi, T. and Tyystjärvi, E. (2005) Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of photosystem II. *Biochim. Biophys. Acta* 1706: 68–80.

Inada, K. (1976) Action spectra for photosynthesis in higher plants. *Plant Cell Physiol.* 17: 355–365.

Kageyama, A., Yokohama, Y., Shimura, S. and Ikawa, T. (1977) An efficient excitation energy transfer from a carotenoid, siphonaxanthin to chlorophyll *a* observed in a deep-water species of chlorophycean seaweed. *Plant Cell Physiol.* 18: 477–480.

Kato, M.C., Hikosaka, K., Hirotsu, N., Makino, A. and Hirose, T. (2003) The excess light energy that is neither utilized in photosynthesis nor dissipated by photoprotective mechanisms determines the rate of photoinactivation in photosystem II. *Plant Cell Physiol.* 44: 318–325.

Koizumi, M., Takahashi, K., Mineuchi, K., Nakamura, T. and Kano, H. (1998) Light gradients and the transverse distribution of chlorophyll fluorescence in mangrove and *Camellia* leaves. *Ann. Bot.* 81: 527–533.

Lambers, H., Chapin, F.S. III., Pons, T.L., (2008) Plant Physiological Ecology. Springer, New York.

Lichtenthaler, H.K., Babani, F.,(2004) Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence parameters and photosynthetic activity. *In* Chlorophyll a Fluorescence: A Signature of Photosynthesis. Edited by Papageorgiou, G.C., Govindjee. pp. 713–736. Springer, Dordrecht.

Losciale, P., Oguchi, R., Hendrickson, L., Hope, A.B., Corelli-Grappadelli, L. and Chow, W.S. (2008) A rapid, whole-tissue determination of the functional fraction of PSII after photoinhibition of leaves based on flash-induced P700 redox kinetics. *Physiol. Plant.* 132: 23–32.

McCree, K.J. (1972) The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agric. Meteorol.* 9: 90–98.

Moss, D.N. (1964) Optimum lighting of leaves. *Crop Sci.* 4: 131–136.

Nishio, J.N. (2000) Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. *Plant Cell Environ.* 23: 539–548.

Nishio, J.N., Sun, J. and Vogelmann, T.C. (1993) Carbon fixation gradients across spinach leaves do not follow internal light gradient. *Plant Cell* 5: 953–961.

Nishiyama, Y., Allakhaverdiev, S.I. and Murata, N. (2006) A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochim. Biophys. Acta* 1757: 742–749.

Ögren, E. and Evans, J.R. (1993) Photosynthetic light-response curves. 1. The influence of CO_2 partial pressure and leaf inversion. *Planta* 189: 182–190.

Ohnishi, N., Allkhverdiev, S.I., Takahashi, S., Higashi, S., Watanabe, M., Nishiyama, Y., et al. (2005) Two-step mechanisms of photodamage to photosystem II: step 1 occurs at the oxygen-evolving complex and step 2 occurs at the photochemical reaction center. *Biochemistry* 44: 8494–8499.

Osmond, C.B. (1994) What is photoinhibition? Some insights from comparisons of shade and sun plants. *In* Photoinhibition of Photosynthesis. Edited by Baker, N. and Bowyer, J.R. pp. 1–24. BIOS Scientific Publishers, Oxford.

Oja, V.M. and Laisk, A.K. (1976) Adaptation of the photosynthesis apparatus to the light profile in the leaf. *Soviet Plant Physiol.* 23: 381–386.

Powles, S.B. (1984) Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant Physiol.* 35: 14–44.

Richter, T. and Fukshansky, L. (1996a) Optics of a bifacial leaf: 1. A novel combined procedure for deriving the optical parameters. *Photochem. Photobiol.* 63: 507–516.

Richter, T. and Fukshansky, L. (1996b) Optics of a bifacial leaf: 2. Light regime as affected by leaf structure and the light source. *Photochem. Photobiol.* 63: 517–527.

Richter, T. and Fukshansky, L. (1998) Optics of a bifacial leaf: 3. Implications for photosynthetic performance. *Photochem. Photobiol.* 68: 337–352.

Stefanov, D. and Terashima, I. (2008) Non-photochemical loss in PSII in high- and low-light-grown leaves of *Vicia faba* quantified by several fluorescence parameters including L_{NP}, F_0/F'_m, a novel parameter. *Physiol. Plant.* 133: 327–338.

Schreiber, U., Kühl, M., Klimant, I. and Reising, H. (1996) Measurement of chlorophyll fluorescence within leaves using a modified PAM fluorometer with a fiber-optic microprobe. *Photosynth. Res.* 47: 103–109.

Sun, J. and Nishio, J.N. (2001) Why abaxial illumination limits photosynthesis carbon fixation in spinach leaves. *Plant Cell Physiol.* 42: 1–8.

Sun, J. Nishio, J.N. and Vogelmann, T.C. (1998) Green light drives CO_2 fixation deep within leaves. *Plant Cell Physiol.* 39: 1020–1026.

Takahashi, K., Mineuchi, K., Nakamura, T., Koizumi, M. and Kano, H. (1994) A system for imaging transverse distribution of scattered light and chlorophyll fluorescence in intact rice leaves. *Plant Cell Environ.* 17: 105–110.

Tanaka, T. and Matsushima, S. (1970) Analysis of yield-determining process and its application to yield-prediction and culture improvement of lowland rice 94: relation between the light intensity on both sides and the amount of carbon assimilation in each side of a single leaf-blade. *Proc. Crop Soc. Jpn.* 39: 325–329.

Terashima, I. (1986) Dorsiventrality in photosynthetic light response curves of a leaf. *J. Exp. Bot.* 37: 399–405.

Terashima, I., (1989) Productive structure of a leaf. *In* Photosynthesis. Edited by Briggs, W.R. pp. 207–226. Alan R. Liss, New York.

Terashima, I., Araya, T., Miyazawa, S.-I., Sone, K. and Yano, S. (2005) Construction and maintenance of the optimal photosynthetic systems of the leaf, herbaceous plant and tree: an eco-developmental treatise. *Ann. Bot.* 95: 507–519.

Terashima, I., Hanba, Y. T., Tazoe, Y., Vyas, P. and Yano, S. (2006) Irradiance and phenotype: comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. *J. Exp. Bot.* 57: 343–354.

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

Terashima, I. and Inoue, Y. (1985) Vertical gradient in photosynthetic properties of spinach chloroplasts dependent on intra-leaf light environment. *Plant Cell Physiol.* 26: 781–785.

Terashima, I., Miyazawa, S. and Hanba, Y.T. (2001) Why are sun leaves thicker than shade leaves?—Consideration based on analyses of CO_2 diffusion in the leaf. *J. Plant Res.* 114: 93–105.

Terashima, I. and Saeki, T. (1983) Light environment within a leaf. I. Optical properties of paradermal sections of *Camellia* leaves with special reference to differences in the optical properties of palisade and spongy tissues. *Plant Cell Physiol.* 24: 1493–1501.

Terashima, I. and Saeki, T. (1985) A new model for leaf photosynthesis incorporating the gradients of light environment and of photosynthetic properties of chloroplasts within a leaf. *Ann. Bot.* 56: 489–499.

Tsuyama, M., Shibata, M. and Kobayashi, Y. (2003) Leaf factors affecting the relationship between chlorophyll fluorescence and the rate of photosynthetic electron transport as determined from CO_2 uptake. *J. Plant Physiol.* 160: 1131–1139.

Vogelmann, T.C. (1993) Plant tissue optics. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44: 233–251.

Vogelmann, T. and Evan, J.R. (2002) Profiles of light absorption and chlorophyll within spinach from chlorophyll fluorescence. *Plant Cell Environ.* 25: 1313–1323.

Vogelmann, T.C., Martin, G., Chen, G. and Buutry, D. (1991) Fiber optic microprobes and measurement of the light microenvironment within plant tissues. *Adv. Bot. Res.* 18: 256–296.

Wang, Y., Noguchi, K. and Terashima, I. (2008) Distinct light responses of the adaxial and abaxial stomata in intact leaves of *Helianthus annuus* L. *Plant Cell Environ.* 31: 1307–1316.

Weis, E. and Lechtenberg, D. (1989) Fluorescence analysis during steady-state photosynthesis. *Philos. Trans. R. Soc. B: Biol. Sci.* 323: 253–268.

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