Chloroplast Movement in the Shade Plant
*Tradescantia albiflora* Helps Protect Photosystem II against Light Stress

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The role of high-light-induced chloroplast movement in the photoprotection of the facultative shade plant *Tradescantia albiflora* was investigated by comparison with pea (*Pisum sativum* L.) leaves, both grown in 50 μmol photons m⁻² s⁻¹. Photoactivation of photosystem II (PSII) in vivo was induced in 1.1% CO₂ by varying either duration (0–2 h) of illumination (fixed at 1800 μmol m⁻² s⁻¹) or irradiance (0–3000 μmol m⁻² s⁻¹) at a fixed duration (1 h) after infiltration of leaves with water or lincomycin (an inhibitor of chloroplast-encoded protein synthesis). At all photon exposures, PSII of *T. albiflora* leaves showed a greater resistance to light stress than pea leaves, although both utilization of absorbed light by photosynthesis and psbA gene product synthesis were smaller than for pea leaves. This greater tolerance was not due to differences in PSII antenna size or the index of susceptibility of PSII to light stress, because these two parameters were comparable in both plants. However, the transmittance increase mediated by chloroplast movement was greater in *T. albiflora* than pea, resulting in a 10% decrease of absorbed light at high light. We suggest that the greater tolerance of PSII against light stress in *T. albiflora* may be partly ascribed to its light-induced chloroplast rearrangement.

PSII is the complex of the photosynthetic apparatus most vulnerable to light stress (Chow, 1994; Osmond, 1994). With peas (*P. sativum* L.) acclimated to various growth light conditions, we demonstrated that photoactivation of PSII is not just an inevitable feature of excess light but occurs in limiting, saturating, and sustained excess light. Furthermore, the reciprocity of irradiance and the duration of illumination in relation to PSII photoactivation in vivo suggests that photoactivation of PSII is a probability event that depends on the photon dosage (Park et al., 1995a, 1995b, 1995c, 1996a). To help overcome this inherent photoactivation of PSII, plants are equipped with various photoprotective mechanisms. Each species may have a different kind and extent of photoprotection, allowing them to survive and even flourish in their natural habitats. Shade plants are more susceptible to light stress than sun plants because of the antenna size, capacity for utilization of absorbed light, D1 protein repair capacity, and nonphotochemical dissipation of absorbed light as heat through ΔpH-mediated protection (with and without xanthophyll cycle activity) (Chow, 1994). Previous investigations of the susceptibility of PSII to light stress using pea leaves grown under various irradiances showed that differing levels of energy-dependent quenching and differing capacities for utilization of absorbed light are partly responsible for the differential susceptibility, although D1 protein synthesis is the most necessary photoprotective strategy (Park et al., 1995a, 1996a, 1996b). However, a main photoprotective strategy such as D1 protein synthesis need not necessarily be the only reason for differential susceptibility within and between species.

Leaves of pea readily acclimate to an increase in growth irradiance, adjusting the amounts of photosynthetic components noticeably within a day and completing the adjustments within several days following the light transfer (Chow and Anderson, 1987a, 1987b). In contrast, *Tradescantia albiflora* does not readily adjust its light-harvesting components in response to growth irradiance and only modulates other photosynthetic components after considerable lag periods (Chow et al., 1991a). These two plants also show differences in leaf anatomy: *T. albiflora* leaves have few layers of cells compared with pea leaves, which have a well-developed palisade parenchyma and multiple cell layers.

Chloroplasts move in the cell in response to the intensity and direction of the incident light. The movement in response to low light ensures maximum light absorption by the chloroplast to maximize photosynthetic utilization below the light saturation of photosynthesis. In contrast, the movement in response to high light is assumed to protect the chloroplast from excessive light (Haupt and Scheurlein, 1990). Although chloroplast movement has a significant effect on the absorption of light (Brugnoli and Björk-
man, 1992; Sinclair and Hall, 1995), there is no experimental evidence yet for a protective function to ameliorate high-light stress (reviewed by Haupt and Scheuerlein, 1990; Björn, 1992; Terashima and Hikosaka, 1995).

The effect of chloroplast movement on the absorption of light would be least pronounced in leaves such as those of pea, which have a well-developed palisade parenchyma and multiple cell layers. In this paper we compare pea and T. albiflora in relation to their ability to cope with short-term light stress, in the hope of uncovering a photoprotective role for chloroplast movement. Our results demonstrate that the high-light-induced chloroplast movement indeed helps protect PSII from light stress and, for this reason, should be regarded as one of the photoprotective strategies used by a facultative shade plant.

MATERIALS AND METHODS

Pea (Pisum sativum L. cv Greenfeast) plants were grown from seed in a compost:perlite mixture (1:1, v/v) and watered every other day with a water-soluble fertilizer (Aquasol; Hortico, Revesby, Sydney, Australia). The plants were cultivated in a growth room (12 h of light/22°C; 12 h of dark/18°C) and illuminated by fluorescent tubes (Philips TLD 58W/86, 50 μmol m⁻² s⁻¹). To ensure uniformity of leaves, the fourth leaf pair from the base, representing the youngest, fully expanded pair of leaves when the plants were 22 to 26 d old, were used. Tradescantia albiflora (Kunth) plants were grown from cuttings in a 1:1 mixture of vermiculite:perlite in a phytotron cabinet (12 h of light/26°C; 12 h of dark/22°C) under metal halide lamps (50 μmol m⁻² s⁻¹; Power Star HQI-T-400 W/D, Osram, Munich, Germany). T. albiflora cuttings were 4 to 6 weeks old, and the third leaves from the growing tip, the youngest, fully developed leaves, were used for experiments.

Inhibitor Uptake

Leaf petioles were cut under water and dipped into a solution of lincomycin (Sigma) in a small Eppendorf tube. The concentration of lincomycin was 0.6 mM for pea leaves and 3 mM for T. albiflora. The reason for the requirement of a higher lincomycin concentration for the inhibition of D1 protein synthesis in T. albiflora is not clear; it could be related to an ability of T. albiflora to sequester lincomycin into a compartment (perhaps the large epidermal cells) where the inhibitor might be rendered ineffective. The leaves were allowed to take up the inhibitors for 2 h in a fume hood with dim light (20 μmol m⁻² s⁻¹). The concentration of lincomycin in the bulk leaf tissue, estimated according to the method of Bilger and Björkman (1994), was 1.2 and 6 mM for pea and T. albiflora, respectively.

Light Treatment

Leaf discs were illuminated in the chamber of an O₂ electrode system (Hansatech, King’s Lynn, Norfolk, UK) through which humidified air containing 1.1% CO₂ at 25°C was passed. Leaf discs were illuminated with a slide projector either at a fixed irradiance (1800 μmol m⁻² s⁻¹) for various durations or for a fixed duration (1 h) at irradiances ranging from 0 to 3000 μmol m⁻² s⁻¹. Results from both combinations were pooled, since we have previously demonstrated reciprocity of irradiance and duration of illumination, i.e. that inactivation of functional PSII depends on the total number of photons absorbed and not the rate of photon absorption (Park et al., 1995a, 1995c, 1996a, 1996b).

Determination of Chl Fluorescence Parameters

F₀, Fₐ, and Fₚ, were measured at room temperature after photoinhibitory light treatments (Plant Efficiency Analyzer, Hansatech). Before measurement, leaf discs were dark treated for 30 min in leaf clips, either directly following illumination or after determination of functional PSII by repetitive flashes. Dark incubation of preilluminated T. albiflora leaves for 30 min was sufficient for chloroplasts to return to the arrangement in low light, so the measured Chl fluorescence parameters F₀ and Fₚ were not affected by chloroplast movement. Excitation light for fluorescence was given at about 2800 μmol m⁻² s⁻¹ for 2 s. To allow for variation in Chl content among leaves, all fluorescence signals were normalized to F₀, values in dark-treated controls prior to light treatment.

Fluorescence quenching parameters during illumination (qP and NPQ) were measured with a fluorometer (PAM, Heinz Walz, Effeltrich, Germany). The calculation of qP was done according to the method of van Kooten and Snel (1990), and NPQ was calculated as Fₚ/Fₚ − 1 (Bilger and Björkman, 1990). The noncyclic electron flux through PSII during steady-state photosynthesis was calculated as (1 − F/Fₚ) × 0.5 × leaf absorptance × irradiance (Schreiber et al., 1994).

Determinations of Functional PSII Reaction Centers, Effective Cross-Section of PSII, and PSI Reaction Centers

The number of functional PSII complexes was determined according to the method of Chow et al. (1989, 1991b) using a leaf-disc O₂ electrode system (Hansatech). After the leaf disc was allowed to come to dark equilibrium for about 10 min, repetitive single-turnover xenon flashes (10 Hz, 2.5 μs of full width at half-peak height; type FX 200; EG & G Electro Optics, Salem, MA) of a saturating intensity were applied for 4 min, followed by 4 min of darkness. This was followed by a second, and sometimes a third, cycle of flashes and darkness. The slight heating artifact due to the flashes was taken into account, and any limitation of electron transport was avoided by the use of background far-red light (Chow et al., 1991b). For the measurement of PSII effective absorption cross-sections (σ), the flash intensity was varied by using neutral density filters. The O₂ yield per flash per mol of Chl (y) with increasing intensity (x) increases according to a general negative exponential curve: y = y_max (1 − exp⁻αx), where y_max is the maximum O₂ yield at saturating flash intensity (Mauzerall and Greenbaum, 1989). The number of PSI reaction centers was determined from the λ₂₀₅ change of thylakoid suspensions induced by blue-green light (500 μmol m⁻² s⁻¹; model no. 4-72; Corning, Corning, NY) after correcting for Chl fluo-
rescence (Chow and Hope, 1987). The Chl content in leaf discs was determined from aqueous buffered 80% acetone extracts (25 mM Heps, pH 7.5), using the extinction coefficients and wavelengths of Porra et al. (1989). The amount of functional PSII reaction centers was expressed in mmol PSII mol⁻¹ Chl. However, most frequently, the content of PSII after a light treatment was calculated as a percentage of the initial value before the light treatment.

Measurements of Photosynthesis

Light-response curves of photosynthesis were determined as rates of O₂ evolution under various irradiances selected by neutral density filters using a leaf-disc O₂ electrode.

Leaf Transmittance, Reflectance, and Absorptance

A homemade integrating sphere was used to measure the transmittance (T) and reflectance (R) of leaf discs. White light illumination (400-700 nm) was provided by a slide projector; the transmitted and reflected light was detected by a quantum sensor. Reflectance was calibrated against standards. Absorption (A) was calculated as: A = 1 - (R + T).

Light Microscopy

For microscopy, tissue pieces approximately 1 mm long and 0.5 mm thick were cut from leaves before and after 30 min of high-light illumination (1300 µmol m⁻² s⁻¹) in humidified air containing 1.1% CO₂. Leaf sections were fixed immediately in ice-chilled 3% glutaraldehyde for 1 h under high light to prevent any chloroplast rearrangement during fixation. Leaf sections were stained with toluidine blue, and then observed and photographed.

RESULTS

Comparison of Chloroplast Composition and Function in Low-Light-Grown Pea and T. albiflora

Since the composition of the photosynthetic apparatus of higher plants is modulated in response to growth irradiance and spectral quality (Anderson, 1986), we grew peas and T. albiflora at the same intensity (50 µmol m⁻² s⁻¹) and quality of light. As shown in Table I, Chl content, Chl a/b ratio, and functional PSII/PSI ratio are comparable between pea and T. albiflora leaves, although the number of functional PSII and PSI is 10 to 15% lower on an area basis in pea leaves. However, pea shows 70% higher maximal photosynthetic O₂ evolution rates than T. albiflora, implying that the utilization efficiency of absorbed light by photosynthesis in saturating light is greater in pea.

The rather similar Chl a/b ratios suggest that there is no more than a 16% difference in the relative size of the light-harvesting PSII antennae between the two plants. The relative size of PSII antennae can be determined by investigating the flash intensity-response curve of O₂ evolution (Mauzerall and Greenbaum, 1989). The yield of O₂ evolution per single-turnover flash as a function of flash intensity follows a single negative exponential increase, which can be expressed as y = ymax (1 - exp⁻α), where y is the O₂ yield per flash at a given flash intensity, ymax is the maximal O₂ yield per flash, α is the effective absorption cross-section of PSII antenna, and x is the flash intensity. Figure 1 clearly shows that the α of pea leaves is comparable to T. albiflora, although the maximal O₂ yield per flash of T. albiflora is slightly higher than that of pea.

T. albiflora Is More Resistant to Light Stress than Pea

To compare the effects of light stress on T. albiflora and peas, leaf discs were initially treated with distilled water (control) or lincomycin, an inhibitor of D1 protein synthesis, and then illuminated in the presence of 1.1% CO₂ in air to avoid any limitation of photosynthesis by CO₂ (Park et al., 1995a). The photochemical yield of PSII (the quantum yield of QA reduction) and the number of functional PSII reaction centers were estimated by the Chl fluorescence yield of O₂ evolution, and the rate of irradiance or the duration of illumination (Park et al., 1995a, 1995b, 1995c).

Figure 2 shows that, with increased photon exposure, Fv/Fm ratios of both plants in the presence of lincomycin decreased biphasically, compared with monophasic declines in untreated pea and T. albiflora leaves. The rate of decline of the Fv/Fm ratio in control leaves was slightly greater than that of T. albiflora at all photon exposures, showing that the susceptibility of PSII to photoinactivation in control T. albiflora leaves is marginally lower than that of pea. This result contrasts with that of Öquist et al. (1992), the discrepancy possibly being due to the different conditions of illumination (e.g., CO₂ concentration) between this study and the previous one.

Since previous comparison of Fv/Fm ratios with the number of functional PSII reaction centers demonstrated that the Fv/Fm ratio may fail to reveal photoinactivation of about 25%

<table>
<thead>
<tr>
<th>Plant</th>
<th>Chl a/b</th>
<th>Chl (mmol m⁻²)</th>
<th>PSII</th>
<th>PSI</th>
<th>PSII/PSI</th>
<th>Pmax</th>
</tr>
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<tbody>
<tr>
<td>Pea</td>
<td>3.17 ± 0.01</td>
<td>0.34 ± 0.08</td>
<td>2.16 ± 0.01</td>
<td>1.55 ± 0.06</td>
<td>1.39</td>
<td>13.9 ± 0.4</td>
</tr>
<tr>
<td>T. albiflora</td>
<td>3.30 ± 0.06</td>
<td>0.35 ± 0.03</td>
<td>2.52 ± 0.06</td>
<td>1.70 ± 0.09</td>
<td>1.48</td>
<td>8.1 ± 0.1</td>
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</table>
Figure 1. The flash-intensity dependence of O₂ yield per flash in pea (●) and T. albiflora (■) leaves grown in 50 μmol m⁻² s⁻¹. A. The fitted curves of pea and T. albiflora leaves followed a negative exponential rise \[ y = y_{\text{max}} (1 - \exp^{-ax}) \], where \( y \) is the O₂ yield at a given flash intensity \( x \) and \( y_{\text{max}} \) is the maximal O₂ yield at saturating flash intensity and where \( y = 0.54 (1 - \exp^{-7.48x}) \) and \( y = 0.63 (1 - \exp^{-7.61x}) \), respectively. B. The fraction of flash O₂ yield \( (y/y_{\text{max}}) \) as a function of increasing flash intensity \( x \). Mean values (±se) for three to four leaf discs are shown.

of the total PSII complexes (Park et al., 1995a, 1996a), we investigated the contents of functional PSII complexes of pea and T. albiflora after various photon exposures. Figure 3 shows that in the absence of lincomycin, the onset of the loss of functional PSII occurred at a lower photon exposure in pea (equivalent to 1 h of illumination at twice the growth irradiance) than in T. albiflora (equivalent to 1 h of illumination at 8 times the growth irradiance). Furthermore, at any given photon exposure, the extent of loss of functional PSII was greater in pea: low-light T. albiflora was distinctly more resistant to photoinactivation of PSII than was low-light pea, a result that again contrasts with our earlier findings (Oquist et al., 1992). It is interesting that in the presence of lincomycin, about 25% of the PSII complexes in low-light-grown pea became nonfunctional after a low photon exposure, consistent with previous results obtained for pea grown under moderate and high light (Park et al., 1995a, 1996a). In contrast, such a “less-stable” subpopulation of PSII was not evident in T. albiflora (Fig. 3).

Comparison of Electron Flux through PSII, Excitation Pressure on PSII, NPQ, and Light Susceptibility of Pea and T. albiflora Leaves

To explain the greater tolerance of PSII in T. albiflora, we need to consider a number of factors that ameliorate photoinactivation. An obvious one is the capacity for the utilization of absorbed light by photosynthetic electron transport to CO₂ and O₂, since an excess of photons occurs only after photosynthesis has been saturated, and the greater the photosynthetic capacity, the smaller the
excess of photons at a given irradiance. To investigate the electron flux through PSII, we carried out Chl fluorescence quenching analyses using leaves exposed to various irradiances. After 5 min of illumination of pea and T. albiflora leaves under humidified air with 1.1% CO₂, allowing maximal electron flux at PSII (Park et al., 1995a), the Chl fluorescence parameters $F_0$ and $F_m$ were obtained and then used for the calculation of noncyclic electron flux at PSII (Schreiber et al., 1994). In pea leaves, the electron flux through PSII increased rapidly at low irradiance and then saturated at 1200 μmol m⁻² s⁻¹ (Fig. 4A). In T. albiflora leaves, however, electron flux through PSII saturated at only 750 μmol m⁻² s⁻¹ with a maximum value 40% lower than that of pea under saturating and excess light.

Since leaves have varying abilities to dissipate excess light by photochemical and nonphotochemical mechanisms, we next measured photochemical quenching and NPQ of Chl fluorescence. The $q_P$ indicates the oxidation state of the QA during steady-state illumination. At all irradiances, $(1 - q_P)$ was lower in pea than in T. albiflora leaves (Fig. 4B) because of the greater electron flux through PSII in pea. The NPQ parameter (NPQ = $F_m/F_n^{'} - 1$) represents nonradiative dissipation of excitation energy as heat and, therefore, is a photoprotective strategy (Osmond, 1994). PSII of T. albiflora might have derived more photoprotection than that of pea, because of T. albiflora's greater NPQ (Fig. 4C). A combined indicator of light stress is given by the ratio $(1 - q_P)/NPQ$, which tends to increase as PSII reaction centers close ($q_P$ lower), but decreases when photoprotective NPQ is enhanced. Figure 4D shows that at a given irradiance the light stress parameter was rather similar for pea and T. albiflora and therefore cannot explain their different sensitivities to photon exposure.

**D1 Protein Synthesis Is Greater in Pea Leaves**

One way to estimate the relative D1 protein synthesis is to determine the increase in the fraction of nonfunctional PSIIIs in lincomycin-treated leaves compared with water-treated (control) leaves at each photon exposure. The capacity for D1 protein synthesis was determined from Figure 3 by subtracting the number of functional PSIIIs in lincomycin-treated leaves from that in control leaves at the same photon exposure (Park et al., 1995a, 1996a). In pea leaves, D1 protein synthesis was saturated at very low photon exposure, but in T. albiflora, it increased asymptotically to the level of pea with increasing photon exposure (Fig. 5), showing that pea leaves have a greater D1 protein synthesis at the photon exposures tested.

**Chloroplast Movement Results in a Decrease of Absorptance**

Another factor that ameliorates photoinactivation of PSII may be chloroplast movement, which can result in changes in absorption of incident light (for review, see Haupt and Scheuerlein, 1990). Therefore, we measured the time course of changes in transmittance and reflectance, and hence of absorptance, of pea and T. albiflora leaves following illumination at 1300 μmol m⁻² s⁻¹ under 1.1% CO₂ in air. Figure 6 depicts the much greater increases in transmittance in T. albiflora compared with pea (Fig. 6A). Chloroplasts in T. albiflora might have moved during illumination, which would decrease the absorptance of incident light.
Flora sorptance in these changes gives rise to a greater decrease in leaf absorptance. The fraction of nonfunctional PSlls was calculated from exposure. The fraction of nonfunctional PSlls was calculated from Figure 3 by subtracting the percentage of nonfunctional PSII in lincomycin-treated leaves from that in control leaves given the same photon exposure.

Albiflora leaves compared with that in pea leaves while the reflectance changes were comparable; therefore, the sum of these changes gives rise to a greater decrease in leaf absorptance in T. albiflora. Upon illumination, the absorptance changes were completed within 20 min and then relaxed completely within 15 min when T. albiflora leaves were darkened or placed in room light (results not shown).

To investigate the apparent ease with which T. albiflora controls light absorption, we examined light micrographs before and after the illumination treatments (Fig. 7). T. albiflora has unusually large epidermal cells on both surfaces of the leaf, with only a thin layer of mesophyll sandwiched between epidermal layers. Examination of T. albiflora leaf sections under the light microscope clearly showed that high light induced very pronounced changes in chloroplast arrangement. In leaves under low light, the chloroplasts were mainly located along walls perpendicular to the direction of the light; whereas in leaves exposed to high light they were lined up along the vertical walls, parallel to the light direction (Fig. 7, C and D). In contrast, chloroplast movement in response to light quantity was not so evident in light micrographs of pea leaves except in the palisade cell layer (Fig. 7, A and B). This change in leaf optics caused by chloroplast movements in T. albiflora was also visible to the eye: areas of leaves that had been illuminated appeared distinctly pale in comparison with unexposed areas, although no Chl bleaching had taken place (data not shown).

DISCUSSION

T. albiflora Is More Resistant to Light Stress than Pea

Despite acclimation to the same growth irradiance, the susceptibility of PSII with respect to photon exposure is greater in pea than in T. albiflora leaves over the entire photon dosage range, as measured by the number of functional PSIIIs (Fig. 3). With untreated leaves, the number of functional PSIIIs in the absence of lincomycin starts to decrease after absorption of 1.6 mmol photons m$^{-2}$ in T. albiflora but after 0.35 mmol photons m$^{-2}$ in pea. This 4-fold difference in the lag phases between two species implies a differential capacity for photoprotection in dealing with absorbed light by PSII, T. albiflora being more resistant to light stress than pea.

Comparable Antenna Size of PSII in T. albiflora and Pea Leaves

Alteration in the effective cross-section of light absorption by PSII is a feasible photoprotective strategy, although its role is still a matter of controversy, even in vitro (Mäenpää et al., 1987; Tyystjärvi et al., 1991). To investigate the role of PSII antenna size, we measured the $\alpha$ by determining the flash intensity-response curve, although similar Chl $a/b$ ratios in the two plants hinted at comparable PSII antenna sizes (Anderson, 1986). The cross-section of a photosynthetic unit is measured by determining the relative yield of O$_2$ as a function of the energy of a homogeneous single-turnover flash of monochromatic light on an optically thin sample (Mauzerall and Greenbaum, 1989). Although in our experiment we used white light and leaf discs, this method still allowed us to estimate qualitatively the relative cross-section of PSII in pea and T. albiflora, simply because the leaves have similar amounts of Chl per unit area (Table I) and leaf absorptance (leaf absorptance of
pea and *T. albiflora* was 0.897 and 0.900, respectively. According to Mauzerall and Greenbaum (1989), the product of optical cross-section and irradiance gives the probability that a PSII unit will absorb a photon. There is only a slight difference in the probability for light absorption by PSII between the two plants, because their \( \sigma \) values are comparable (Fig. 1). Hence, the antenna size of PSII in vivo may not be related to the differential susceptibility between the two plants.

**Utilization of Absorbed Light by Photosynthesis Is Lower in *T. albiflora* than in Pea**

It is assumed that a greater utilization of absorbed light by photosynthesis should result in PSII being under less excess light, thereby causing lower photoinactivation (Osmond, 1994). However, in spite of the maximal photosynthetic capacity for \( \text{O}_2 \) evolution and the maximal photosynthetic electron flux through PSII in *T. albiflora* being 30 to 40% lower than in pea (Table I; Fig. 4A), *T. albiflora* showed greater tolerance to PSII photoinactivation. Therefore, the greater tolerance of *T. albiflora* to light stress cannot be ascribed to its lower utilization efficiency of absorbed light by photosynthetic electron transport to \( \text{CO}_2 \) or \( \text{O}_2 \).

**Index of Susceptibility to Light Stress Is Similar for the Two Plants**

Chl fluorescence quenching analyses were performed to assess PSII photoinactivation under steady-state photosynthesis, since they are indicative of the photochemical and nonphotochemical processes of dissipation of absorbed light. \( q_P \) is indicative of the use of light energy for driving PSII photochemistry. The proportion of reduced \( Q_A \) during steady-state photosynthesis is estimated by \( 1 - q_P \), which reflects the excitation pressure on PSII (Öquist and Huner, 1993). The excitation pressure on PSII, which increases with increasing irradiance, is greater in *T. albiflora* than in pea (Table I; Fig. 4A). *T. albiflora* showed greater tolerance to PSII photoinactivation. Therefore, the greater tolerance of *T. albiflora* to light stress cannot be ascribed to its lower utilization efficiency of absorbed light by photosynthetic electron transport to \( \text{CO}_2 \) or \( \text{O}_2 \).
albiflora than in pea (Fig. 4B), suggesting that T. albiflora should be more photoactivated than pea. On the other hand, the NPQ, the probability for thermal deactivation of the absorbed light, showed that T. albiflora has a greater capacity to dissipate the absorbed light as heat during steady-state photosynthesis (Fig. 4C). However, the nonradiative dissipation of absorbed light in T. albiflora leaves may be overestimated since chloroplast movement (see “Chloroplast Movement Helps Protect PSII from Photoinactivation”) will decrease Chl fluorescence emission (Fm') as a result of a light-induced decrease of leaf absorbance (Brugnoli and Bjorkman, 1992). Indeed, the apparent 8 to 20% greater NPQ in T. albiflora compared with pea (Fig. 4C) is partly accounted for by an 8% greater light-induced decrease of leaf absorbance in T. albiflora (see below). Hence, NPQ was very comparable in the two plants, and any difference was certainly too small to account for the greater resistance of T. albiflora to photoinactivation of PSII.

There has been a tendency to consider independently either the excitation pressure on PSII or the NPQ as a determining factor for the photoinactivation or photoprotection of PSII, respectively (Deming-Adams and Adams, 1992; Öquist and Huner, 1993; Chow, 1994; Osmond, 1994). However, from our above data, it is clear that these individual parameters fail to explain the differential susceptibility between species in this study and within species (Park et al., 1996a, 1996b), because two opposing functions may operate simultaneously with changing irradiance.

The ratio (1 - qP)/NPQ is regarded as indicative of the susceptibility of PSII to light stress (Park et al., 1995c, 1996b). At all irradiance levels, there is little difference in this parameter between the two plants (Fig. 4D), suggesting that all else being equal the two plants should have the same probability of photoinactivation of PSII. Therefore, the greater susceptibility of pea leaves to photoinactivation cannot be explained by events mediated by either excitation pressure on PSII or NPQ.

D1 Protein Synthesis Is Apparently Lower in T. albiflora than in Pea

To investigate the role of D1 protein synthesis as a possible factor responsible for the differential susceptibility of PSII to light stress, the difference between the number of nonfunctional PSIIIs in lincomycin-treated and control leaves was determined at the same photon exposure (Fig. 5). The effect of lincomycin in pea leaves was maximal at very low photon exposure and was maintained over most of the light regime. Conversely, in T. albiflora, the lincomycin effect was lower at low incident photon exposure and then slowly increased to the same level as pea. Hence, D1 protein synthesis in T. albiflora leaves started to operate rather slowly and is likely to require a higher irradiance to be saturated, compared with pea, which showed that D1 protein synthesis is saturated at low irradiance (Fig. 6; Park et al., 1996a). This slower and lower operation of D1 protein synthesis in T. albiflora may be due to the lower extent of PSII photoinactivation, given that some other photoprotective mechanism could sufficiently reduce the actual number of photons arriving at PSII. Indeed, this may be deducible from Figure 3, because the lag period before any PSII becomes photoinactivated is greatly extended up to 4-fold after photon exposure in T. albiflora compared with pea. This extended lag period is probably related to the overall photoprotective capacity of a plant, since pea leaves grown in varying irradiance also show differential lag phases (Park et al., 1996a, 1996b). Hence, the maximal capacity for protection of PSII in plants against light stress should be variable and regulated by genomic information as well as the light growth environment. In any case, the smaller effect of lincomycin in T. albiflora, if indeed a measure of D1 protein synthesis capacity, does not explain the greater resistance of T. albiflora PSII to light stress.

Chloroplast Movement Helps Protect PSII from Photoinactivation

Exposure of leaves to strong white (Terashima and Hikosaka, 1995) or blue (Inoue and Shibata, 1974; Brugnoli and Bjorkman, 1992) light induces absorption changes due to chloroplast movements. In our study, exposure to high white light caused significant changes in leaf optics in T. albiflora, but only slight changes in pea (Fig. 6). The decrease in leaf absorbance was caused by concomitant increases in both transmittance and reflectance, resulting from marked chloroplast movement in T. albiflora (Fig. 7). Chloroplast rearrangement in T. albiflora, therefore, can cause a significant change in light absorption. In shade plants, an increase of light absorption up to 10% in low light is likely to be very important to ensure that photosynthesis functions fully on the forest floor. On the other hand, a 10% decrease of light absorption in high light is likely to have a significant photoprotective effect in T. albiflora, in which the photoprotection of PSII against light stress via the utilization of absorbed light and D1 protein synthesis is less effective than in pea.

In conclusion, compared with pea, T. albiflora shows a lower capacity for the utilization of absorbed light of saturating irradiance and a slower engagement of D1 protein synthesis, both of which are known to be major photoprotective mechanisms. On this basis, T. albiflora might be expected to be more susceptible to light stress than pea. But this is not the case, because another photoprotective strategy helps to protect the PSII of T. albiflora from high-light stress by decreasing the absorption of light by PSII through marked chloroplast movement. Chloroplast movement in a shade plant such as T. albiflora may be a better strategy for coping with the fluctuating light environment within the canopy than D1 protein repair, which is probably a more energy-consuming process. For some shade plants with lower maximal utilization of absorbed light by photosynthesis, it appears that avoidance of excess light by marked chloroplast movement is one of the photoprotective strategies.

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