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Response of *Tradescantia albiflora* to growth irradiance: Change *versus* changeability

Jan M. Anderson[1,*], Wah Soon Chow[1], Youn-Il Park[2], Linda A. Franklin[1], Sharon P.-A. Robinson[3] & Philip R. van Hasselt[4]

[1]*Research School of Biological Sciences, Australian National University, Canberra ACT 2601, Australia;* [2]*Department of Biology, Chungnam National University, Taejon 305764, Korea;* [3]*Department of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia;* [4]*Laboratory of Plant Physiology, University of Groningen, 9750 AA Haren, The Netherlands;* [*]*Author for correspondence (e-mail: anderson@rsbs.anu.edu.au)*

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Abstract

Most chloroplasts undergo changes in composition, function and structure in response to growth irradiance. However, *Tradescantia albiflora,* a facultative shade plant, is unable to modulate its light-harvesting components and has the same Chl a/Chl b ratios and number of functional PS II and PS I reaction centres on a Chl basis at all growth irradiances. With increasing growth irradiance, *Tradescantia* leaves have the same relative amount of chlorophyll–proteins of PS II and PS I, but increased xanthophyll cycle components and more zeaxanthin formation under high light. Despite high-light leaves having enhanced xanthophyll cycle content, all *Tradescantia* leaves acclimated to varying growth irradiances have similar non-photochemical quenching. These data strongly suggest that not all of the zeaxanthin formed under high light is necessarily non-covalently bound to major and minor light-harvesting proteins of both photosystems, but free zeaxanthin may be associated with LHC II and LHC I or located in the lipid bilayer. Under the unusual circumstances in light-acclimated *Tradescantia* where the numbers of functional PS II and PS I reaction centres and their antenna size are unaltered during growth under different irradiances, the extents of PS II photoinactivation by high irradiances are comparable. This is due to the extent of PS II photoinactivation being a light dosage effect that depends on the input (photon exposure, antenna size) and output (photosynthetic capacity, non-radiative dissipation) parameters, which in *Tradescantia* are not greatly varied by changes in growth irradiance.

Abbreviations: Car – total carotenoids; Chl – chlorophyll

Introduction

Terrestrial plants being sessile must cope with momentary, diurnal and seasonal light changes that may vary over three orders of magnitude. For example, shade plants on a rainforest floor must grow with less than 0.5% of the irradiance of the top of the canopy, apart from transitory illumination from sunflecks (Björkman and Ludlow 1972). Above light-limiting irradiance, however, plants absorb more incident light than they are able to use for photosynthesis. Environmental stresses further limit light utilisation. Plants subjected to excessive irradiance may become photoinhibited with a loss of PS II function (Chow 1994; Osmond 1994). Hence, plants have developed many photoprotective strategies to minimise the deleterious effects of absorbed excess excitation energy and its consequences. A major strategy designed to function during periods where light irradiance exceeds the photosynthetic capacity of leaves, the xantho-

phyll cycle, involves the reversible, light-dependent de-epoxidation of violaxanthin to antheraxanthin and zeaxanthin (Demmig-Adams and Adams 1992, 1996; Horton et al. 1996; Gilmore 1997; Niyogi 1999). Xanthophyll cycle carotenoids are associated with both photosystems: Major and minor LHC II (45–60%), and LHC I (55–40%) (Thayer and Björkman 1992; Lee and Thornber 1995; Hurry et al. 1997), but neither the nature of the binding, nor whether all are non-covalently bound to specific chlorophyll–proteins or other proteins is known. Within LHC II, about 50% of violaxanthin is located in the minor Lhcbs, CP29, CP26 and CP24 in C_3 chloroplasts (spinach (Ruban et al. 1994), *Arabidopsis* (Hurry et al. 1997) and barley (Lee and Thornber 1995)) and 80% in a C_4 maize mesophyll chloroplast (Bassi et al. 1993).

While many studies have shown that the extent of deepoxidation of violaxanthin to antheraxanthin and zeaxanthin is correlated with rapid nonphotochemical quenching of chlorophyll fluorescence (Demmig-Adams and Adams 1992, 1996; Horton et al. 1996; Gilmore 1997), the molecular mechanisms of quenching of chlorophyll fluorescence by zeaxanthin are unknown. Furthermore, there is no agreement as to how much zeaxanthin is involved in nonradiative dissipation by PS II. A single molecule in a minor Lhcb may be an efficient quencher (Crofts and Yerkes 1994), several zeaxanthin molecules associated with each PS II antenna may be needed (Gilmore 1997), or NPQ is a property of the complete PS II antenna, including major Lhcbs (Horton et al. 1996). Moreover, NPQ occurs in mutants that lack zeaxanthin (Pogson et al. 1998).

Acclimation of plants to varying light environments has a profound effect on the composition, photosynthetic function and nonradiative dissipation of excess energy, as well as structural organization of the photosynthetic apparatus (Anderson and Osmond 1987; Anderson et al. 1988). In higher plants, there is a marked lateral heterogeneity in the distribution of the photosystems between appressed and nonappressed membrane domains: Most PS II complexes are located in appressed membranes while PS I complexes are found only in nonappressed membranes. This distribution results in an inverse relationship between Chl *a*/Chl *b* ratios of sun and shade plants, membrane stacking, and even extent of PS II photoinactivation (Anderson and Aro 1994). However, modulation of the photosynthetic apparatus of the facultative shade plant, *Tradescantia albiflora* grown under varying irradiances is very limited. Light-acclimated *Tradescantia* has constant Chl *a*/Chl *b* ratios, PS II to PS I stoichiometry (Chow et al. 1991) and the extent of membrane stacking is unaltered (Adamson et al. 1991).

Sun or high-light plants typically have a larger total pool size of xanthophyll cycle components, as well as a greater ability to convert violaxanthin to antheraxanthin and zeaxanthin under high light (e.g. Thayer and Björkman 1990; Demmig-Adams and Adams 1992, 1996; Brugnoli et al. 1994, 1998). Sun leaves of *Lingustrum ovalifolium* with lower chlorophyll and higher carotenoid content, higher Chl *a*/Chl *b* ratios and less appressed to non-appressed membranes, have both larger pools of V + A + Z and more photoconvertible violaxanthin compared with shade leaves (Brugnoli et al. 1994). In several species, both higher levels of xanthophyll cycle components and amount of photoconvertible violaxanthin are strongly correlated with Chl *a*/Chl *b* ratios (Brugnoli et al. 1994, 1998), an indicator of the amount of membrane stacking (Anderson and Aro 1994).

Since light-acclimated *Tradescantia* chloroplasts are exceptional with the same degree of membrane stacking (Adamson et al. 1991), and limited modulation of the photosynthetic apparatus, what are the expected consequences for xanthophyll cycle capacity and NPQ, and PS II photoinactivation? Our aims were to determine (i) the capacity of the xanthophyll cycle in relation to nonphotochemical quenching, and (ii) the extent of photoinactivation of PS II in light-acclimated *Tradescantia.*

Materials and methods

Plant growth conditions

Tradescantia albiflora plants were cultivated in compost:perlite mixture in a growth chamber under photon flux densities of 10, 50, 250 and 500 μmol photons m^{-2} s^{-1} supplied by fluorescent and incandescent lights for a 12 h photoperiod, 25 °C day/18 °C night. Plants were watered with nutrient solution each morning and with water each afternoon. *Tradescantia* cuttings were grown for 12 weeks and the leaves used for experiments were two pairs of the youngest fully-developed leaves of shoots.

Photoinhibitory light treatments

Leaf discs or pieces, floating adaxial side up on water or inhibitor solutions were illuminated with an HMI

lamp. Light (1600 μmol photons m^{-2} s^{-1}) was passed through a heat filter (Schott 115, Tempax) and the temperature was controlled at 23 °C. Leaves (with their petioles in water) were illuminated with an HMI lamp, prior to the isolation of photoinhibited thylakoids.

To prevent D1 protein synthesis in the light-acclimated *Tradescantia* leaves, the petioles were immersed in 0.6 mm lincomycin in the fumehood for 2–3 h prior to punching leaf discs for photoinhibitory treatments. During photoinhibitory treatment, leaf discs were floated on a solution of 1 mm lincomycin.

Photosynthetic pigments and pigment-protein complexes

Leaf discs were extracted in buffered 80% acetone and total Chls *a* and *b* and Chl *a*/Chl *b* ratios were quantified using the extinction coefficients and wavelengths determined by Porra et al. (1989). Carotenoids were measured in samples taken before and during light treatments. Three discs (each 0.98 cm^2) were punched from control and photoinhibited leaves and stored in liquid nitrogen. Samples were subsequently ground in liquid N_2 and extracted with 100% acetone. Thylakoids or subchloroplast membrane fractions were also extracted with 100% acetone using minimal volumes of fractions. Pigments were measured using the HPLC method and solvents A and B described by Gilmore and Yamamoto (1991). The pigments were separated on a Spherisorb ODS1 column (Alltech Associates, Sydney, Australia) and concentrations were calculated as described previously (Robinson et al. 1993).

Pigment–protein complexes in isolated thylakoids were resolved by non-denaturing Deriphat gel electrophoresis (Lee and Thornber 1995). The identity of bands was established by absorption spectroscopy and polypeptide composition following SDS–PAGE. The chlorophyll content was determined by averaging the results of two gel scans at 675 and 650 nm.

Fluorescence and O_2 evolution

Fluorescence parameters (Fv/Fm and Fo) were measured with a fluorometer (Plant Efficiency Analyser, Hansatech Ltd, King's Lynn, UK) to assess photoinhibition during high irradiance conditions. Six replicate discs were removed from the high light after varying periods, and dark-adapted for 30 min in individual leaf clips prior to measurement of chlorophyll a fluorescence. Chlorophyll fluorescence yield during actinic illumination with white light was determined using a pulse-modulated chlorophyll fluorometer (PAM 101; Heinz Walz, Effeltrich, Germany), coupled with a Hansatech O_2 electrode chamber. An individual leaf rested on a moist sponge and air containing 1.1% CO_2 was flushed through the chamber to avoid CO_2 limitations during measurements. Fm was determined on leaves after 30 min dark incubation. Calculations of photochemical quenching q_P were made according to Van Kooten and Snell (1990), and nonphotochemical quenching (NPQ), according to the Stern-Volmer equation with $NPQ = Fm/Fm' - 1$, where Fm and Fm′ are maximum fluorescence yield after dark treatment and during illumination, respectively.

Results

Content and capacity of the xanthophyll cycle and nonphotochemical quenching

When *Tradescantia albiflora* was grown under varying irradiance [μmol photons m^{-2} s^{-1}: 50, (low); 250 (medium) and 500 (high)] of constant quality, the Chl *a*/Chl *b* ratios were comparable (Table 1) as demonstrated earlier (Chow et al. 1991). This contrasts with acclimation under varying light qualities of constant irradiance, where changes in Chl *a*/Chl *b* ratios are invoked (Liu et al. 1993). Identical Chl *a*/Chl *b* ratios (Table 1) and amounts of PS II and PS I on a chlorophyll basis in high-and low-light *Tradescantia* (Adamson et al. 1991) suggest a similar relative distribution of chlorophyll between chlorophyll–proteins of PS II and PS I. Following non-denaturing gel electrophoresis, the relative amounts of chlorophyll associated with specific chlorophyll–proteins were indeed identical in high-and low-light *Tradescantia* (Table 2; Chow et al. 1991). After high irradiance treatment (1600 μmol photons m^{-2} $s^{-1}$for 3 h) of leaves from both growth conditions, the distribution of chlorophyll between the chlorophyll–proteins was unaltered (Table 2).

Given identical Chl *a*/Chl *b* ratios, amounts of PS II and PS I on a chlorophyll basis (Adamson et al. 1991) and chlorophyll distribution amongst the various chlorophyll–proteins of thylakoids isolated from *Tradescantia* grown under varying irradiance (Tables 1 and 2), we next analysed the pigment content by HPLC. As the growth irradiance increased, there was a 3.3-fold decrease of total chlorophyll on a leaf area basis at the highest growth irradiance (Table 1). Although the total thylakoid membrane content per

Table 1. Chlorophyll and carotenoid content (μmol m^{-2}) $\pm$ S. D. (n = 2) of light-acclimated *Tradescantia* leaves. Growth irradiance in μmol photons m^{-2} s^{-1}: low-light, 50; medium-light, 250; high-light, 500

Leaves	Chlorophyll	Carotenoids	Chl/Car	Chl *a*/Chl *b*
Low	453 ± 13	93 ± 6	4.9	3.49
Medium	354 ± 7	93 ± 3	3.8	3.55
High	137 ± 28	49 ± 8	2.9	3.59

chloroplast decreases with increasing growth irradiance, there was no change in the ratio of appressed to nonappressed membranes (Adamson et al. 1991). Low- and medium-light *Tradescantia* leaves had comparable amounts of total carotenoids on a leaf area basis, but less in high-light *Tradescantia.* However, the decline in carotenoid content was not as severe as that of chlorophyll: hence chlorophyll/carotenoid ratios decreased with increasing growth irradiance, despite comparable Chl *a*/Chl *b* ratios (Table 1). On a chlorophyll basis, low-light leaves had more α-carotene (Table 3). Higher α-carotene content is found in some shade plants (Thayer and Björkman 1990). The relative content of β-carotene increased from low- to medium- to high-light leaves (Table 3). With increasing growth irradiance, there was no significant change in neoxanthin, but a 1.8-fold increase in lutein and a 3-fold increase in xanthophyll cycle components (V + A + Z) (Table 3). As the total pool of xanthophyll cycle carotenoids increased, more violaxanthin was photoconverted to antheraxanthin and zeaxanthin under high light. In medium- and particularly in high-light *Tradescantia*, some antheraxanthin and zeaxanthin were also retained overnight.

In addition to useful photochemical conversion, nonphotochemical quenching is a major pathway for the harmless dissipation of excess excitation energy. Net P_{max}, the maximum capacity for light-and CO_2-saturated oxygen evolution, corrected for the dark respiration and instrument drift, was only marginally greater in high-light than low-light *Tradescantia* (Figure 1A). This is consistent with a previous finding that P_{max} is optimal at intermediate growth irradiance in a glasshouse (Adamson et al. 1991). Similarly, the average quantum efficiency of PS II photochemistry during steady-state photosynthesis, ϕ_{PSII}, was slightly higher in high-light leaves (Figure 1B), probably due mainly to a less reduced state of $Q_A(1 - q_P)$ being equivalent to reduced Q_A (Figure 1C). NPQ, however, was fairly similar for leaves from both growth conditions (Figure 1D), despite the marked differences in Z + A as a fraction of V + A + Z (Table 3).

Extent of photoinactivation of PS II in light-acclimated Tradescantia

Next, we investigated the time course of photoinhibition with light-acclimated *Tradescantia* leaves which have varying total V + A + Z content and xanthophyll cycle capacity (Table 3), but similar NPQ (Figure 1D) and the same ratio of stacked to non-stacked membrane (Adamson et al. 1991). With peas acclimated to light intensity by varying growth irradiance, there is a linear relationship between many parameters, including lower Chl *a*/Chl *b* ratios, higher extent of membrane stacking, and greater photoinhibition in low-light plants (Anderson and Aro 1994).

The decline in Fv/Fm ratios of light-acclimated *Tradescantia* leaf discs was compared during illumination with high light (1000 or 1800 μmol photons m^{-2} s^{-1}) for varying times. Following light-treatments, the discs were dark-adapted for 30 min to allow relaxation of easily reversible fluorescence quenching components. The time courses of photoinhibition of high- and low-light-acclimated leaves were comparable at room temperature (Figure 2B). This was always observed with plants grown from year to year. The fluidity of thylakoid membranes might be decreased at low temperature or enhanced at high temperature compared to room temperature. Even at low temperature (6 °C) (Figure 2A), the extents of PS II photoinactivation were similar. This was observed also at high temperature (35 °C) with plants grown at 10 μmol photons m^{-2} s^{-1} (Figure 2C). D1 protein turnover is prevented in leaves that have previously taken up a low concentration of the chloroplast-encoded protein synthesis inhibitor, lincomycin that specifically inhibits D1 protein synthesis. In the absence of D1 protein turnover, the time-course of decline in Fv/Fm was slightly slower in low-light lincomycin-treated *Tradescantia* grown at very low irradiance (10 μmol photons m^{-2} s^{-1}) (Figure 2D). Despite the lower photosynthetic capacity, small xanthophyll cycle pool and less photoconversion of zeaxanthin, low-light *Tradescantia* did not seem any more susceptible to light stress than high-light grown plants.

Table 2. Distribution (%) of chlorophyll amongst the chlorophyll–proteins of thylakoids isolated from light-acclimated *Tradescantia* leaves grown under low (50 μmol photons m^{-2} s^{-1}) and high (500 μmol photons m^{-2} s^{-1}) light. Thylakoids were isolated from leaves (control thylakoids: Chl *a*/Chl *b* ratio of 3.32 for low-light and 3.42 for high-light) and from leaf discs that had been treated with high irradiance of 1600 μmol photons m^{-2} $s^{-1}$for 3 h (photo-inhibited samples). The chlorophyll–proteins were resolved by non-denaturing Deriphat gel electrophoresis, and the chlorophyll content determined by scanning gels at 675 and 650 nm

Chlorophyll-proteins	Low-light		High-light	
	Control	Photoinhibited	Control	Photoinhibited
PS I-LHC I (∗tri)	15	15	15	15
PS I-LHC I (core)	30	28	29	30
PS II (core)	7	7	7	7
LHC II (∗tri)	11	11	11	11
LHC II (∗mono)	27	29	30	28
FP	10	9	8	9

∗tri – trimer; ∗mono – monomer; FP – free pigment.

Discussion

Xanthophyll cycle and nonphotochemical quenching

In contrast to the striking sun/shade acclimation of most higher plants, *Tradescantia albiflora* grown under varying irradiances, showed very limited modulation of its photosynthetic apparatus. Chl *a*/Chl *b* ratios, PS II/PS I stoichiometry, and the relative distribution of chlorophyll amongst the various chlorophyll–protein complexes were identical in low-, medium- and high-light *Tradescantia*. Thus, neither the size of the light-harvesting antennae nor the amounts of PS II and PS I on a chlorophyll basis were altered. However, this lack of modulation with respect to the distribution of chlorophyll between reaction centre and light-harvesting complexes was not matched by an unaltered carotenoid content in light-acclimated *Tradescantia* leaves. Not only did the total amount of carotenoids on a chlorophyll basis increase with increasing growth irradiance, but also there was a 1.8-fold increase in lutein and a 3-fold increase in xanthophyll cycle components (V + A + Z) (Table 3). Enhanced photoconversion of violaxanthin was also observed with increasing growth irradiance; medium- and particularly high-light leaves also retained some overnight zeaxanthin.

The capacity for photoconversion of violaxanthin to zeaxanthin is strongly correlated with nonphotochemical quenching under non-stressed conditions (Demmig-Adams and Adams 1992, 1996; Horton et al. 1996; Gilmore 1997). Our results with light-acclimated *Tradescantia* having enhanced xanthophyll capacity and V + A + Z content but comparable NPQ (Figure 1D) with increasing growth irradiance, may now be rationalised. Recently, Li et al. (2000) elegantly demonstrated that an *Arabidopsis* thaliana mutant, deficient in an intrinsic PS II Chl *a/b*-protein PsbS (CP22) but with the usual xanthophyll cycle components and activity, was highly defective in NPQ. Hence stoichiometric amounts of PsbS per core PS II complex would account for light-acclimated *Tradescantia* thylakoids that all possess identical amounts of PS II on a chlorophyll basis (Adamson et al. 1991) having comparable NPQ (Figure 1D).

Despite enhanced zeaxanthin and antheraxanthin and lutein in medium- and high-light leaves under high light (Table 3), there was no significant increase in NPQ compared to low-light *Tradescantia* (Figure 1D). This is consistent with the current view that not all zeaxanthin and antheraxanthin molecules are involved in NPQ (Croft and Yerkes 1994; Horton et al. 1996; Gilmore 1997). Since time-resolved Chl fluorescence lifetimes are independent of PS II antenna size (Gilmore et al. 1996), Gilmore (1997) suggested that antheraxanthin and zeaxanthin enrichment around Chl-proteins might explain why changes in peripheral PS II antenna size do not necessarily affect either levels of xanthophyll cycle pigments on a PS II basis or NPQ.

Since light-acclimated *Tradescantia* thylakoids increase both xanthophyll pool size and the amount of photoconvertible violaxanthin and lutein under high

Table 3. Carotenoid content (per unit chlorophyll) ± S. D. (n = 2) of light-acclimated *Tradescantia* at growth irradiance and following high light treatment. Plants were grown at low-light, 50 μmol photons m^{-2} s^{-1}; medium-light, 250 μmol photons m^{-2} s^{-1}; high-light, 500 μmol photons m^{-2} s^{-1}. Leaves were collected 3 h into the photoperiod. Photoconversion of xanthophyll cycle pigments is also shown for comparison with similar leaves that were illuminated with high light (1600 μmol photons m^{-2} s^{-1} for 15 min)

Carotenoids (mmol mol chl^{-1})	Low-light	Medium-light	High-light
α-carotene	27.3 ± 2.4	11.9 ± 5.6	3.7 ± 0.8
β-carotene	44.8 ± 0.1	65.3 ± 1.5	89.5 ± 5.6
Lutein	69.1 ± 4.3	94.2 ± 2.0	122.7 ± 0.3
Neoxanthin	31.6 ± 0.8	39.0 ± 7.2	36.6 ± 3.6
(V + A + Z)	33.0 ± 1.6	51.9 ± 1.0	100.1 ± 12.1
Total carotenoids	206 ± 7	262 ± 13	353 ± 13
Photoconversion of xanthophyll pigments			
Leaves 3 h into normal photoperiod			
(A + Z)/(V + A + Z) (%)	0	47 ± 12	58 ± 26
Leaves exposed to 1600 μmol photons m^{-2} s^{-1} for 15 min			
(A + Z)/(V + A + Z) (%)	64 ± 0.1	77 ± 4	89 ± 3

growth irradiance (Table 3), without a significant increase in NPQ, multiple roles for zeaxanthin and possibly lutein within PS II are likely. These may include the following: (i) Direct thermal dissipation of excess light energy absorbed by chlorophylls by singlet–singlet energy transfer from chlorophyll to zeaxanthin may occur (Chow 1994; Frank et al. 1994; Owens 1994). (ii) Indirect energy quenching by free excess zeaxanthin associated with LHC II aggregation is possible (Horton et al. 1996). (iii) Zeaxanthin and possibly lutein may be attached to other stress proteins to dissipate excess light energy and thereby protect the developing photosynthetic apparatus that is depleted in LHC II and LHC I. These proteins include ELIPs formed during early greening of etiolated plants (Kröl et al. 1999), an undefined protein in intermittantly-lit plants with few minor or major Lhcbs (Jahns and Krause 1994), or Cbr (an ELIP homologue) which may bind zeaxanthin and is specifically associated with LHC II in *Dunaliella* (Banet et al. 2000). When light stress is exacerbated by very low temperature, enhanced amounts of both zeaxanthin and PsbS are observed (Ottander et al. 1995). (iv) Free zeaxanthin that spans both halves of the lipid bilayer may protect against heat stress by reducing thylakoid fluidity (Havaux 1998). Extra zeaxanthin content in the lipid bilayer of high- compared to low-light *Tradescantia* may partly account for the similar time course of PS II photoinactivation at 35 °C (Figure 2C).

Are all zeaxanthin molecules formed following photoconversion of violaxanthin associated with specific chlorophyll-proteins?

The difficulty of isolating chlorophyll–protein complexes without loss of carotenoids, especially weakly bound violaxanthin and zeaxanthin (e.g. Lee and Thornber 1995; Ruban et al. 1999) has prevented definitive location of xanthophyll cycle components. With light-acclimated *Tradescantia* we were unable to demonstrate the location of the 'extra' zeaxanthin and antheraxanthin molecules in medium- and high-light thylakoids (data not shown). Variable amounts of xanthophyll cycle carotenoids associated with identical distribution of chlorophyll–proteins in light-acclimated *Tradescantia*, however, strongly imply that not all A + Z are necessarily non-covalently bound to specific Lhcb and Lhca proteins. Some free zeaxanthin molecules may also be associated with chlorophyll-proteins either as cleft fillers between non-perpendicularly aligned transmembrane α-helices or as space fillers (boundary lipids) around the circumference of core PS II, PS II dimers or LHC II trimers. Since specific lipids are known to be involved in the dimerization of PS II (Kruse et al. 2000) and trimeriz-

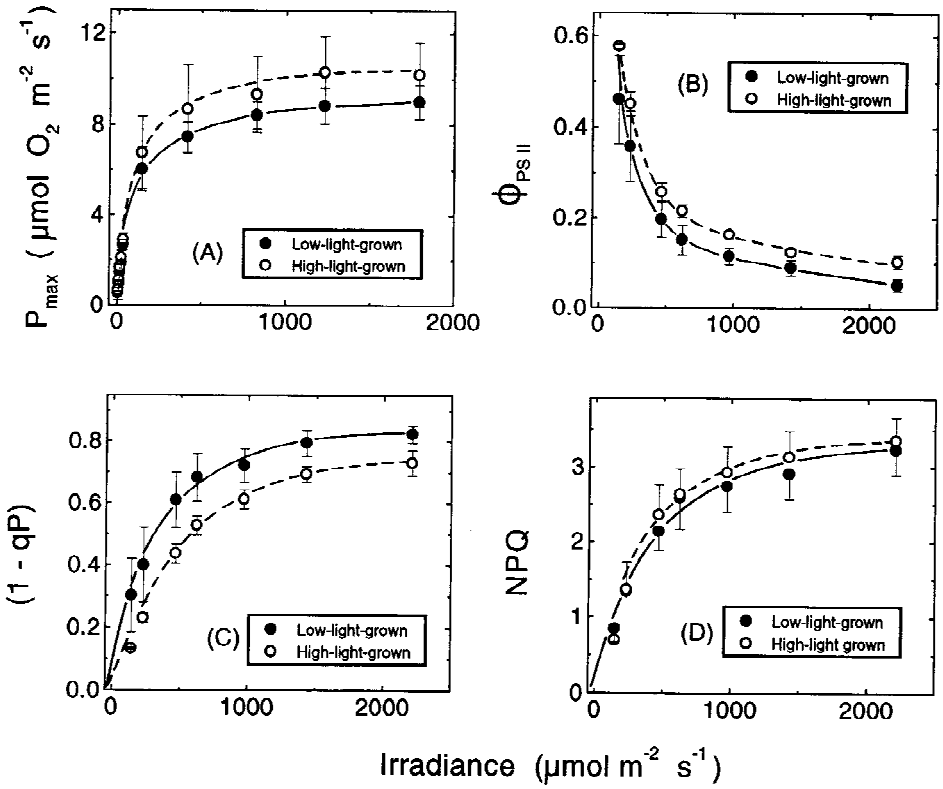

Figure 1. Irradiance response curves of light- and CO_2-saturated photosynthetic oxygen evolution per leaf area (A), and chlorophyll fluorescence parameters [Φ_{PSII}, average quantum efficiency of PS II at steady-state photosynthesis (B); $(1 - q_P)$ which is equivalent to reduced Q_A (C); NPQ (D)] in high- and low-light acclimated *Tradescantia* leaves. Growth irradiance of high-light plants was 500 and low-light plants was 50 μmol photons m^{-2} s^{-1}.

ation of LHC II (Hobe et al. 1994), free carotenoid molecules may also be involved in the aggregation state of complexes.

Similar PS II photoinactivation in light-acclimated Tradescantia *thylakoids with somewhat different photoprotective strategies*

Despite somewhat different photosynthetic capacity (Figure 1A), reduction of Q_A (Figure 1C) and D1 protein turnover (Figure 2D) between low-and high-light acclimated *Tradescantia*, the extent of PS II functional loss was identical over a wide temperature range [low (6 °C) (Figure 2A); room (22 °C) (Figure 2B); high (35 °C) (Figure 2C)]. Since reciprocity is observed between irradiance and time of illumination, the photoinactivation of PS II is a probability and light dosage event that depends on the number of photons absorbed by the leaf and not the rate of photon absorption (Park et al. 1995). When the leaf has absorbed some 10^6–10^7 photons, there is inevitable photoinactivation of one PS II. With the same number of PS IIs on a chlorophyll basis, identical effective PS II antenna size and the same NPQ in light-acclimated *Tradescantia*, the input pressure on PS II is comparable. Based on the radical pair model and our unifying model (Anderson et al. 1988), this input pressure depends mainly on antenna size. Since PS II photoinactivation is a light dosage effect that depends on both the input into PS II (photon exposure and antenna size) and the output from PS II (photosynthetic capacity and non-radiative dissipation) and these parameters are not greatly changed with growth irradiance in *Tradescantia*, comparable extents of PS II photoinactivation are expected.

Some plants, particularly shade plants, limit the amount of light absorbed by chloroplast movement in sustained high light (Brugnoli and Björkman 1992). This allows low-light plants to deal with transitory high-light exposure characteristic of an understory environment. Low-light *Tradescantia* leaves showed marked movement of chloroplasts in response to high light resulting in a 10% decrease in absorbed light

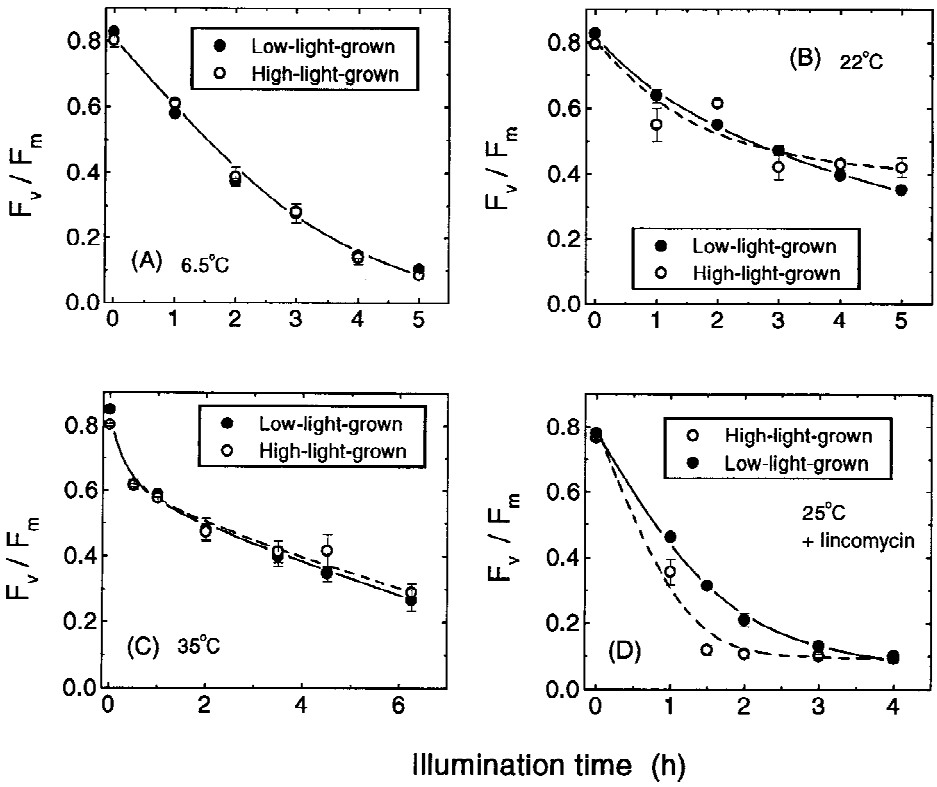

Figure 2. Time-course of photoinactivation of PS II during illumination at 1000 (A and B) and 1800 (C and D) μmol photons m^{-2} s^{-1}, determined as the decline in Fv/Fm ratios, in light-acclimated *Tradescantia* leaves at varying temperature and when D1 protein synthesis is prevented. (A) 6.5°C; (B) 22°C; (C) 35°C; and (D) with leaves that were pretreated with lincomycin to prevent D1 protein synthesis prior to light treatment. Growth irradiance for high-light plants was 500 μmol photons m^{-2} s^{-1}, and low-light plants was 50 (A and B) or 10 μmol photons m^{-2} s^{-1} (C and D).

compared to peas grown at comparable irradiance (50 μmol photons m^{-2} $s^{-1)}$ (Park et al. 1996). Chloroplast movement may be more prominent in low- than high-light *Tradescantia.* If so, it might help compensate for lower capacity of the xanthophyll cycle (Table 3) and D1 protein turnover (Figure 2D) in low- compared to high-light *Tradescantia*.

In the multifaceted dynamic spatial and temporal regulation of PS II function, many interacting factors must be considered. These include photosystem stoichiometry, effective antenna sizes, energy distribution between the photosystems, electron transport rate, light-activation of zeaxanthin formation, the entire processes of non-radiative dissipation, cyclic events in PS II and PS I, and the heterogeneity of PS II and PS I units that are located in appressed and non-appressed domains. Clearly, plants acclimated to sun/high-light and shade/low-light employ varying amounts of many strategies to regulate resistance to irreversible light damage. For balanced excitation distribution to both photosystems at limiting light, the product of the number of functional PS II reaction centres and the effective PS II antenna size must equal the product of the number of PS I functional reaction centres and the effective PS I antenna size. Most plants, in response to growth irradiance, have marked modulations in photosystem stoichiometry, effective antenna size and NPQ but no such modulation is found in light-acclimated*Tradescantia* leaves. Maybe the larger xanthophyll cycle pool size helps *Tradescantia*, a facultative shade plant, to adapt to growth in higher light by several factors yet to be defined, such as chloroplast movement or extra zeaxanthin.

In summary, our results with light-acclimated *Tradescantia* support the emerging view that multiple zeaxanthin energy quenching mechanisms operate. Furthermore, not all the zeaxanthin formed in the xanthophyll cycle under high photon exposure need necessarily be non-covalently bound to specific light-harvesting chlorophyll–proteins of PS II and PS I to

promote direct and indirect energy quenching. Under certain stress situations, extra free zeaxanthin may be associated with LHC II and LHC I, other proteins such as ELIPS or extra PbsS, or even occur free in the lipid bilayer.

The comparable extents of PS II inactivation in light-acclimated *Tradescantia* leaves having similar PS II/PS I stoichiometry, light-harvesting antenna size, number of PS II reaction centres per Chl and comparable capacity for nonphotochemical quenching, because PS II photoinactivation is a light dosage effect. Most plants, however, modulate the number of PS II reaction centres and their effective antenna size, to allow PS II to function with efficacy under the conflicting demands of efficient use of low irradiance so that all plants may have the same quantum efficiency of photosynthesis, and protection from sustained high light, against the background of the very low but inevitable probability of PS II photoinactivation which depends on photons absorbed, and not irradiance *per se* (Park et al. 1995; Anderson et al. 1998).

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