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An exceptional irradiance-induced decrease of light trapping in two *Tradescantia* species: an unexpected relationship with the leaf architecture and zeaxanthin-mediated photoprotection

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Abstract

Leaf anatomy and irradiance-dependent leaf transmittance changes serving as irradiance acclimation mechanisms in leaves were studied in two ecologically contrasting *Tradescantia* species, a shade plant *T. fluminensis* Vell. and a sun plant *T. sillamontana* Matuda, grown at different irradiances. A dramatic increase in leaf thickness (2 to 4-fold) under a high growth irradiance (800 μmol m^{-2} s^{-1}) compared with a low growth irradiance (60 μmol m^{-2} s^{-1}), achieved mainly by expansion of the epidermis, was recorded in both species. The effect took place on the background of modest changes in mesophyll thickness (1.8-fold in *T. fluminensis* and 1.15-fold in *T. sillamontana*) and chloroplast size (0.8-fold in *T. fluminensis* and an insignificant change in *T. sillamontana*). Mesophyll structure and growth irradiance response did not seem to facilitate significantly light-dependent chloroplast (avoidance) movement in these species. Nevertheless, an exceptionally large (2 to 4-fold) irradiance-induced increase in light transmittance attributable to chloroplast avoidance movement was revealed. This increase by far exceeded that in other higher plants according to available literature**.** The magnitude of the irradiance-dependent transmittance changes positively correlated both with the rate of photosystem II recovery and with the extent of xanthophyll deepoxidation in the leaves. This was opposite to a negative correlation observed between the same parameters in different plant species. We hypothesize that, at the evolutionary timescale, chloroplast avoidance movement might adjust independently from other photoprotective mechanisms, *e.g.*, non-photochemical quenching, whereas, on the ontogenetic timescale, adjustment of these mechanisms inevitably follows the same trend.

Additional key words: leaf anatomy, chloroplast movements, light transmittance, non-photochemical quenching, xanthophyll cycle.

Introduction

In nature, plants need to survive under and cope with a wide irradiance range. This is possible due to operation of diverse mechanisms serving long- or short-term acclimation/regulation of the photosynthetic apparatus. These mechanisms include adjustment of pigment content and composition in photosystems (PSs) I and II (Anderson 1986, Anderson *et al.* 1988, Kouřil *et al.* 2013), ATP-synthase, Calvin-Benson cycle enzymes (Seemann 1989, Tikhonov 2013, 2015), violaxanthin deepoxidase activity (Demmig-Adams 1990), and accumulation of non-photosynthetic protective pigments (Solovchenko and Merzlyak 2008, Solovchenko 2010). These mechanisms provide an efficient irradiance acclimation in most plants, however, a remarkable lack of the plasticity of irradiance responses was discovered in *Tradescantia albiflora* (Chow *et al.* 1991). This lack of plasticity manifests itself as a relatively constant chlorophyll *a*/*b* ratio, photosynthetic antenna size, and PS I / PS II ratio, whereas

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Abbreviations: DE - coefficient of deepoxidation; HI - high irradiance; IIT - irradiance-induced increase in leaf light transmittance; LI - low irradiance; NPQ - non-photochemical quenching; PS - photosystem; q_{NPQ} - coefficient of non-photochemical quenching; T - transmittance; Φ_{PSII} - quantum yield of photochemical reaction in photosystem II.
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ATP synthase and Rubisco content display a certain degree of adjustment to growth irradiance. Thus, the *T. albiflora* light-harvesting apparatus seems to be permanently locked in the "shade-plant mode" (Adamson *et al.* 1991), probably reflecting the fact that *T. albiflora* evolved as plant of tropical forests (Hunt 1980, Weber 2003). Nevertheless, this species becomes widespread in the world conquering a variety of habitats over recent decades (Berger 1993, Global Invasive Species Database 2015).

The genus *Tradescantia* contains species with contrasting environmental preferences, therefore, it represents a suitable model for comparative study of different modes of irradiance response in species featuring a limited plasticity of their photosynthetic apparatus. Unfortunately, literature virtually lacks data on photosynthesis of *Tradescantia* species, except for *T. albiflora.* Previously, comparing a high irradiance response in several *Tradescantia* species (*T. albiflora, T. fluminensis , T. sillamontana*, and *T. navicularis*), we showed that the sun-type species possess two specific features compared to shade-type: a great tolerance to high irradiance manifesting itself as a higher photochemical quantum yield of PS II (Φ_{PSII}) at high actinic irradiances and a more rapid response to short-term (in the time scale of minutes) changes in irradiance, including Φ_{PSII} recovery and non-photochemical quenching (NPQ) relaxation in darkness after exposure to radiation (Samoilova *et al.* 2011, Ptushenko *et al.* 2013). Considering the findings outlined above, one can raise two questions: *1*) which features of the photosynthetic apparatus give rise to the marked difference between the shade- and sun-type *Tradescantia* species in the rate and extent of irradiance acclimation?; *2*) what are possible contributions of adaptive (evolved over a long time and pre-programmed in the genome) or acclimatory (expressed in response to environmental stimuli on the ontogenetic time-scale) components to the irradiance acclimation strategy? In this context, it would be interesting to know if the remarkable lack of plasticity is typical of other *Tradescantia* species "locked" in the shade- or sun-plant mode, or this is a peculiarity of *T. albiflora*.

Chloroplast movement is a widespread short-term irradiance acclimation mechanism of plants (Zurzycki 1955, Kasahara *et al.* 2002, Suetsugu and Wada 2012, Königer 2014). Chloroplasts are arranged along the upper and lower cell walls under a low irradiance (the accumulation response) or along the anticlinal cell walls under a high irradiance (the avoidance response). Transition from the accumulation to avoidance response is accompanied by an increase in leaf light transmittance. It was previously shown that in a facultative shade plant *T. albiflora* grown at a low (50 μmol m^{-2} s^{-1}) irradiance, chloroplast avoidance plays a significant role in protection from excessive radiation (Park *et al.* 1996). Indeed, an irradiance-induced increase in leaf light transmittance (IIT) found in *T. albiflora* by Park *et al.* (1996) was significantly higher (up to 1.9-fold) than in other species studied (Königer and Bollinger 2012). Moreover, chloroplast avoidance in *T. albiflora* plants grown under a low irradiance (LI) was suggested to provide a sufficient photoprotection compensating for lack of xanthophyll and PS II D1 protein turnover (as compared with high irradiance (HI)-grown plants of the same species (Anderson *et al.* 2001). In the frame of this hypothesis, it is also supposed that chloroplast avoidance may be more prominent in LI- than HI-grown *Tradescantia* plants.

Chloroplast avoidance, as manifested by the amount of light transmitted by a leaf, is modulated by overall leaf architecture including mesophyll thickness as well as by sizes of cells and chloroplasts (Davis *et al.* 2011). As noted above, chloroplast avoidance is a short-term acclimation response, whereas a change in leaf anatomy is a long-term acclimation response to changing irradiance. Accordingly, one may ask if, and to which extent, the extreme ability of *Tradescantia* to increase transmittance (or to reduce absorption) of its leaves in response to actinic light (Park *et al.* 1996) is determined by their anatomy, and how it is regulated by a long-term growth irradiance.

In the present study, we focused on the anatomy and IIT of leaves of two *Tradescantia* species. We attempted to resolve the contributions of adaptation (acquired in the course of evolution) and long-term acclimation (formed in the course of plant ontogenesis) components to irradiance responses. Toward this aim, we examined two ecologically contrasting *Tradescantia* species, a shade plant *T. fluminensis* and a sun plant *T. sillamontana*, both grown at two substantially different irradiances: LI, typical for *T. fluminensis* natural habitats, and HI, typical for *T. sillamontana*. Specifically, we attempted: *1*) to highlight the architectural peculiarities of *Tradescantia* leaves and estimate their plasticity; *2*) to assess the magnitude of chloroplast avoidance response and its possible relationships with the peculiarities of the leaf anatomy; *3*) to outline a role of chloroplast avoidance response in protection of the *Tradescantia* species against excessive radiation.

Materials and methods

Plants and growth conditions: *Tradescantia fluminensis* Vell. and *Tradescantia sillamontana* Matuda were grown from seedlings obtained from the Department of Tropical and Subtropical Plants of N.V. Tsitsin Main Botanic Gardens of the Russian Academy of Sciences (Moscow, Russia). The plants were cultivated in soil at a temperature of 22 - 24 °C, a 16-h photoperiod and two irradiances 60 (LI) or 800 (HI) μmol(PAR) m^{-2} s^{-1} provided by white LEDs (*USS-90 Highway W*, *Focus LLC*, Moscow Region, Russia). The growth irradiance

was measured with a *Li-250A* light meter equipped with a *Li-190SA* quantum sensor (*Li-COR Biosciences*, Lincoln, NE, USA). All measurements were carried out with fully expanded mature leaves of the same age (3 - 4 weeks).

Leaf anatomy measurements were performed with a motorized digital photomicroscope *Eclipse 90i* (*Nikon*, Tokyo, Japan). Leaf cross-sections were prepared from fresh leaves (for chloroplast size measurements) or from leaves dehydrated with 80 % (v/v) ethanol (for the whole leaf or mesophyll layer thickness and mesophyll cell size measurements). Three leaves for each species and treatment were examined, 10 - 40 (for leaf or mesophyll thickness) or 40 - 110 (for chloroplast or cell size) measurements on 3 - 7 cross-sections from each leaf were made for subsequent averaging.

Chlorophyll fluorescence measurements on intact plants were performed using a PAM-fluorometry protocol (Schreiber *et al.* 1986, Baker 2008). The plants were adapted to darkness for 3 - 4 h before measurements, then an attached leaf was subjected to a course of a 30 min actinic radiation (λ = 475 nm) of 150 μmol m^{-2} s^{-1} and a subsequent 60 min dark adaptation. Chlorophyll fluorescence was recorded from the adaxial (upper) surface of a leaf using a *PAM-fluorometer FluorPen FP100* (*Photon Systems Instruments*, Brno, Czech Republic). Saturating pulses of 3000 μmol m^{-2} s^{-1} were applied for 0.5 s and a measuring irradiance was less than 0.1 μmol m^{-2} s^{-1} on average.

Photosystem II operating efficiency, Φ_{PSII}^{Light}, was assessed from a steady state Φ_{PSII} value under irradiance [$\Phi_{PSII} = (F_m' - F_t)/F_m'$, where F_m' is fluorescence from actinic light-adapted leaf during its exposition to saturating flash and F_t is fluorescence immediately before the saturating flash]. Recovery of PS II, Φ_{PSII}^{Recov}, was assessed from a Φ_{PSII} value in darkness 5 min ($\Phi_{PSII}^{5\,min}$) or 1 h (Φ_{PSII}^{1h}) after exposure to actinic irradiance. An initial Φ_{PSII} value of a 1 - 2 h dark adapted leaf is designated $\Phi_{PSII}^{Initial}$. Non-photochemical quenching was assessed with a coefficient $q_{NPQ} = (F_m - F_m')/F_m'$, where F_m is fluorescence from dark-adapted leaf during its exposition to the saturating flash (Maxwell and Johnson 2000, Ptushenko *et al.* 2014). All leaves remained attached to the plant in the course of the measurement described above.

The same leaves were then used for light transmittance measurements and xanthophyll assay. A time interval between chlorophyll fluorescence and transmittance measurements was 12 - 16 h.

Leaf transmittance measurements: As avoidance response manifested by increases in leaf transmittance is saturated at 20 - 90 μmol m^{-2} s^{-1} in most species (Königer and Bollinger 2012), we chose an actinic irradiance of 150 μmol m^{-2} s^{-1} (which is sufficient for induction of avoidance response). The plants were adapted to darkness for 4 - 5 h before measurements, then a leaf was subjected to an actinic irradiation (150 μmol m^{-2} s^{-1}, λ = 475 nm) for 30 min. Leaf transmittance spectra were recorded in the course of irradiation and subsequent 90 min of dark adaptation of a leaf in a range of 400 - 800 nm using an *Agilent Cary 300* (*Agilent*, Santa Clara, USA) spectrophotometer with a 150 mm integrating sphere *CA-30I* (*Agilent*). Actinic and measuring beams were incident at the adaxial side of a leaf. Transmittance (T) changes (measured as difference ΔT, or ratio T_{Light}/T_{Dark}) were wavelength dependent being higher within the regions of strong pigment absorption peaking at 680 nm and lower in the regions of weak absorption. Hence, ΔT_{680} or $T_{Light,680}/T_{Dark,680}$ were employed as measure of transmittance changes. The index "680" is omitted hereafter for clarity. To avoid confusion in description of relative changes in transmittance, T is expressed as fraction of unity.

Xanthophyll pigments assay: A spot (6 mm diameter) on a half of a dark adapted leaf (see above) was irradiated by an actinic radiation (λ = 475 nm, 150 μmol m^{-2} s^{-1}) for 30 min. The other half of the leaf blade remained in darkness. After the irradiation, acetone extracts were prepared from both the irradiated part and the darkened part of the leaf (Solovchenko *et al.* 2001). The extracts were then injected into a *Waters Alliance 2695* chromatograph equipped with a *Waters Sunfire RP C18* column (150 × 4.6 mm, 3.5 μm) and *Waters 2995* diode-array detector (*Waters*, Milford, USA) according to an earlier published protocol (Merzlyak *et al.* 2005). Xanthophyll deepoxidation DE = (Z +0.5A)/(Z + A + V), where Z, A, and V are zeaxanthin, antheraxanthin, and violaxanthin content, respectively. Values of DE of leaves dark-adapted for 5 - 6 h and light- adapted for 30 min are denoted as DE_{Dark} and DE_{Light}, respectively.

Data treatments and statistics: Data on leaf anatomy are means of 10 - 110 measurements. Leaf transmittance values are means of 3 - 7 measurements. Curve-fitting and linear regression coefficient calculations were performed with the *Origin* software (*OriginLab*, Northampton, USA), Pearson's sample correlation coefficients r_{xy} were determined with online calculators (http://www.socscistatistics.com/tests/pearson/Default. aspx, http://math.semestr.ru/corel/pearson-correlation-coefficient.php).

Results

Leaves of *T. fluminensis* and *T. sillamontana* possessed, on both leaf surfaces, a thick epidermis (80 - 515 μm on adaxial and 45 - 175 μmon abaxial,) consisting of one or two cell layers (Fig. 1 Suppl.). Epidermal cells were

prism shaped (usually penta- or hexagonal almost regular or slightly flattened across the axis), much larger (4 - 6-fold in width and up to 25-fold in length) than mesophyll cells. Mesophyll thickness comprised only a small part of the whole leaf thickness: 10 - 13 % (in the HI-grown leaves) to 24 % (in the LI-grown leaves; Figs. 1*A*,*B* and 2*A*). The mesophyll was undifferentiated, comprised predominantly rounded, almost isodiametric cells with an elliptic axe ratio of 0.8 - 1.0 (Fig. 1 and Fig. 2 Suppl.), with the cell diameter to mesophyll thickness ratio varying from *ca.* 0.2 to 0.4 (Fig. 2*B*). Thus, the mesophyll layer contained on average two to three (*T. fluminensis*) or two to five (*T. sillamontana*) mesophyll cells along the normal to the layer plane, but they did not form pronounced paradermal layers (Fig. 1 Suppl.).

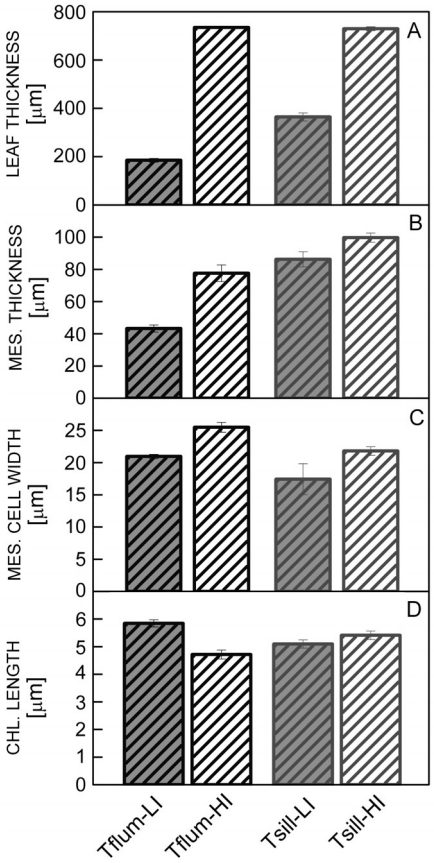

Fig. 1. Leaf thickness (*A*), mesophyll (Mes.) thickness (*B*), Mes. cell width (*C*), and Mes. chloroplast (Chl) length (*D*) of *Tradescantia* leaves. Means ± SEs, n = 10 - 110.

Mesophyll chloroplast shapes were ellipsoids (an axe ratio of 0.7 - 0.8). The diameter (*i.e.*, the largest dimension) of the chloroplasts was *ca*. 20 or 30 % (in the HI- and LI-grown leaves, respectively) of a mesophyll cell diameter (Figs. 1*C*,*D* and 2*C*).

The most notable differences in leaf anatomy between *T. fluminensis* and *T. sillamontana* were found in mesophyll and whole leaf thicknesses, being especially apparent in the case of the LI-grown leaves (Fig. 1*A*,*B*). The LI-grown *T. sillamontana* plants possessed two times thicker leaves (360 ± 17 μm) with a two times thicker mesophyll (86 ± 5 μm) compared to the LI-grown *T. fluminensis* plants (180 ± 7 μm and 43 ± 2 μm, respectively). High irradiance-grown *T. fluminensis* attained the leaf thickness characteristic of *T. sillamontana* (730 ± 8 μm), but the mesophyll remained slightly (by 25 - 30 %) thicker in *T. sillamontana* leaves. Notably, the mesophyll thickness expressed as fraction of the whole leaf thickness was similar in both species, changing in the same manner as growth irradiance changed (Fig. 2*A*). The size and shape of the mesophyll cells and chloroplasts were virtually identical in the two species studied.

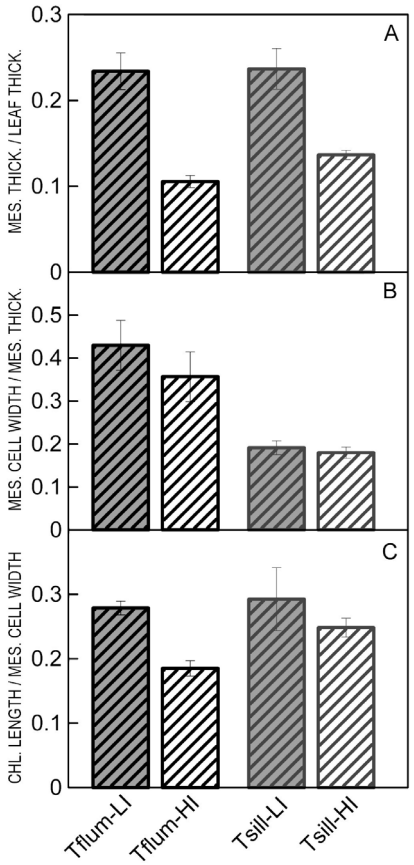

Fig. 2. Ratios of mesophyll (Mes.) thickness to the whole leaf thickness (*A*); Mes. cell width to Mes. thickness (thick.) (*B*); and Mes. chloroplast (Chl) length to Mes. cell width (*C*). Means ± SEs, n = 10 - 110.

A difference between the LI- and HI-grown leaves was especially apparent in the whole leaf thickness. It was substantially (*ca*. 4-fold for *T. fluminensis*, 730 ± 8 *vs*. 180 ± 7 μm; 2-fold for *T. sillamontana*, 730 ± 8 *vs*. 360 ± 17 μm) higher under the HI than LI. The mesophyll thickness was somewhat less responsive to growth irradiance, increasing *ca*. 1.8 times in *T. fluminensis* (from 40 to 70 μm), and 1.15 times in *T. sillamontana* (from 85 to 100 μm) with growth irradiance. As result, the proportion of mesophyll within leaf thickness

dropped in both species *ca*. two times (from 24 to 10 - 13 %) along with an increase in growth irradiance. The mesophyll cell size was slightly (*ca*. 1.20 - 1.25 times) higher under the HI than LI. On the contrary, the chloroplast size of the mesophyll cells remained almost unchanged (in *T. sillamontana*) or even decreased (from 5.8 to 4.7 μm in *T. fluminensis*). This led to a slight decrease in chloroplast-to-cell diameter ratio (*ca*. 1.17-fold in *T. sillamontana* cells and up to 1.5-fold in *T. fluminensis* cells; Fig. 2*C*).

Irradiation of a leaf led to an IIT with a typical time of 10 - 15 min. The IIT was reversible after 15 to 35 min in darkness (Fig. 4 Suppl.). These transmittance changes followed nearly the same trend in both species regardless of growth irradiance reaching a steady state ΔT of *ca*. 0.04 starting from T_{Dark} = 0.035 - 0.04. This corresponded to a *ca*. two-fold increase in T (except for LI-grown *T. fluminensis* leaves which were the most "opaque" with T_{Dark} = 0.016 % and hence displaying T_{Light}/T_{Dark} = 4; see Fig. 3).

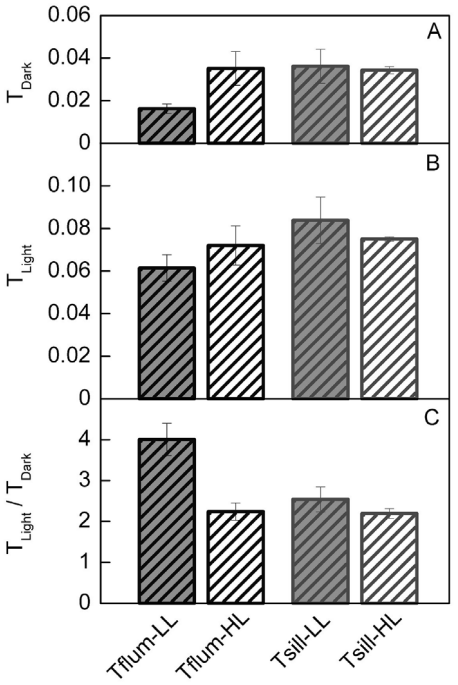

Fig. 3. Steady-state leaf transmittance in the dark, T_{Dark} (*A*), in the light (150 μmol photons $m^{-2}s^{-1}$), T_{Light}, (*B*), and its ratio, T_{Light}/T_{Dark} (*C*). Means ± SEs, *n* = 3 - 7.

The actinic radiation caused simultaneous changes in T, q_{NPQ}, and DE (xanthophyll de-epoxidation is considered as key mechanism of NPQ and hence leaf protection). The q_{NPQ} changed in a range of 1 - 2.7 in different samples, T_{Light}/T_{Dark} (at 680 nm) varied in a range of 2 - 4, and DE_{Light}/DE_{Dark} ranged from 1.1 to 9. Our data show that greater changes in transmittance T_{Light}/T_{Dark} correspond to greater changes in deepoxidation DE_{Light}/DE_{Dark}, to a greater q_{NPQ}, and also to lower steady state values of Φ_{PSII}^{Light} (Fig. 4, Fig. 5 Suppl., Table 1). Hence, higher q_{NPQ} values correspond to a higher DE_{Light}/DE_{Dark} and lower Φ_{PSII}^{Light} (Table 1). We also recorded a positive correlation between Φ_{PSII}^{Recov} and T_{Light}/T_{Dark}, and a weaker correlation between Φ_{PSII}^{Recov} and DE_{Light}/DE_{Dark} (Fig. 4, Fig. 6 Suppl., Table 1). Thus, one can assume that chloroplast avoidance movement is more essential for photoprotection at the irradiance used in this work than deepoxidation of violaxanthin.

Table 1. Pearson's sample correlation coefficients (r_{xy}) for values of changes in transmittance, xanthophyll deepoxidation and photosystem II activity. Correlation is significant at a 5 % level or 1% level (*).

Parameters x	y	r_{xy}	*n*
T_{Light}/T_{Dark}	DE_{Light}/DE_{Dark}	0.784*	12
T_{Light}/T_{Dark}	q_{NPQ}	0.599	13
T_{Light}/T_{Dark}	$\Phi_{PSII}^{5\,min}$	0.790*	16
T_{Light}/T_{Dark}	Φ_{PSII}^{1h}	0.585	16
DE_{Light}/DE_{Dark}	q_{NPQ}	0.718	11
DE_{Light}/DE_{Dark}	$\Phi_{PSII}^{5\,min} / \Phi_{PSII}^{Dark}$	0.643	17
T_{Light}/T_{Dark}	$\Phi_{PSII}^{5\,min} / \Phi_{PSII}^{Dark}$	0.473	17
q_{NPQ}	Φ_{PSII}^{Light}	-0.934*	15

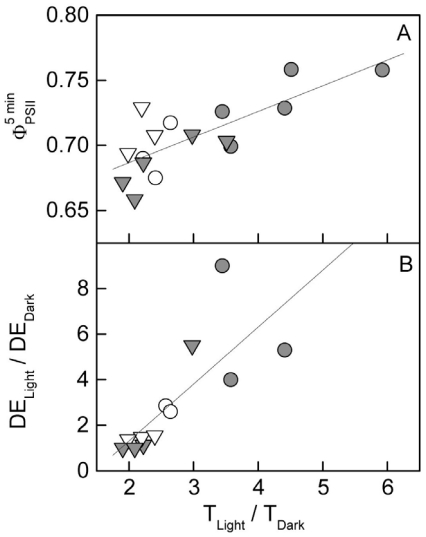

Fig. 4. Relationships between light dependent steady-state values of leaf light transmittance changes T_{Light}/T_{Dark} and photosystem II activity restored in 5 min after irradiation $\Phi_{PSII}^{5\,min}$ (*A*), or xanthophyll deepoxidation changes DE_{Light}/DE_{Dark} (*B*) in *T. fluminensis* (*circles*) and *T. sillamontana* (*triangles*) grown at low irradiance (*grey symbols*) or high irradiance (*white symbols*).

Since the actinic irradiance employed in our experiments does not lead to a significant photoinhibition (Powles and Thorne 1981, Demmig *et al.* 1987, Tyystjärvi and Aro 1996), recovery of PS II activity was

relatively fast. Therefore, we selected Φ_{PSII} values reached after a relatively short period (5 min) of dark recovery ($\Phi_{PSII}^{5\,min}$); Φ_{PSII} values reached after longer dark recovery periods (*e.g.*, 1 h) were less variable among individual leaves belonging to the same or even different species, and therefore were less suitable for the analysis. Nevertheless, correlation remained when $\Phi_{PSII}^{1\,h}$ was used instead of $\Phi_{PSII}^{5\,min}$ (though with lower Pearson's r_{xy} value; see Table 1). One can also use $\Phi_{PSII}^{Recov}/\Phi_{PSII}^{Initial}$ (*e.g.*, $\Phi_{PSII}^{5\,min}/\Phi_{PSII}^{Dark}$) instead of a Φ_{PSII}^{Recov} (*e.g.*, $\Phi_{PSII}^{5\,min}$) value; correlation with T_{Light}/T_{Dark} also persisted in this case (Table 1).

Discussion

Our previous studies showed that *T. fluminensis* and *T. sillamontana* belonging to disparate ecological groups (mesophytes from shaded habitats and succulents from semideserts) differ significantly in their ability to protect from photoinhibition and in the rate of responses to changes in inirradiance (Samoilova *et al.* 2011, Ptushenko *et al.* 2013). We tried to relate these functional differences with marked differences in leaf morphology and anatomy revealed in *T. fluminensis* and *T. sillamontana*.

The most remarkable differences between the shade and sun *Tradescantia* species were found in the mesophyll and whole leaf thickness: *T. fluminensis* LI-grown leaves and their mesophyll were considerably thinner than those of *T. sillamontana* (Fig. 2*A*). High irradiation-grown leaves had the same thickness in both species, still mesophyll thickness remained less in *T. fluminensis* compared with *T. sillamontana*. *T. fluminensis* mesophyll possessed a slightly lower chloroplast-to-cell size ratio, which may manifest the presence of an additional room for chloroplast movement in a cell, improving its ability to adjust to irradiance changes (Fig. 2*C*). However, this difference was essential only in the HI-grown *Tradescantia* plants being almost negligible in the LI-grown plants.

The most striking feature of the *Tradescantia* HI response as manifested by leaf anatomy was a large increase in leaf thickness in comparison with other species (Table 1 Suppl.). Published data on growth irradiance responses of leaf anatomy in herbaceous plants, deciduous trees of the cool temperate region, moist temperate deciduous forests, and evergreen trees of rain forests are collected in this table. For all the species presented, a HI-induced increase in leaf thickness ranged in an interval of 5 - 71 %, or 5 to 127 μm in the absolute scale (with the most typical values varying within a 30 - 40 % interval). At the same time, *T. sillamontana* manifested *ca*. two-fold and *T. fluminensis* four-fold increase in leaf thickness. Our data on the HI response of *Tradescantia* leaf anatomy are in accord with those of Adamson *et al.* (1991) obtained for *T. albiflora*, a close relative of *T. fluminensis*. The authors presented no details on leaf thickness or anatomy but reported a 1.9 - 2.7-fold increase in specific leaf mass in response to an increase in growth irradiance (from 25 - 70 to 220 - 1800 μmol photons $m^{-2}s^{-1}$), which may be ascribed, at least partially, to increase in leaf thickness.

Given little change in cell number, such a significant increase in leaf thickness was mainly due to expansion of the epidermal cell layers, whereas the contribution of mesophyll cells was smaller (Figs. 1 and 2). Still, leaf architecture features responsible for the remarkably higher IIT in the LI-grown *Tradescantia* in comparison with the HI-grown *Tradescantia* (especially *T. fluminensis*) and especially with other species remain unclear.

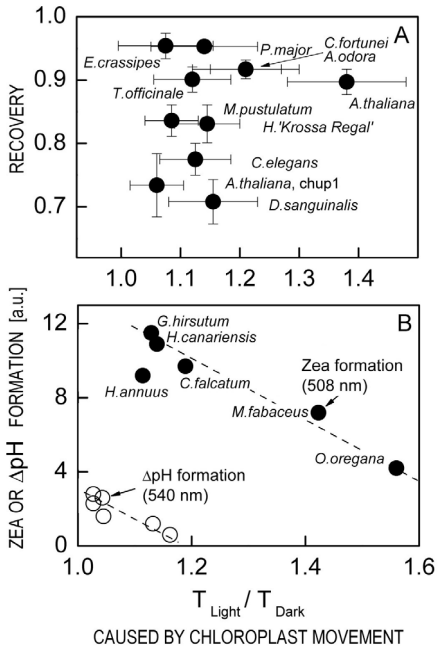

Fig. 5. Relationships between irradiance dependent steady-state leaf transmittance changes T_{Light}/T_{Dark}, caused by chloroplast avoidance movement, and recovery of photosystem (PS) II activity 1 h after irradiation $\Phi_{PSII}^{1\,h}/\Phi_{PSII}^{Dark}$ in 10 plant species (*A*), or zeaxanthin formation (xanthophyll deepoxidation) and ΔpH formation in 6 plant species (*B*). Irradiance was 1000 or 940 μmol m^{-2} s^{-1} (for measuring PS II recovery and xanthophyll deepoxidation, respectively). Data from Königer and Bollinger (2012) in *A* and Brugnoli and Björkman (1992) in *B*.

Mesophyll cell size was suggested as anatomical trait related to the extent of IIT. Davis *et al.* (2011) showed that the IIT correlates with the mesophyll cell size: the larger the cell, the more room for chloroplast movement and hence a more pronounced effect on leaf transmittance. The highest IIT values are found in plants with cells larger than 30 - 40 μm. However, *Tradescantia* species having relatively small (17 - 25 μm) cells appa-

rently did not fit this pattern. Moreover, the mesophyll cell size was slightly lower in LI- than HI-grown leaves of both species (Figs. 2 and 3), whereas the IIT extent was slightly (in *T. sillamontana*) or even remarkably (in *T. fluminensis*) higher in LI-grown ones (Fig. 4).

Another factor of a high IIT is chloroplast size. Jeong *et al.* (2002) showed a severe retardation of a large chloroplast rearrangement. Taking into account both factors, one might also consider the chloroplast-to-cell size ratio (rather than the absolute cell size) as leaf architecture feature related to capability to provide a high extent of IIT. This ratio could express the expectation of less limited moving of relatively small particles within a large volume. Still, the LI-grown plants of both *Tradescantia* species possessed higher chloroplast to cell size ratios than the HI-grown (Fig. 3*C*), thus compromising this parameter, too. Our finding is apparently confirmed by results of Adamson *et al.* (1991) who also showed a slight decrease in chloroplast size of HI-grown *T. albiflora* leaves compared with LI-grown ones.

Remarkably, a slight (5 - 8 %) growth irradiance-dependent change of mesophyll cell size in *Tradescantia* is comparable with that in *Begonia* × *semperflorens* or two *Shorea* species (arboreous evergreens), whereas in some species, *e.g.*, in *Nicotiana benthamiana* mesophyll cells increase *ca*. 1.5-fold in width and *ca*. 2-fold in height at HI. And, paradoxically, *N. benthamiana* plants exhibit almost no changes in IIT in response to changes in growth irradiance (*ca*. $\Delta T \approx 0.035$ both in sun- and shade-grown leaves), whereas *B. semperflorens* leaves manifest noticeable differences between shade-grown ($\Delta T = 0.089$) and sun-grown ($\Delta T = 0.058$) plants (Davis *et al.* 2011).

One can conclude that *Tradescantia* leaf anatomy is far from an “ideal architectural pattern” from the standpoint of avoidance response. Thus, according to Davis *et al.* (2011), this pattern implies large mesophyll cells (40 - 50 μm in diameter), whereas cells smaller than 25 μm in diameter provide for modest IIT. Both *Tradescantia* species studied in the present work possessed relatively small mesophyll cells (17 - 21 μm in diameter) of the LI-grown plants. Even HI did not lead to substantially larger mesophyll cells (22 - 25 μm in diameter), so acclimation to gowth irradiance apparently did not “improve” leaf anatomy to make it more suitable for the high IIT. By contrast, the IIT in *Tradescantia* leaves amounted to 2 - 4-fold, substantially exceeding in magnitude changes described by Königer and Bollinger (2012) for other 10 species (1.07 - 1.40-fold). The reason(s) for the apparent mismatch between the irradiance-dependent chloroplasts rearrangement, as revealed by confocal microscopy in a work by Königer and Bollinger, and simultaneous changes in leaf transmittance for some species remain so far elusive.

It is so far unknown whether the IIT and high radiation tolerance are related and to what extent. The hypothesis of Park *et al.* (1996) relating a sizeable IIT in *T. albiflora* with its increased HI tolerance lacks a solid experimental proof. Furthermore, Königer and Bollinger (2012) concluded (based on analysis of 10 species from different ecological groups) that "there is no correlation between the speed or the degree of transmission changes and the high light stress tolerance". On the other hand, there is a number of reports demonstrating that blocking avoidance response of chloroplasts by a mutation (in an irradiance sensing system or an actin-binding protein) exacerbates leaf photodamage (Jeong *et al.* 2002, Kasahara *et al.* 2002, Königer *et al.* 2008, Sztatelman *et al.* 2010). One can suggest that avoidance response might exert a dominant contribution to overall HI tolerance in these species.

Apparent discrepancy between the conclusions of Königer and Bollinger and the data on chloroplast avoidance mutants can be avoided by an assumption that chloroplast avoidance is integrated, with other photoprotective reactions, into a complex irradiance response, and hence a modest avoidance response of a plant species may be compensated by other protective mechanisms. At the same time, the acute impairment of avoidance response by mutations or inhibitors cannot be compensated for by the other photoprotective mechanisms, as their plasticity on the ontogenetic or shorter timescale is restricted compared to that on the phylogenetic timescale.

To elucidate a possible contribution of chloroplast movement to the tolerance of excessive irradiance in *Tradescantia*, we attempted to compare the extent of chloroplast avoidance movement and other parameters of the photosynthetic apparatus in the *Tradescantia* species. To estimate the ontogenetic plasticity of chloroplast avoidance response, we studied acclimation of the shade-type *Tradescantia* to the HI conditions and *vice versa*.
Excessive irradiation leads to inhibition of PS II activity apparent as decline in Φ_{PSII} (Barber and Andersson 1992, Tyystjärvi and Aro 1996). Therefore, a Φ_{PSII} value after exposure of a leaf to excessive irradiance, Φ_{PSII}^{Recov}, may serve as measure of a high radiation stress. Thus, a Φ_{PSII} value after 5 min dark recovery, $\Phi_{PSII}^{5\,min}$, was the highest in LI-grown *T. fluminesis* leaves. Taking into account that these leaves also showed the most pronounced (4-fold) IIT (Fig. 4), one can suppose that chloroplast avoidance movement played a significant role in protection of the photosynthetic apparatus of the *Tradescantia* leaves. Nevertheless, the difference in the average $\Phi_{PSII}^{5\,min}$ values between the two *Tradescantia* species acclimated to the LI and the HI was insubstantial. Taking into account the above mentioned limitations of the estimation of the avoidance contribution to overall photoprotection, we compared the variation in $\Phi_{PSII}^{5\,min}$ with the variations in T_{Light}/T_{Dark} and DE_{Light}/DE_{Dark} (Fig. 5 Suppl.).
There is a positive correlation between the $\Phi_{PSII}^{5\,min}$ and T_{Light}/T_{Dark} values in the studied *Tradescantia* species

(Fig. 5*A*). Remarkably, this is not the case, according to Königer and Bollinger (2012),, in other 10 plant species (see Fig. 6*A*). However, in all the 10 species, T_{Light}/T_{Dark} values are mainly within the range 1.05 - 1.2 (only one species, *A. thaliana*, possesses a greater value of 1.4), whereas the *Tradescantia* plants showed a much larger T_{Light}/T_{Dark} (2 - 6).

The existence of this correlation supports a hypothesis relating the tolerance to excessive irradiance to the magnitude of IIT. Still, it is not clear whether they simply correlate with up-regulation of other photoprotective mechanisms. Accordingly, the contribution of the IIT to leaf photoprotection remains uncertain. Anderson *et al.* (2001) suggested that chloroplast avoidance movement might supplement the xanthophyll cycle in LI-grown *Tradescantia* leaves taking into account that photoprotective capacity of the xanthophyll cycle is lower in comparison with that of HI-grown leaves. Moreover, Brugnoli and Björkman (1992) demonstrated a negative correlation between the IIT and the zeaxanthin and pH build-up in six plant species (Fig. 6*B*). In other words, plant species with a more pronounced IIT exhibit a weaker deepoxidation response to irradiance and *vice versa*. Thus, in a broad group of taxonomically distant species, these two types of photoprotective mechanisms compensate an insufficient activity of each other.

However, we recorded a completely different picture in the leaves of the same species as well as in the two different (but closely related) species, *T. fluminensis* and *T. sillamontana*: a positive correlation of the T_{Light}/T_{Dark} and the DE_{Light}/DE_{Dark} ratio (Fig. 5*B*). The following hypothesis reconciles the apparent controversy caused, as outlined above, by inter- and intra-specific variability. Namely, an independent adjustment of chloroplast avoidance response and other photoprotective mechanisms (*e.g.*, xanthophyll deepoxidation) can take place only at the evolutionary timescale. By contrast, irradiance acclimation of a plant on the ontogenetic time scale is feasible only by concerted regulation of radiation trapping by a leaf (implemented *via* chloroplast avoidance response and IIT) and other (*e.g.*, NPQ-based) photoprotective mechanisms (Konert *et al.* 2013).

Notably, *T. albiflora*, a close relative of *T. fluminensis*, shows a very limited modulation of its photosynthetic apparatus by growth irradiance in contrast to striking sun/shade acclimation responses characteristic of many higher plant species (Chow *et al.* 1991, Anderson *et al.* 2001). One can also assume that regulation of different photoprotective mechanisms proceeding exclusively in a concerted manner is another manifestation of a low acclimation plasticity. This trait might also be peculiar of the *Tradescantia* species studied.

Conclusion

Until now, *Tradescantia* species remain largely untouched in the field of photosynthesis research. To the best of our knowledge, the only exception is *T. albiflora* exhibiting a number of remarkable photosynthetic features. These features comprise a combination of tolerance to an extraordinary wide growth irradiance range and an unusually low plasticity of photoprotective responses of photosynthetic apparatus together with an extremely high IIT in comparison with other species. These features imply that an IIT is key mechanism of photoprotection and growth irradiance acclimation of *Tradescantia* plants, whereas other mechanisms are limited.

On the other hand, the efficiency of chloroplast avoidance response is related to the specific features of leaf anatomy, especially a large cell diameter and small chloroplasts. However, the *Tradescantia* leaf architecture was, against expectations, far from an "ideal architectural pattern" from the standpoint of avoidance response. The *Tradescantia* leaf anatomy displays an unusually high responsiveness to growth irradiance as compared with many other higher plants species. Still, the overall leaf architecture of the studied *Tradescantia* species remains qualitatively the same showing no striking changes facilitating chloroplast avoidance response.

Nevertheless, both the shade-plant *T. fluminensis* and the sun-plant *T. sillamontana* demonstrated a very high IIT evidently due to extensive chloroplast avoidance movements. It is essential that the magnitude of the IIT positively correlated with other parameters manifesting the protection state of the photosynthetic apparatus. Collectively, these features suggest that: *1*) the high magnitude of the IIT is a feature of at least some *Tradescantia* species appearing at diverse growth irradiances; *2*) it is not strictly determined by leaf architecture; and *3*) it is modulated in concert with other photoprotective mechanisms. Further investigations are needed to find out whether the IIT serves as mechanism compensating an insufficient activity of other photoprotective mechanisms.

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