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Responses of photosystem I compared with photosystem II to high-light stress in tropical shade and sun leaves

C. Barth,[1] G. H. Krause[1] & K. Winter[2]

ABSTRACT

Sun and shade leaves of several plant species from a neotropical forest were exposed to excessive light to evaluate the responses of photosystem I in comparison to those of photosystem II. Potential photosystem I activity was determined by means of the maximum P700 absorbance change around 810 nm (ΔA_{810max}) in saturating far-red light. Leaf absorbance changes in dependence of increasing far-red light fluence rates were used to calculate a 'saturation constant', K_s, representing the far-red irradiance at which half of the maximal absorbance change ($\Delta A_{810max}/2$) was reached in the steady state. Photosystem II efficiency was assessed by measuring the ratio of variable to maximum chlorophyll fluorescence, F_v/F_m, in dark-adapted leaf samples. Strong illumination caused a high degree of photo-inhibition of photosystem II in all leaves, particularly in shade leaves. Exposure to 1800-2000 μmol photons m^{-2} s^{-1} for 75 min did not substantially affect the potential activity of photosystem I in all species tested, but caused a more than 40-fold increase of K_s in shade leaves, and a three-fold increase of K_s in sun leaves. The increase in K_s was reversible during recovery under low light, and the recovery process was much faster in sun than in shade leaves. The novel effect of high-light stress on the light saturation of P700 oxidation described here may represent a complex reversible mechanism within photosystem I that regulates light-energy dissipation and thus protects photosystem I from photo-oxidative damage. Moreover, we show that under high-light stress a high proportion of P700 accumulates in the oxidized state, $P700^+$. Presumably, conversion of excitation energy to heat by this cation radical may efficiently contribute to photoprotection.

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INTRODUCTION

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When light energy absorbed by plants exceeds the capacity of light utilization in photosynthesis, photo-inhibition may occur. It has been shown in numerous studies that photosystem (PS) II is a primary site of inhibition (e.g. Krause 1988; Aro, Virgin & Andersson 1993). Photo-inhibition of PSII can be easily detected *in vivo* by a decrease in the 'dark-adapted' ratio of variable to maximum chlorophyll *a* fluorescence (F_v/F_m) and represents a reversible increase in thermal dissipation of excitation energy (Krause & Weis 1991). The xanthophyll cycle, known as a protective mechanism for PSII, facilitates dissipation of excess light energy via conversion of violaxanthin (V) to antheraxanthin (A) and zeaxanthin (Z) in the thylakoid membrane (Demmig-Adams & Adams 1992a; Pfündel & Bilger 1994; Gilmore 1997; Niyogi, Grossman & Björkman 1998). Depending on the acclimation state of the plant, a major part of the persistent decline in F_v/F_m appears to be based on the presence of Z (and A), and the inactivation of the D1 protein in the PSII reaction centres can be minimized (Thiele *et al.* 1996; Thiele, Winter & Krause 1997).

Recent publications provide evidence that in certain circumstances PSI can be photo-inhibited as much as or even faster than PSII. Potential activity of PSI *in vivo* can be assessed by measuring the P700 absorbance change around 810-830 nm (Harbinson & Woodward 1987; Weis & Lechtenberg 1989; Klughammer & Schreiber 1991, 1994). A preferential photo-inactivation of PSI was observed at chilling temperatures in potato (*Solanum tuberosum*) leaves (Havaux & Davaud 1994) and in cold-sensitive *Cucumis sativus* L., when leaves were chilled under low light (Terashima, Funayama & Sonoike 1994; Sonoike 1996; Terashima *et al.* 1998) or both under low and high light (Barth & Krause 1999). In leaves of chilling-sensitive pumpkin (*Cucurbita maxima* L.) and tobacco (*Nicotiana tabacum* L.), high light at 4 °C caused inhibition of both photosystems to a similar degree (Barth & Krause 1999).

There is strong evidence that active oxygen species are involved in the inactivation of PSI (Havaux & Davaud 1994). Destruction of the iron sulphur centres (F_A, F_B, F_X), is thought to be a primary event of PSI photo-inhibition and supposedly triggers proteolysis of the PSI-A/B reaction centre proteins and of extrinsic polypeptides of the PSI complex (Inoue, Sakurai & Hiyama 1986; Sonoike *et al.* 1995, 1997; Sonoike 1996; Terashima *et al.* 1998; Tjus, Møller & Scheller 1999).

Several mechanisms, such as the antioxidative scavenging system, the xanthophyll cycle and cyclic electron flow around PSI, are discussed to protect PSI from photo-inhibition. It has been reported that PSI and PSII contain similar amounts of xanthophyll cycle pigments and that in high light A and Z are formed in PSI (Thayer & Björkman 1992; Färber *et al.* 1997). However, it is unknown whether antheraxanthin (A) and zeaxanthin (Z) protect PSI. The cyclic electron flow around PSI is considered to protect PSI by preventing

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Image Previews

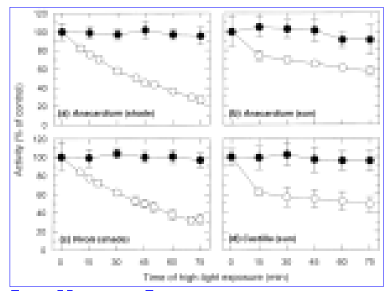

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Figure 1. Time course of photo-inhibition of detached shade leaves of *Anacardium* and *Virola* and sun le...

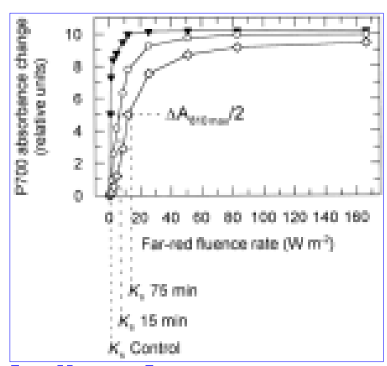

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Figure 2. Demonstration of light saturation curves of P700

overreduction of its acceptor side and by maintaining a pH gradient across the thylakoid membrane that downregulates PSII (Manuel *et al.* 1999; Cornic *et al.* 2000).

Plants in the tropical forest are periodically exposed to highly excessive sunlight. Outer-canopy sun leaves of trees have to cope with extreme solar irradiances on clear or partly cloudy days. Shade plants growing in the forest understorey are exposed to high light when gaps are created by fallen trees. In addition to visible light, the high UV irradiance in the tropics is known to promote photo-inhibition of PSII (Krause *et al.* 1999a). Photo-inhibition of PSII in tropical plants has been described for leaves growing in the shade, leaves acclimated to conditions of natural gaps of the tropical forest and in young and mature sun leaves of various tropical tree species (Krause, Virgo & Winter 1995; Krause & Winter 1996; Thiele *et al.* 1996; Thiele, Krause & Winter 1998; Krause *et al.* 1999a). So far, the response of PSI in shade and sun leaves of tropical forest plants has not been investigated.

Sun and shade leaves differ in their organization and function of the photosynthetic apparatus (Anderson, Chow & Goodchild 1988; Walters & Horton 1999). Leaves that are well acclimated to conditions of excess light possess an increased pool size of xanthophyll cycle pigments and exhibit faster kinetics of de-epoxidation of V, as well as significantly higher levels of A and Z when exposed to extreme light conditions (Königer *et al.* 1995; Krause *et al.* 1995). Moreover, sun leaves are characterized by a lower α-carotene to β-carotene ratio in comparison with shade leaves (Thayer & Björkman 1990; Demmig-Adams & Adams 1992b; Königer *et al.* 1995; Brugnoli *et al.* 1998; Demmig-Adams 1998).

The aim of the present study was to investigate responses of PSI to extreme light conditions in tropical shade and sun leaves. For this purpose, leaves of various tropical forest species were exposed to high light under controlled conditions. In addition to PSI activity, photo-inhibition of PSII was assayed for comparison. To characterize the shade and sun leaves tested, their pigment composition and in particular the xanthophyll cycle activity was determined.

MATERIALS AND METHODS

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The experiments were carried out at the **Smithsonian Tropical Research Institute** in Panama City, Republic of Panama. Pigment analyses were performed at the Institute of Plant Biochemistry, Düsseldorf University.

absorbance changes around 810 nm as a funct...

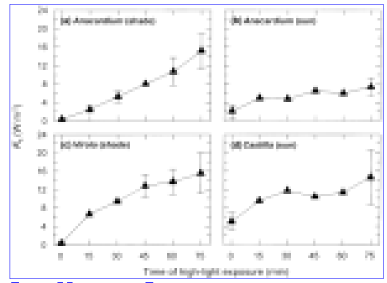

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Figure 3. Effects of high-light exposure on the saturation constant, K_s, of PSI in shade leaves of *Ana*...

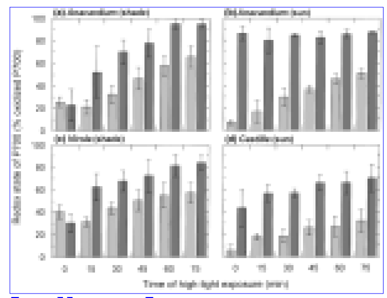

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Figure 3. Redox state of P700 in moderate (light bars) and high (dark bars) white light in shade leave...

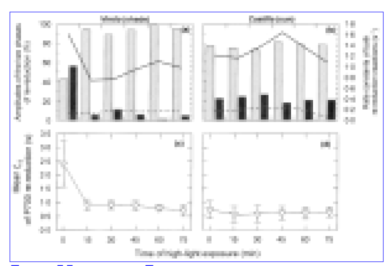

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Figure 5. Effects of high-light exposure on the re-reduction kinetics of oxidized P700 in the dark ana...

Plant material

Tree seedlings of *Anacardium excelsum* (Bertero & Balb.) Skeels (Anacardiaceae) and *Virola surinamensis* (Rol.) Warb. (Myristicaceae) were cultivated in pots in a shaded greenhouse (10-60 μmol m $^{-2}$ s $^{-1}$ PAR) at 25-30 °C. Leaves of 9-12-month-old plants were used. Additionally, mature leaves of *Dieffenbachia longispatha* L. (Araceae; also grown in the greenhouse) and of the understorey shrub *Piper carrilloanum* L. (Piperaceae), growing in the humid, seasonally dry lowland forest (10-150 μmol m $^{-2}$ s $^{-1}$ PAR) of the Metropolitan Natural Park near Panama City, were used for experiments. Young but fully expanded sun leaves from the tree crowns of *Anacardium excelsum* L. and *Castilla elastica* L. (Moraceae) and mature sun leaves of *Luehea seemannii* L. (Tiliaceae) were obtained from trees of the Metropolitan Natural Park, Panama. The outer crown leaves were accessible by means of a construction crane and were harvested in the early morning, kept in a bucket with the petioles immersed in water, transported to the laboratory within 30 min and adapted for at least 45 min to shade in the greenhouse. The sun-acclimated canopy leaves had received approximately 2000-2200 μmol m $^{-2}$ s $^{-1}$ PAR on clear days.

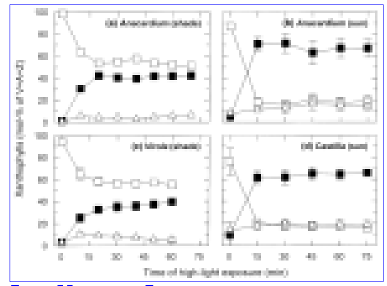

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Figure 6. Kinetics of de-epoxidation of V and formation of A and Z in shade leaves of *Anacardium* (a) a...

Photo-inhibition and recovery treatments

Before photo-inhibitory treatment, three leaf discs (1·65 cm^2) were punched from the leaf blade for control. One disc was immediately frozen with liquid nitrogen in order to analyse chloroplast pigments in the dark-adapted state. The other two control discs were kept in darkness on wet tissue paper until the measurements. Photo-inhibition treatment was carried out in a controlled-environment chamber (EGC, Chagrin Falls, OH, USA) where air temperature was set to 24 °C. Leaf blades were placed horizontally on a metal grating and the upper leaf surface was exposed to 1800 (shade leaves) or 2000 μmol m $^{-2}$ s $^{-1}$ PAR (sun leaves). PAR was measured with a quantum sensor (LI-189; LI-Cor, Lincoln, NE, USA). An air moistener was installed below the leaves. Leaf temperature was 25-29 °C, measured on the lower leaf side. At the times given in the graphs, one leaf disc each was sampled for determination of potential PSII and PSI activities and for pigment analysis.

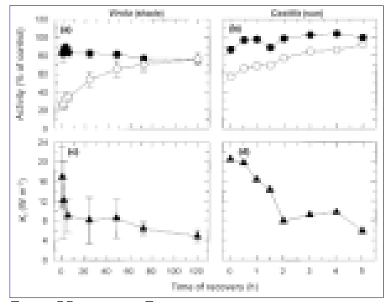

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Figure 7. Potential activity of PSI (●, ΔA_{810max}), potential efficiency of PSII (○, F_v/F_m ratio) (a, b) a...

To assess recovery from photo-inhibition, attached leaves of shade-grown *Virola surinamensis* tree seedlings and of detached canopy sun leaves of *Castilla elastica* were photo-inhibited for 75 min in the climate chamber as described above. For recovery, plants of *Virola* were placed in the shaded greenhouse; recovery was followed for 5 d. Leaves of *Castilla* were illuminated with 30-50 μmol m $^{-2}$ s $^{-1}$ PAR for 5 h. During recovery, leaf discs were removed for determination of potential PSII and PSI activity and pigment composition.

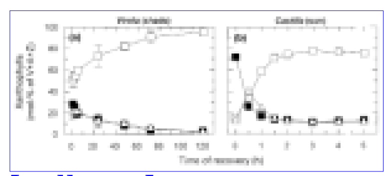

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Figure 8. Time course of epoxidation of xanthophyll cycle pigments in shade leaves of *Virola* and canop...

Determination of potential PSII efficiency

The ratio of maximum variable to maximum total Chl *a* fluorescence (F_v/F_m) determined after 10 min dark-adaptation served as a measure for potential PSII efficiency. The decrease in F_v/F_m indicates the degree of inactivation of PSII (Demmig-Adams & Björkman 1987; Krause & Weis 1991) and was measured with a PAM 2000 fluorometer (Walz, Effeltrich, Germany). For data analysis a portable PC (Poquet Computer Corp., Santa Clara, CA, USA), equipped with data acquisition software DA-2000 (Walz) was used. The measuring procedure has been described elsewhere (Barth & Krause 1999).

Determination of potential PSI activity

After the F_v/F_m determination, potential activity of PSI was determined by P700 absorbance change measurements at 810 nm (Klughammer & Schreiber 1998). A PAM 101 fluorometer (Walz) was connected with a dual-wavelength emitter-detector unit ED-P700DW consisting of a LED-driver unit, an emitter-detector unit and an AC/DC adapter (Walz). The LED-driver possessed infrared emitting diodes with peak emission at 810 nm (sample) and 860 nm (reference) and was connected to the PAM 101 using two arms of a five-arm fibre optic system (101-F5, Walz). Contributions of plastocyanin to the absorbance changes were minimized by this novel measuring system. The third and the fourth arm of the fibre optics were connected to a KL-150 lamp (Schott, Mainz, Germany) and to a KL-1500 lamp (Schott) to provide far-red light and actinic white light, respectively. Saturating far-red light (166 W m^{-2}, uncorrected for wavelength dependence of the pyranometer sensor LI-200SA, LI-COR) was obtained by mounting a 720 nm cut-off filter (Schott) directly on the end of the fibre optics. For signal recording, a chart recorder (Kipp & Zonen, Delft, Netherlands) was used. Leaf discs placed on a small piece of wire net and wet tissue paper below were enclosed in a cuvette (LSC-2; ADC Ltd, Hoddesdon, UK) and ventilated with a moistened air stream. Temperature in the cuvette was kept at 24 °C using a thermostat. The upper leaf surface was illuminated via the main arm of the fibre optics through a window in the cuvette. After 5 min pre-illumination with 120 μmol m^{-2} s^{-1} actinic white light, the steady-state A_{810} signal was measured; then the actinic light was switched off and the signal in the state of fully reduced P700 was recorded in the dark. Saturating far-red light (30 s) was given to trigger the oxidation of P700.

The signal difference between reduced and oxidized state of P700 (ΔA_{810max}) served as a relative measure for the photochemical capacity of PSI, in the following termed 'potential PSI activity'. (cf. Harbinson & Woodward 1987; Weis & Lechtenberg 1989). It should be noted that ΔA_{810max} does not provide information on quantum yield of PSI photochemistry. The signal ΔA_{810} was also used to analyse the kinetics of P700 oxidation in far-red light and P700 re-reduction in the dark. After recording the signal in far-red light, high actinic white light (1750 μmol m^{-2} s^{-1}) was applied for 1 min to determine the redox state of P700 under this condition. Finally, high far-red light was applied again and varied in three steps to confirm that the maximum far-red fluence rate was

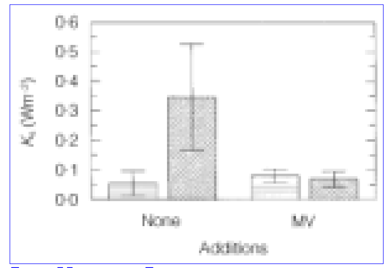

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Figure 9. Saturation constant, K_s, of P700 oxidation in isolated thylakoids of spinach. Control thylak...

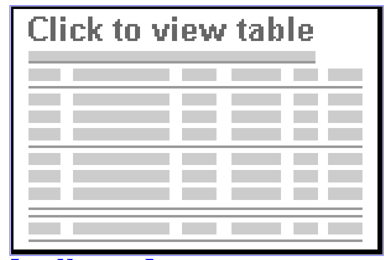

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Table 1. Effects of photo-inhibitory treatment on the saturation constant, K_s, of PSI in shade leaves ...

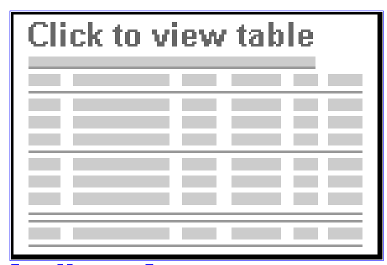

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Table 2. Content of chlorophyll (Chl) *a* + *b*, Chl *a/b* ratio and pools of xanthophyll cycle pigments (VA...

saturating. We assume that ΔA_{810max} represents the state of nearly complete oxidation of P700. The pre-illumination with white light (see above) should have overcome a possible acceptor side limitation of PSI that might have prevented full P700 oxidation (cf. Klughammer & Schreiber 1994). Data of P700 oxidation kinetics in saturating far-red light were fitted to a first-order reaction: $P700_{ox} = P700_{red} [1 - \exp(-kt)]$ using the software program GraFit 3·0. Re-reduction kinetics of oxidized P700 in the dark after saturating far-red illumination followed a first order reaction with two components: $P700_{red} = P700_{ox1} [1 - \exp(-k_1 t)] + P700_{ox2} [1 - \exp(-k_2 t)]$. The χ^2 of the fitted curves ranged between 0·0961 and 0·0001. The re-reduction occurred in the range of seconds; the absence of a very fast phase (ms range) indicated that reduced intersystem electron carriers did not accumulate in far-red light. (see Harbinson & Woodward 1987).

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Determination of a saturation constant, K_s, of P700 oxidation

Leaf absorbance changes at 810 nm were measured as a function of increasing fluence rates of far-red light to calculate a 'saturation constant', K_s, representing the far-red irradiance at which half of the maximum absorbance change ($\Delta A_{810max}/2$) was reached. Far-red intensities were varied in 10 steps. A characteristic saturating curve was obtained corresponding to the Michaelis-Menten kinetics of enzymatic reactions, where the K_m value (the substrate concentration) corresponds to K_s (the far-red intensity) and *v* (the reaction velocity) is represented by the absorbance change ΔA_{810}. The value of K_s was determined by using the linear plot of Hanes: the quotient of far-red intensity/ΔA_{810} is plotted against far-red intensity; the point of intercept with the abscissa equals $-K_s$. The correlation coefficient *r* for linear regression was between 0·97 and 1·00.

Chloroplast pigment analysis

Quantitative chloroplast pigment analysis was performed by homogenizing leaf segments in liquid nitrogen in a mortar in the presence of a small amount of Na_2CO_3. The pigments were extracted with 1·0 mL 99·5% acetone. After centrifugation of the extract for 3 min at 14 000 r.p.m. (above 10 000 *g*) (Microcentrifuge 5415 C; Eppendorf, Hamburg, Germany), the supernatant was filtered through 0·2 µm Minisart SRP 15 Syringe microfilters (Sartorius, Göttingen, Germany). The pigments were analysed by means of high-performance liquid chromatography according to the method of Färber *et al.* (1997).

Thylakoid membrane isolation and determination of potential activities of PSII and PSI and of K_s *in vitro*

Thylakoid membranes from spinach (*Spinacia oleracea* L. *cv.* Subito) grown in the greenhouse at 70-120 μmol m $^{-2}$ s $^{-1}$ PAR (16 h light : 8 h dark cycle) were isolated at 4 °C as described by Krause, Köster & Wong (1985). Thylakoids were isolated from dark-adapted leaf discs and from leaf segments illuminated with approximately 2000 μmol m $^{-2}$ s $^{-1}$ at 4 °C for 4 h. The isolation medium contained 330 m M sucrose, 10 m M NaCl, 2 m M EDTA, 1 m M $MnCl_2$, 1 m M $MgCl_2$, 0·8 m M KH_2PO_4, 20 m M sodium ascorbate, 0·4% (w/v) bovine serum albumin, 0·05% (w/v) cystein and 44 m M MES buffer/NaOH, pH 6·1. Thylakoids were released by osmotic shock in 5 m M $MgCl_2$ and resuspended in a 'double strength' medium to obtain final concentrations of 330 m M sucrose, 5 m M KCl, 5 m M $MgCl_2$, 1 m M KH_2PO_4 and 40 m M HEPES buffer/KOH, pH 7·6. The Chl concentrations were determined in 80% acetone according to Arnon (1949).

For determination of F_v/F_m ratios, thylakoids equivalent to 20 μg Chl were suspended in a total sample volume of 1 mL of the above resuspension medium. The suspension was placed in a cuvette (KS 101; Walz) connected to PAM 101/102/103 units (Walz) via a translucent stopper and a five-arm fibre optic system (101-F5; Walz). Samples were stirred during fluorescence measurements. Initial fluorescence, F_0, was recorded in low measuring light (PAM 101). Then far-red light (0·4 W m $^{-2}$) was applied for 3 s. Finally, a saturating pulse of white light (5000 μmol m $^{-2}$ s $^{-1}$) was given in order to determine F_m. In each sample, F_v/F_m ratios were measured twice.

The ΔA_{810max} (potential PSI activity) was determined in the presence and absence of 50 μ M methyl viologen (MV). Samples in the suspension cuvette (KS 101; Walz) contained 200 μg Chl in 1 mL resuspension medium. They were pre-illuminated with 300 μmol m $^{-2}$ s $^{-1}$ actinic white light for 3 min and darkened for 30 s before far-red light was given. Saturating far-red light (46 W m $^{-2}$) was obtained by combining two far-red diodes (102-FR; Walz) and a KL-150 lamp (Schott) equipped with a RG 9 filter (Schott). With this set-up, increasing far-red fluence rates were produced to determine the saturation constant K_s of P700 oxidation.

RESULTS Go to: Choose Go

Photo-inhibition of shade and sun leaves

In shade leaves of *Anacardium* (Fig. 1a) and *Virola* (Fig. 1c), the potential efficiency of PSII measured as F_v/F_m ratio decreased during 75 min strong illumination by about 70% with respect to controls. In contrast, potential PSI activity determined as P700 absorbance change at 810 nm was not affected. In young sun leaves of *Anacardium* (Fig. 1b) and *Castilla* (Fig. 1d), a 60% (Fig. 1b) and 50% (Fig. 1d) inhibition of PSII was reached within 75 min. The PSII data from sun leaves are consistent with results reported by Krause *et al.* (1995) and Thiele *et al.* (1996). Potential PSI activity in the sun leaves was either not or only slightly inhibited (Fig. 1b & d).

Compared with the shade leaves of *Anacardium* and *Virola*, very similar results were obtained with the shade leaves of *Dieffenbachia*. In leaves of the understorey shrub *Piper*, potential PSII efficiency was reduced by about 50% after 75 min illumination; potential PSI activity remaining unaffected. Mature sun leaves of *Luehea* were particularly resistant against high-light stress. PSII efficiency was diminished within 75 min by 25% only. Potential PSI activity was not decreased (data not shown).

Saturation constant K_s of P700 oxidation

Although potential activity (i.e. capacity) of PSI measured in saturating far-red light was not affected by high-light exposure in all the species tested (cf. Figure 1), a conspicuous effect on PSI was induced. When P700 absorbance changes were measured as a function of increasing far-red fluence rates, a marked change in the light-saturation curve was observed, as demonstrated in Fig. 2 for shade leaves of *Virola*. The 'saturation constant', K_s, strongly increased depending on the time in high light.

In all leaves tested, a significant increase in K_s was observed already after 15 min in high light. The increase in K_s was most pronounced in shade leaves of *Anacardium* and *Virola* (Fig. 3a & c); after 75 min, a more than 40-fold increase in K_s was obtained. A strong increase in K_s was also seen in shade leaves of *Dieffenbachia* and *Piper* (Table 1). In sun leaves of *Anacardium* and *Castilla* (Fig. 3b & d), K_s increased significantly, but to a lesser extent (about three-fold in 75 min). The least effect on K_s was observed in sun leaves of *Luehea* (Table 1). It should be noted that K_s was much lower in non-stressed controls of shade than of sun leaves (Fig. 3, Table 1).

Linear plots of K_s versus F_v/F_m showed a correlation between these two parameters in shade leaves of *Anacardium*, *Virola* and *Piper*, as well as in sun leaves of *Anacardium* and *Castilla* (correlation coefficient, *r*, between -0·86 and -0·99), but not in *Luehea* (r = -0·62).

Redox state of P700 in white light

Determination of the redox state of P700 (i.e. the accumulation of the radical cation $P700^+$) in continuous actinic light can be used to evaluate the control of linear electron transport from PSII to PSI (Harbinson, Genty & Baker 1989; Weis & Lechtenberg 1989). The redox state of P700 was recorded under moderate (120 µmol m^{-2} s^{-1}) and strong (1750 µmol m^{-2} s^{-1}) white light (Fig. 4) in control leaves and immediately after high-light exposure of leaves from all species investigated. In dark-adapted controls of shade-grown *Anacardium* and *Virola* (Fig. 4a & c), about 25 and 40% $P700^+$, respectively, accumulated after 5 min illumination with 120 µmol m^{-2} s^{-1} white light. In shade leaves of *Dieffenbachia* (not shown), the redox state was comparable (30% $P700^+$). In contrast, dark controls of *Piper* (below 10% $P700^+$) and of sun leaves of *Anacardium* and *Castilla* (Fig. 4b & d) revealed a significantly lower percentage of $P700^+$. In control leaves of sun-acclimated *Luehea*, no $P700^+$ could be detected. When 1750 µmol m^{-2} s^{-1} white light was applied for 1 min, considerably more P700 was oxidized in controls of the sun leaves (Fig. 4b & d) and of *Piper* (data not shown), whereas in control shade leaves of *Anacardium* and *Virola* (Fig. 4a & c), the redox state of P700 was not significantly different from that determined in moderate light.

Upon photo-inhibition treatment, the proportion of $P700^+$ determined in moderate white light increased significantly in all species (Fig. 4). This can be explained by the decrease in PSII activity due to photo-inhibition (cf. Figure 1) which diminishes electron flow to PSI. In strong white light, a significantly increased proportion of oxidized P700 compared to controls was observed in photo-inhibited samples of shade-grown *Anacardium*, *Virola* (Fig. 4a & c) and *Dieffenbachia* (data not shown), but not in *Piper* (not shown) and in all the sun leaves tested, except for a tendency to an increase in *Castilla* (Fig. 4d).

Oxidation and reduction kinetics of P700

In order to clarify whether the increase in K_s was related to altered rates of P700 oxidation and re-reduction, the kinetics of the ΔA_{810max} signal was analysed. The oxidation kinetics of P700 in saturating far-red light followed a first order reaction (Harbinson & Woodward 1987). The mean half-time, $t_{1/2}$, of P700 oxidation measured in controls ranged between 0·2 and 0·8 s. In photo-inhibited samples, $t_{1/2}$ was decreased in *Castilla* and in *Piper*, respectively, indicating an acceleration of P700 oxidation. However, $t_{1/2}$ did not change significantly in the leaves of the other species tested (data not shown).

The re-reduction kinetics of $P700^+$ in the dark was fitted to a first-order reaction with two components (see MATERIALS AND METHODS). In the control shade leaves of *Virola* (Fig. 5a), the amplitude of the first phase (rate constant k_1 = 1·6 s^{-1}) was slightly smaller than the amplitude of the second phase (k_2 = 0·2 s^{-1}). Upon high-light exposure, an increase of the amplitude of the fast phase and a decrease in the amplitude of the slow phase was observed. Whereas the rate constant of the fast phase decreased by about 50%, that of the slow phase did not change (Fig. 5a). Similar results were obtained in shade leaves of *Anacardium* (data not shown), although the rise in the amplitude of the fast phase proceeded more gradually (from 25% to 80% during 75 min in high light). In contrast, the fast phase predominated in control sun leaves of *Castilla* and remained more or less constant upon high-light exposure. Rate constants of both phases did not seem to alter significantly (Fig. 5b). Re-reduction kinetics analysed in shade leaves of *Piper* and sun-acclimated leaves of *Anacardium* and *Luehea* (all not shown) were comparable with those of *Castilla*.

Due to the increase in the amplitude of the fast phase, $t_{1/2}$ of P700 re-reduction in the dark decreased strongly in shade leaves of *Virola* (Fig. 5c) and *Anacardium* (not shown). In sun-acclimated canopy leaves of *Castilla* (Fig. 5d) and *Anacardium* (not shown), $t_{1/2}$ remained approximately constant. On the whole, a correlation of the kinetic data of P700 oxidation and re-reduction with the increase in K_s was not seen.

Xanthophyll cycle activity and chloroplast pigment composition

Leaves of shade grown plants of *Anacardium* (Fig. 6a) revealed a lower xanthophyll cycle activity compared to *Piper* (not shown) and the canopy sun leaves (Fig. 6b & d). In the latter, the kinetics of de-epoxidation of V was faster and a significantly higher proportion of V was de-epoxidized to A and Z. In shade leaves of *Virola* (Fig. 6c) and *Dieffenbachia* (data not shown), de-epoxidation kinetics of V was even slower than in *Anacardium* shade leaves. In *Piper* (data not shown), xanthophyll cycle activity was higher than in shade leaves of the other species tested, but lower than in the sun leaves.

Table 2 summarizes the pigment composition of each species and reflects characteristic properties of shade and sun leaves. The Chl *a* + *b* content per leaf area unit was higher in the shade than in the sun leaves. In case of sun leaves of *Anacardium* and *Castilla*, the Chl content was relatively low, because young, light green (but fully expanded) leaves were used. The Chl *a*/Chl *b* ratio was significantly lower in shade than in sun leaves, indicating a smaller LHCII in the latter. The pool of xanthophyll cycle pigments (VAZ) based on Chl *a* + *b* was significantly higher in the understorey plant *Piper* and particularly in the canopy sun leaves in comparison to the shade-grown plants. Whereas the content of neoxanthin was similar in all the species tested, the content of lutein was higher in sun leaves compared to shade leaves (cf. Thayer & Björkman 1990; Brugnoli *et al.* 1998; Demmig-Adams 1998). Sun-acclimated leaves contained small amounts of α-Car and revealed a markedly higher content of β-Car than shade leaves, but the sum of α-and β-Car per Chl *a* + *b* was approximately the same in all species (cf. Königer *et al.* 1995; Krause *et al.* 1995, 1999a).

PSI, PSII and K_s under recovery conditions

Recovery from photo-inhibition was tested in shade leaves of *Virola* and in sun leaves of *Castilla*. After high-light exposure for 75 min, recovery was followed in low light (see MATERIALS AND METHODS). Although no inhibition of potential PSI activity could be observed in most experiments with *Virola* (cf. Fig. 1a), in the experimental series of the recovery study, a reduction in PSI capacity by about 15% after 75 min high light was detected. Subsequently, potential PSI activity declined slightly further under shade conditions (Fig. 7a). Potential PSII activity was diminished by high-light exposure to a similar degree as shown above (cf. Fig. 1a) and recovered very slowly. Sun-acclimated canopy leaves of *Castilla* revealed a much faster recovery of PSII activity (Fig. 7b). The data are in good agreement with results reported earlier by Krause *et al.* (1995, 1999a).

The increase in K_s of PSI caused by high-light stress was reversible, as shown for shade leaves of *Virola* (Fig. 7c) and sun leaves of *Castilla* (Fig. 7d). The decrease in K_s and increase in F_v/F_m during recovery were not closely correlated (r = -0·76 and -0·93 for *Virola* and *Castilla*, respectively). But similar to PSII activity, recovery of K_s was much slower in *Virola* (except for a fast initial recovery phase during the first 5 h) than in *Castilla*. In the latter, the control level of K_s was reached after 5 h in low light, but this was not the case in *Virola* even after 120 h recovery treatment.

Xanthophyll cycle during recovery

The slow recovery of PSII in *Virola* was associated with a slow epoxidation of Z to A and V (Fig. 8a). After 120 h, the Z and A levels of dark-adapted control leaves were reached. However, at this time PSII recovery was still incomplete (cf. Fig. 7a). In sun leaves of *Castilla*, Z was epoxidized very rapidly close to the level of dark-adapted leaves during the first hour under low light (Fig. 8b). A transient increase in the level of A w as seen during this time. In the following slow recovery phase, Z did not decrease significantly further. This kinetics is in agreement with data from Thiele *et al.* (1996).

The K_s effect in isolated thylakoids

In order to exclude the possibility that the increase in K_s seen in light-stressed leaves is based on redox components of the chloroplast stroma, K_s was determined in thylakoids isolated from photo-inhibited spinach leaves (Fig. 9). In the thylakoid preparations, the potential PSII efficiency (F_v/F_m) was substantially reduced (66% of control). However, potential PSI activity was not significantly changed in comparison to non-inhibited control thylakoids.

The K_s values determined in isolated control thylakoids were much lower than K_s values measured in control leaves where K_s was around 0·5 W m^{-2}. This was probably due to different absorption properties of thylakoid suspensions. An about six-fold increase in K_s was found in photo-inhibited thylakoids compared to controls (Fig. 9). The same factor of increase was seen *in vivo* in the same leaf material of *Spinacia* (data not shown). Methyl viologen (MV), which efficiently accepts electrons from the reducing side of PSI, had no effect on K_s in control thylakoids, but fully suppressed the increase in K_s in photo-inhibited thylakoids (Fig. 9). It should be noted that the re-reduction of $P700^+$ in isolated thylakoids was extremely slow ($t_{1/2} \approx 8$ s) and was even further slowed down in the presence of MV ($t_{1/2} \approx 35$ s).

DISCUSSION Go to: Choose Go

DISCUSSION

Potential activities of PSI and PSII and xanthophyll cycle activity during photo-inhibition and recovery

Potential PSI activity exhibited a high tolerance to extreme light conditions. Essentially, neither in shade nor in sun leaves, a decrease in PSI capacity was observed upon high-light stress (Fig. 1). In contrast to PSI, potential efficiency of PSII was strongly affected upon high-light exposure. The decrease of potential PSII efficiency proceeded considerably faster in shade than in sun leaves (compare Fig. 1a & c and Fig. 1b & d), as has been reported earlier (e.g. Öquist *et al.* 1992; Krause *et al.* 1999a). The lower susceptibility to photo-inhibition of PSII in sun leaves was related to faster kinetics and higher degree of V de-epoxidation (Fig. 6) and an increased pool size of xanthophyll cycle pigments (Table 2). Characteristic differences between sun and shade leaves in xanthophyll cycle activity and pools of the pigments involved have been observed before (e.g. Thayer & Björkman 1990; Königer *et al.* 1995; Demmig-Adams 1998; Krause *et al.* 1999a). De-epoxidized xanthophylls are supposed to facilitate dissipation of excess excitation energy (see INTRODUCTION). Moreover, the higher level of β-Car (decreased α/β-Car ratio) and the increased amount of lutein in sun-acclimated leaves (Table 2) may provide improved photoprotection against triplet Chl and singlet oxygen generated under high-light stress as discussed recently by Niyogi, Björkman & Grossman (1997) and Krause, Carouge & Garden (1999b). Due to its larger conjugated π-electron system β-Car might be a more efficient photoprotectant than α-Car, whereas the latter may be important for light-harvesting in the inner (Chl *a*-binding) antennae of plants growing in the shade. The change in the α/β-Car ratio appears to be an important acclimative response to low/high light. In mature leaves of shade-grown *Anacardium excelsum* and *Ficus insipida* a drastic increase in β-Car and decrease in α-Car was observed when the plants were exposed daily to full natural sunlight for short periods (unpublished results).

Recovery of PSII from photo-inhibition was much faster in sun-acclimated leaves than in shade leaves (compare Fig. 7a & b). The fast recovery of PSII in sun leaves was associated with a fast epoxidation of Z in the xanthophyll cycle (Fig. 8b) as reported previously, for example, by Krause *et al.* (1995) and Thiele *et al.* (1996).

The K_s effect - a novel photoprotective mechanism for PSI?

The increase in K_s observed upon exposure to excessive light indicates that higher far-red light is required to oxidize P700. Related to controls, the increase in K_s was more pronounced in shade-grown leaves than in sun-acclimated leaves (Fig. 3). The significantly lower K_s measured in controls of shade compared to sun leaves (Fig. 3) may be explained by an enlarged LHCI in the shade leaves (Maxwell *et al.* 1999) and accordingly higher amount of 'Chl red forms' that absorb at wavelengths λ> 700 nm (Rivadossi *et al.* 1999).

The effect on K_s was also observed in chilling-sensitive and chilling-tolerant crop plants when leaves were exposed to high light at 20 °C or at 4 °C. The increase in K_s was less pronounced in chilling-tolerant spinach than in chilling-sensitive plant species, but generally higher at 4 °C than at 20 °C (unpublished results). Apparently, the increase in K_s is a universal response of PSI to conditions of excessive light. Parallel investigations by Manuel *et al.* (1999) and Cornic *et al.* (2000) have demonstrated a similar, but kinetically different change in the light saturation of P700 oxidation caused by strong illumination of leaves of the high-alpine species *Geum montanum* and other C_3 plants, respectively. Interestingly, studies of heat stress revealed a strongly reduced efficiency of P700 oxidation by far-red light in leaves heat-treated in the dark (Havaux, Greppin & Strasser 1991; Bukov *et al.* 1999). This effect resembles the response to high light studied here only on first sight. The heat-induced alterations in PSI photochemistry were associated with a drastic decrease in the half-times of the two phases of $P700^+$ re-reduction that was not observed in light-stressed leaves. In the present study, heat stress was avoided, as the exposure was carried out under controlled temperature conditions. When shade leaves of *Piper* and sun leaves of *Luehea* were kept in darkness for 75 min at either 30 or 24 °C, no differences in K_s were found (data not shown).

Although the increase in K_s caused by high-light stress was correlated in most (but not all) experiments with a decrease in F_v/F_m, there was no close correlation between the two parameters under recovery conditions (Fig. 7). The data suggest that in response to excessive light, reversible changes in potential PSII efficiency and PSI photochemistry occur in parallel, but represent independent processes.

Several alternative mechanisms can conceivably explain the decreased efficiency of P700 oxidation by far-red light (increase in K_s): (i) enhanced dissipation of excitation energy in the antenna of PSI by means of Z and A formed in the xanthophyll cycle; (ii) increased cyclic electron flow around PSI; (iii) increased rate of charge recombination reactions between oxidized P700 and reduced acceptors.

(i) A role of energy dissipation mediated by the xanthophyll cycle appears unlikely as the shade leaves, which exhibited a much higher increase in K_s than the sun leaves, formed substantially lower amounts of Z and A. In experiments with *Cucurbita maxima*, complete inhibition of V de-epoxidation by incubation of leaves with dithiothreitol strongly enhanced photo-inhibition of PSII under 2000 μmol photons m^{-2} s^{-1} at 20 °C; but potential PSI activity (ΔA_{810max}) was not affected (Barth & Krause 1998) and the degree of K_s increase not influenced by the absence of Z and A (unpublished results). Overall, the present data do not provide evidence for photoprotection of PSI by the xanthophyll cycle.

(ii) Accelerated cyclic electron transport around PSI was suggested to be the cause of the decreased efficiency of P700 oxidation by far-red illumination, as reported very recently for light-stressed leaves (Manuel *et al.* 1999; Cornic *et al.* 2000). That conclusion was deduced from a faster re-reduction kinetics of oxidized P700 in the dark. However, in terms of kinetics the effect on PSI described by these authors appears to differ from the increase in K_s investigated here. Cornic *et al.* (2000) found that the full change in the sensitivity of P700 to far-red light occurs in only 5 min high-light exposure, the K_s value in our study increased steadily with exposure time. Our data confirm the finding of Manuel *et al.* (1999) and Cornic *et al.* (2000) that $P700^+$ reduction in the dark is biphasic. In shade leaves of *Virola*, the amplitude of the fast phase strongly increased upon short-term (15 min) illumination, causing a drop in the mean $t_{1/2}$ of P700 re-reduction (Fig. 5a & c). This change was not correlated with the increase in K_s. In tropical sun leaves (Fig. 5b), a predominant first phase of P700 re-reduction was found in controls, and no significant alterations in kinetics were caused by high-light stress (Fig. 5d). Nevertheless, these leaves exhibited a significant increase in K_s (Fig. 3b & d). In contrast to observations made here, Cornic *et al.* 2000) reported strong increases in the rate constant of the fast phase.

The two phases of P700 re-reduction seem to indicate that there are two pools of electrons (or alternatively two populations of PSI) characterized by different rates of electron donation to $P700^+$ as recently discussed by Bukov *et al.* (1999). The donors cannot be reduced intersystem carriers, such as the cytochrome b_6f complex and plastocyanin, as those would reduce $P700^+$ in a microsecond time scale (Haehnel 1984). This would mean that in photo-inhibited shade leaves, the fast donating pool is enlarged (higher amplitudes of the fast phase, Fig. 5a), but rate of donation is diminished, as indicated by the decreased rate constant of the fast phase.

In total, our data show a lack of correlation between increase in K_s and re-reduction kinetics of P700 *in vivo*. In addition, experiments *in vitro* clearly demonstrated that electron transport around PSI is not responsible for the increase in K_s. In isolated thylakoids, cyclic electron flow is largely suppressed due to the loss of stromal components after osmotic shock of the chloroplasts. But an increase in K_s value comparable to that obtained *in vivo* was found in thylakoids isolated from photo-inhibited leaves of *Spinacia* (Fig. 9).

(iii) Enhanced rates of charge recombinations between $P700^+$ and reduced electron acceptors may give a plausible explanation of the increase in K_s.

Forward electron transfer reactions compete with physiologically unproductive charge recombination reactions (for a recent review see Brettel 1997). When the secondary acceptor A_1 is pre-reduced, recombination between the primary radical pair, $P700^+A_0^-$ at room temperature yields the singlet ground state of P700 (yield ≈ 70%) and the P700 triplet state, $^3P700$ (yield ≈ 30%). When all three FeS centres are pre-reduced, recombination between $P700^+$ and A_1^- takes place mainly yielding $^3P700$, which then decays to the singlet ground state (Polm & Brettel 1998).

It was proposed that light and low temperature stress cause an accumulation of reducing power on the acceptor side of PSI (Havaux & Davaud 1994; Terashima *et al.* 1994; Sonoike 1996). Hence, when the FeS centres F_X, F_A and F_B are reduced, recombination in the radical pairs $P700^+A_0^-$ and/or $P700^+A_1^-$ can occur; thereby P700 returns, in part via $^3P700$, back to the ground state. Thus, excessive photosynthetic energy may be dissipated in PSI via charge recombination when FeS centres are not oxidized by an external acceptor within the time of the back-reaction. One has to assume that enhanced charge recombination persists for some time (depending on the type of leaf) in low light as seen by the 'recovery' kinetics of K_s (Fig. 7c & d). Such effect could possibly be caused by functional alterations of electron acceptors in the PSI reaction centre. As the FeS centres have been shown to be primary targets of photo-inactivation of PSI (Sonoike *et al.* 1995; Sonoike 1996; Tjus *et al.* 1999), they might in the still active centres be altered in a manner leading to faster charge recombination. Increased charge recombination between $P700^+$ and A_0^- as a result of destruction of the three FeS centres has been previously suggested to occur in cucumber leaves exposed to low light at chilling temperatures (Sonoike *et al.* 1995).

When an efficiently acting electron acceptor of PSI, such as MV, is added, charge recombination between $P700^+$ and A_0^-/A_1^- as well as P700 triplet formation are prevented (Takahashi & Katoh 1984). This could explain the increase in the efficiency of P700 oxidation by far-red light (i.e. decrease in K_s; Fig. 9) observed in photo-inhibited thylakoids when MV was added.

In summary, the effect on the light saturation of P700 oxidation in photo-inhibited leaves may at present be explained best by charge recombination reactions that are favoured when the acceptor side of PSI becomes reduced. The triplet P700 that in part derives from recombination between $P700^+$ and A_0^-/A_1^- apparently returns to the ground state by harmless energy dissipation. P700 seems to be shielded from O_2 so that the formation of $^1O_2$ is avoided (Brettel 1997). In fact, production of $^1O_2$ in PSI under photo-inhibitory conditions was not found (Hideg & Vass 1995). One might consider a quenching of $^3P700$ by β-carotene that is present in the PSI core (Lichtenthaler, Prenzel & Kuhn 1981; Färber *et al.* 1997). But the mechanism of $^3P700$ decay is still not clear.

Accumulation of $P700^+$ under high-light stress

The high proportion of $P700^+$ observed in controls of sun leaves (Fig. 4b & d) under high light (1750 μmol m $^{-2}$ s $^{-1}$) may be explained by the formation of a high ΔpH across the thylakoid membrane. This is known to induce the ΔpH-dependent quenching mechanism, qE, that downregulates PSII, i.e. electron transport from PSII to PSI is limited (Harbinson *et al.* 1989; Weis & Lechtenberg 1989). In addition, the high ΔpH may restrict the electron transfer from plastoquinone to the cytochrome b_6f complex. The very low proportion of $P700^+$ measured in moderate white light (120 μmol photons m $^{-2}$ s $^{-1}$) in controls of sun leaves (Fig. 4b & d) indicates that the ΔpH is low and strong qE is not induced by such light intensity, which is consistent with the function of sun leaves. In contrast, control shade leaves exhibited a redox state of P700 in high light similar to that determined in moderate light (Fig. 4a & c). In comparison to sun leaves, shade leaves are known for their low capacity of qE associated with a low xanthophyll cycle activity (Fig. 6). As indicated by quenching analyses with shade leaves of *Anacardium*, the moderate light intensity was sufficient to induce maximum qE (data not shown). Therefore, strong white light did not cause a higher oxidation of P700 in control leaves. But with progressing photo-inhibition of PSII, very high proportions of $P700^+$ were reached (Fig. 4a & c).

It is known that the cation radical $P700^+$ converts excitation energy to heat (Nuijs *et al.* 1986). Presumably, the accumulation of $P700^+$ observed under light stress is an important factor in preventing damage to the reaction centre. The present study documents that $P700^+$ indeed strongly accumulates under excessive light when PSII activity is restricted by non-photochemical quenching processes (see also Weis & Lechtenberg 1989). Apparently, such restriction is based predominantly on a high ΔpH and associated qE in sun leaves and on photo-inhibition of PSII in shade leaves.

CONCLUSIONS

Go to: Choose Go

It is evident from the study presented here that both in shade and sun leaves, PSI potential activity is remarkably stable in excessive light as compared to PSII. However, upon high-light stress, leaves of all species studied, particularly shade leaves, exhibited a conspicuous reversible decrease in the efficiency of P700 oxidation by far-red light, expressed as an increase in the saturation constant K_s. This effect might represent a protective mechanism of thermal energy dissipation by enhanced charge recombination in the reaction centre of PSI. The physiological significance of charge recombination as a photoprotection of PSI has not been suggested previously. Furthermore, under excessive light, a high proportion of $P700^+$ accumulates that may dissipate excitation energy and thus contribute to stabilization of PSI. The xanthophyll cycle seems to protect PSII rather than PSI, but photo-inhibition and downregulation of PSII by means of Z and A may indirectly protect PSI by restricting electron flow and thereby favouring $P700^+$ accumulation.

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