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How to cite

HAVAUX, Michel, GREPPIN, Hubert, STRASSER, Reto. Functioning of photosystems I and II in pea leaves exposed to heat stress in the presence or absence of light. In: Planta, 1991, vol. 186, n° 1, p. 88–98. doi: 10.1007/BF00201502

This publication URL: https://archive-ouverte.unige.ch/unige:178469
Publication DOI: 10.1007/BF00201502

Planta (1991) 186:88–98

Planta

Functioning of photosystems I and II in pea leaves exposed to heat stress in the presence or absence of light

Analysis using in-vivo fluorescence, absorbance, oxygen and photoacoustic measurements

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Received 21 February; accepted 28 June 1991

Abstract. Fluorimetric, photoacoustic, polarographic and absorbance techniques were used to measure in situ various functional aspects of the photochemical apparatus of photosynthesis in intact pea leaves (*Pisum sativum* L.) after short exposures to a high temperature of 40° C. The results indicated (i) that the in-vivo responses of the two photosystems to high-temperature pretreatments were markedly different and in some respects opposite, with photosystem (PS) II activity being inhibited (or down-regulated) and PSI function being stimulated; and (ii) that light strongly interacts with the response of the photosystems, acting as an efficient protector of the photochemical activity against its inactivation by heat. When imposed in the dark, heat provoked a drastic inhibition of photosynthetic oxygen evolution and photochemical energy storage, correlated with a marked loss of variable PSII-chlorophyll fluorescence emission. None of the above changes were observed in leaves which were illuminated during heating. This photoprotection was saturated at rather low light fluence rates (around $10\ W \cdot m^{-2}$). Heat stress in darkness appeared to increase the capacity for cyclic electron flow around PSI, as indicated by the enhanced photochemical energy storage in far-red light and the faster decay of P_{700}^{+} (oxidized reaction center of PSI) monitored upon sudded interruption of the far-red light. The presence of light during heat stress reduced somewhat this PSI-driven cyclic electron transport. It was also observed that heat stress in darkness resulted in the progressive closure of the PSI reaction centers in leaves under steady illumination whereas PSII traps remained largely open, possibly reflecting the adjustment of the photochemical efficiency of undamaged PSI to the reduced rate of photochemistry in PSII.

Key words: Chlorophyll fluorescence – Heat stress – Leaf (light absorbance) – Photoprotection – Photosystem I, II – *Pisum* (photosynthesis)

Introduction

The responses of the photosynthetic machinery to a particular environmental constraint are modulated by the other physico-chemical parameters of the environment. A combination of different stress factors can result in intensification, overlapping or reversal of the stress effects (Osmond et al. 1987). Under natural conditions where various environmental factors normally change in unison and interact, stress conditions may elicit more pronounced effects than a single stressor in a controlled environment (Larcher et al. 1990). This is relatively well documented for the interaction between light and physico-chemical stresses such as chilling, freezing, drought or salinity (for reviews, see Kyle et al. 1987). It is believed that the reduced rate of photosynthesis in stressed leaves can result in the light absorption by the antenna pigments being in excess of that which can be dissipated by the electron-transport chain, thus creating conditions for photoinhibition to occur. Photoinhibition of photosynthesis corresponds to specific alterations in photosystem (PS) II (Powles 1984; Kyle et al. 1987), the typical symptoms being a reduction of the quantum yield of oxygen evolution and a modification of the characteristics of chlorophyll-a fluorescence (Baker and Horton 1987).

The role of light in modifying the heat stability of the photosynthetic apparatus is less clear. In isolated chloroplasts incubated at high temperatures, light has been shown to produce an injurious effect on the electron-transport chain (Ageeva 1977) and to cause a selective bleaching of photosynthetic pigments (Gounaris et al. 1983; Thomas et al. 1984). Similarly, in wheat plants, thermal injury of in-vivo photosynthesis was strongly

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Abbreviations: B_1 and B_2 = fraction of closed PSI and PSII reaction centers, respectively; ES = photoacoustically measured energy storage; F_0, F_m and F_s = initial, maximal and steady-state levels of chlorophyll fluorescence; P_{700} = reaction center of PSI; PS (I, II) = photosystem (I, II); $V = (F_s - F_0)/(F_m - F_0)$ = relative variable chlorophyll fluorescence

aggravated by bright light (Al-Khatib and Paulsen 1989). Various other examples of light stress at high temperature are documented by Ludlow (1987). In contrast, a few reports have indicated that, rather than causing injury, light can alleviate the heat-induced inhibition of leaf or chloroplast photosynthesis (Schreiber and Berry 1977; Kislyuk 1979; Weis 1981, 1982a, b). In a short fluorimetric study of intact pea leaves placed at high temperature (Havaux and Strasser 1990), we have recently confirmed this observation and demonstrated that light at high temperature offers a full protection of PSII against inactivation over a large range of irradiances, up to around 100 $W \cdot m^{-2}$. The present paper is a comprehensive extension of our preliminary work, with special emphasis on the in-vivo PSI response to high temperature. Here, the aftereffects of relatively mild heat stress (40° C) on the functioning of the photosystems were studied using a combination of several in-vivo photosynthetic techniques: measurements of PSII chlorophyll fluorescence were combined with polarographic, photoacoustic and far-red-absorbance measurements which reflect properties of PSI and-or its interaction with PSII. Our results indicate that, in contrast to PSII, PSI is robust regarding heat stress in the dark, exhibiting an increased capacity for cyclic electron transfer – a phenomenon which may represent a compensatory mechanism for the reduced PSII activity. Light at high temperature prevented the loss of oxygen evolution and variable fluorescence and reduced the stimulation of PSI cyclic transport.

Material and methods

Plant material and stress treatments. All experiment were carried out on intact leaves of pea (*Pisum sativum* L., cv. Petit Provençal) grown under controlled environmental conditions (temperature, 25° C; irradiance, around 60 $W \cdot m^{-2}$; photoperiod, 13 h; relative air humidity, 60%). Heat stress was induced in attached leaves as described in a previous paper (Havaux and Strasser 1990). Water at a constant temperature (45° C) was pumped from a Colora WK3DS thermostatted water bath (Colora Messtechnik, Lorch/Württ., Germany) into a block of Plexiglas which was placed directly in contact with the leaf sample, ensuring the maintenance of the leaf temperature, monitored with a Digi-Sense 8528–20 thermocouple thermometer (Cole-Parmer Instruments, Chicago, Ill., USA), between 39.5° C and 40° C. During heating, leaves were either kept in darkness or illuminated with 300 to 600-nm light of moderate fluence rate (30 $W \cdot m^{-2}$ unless specified otherwise) supplied by a halogen lamp (KL1500 light source; Schott, Mainz, Germany) via fiber optics.

Measurements of chlorophyll fluorescence in modulated light. In-vivo PSII-chlorophyll fluorescence emitted at 685 nm was measured at room temperature (± 22° C) using a Hansatech twin-channel modulated fluorescence-measuring system (MFMS–2; Hansatech, Kings Lynn, Norfolk, UK), the technical characteristics of which can be found elsewhere (Bolhàr-Nordenkampf et al. 1989). The experimental protocol used to measure the initial (F_0), maximal (F_m) and steady-state (F_s) levels of modulated chlorphyll fluorescence has been described at length in a previous paper (Havaux et al. 1991). The steady-state level of chlorophyll fluorescence (F_s) was measured in attached pea leaves adapted to an actinic light (300–600 nm) of 30 $W \cdot m^{-2}$. The F_0 level was obtained by simultaneously turning off the actinic light and applying a short pulse of far-red light (730 nm, 25 $W \cdot m^{-2}$). It should be kept in mind that the determination of the F_0 level with the modulated-fluorescence technique can possibly be affected by several factors such as the presence of inactive PSII reaction centers (Cao and Govindjee 1990), the reduction of some Q_A by the far-red light itself or a slow relaxation of Q_A reduction which can mask F_0 quenching. The F_m level was determined by applying an intense, photosynthetically saturating, light (300–600 nm, 500 $W \cdot m^{-2}$) pulse for 1 s. The relative variable chlorophyll fluorescence $V = (F_s - F_0)/(F_m - F_0)$ was used as a qualitative indicator of the fraction B_2 of closed PSII reaction centers. In reality, V is a hyperbolic function of B_2 (Strasser 1981):

$$V = B_2/(1 + G \cdot ((F_m - F_0)/F_0) \cdot (1 - B_2)) \quad \text{Eq. (1)}$$

where G is the probability for energy exchanges between different PSII units. The relative variable fluorescence V is equal to $1\text{-}q_P$ where q_P is the so-called photochemical quenching coefficient (Schreiber et al. 1986).

Measurements of oxygen evolution. Oxygen exchanges by leaf disks of 8 mm diameter were measured at 22° C with a Clark-type oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA) as described elsewhere (Strasser and Sironval 1972). The leaf sample was placed directly in contact with the electrode and was covered with a Plexiglas ring forming a small assimilation chamber (≈0.3 ml) which was wrapped with a Teflon membrane. The oxygen and CO_2 concentrations were equilibrated by diffusion through the Teflon membrane from the outside atmosphere to the small assimilation chamber. The CO_2 concentration in the chamber was then similar to the CO_2 concentration of the air. Photosynthetic oxygen evolution was monitored in broadband light (300–600 nm), the fluence rate of which was adjusted using neutral density filters (Schott).

Measurements of leaf absorbance changes at 820 nm. Light-induced changes in leaf absorbance at around 820 nm were monitored at room temperature with a Hansatech P_{700}^{+} measuring system employed in the reflection mode. The upper surface of the leaves was illuminated with a 820-nm light pulsed at 4.8 kHz. The lower surface of the leaves was covered with an aluminium foil acting as a reflecting surface and thus eliminating transmitted light, so that changes in leaf reflectance were directly indicative of changes in leaf absorbance. Light reflected by the leaves was monitored by a photodiode screened by a 820-nm interference filter. The signal from the detector was analyzed by an Arete 200 computer (North Star Horizon, San Diego, Cal., USA). In contrast to measurements of P_{700} oxidation in (low-fluence-rate) far-red light, irradiation with a 300 to 600-nm actinic light caused slow changes (in the order of second to minutes) in leaf absorbance at around 820 nm for reasons other than the comparatively fast P_{700} redox changes. Because of those additional changes as well as the scattering by the leaf sample of the 820-nm measuring beam, the relationship between changes in 820-nm absorbance and P_{700}^{+} concentration is complex and, consequently, it is difficult to interpret the data in strictly absolute terms. The P_{700} redox state was estimated as described by Weis and Lechtenberg (1988): steady-state illumination was interrupted and the fast absorbance decrease (ΔS) occurring in the dark was taken as representative of P_{700}^{+} spontaneous re-reduction. The maximal change in absorbance $(\Delta S)_{max}$ (corresponding to a complete oxidation of P_{700}) was obtained by applying far-red light (730 nm) of saturating irradiance (approx. 25 $W \cdot m^{-2}$). The fraction of P_{700} in the oxidized state (B_1) was calculated as follows:

$$B_1 = \Delta S/(\Delta S)_{max} \quad \text{Eq. (2)}$$

Photoacoustic measurements. The photoacoustic signals generated by small leaf discs were measured at 25° C with a custom-made photoacoustic spectrometer which has been described in a previous paper (Havaux 1989). The photothermal signals were measured with a blue-green light (300–600 nm, 16.5 $W \cdot m^{-2}$) or a far-red light (> 700 nm, 21.5 $W \cdot m^{-2}$) modulated at 367 Hz. Photochemi-

cal energy storage (ES) was estimated by comparing the amplitude of the photothermal signal in the presence (A_+) and in the absence (A_-) of a background, photosynthetically saturating, white light (520 W · m^{-2}), as in Bults et al. (1982) and Poulet et al. (1983):

$$ES = 1-(A_-/A_+) \quad \text{Eq. (3)}$$

All irradiances were measured with a YSI-Kettering 65A lightmeter (Yellow Springs Instrument Co.).

Results

Photosynthetic oxygen evolution and PSII-chlorophyll fluorescence emission. The data presented in Fig. 1 show the heat-sensitivity of photosynthesis in pea leaves and its modulation by the light environment. Intact pea leaves were pretreated at 40° C in the presence or the absence of a blue-green light of moderate irradiance (30 W · m^{-2}). After this treatment, leaf oxygen exchanges were measured at 22° C using a conventional Clark-type oxygen electrode (Fig. 1A). One can see that heat stress in the dark caused a drastic inhibition of both light-limited and -saturated rates of photosynthetic oxygen production. After around 1 h, oxygen evolution was reduced to approx. 20% of the rate measured before exposure to heat. In contrast, when the leaves were exposed to high temperature in the presence of light, no inhibition of oxygen evolution was detected, clearly showing the protective nature of light against heat injury of the linear electron transport in the chloroplasts of whole leaves.

In vivo PSII-chlorophyll fluorescence was also measured in pea leaves after heat treatment (Fig. 1B). As previously reported (Havaux and Strasser 1990), heat stress in the dark had a marked effect on PSII of pea leaves, causing a drastic loss of the maximal variable PSII-chlorophyll fluorescence (F_m–F_0) measured in the light – an effect which was due exclusively to a decrease in the height of the F_m peak since no measurable change in F_0 fluorescence level was observed. As far as fluorescence measurements in *dark-adapted* samples are concerned (data not shown), a slight increase in F_0 was noticed in heat-pretreated pea leaves. Comparison of Figs. 1A and 1B shows that the extent of the reduction in the maximal variable PSII-chlorophyll fluorescence as a function of the treatment time was very similar to that of oxygen evolution. This close correlation could indicate that the inhibition of the linear electron flow was related specifically to PSII (or vice versa).

When light and high temperature were combined, in-vivo chlorophyll fluorescence emission was not affected and maximal variable fluorescence was fully preserved. As shown in the inset of Fig. 1B, even very weak light was able to preserve variable fluorescence. This photoprotection phenomenon was saturated at around 10 W · m^{-2}.

P_{700} redox changes in far-red light. One of the problems we wanted to address was whether the in-vivo functioning of PSI exhibited the same light-dependent response to heat as PSII. To this end, absorbance measurements were performed at around 820 nm, reflecting changes in the redox state of the reaction center P_{700} of PSI (Inoue et al. 1973). Illumination of an intact leaf with far-red

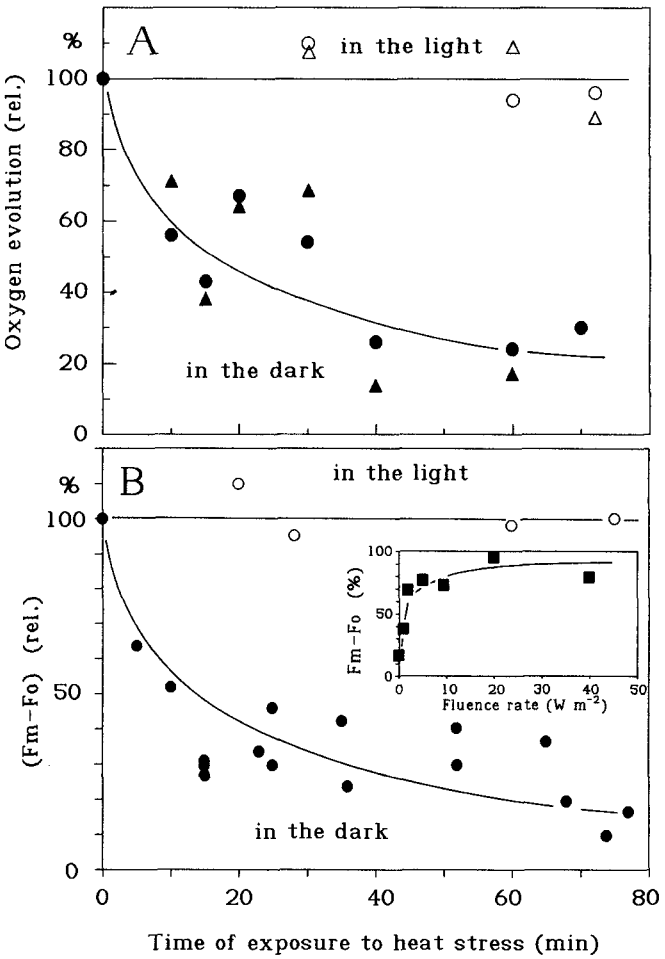

Fig. 1A, B. Photosynthetic oxygen evolution **A** and amplitude (F_m-F_0) of maximal variable chlorophyll fluorescence **B** in pea leaves pretreated for various times at a high temperature of 40 ± 0.5° C in the dark *(closed symbols)* or in the light (30 W · m^{-2}, *open symbols*). Oxygen exchanges were monitored at 22° C in leaves illuminated with a 300 to 600-nm light of saturating (500 W · m^{-2}, ○–○ and ●–●) or limiting (6 W · m^{-2}, △–△ and ▲–▲) irradiance. Modulated chlorophyll fluorescence was measured in leaves adapted for about 15 min to an actinic light of 30 W · m^{-2}. No significant change was observed in the F_0 level after heat stress in either light or darkness. *Inset:* F_m-F_0 in pea leaves pretreated at 40° C for 15 min in the presence of various fluence rates of a broadband light (300–600 nm). Variable fluorescence was measured under steady-state conditions in leaves adapted to the light used during the heat treatments (for the samples heated in the dark, fluorescence measurements were performed with an actinic light of 5 W · m^{-2}). All data are expressed as a percentage of the (F_m-F_0) or oxygen-evolution values measured in control leaves before heat treatments

light (e.g. 730 nm) absorbed by the PSI pigments results in the transformation of P_{700} into oxidized P^+_{700}, producing a measurable increase in the leaf absorbance at around 820 nm (Schreiber et al. 1988; Weis and Lechtenberg 1988; Harbinson and Hedley 1989; see also trace 1 in Fig. 3). Figure 2 shows the dependence of the steady-state amount of P^+_{700} on the fluence rate of the exciting far-red light. A typical light-saturation curve was obtained, with a linear part at low fluence rates of far-red light (inset of Fig. 2) and a plateau at fluence rates higher

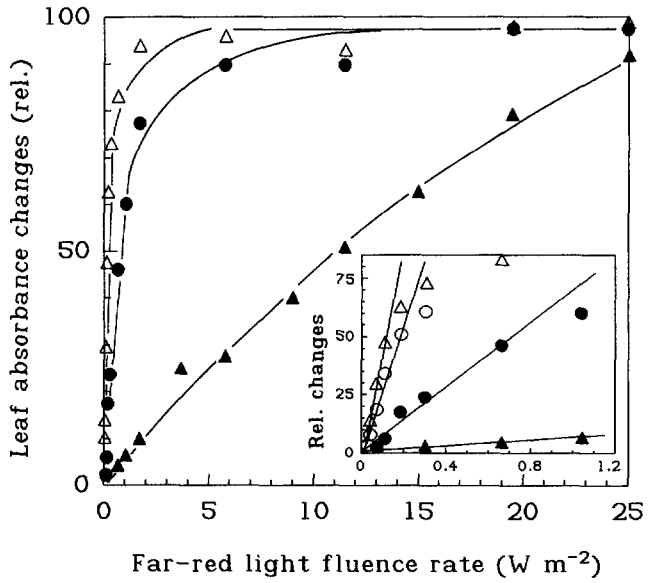

Fig. 2. Effects of heat stress (40° C for 20 min) in the presence or the absence of light (22.5 W · m^{-2}) on the changes in leaf absorbance at around 820 nm induced by different fluence rates of far-red light (730 nm). Absorbance changes at 820 nm were monitored at 25° C. Data are expressed as a percentage of the maximal absorbance decrease measured in unstressed leaves with saturating far-red light (100% corresponds to a relative absorbance change ΔS/S of 9.25 · 10^{-3}). Δ, dark-adapted control leaves; ○, light-adapted control leaves; ▲, leaves heated in the dark; ●, leaves heated in the light. *Inset:* an expansion of the low-fluence-rate region

than around 2 W · m^{-2} (in controls). At very low fluence rates (see inset), photoinduced changes in 820-nm absorbance were systematically smaller in control pea leaves previously adapted to broadband light (for 15 min) than in leaves dark-adapted for a prolonged time, although the differences were not very marked. Heat stress in the dark markedly changed the shape of the curve of absorbance changes versus irradiance: the leaf absorbance increase induced by a given fluence rate of far-red light was much smaller than in control leaves and P_{700} photooxidation became saturated at much higher irradiances. High fluence rates of around 25 W · m^{-2} resulted, however, in almost similar accumulations of P^{+}_{700} in stressed and unstressed leaves, indicating that high temperature did not appreciably alter the size of the P_{700} pool. Light at high temperature clearly reduced the changes observed in the saturation curve of P_{700} photooxidation; the saturation profile was intermediate between that of controls and leaves warmed in darkness.

Figure 3 shows the kinetics of P^{+}_{700} re-reduction in the dark upon interruption of far-red light in control pea leaves and in leaves previously heated either in the dark (panel A) or in the light (panel B). In unstressed leaves, the kinetics of P^{+}_{700} reduction in the dark were relatively slow, showing some dependency on the fluence rate of far-red light with a faster absorbance decrease at high (28 W · m^{-2}) than at low irradiance (5.8 W · m^{-2}). A striking effect of heat stress in the dark (traces 1 and 2) was a considerable acceleration of the dark reduction

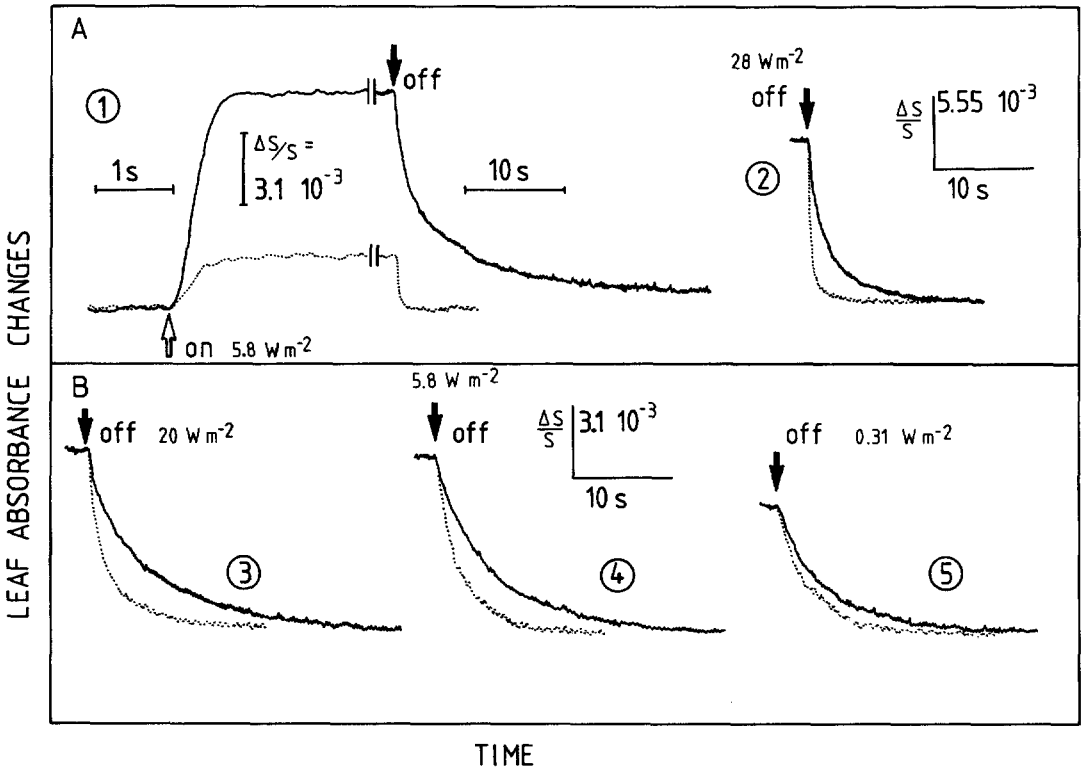

Fig. 3A, B. Kinetics of in-vivo P_{700} oxidation by far-red light and spontaneous P^{+}_{700} reduction in the dark (as reflected by leaf absorbance changes at 820 nm) in control (———) or heat-stressed (40° C for 15 min, - - - - -) pea leaves. **A** Leaves heated in the dark and dark-adapted control leaves. **B** Leaves heated in the light (30 W · m^{-2}) and light-adapted control leaves. For purposes of comparison, absorbance traces of stressed leaves shown in 2, 3, 4 and 5 were normalized to the height of the control signals

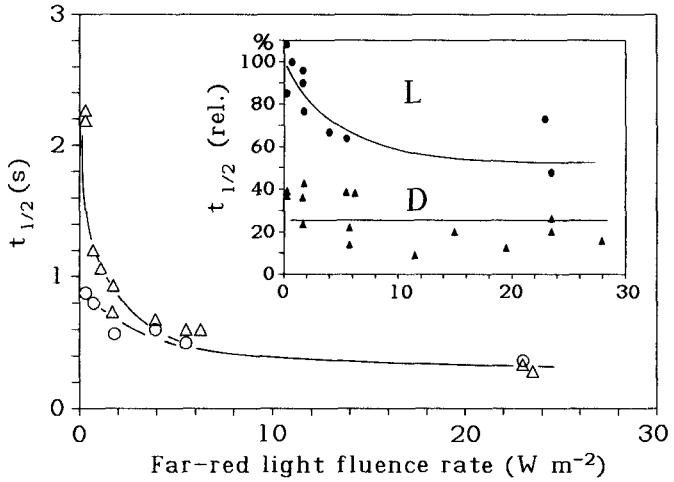

Fig. 4. Half-time ($t_{1/2}$) for the reduction of P_{700}^+ in the dark, monitored in attached pea leaves upon interruption of illumination by far-red light. Pea leaves, previously adapted to darkness (Δ) or to an actinic light of 30 W · m^{-2} for 15 min (○), were illuminated with far-red light (730 nm) of various fluence rates. Far-red light was suddenly switched off and the decrease in leaf absorbance at around 820 nm was monitored in the dark, as in Fig. 4. *Inset:* effects of heat stress (40° C for 15 min) in the dark (*D*, ▲) or in the light (*L*, ●, 30 W · m^{-2}) on the half-time $t_{1/2}$ for P_{700}^+ dark reduction, expressed as a percentage of the values measured in the corresponding, dark- or light-adapted, unstressed leaves

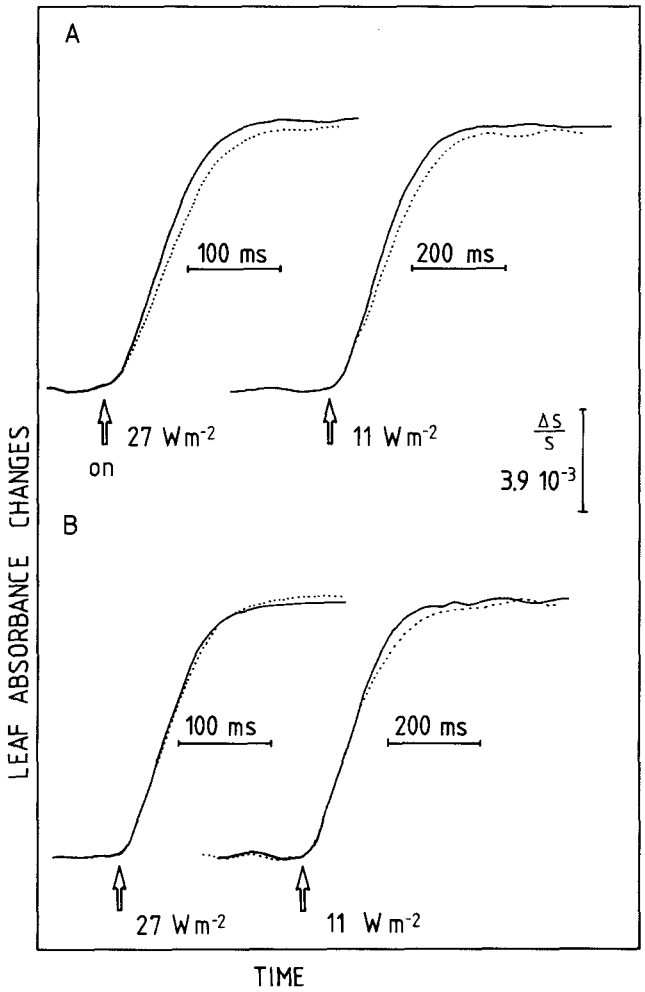

Fig. 5A, B. Kinetics of the increase in leaf absorbance at around 820 nm induced by high-irradiance far-red light in control pea leaves (———) and in leaves heated at 40° C for 15 min **A** in the dark or **B** in the light (30 W · m^{-2}) (- - - -)

of P_{700}^+. Though substantially less pronounced, the stimulation of P_{700}^+ dark reduction was also observed after heat stress in the light (traces 3–5). These effects are summarized in Fig. 4 which shows the half-time ($t_{1/2}$) of the absorbance decrease monitored after a light-to-dark transition in pea leaves illuminated with various fluence rates of far-red light. In control pea leaves kept at 25° C, $t_{1/2}$ was approx. 0.5 s at fluence rates higher than around 2 W · m^{-2}. Below this, the $t_{1/2}$ values noticeably increased with decreasing fluences of far-red light, indicating that P_{700}^+ reduction is not a first-order reaction. The dependence of the half-time on low irradiances was observed to be much stronger (with higher $t_{1/2}$ values) in control leaves which were previously dark-adapted than in those which were adapted to a blue-green light prior to leaf absorbance measurements. Possibly, the build-up of reductant pool(s) (e.g. ascorbate, glutathione, NADPH,...) during preillumination contributed to (and accelerated) P_{700}^+ reduction.

The inset of Fig. 4 shows the effects of exposing pea leaves to 40° C on the $t_{1/2}$ values. Heat stress in the dark drastically reduced $t_{1/2}$ by around 80% at all fluence rates of far-red light. After heat treatment in the light, a reduction of $t_{1/2}$ was also observed but its extent was substantially smaller as compared to samples heated in the dark.

The kinetics of the 820-nm absorbance increase induced by far-red light is a function of both P_{700} photooxidation and the simultaneous re-reduction of oxidized P_{700}^+. As this latter reaction was strongly influenced by the stress pretreatments, it was not possible to use such kinetics to directly examine stress effects on the efficiency of P_{700}^+ oxidation in PSI. However, information on P_{700} photooxidation could be obtained by measuring the initial rate of leaf absorbance increase induced by a supersaturating fluence rate of far-red light because, under such conditions, P_{700}^+ reduction will not compete significantly with the oxidation reaction. Although no clear effect of the heat treatments was observed on the initial part of the leaf-absorbance increase induced by high-irradiance far-red light (Fig. 5), it was unfortunately not possible to calculate accurately the slope of the tangent at the origin, because of an apparent lag-phase. It is difficult to say whether this lag-phase had a real physiological cause (influence of the plastoquinone PQH_2 pool?) or whether it was due to the time resolution of our instrument (determined by the speed of the shutter opening). In any case, the data presented in Fig. 5 have only a qualitative value.

P_{700} redox changes in broadband light. In contrast to far-red light, broadband light (300–600 nm) excites chlorophylls in both PSI and PSII, allowing PSII-chlorophyll fluorescence and P_{700} redox changes to be

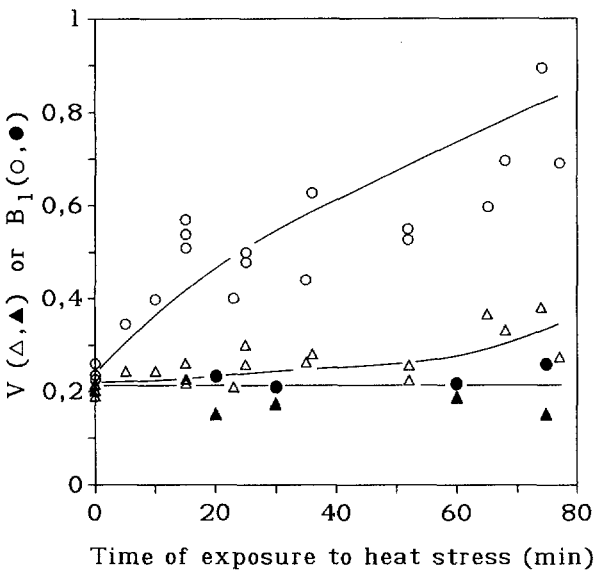

Fig. 6. Fraction (B_1) of closed PSI reaction centers *(circles)* and relative variable chlorophyll fluorescence (V) *(triangles)* as experimental approximate of B_2, the fraction of closed PSII centers, in attached pea leaves adapted to an actinic blue-green light of 30 W · m^{-2} after pretreatment at 40° C in the presence *(closed symbols)* or the absence *(open symbols)* of light (30 W · m^{-2}). Steady-state modulated fluorescence and 820-nm absorbance measurements were performed at room temperature (22° C)

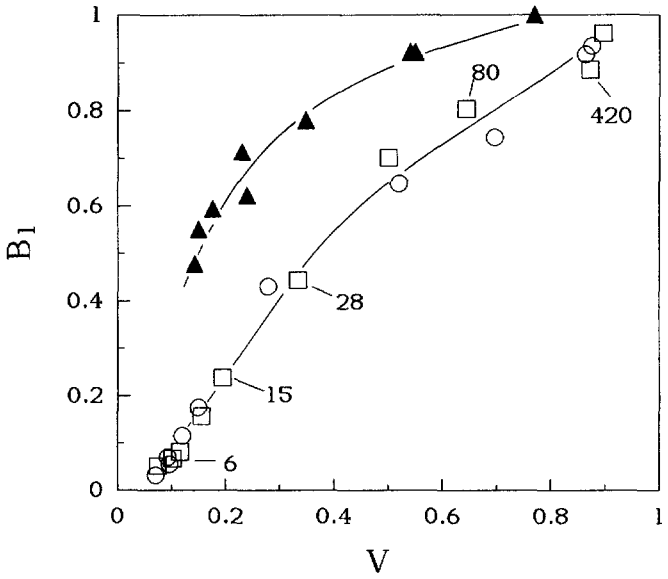

Fig. 7. Effects of heat stress (40° C for 15 min) in the dark (▲) or in the light (11 W · m^{-2}, ○) on the plot of B_1 versus V ($\approx B_2$) in pea leaves adapted to various fluence rates of an actinic blue-green light. Unstressed control leaves = □. The light fluence rates (in W · m^{-2}) used to obtain some experimental points in control samples are indicated by the numbers

measured simultaneously in leaves photosynthesizing under steady-state conditions. In Fig. 6 are shown the time courses of the changes in the fraction B_1 of closed PSI centers and in the relative variable chlorophyll fluorescence (V), which is used as an approximation of the fraction B_2 of closed PSII traps (cf. Eq. 1), in pea leaves exposed at 40° C. For the relatively low-light conditions used here (30 W · m^{-2}), both B_1 and V were close to 0.2. Heat stress in the light did not change this value. When heat stress was imposed in the dark, V remained largely unaffected, whereas B_1 was dramatically modified. The fraction of closed PSI centers increased markedly and almost linearily with increasing time of exposure to 40° C. After 80 min, almost 90% of the PSI reaction centers were closed whereas most of the PSII reaction centers remained open. Data of Fig. 7 confirm this differential behavior of PSI and PSII traps under steady illumination. Both V and B_1 were measured in the differently treated leaves illuminated with various fluence rates of the actinic blue-green light. The plot of B_1 versus V was similar in unstressed control leaves and in leaves heated in the light; an almost linear relationship was obtained, indicating a strong correlation between the redox states of PSI and PSII. Harbinson et al. (1989) have previously observed that the relationship between V and B_1 can vary considerably with the environmental conditions and, indeed, Fig. 7 shows that the good correlation between the redox states of the photosystems was lost after heating the leaves in the dark.

Photoacoustic measurements. Figure 8 shows the photoacoustically monitored photochemical energy storage (ES) in pea leaves illuminated with a 320 to 640-nm light exciting both photosystems or with a far-red light (> 700 nm) absorbed in PSI. In pea leaves illuminated with the broadband light, ES exceeded 15% of the absorbed light energy. Although the exact nature of the component(s) responsible for the energy storage monitored during photoacoustic measurements is unknown, it has been shown that ES reflects photochemical events involving both PSI and PSII (Carpentier et al. 1990) and it is believed that, under conditions where the light distribution between the two photosystems is well balanced, PSI and PSII contribute equally to the energy storage (S. Malkin, Weizmann Institute of Science, Rehovot, Israel, personal communication). Heat stress in darkness reduced rapidly and drastically the photochemical energy conversion to around 5%. Again, no effect of heat stress in the light was observed.

When monitored in far-red light, ES in control pea leaves was low (approx. 3%), as it is typically in C_3 higher plants (Herbert et al. 1990). Under PSI-exciting far-red light conditions, ES is specifically related to the photofunctioning of PSI, most probably reflecting the production of photochemical products by means of cyclic electron flow around this photosystem (Carpentier et al. 1984; Canaani et al. 1989; Herbert et al. 1990). Excitation of PSII by the longwave light used here appeared to be insignificant for several reasons: no oxygen production was photoacoustically detected in far-red light; inhibition of PSII by diuron did not reduce the energy storage in far-red light (see also Herbert et al. 1990); reduction of PSII activity in heated leaves was accompanied by an increase (and not decrease) in ES (cf. below).

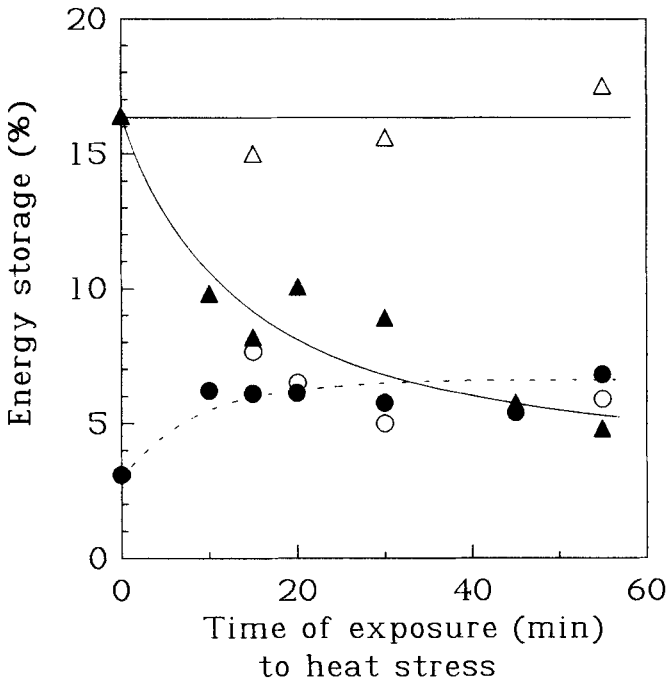

Fig. 8. Effects of a high-temperature treatment (40° C) in the dark *(closed symbols)* or in the light (30 W · m^{-2}, *open symbols*) on the photoacoustically measured extent of photochemical energy storage in pea leaves illuminated with a modulated broadband (300–600 nm, △ and ▲) or far-red (> 700 nm, ○ and ●) light

The preexistence of a substantial internal pool of active electron donors capable of supporting electron transfer in PSI can also be excluded since it was possible to measure energy storage after prolonged illumination of the sample with far-red light (several hours) which would have probably exhausted this pool completely. Figure 8 shows that both heat stress in the light and in the dark noticeably stimulated this process; indeed the energy storage increased from 3% approx. 6%.

Discussion

When imposed in darkness, relatively short and moderate heat treatments (40° C) severely impaired the in-vivo functioning of the photochemical apparatus of the chloroplasts. The presented data have shown that light strongly interacts with high temperature, greatly reducing the extent of the heat-induced damage to the photosynthetic apparatus. This interaction was observed for all the measured parameters which provide information on the properties of PSII, PSI or their interaction.

Photosystem II. Exposure of intact pea leaves to a temperature of 40° C in the dark provoked a dramatic loss of the 685-nm chlorophyll fluorescence emission above the ground level F_0 (Fig. 1B), as previously observed under different experimental conditions (Havaux and Strasser 1990). If we exclude the rather improbable case where a decrease in the rate constant for photochemistry in PSII was exactly compensated by an inverse change in the rate constant for nonphotochemical energy dissipation in the PSII-chlorophyll antennae, the quenching of F_m associated with unchanged F_0 can be interpreted from theoretical considerations (for details see Björkman 1987; Havaux and Strasser 1990) as the manifestation of an increased rate constant (k_{bH}) of thermal energy dissipation at the PSII reaction center. This k_{bH} increase will reduce the probability p_{b2} of energy transfer from the excited reaction-center pigment back to the chlorophyll antenna from which the variable PSII-chlorophyll fluorescence originates (Butler 1978):

$$p_{b2} = k_{b2}/(k_{b2} + k_{bH} + k_{bF}) \qquad \text{Eq. (4)}$$

with k_{b2} and k_{bF} being, respectively, the rate constant for this back-transfer and for radiative deexcitation of the reaction-center pigment. A practical consequence of this p_{b2} change is that in-vivo chlorophyll fluorescence measurements lose much of their predictive value for the estimation of the photochemical efficiency of PSII. Indeed, in the well known Butler's model (Kitajima and Butler 1975; Butler 1978) and all the related models, the familiar fluorescence parameter $(F_m - F_0)/F_m$ is a direct estimate of the maximal quantum yield for photochemistry in PSII (Φ_{If}^{open}) under the express assumption that $p_{b2} \approx 0$ in open centers and $p_{b2} \approx 1$ in closed centers (in other words, when $k_{b2} \gg k_{bH} + k_{bF}$) so that $(F_m - F_0)/F_m = p_{b2} \cdot \Phi_{If}^{open}$ can be approximated to Φ_{If}^{open}. As it is presumably not the case under the stress conditions used here, it is hazardous to interpret changes in $(F_m - F_0)/F_m$ only in terms of changes in quantum yield and, consequently, our discussion of the fluorescence data will be limited to the analysis of the relative variable fluorescence, V, which compares the relative heights of the minimal, maximal and steady-state fluorescence levels and provides qualitative information on B_2 (Eq. 1).

As the proton and metal-cation concentrations in the intrathylakoid space have been recognized to play an important role in the heat tolerance of the chloroplasts (Weis 1982a, b), it is conceivable that photoprotection of PSII operated through the light-induced intrathylakoid acidification and related cation exchanges which somehow stabilized the thylakoid membrane and maintained the PSII reaction center in its normal conformation, preventing the stimulation of its thermal deactivation. The saturation curve of the photoprotection (inset of Fig. 1B) is compatible with this suggestion since saturation of the proton gradient is known to occur at low irradiance (Heldt et al. 1973). An alternative explanation can however be proposed, considering the high heat-sensitivity of ribulose-1,5-biphosphate carboxylase. It has been shown that, after mild heat stress, light-activation of the carboxylase enzyme and consequently the overall photosynthetic fixation of CO_2 are markedly inhibited – a phenomenon which can also be prevented, at least partially, by the presence of light during the treatment (Weis 1981, 1982a). It is then possible that the above k_{bH} change could actually be a regulatory response of PSII to the reduced Benson-Calvin cycle activity, perhaps avoiding potentially harmful overexcitation of the PSII reaction center. In this case, the differential behavior of the two photosystems is an intriguing phenomenon indicating the

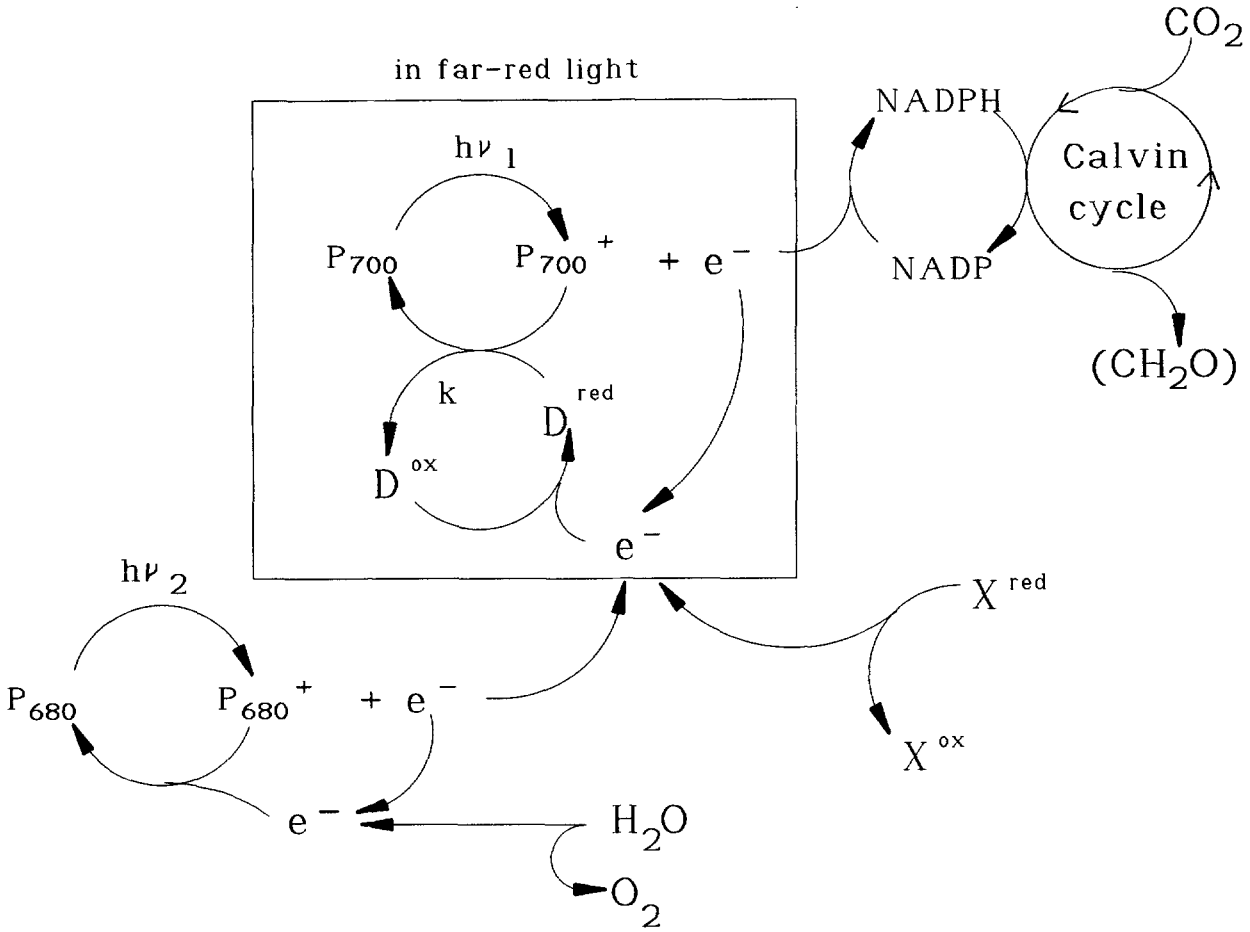

Fig. 9. Hypothetical scheme of the electron pathways involved in the reversible photooxidation/dark reduction of the PSI reaction center (P_{700}) in leaves. D^{red} represents a reductant or a group of reductants. k is the rate constant for the P_{700}^+ reduction. P_{680} is the reaction center of PSII. X^{red} represents a pool of reductants not involved in the PSI-cyclic electron flow. Seemingly, there is appreciable electron donation by X^{red} in leaves preilluminated before the leaf absorbance measurements (see text)

existence of a complex control system affecting differently the activities of PSI and PSII. How a reduced CO_2-fixation activity can modulate the thermal deactivation of the PSII traps remains, however, an open question.

Photosystem I. Based on in-vitro studies, PSI is generally considered to be more tolerant to heat injury than PSII (Berry and Björkman 1980; Quinn and Williams 1985). The present work confirms that this conclusion is valid in vivo too.

Under far-red light conditions, photochemical energy storage implies the cycling of electrons around PSI (Carpentier et al. 1984; Canaani et al. 1989; Herbert et al. 1990). It has been shown in various plant species that ES in far-red light is insensitive to the herbicide diuron but is abolished by an inhibitor of plastoquinol oxidation (Canaani et al. 1989; Herbert et al. 1990), indicating the involvement of this latter electron carrier in the cycle around PSI. It was also observed that this energy storage is partially inhibited by CCCP (carbonylcyanide *m*-chlorophenylhydrazone), an inhibitor of photophosphorylation (Herbert et al. 1990). The inhibitory effect of CCCP indicates that the bulk of the energy storage by PSI-cyclic electron flow represents photophosphorylation of ADP – an idea which is compatible with the supposed lifetime in the millisecond range of the measured photochemical products (Malkin and Cahen 1979). An interesting finding was that heat stress did not inhibit the photoacoustically monitored photochemical energy conversion in PSI (Fig. 8). In fact, it was even stimulated in heated leaves, indicating an increased capacity of pea leaves for PSI-driven, energy-storing electron flow. This observation is in agreement with previous in-vitro studies showing higher rates of electron transport mediated by PSI in isolated chloroplasts incubated at high temperature (Katoh and San Pietro 1967; Armond et al. 1978; Thomas et al. 1984). Possibly, this heat-induced stimulation of cyclic electron transport through PSI could be an adaptive process, producing ATP under conditions when the PSII activity was severely diminished. This ATP synthesis could be important for the survival of the plant and necessary for repair of stress-damaged processes, as suggested by Canaani et al. (1989).

When one analyzes the dark re-reduction of P_{700}^+ oxidized by far-red light, it is also necessary to consider

cyclic pathways in PSI. The simplest scheme for this electron cycling is shown in Fig. 9: the reductant(s) D^{red} provide(s) electrons for the reduction of P_{700}^+ and oxidized D^{ox} is reduced by electrons derived from the light-induced transformation of P_{700} into P_{700}^+, possibly via several electron carriers. The complete reversibility in the dark of the P_{700} photooxidation excludes the possibility of a substantial "loss" of electrons to the Benson-Calvin cycle. It is clear that there are several pools of reductants in the chloroplasts (denoted X^{red} in Fig. 9) that could possibly reduce P_{700}^+ independently of the above electron cycle, e.g. ascorbate, glutathione, NADPH. In dark-adapted pea leaves, the influence of those pools appeared to be negligible, as succesive P_{700} reduction-oxidation by repetitively switching on and off the far-red light (a procedure which should exhaust those pools) can be performed virtually indefinitely without any apparent effect. Another argument supporting this idea is given below. By contrast, in leaves pre-illuminated with actinic blue-green light before the absorbance measurements, it seems that electron-donor pools built-up during the light phase significantly competed with the cyclic pathway, accelerating the P_{700}^+ reduction (see below).

The data of Figs. 3 and 4 indicated that the reduction of P_{700}^+ by the putative cyclic system operating in far-red light is slow compared with the in-vivo P_{700}^+ decay monitored upon interruption of radiation exciting both photosystems; this decay is caused by electrons derived from PSII via plastocyanin (Haehnel 1984) and has a $t_{1/2}$ of a few milliseconds (Harbinson and Hedley 1984). Dark reduction of P_{700}^+ accumulated in far-red light was, however, considerably accelerated in leaves which were previously heated (Figs. 3, 4), indicating a heat effect on the PSI environment which stimulated the reaction between P_{700}^+ and D^{red}. This could be the consequence of a change in the rate constant of the reaction k and-or the size of the electron-donor pool ($[D^{red}]$). Qualitative information on both aspects can be obtained from a knowledge of the half-time of P_{700}^+ reduction ($t_{1/2}$) (see *Appendix*). Using the data of Fig. 4 and Eqs. 3A and 4A, we have calculated at time 0 (when the far-red light was turned off) (i) the product $k \cdot [D^{red}]_0$ and (ii) the normalized size of the electron donor pool $[D^{red}]_0/[D^{red}]_0^{sat}$ for the stressed and unstressed pea leaves (Figs. 10A, B). Figure 10A shows that heat pretreatment increased the $k \cdot [D^{red}]_0$ values, with a more pronounced effect after heat stress in the dark (Fig. 10A). It is very likely that this heat effect was due to a change in k rather than in the electron-donor pool size since no difference was seen in the fluence-rate dependence of the relative pool size between heated leaves and their respective controls (Fig. 10B). In addition, for the leaves kept in the dark (at 25 or 40° C), the extrapolation of the plots of pool size versus I to the ordinate (inset of Fig. 10B) gave a value close to 0, indicating that there were no active electron donors before far-red light illumination. Consequently, we can consider that, in this case, all the electrons available for the P_{700}^+ reduction originally came from the photooxidation of P_{700} and therefore $[D^{red}]_0 = [P_{700}^+]$ in the steady state. As the steady-state level of P_{700}^+ was diminished by the dark exposure at 40° C (Fig. 2), the

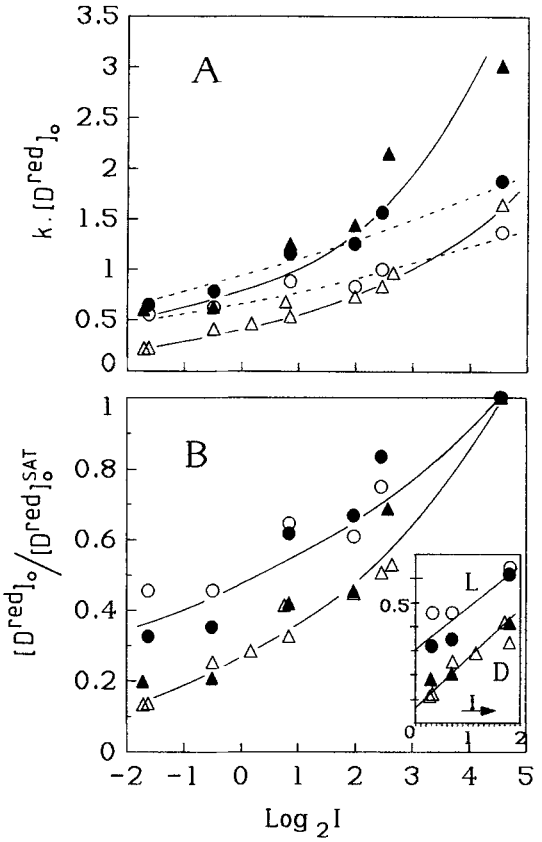

Fig. 10A, B. Semi-log plot of **A** the product $k \cdot [D^{red}]_0$ and **B** the relative size of the electron-donor pool $[D^{red}]_0/[D^{red}]_0^{sat}$ versus the irradiance of far-red light I (in $W \cdot m^{-2}$) in control and heat-stressed (40° C for 15 min) pea leaves. Data were calculated from the $t_{1/2}$ values of Fig. 4 according to the equations given in the *Appendix*. Log to the base 2 was used because the irradiances of far-red light used in this experiment differed by a factor of around 2. △, control dark-adapted leaves; ○, control light-adapted leaves; ▲, leaves heated in the dark; ●, leaves heated in the light. *Inset:* the relative electron-donor pool size versus I ($W \cdot m^{-2}$) for dark-adapted controls or leaves heated in the dark (*D*) and light-adapted leaves or leaves heated in the light (*L*)

increased magnitude of $k \cdot [D^{red}]_0$ necessarily implies a marked increase in the rate constant k. In contrast, the extrapolated value of the relative D^{red} pool size (to $I=0$) was around 0.3 in (control or heated) leaves preilluminated with the blue-green light (inset of Fig. 10B). Thus, in those leaves, there were additional electron-donor pools built-up during preillumination (possibly, ascorbate, glutathione,...) which were probably responsible for the slight changes in P_{700}^+ decay rate and level observed in preilluminated control samples as compared to dark-adapted controls (Figs. 3, 4).

In the light of the photoacoustic data of Fig. 8 and the absorbance curves presented in Fig. 5, it seems likely that P_{700}^+ oxidation in far-red light was not significantly affected by warming the leaves. In this context, it has been reported (Rumberg 1964) that the PSI reaction center in vitro is stable up to relatively high temperatures (at least 60° C). Therefore, the modification of the steady-state levels of P_{700}^+ (Fig. 2) can be explained only in terms of changes in the turnover rate of P_{700}.

Interplay between PSI and PSII. Heat treatments in the dark caused a progressive steady-state closure of the PSI reaction centers in leaves illuminated with a blue-green light, with no similar changes in PSII (Figs. 6, 7). The degree of "opening" $(1-B_1)$ of the PSI reaction centers is related to the photochemical efficiency of PSI (Φ_1) as follows:

$$\Phi_1 = (1-B_1)E_{1a}/J_1 \qquad \text{Eq. (5)}$$

with

$$E_{1a} = p_{1a}E_1 \qquad \text{Eq. (6)}$$

where J_1 is the light absorption in PSI and E_{1a} is the energy flux from the absorbing PSI pigments to the PSI reaction centers, p_{1a} is the probability of this latter energy transfer and E_1 is the total excitation rate in the PSI-chlorophyll antennae (essentially, J_1 and spillover of energy from PSII to PSI). The probability p_{1a} can be expressed in terms of rate constants (k_{1i}) as follows:

$$p_{1a} = k_{1a}/\sum_i k_{1i} \qquad \text{Eq. (7)}$$

Consequently, combination of Eqs. 5, 6 and 7 indicates that it is only when there is no change in the rate constants of energy dissipation in PSI (E_{1a}/J_1 = constant) that the fraction of open PSI centers $(1-B_1)$ is directly proportional to Φ_1. It is clear that this condition is not necessary fullfilled in stressed leaves. Nevertheless, from a *qualitative* point of view, the closure of the PSI centers during heat stress in the dark could be interpreted as the adjustment of the PSI photochemical efficiency to the decreased rate of photochemistry in PSII. Interestingly, a relationship between B_1 and V similar to that observed during heat stress in the dark was measured after a photoinhibitory treatment affecting specifically PSII (Havaux and Eyletters 1991).

Overall, it can be concluded that short exposures of intact pea leaves to high temperature affected both PSI and PSII. In many respects, the response of PSI towards heat appeared to be the inverse of that of PSII: elevated temperatures caused profound inhibitory changes in PSII whereas PSI functioning was stimulated in heat-treated leaves. Our data do not allow us to decide if damage of PSII was responsible for the loss of photosynthetic activity or, on the contrary, if damage located in the dark reactions of photosynthesis resulted in a down-regulation of PSII. Further studies will have to determine whether or not the above-mentioned changes represent an adaptive mechanism by which proton pumping and ATP synthesis are partially maintained via PSI cyclic flow, and deactivated PSII is protected against potential overexcitation associated with reduced Benson-Calvin cycle activity. The heat-induced effects on the functioning of PSII were completely suppressed when leaves were illuminated during heating, indicating that a low to moderate light environment plays an ecologically important role of stabilizer. In contrast to the changes in the variable PSII-chlorophyll fluorescence, the heat-induced stimulation of PSI activity was only partially reversed by light, indicating that the two sets of phenomena do not reflect a common perturbation of thylakoid membrane organisation, in contrast to previous suggestion (Thomas et al. 1984).

We wish to thank Professor R. Lannoye (ULB, Brussels) for the use of this photoacoustic spectrometer and Mrs. M. Eyletters for her help.

Appendix

If we consider the dark reduction of P_{700}^+ accumulated in leaves illuminated with far-red light as a second-order reaction

$$P_{700}^+ + D^{red} \rightarrow P_{700} + D^{ox}$$

the rate of P_{700}^+ decay at time 0 (when the far-red light is switched off) is given by the following expression:

$$-(d\,P_{700}^+/dt)_0 = k[P_{700}^+]_0[D^{red}]_0 \qquad \text{Eq. (1A)}$$

where k is the rate constant of the reaction and $[P_{700}^+]_0$ and $[D^{red}]_0$ are the concentrations (at time 0) of P_{700}^+ and reduced electron donors D^{red}, respectively (see Fig. 9). In first approximation, we can write

$$d\,P_{700}^+/dt = ([P_{700}^+]_{t1/2} - [P_{700}^+]_0)/t_{1/2} \qquad \text{Eq. (2A)}$$

where $[P_{700}^+]_{t1/2}$ is the P_{700}^+ concentration at time $t_{1/2}$ (which is equal per definitio to 1/2 $[P_{700}^+]_0$).
The combination of Eqs. 1A and 2A gives

$$k \cdot [D^{red}]_0 = 1/(2t_{1/2}) \qquad \text{Eq. (3A)}$$

This expression allows changes in k and-or $[D^{red}]_0$ to be detected. Comparing expression 3A calculated for a given irradiance of far-red light I with that corresponding to the saturating irradiance I_{sat}, we have

$$[D^{red}]_0/[D^{red}]_0^{sat} = (t_{1/2})^{sat}/(t_{1/2}) \qquad \text{Eq. (4A)}$$

Eq. 4A allows the determination of the fraction of the maximal D^{red} pool built-up by a given value of I.

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