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Stress Tolerance of Photosystem II in Vivo

Antagonistic Effects of Water, Heat, and Photoinhibition Stresses

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ABSTRACT

The in vivo photochemical activity of photosystem II was inferred from modulated chlorophyll fluorescence and photoacoustic measurements in intact leaves of several plant species (*Lycopersicon esculentum* Mill., *Solanum tuberosum* L., *Solanum nigrum* L.) exposed to various environmental stresses (drought, heat, strong light) applied separately or in combination. Photosystem II was shown to be highly drought-resistant: even a drastic desiccation in air of detached leaf samples only marginally affected the quantum yield for photochemistry in photosystem II. However, water stress markedly modified the responses of photosystem II to superimposed constraints. The stability of photosystem II to heat was observed to increase strongly in leaves exposed to water stress conditions: heat treatments (e.g. 42°C in the dark), which caused a complete and irreversible inhibition of photosystem II in well-watered (tomato) leaves, resulted in a small and fully reversible reduction of the photochemical efficiency of photosystem II in drought-stressed leaves. In vivo photoacoustic data indicated that photosystem I was highly resistant to both heat and water stresses. When leaves were illuminated with intense white light at 25°C, photoinhibition damage of photosystem II was more pronounced in water-stressed leaves than in undesiccated controls. However, in nondehydrated leaves, photoinhibition of photosystem II was strongly temperature dependent, being drastically stimulated at high temperatures above 38 to 40°C. As a consequence, when exposed to strong light at high temperature, photosystem II photochemistry was significantly less inhibited in dehydrated leaves than in control well-hydrated leaves. Our results demonstrate the existence of a marked antagonism between physicochemical stresses, with water stress enhancing the resistance of photosystem II to constraints (heat, strong light at high temperature) that are usually associated with drought in the field.

Leaf photosynthesis is abolished easily by elevated temperatures. Within the photosynthetic apparatus, PSII seems to be the most heat-sensitive function, whereas PSI activity, stromal enzymes, or chloroplast envelope are comparatively much more thermostable (3, 5, 12, 18, 29). It is believed that increasing temperature leads first to a blockage of PSII reaction centers and then to a dissociation of antennae pigment-protein complexes from the central core of the PSII light-harvesting apparatus (2, 3, 10). Gounaris et al. (10) have suggested that this dissociation event may be related to the phase separation of non-bilayer-forming lipids in the thylakoid membranes.

For a given plant material, the thermolability of PSII has been reported to vary substantially due to influence from various environmental factors. For instance, light has been shown to markedly reduce damage to PSII during heat stress depending on its intensity and spectral characteristics (12, 31, 36). Increased thermostability of PSII has been observed in leaves exposed to physicochemical stresses such as high salinity (21) or hypertonic stress (17). It was also reported that changes in the leaf water potential and osmotic potential influence the thermal tolerance of photosynthesis (14, 32). Observations of this nature suggest the existence of antagonistic interactions between environmental stresses, with one constraint enhancing the tolerance of photosynthesis toward another, superimposed constraint. With regard to temperature stress, this is clearly very important from an ecophysiological viewpoint because under natural conditions, heat stress is often combined with other constraints such as water deficit and strong light. For this reason, we examined the effects of multiple-stress conditions on PSII. To this end, modulated fluorometry and photoacoustic spectroscopy were used to monitor in situ the PSII photofunctioning in intact leaves of various plant species (*Lycopersicon esculentum, Solanum* sp.) exposed to a combination of water stress and heat stress in the dark or in strong light. Here we show that although PSII photochemistry remains virtually unchanged in water-stressed leaves, leaf desiccation considerably enhances the resistance of PSII to high-temperature stress in the dark and (to a lesser extent) in bright light.

MATERIALS AND METHODS

Plant Material and Stress Treatments

All the experiments were performed on mature leaves of tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), and *Solanum nigrum* L. plants grown in a glasshouse (night/day temperature approximately 15–25°C) under natural sunlight conditions. Rapid water stress was imposed on detached leaves as described in ref. 11: leaf samples were placed on filter paper in an open Petri dish and dehydrated in air of about 30% RH. This treatment was done in the dark at room temperature (24 ± 2°C); control leaf samples were kept on moist filter paper under the same conditions. The

resulting water stress was characterized by determining leaf water potential and/or RWC.[1] RWC was measured according to the ratio (fresh weight − dry weight)/(water-saturated weight − dry weight), as previously described (11). Ψ was determined in leaf disks of 5.5 mm diameter placed in Wescor thermocouple psychrometers connected to a Wescor HR33 Dew point microvoltmeter. This rapid leaf dehydration treatment was compared with slow water stress induced by withholding irrigation to the plants; control plants were watered daily.

Heat stress was induced in intact (detached) leaves as described in ref. 12. Water was pumped from a thermostatted water bath (LKB 2219 Multitemp II) into a block of plexiglass that was placed directly in contact with the lower side of the leaf. Leaf temperature, monitored by a thermocouple thermometer (YSI-tele thermometer + YSI 427 probe) stuck on the upper leaf side, was increased at a rate of approximately 1°C min^{-1}. Heat treatments were done in the dark. No difference was observed in the rate of temperature increase between water-stressed and -unstressed leaves.

Chl Fluorescence Measurements

Information on the functioning of PSII was derived from measurements of in vivo variable PSII-Chl fluorescence. Chl fluorescence emission from the upper surface of the leaves was measured with a pulse amplitude modulation fluorometer (PAM 101–103, H. Walz, Effeltrich, FRG). F_o, F_m, and F_s levels of modulated Chl fluorescence were determined in dark- or light-adapted leaves as described in ref. 13. F_o was excited with a dim, nonactinic, 650-nm light beam modulated at 1.6 kHz. Variable fluorescence was induced by a white actinic light provided by a Schott KL1500 light source. The fluence rate of this light was adjusted by neutral density filters. F_m was induced by a 800-ms pulse of intense white light (about 1500 W m^{-2}). In light-adapted leaves, the F_o level was obtained by simultaneously switching off the actinic light and applying a pulse of saturating (for PSI) far red light (740 nm). The maximal photochemical efficiency, ϕ_P^{open}, of PSII in dark- or light-adapted leaves was estimated by the fluorescence ratio $(F_m - F_o)/F_m$; ϕ_P^{open} is the photochemical efficiency of PSII when all the PSII reaction centers are (or would be) in the open (active) configuration. The actual quantum yield of PSII photochemistry, ϕ_P, in the light-adapted state was calculated by the $(F_m - F_s)/F_m$ ratio. ϕ_P is the photochemical efficiency of PSII when a fraction of reaction centers are open, the other traps being in the closed (inactive) configuration with the primary electron acceptor Q_A in the reduced state. A Li-Cor radiometer (LI-185A) was used to measure light fluence rates.

[1] Abbreviations: RWC, relative water content; Ψ, water potential; ϕ_P, ϕ_P^{open}, actual and maximal quantum yield of photochemistry in PSII, respectively; PES, photoacoustically monitored photochemical energy storage; T_c, critical temperature for heat-induced fluorescence rise; T_P, temperature of heat-induced peak fluorescence; F_o, F_m, F_s, initial, maximal, and steady-state levels of Chl fluorescence, respectively; Q_A, primary electron acceptor of PSII.

Photoacoustic Measurements

The photoacoustic signals generated by small leaf discs (diameter, 1 cm) at 25°C were measured with a custom-made photoacoustic spectrometer that has been described (12). The photothermal signals were measured with a broadband light (320–640 nm, 30 W m^{-2}) or a far red light (>715 nm, approximately 20 W m^{-2}) modulated at 381 Hz. PES was estimated by comparing the amplitude of the photothermal signal measured in the presence (A_+) and in the absence (A_-) of a background, photosynthetically saturating, white light (approximately 500 W m^{-2}) as in (12, 15): PES = $1 - (A_-/A_+)$.

RESULTS

Rapid Water Stress

Detached tomato leaves were subjected to dehydration in air for several hours, resulting in a rapid and pronounced decrease in both RWC and Ψ of the samples (Fig. 1). For instance, after 24 h of dehydration in the dark, the leaf RWC fell to less than 50% and Ψ reached values between −20 and −30 bars. The effects of this treatment on the PSII function were monitored in vivo by measuring the characteristics of variable Chl fluorescence emission.

Although the imposed water stress was quite severe, the maximal photochemical efficiency of PSII (ϕ_P^{open}, calculated from the F_o and F_m fluorescence levels; $\phi_P^{open} = [F_m - F_o]/F_m$) was only marginally affected: ϕ_P^{open} remained virtually unchanged during the first 24 h of dehydration and slightly decreased for longer treatment times (Fig. 2A). For example, a drastic desiccation treatment of 3 d, which resulted in RWC <40% and Ψ <−40 bars, caused a small decrease of only −15% in ϕ_P^{open}, suggesting a high tolerance of PSII toward water stress. This robustness of PSII is also shown by the experiments presented in Figure 2B, where the actual quantum yield ϕ_P for photochemistry in PSII (=$[F_m - F_s]/F_m$) was measured in control and dehydrated (for 24 h in darkness) tomato leaves photosynthesizing under steady-state conditions at different fluence rates of a white actinic light. Clearly, over the whole range of light irradiances examined, there was no significant difference in ϕ_P between water-stressed and unstressed leaves.

Heat Stress

Schreiber and Berry (31) discovered that a slow elevation of leaf temperature (at a rate of around 1°C min^{-1}) brings about a progressive increase in the Chl *a* fluorescence intensity under low excitation light, reaching a peak at a temperature presumably corresponding to a complete destruction of PSII activity. Later studies have demonstrated that T_P and T_C are direct indexes of the chloroplast thermostability (3, 5) and can be used to estimate the relative heat tolerance of plants (6, 32).

Figure 3 shows examples of such fluorescence thermographs recorded in tomato leaves dehydrated for various times. It can be seen that water stress caused a marked shift in both T_C and T_P values toward higher temperatures; T_P was around 46°C in well-hydrated control leaves and increased

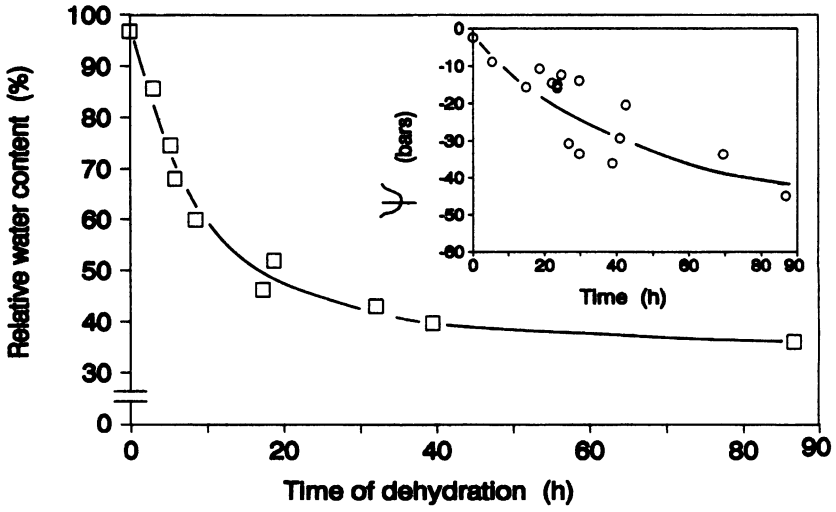

Figure 1. Time course of the changes in RWC and Ψ (inset) of tomato leaves submitted to rapid dehydration in air.

to 48.5, 50.0, and 52.5°C in leaves dehydrated for 3.5, 7.5, and 24 h, respectively. Increased T_P (and T_C) values indicate that water stress was associated with enhanced heat tolerance of photosynthesis in tomato leaves. A similar effect was observed in other plant species (data not shown), including wheat and potato (see below).

The heat-induced fluorescence rise probed with a low excitation light has previously been interpreted as the result of a physical dissociation between the light-harvesting Chl-protein complexes and the PSII reaction centers (2, 3). Recent works have, however, questioned this interpretation, suggesting that the Chl fluorescence increase in heated leaves is associated with a shift in the redox state of Q_A (7). The observation (inset of Fig. 3) that illumination of the leaf samples with far red background light during heating significantly reduced the amplitude of the fluorescence rise monitored with a weak modulated light confirmed that part of the observed fluorescence changes are indeed due to Q_A reduction.

For this reason, in all the experiments presented below, determination of the true F_o was done in the presence of far red light in order to reoxidize the acceptors reduced during heat stress in darkness. Using short pulses of intense light, the F_m was also monitored during heat treatment in the dark, thus allowing the ϕ_P^{open} to be determined from the $(F_m - F_o)/F_m$ fluorescence ratio (Fig. 4).

It was observed that photochemistry sharply decreased above a 38°C threshold temperature in well-hydrated leaves. Interestingly, this temperature appeared to correspond to the T_C values previously measured in the fluorescence-temperature plots of Figure 3. Clearly, water stress shifted the threshold temperature toward much higher values (around 45°C in leaves previously dehydrated for 6.5 h—a temperature that resulted in a complete inactivation of PSII in control leaves), thus confirming the stabilization of PSII to high temperature by water-stress conditions. It can also be seen that slowly increasing temperature of control tomato leaves to 42°C caused a 40% inhibition of PSII photochemistry, and maintenance of the leaves at this high temperature for around 15 min resulted in a complete destruction of PSII activity

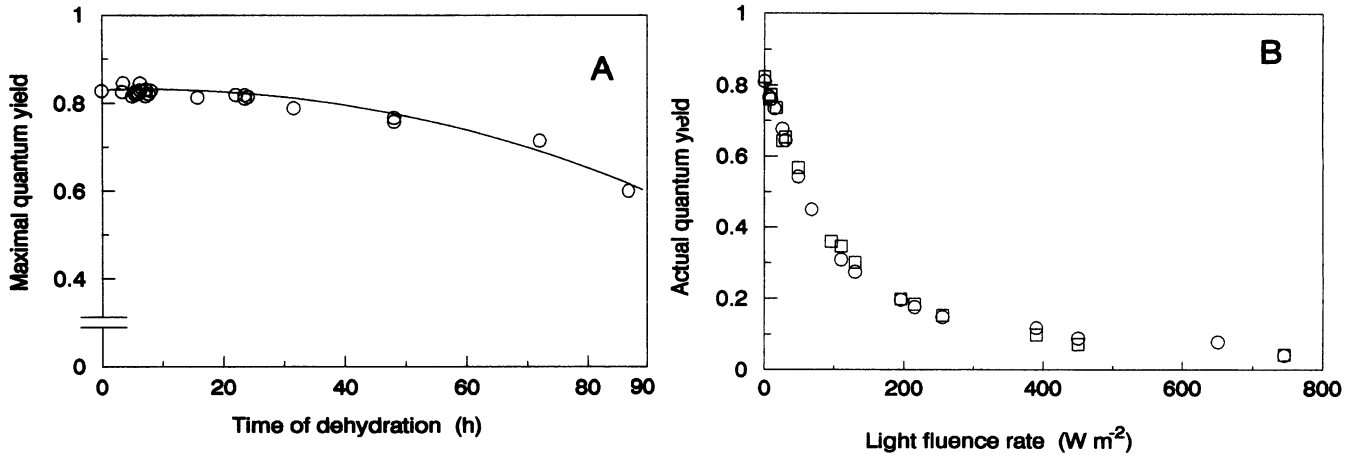

Figure 2. A, ϕ_P^{open} (as estimated by the Chl fluorescence ratio $[F_m - F_o]/F_m$) in dark-adapted tomato leaves during dehydration stress. B, ϕ_P (as estimated by the Chl fluorescence ratio $[F_m - F_s]/F_m$) in tomato leaves adapted (for 5–10 min) to various fluence rates of white actinic light after dehydration for 0 (□) or 24 h (○).

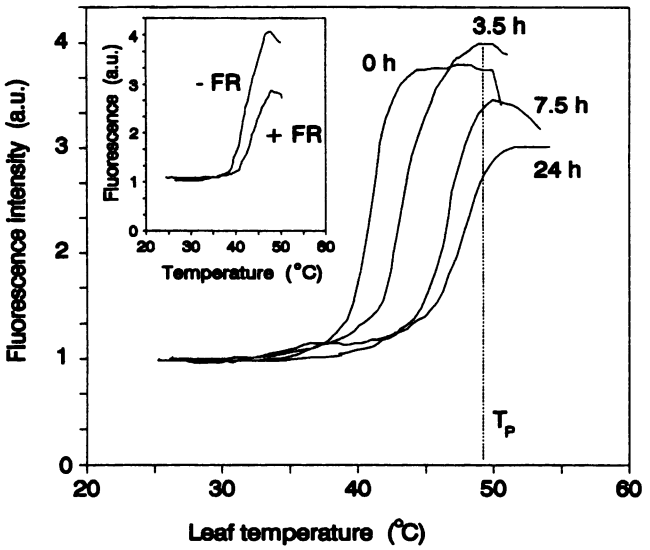

Figure 3. Temperature dependence of the Chl fluorescence intensity (arbitrary units) in tomato leaves dehydrated for 0, 3.5, 7.5, or 24 h. Leaves were placed first at 25°C and the F_o level was excited at this temperature by a weak modulated light beam. The leaf temperature was then slowly increased at a rate of approximately 1°C min^{-1} and the modulated fluorescence emission was monitored during heating. Inset, Fluorescence-temperature plot of control tomato leaves illuminated (+FR) or not (−FR) with far red background light (740 nm).

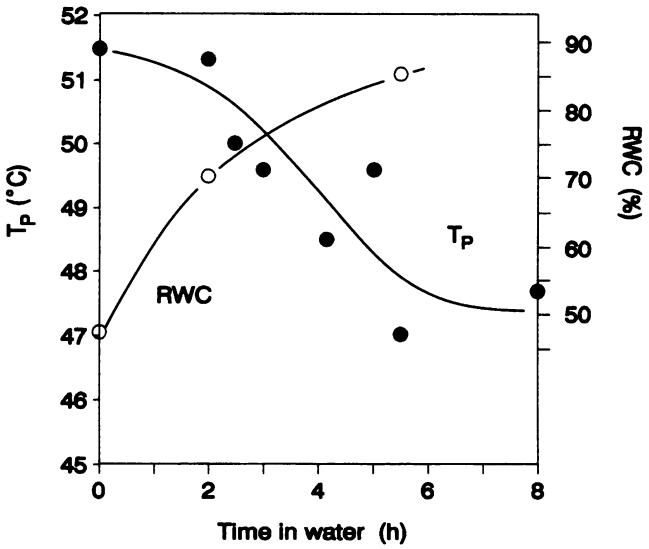

Figure 5. Time courses of the changes in PSII thermotolerance (as indicated by the peak temperature T_P of the fluorescence-temperature plot, see Figure 3) and RWC of dehydrated tomato leaves (for 16 h in the dark) upon transfer to water.

(inset of Fig. 4); this inhibition was not reversible at 25°C (data not shown). In contrast, water-stressed leaves placed at 42°C exhibited a much smaller decrease (−15%) in the photochemical efficiency of PSII (Fig. 4), which was observed to be time-independent (inset) and fully reversible upon return to more favorable temperature conditions (not shown).

Figure 5 shows that the PSII thermotolerance acquired during leaf dehydration is reversible upon transfer of the leaf samples into water. Leaves were dehydrated for 16 h, resulting in an increase in T_P of around 5°C. This increase in PSII thermotolerance was observed to be completely lost after 5 h in water.

The photochemical activity of leaves exposed to water stress and/or heat stress was also examined with the photoacoustic technique. The amplitude of the photoacoustic signals generated by leaf discs illuminated with a light pulsed at a high frequency (e.g. 381 Hz) is directly proportional to the fraction of absorbed light energy dissipated as heat. Thus, neglecting the low-yield Chl fluorescence emission, the energy stored in intermediates of the photochemical processes can be estimated by comparing the photothermal signal amplitude with the maximal photothermal signal obtained by applying an additional strong (photosynthetically saturat-

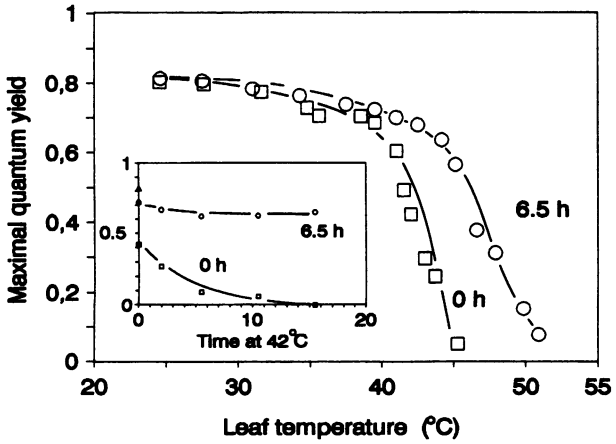

Figure 4. ϕ_P^{open} in dark-adapted tomato leaves (dehydrated for 0 or 6.5 h) during slow heating at an approximate rate of 1°C min^{-1}, as explained in the legend of Figure 3. Inset, changes in ϕ_P^{open} in leaves maintained at 42°C.

Table I. *PES in Blue-Green and Far Red Light of Solanum nigrum Leaves Exposed to Heat (50°C for 16 min in the Dark) and/or Water Stress (Leaf Desiccation in Air for 6 or 17 h; RWC: 58 and 45%, Respectively)*

Data are mean values ± SD.

	PES	
	Blue-green light	Far red light
	%	
Control	11.6 ± 2.1	4.1 ± 1.6
Heat stress	2.6 ± 0.9	6.0 ± 1.8
Water stress		
6 h	11.9 ± 0.7	4.9 ± 2.5
17 h	9.6 ± 1.1	3.9 ± 0.4
Water stress + heat stress		
6 h	8.3 ± 0.1	4.3
17 h	6.5 ± 1.6	4.1 ± 0.7

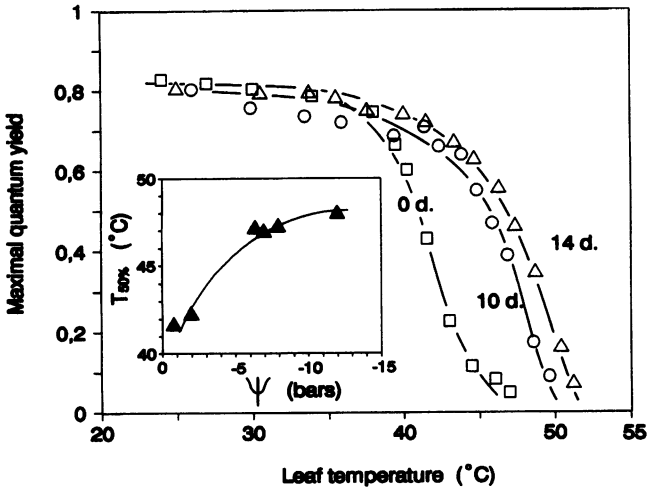

Figure 6. Temperature dependence of the ϕ_P^{open} of PSII in potato leaves exposed to slow water stress induced by withholding irrigation to the plants for 0, 10, and 14 d (Ψ = −2, −10, and −12 bars, respectively). Inset, Plot of the temperature ($T_{50\%}$) corresponding to 50% inhibition of ϕ_P^{open} versus leaf Ψ during slow water stress. Heat treatments and fluorescence measurements were done as in Figures 3 and 4.

ing) background light (12, 15). In control *Solanum* leaves illuminated with a broadband light (320–640 nm) exciting both photosystems, the PES represented about 12% of the absorbed light energy (Table I). When monitored in far red light (>715 nm), PES 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 (15); in control *Solanum* leaves, PES in far red light was rather low (around 4%). Rapid leaf dehydration for 6 h (or 17 h) exerted no, or very little, influence on PES in both blue-green and far red lights, confirming the resistance of the photochemical apparatus of the chloroplasts to drought. In contrast, heat stress drastically reduced PES monitored in blue-green light and noticeably stimulated PES in far red light, indicating a marked inhibition of PSII and a stimulation of PSI. This selective inhibition of PSII by high temperature was considerably less pronounced in water-stressed leaves, thus confirming our Chl fluorescence data.

Slow Water Stress

For purposes of comparison, heat resistance of PSII was also tested in leaves exposed to more realistic water-stress conditions created by withholding irrigation to the plants. In this case, low Ψ of −12 bars, which was usually obtained in detached leaves within less than 15 h of dehydration, required around 15 d. Figure 6 presents experimental data obtained with potato plants showing that slowly developing water stress induced a progressive increase in chloroplast thermostability, thus confirming our results obtained with rapidly desiccated tomato leaf samples. The temperature for 50% inhibition of the PSII photochemical activity was shifted by +5°C in stressed leaves with a Ψ of −10 bars (corresponding to 10 d of water deprivation) and +6.5°C under more severe drought conditions (Ψ = −12 bars, 14 d after stopping watering the plants). The inset in Figure 6 shows the experimental relationship between leaf water potential and the temperature for 50% inhibition of the maximal photochemical efficiency of PSII.

Photoinhibition

Figure 7 shows the repercussions of a strong-light treatment on the PSII functioning (as indicated by the fluorescence ratio $\phi_P^{open} = [F_m - F_o]/F_m$ measured under steady-state conditions in light of 30 W m^{-2}) in tomato leaves exhibiting different levels of water stress. Exposure of unstressed leaves to bright light (800 W m^{-2}) at a temperature of 25°C provoked

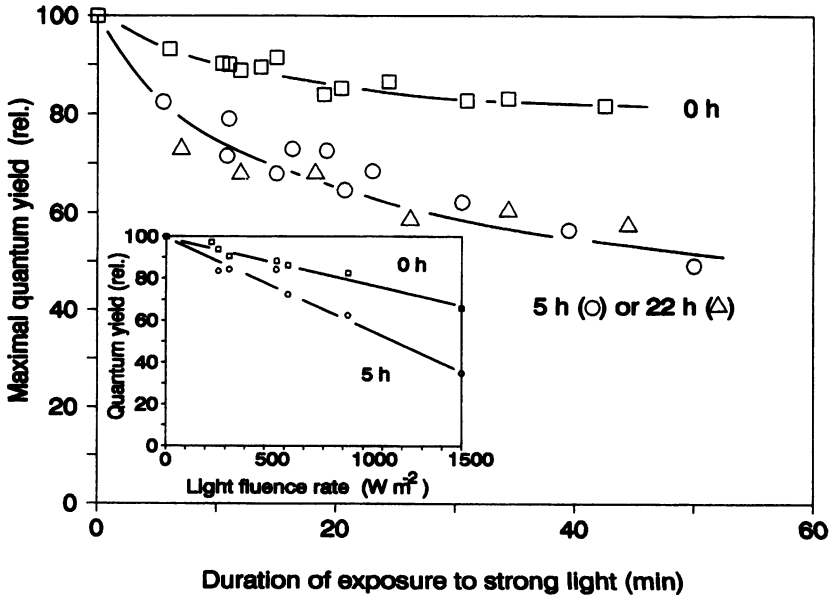

Figure 7. Photoinhibition stress in tomato leaves dehydrated for 0, 5, or 22 h. ϕ_P^{open} was measured in leaves adapted to a light of 30 W m^{-2} before or after exposure for various times to a strong light of 850 W m^{-2} at 25°C. Inset, Effects of a 30-min exposure to various fluence rates of white light on the maximal photochemical efficiency of PSII in leaves dehydrated for 0 or 5 h.

a substantial decrease in the PSII quantum yield. After a 30-min exposure, ϕ_P^{open} was reduced by around 20%; this decrease was directly proportional to the fluence rate of the white light (inset of Fig. 7). Leaf desiccation significantly enhanced the susceptibility of PSII to photoinhibition: a 30-min exposure to 800 W m^{-2} brought about a decrease of 40% in ϕ_P^{open}.

We have examined whether this water-stress-induced sensitization to photoinhibition damage manifested similarly at 25°C and at elevated temperatures: tomato leaves were exposed for a given time (20 min) to intense light (300 W m^{-2}) at various temperatures ranging from 25 to 42.5°C. Figure 8 shows the ϕ_P^{open} of photochemistry in PSII after these treatments. In nondesiccated leaves (RWC = 90%), the inhibition of PSII was very limited at temperatures between 25 and 35°C. Above this, ϕ_P^{open} drastically decreased, falling to very low values closed to 0.1 after strong illumination at 42°C. This loss of PSII activity is due to the simultaneous effects of heat stress and photoinhibition stress. These two effects can be separated by expressing ϕ_P^{open} measured after exposure to the combination of light and temperature stresses as a percentage of the ϕ_P^{open} value measured under heat stress conditions only (i.e. without the photoinhibitory light).

Using this representation (inset of Fig. 8), it was observed that in control tomato leaves, photoinhibition was strongly temperature dependent, sharply increasing at temperatures higher than 38 to 40°C. In contrast (inset of Fig. 8), photoinhibition was much less dependent on the leaf temperature in water-stressed leaves characterized by a low RWC of around 65%. Consequently, at temperatures higher than 40°C, PSII photochemistry was significantly less inhibited by strong light in dehydrated leaves than in nondehydrated leaves (Fig. 8). Thus, when the strong illumination occurred at elevated temperature, water-stressed leaves exhibited similar or reduced damage of PSII (depending on the temperature) as compared to undesiccated leaves.

DISCUSSION

PSII is believed to play a key role in the response of leaf photosynthesis to environmental perturbations (4). In particular, several physicochemical constraints such as heat or strong illumination are supposed to have their primary target in, or close to, the reaction center of this photosystem (5, 20). This study has confirmed the susceptibility of PSII to heat and light stresses in both tomato and *Solanum* leaves, with a strong modulating effect of other environmental factors. In contrast, PSII was observed to be extremely robust to drought conditions. Drastic desiccation treatments resulting in leaf RWC and Ψ as low as 40% and −40 bars, respectively, did not significantly perturb the PSII functioning in dark- and light-adapted leaf samples (Fig. 2).

On this point, our data are in agreement with the recent view (33) that the photosynthetic machinery can tolerate high levels of leaf water deficit and that the inhibition of CO_2 fixation typically observed in water-stressed leaves is almost exclusively due to reduced CO_2 supply resulting from stomatal closure. Inhibition of primary reactions of photosynthesis observed in early studies of water stress was probably the result of photoinhibition to which water-stressed plants are sensitized (see Fig. 7), or could possibly be an artefact of the preparation of isolated chloroplasts. It is clear that lowered CO_2 fixation activity associated with water deficit will cause a decreased demand for NADPH and ATP in the chloroplasts, which should cause a down-regulation of the photosynthetic electron transport system. Therefore, one can be surprised by the fact that no difference was observed in the quantum efficiency of electron transfer in PSII between severely dehydrated and well-hydrated leaves exposed to various light irradiances (Fig. 2B).

As a possible explanation, one can suggest that water stress opened a new route for utilization of photosynthetic electrons with a final acceptor other than carbon dioxide, e.g. molecular oxygen. In favor of this suggestion is the experimental obser-

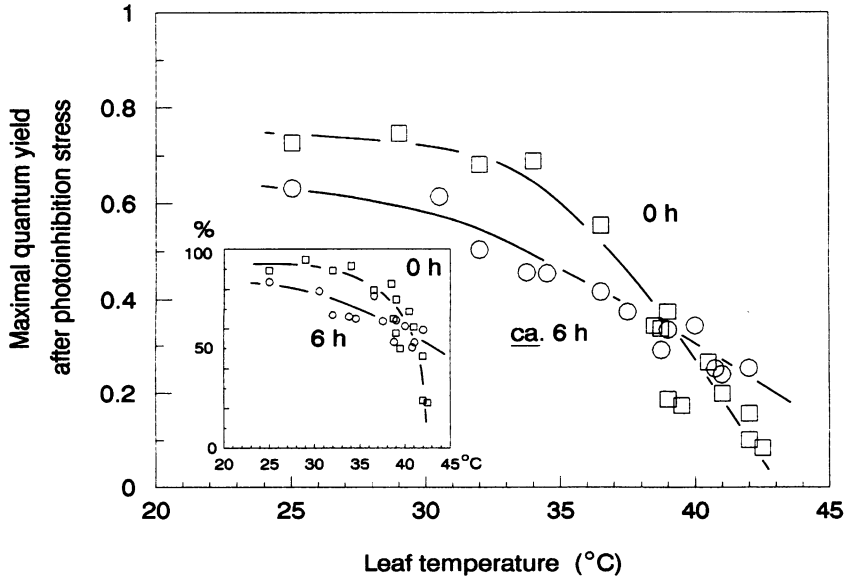

Figure 8. ϕ_P^{open} in tomato leaves adapted to a light of 30 W m^{-2} after exposure for 20 min to a strong light (300 W m^{-2}) at different temperatures. Inset, ϕ_P^{open} expressed in percent of the quantum yield measured before photoinhibition.

vation (27) that dehydration of plants is accompanied by an increase in $^{18}O_2$ uptake relative to CO_2 uptake, as monitored by MS, and the recent calculations (8) of the partitioning of photosynthetic electron flow between CO_2 and O_2 reduction from simultaneously measured Chl fluorescence and CO_2 exchange data, indicating increased allocation of electrons to O_2 in desiccated leaves. Analysis of the $^{18}O_2$ uptake activity of the tomato leaves used here for the Chl fluorescence measurements (data not shown) has effectively shown a marked increase in O_2 uptake during desiccation stress. Further studies will have to determine the O_2-consuming mechanism(s) (photorespiration, chlororespiration, direct O_2 photoreduction by the photosynthetic electron transfer chain) that could be responsible for the maintenance of electron flux in PSII in water-stressed C_3 leaves.

The present study has shown the existence of a marked interaction between physicochemical stresses: one constraint (water deficit) increases the resistance of PSII photochemistry to superimposed constraints, namely, heat combined or not with photoinhibitory light. Our results confirm previous data of Seemann et al. (32), who have observed a correlation between the leaf water potential and osmotic potential and the heat tolerance of photosynthesis in plants grown in the field. As mentioned above, increased stability of PSII to heat is of great ecophysiological significance because both stresses are usually combined in the field: dry areas are also hot areas. Our results show that water stress can induce a shift of more than +5°C in the heat resistance of PSII (Figs. 4 and 6). An increase in heat resistance of a few degrees can be extremely important for a plant's survival because heat-induced inactivation of photosynthesis occurs in a very narrow temperature range, as shown in Figures 4 and 6. Consequently, certain heat-stress conditions can cause a complete and irreversible destruction of PSII in well-watered plants and only a small, fully reversible reduction of the PSII activity in water-stressed plants (Fig. 4, inset). Incidentally, this study has confirmed that the in vivo PSI function is very robust to stress conditions because neither water stress nor heat stress inhibited PES in PSI (Table I).

How can water stress provide protection to PSII against heat injury? Several possible mechanisms can be proposed. It is believed that thermal denaturation of PSII is linked to major physical changes occurring in the lipid matrix of thylakoid membranes during heating (in particular, increased fluidity and formation of cylindrical inverted lipid micelles), which are likely to alter lipid-protein interactions (10) and, hence, cause conformational changes in thylakoid proteins (1). The observation that various treatments, such as acclimation of plants to growth at elevated temperature (26, 34), catalytic hydrogenation of thylakoids (35), or mutation causing deficiency in the activity of a chloroplast fatty acid desaturase (19), modify at the same time the lipid phase of the thylakoid membranes and the thermostability of PSII supports this view. Considering the apparent correlation between heat tolerance of photosynthesis and chloroplast lipids, one can propose that water stress increases the stability of PSII to heat by strengthening the interactions between PSII proteins and their lipid environment. This could be achieved via the alteration of the lipid composition of the thylakoid membranes, which has been reported in plants exposed to water stress (9, 25, and others). However, the available experimental data showing changes in leaf/chloroplast lipids during drought were obtained in plants stressed for periods of several days to several weeks. Further studies will determine whether those lipid changes can also occur during shorter periods of time (a few hours) corresponding to the kinetics of drought-induced increase in PSII thermoresistance and its reversal upon rewatering presented in this article (Figs. 3 and 5).

Various in vitro investigations have suggested the existence of soluble protective compounds in the chloroplast stroma stabilizing thylakoid membranes under stress conditions. For instance, when the stroma of the chloroplasts was removed, the thermostability of the thylakoids from heat-hardened leaves was decreased and comparable to the heat resistance of chloroplast membranes obtained from nonhardened control plants (30). When isolated chloroplasts were heated in the presence of water-soluble compounds such as sugars or proteins (18, 24, 29, 32), thylakoid membranes were partially or completely protected against heat damage. It is interesting that those soluble compounds have been shown to accumulate in leaves submitted to drought stress (22, 28).

On the other hand, using vacuum-infiltrated leaf slices in solution, Kaiser (17) has shown that high osmotic potential partially prevents photosynthesis from inactivation at supraoptimal temperature, and suggested that intracellular salt concentration could be an important agent for adaptation to high temperature, which is in agreement with the observation that photosynthesis of salt-stressed leaves is more resistant to severe temperature stress as compared to nonstressed leaves (21). In this context, in vitro studies have also demonstrated that proton and metal-cation concentrations of the suspension medium play a crucial role in the heat stability of isolated chloroplasts and the maintenance of the molecular assembly of the PSII reaction center (16, 36). Those laboratory studies are corroborated by field studies (32) that have shown a good correlation between the thermal tolerance of leaves and the osmotic potential of leaf water. Consequently, it can be suggested that the increased tolerance of PSII toward heat stress observed in this study was possibly caused by a desiccation-related accumulation of some protective compounds in the surroundings of the heat-sensitive thylakoid membranes.

Sensitization of photosynthesis to photoinhibition damage by environmental stress conditions is well documented in a number of plant species exposed to various kinds of physicochemical stresses (20, 23). This work has confirmed the exacerbation of photoinhibition damage by heat (inset of Fig. 8) and water stress (Fig. 7). The new result shows that at elevated temperatures (>40°C), photoinhibitory light induced less damaging effects in water-stressed leaves than in well-watered control leaves (Fig. 8), indicating that water stress counteracts the negative effects of high light when combined with elevated temperature, as frequently occurs. Although the protective effects of water stress against heat injury reported here were observed in various *Solanaceae*, those phenomena could be species dependent. Indeed, in a detailed study of the tropical pasture legume Siratro using 77K Chl fluorescence, Ludlow and Björkman (23) have observed that high temperatures potentiate photoinhibition

(as shown here) and that this effect is exacerbated by water stress (in contrast to the results obtained in this work); furthermore, in this species, the leaf water status did not appear to affect the threshold temperature for direct heat damage to primary photosynthetic reactions. Different plant species have evolved different strategies for adapting to stressful environments (e.g. stress avoidance by leaf movements in Siratro versus stress tolerance via the protective mechanisms presented here in tomato and potato leaves).

In conclusion, the presented data illustrate the complexity of photosynthetic responses to environmental stresses, with the effects of a given constraint being markedly modulated by the other environmental factors. This study focuses on the antagonism/synergism between temperature, light, and water availability, and shows that a combination of heat and water stresses elicits less injurious effects on the in vivo PSII function than heat stress alone. As a consequence, the in vivo PSII activity could be substantially more heat resistant in the field than previously estimated from laboratory experiments. The interaction between stressors suggests that the behavior of plants monitored under controlled conditions in the laboratory, where the effects of a defined constraint are studied in one factor-one response tests, might be quite different from plants' responses in the field, where several factors usually change simultaneously and interact.

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