Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants

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Received 23 October 1998; Accepted 22 February 1999

Abstract

Modulated chlorophyll fluorescence, rapid fluorescence induction kinetics and the polyphasic fluorescence transients (OJIP) were used to evaluate PSII photochemistry in wheat plants exposed to water stress and/or heat stress (25–45 °C). Water stress showed no effects on the maximal quantum yield of PSII photochemistry ($F_v/F_m$), the rapid fluorescence induction kinetics, and the polyphasic fluorescence transients in dark-adapted leaves, indicating that water stress had no effects on the primary photochemistry of PSII. However, in light-adapted leaves, water stress reduced the efficiency of excitation energy capture by open PSII reaction centres ($F_∞/F_{m}$) and the quantum yield of PSII electron transport ($\phi_{\text{psii}}$), increased the non-photochemical quenching ($q_N$) and showed no effects on the photochemical quenching ($q_P$). This suggests that water stress modified the PSII photochemistry in the light-adapted leaves and such modifications may be a mechanism to down-regulate the photosynthetic electron transport to match a decreased CO$_2$ assimilation. In addition, water stress also modified the responses of PSII to heat stress. When temperature was above 35 °C, thermostability of PSII was strongly enhanced in water-stressed leaves, which was reflected in a less decrease in $F/F_{m}$, $q_m$, $F_∞/F_{m}$, and $\phi_{\text{psii}}$ in water-stressed leaves than in well-watered leaves. There were no significant variations in the above fluorescence parameters between moderately and severely water-stressed plants, indicating that the moderate water stress treatment caused the same effects on thermostability of PSII as the severe treatment. It was found that increased thermostability of PSII may be associated with an improvement of resistance of the O$_2$-evolving complex and the reaction centres in water-stressed plants to high temperature.

Key words: Chlorophyll fluorescence, heat stress, photosystem II photochemistry, water stress, wheat (Tritium aestivum L.).

Introduction

Water stress is one of the most important environmental factors inhibiting photosynthesis (Bradford and Hsiao, 1982). Many studies have shown that the decreased photosynthesis under water stress can be associated with the perturbations of the biochemical processes (Graan and Boyer, 1990; Lauer and Boyer, 1992). In particular, PSII has been shown to be very sensitive to water stress. Several in vivo studies demonstrated that water stress resulted in damage to the oxygen-evolving complex of PSII (Canaani et al., 1986; Toivonen and Vidaver, 1988) and to the PSII reaction centres (Havaux et al., 1987; He et al., 1995).

On the other hand, other studies have shown that the inhibited CO$_2$-dependent O$_2$ evolution and net CO$_2$ assimilation induced by water stress can be recovered by high external CO$_2$ concentration (Frederik et al., 1990; Cornic, 1994), implying that the perturbations of the biochemical processes are not responsible for the inhibited CO$_2$ assimilation, and stomata instead may play a dominant role in the decreased CO$_2$ assimilation under water stress. The conclusion can be further supported by the fact that PSII photochemistry is hardly affected by water stress (Cornic and Briantais, 1991; Cornic, 1994; Liang et al., 1997). Thus, it is still a matter of uncertainty how water stress affects PSII photochemistry.

Under natural conditions, multiple environmental stresses co-occur frequently. It has been reported that the responses of plants to several simultaneous stresses are usually not predictable by single-factor analysis and a combination of different environmental stress factors can

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result in intensification, overlapping or antagonistic effects (Osmond et al., 1986). The effects of interaction of drought and high light on PSII have been investigated widely, showing that drought predisposes photoinhibitory damage to PSII (Masojidek et al., 1991; Giardi et al., 1996). However, much less attention has been paid to the responses of PSII to a combination of water stress and heat stress. It is obviously important to know the mechanisms of the interaction of these two stresses on PSII functions from the view of an ecophysiological aspect, since water stress is often combined with heat stress, in particular in arid and semi-arid regions.

In this study, the effects of water stress on PSII photochemistry and thermostability of PSII in wheat plants by analyses of fluorescence quenching, the rapid fluorescence induction kinetics (i.e. Kautsky curve), and the polyphasic rise of fluorescence transients were examined (Krause and Weis, 1991; Schreiber et al., 1994; Govindjee, 1995; Strasser et al., 1995; Strasser, 1997). It is demonstrated that, although water stress showed no effect on the primary photochemistry of PSII, it increased thermostability of PSII. It is further demonstrated that such increased thermostability may be associated with an improvement on resistance of the O₂-evolving complex and the reaction centres to high temperature.

Materials and methods

Plant material and stress treatments

Wheat (Triticum aestivum L. cv. Shannong 229) seedlings were grown in plastic pots (14 cm in diameter and 13 cm in height) in a growth chamber. The pots were filled with soil which was composed of loam, peat, and coarse sand in 7:3:2 volume ratio and added with NPK (15:15:15, by vol.) complete fertilizer. Light irradiance at the top of plants was c. 180 μmol m⁻² s⁻¹ as provided by high pressure sodium lamps (SON-T AGRO, Philips, Belgium) with a photoperiod of 16:8 h. Air temperature during day and night was kept at 20±1°C and relative humidity at 80±3%. All plants were watered daily.

After 4 weeks, the seedlings were used for the water stress treatment, which was developed gradually by withholding water. After a period of 10 d, leaf water potential reached −2 MPa, measured with a pressure chamber (Model 3000, Soil Moisture Equipment Co., USA). The youngest and fully expanded leaves were used for the measurements.

Heat stress was applied on leaf discs (2 cm). The leaf disc was directly placed into the smooth bottom of a small hole (5.5 cm in height × 3 cm in diameter) in a block of brass, of which the temperature was regulated by circulation of water from a thermostated water bath. At the same time, the up-face of the leaf discs was pressed directly by a block of glass so that the water evaporation of the leaf could be prevented and the heat equilibrium between the leaf disc and the brass block could be reached immediately. The hole of the brass was also covered during treatment. The leaf discs were exposed to different elevated temperatures in the dark for 15 min and then their fluorescence characteristics were measured at 25°C before and after temperature treatment. No difference was observed in the rate of temperature increase between drought-stressed and well-watered leaves. Also, no changes were observed in various fluorescence parameters during the measurements of fluorescence after dark-adaptation of heat-stressed leaves.

Analysis of gas exchange

Gas exchange analysis was made using an open system (Ciras-1, PP system, UK). Net CO₂ assimilation rate was determined at a CO₂ concentration of 360 cm⁻³ m⁻³, 80% relative humidity, and 180 μmol m⁻² s⁻¹ light intensity. Leaf stomatal conductance (Gₛ) was measured under the same conditions with a steady-state porometer (Li-1600, Li-Cor, Lincoln, NE, USA).

Measurements of chlorophyll fluorescence

Chlorophyll fluorescence was measured at room temperature with a portable fluorometer (PAM-2000, Walz, Germany). The fluorometer was connected to a leaf-clip holder (2030-B, Walz) with a tri-furcated fibre optic (2010-F, Walz) and to a computer with data acquisition software (DA-2000, Walz). The experimental protocol of Genty et al. (1989) was basically followed.

The minimal fluorescence level (F₅₀) with all PSII reaction centres open was measured by the measuring modulated light which was sufficiently low (<0.1 μmol m⁻² s⁻¹) not to induce any significant variable fluorescence. The maximal fluorescence level (Fm) with all PSII reaction centres closed was determined by a 0.8 s saturating pulse at 8000 μmol m⁻² s⁻¹ in dark-adapted leaves. The leaf disc was then continuously illuminated with white actinic light at an intensity of 180 μmol m⁻² s⁻¹ which was equivalent to growth PPFD of wheat seedlings in the growth chamber. The steady-state value of fluorescence (F₀) was thereafter recorded and a second saturating pulse at 8000 μmol m⁻² s⁻¹ was imposed to determine the maximal fluorescence level in light-adapted leaves (Fmₑ). The actinic light was removed and the minimal fluorescence level in the light-adapted state (F₅₀) was determined by illuminating the leaf disc with 3 s far-red light. All measurements of F₀ and F₅₀ were performed with the measuring beam set to a frequency of 600 Hz, whereas all measurements of F₅₀ and Fmₑ were performed with the measuring beam automatically switching to 20 kHz during the saturating flash.

By using fluorescence parameters determined in both light- and dark-adapted leaves, the following calculations were made (1) the maximal quantum yield of PSII photochemistry, F₅₀/Fmₑ (2) the photochemical quenching coefficient, qₑ = (F₅₀ − F₅₀)/(Fmₑ − F₅₀), and the non-photochemical quenching coefficient, qₑ = (F₅₀−F₀)/(Fmₑ−F₀), (3) the efficiency of excitation capture by open PSII reaction centres, F₀/Fmₑ, and the actual quantum yield of PSII electron transport, Ÿₑ. Fluorescence nomenclature was according to van Kooten and Snel (1990).

The rapid fluorescence induction kinetics (Kautsky curve) were measured in dark-adapted leaves suddenly illuminated with moderate red light (peak at 655 nm) of 45 μmol m⁻² s⁻¹ at a sampling rate of 1000 μs point⁻¹. In order to avoid an incomplete reoxidation of the plastoquinone pool in the dark phase which could result in an increase in fluorescence level at phase I, the dark-adapted leaves were illuminated with 3 s far-red light prior to measurement of the fluorescence induction kinetics.

The polyphasic rise of fluorescence transients (OJIP) was measured by a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., King’s Lynn, UK) according to Strasser et al. (1995). The transients were induced by red light of 3000 μmol m⁻² s⁻¹ provided by an array of six light-emitting diodes (peak 650 nm), which focused on the sample surface to give homogeneous illumination over the exposed area of the
sample (4 mm in diameter). The fluorescence signals were recorded within a time scan from 10 μs to 1 s with a data acquisition rate of 100,000 points s⁻¹ for the first 2 ms and of 1000 points s⁻¹ after 2 ms. The fluorescence signal at 40 μs was considered as a true \( F_0 \) since the fluorescence yield at this time was shown to be independent of light intensity.

All samples were dark-adapted for 30 min prior to fluorescence measurements.

**Results**

**Effects of water stress on water status, net CO₂ assimilation and stomatal conductance**

Water stress was induced gradually in wheat plants by withholding water. Two levels of water stress were examined, moderate and severe water stress, which were reached c. 3–4 d and 10 d, respectively, after withholding water. Plants wilted if further water stress was imposed after 10 d. Water stress decreased the relative water content of leaves (RWC) and the leaf water potential \( (\psi_w) \). RWC decreased from 92.1% in well-watered plants to 88.3% and 80.5% in moderately and severely water-stressed plants, respectively. Accordingly, \( \psi_w \) decreased from −0.56 in well-watered plants to −1.12 and −1.95 MPa in moderately and severely water-stressed plants, respectively (Table 1).

The rate of net CO₂ assimilation decreased by c. 52% and 89% in moderately and severely water-stressed plants, respectively. Leaf stomatal conductance also decreased significantly as water stress developed (Table 1).

**Effects of water stress on PSII photochemistry**

No changes were observed in the maximal quantum yield of PSII photochemistry \( (F_0/F_m) \) measured in dark-adapted leaves in either moderate or severely stressed plants (Table 2).

Although \( F_0/F_m \) is often used to assess the maximal quantum yield of PSII photochemistry and shows no change during water stress, it gives no direct information on the heterogeneity of PSII reaction centres. In order to examine further whether water stress induces the changes in the heterogeneity of PSII reaction centres, the rapid fluorescence induction kinetics was determined.

When a dark-adapted leaf was illuminated with red light of moderate intensity (40 μmol m⁻² s⁻¹), a typical Kautsky curve was observed, which displayed a rapid rise of chlorophyll fluorescence from the minimal level (O) to an intermediate level (I) followed by a very fast rise to the maximum level (P) (the inset in Fig. 1). The O-I phase has been attributed to \( Q_A \) reduction in the \( Q_B \)-non-reducing PSII reaction centres, in which the electron transfer from \( Q_A \) to \( Q_B \) is inhibited and phase I-P reflects the accumulation of \( Q_A \) in the active PSII reaction centres with efficient electron transfer to the plastoquinone pool (Chylla and Whitmarsh, 1989; Cao and Govindjee, 1990). The ratio \( (F_t - F_0)/(F_p - F_0) \) can thus be considered a measure of the percentage of those \( Q_B \)-non-reducing PSII reaction centres. Table 2 and Fig. 1 show that water stress induced no changes in this ratio, suggesting that water stress exerted no effect on the proportion of \( Q_B \)-non-reducing PSII reaction centres.

Recently, it has been demonstrated that when a dark-adapted leaf is illuminated suddenly with high intensity actinic light (3000 μmol m⁻² s⁻¹), the fluorescence transient shows a polyphasic rise, including phases O, J, I, and P (Strasser et al., 1995). The initial Chi fluorescence at O level reflects the minimal fluorescence yield when all molecules of \( Q_A \) are in the oxidized state. The P level corresponds to the state in which all molecules of \( Q_A \) are in the reduced state. Steps J and I occur at about 2 ms and 30 ms, respectively, between the commonly observed

| Well-watered | 0.56 ± 0.07 | 92.1 ± 4.3 | 220.2 ± 8.9 | 15.5 ± 0.3 |
| Moderately stressed | 1.12 ± 0.12 | 88.3 ± 5.2 | 105.9 ± 12 | 7.5 ± 0.3 |
| Severely stressed | 1.95 ± 0.09 | 80.5 ± 8.0 | 50.7 ± 6.5 | 1.7 ± 0.06 |

**Table 2. Effects of water stress on the maximal quantum yield of photochemistry of PSII \( (F_t/F_0) \) and the ratio \( (F_t - F_0)/(F_p - F_0) \) determined from the rapid fluorescence induction kinetics in dark-adapted leaves, photochemical \( (q_P) \) and non-photochemical \( (q_N) \) quenching, the efficiency of excitation capture by open PSII reaction centres \( (F_t/F_m) \) and the actual quantum yield of PSII electron transport \( (\Phi_{PSII}) \) in light-adapted leaves, which were illuminated by white actinic light at an intensity of 180 μmol m⁻² s⁻¹; values are means ± SE of four replicates**

| Well-watered | 0.821 ± 0.007 | 0.232 ± 0.012 | 0.781 ± 0.008 | 0.321 ± 0.005 | 0.752 ± 0.008 | 0.601 ± 0.009 |
| Moderately stressed | 0.824 ± 0.008 | 0.221 ± 0.020 | 0.790 ± 0.005 | 0.367 ± 0.003 | 0.676 ± 0.003 | 0.545 ± 0.005 |
| Severely stressed | 0.828 ± 0.006 | 0.226 ± 0.017 | 0.785 ± 0.008 | 0.448 ± 0.003 | 0.610 ± 0.006 | 0.501 ± 0.006 |
steps O and P. The rise from phase O to phase J results from the reduction of $Q_A$ to $Q_A^-$ and is associated with the primary photochemical reactions of PSII. The intermediate step I and the final step P reflect the existence of fast and slow reducing PQ centres as well as to the different redox states of PSII reaction centres (Strasser et al., 1995). Thus, the polyphasic rise of fluorescence transients can give more information on PSII photochemistry.

The polyphasic Chl $a$ fluorescence transients in wheat plants during water stress were followed. The polyphasic rise in Chl $a$ fluorescence transients in well-watered plants demonstrated a typical polyphasic rise, including phases O, J, I, P, described in details by Strasser et al. (1995). It was observed that water stress had no significant effect on these phases in the polyphasic rise of fluorescence transients (Fig. 2A, curve a, b).

The above results show that water stress had no effects on the primary photochemistry of PSII determined in dark-adapted leaves. It was further investigated whether water stress induces modifications in PSII photochemistry in light-adapted leaves. The changes in fluorescence characteristics in light-adapted leaves were examined by the use of pulse-modulated fluorescence.

Table 2 shows that water stress decreased the efficiency of excitation energy capture by open PSII reaction centres ($F_o/F_m$) and the quantum yield of PSII electron transport ($\phi_{PSII}$), increased the non-photochemical quenching coefficient ($q_N$). However, it had no effects on the photochemical quenching coefficient ($q_P$).

Effects of water stress on thermostability of PSII

The effects of water stress on thermostability of PSII were investigated. First, the effects of the degree of water stress on thermostability of PSII were examined. No significant difference was observed in the responses of PSII photochemistry (the first parameters in dark- and light-adapted leaves, such as $F_/F_m$, $F_/F_m$ in PSII, $\phi_{PSII}$, $q_P$, and $q_N$, data not shown) to heat stress between moderate and severely water-stressed plants, suggesting that the moderate water stress treatment caused the same effects on thermostability of PSII as the severe treatment. Therefore, only the results in severely water-stressed plants are presented here.

The responses of the maximum quantum yield of PSII
photochemistry ($F_v/F_m$), the minimal fluorescence ($F_0$) and the maximum fluorescence ($F_m$) in dark-adapted leaves to elevated temperatures are shown in Fig. 3. When temperature was increased to 40 °C, $F_v/F_m$ began to decrease significantly in both well-watered and water-stressed plants. More importantly, water-stressed plants exhibited higher values of $F_v/F_m$ than well-watered plants under high temperatures. For example, at 45 °C, $F_v/F_m$ decreased by 38% in well-watered plants but only 18% in water-stressed plants, indicating that water stress induced an increase in resistance of PSII to heat stress (Fig. 3A).

A larger increase in $F_0$ and a greater decrease in $F_m$ were observed in well-watered leaves than in water-stressed leaves (Fig. 3B, C).

It has been suggested that PSII reaction centre is one of the damage sites induced by heat stress (Havaux, 1993). Therefore, this study investigated whether the greater decrease in $F_v/F_m$ in well-watered plants during heat stress was associated with a larger increase in the proportion of $Q_B$-non-reducing PSII reaction centres, expressed as the ratio ($F_v-F_0$)/($F_p-F_0$), which has been shown to be sensitive to heat stress (Klinkovsky and Naus, 1994). Figure 1 shows that indeed a larger increase in this ratio was observed in well-watered plants than in water-stressed plants, suggesting that water stress also increased thermostability of PSII reaction centres.

It has been shown that the $O_2$-evolving complex (OEC) is most susceptible in PSII apparatus to heat stress (Berry and Björkman, 1980). This study further examined whether the greater decrease in $F_v/F_m$ in well-watered leaves during heat stress was associated with the greater inhibition of the OEC activity.

As shown in Fig. 2, well-watered plants show a typical polyphasic rise of fluorescence transients, including phases O, J, I, P. It has been shown that heat stress can induce a rapid rise in the polyphasic fluorescence transients. This phase, occurring at around 300 μs, has been labelled as K, and is the fastest phase observed in the OJIP transient which, in consequence, becomes an OKJIP transient (Guisset et al., 1995; Srivastava and Strasser, 1996; Srivastava et al., 1997). It has also been shown that phase K is caused by an inhibition of electron donor to the secondary electron donor of PSII, $Y_Z$, which is due to a damaged OEC, phase K can therefore be used as a specific indicator of damage to the OEC (Guisset et al., 1995; Strasser, 1997).

Figure 2A shows that when exposed to 45 °C for 15 min, a very clear K step appeared in well-watered plants. But, this heat-induced K step did not get pronounced in water-stressed plants. The changes in the amplitude in the K step, expressed as the ratio $F_K/F_J$, was shown in Fig. 2B. A higher ratio $F_K/F_J$ was observed in well-watered plants than in water-stressed plants during 35–45 °C, indicating that water stress can increase resistance of the OEC to heat stress.

Further experiments were carried out to examine the responses of PSII photochemistry in light-adapted leaves, i.e. under steady-state photosynthesis, to high temperatures. Figure 4 shows that a greater decrease in $\phi_{PSII}$, $F_v/F_m$, and $q_p$ was observed in well-watered plants than in water-stressed plants in the range 35–45 °C, indicating that PSII in water-stressed plants also shows better thermostability in light-adapted leaves.

**Discussion**

*Water stress has no effect on the primary photochemistry of PSII*

These results show that no significant differences in the maximal quantum yield of PSII photochemistry ($F_v/F_m$),
and in the rapid fluorescence induction kinetics and the polyphasic rise of fluorescence transients were observed between well-watered and water-stressed plants, indicating that water stress had no effect on the primary photochemistry of PSII (Table 2; Figs 1, 2). However, these results show that water stress induced a decrease in the quantum yield of PSII electron transport ($\Phi_{PSII}$) (Table 2), suggesting that water stress resulted in modifications in PSII photochemistry in light-adapted leaves. The decreased $\Phi_{PSII}$ was a result of the decrease in the efficiency of excitation energy capture by open PSII reaction centres ($F_v/F_m$) (Fig. 3A) as previously observed by Baker (1991). A decrease in $F_v/F_m$ may reflect light-induced non-photochemical quenching ($q_P$) ($\Phi_{PSII} = F_v/F_m \times q_P$, Genty et al., 1989) (Table 2).

A decrease in $F_v/F_m$ may reflect light-induced non-photochemical quenching (Brestic et al., 1995). Thus, a high $q_N$ in water-stressed leaves may be a mechanism to down-regulate photosynthetic electron transport so that production of ATP and NADPH would match with the decreased CO$_2$ assimilation due to the closure of stomata (Table 1).

**Water stress increases thermostability of PSII**

The results in this study demonstrate the existence of an antagonism between water stress and heat stress in wheat plants, with water stress enhancing the resistance of PSII to heat stress. It was found that increased resistance of PSII to heat stress in water-stressed leaves was exhibited not only in the resistance of the maximal quantum efficiency of PSII photochemistry in the dark-adapted state (i.e. $F_v/F_m$) (Fig. 3A) as previously observed by Havaux (1992) and Epron (1997), but also in the resistance of PSII photochemistry in the light-adapted state, i.e. under steady-state photosynthesis, such as $F_v/F_m$, $q_P$, and (PSII, to high temperatures (Fig. 4).

These results show that such an increased resistance of PSII to heat stress in water-stressed leaves was associated with an improvement of the resistance of PSII reaction centres to high temperatures, as indicated by a smaller increase in the proportion of $Q_P$-non-reducing PSII reaction centres during heat stress in water-stressed leaves than in well-watered plants (Fig. 1). A smaller decrease in photochemical quenching ($q_P$) also suggests increased thermostability of PSII reaction centres in water-stressed plants since $q_P$ can represent the fraction of open PSII reaction centres (Genty et al., 1989; van Kooten and Snel, 1990) (Fig. 4C). In addition, these results demonstrate that increased thermostability of PSII in water-stressed plants was also associated with an improvement in thermostability of the O$_2$-evolving complex (OEC), as shown by a less pronounced phase K in the polyphasic fluorescence transients in water-stressed plants than in well-watered plants (Fig. 2).

How water stress can protect PSII against heat damage is not clear. One possibility is that increased thermostability of PSII may be associated with the accumulation of some soluble compounds in water-stressed leaves. It has been shown that theromeresistance of the thylakoids to high temperature stress is enhanced, when the chloroplasts are incubated in the presence of some soluble compounds, such as sugars and proteins (Krause and Santarius, 1975; Santarius, 1975). It is interesting that these soluble compounds indeed accumulate in water-stressed leaves (Seemann et al., 1986; Sachs and Ho, 1986).

Water stress usually results in a decrease in leaf transpiration because of the stomatal closure which commonly occurs under water stress. Such a decrease in leaf transpiration results in an increase in leaf temperature. However, it has been shown that leaf photosynthesis is thought to be the main target of high temperatures and PSII within
the photosynthetic apparatus appears to be most heat sensitive (Berry and Björkman, 1980). Increased thermos-
stability of PSII in water-stressed leaves may thus help to improve the resistance of the photosynthetic metabolism to high temperature and thus may increase the resistance of the whole plant to high temperature.

Acknowledgements

We are grateful to Hong Kong Baptist University (FRG grant) and the Croucher Foundation for their financial support to this study.

References


PSII thermostability in water-stressed plants


