Enhanced photosystem 2 thermostability during leaf growth of elm (*Ulmus pumila*) seedlings

C.-D. JIANG[*,+], G.-M. JIANG[*,+], X. WANG[**], L.-H. LI[*], D.K. BISWAS[*], and Y.-G. LI[*]

Laboratory of Quantitative Vegetation Ecology, Institute of Botany, the Chinese Academy of Sciences, Beijing 100093, P.R. China[*]
Department of Biology, Indiana University-Purdue University Indianapolis, 723 West Michigan St., Indianapolis, IN 46202-5132, USA[**]

Abstract

We examined photosynthetic activities and thermostability of photosystem 2 (PS2) in leaves of elm (*Ulmus pumila*) seedlings from initiation to full expansion. During leaf development, net photosynthetic rate (P_N) increased gradually and reached the maximum when leaves were fully developed. In parallel with the increase of P_N, chlorophyll (Chl) content was significantly elevated. Chl *a* fluorescence measurements showed that the maximum quantum yield of PS2 (φ_{PS2}), the efficiency a trapped exciton, moved an electron into the electron transport chain further than Q_A^- (Ψ_o), and the quantum yield of electron transport beyond Q_A (φ_{Eo}) increased gradually. These results were independently confirmed by our low irradiance experiments. When subjected to progressive heat stress, the young leaves exhibited considerably lower φ_{PS2} and higher minimal fluorescence (F_0) than the mature leaves, revealing the highly sensitive nature of PS2 under heat in the newly initiating leaves. Further analysis showed that PS2 structure in the newly initiating leaves was strongly altered under heat, as evidenced by the increased fluorescence signals at the position of the K step. We therefore demonstrated an inhibition in the oxygen-evolving complex (OEC) in the young leaves. This resulted in decrease in amount of the functional PS2 reaction centres and relative increase in the PS2 reaction centres with inhibited electron transport at the acceptor side under heat. We suggest that the enhanced thermostability of PS2 during leaf development is associated with improved OEC stability.

Additional key words: chlorophyll *a* fluorescence; JIP-test; leaf growth; thermostability of photosystem 2.

Introduction

Formation of chloroplast ultrastructure, accumulation of chlorophylls, and synthesis of major components of photosynthetic apparatus proceed almost in parallel during leaf development. These synchronized processes are often reflected in proportional increase in net photosynthetic rate (P_N) (Šesták 1985). Photosystem 2 (PS2) is one of the major protein complexes in the photosynthetic apparatus in higher plants. As a result, more and more research effort has been devoted to understanding development of PS2 complex in the expanding leaves (Guenther and Melis 1990, Lebkuecher *et al.* 1999, Srivastava *et al.* 1999, Choinski *et al.* 2003) and a lot of progress has been made in this field. Guenther and Melis (1990) found different developmental status of PS2 complex at various stages of chloroplast maturity. More recently, Choinski *et al.* (2003) noticed that the maximal quantum yield of PS2 (φ_{PS2}) increases during leaf growth. Most previous investigations, however, were focused primarily on plants growing under artificial conditions with suboptimal irradiance levels (Guenther and Melis

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[+]Corresponding authors; fax: +86-10-62590348; e-mail: jcdao@ibcas.ac.cn
Abbreviations: F_0 – minimum fluorescence intensity; F_m – maximum fluorescence intensity; F_P – the fluorescence peak; OEC – oxygen-evolving complex; P_N – net photosynthetic rate; PS2 – photosystem 2; V_I – relative variable florescence at about 30 ms (plateau I of the OIP transient); V_t – relative variable fluorescence at time t; φ_{Eo} – quantum yield of electron transport beyond Q_A; φ_{PS2} – maximum quantum yield of PS2; Ψ_o – efficiency a trapped exciton can move an electron into the electron transport chain further than Q_A^-.
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1990, Lebkuecher *et al.* 1999, Srivastava *et al.* 1999). The development of PS2 in leaves grown under field conditions has not been adequately investigated. We examined development of PS2 in the expanding leaves of the elm seedlings growing under natural irradiance in the field.

PS2 is highly sensitive to various environmental stresses (Verhoeven *et al.* 1999, Munné-Bosch *et al.* 2000, Jiang *et al.* 2002). This is especially true about PS2 in the newly initiating leaves (Krause *et al.* 1995, Bertamini and Nedunchezhian 2003), because the young leaves are often exposed to high irradiance at the topmost canopy or branches and are therefore exposed to extreme irradiance. Photoprotective mechanisms are preferentially developed at the early stages of leaf expansion to protect the young leaves against high irradiance (Yoo *et al.* 2003, Jiang *et al.* 2005). High irradiance typically occurs with high leaf temperature (Long *et al.* 1994). Leaf temperature reached 40 ºC in the seedlings growing in forest gaps (Mulkey and Pearcy 1992, Koniger *et al.* 1995, 1998), and could even exceed 42 ºC in plants growing in Inner Mongolia highland (Jiang and Zhu 2001). At present, it is still unknown if the young and old leaves differ in their PS2 thermostability at high temperature.

PS2 is generally one of the most thermolabile components in the electron transport chain (Weis and Berry 1988, Havaux 1996, Lu and Zhang 1999). The oxygen-evolving complex (OEC) at the donor side of PS2, however, is extremely sensitive to heat (Weis and Berry 1988, Havaux 1993, Yamane *et al.* 1998, Lu and Zhang 1999). As a result, exposure of plants to heat even for a short time period may induce significant inactivation of the OEC (Havaux 1993, Yamane *et al.* 1998, Lu and Zhang 1999, Chen *et al.* 2004). Similarly, heat can significantly reduce electron transfer from Q_A to Q_B at the acceptor side of PS2 (Bukhov *et al.* 1990, Cao and Govindjee 1990, Joshi *et al.* 1995, Lazár *et al.* 1999). The inhibition of electron transport at the acceptor side of PS2 was attributed to the changes in structure of PS2 reaction center and to a shift in redox potential of Q_A (Ducruet and Lemoine 1985, Pospíšil and Tyystjärvi 1999). We examined responses of PS2 to heat at both the donor and acceptor sides by analyzing fast fluorescence rise during leaf development in order to elucidate differences in responses to heat in PS2 of the young and mature leaves.

Materials and methods

Plants: We conducted the experiments in 2004 at Duolun Ecosystem Research Station of the Chinese Academy of Sciences, located in Duolun County, the Inner Mongolia (42º23´N, 112º23´E). Elm (*Ulmus pumila* L.), one of the most widely distributed tree species in the Inner Mongolia highland, was selected for this study. Elm seedlings were grown in sandy soil in the field under natural irradiance. Sufficient nutrients and water were supplied throughout the experiment to avoid potential nutrient and water stresses. All measurements were taken from early June to early July. During the experiment, daily mean air temperature ranged from 17 to 25 ºC and the maximum photosynthetic photon flux density (PPFD) was approximately 2 200 μmol m^{-2} s^{-1} at midday on sunny days. The newly initiating leaves at the top of stems were followed individually for examination from budbreak to full expansion.

Heat treatment: Leaves from elm seedlings were cut at 07:00 just before the start of heat treatment and placed on the external surface of an aluminum chamber with a water bath for temperature control. To avoid dehydration, the borders and petioles of the excised leaves were covered with wet filter paper. All leaf samples were heated at 25, 30, 35, 40, and 45 ºC for 15 min at each temperature, respectively, in dark before measurement of Chl *a* fluorescence.

P_N was measured at about 25 ºC and at ambient CO_2 concentration (350 μmol mol^{-1}) with a portable photosynthesis system (*LCA-4, ADC*, Hoddesdon, UK). Measurements were taken at 10:00 and PPFD was controlled at 1 500 μmol m^{-2} s^{-1}.

Fluorescence induction OIP kinetics at low irradiance: Chl *a* fluorescence transients (OIP) were measured using a Plant Efficiency Analyzer (*PEA*, *Hansatech Instruments*, King's Lynn, Norfolk, UK) in the dark-adapted leaves treated with low red radiation (30 μmol m^{-2} s^{-1}). All fluorescence transients were recorded during a 5-s pulse of low red radiation provided by an array of six light-emitting diodes. In typical OIP curves, step I shows the accumulation of Q_A^-. The initial rise from level O to I is related to a reduction of the primary quinone electron acceptor Q_A in the PS2 centers, which are unable to reduce the subsequent quinone acceptor Q_B (Melis 1985, Chylla and Whitmarsh 1989, Cao and Govindjee 1990). These PS2 centers are termed "Q_B-non-reducing" PS2 centers (Melis 1985, Chylla and Whitmarsh 1989, Cao and Govindjee 1990). The relative variable florescence at about 30 ms (plateau I of the OIP transient, $V_I = [F_{30ms} - F_{50\mu s}]/[F_P - F_{50\mu s}]$) approximates the fraction of the Q_B-non-reducing PS2 centers (Melis 1985, Chylla and Whitmarsh 1989, Cao and Govindjee 1990, Lebkuecher *et al.* 1999, Tomek *et al.* 2003, Chen *et al.* 2004). While increase in the intermediate fluorescence level V_I can be additionally attributed to the equilibrium between Q_A and Q_B in the functional reaction centers (Hsu and Lee 1991, Tomek *et al.* 2003) and initial rise from level O to I can be slightly modulated by the state of the oxygen-evolving complex (Hsu 1993, Lavergne and Leci 1993), their contribution is relatively small. Therefore, we attributed the

observed changes in V_I mainly to Q_B-non-reducing centers.

Fluorescence induction OJIP kinetics at high irradiance: The polyphasic rise of fluorescence transients (OJIP) was measured with a Plant Efficiency Analyzer (*PEA, Hansatech Instruments*, King's Lynn, Norfolk, UK) on the fully dark-adapted leaves. All fluorescence transients were recorded during a 60-s pulse of red radiation (2 880 μmol m^{-2} s^{-1}) provided by an array of six radiation-emitting diodes. The fluorescence signals were recorded within a time scan from 10 μs to 1 s with a data acquisition rate of 10^5 readings per s for the first 2 ms, and a rate of 10^3 readings per s thereafter.

Each stage in the polyphasic rise of fluorescence transient, O, J, I, and P was analyzed according to the *JIP-test* (Strasser *et al.* 1995, 2000, Strasser and Strasser 1997). The original recorded data include maximum fluorescence intensity (F_m), fluorescence intensity at 50 μs, *i.e.* the minimum intensity (F_0), fluorescence intensity at 300 μs (K step), and fluorescence intensity at 2 ms (J step). The maximum quantum yield of primary photochemistry ($\varphi_{PS2} = 1 - F_0/F_m$), the efficiency a trapped exciton can move an electron into the electron transport chain further than Q_A^- ($\Psi_o = 1 - V_J$), the quantum yield of electron transport beyond Q_A ($\varphi_{Eo} = \varphi_{PS2}\ \Psi_o$), and the relative variable fluorescence [V_t] at 300 μs (K step) and at 2 ms (J step), defined as $V_t = (F_t - F_{50\mu s})/(F_m - F_{50\mu s})$ were all calculated according to Strasser *et al.* (1995, 2000).

Chl analysis: Leaf Chl was extracted with 80 % acetone at room temperature and the extract was analyzed for pigments using a spectrophotometer (*UV-120 system Shimadzu*, Japan) according to Arnon (1949). The absorbance was measured at 663 and 645 nm, respectively. The leaves were analyzed with at least three independent replicates at each sampling.

Results

Changes in P_N and Chl content during leaf development: Leaf development was evaluated by measuring P_N and Chl content on an area basis during leaf growth (Fig. 1). Three days after initiation, the elm leaves showed little photosynthetic activity. There was an abrupt increase in P_N in 5-d-old leaves compared with 3-d-old leaves. P_N increased with leaf growth until full expansion on the 11th d after bud-break, when it reached maximum (Fig. 1*A*). Total Chl content increased in parallel to changes in P_N as elm leaves expanded (Fig. 1*B*).

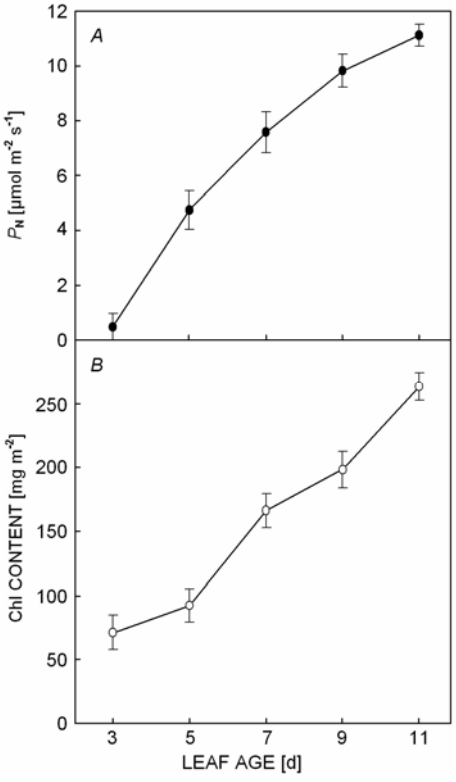

Fig. 1. Net photosynthetic rate (P_N) (*A*) and chlorophyll (Chl) content (*B*) in elm (*Ulmus pumila*) leaves from bud-break to full expansion. Measurements were performed at 10:00. The photosynthetic photon flux density (PPFD) was 1 500 μmol m^{-2} s^{-1}. Means ± SE (n = 5).

Changes in PS2 during leaf development: The Chl *a* fluorescence transient was a rich source of information about the structure and function of PS2 as leaves grew (Fig. 2*A,B*). The maximum quantum yield of primary photochemistry (φ_{PS2}) measured in fully dark-adapted leaves increased slightly with leaf development, and fully developed leaves had slightly higher φ_{PS2} than the youngest leaves (Fig. 2*C*). Parameters calculated from the polyphasic rise of fluorescence transients included the efficiency a trapped exciton can move an electron into the electron transport chain further than Q_A^- (Ψ_o) and the quantum yield of electron transport beyond Q_A (φ_{Eo}) (Fig. 2*C*). Both Ψ_o and φ_{Eo} increased gradually but in parallel to each other with leaf growth (Fig. 2*C*). The fluorescence induction OIP kinetics showed a gradual but steady decline in V_I as the elm leaves expanded (Fig. 3).

Changes in φ_{PS2} and F_0 under heat stress: From 25 to 30 ºC, neither the young nor the mature leaves had remarkable changes in φ_{PS2} (Fig. 4). When temperature was elevated to 35 ºC, φ_{PS2} in the youngest leaves began to decrease precipitously, while φ_{PS2} in the mature leaves declined much more gradually. More importantly, the younger leaves always exhibited lower φ_{PS2} than the mature leaves when leaf temperature was over 35 ºC (Fig. 4*A*). F_0 also showed greater increase in the younger leaves than in the mature leaves with rising temperature (Fig. 4*B*), indicating a higher proportion of the inactivated

PS2 reaction centers in the young leaves (Ducruet and Lemoine 1985, Yamane *et al.* 1998).

Changes of V_K/V_J and V_I under heat stress: Further analyses were carried out to investigate the responses of fluorescence signals at positions of the K and I steps of Chl *a* fluorescence transients to elevated temperatures. As shown in Fig. 5, there was a large enhancement of fluorescence signal at the position of the K step when leaves were subjected to a temperature of 45 ºC. This phase, occurring at around 300–500 µs, could be used as a specific indicator of heat damage to the OEC (Strasser *et al.* 1995, 2000, Lazár *et al.* 1999, Lazár and Pospíšil 1999). The changes in the amplitude in fluorescence signal at the position of the K step, expressed as the ratio of V_K to V_J, showed that the young leaves exhibited a greater increase in V_K/V_J than the mature ones in the temperature range of 35–45 ºC (Fig. 6).

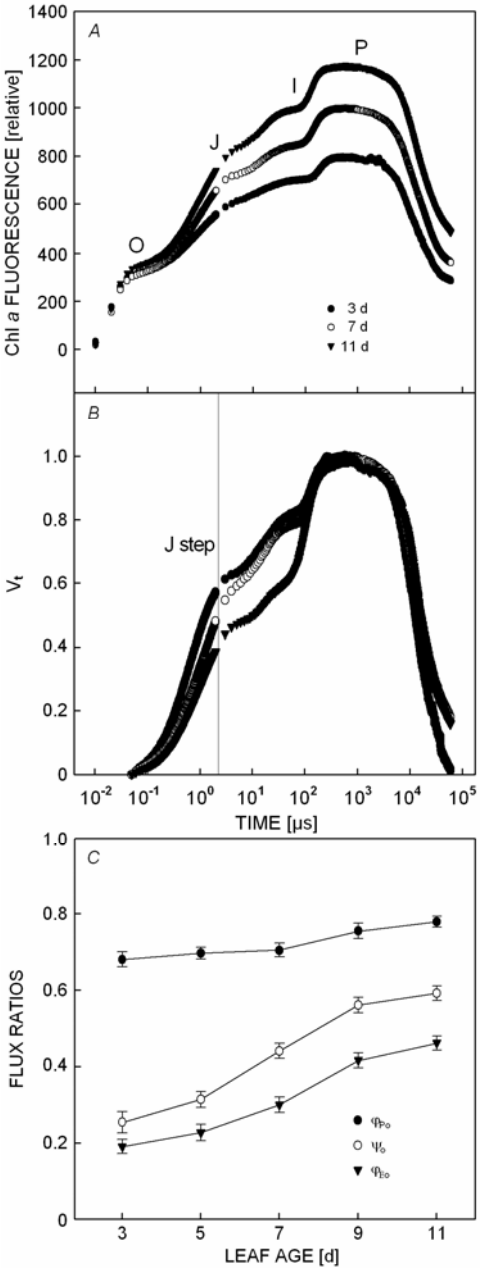

Fig. 2. Polyphasic chlorophyll (Chl) fluorescence transients in the dark-adapted elm leaves during leaf growth (*A*), relative variable fluorescence V_t between F_0 and F_m (*B*), and the flux ratios (φ_{PS2}, Ψ_o, and φ_{Eo}) (*C*). Measurements were performed at 07:00 after dark adaptation. Plants were day-light-adapted at 07:00 before leaves were dark-adapted within leaf clips for 20 min. Means ± SE (n = 6).

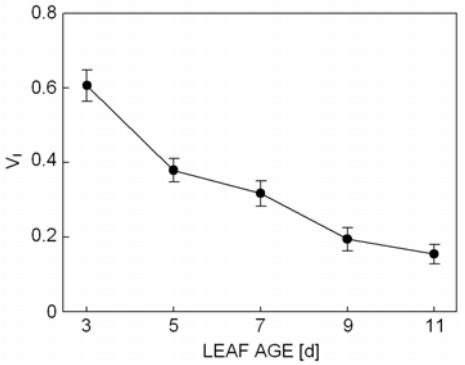

Fig. 3. The proportion of relative variable fluorescence at 30 ms (V_I) during leaf growth. The plants were day-light-adapted at 07:00 and then leaves were dark-adapted within the leaf clips for 20 min. Measurements were performed at 07:00. Means ± SE (n = 6).

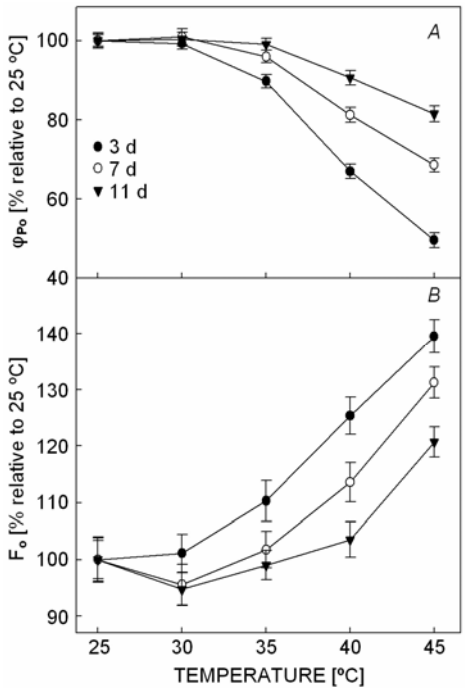

Fig. 4. The maximum quantum yield of primary photochemistry (φ_{PS2}) (*A*) and minimal fluorescence (F_0) (*B*) in the young and mature leaves exposed to various temperatures in dark for 15 min. Means ± SE (n = 5).

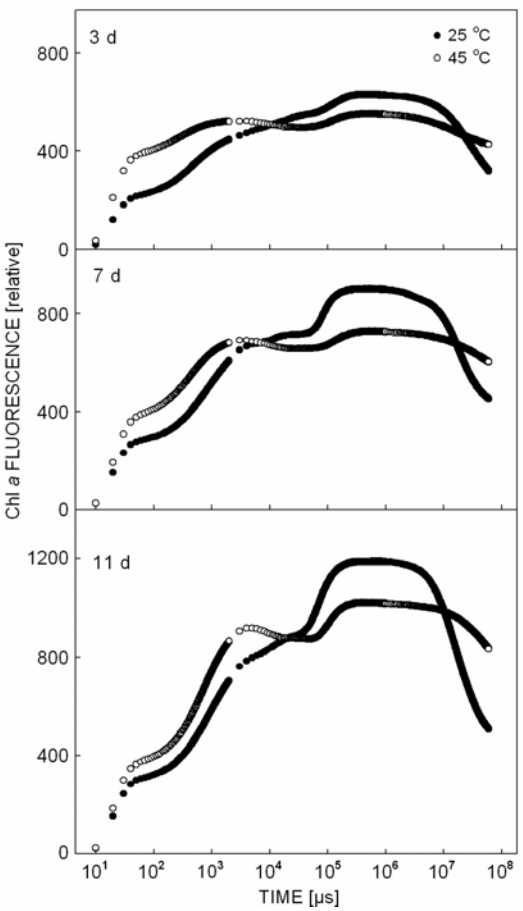

Fig. 5. Effect of high temperature on polyphasic chlorophyll (Chl) fluorescence transients in the young and mature leaves. Leaves were subjected to heat stress for 15 min in darkness.

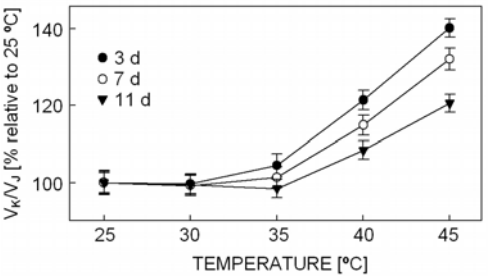

Fig. 6. Effect of high temperature on the ratio of V_K to V_J in the young and mature leaves. Leaves were treated for 15 min in darkness at various temperatures. Means ± SE (n = 5).

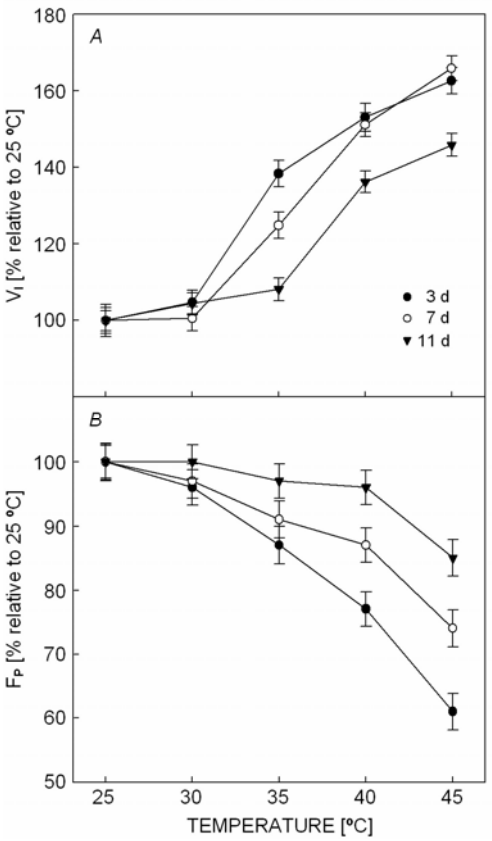

Fig. 7. Effect of high temperature on the proportion of the relative variable fluorescence at 30 ms (V_I) (*A*) and F_P (*B*) in the young and mature leaves. Leaves were treated for 15 min in darkness at various temperatures. Means ± SE (n = 5).

The relative variable fluorescence at 30 ms (V_I) at low irradiance and elevated temperatures was measured during leaf expansion (Fig. 7). No remarkable changes in V_I were observed between 25 and 30 ºC either in the young or mature leaves (Fig. 7*A*). V_I in the younger leaves started to increase significantly when temperature rose above 30 ºC. When leaf temperature exceeded 35 ºC, the younger leaves had much higher V_I than the more mature ones (Fig. 7*A*). Since V_I is calculated as $V_I = (F_{30ms} - F_{50\mu s})/(F_P - F_{50\mu s})$, its value will be influenced by the level of F_P to a great extent. As a matter of fact, noticeable decrease in F_P was observed under high temperatures (Fig. 7*B*).

Discussion

Development of PS2 complex: We found that photosynthetic activities and Chl content showed synchronized increase in the developing elm leaves, which is a finding consistent with other studies on dicotyledonous species (Šesták 1985, Choinski *et al.* 2003). Choinski *et al.* (2003) found that the younger leaves had lower φ_{PS2} ratios than the mature ones, regardless of measuring time, *i.e*. midday or 2 h after the sunset. They argued that lower φ_{PS2} ratios could reflect some degree of chronic photoinhibition during leaf development. We made the determination of φ_{PS2} in the early morning on the dark-adapted leaves and therefore the effect of strong irradiance was clearly avoided. The slightly lower φ_{PS2} in the younger leaves could have been caused by the

differential developmental status of PS2 complex components during leaf growth, rather than by chronic photoinhibition of PS2.

In the elm seedlings, φ_{PS2} in the mature leaves was only 10 % higher than that in the youngest leaves, while in the sunflower seedlings, Lebkuecher *et al.* (1999) reported drastic increase in φ_{PS2} during PS2 development. The most likely reason for the large difference in change in φ_{PS2} during leaf development in these two studies was irradiation the seedlings were exposed to before treatment. Lebkuecher *et al.* (1999) used the etiolated sunflower seedlings that had been grown in continuous darkness for 6 d, while we used dark-adapted leaves from the elm seedlings grown in the field under natural irradiance. Obviously, the different pre-conditions of the seedlings could account for the observed differences in change in φ_{PS2} as leaves grew. Nevertheless, φ_{PS2} in both species increased as PS2 in the expanding leaves developed.

While φ_{PS2} was only slightly lower in the young leaves, Ψ_o and φ_{Eo} were significantly lower in the newly initiating leaves than in the fully expanded ones. These results suggest that electron transport beyond Q_A was not fully functional in the young leaves. Q_B-non-reducing reaction centres are the ones in which the ability to transfer electrons from Q_A to Q_B is lost or has not been set up (Chylla and Whitmarsh 1989, Cao and Govindjee 1990, Lebkuecher *et al.* 1999). During leaf development, V_I at low irradiance was found to decrease significantly while Ψ_o and φ_{Eo} increased significantly, suggesting a higher proportion of Q_B-non-reducing reaction centers in the young leaves than in the mature ones. However, changes in V_I can not be caused only by increase in fluorescence signal at the I step, but by decrease in F_P as well. Therefore, higher V_I does not necessarily mean more Q_B-non-reducing PS2 reaction centers in the younger leaves. Instead, it could indicate less functional (Q_B-reducing) PS2 reaction centers in the younger than in the fully developed leaves. The relatively higher ratio of Q_B-non-reducing reaction centers and less functional PS2 reaction centers in the young leaves might be responsible for the overall low electron transport beyond Q_A in these immature leaves. Considering the gradual increase of electron transport in parallel with development of the photosynthetic apparatus during leaf growth, we believe that steady increase in electron transport partly explains gradual increase in P_N as leaves mature.

Thermostability of PS2 at different developing stages of leaf can be examined by analyzing fluorescence induction kinetics, which is a useful, reliable, and noninvasive method to assess PS2 functions (Krause and Weis 1991, Strasser and Strasser 1997, Lazár *et al.* 1999, Strasser *et al.* 2000). We found that heat stress had a significant effect on φ_{PS2} and F_0 in the young leaves, especially in the newly initiating leaves, demonstrating that PS2 in the young leaves was more sensitive to heat than that in the mature leaves.

The lower thermostability of PS2 reaction centers in the young leaves may be associated with the OEC of PS2, as reflected by a greatly pronounced fluorescence signal at the position of the K step in the polyphasic fluorescence transients in the young leaves. The OEC of PS2 in the young leaves was particularly sensitive to heat, but sensitivity was progressively alleviated as leaves developed. Because PS2 in the young leaves was not fully developed, we suggest that particularly high sensitivity of the OEC to heat in the young leaves may be associated with development of PS2. As leaves expanded and matured, the thermostability of PS2 reaction centers gradually improved and leaves became less sensitive to heat.

Both the donor and acceptor side of PS2 reaction centers are sensitive to heat, regardless of being measured *in vitro* or *in vivo* (Joshi *et al.* 1995, Pospíšil and Tyystjärvi 1999, Lu and Zhang 1999, Sinsawat *et al.* 2004). Increased V_I has been used to assess the extent of heat inhibition on electron transport at the acceptor side of PS2 (Lu and Zhang 1999, Chen *et al.* 2004). We found that V_I in the young leaves was significantly higher under high temperatures, suggesting that heat markedly reduced electron transport at the acceptor side of PS2. However, it is noteworthy that V_I is a derived ratio parameter ($V_I = (F_{30ms} - F_{50\mu s})/(F_P - F_{50\mu s})$) and its value can be equally influenced by increase of the fluorescence signal at the position of the I step and by decrease in F_P. We found that heat significantly reduced F_P, especially in the young leaves. As a result, the increased V_I in the high-temperature-stressed leaves may indicate a relative change in the proportion of Q_B-non-reducing PS2 reaction centers, not necessarily an increase in the absolute amount of the Q_B-non-reducing PS2 centers.

Suppression of P level could serve as a useful qualitative indicator for inhibition of OEC activity under heat (Pospíšil and Tyystjärvi 1999, Pospíšil and Dau 2000). We examined the simultaneous decline in F_P and increase in V_K/V_J. Our results indicated damage to OEC under heat in the leaves of the elm seedlings. We believe that high temperatures may damage OEC and alter the structure of PS2, resulting in decrease in amount of the functional PS2 reaction centers and suppression of electron transport at the acceptor side of PS2. Our results are in agreement with earlier findings of Pospíšil and Tyystjärvi (1999). Since OEC was more vulnerable to heat in the young leaves than in the mature ones, heat damaged OEC in the young leaves might account for significant inhibition of electron transport at the acceptor side of PS2 in the young leaves.

Actually, high irradiance is often coupled with high leaf temperature in a field, especially in dry and hot weather (Long *et al.* 1994, Mulkey and Pearcy 1992, Koniger *et al.* 1995, Koniger *et al.* 1998, Jiang and Zhu 2001). Photoprotective mechanisms in the newly initiating leaves can be timely developed to protect the photosynthetic apparatus against high irradiance (Yoo *et al.* 2003, Jiang *et al.* 2005). However, destruction to photo-

synthetic apparatus still occurred in the newly expanding leaves under field conditions in summer. We believe that higher sensitivity of PS2 to heat in the young leaves may thus help to explain why the young leaves are easy to be damaged in the field.

In summary, we showed that PS2 develops gradually but in parallel with increase of photosynthetic activities in the expanding elm leaves. Enhanced thermostability of PS2 in more mature leaves may be associated with increased stability of the donor side of PS2 complex.

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