Increased photosynthetic activities and thermostability of photosystem II with leaf development of elm seedlings (Ulmus pumila) probed by the fast fluorescence rise OJIP

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Abstract

Experiments were conducted to investigate the photosynthetic activity and thermostability of photosystem II (PSII) in elm seedling (Ulmus pumila) leaves from initiation to full expansion. During leaf development, photosynthesis, measured as CO₂ fixation, increased gradually and reached a maximum value when leaves were fully developed. In parallel with the increase of carbon assimilation, chlorophyll content increased. The chlorophyll a fluorescence measurements showed that the maximum quantum yield of PSII primary photochemistry (ϕp), the efficiency with which the energy of trapped excitons is converted into the electron transport beyond QA (Ψo) and the quantum yield of electron transport beyond QA (ϕEo) increased gradually. The low light experiments confirmed these results independently. When subjected to heat stress, young leaves exhibited progressively lower ϕp and maximal fluorescence (Fm) values with considerably higher minimal fluorescence (Fo) than mature leaves, demonstrating that PSII in newly initiating leaves is more sensitive to heat stress. Further analysis revealed that PSII structure in newly initiating leaves showed a robust alteration under heat stress, which was reflected by the clear K phase in the OJIP curves. Therefore, we suggest that the enhanced thermostability of PSII in the case of leaf growth might be associated with an improvement of the stability of the oxygen-evolving complex (OEC) to heat stress during leaf development.

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1. Introduction

During leaf development, the formation of chloroplast ultrastructure, chlorophyll accumulation and the synthesis of the major components of the photosynthetic apparatus proceed almost in parallel and often result in a proportional increase of net photosynthesis (Šesták, 1985). Photosystem II (PSII) is one of the major protein complexes of the photosynthetic apparatus of higher plants. During chloroplast maturation, Guenther and Melis (1990) observed different developmental states of the PSII complex. Recently, Choinski et al. (2003) noticed that the maximum quantum yield of PSII primary photochemistry increases along with leaf growth. Here, attention has been paid to the development of PSII complexes under natural conditions. In contrast, most previous investigations concentrated on plants growing under artificial conditions with suboptimal irradiance levels (Guenther and Melis, 1990; Lebkuecher et al., 1999; Srivastava et al., 1999). Therefore, the development of photosystem II during leaf growth under field conditions is not completely understood. In this study, we grew plants under natural irradiance to explore the development of the PSII during the process of leaf growth.

Photosystem II is very sensitive to environmental stresses (Verhoeven et al., 1999; Jiang et al., 2002). Photosystem II complexes in newly initiating leaves are not yet fully devel-
2. Material and methods

2.1. Plant materials

Experiments were performed at the Duolun Ecosystem Research Station of the Chinese Academy of Sciences, which is located in Dunlun County, the Inner Mongolia. Elm (Ulmus pumila), one of the widely distributed tree species in the Inner Mongolian highland, was selected as plant materials. Elm seedlings were grown in the field, where a sandy soil was used. Nutrients and water were supplied sufficiently throughout, thus potential nutrient and drought stresses were avoided. All measurements were conducted in the early days from June to July. Several newly initiating single leaves, which developed on midday, were used. This means that chlorophyll a fluorescence was measured.

2.3. Measurement of photosynthesis

Photosynthetic CO₂ fixation (Pn) was measured at a temperature of about 25 °C and at ambient CO₂ concentration (350 μmol mol⁻¹) with a portable system (LCA-4, ADC, Hoddesdon, UK). Measurements were performed at 10:00 a.m. at a photosynthetic photon flux density of 1500 μmol m⁻² s⁻¹.

2.4. Prompt fluorescence induction OIP kinetics at low light intensity

Chlorophyll a fluorescence transients (OIP) were measured by a plant efficiency analyzer (PEA, Hansatech, UK) in dark-adapted samples suddenly illuminated with low red light (30 μmol m⁻² s⁻¹). Fluorescence transients were recorded during a 5 s light pulse provided by an array of six light-emitting diodes. For typical OIP curves, the step I shows the accumulation of Q₅⁻ as a multiple turn over event. It is suggested that the initial rise from O to I level is related to a reduction of the primary quinone electron acceptor Q₅ in those PSI centers, which cannot reduce the subsequent quinone acceptor QＢ. These PSI centers are termed as "Q₅-non-reducing" PSI centers (Melis, 1985; Chylla and Whitmarsh, 1989). Cao and Govindjee, 1990. Thus, the relative variable fluorescence at about 30 ms (plateau I of the OIP transient, \(V₁ = (F₉₀₋₃₀ − F₀₋₃₀)/F₀₋₃₀\)) is suggested as an approximate estimation of the fraction of Q₅-non-reducing PS II centers (Melis, 1985; Chylla and Whitmarsh, 1989; Cao and Govindjee, 1990; Lebkuecher et al., 1999; Tomek et al., 2003; Chen et al., 2004). In fact, the increase of the intermediate fluorescence level \(V₁\) is not only attributed to...
Qb-non-reducing centers but also attributed to the equilibrium between Qb and Qh in functional reaction centers (Hsu and Lee, 1991). In addition, the initial rise from the O to I level is also slightly modulated by the state of the oxygen-evolving complex (Hsu, 1993). Considering both of the contributions are relatively small and constant, we assumed in this study that the changes of Vt were mainly attributed to Qb-non-reducing centers.

2.5. Polyphasic fluorescence induction OJIP at high light intensity

The polyphasic rise of fluorescence transients (OJIP) were measured with a plant efficiency analyzer (Hansatech). Fluorescence transients were recorded during a 60 s pulse of red radiation \(2880 \mu\text{mol (photon) m}^{-2} \text{s}^{-1}\) provided by an array of six light-emitting diodes. The fluorescence signals were recorded within a time scan from 10 \(\mu\text{s}\) to 1 s with a data acquisition rate of 100 readings/ms for the first 2 ms, and 1 readings/ms after 2 ms. All the measurements were done on fully dark-adapted leaves.

Each polyphasic rise of fluorescence transients were O-J-I-P, which was analyzed according to the JIP-test (Strasser et al., 1995, 2000; Strasser, 1997). The following original data were retained: maximum fluorescence intensity \((F_m)\); the fluorescence intensity at 50 \(\mu\text{s}\) considered as minimum intensity \((F_o)\); the fluorescence intensity at 300 \(\mu\text{s}\) (K step) and the fluorescence intensity at 2 ms (J step). The maximum quantum yield of primary photochemistry \((\varphi_{po} = 1 - F_o/F_m)\), the efficiency with which the energy of a trapped exciton is converted into the electron transport beyond Qh \((\psi_o = 1 - V_j)\), the quantum yield of electron transport beyond Qh \((\varphi_Eo = \varphi_{po} \cdot \psi_o)\) and the relative variable fluorescence \((V_t)\) at 300 \(\mu\text{s}\) (K step) and at 2 ms (J step) as \(V_t = (F_t - F_{50\mu})/(F_m - F_{50\mu})\) were all calculated according to Strasser et al. (1995, 2000).

2.6. Pigment analysis

Leaf chlorophyll was extracted with 80% acetone and the extracts were analyzed with an UV-120 system (SHIMADZU, Japan) according to Arnon (1949).

Each experiment was conducted at least three times independently.

3. Results

3.1. Changes in photosynthesis and chlorophyll content during leaf development

Leaf development was evaluated by the measurement of photosynthetic CO2 fixation and chlorophyll content. Fig. 1 showed the changes in photosynthetic activity and total chlorophyll content on a leaf area basis during leaf growth. Elm leaves at 3 days after initiation had a low photosynthetic activity \((P_n)\) that increased continually with leaf growth. The value of \(P_n\) reached the maximum level on the 11th day after leaf emergence (Fig. 1A). Correspondingly, the total chlorophyll content per unit leaf area increased steadily with leaf development (Fig. 1B), which exhibited a similar pattern to the photosynthetic activity.

3.2. Changes in photosystem II during leaf development

The development of photosystem II complexes (PSII) during leaf growth was characterized in terms of JIP-test parameters. The JIP-test is a screening procedure that is based on the measurement of the fast fluorescence rise. It provides information about the structure and function of photosystem II (Strasser et al., 1995, 2000).

The maximum quantum yield of primary photochemistry \((\varphi_{po})\) measured in fully dark-adapted leaves increased slightly with leaf development, with the values in the fully developed leaves being a little higher than that in the youngest leaves (Fig. 2). The chlorophyll a fluorescence transient is a rich and complex signal (Fig. 2A and B). Besides \(\varphi_{po}\), other parameters calculated through the polyphasic rise of fluorescence transients are also shown in Fig. 2C. Evidently, both the efficiency with which the energy of a trapped exciton is converted...
Fig. 2. (A) The polyphasic chlorophyll fluorescence transients in fully dark-adapted leaves during leaf growth. (B) The relative variable fluorescence $V_t$ between the minimal fluorescence ($F_o$) and the maximum fluorescence ($F_m$), (C) The maximum quantum yield of primary photochemistry ($\phi_{po}$), the efficiency with which the energy of a trapped exciton is converted into electron transport beyond $Q_A$ ($\Psi_o$) and the quantum yield of electron transport beyond $Q_A$ ($\phi_{Es}$) during leaf growth. Measurements were performed at 7:00 a.m. after dark-adaptation. The plants were day light adapted at 7:00 a.m. and then leaves were dark-adapted within the leaf clips for 20 min. Values are means ± S.E. (n = 6).

Fig. 3. The proportion of the relative variable fluorescence at 30 ms ($V_t$) during leaf growth. The insert: the prompt fluorescence induction OIP kinetics at low light intensity was also measured during leaf growth. The OIP kinetics showed that the $V_t$ declined considerably with leaf growth (Fig. 3). Indicating that young leaves contained a significantly higher proportion of $Q_B$-non-reducing reaction centers during the initial stages of leaf growth.

3.3. Changes of the maximum quantum yield of primary photochemistry, minimum fluorescence and maximum fluorescence under heat stress

Fig. 4 showed changes in the maximum quantum yield of PSII primary photochemistry ($\phi_{po}$), minimum fluorescence ($F_o$) and maximum fluorescence ($F_m$) in dark-adapted leaves with increasing temperature. From 25 to 30 °C, neither young nor mature leaves showed a reduction in $\phi_{po}$. When the temperature was increased to 35 °C, $\phi_{po}$ in the youngest leaves began to decrease strongly. In mature leaves, a significant decrease of the $\phi_{po}$ was only observed after a further increase of the temperature to 40 °C. More importantly, at leaf temperature higher than 35 °C young leaves always exhibited lower $\phi_{po}$ levels than mature leaves (Fig. 4A). With increasing temperature young leaves also showed a larger increase in $F_o$ and a greater decrease in $F_m$ than in mature leaves (Fig. 4B and 4C), indicating that the lower values of $\phi_{po}$ in young leaves resulted from a considerable increase in $F_o$ and a significant decrease in $F_m$.

3.4. Changes of the $V_{J}/V_{I}$ and $V_t$ under heat stress

Further analyses were carried out to explore the responses of the K and I steps of the chlorophyll $a$ fluorescence transients to elevated temperatures. As showed in Fig. 5, a great enhancement of the K step was clearly noted when subjected to a leaf temperature of 45 °C. This phase, occurring at around
Fig. 4. The maximum quantum yield of primary photochemistry (\(\phi_{po}\)) (A), the minimal fluorescence (\(F_o\)) (B) and the maximum fluorescence (\(F_m\)) (C) in young and mature leaves exposed to various temperatures in the dark for 15 min. Values are means ± S.E. (\(n = 5\)).

300 μs, could be used as a specific indicator of damage to the OEC (Strasser, 1997; Lazar et al., 1999; Lazar and Pospíšil, 1999; Strasser et al., 2000). The changes in the amplitude in the K step, expressed as the ratio \(V_K/V_J\), are presented in Fig. 6. In the 35–45 °C temperature range young leaves exhibited a higher increase in the ratio of \(V_K/V_J\) than mature ones, indicating that OEC of PSII in young leaves was more sensitive to heat stress, but that this sensitivity declined during leaf development.

Fig. 7 shows changes in the relative variable fluorescence at 30 ms at low light intensity when subjected to elevated temperatures. No clear changes in \(V_1\) were observed in the range of 25–30 °C both in young and mature leaves, but \(V_1\) in young leaves started to increase significantly when the temperature was further increased. At leaf temperatures above 35 °C, young leaves exhibited much higher \(V_1\) levels than mature ones (Fig. 7A). Since \(V_1\) is determined as: \(V_1 = (F_{30\text{ms}} - F_{50\text{ms}})/(F_P - F_{50\text{ms}})\), \(V_1\) may be greatly influenced by the level of \(F_P\). In fact, it was observed in this study that the increased \(V_1\) under the high temperature was accompanied by a noticeably decrease in \(F_P\). If the values of \(F_P\) at various temperatures were assumed to be constant, equaling the value at 25 °C, then quite different results were observed (Fig. 7B). In that case the values of \(V_1\) increased only slightly as a consequence of the heat stress and no difference between young and mature leaves was observed.
Fig. 6. Effect of high temperature on the ratio of $V_K$ to $V_J$ in young and mature leaves. Leaves were treated for 15 min in darkness at various temperatures. Values are means ± S.E. ($n=5$).

Fig. 7. Effect of high temperature on the proportion of the relative variable fluorescence at 30 ms ($V_I$) in young and mature leaves. $V_I$ calculated using $F_P$ at various temperatures (A), $V_I$ calculated using $F_P$ at 25°C (B). Leaves were treated for 15 min in darkness at various temperatures. Values are means ± S.E. ($n=5$).

4. Discussion

4.1. Development of PSII complex

Most dicotyledonous plant leaves show increases in photosynthetic activities and chlorophyll content during leaf development (Choinski et al., 2003). Our observations confirmed this (Fig. 1). Choinski et al. (2003) observed that in younger leaves $\phi_{po}$ was lower than in mature ones, whether the measurements were done at midday or 2 h after sunset. They argued that lower $\phi_{po}$ values could reflect some degree of chronic photo-inhibition during leaf development. In our case, the determination of $\phi_{po}$ was carried out at 7:00 a.m. with dark-adapted leaves. Therefore, the effect of strong irradiance was avoided. Our results suggest that the slightly lower $\phi_{po}$ in young leaves compared to mature ones can not be attributed to chronic photo-inhibition, but is a reflection of the different developmental states of PSII complexes during leaf growth.

Interestingly, Lebkuecher et al. (1999) reported that $\phi_{po}$ increased strongly during PSII development, which is somewhat inconsistent with the result in this study. Our data revealed that $\phi_{po}$ in mature leaves was only 10% higher than that in the youngest leaves (Fig. 2). What is the cause of this difference? We noticed that etiolated seedlings were tested in the experiment of Lebkuecher et al. (1999), thus their results may not correctly reflect the development of PSII in the real field-grown plants.

Though young leaves had only slightly lower $\phi_{po}$ than mature ones (Fig. 2), the values of $\Psi_o$ and $\phi_Eo$ in young leaves, especially in newly initiating leaves, were significantly lower than in the mature leaves (Fig. 2), revealing that the activity of electron transport beyond $Q_A$ was considerably hindered. In $Q_B$-non-reducing reaction centers, electron transfer between $Q_A$ and $Q_B$ does not function (Chylla and Whitmarsh, 1989; Cao and Govindjee, 1990; Lebkuecher et al., 1999). During leaf development, $V_I$ at low light intensity, which reflects the proportion of the $Q_B$-non-reducing reaction centers, significantly decreased in parallel with a pronounced improvement in $\Psi_o$ and $\phi_{po}$ (Figs. 2 and 3). Therefore, it is easily deduced that the higher proportion of $Q_B$-non-reducing reaction centers in young leaves could be responsible for the low electron transport beyond $Q_A$ (Fig. 3). Considering that the activity of electron transport increased gradually in parallel with the development of photosynthesis during leaf growth (Figs. 1 and 2), we believe that the conversion of $Q_B$-non-reducing reaction centers to functional centers might partly explain the gradual increase of photosynthesis.

4.2. Thermostability of PSII complex to heat stress

The thermostability of PSII at different developmental stages was examined by analyzing the prompt fluorescence induction kinetics, that have been verified to be a useful, reliable and non-invasive method to assess PSII functions (Krause and Weis, 1991; Lazár et al., 1999; Strasser et al., 2000). In this study, heat stress had a significant effect on $\phi_{po}$ in young leaves (especially in newly initiating leaves), revealing that PSII in young leaves was more sensitive to heat stress than in mature ones (Fig. 4). The decreased thermostability of PSII reaction centers in young leaves may be associated with the OEC of PSII, as reflected by a pronounced step...
K in the polyphasic fluorescence transients of young leaves (Figs. 6 and 7). Because PSII in the young leaves was not yet fully developed (Figs. 2 and 3), it is reasonable to deduce that the higher sensitivity of the OEC of PSII in young leaves to heat stress may be associated with the developmental state of PSII. Along with leaf maturation, the thermostability of PSII reaction centers was gradually improved (Fig. 7).

It has been shown that not only the donor side but also the acceptor side of PSII reaction centers is sensitive to heat stress, whether measured in vitro or in vivo (Joshi et al., 1995; Pospíšil and Tyystjärvi, 1999; Lu and Zhang, 1999; Sinsawat et al., 2004). Thus, the variations of the electron transport at the acceptor side of PSII were examined at various temperatures. In some papers, an increased Vf was used as a probe for the inhibition of electron transport at the acceptor side of PSII under heat stress (Lu and Zhang, 1999; Chen et al., 2004). On the basis of this parameter, the significantly increased Vf in young leaves indicated that heat stress strongly reduced electron transport at the acceptor side of PSII with increasing temperature (Fig. 7A). However, the large increase in Vf under high temperatures was strongly reduced when calculated on the assumption of a constant Fv/Fm (Fig. 7B), indicating the enhanced Vf values may result from a decrease in Fv. It has been shown that the suppression of the P level could also serve as a useful qualitative indicator for inhibition of OEC activity as a consequence of heat stress (Pospíšil and Tyystjärvi, 1999; Pospíšil and Dau, 2000). Simultaneously, the decline in Fv/Fm was accompanied by an increase in Vf/Vm in this study (Figs. 4 and 6). Therefore, we deduced that strongly decreased electron transport activity at the acceptor side of PSII might be correlated damage to the OEC under heat stress. High temperatures may damage the OEC, then alter the structure of PSII and result in an inhibition at the acceptor side of PSII (Pospíšil and Tyystjärvi, 1999). Since the OEC was more vulnerable to heat stress in young leaves than in mature ones (Figs. 5 and 6), we believe it might be responsible for the significant inhibition of electron transport at the acceptor side of PSII in young leaves (Fig. 7).

5. Conclusions

During leaf growth, PSII develops gradually paralleled by an increase of photosynthesis. The enhanced thermostability of photosystem II in the case of leaf growth may be associated with the increased stability of the donor side of the PSI complex.

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