Development of photosystems 2 and 1 during leaf growth in grapevine seedlings probed by chlorophyll *a* fluorescence transient and 820 nm transmission *in vivo*

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Abstract

Chlorophyll (Chl) *a* fluorescence transient and 820-nm transmission kinetic were investigated to explore the development of photosynthetic apparatus in grapevine leaves from emergence to full expansion. In this study, all leaves at various developing stages exhibited typical Chl *a* fluorescence transient. In newly initiating leaves, the maximum quantum yield of primary photochemistry (φ_{P0}) was slightly lower (<10 %) than that in fully expanded leaves. Nevertheless, the fluorescence rise from O to J step was clearly speeded up in young leaves compared with that in fully expanded leaves. Additionally, a distinct K step appeared in young leaves at high irradiances. With leaf development, the efficiency that a trapped exciton can move an electron into the electron transport chain further than Q_A^- (Ψ_0), the quantum yield of electron transport beyond Q_A (φ_{E0}), electron transport flux per excited cross section (ET_0/CS_0), the amount of active photosystem (PS) 2 reaction centres per excited cross section (RC/CS_0), and the performance index on cross section basis (PI_{CS}) increased gradually and rapidly. Young leaves had strikingly lower amplitude of transmission at 820 nm. A linear relationship between Ψ_0 and the transmission at 820 nm (I_{30}/I_0) was evident. Based on these data, we suggest that (*1*) the primary photochemistry of PS2 may be not the limiting step of the photosynthetic capacity during leaf growth under natural irradiance; (*2*) oxygen evolving complex (OEC) might be not fully connected to PS2 at the beginning of leaf growth; (*3*) though there are a few functional PS1 and PS2 at the early stages of leaf development, they match perfectly.

Additional key words: irradiance; leaf age; oxygen evolving complex; performance index; reaction centre; *Vitis*.

Introduction

The development of the photosynthetic apparatus has been widely studied in both higher plants and algae. Photosystem (PS) 2, as one of the major protein complexes in photosynthetic apparatus in higher plants, has been carefully investigated during leaf development and much advancement has been made in this field. Guenther and Melis (1990) observed different developmental status of PS2 complex and quick increase in the maximum quantum yield of PS2 primary photochemistry during chloroplast maturation. After that, Srivastava *et al.* (1999) found that plants growing under 1-ms flashes every 15 min fully construct small PS2 units with inner antenna, while oxygen evolving complex (OEC) is not functionally connected to PS2. The development of Hill activity and fully functional PS2 reaction centres (RCs) can be finished in few minutes after transferring plant grown under flash to continuous irradiance (Ogawa *et al.* 1973, Franck *et al.* 1984, Srivastava *et al.* 1999). We noticed that most previous investigations, however, were focused primarily on etiolated plants growing in darkness or flashes (Guenther and Melis 1990, Lebkuecher *et al.* 1999, Srivastava *et al.* 1999). The development of PS2 in plants growing in natural irradiance has not been adequately investigated. Recently, Bertamini and Nedunchezhian (2003a,b) noticed that high irradiance could easily inactivate the donor side of PS2 in young leaves growing under natural irradiance. They found that the inactivation of the donor side of PS2 is due to the marked loss of 33 kDa protein of the water-splitting complex in young leaves under high irradiance. Yet, they did

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not give clear reasons why the water splitting complex in young leaves is prone to be damaged under high irradiance. In this study, we hypothesized that there might be some differences in the structure and function of PS2 between young and mature leaves even though plants grow under normal irradiance.

Monitoring transmission changes at 820 nm *in vivo*, that reflect the redox states of plastocyanin (PC) and P700, is a good technique for the measurements of PS1 activity (Schreiber *et al.* 1988, Strasser and Tsimilli-Michael 2001, Schansker *et al.* 2003). Currently, more and more attention has been focused on the fluorescence characteristics of PS1, while there is little information on the simultaneous measurement of chlorophyll (Chl) *a* fluorescence of PS1 and PS2 *in vivo*. In this study, advanced *PEA Senior* instrument measuring Chl *a* fluorescence transient and transmission at 820 nm simultaneously was utilized to evaluate the cooperation between PS2 and PS1 in leaves from initiation to full expansion.

Therefore, the objectives of this investigation were (*1*) to reveal the differences in the structure and function of PS2 between young and mature leaves growing under natural irradiance, and (*2*) to follow the cooperation between PS2 and PS1 during leaf growth.

Materials and methods

Plants: Grapevine plants (*Vitis vinifera* L.) were grown in greenhouse where the temperature was 20–25 °C during the day and 14 –16 °C at night. Plant density was 1 plant per pot. Sufficient nutrients and water were supplied throughout the experiment to avoid potential nutrient and water stresses. Measurements were performed with single leaves from emergency to full expansion. Area of leaves was determined by a leaf area meter (*LI-3000A*, *LiCor*, USA). The area of fully expanded leaf was taken as 100 %, the relative area of unexpanded leaves was expressed as percentage of fully expanded leaves.

Chl *a* fluorescence transient and 820 nm transmission were measured simultaneously using a dual channel *PEA Senior* instrument (*Hansatech Instruments*, UK). Irradiation consisted of a 1-s pulse of continuous red radiation (650-nm peak wavelength) provided by an array of four light-emitting diodes (LEDs) focused on a leaf surface with a diameter of 5 mm. Three measuring irradiances, *i.e.* 1 000, 2 000, and 3 000 μmol m^{-2} s^{-1} were utilized. The far red radiation source was a *QDDH73520 LED* (*Quantum Devices Inc.*) filtered at 720±5 nm. The modulated (33.3 kHz) far-red measuring radiation was provided by an *OD820LED* (*Opto Diode Corp.*) filtered at 830±20 nm. Chl *a* fluorescence transient and 820 nm transmission measurements were carried out according to Strasser and Tsimilli-Michael (2001) and Schansker *et al.* (2003).

Table 1. Formulae and terms used in the analysis of the O-J-I-P fluorescence induction dynamics curve.

Term and formula	Definition
$F_0 = F_{50\mu s}$	minimal fluorescence, when all PS2 RCs are open (at t = 0)
$F_m = F_p$	maximal fluorescence, when all PS2 RCs are closed
$V_t \equiv (F_t - F_0)/(F_m - F_0)$	relative variable fluorescence at time t
$V_J \equiv (F_J - F_0)/(F_m - F_0)$	relative variable fluorescence at the J-step
$M_0 \equiv 4\,(F_{300\mu s} - F_0)/(F_m - F_0)$	approximated initial slope [ms^{-1}] of the fluorescence transient
Yields or flux ratios	
$\varphi_{P0} \equiv TR_0/ABS = [1 - (F_0/F_m)]$	maximum quantum yield of primary photochemistry (at t = 0)
$\Psi_0 \equiv ET_0/TR_0 = (1 - V_J)$	probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\varphi_{E0} \equiv ET_0/ABS = [1 - (F_0/F_m)]\,\psi_0$	quantum yield of electron transport (at t = 0)
Phenomenological energy fluxes (per excited cross section – CS)	
$ABS/CS_0 \approx F_0$	absorption flux per CS, approximated by F_0
$TR_0/CS_0 = \varphi_{P0}\,(ABS/CS_0)$	trapped energy flux per CS (at t = 0)
$ET_0/CS_0 = \varphi_{E0}\,(ABS/CS_0)$	electron transport flux per CS (at t = 0)
$DI_0/CS_0 = (ABS/CS_0) - (TR_0/CS_0)$	dissipated energy flux per CS (at t = 0)
Density of reaction centres	
$RC/CS_0 = \varphi_{P0}\,(V_J/M_0)(ABS/CS_0)$	density of RCs (Q_A-reducing PS2 reaction centres)
Performance index	
$PI_{CS} \equiv (RC/CS_0)\,[\varphi_{P0}/(1 - \varphi_{P0})][\psi_0/(1 - \psi_0)]$	performance index on cross section basis
The probability for energy transfer among PS2 units (*p*)	
$P = F_{0.05ms}\,C/(F_m - F_{0.05ms})$	PS2 connectivity among PS2 units

Recorded original data include fluorescence intensity at 50 μs (the minimum intensity F_0), fluorescence intensity at 300 μs (K step), fluorescence intensity at 2 ms (J step), and peak fluorescence intensity (F_p). Terms and formulae used in the analysis of the O-J-I-P fluorescence induction dynamics curve were defined and calculated as in Table 1 according to Strasser *et al.* (1995, 2000, 2001).

Transmission of PS1 at 820 nm ($I_t/I_{t=0}$) was calculated according to Strasser *et al.* (2001) and Schansker *et al.* (2003).

Results

Changes in the maximum quantum yield of primary photochemistry (φ_{P0}): Chl *a* fluorescence induction kinetics is a good indicator for the functioning of photosynthesis in intact leaves. Thus, photosynthetic capacity of leaves at various expanding stages was examined by Chl *a* florescence transient. Fig. 1 shows the florescence induction curves plotted on linear and logarithmic time scales measured at 3 000 μmol m^{-2} s^{-1}. F_0 and the maximum fluorescence (F_m) increased significantly with the increase of leaf area from the relative area of 10 to about 40 %. Then F_0 and F_m increased slowly with increasing leaf area, and the values of F_0 and F_m levelled off when leaf area expanded to approximately 80 % of its final size (Fig. 1, Table 2). These results demonstrated the improvement of photosynthetic apparatus with leaf expansion. However, we noticed that the maximum quantum yield of primary photochemistry (φ_{p0}), calculated according to $(F_m - F_0)/F_m$, increased only slightly (<10 %) during the course of leaf growth (Table 1), suggesting that primary photochemistry of PS2 was almost fully built up at the early beginning of leaf development.

Changes of the Chl *a* fluorescence rise kinetic: The relative variable fluorescence at any time t is defined as $V_t = (F_t - F_0)/(F_P - F_0)$. This expression is taken as a measure of the fraction of the primary quinone electron acceptor of PS2 being in its reduced state. Fig. 2 depicts the variable fluorescence curves (V_t) presented on a logarithmic time scale. Obviously, the fluorescence rise from O to J step in young leaves was speeded up compared with that in fully expanded leaves (Fig. 2), reflecting that a larger fraction of the primary quinone electron acceptor of PS2 was reduced in young leaves. Here, the fast rise can be ascribed to a slowdown of electron transport beyond Q_A^-.

Under various stresses, an early step appears in the fluorescence rise at about 300–500 μs and is labelled as the K-step (Strasser *et al.* 2004). Generally, the K-step is usually 'hidden' in the O-J rise (Strasser *et al.* 2004). In order to compare the amplitude of the K-step during leaf development, the fluorescence curves were normalized between F_0 and F_J, *i.e.* $W = (F - F_0)/(F_J - F_0)$. Fig. 3 shows that the K-step in newly initiating leaves did not

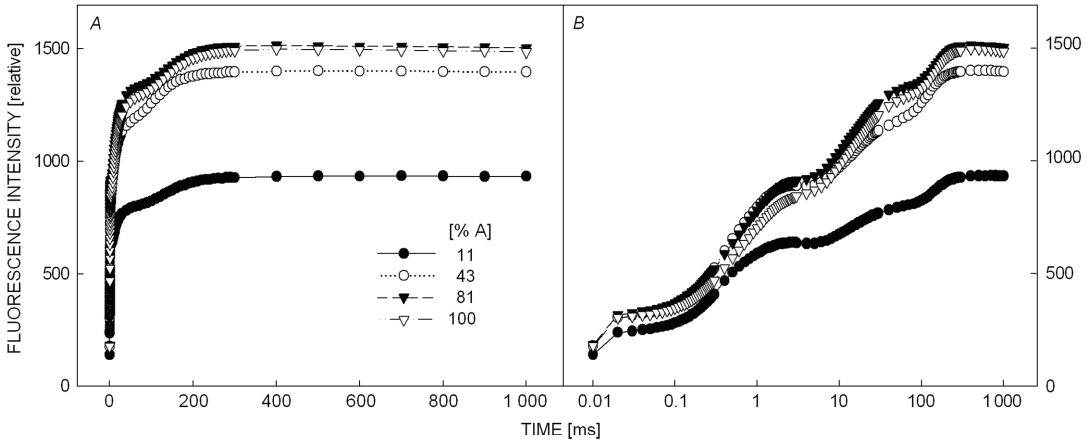


Fig. 1. The chlorophyll *a* fluorescence transients in newly expanding and fully expanded leaves of grapevine seedlings measured at different irradiances. The curves are presented on a linear (*A*) and a logarithmic (*B*) time scale. Each curve represents the averages of 5 independent measurements.

Table 2. Changes in the initial fluorescence (F_0), the maximum fluorescence (F_m), and the maximum quantum yield of primary photochemistry (φ_{P0}) in grapevine leaves from emergency to full expansion. Leaves were dark-adapted for 12 h. Each point represents the average of 5 independent measurements.

	11 % A	43 % A	81 % A	100 % A
F_0	224±16 (75.2)	288±15 (96.6)	300±11 (100)	298±18 (100)
F_m	934±35 (62.5)	1400± 47 (93.6)	1512± 31 (100)	1495±42 (100)
φ_{p0}	0.760±0.010 (94.9)	0.794±0.01 (99.1)	0.802±0.010 (100)	0.801±0.01 (100)

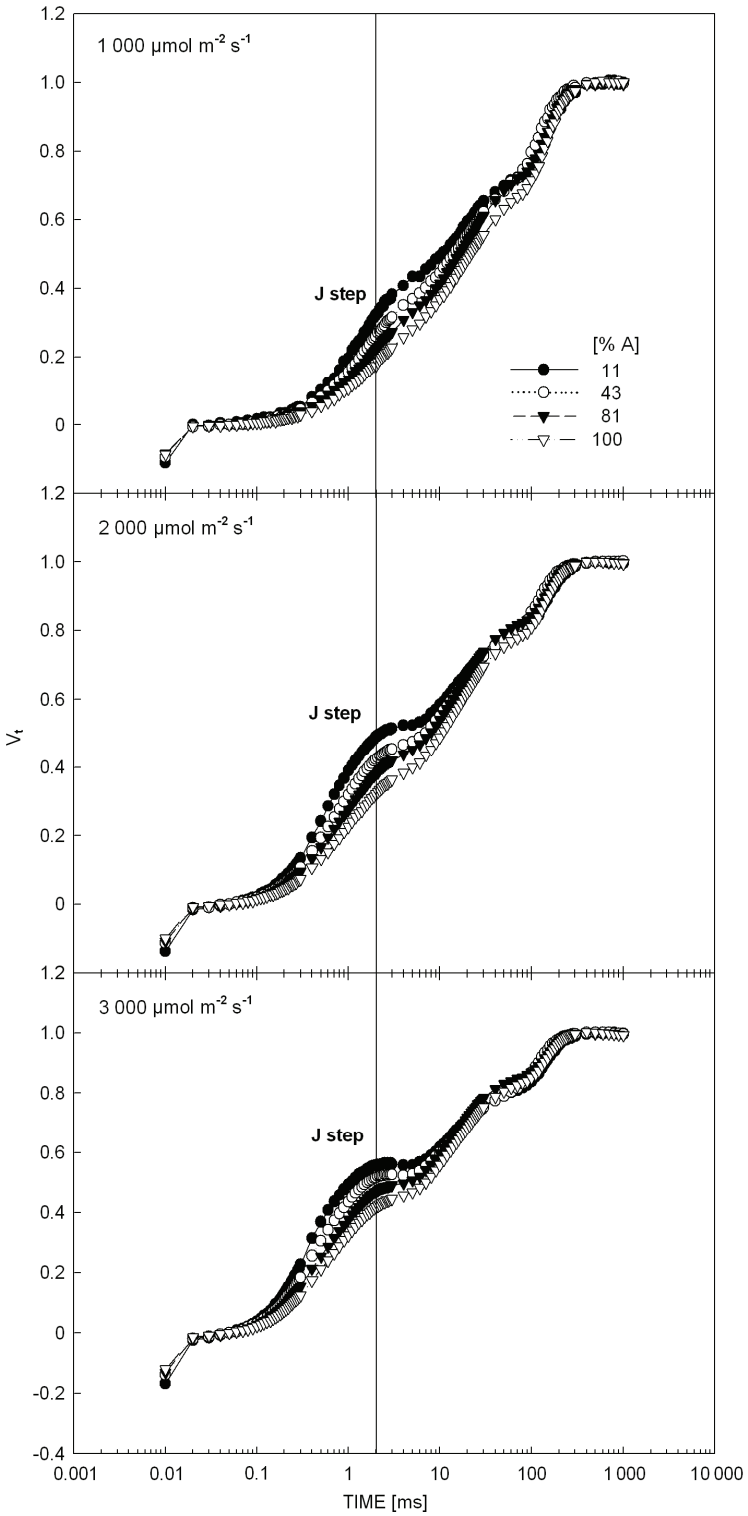


Fig. 2. The rise kinetics of the relative variable fluorescence $V_t = (F_t - F_0)/(F_m - F_0)$ in grapevine leaves from emergency to full development measured at different irradiances. Each curve represents the average of 5 independent measurements.

appear at 1 000 µmol m^{-2} s^{-1}, while it became apparent at 2 000 µmol m^{-2} s^{-1}, and became even more pronounced at 3 000 µmol m^{-2} s^{-1}. In addition, as shown in Fig. 4 young leaves indeed had a higher ratio of V_K/V_J than fully expanded leaves when measured at 2 000 and 3 000 µmol m^{-2} s^{-1}, while there were no significant differences at 1 000 µmol m^{-2} s^{-1}.

PS2 connectivity, reflecting the excitation energy transfer among PS2 units, also influences the shape of the induction curve (Stirbet *et al.* 1998). In this study, the probability for energy transfer among PS2 units (*p*) in newly initiating leaves was distinctly higher than that in mature ones. With leaf growth, the relatively higher *p* decreased rapidly (Fig. 5), indicating that PS2 was tightly

connected and could efficiently exchange excitation energy at the early developmental stages of leaf growth.

Changes in the parameters of JIP test: To exactly express the development of PS2 in leaves at various expanding stages, several parameters were calculated according to the JIP-test. The efficiency that a trapped exciton can move an electron into the electron transport chain further than Q_A^- (Ψ_0), the quantum yield of electron transport beyond Q_A (φ_{E0}), electron transport flux per excited cross section (ET_0/CS_0), the Q_A-reducing PS2 RCs per excited cross section (RC/CS_0), and the performance

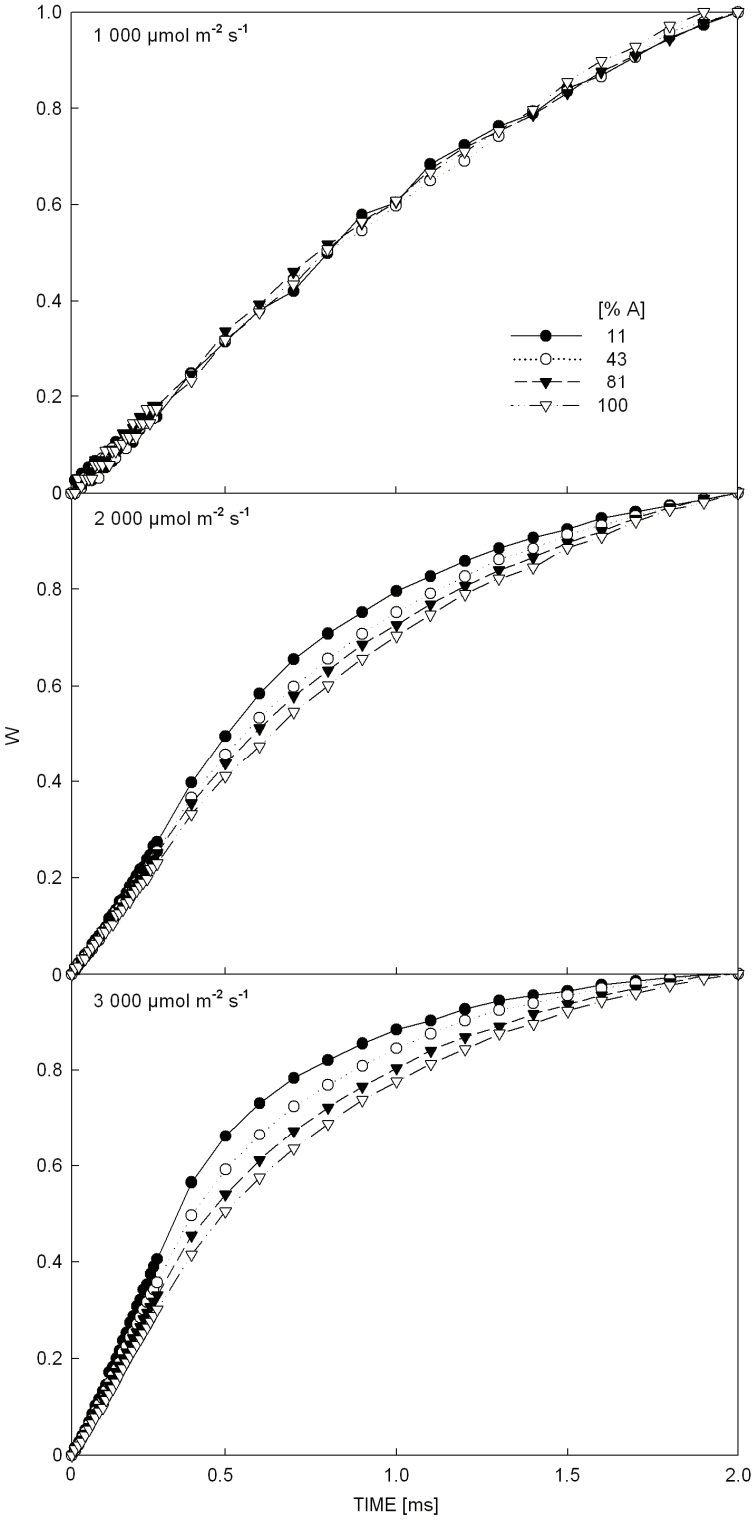


Fig. 3. Changes in the shape of the fluorescence transient curves measured at different irradiances, expressed as $W = [(F_t - F_0)/(F_J - F_0)]$, in grapevine leaves from emergency to full expansion. Each curve represents the average of 5 independent measurements.

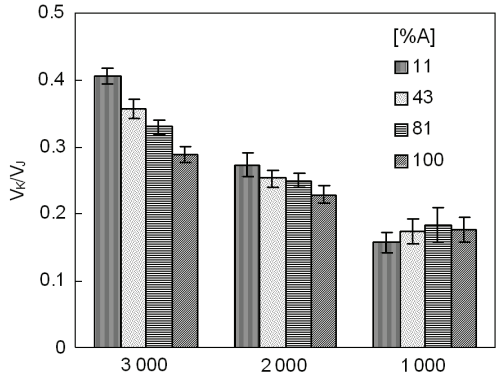


Fig. 4. Changes in the ratio of relative fluorescence at 300 μs and 2 ms (expressed as V_K/V_J) in grapevine leaves from emergency to full expansion measured at various irradiances. Each point represents the average of 5 independent measurements. Values were obtained from Fig. 2 and calculated according to $V_K/V_J = (F_K - F_0)/(F_J - F_0)$.

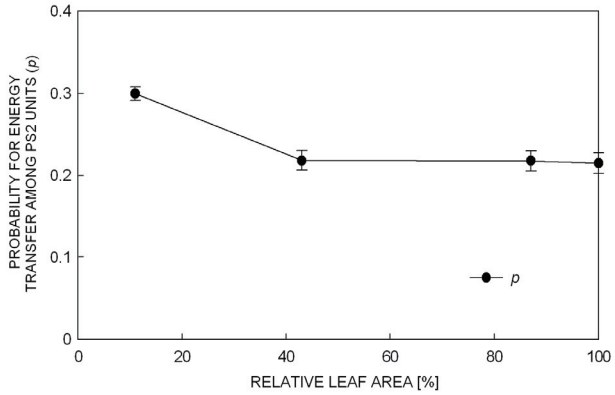


Fig. 5. Changes in the probability for energy transfer among PS2 units (*p*) in grapevine leaves from emergency to full expansion measured at irradiance of 3 000 μmol m^{-2} s^{-1}. Each point represents the average of 5 independent measurements. Values were obtained from Fig. 2*C* and calculated according to Strasser and Stirbet (2001).

index on absorption basis (PI_{CS}) are shown in Fig. 6. Evidently, young leaves had lower Ψ_0, φ_{E0}, and ET_0/CS_0 than fully expanded leaves (Fig. 6); whereas Ψ_0, φ_{E0}, and ET_0/CS_0 increased gradually with leaf development, reflecting the accomplishment of photosynthetic electron transport in PS2 complex which was strongly confirmed by progressive increase of the Q_A-reducing PS2 RCs per excited CS. In addition, all the above parameters, which describe leaf development, can be quantified by the so-called performance index on leaf area basis, PI_{CS} (Fig. 6).

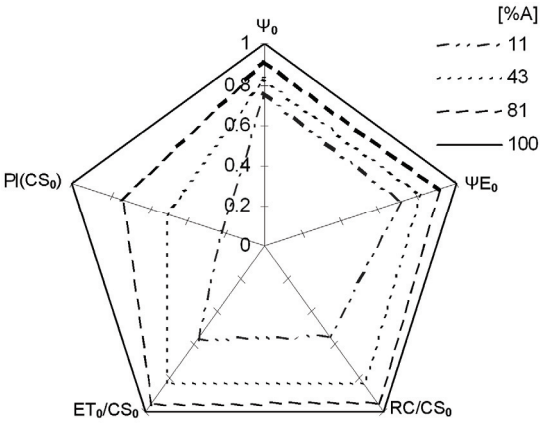


Fig. 6. Changes in the efficiency that a trapped exciton can move an electron into the electron transport chain further than Q_A^- (Ψ_0), the quantum yield of electron transport beyond Q_A (φ_{E0}), the amount of active PS2 reaction centres per excited cross section (RC/CS_0), electron transport flux per excited cross section (ET_0/CS_0), and the performance index on cross section basis (PI_{CS}) in grapevine leaves from emergency to full expansion. Each point represents the average of 5 independent measurements.

Changes in 820 nm transmission: Obviously, there were clear differences in the amplitude of transmission at 820 nm ($I_t/I_{t=0}$) in leaves at various developing stages (Fig. 7). With the increase of irradiance, the differences in the amplitude of transmission at 820 nm were enhanced. We found that newly expanding leaves always had noticeably lower amplitude of transmission at 820 nm than fully expanded leaves at different irradiances (Figs. 7 and 8), implying that less functional PS1 was developed at the early stages of leaf development. To exclude the effect of PS2 on PS1 and further test the result, far-red radiation was utilized to excite PS1 preferentially. Similarly, the amplitude of transmission at 820 nm in newly initiating leaves was also distinctly lower than that in fully expanded leaves (Fig. 9).

Discussion

The performance index (PI) is a sensitive indicator of photosynthesis (Strasser *et al.* 2000, 2001). In this study, the gradual increase of PI_{CS} with leaf expansion reflected the accomplishment of photosynthesis. This is consistent with our conclusion obtained in field grown soybean plant (Jiang *et al.* 2005). However, we noticed that φ_{P0} in fully developed leaves was only slightly higher (<10 %) than that in newly initiating leaves, though F_m values in fully developed leaves were significantly higher than in newly initiating leaves. This indicates that the primary photochemistry of PS2 is not the limiting step of photosynthetic capacity during leaf growth. Probably, the lower photosynthetic capacity in young leaves is due to absence of functional photosynthetic apparatus. The substantial increase in the number of RC/CS_0 and the accomplishment of photosynthetic electron transport in PS2 complex with leaf expansion strongly supported this opinion.

In an independent study, we found that the primary

photochemistry of PS2 is built up at the early beginning of leaf development in field (Jiang *et al.* 2005). However, Lebkuecher *et al.* (1999) reported that the primary photochemistry of PS2 drastically increases in sunflower seedlings with maturation of chloroplast. Srivastava *et al.* (1999) also obtained similar results in pea seedlings.

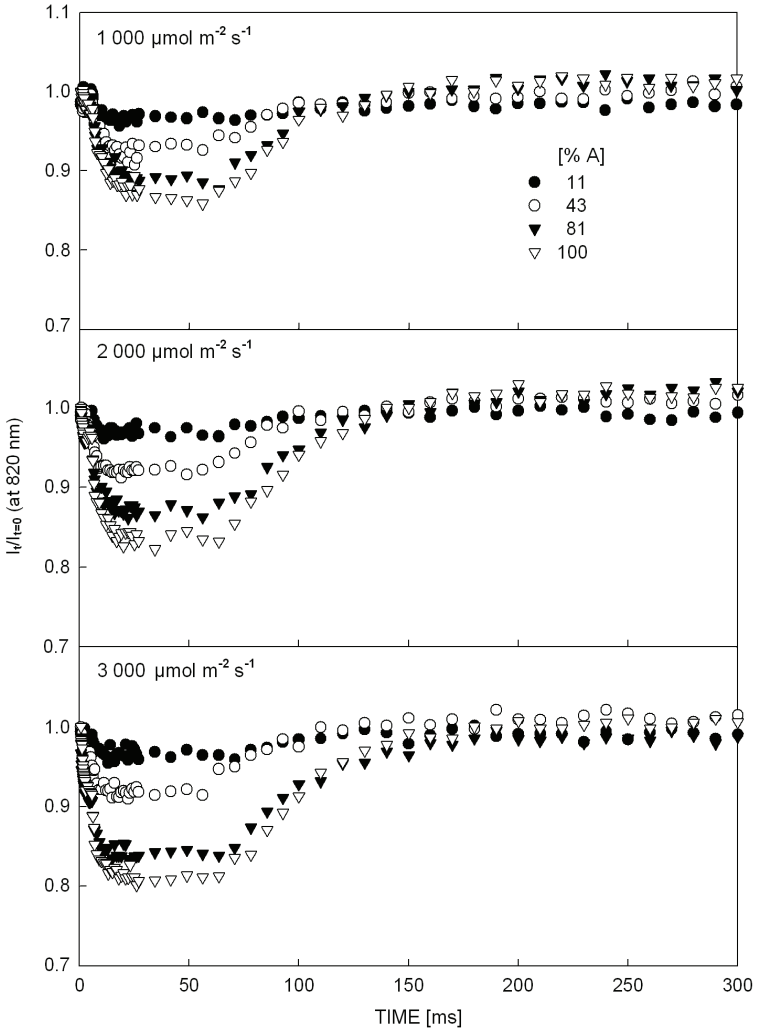


Fig. 7. Changes in the transmission of photosystem 1 at 820 nm (expressed as $I_t/I_{t=0}$) in grapevine leaves from emergency to full expansion measured at different irradiances. Each curve represents the average of 5 independent measurements.

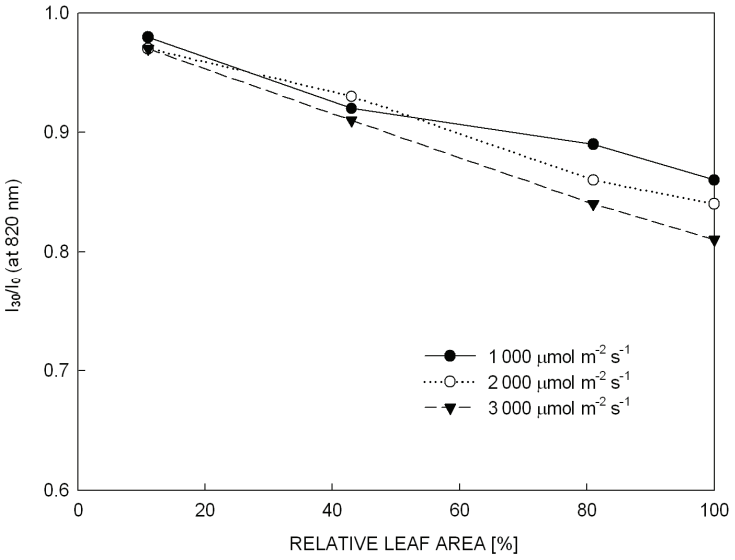


Fig. 8. Changes in the transmission of photosystem 1 at 820 nm (expressed as I_{30}/I_0) in grapevine leaves from emergency to full expansion at different irradiances. Each curve represents the average of 5 independent measurements. Values were obtained from Fig. 7 and calculated according to Schansker *et al.* (2003).

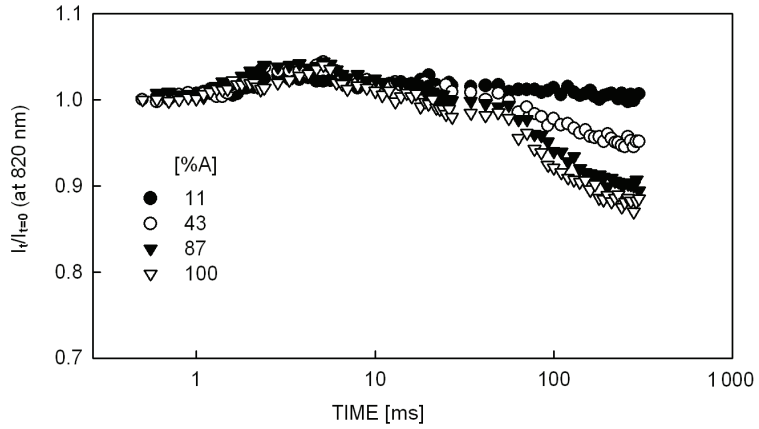
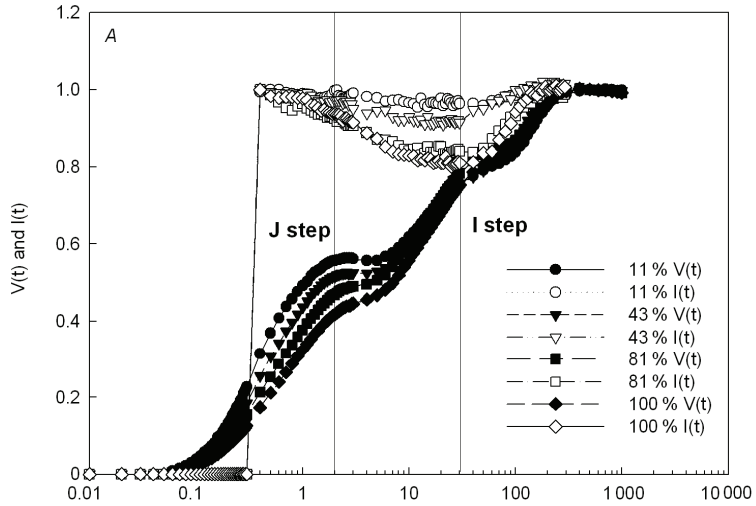


Fig. 9. Changes in the transmission of photosystem 1 at 820 nm in grapevine leaves from emergency to full expansion at far-red radiation. Each curve represents the average of 5 independent measurements.



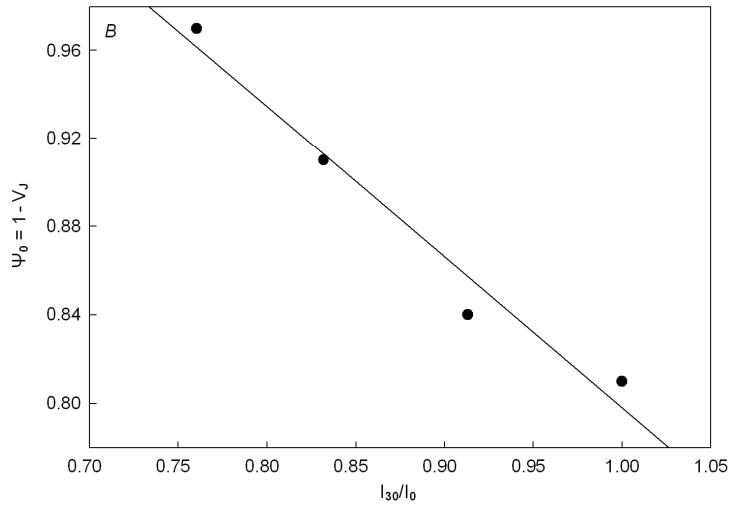


Fig. 10. (*A*) Simultaneously measured induction curves of chlorophyll *a* fluorescence and the transmission at 820 nm in grapevine leaves from emergency to full expansion. Signals plotted on a logarithmic time scale. Each curve represents the average of 5 independent measurements. (*B*) Linear relationship between the efficiency that a trapped exciton can move an electron into the electron transport chain further than $Q_A -$ ($\Psi_0 = 1 - V_J$) and the transmission at 820 nm (expressed as I_{30}/I_0). Data were obtained from Fig. 10*A*.

Evidently, these conclusions are inconsistent with our result. Photon energy is important for the development of photosynthetic apparatus (Barnes *et al.* 1996, Srivastava *et al.* 1999). Lebkuecher *et al.* (1999) and Srivastava *et al.* (1999) used etiolated seedlings that had been grown in continuous darkness or flashes, but we used grapevine seedlings grown in the greenhouse under natural irradiance. Therefore, the most likely reason for the large difference in primary photochemistry of PS2 during leaf development in our studies is due to the irradiance the seedlings were exposed to before experimental irradiation.

We found a distinct K step in young leaves at high irradiance. The appearance of K phase may be influenced by factors such as S-state of oxygen evolving complex (OEC), the acceptor side of PS2, the connectivity among PS2 units, *etc*. In our study, all leaves examined have been fully dark-adapted (12 h), which meant the OEC was in its S1 state. Thus, the K step should not be influenced by S-state. We also noticed that the fluorescence yield at J level was clearly enhanced in newly expanding leaves, reflecting the accumulation of Q_A^- at the acceptor side of PS2. In order to exclude the effect of acceptor side of PS2, the fluorescence curves were normalized between F_0 and F_J. In the normalized curves, there were still clear differences at K step between young and mature leaves at high irradiances. Meanwhile, the ratios of V_K to V_J in young leaves were also clearly higher than in fully expanded leaves. Therefore, we thought the appearance of K step is independent of the redox state of acceptor side of PS2. We also found that newly expanding leaves had relative higher *p* than fully expanded leaves, then the obvious K phase in young leaves might not result from lower connectivity among PS2 units. Considering that the appearance of the K step can be attributed to the inactivation of the OEC (Strasser *et al.* 1995, 2000, Lazár and Pospíšil 1999, Lazár *et al.* 1999), we suggest the distinct increase in the K step in newly expanding leaves at high irradiance might reflect that the functional connection between OEC and PS2 has not been fully built, or OEC might be not fully functionally connected to PS2 at the beginning of leaf growth. Probably, this is the reason why the 33 kDa protein of the water-splitting complex in young leaves is prone to be damaged by high irradiance (Bertamini and Nedunchezhian 2003a,b). Our other experiments also demonstrated that OEC of PS2 in young leaves was strikingly sensitive to heat stress, and such sensitivity was alleviated with leaf growth (Jiang *et al.* 2006). With leaf development, the connection between PS2 and OEC was improved steadily, thus the K step disappeared gradually.

The measurement of transmission at 820 nm revealed that only a few functional PS1 were developed at the initial stages of leaf growth. A comparison of Chl *a* fluorescence transient and transmission at 820 nm shows that during the fluorescence rise the transmission at 820 nm decreased firstly, and then increased again (Fig. 10*A*), indicating the initial oxidation of PC and P700 induced by red irradiation can be fully reversed in young leaves. Therefore, the electrons coming in from PS2 might not limit the reduction of PS1. The rise of transient from O to J is due to the net photochemical reduction of Q_A to Q_A^-, the efficiency by which a trapped exciton can move an electron into the electron transport chain further than Q_A^- can be calculated as $\Psi_0 = 1 - V_J$. Considering the linear relationship between Ψ_0 and the transmission at 820 nm (I_{30}/I_0) (Fig. 10*B*), we thought less functional PS1 and PS2 match perfectly at the early stages of leaf growth. With leaf expanding, the number of functional PS1 and PS2 increased rapidly and steadily.

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