Photosynthetic Responses to Heat Treatments at Different Temperatures and following Recovery in Grapevine (*Vitis amurensis* L.) Leaves

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**Abstract**

**Background:** The electron transport chain, Rubisco and stomatal conductance are important in photosynthesis. Little is known about their combined responses to heat treatment at different temperatures and following recovery in grapevines (*Vitis spp.*) which are often grown in climates with high temperatures.

**Methodology/Findings:** The electron transport function of photosystem II, the activation state of Rubisco and the influence of stomatal behavior were investigated in grapevine leaves during heat treatments and following recovery. High temperature treatments included 35, 40 and 45°C, with 25°C as the control and recovery temperature. Heat treatment at 35°C did not significantly (*P*>0.05) inhibit net photosynthetic rate (*Pn*). However, with treatments at 40 and 45°C, *Pn* was decreased, accompanied by an increase in substomatal CO2 concentration (*Ci*), decreases in stomatal conductance (*gs*) and the activation state of Rubisco, and inhibition of the donor side and the reaction center of PSII. The acceptor side of PSII was inhibited at 45°C but not at 40°C. When grape leaves recovered following heat treatment, *Pn*, *gs* and the activation state of Rubisco also increased, and the donor side and the reaction center of PSII recovered. The increase in *Pn* during the recovery period following the second 45°C stress was slower than that following the 40°C stress, and these increases corresponded to the donor side of PSII and the activation state of Rubisco.

**Conclusions:** Heat treatment at 35°C did not significantly (*P*>0.05) influence photosynthesis. The decrease of *Pn* in grape leaves exposed to more severe heat stress (40 or 45°C) was mainly attributed to three factors: the activation state of Rubisco, the donor side and the reaction center of PSII. However, the increase of *Pn* in grape leaves following heat stress was also associated with a stomatal response. The acceptor side of PSII in grape leaves was responsive but less sensitive to heat stress.

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**Introduction**

High temperature negatively affects plant growth and survival and hence crop yield. Photosynthesis is known to be one of the most heat-sensitive processes, and it can be inhibited by high temperature before other symptoms of stress are detected [1,2]. Inhibition of photosynthesis by heat stress has long been attributed to an impairment of electron transport activity, especially the inhibition of photosystem II (PSII) activity [3,4]. Heat stress not only damages the oxygen-evolving complex of PSII [5,6], but also impairs electron transfer within the PSII reaction centres [7,8,9] and downstream of PSII. Some authors [10,11] have suggested that the initial site of the inhibition is associated with a Calvin cycle reaction, especially inactivation of Rubisco [12,13,14,15]. However, for different species, the specific effects of heat stress maybe different.

Worldwide, grape has become one of the most productive and important specialty crops. In many production regions, the maximum midday air temperature can reach more than 40°C, which is especially critical at berry ripening. Some researchers suggested the optimum temperature for photosynthesis is between 25°C and 35°C for some grape cultivars [16,17]. Temperatures above 35°C generally reduce photosynthesis in grape leaves. Climate change may produce more frequent high temperature conditions close to the current northern limit of grape cultivation [18]. Extreme temperatures may therefore endanger berry quality and economic return [19]. Although there are many reports dealing with the influence of heat stress to photosynthesis in grape...
Figure 1. $P_n$, $C_i$ and $g_s$ in grape leaves under different heat treatments and following recovery. 25°C: normal growth and recovery temperature; 35, 40 and 45°C: high temperature treatments. Each value represents the mean ± S.E. of four replicates. At the same time point, the numerical values with different letters are significantly different ($P<0.05$) according to Duncan’s multiple comparison.

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photosynthesis [20,21,22,23], a very limited number of papers consider the combined response of the components of PSII, Rubisco and stomatal conductance to heat stress [24,25]. Recently, Kadir [24] and Kadir et al. [25] determined the response of Vitis species to high temperature under controlled environmental conditions through chlorophyll fluorescence measurements such as Fv/Fm, Fv/Fo, and Fp. It is not known if inhibition of grape photosynthesis by heat stress is caused by an impairment of electron transport or Rubisco activity. Moreover, the effect of heat on the donor side, acceptor side, reaction center, and energy partitioning of PSII in grapevine is not clear. In contrast, studies about effects of low temperatures on photosynthetic performance of grape leaves from electron transport and energy partitioning are relatively abundant [23,26,27]. In addition, recovery from heat stress is an important factor of heat tolerance in plants. Including grapevine, may be most likely to experience heat stress around midday, and relatively normal temperatures otherwise. Thus, plants may be exposed to a heat stress – recovery – heat stress – recovery cycle. However, less information is available on the plant behavior under the heat-stress recovery compared with under heat stress. In the past, attention usually was focused on the plant’s direct response to stress. Consequently, more definitive studies on the plant traits for heat tolerance must be conducted to understand the mechanism of the recovery from heat. These results may help develop modern and acceptable technologies to increase and stabilize berry yield and qualities.

Inhibition of PSII leads to a decrease in variable chlorophyll fluorescence. Thus, in vivo chlorophyll fluorescence may be used to detect changes in the photosynthetic apparatus [28,29]. Strasser et al. have developed a method for the analysis of the kinetics of fast fluorescence increases since the non-destructive measurements can be done with a high resolution of 10 μs [30]. All oxygenic photosynthetic materials investigated so far show a polyphasic fluorescence rise consisting of a sequence of phases, denoted as O, J, I, and P (OJIP test). With this test, it is possible to calculate several phenomenological and biophysical expressions of PSII. The kinetics of OJIP are considered to be determined by changes in the redox state of Qs, but at the same time, the OJIP transient reflects the reduction of the photosynthetic electron transport chain [31]. The OJIP test has been a powerful tool for the in vivo investigation of the behavior of PSII function including energy absorption, trapping, and electron transport [30,32,33].

In the present study, gas exchange parameters, chlorophyll fluorescence parameters and the activity state of Rubisco in grape leaves during high temperature (35, 40 or 45°C) treatments and following recovery (stress-recovery-stress-recovery) were investigated. Our objective was to determine the importance of electron transport, Rubisco and stomatal factors to maintain photosynthesis and the sensitivity of components of the photosynthetic apparatus in grape leaves under high temperature stress and during recovery.

Results

Net photosynthesis rate (Pn), substomatal CO2 concentration (Ci) and stomatal conductance (gs) At normal growth conditions of 25°C, Pn, Ci and gs of grape leaves did not change during the experiment (over the 3 days of the growth period monitored). Heat stresses at 35°C at two times did not significantly (P>0.05) influence Pn, Ci and gs of grape leaves compared with the control (at 25°C). A decline of Pn and gs after 40 and 45°C treatments was observed, accompanied with a Ci increase. Heat stress at 45°C had stronger negative impact on Pn and gs than 40°C and recovered more slowly. On the fourth day of recovery (Day 6) after the second heat stress, Pn, Ci and gs of plants that had received a 40°C treatment recovered to the control levels, but those exposed to 45°C were still exhibiting an effect of heat stress (Fig. 1).

Donor side, reaction centre and acceptor side of PSII and PSI It has been shown that heat stress can induce a rapid rise in the OJIP polyphasic fluorescence transients. This phase, occurring at around 300 μs and labeled K, is caused by an inhibition of the oxygen evolving complex (OEC). The amplitude of step K can therefore be used as a specific indicator of damage to PSII donor side [26]. Fig. 2 shows the changes in the amplitude in the K step expressed as the ratio Wk. Compared with the control (25°C), heat stress at 35°C did not alter Wk of grape leaves. After the first heat treatment of 40°C or 45°C for 5 h, Wk of grape leaves increased steeply, and Wk was higher at 45°C than at 40°C. During the following recovery (on Day 2), Wk values of these treatments were similar to the control level. However, they rapidly increased again after the second heat stress. On the first day of recovery (Day 3), they declined to some extent, but Wk of the 45°C treatment was bigger than that of the 40°C treatment. On the fourth day of recovery (Day 6), Wk of the 40°C and 45°C treatments recovered to the control level.

RCQA shows the density of the Qx-reducing PSII reaction centers. Fig. 2 demonstrates that heat stress at 35°C did not influence the RCQA during the experiment. The first (on Day 1) and second (on Day 2) stresses of 40°C or 45°C significantly (P<0.05) reduced the RCQA. The RCQA values of the two treatments returned to control values during the first recovery. After the second stress, RCQA values of the two treatments basically reached the level of controls during recovery of the first day.

Fig. 3 demonstrates the changes in maximum quantum yield for primary photochemistry (ϕps), the quantum yield for electron transport (ϕet), the probability that a trapped exciton moves an electron into the electron transport chain beyond QA (ϕDi), the quantum yield for dissipated energy (ϕdi) in grape leaves during high temperature stress and recovery. Heat stress at 35°C did not significantly (P>0.05) alter ϕps, ϕet, ϕDi and ϕdi in grape leaves. ϕps significantly declined while ϕet was enhanced at the end of the first and second heat stress of 40°C and 45°C. However, at the same time, ϕet and ϕDi showed no change at 40°C, but decreased at 45°C compared with the control. ϕps decreased and ϕDi rose at 40°C less than at 45°C at the first stress. However, ϕps and ϕDi at 40°C was similar to those at 45°C at the second stress. After both stress periods, these parameters recovered to control levels by the first day (Day 2 and Day 3) of recovery.

δRo expresses the redox state of PSI, i.e., the efficiency with which an electron from PQ through PS I to reduce PS I end electron acceptors. Heat stress at 35°C and 40°C did not change the δRo in grape leaves, but the δRo at 45°C rose significantly (P<0.05). However, these parameters recovered to control levels in the first day of recovery (Fig. 4).

PSII efficiency and excitation energy dissipation PSII efficiency and excitation energy dissipation in grape leaves was examined by modulated fluorescence techniques. Fig. 5 shows that heat stress at 35°C had no effect on the actual PSII efficiency (ϕPSII), photochemical quenching coefficient (ϕq), as well as non-photochemical quenching (NPQ). Heat stress at 40 and 45°C led to a sharp decrease of ϕPSII and ϕq, and a striking increase of NPQ. After the first 40°C stress, NPQ, ϕPSII and ϕq recovered to the control levels the following day (Day 2). With a 1 d recovery after the second 40°C stress, ϕPSII slowly rose while NPQ declined.
to some extent, but $q_p$ reached the control level. On the fourth day of recovery (Day 6), NPQ, $\Phi_{PSII}$ and $q_p$ had recovered to control levels. With the 45°C stress, NPQ, $\Phi_{PSII}$ and $q_p$ changed more dramatically, and recovered more slowly after the second stress although they had recovered to the control levels after the first stress.

The activation state of Rubisco

As shown in Fig. 6, heat stress at 35°C had no influence on the activation state of Rubisco in grape leaves compared with 25°C. When the grape leaves were exposed to 40 or 45°C the first time, the Rubisco activation state declined significantly ($P<0.05$), and 45°C led to the bigger decline. However, after 1 d of recovery, the Rubisco activation state recovered to the control level. When these grape leaves were exposed to 40 or 45°C a second time, the Rubisco activation state declined more than after the first stress, with 45°C resulting in a sharper decrease. On the first day during the second recovery (Day 3), the Rubisco activation state of both treatments had not recovered to the control level although the 40°C treatment recovered more rapidly than the 45°C treatment. On Day 6, the Rubisco activation state of both the 40°C and 45°C treatments reached the control level.

Discussion

The step limiting photosynthesis at high temperatures has been debated recently. One proposed limitation is heat-induced deactivation of Rubisco [12,13,34,35]. The other proposed limitation is impairment of the entire electron transport chain [36,37,38,39]. In fact, different high temperatures may have different effects. This study clearly shows that $P_n$ was not limited at 35°C in grape leaves, but it was limited at 40°C and 45°C. This
result is similar to views that the optimum temperature for photosynthesis is between 25 and 35°C for some grape leaves [16,17]. When the grape leaves were stressed at 40°C or 45°C, $P_a$ and the activation state of Rubisco were markedly reduced while $C_i$ increased, indicating that the inhibition of photosynthesis is non-stomatal and associated with Rubisco (Fig. 1 and 6). The reduction of $P_a$ increase proportionally with the increasing of treatment temperature. However, when the grape leaves had recovered from heat stress, the increase of $P_a$ was accompanied by increases of $g_s$ and the activation state of Rubisco, indicating that $P_a$ recovery was also associated with stomatal factors and the activation state of Rubisco [Fig. 1 and 6]. Recent studies with cotton, wheat, tobacco, and maize have confirmed earlier observations that Rubisco is deactivated markedly in response to moderate heat stress [12,13,14,40,41]. However, heat stress at 35°C did not significantly ($P>0.05$) influence the activation state of Rubisco in grape leaves, which is similar to the effect on $P_a$ (Fig. 6). It has been shown that the inhibition of Rubisco activation by moderately elevated temperatures up to 40°C was fully reversible after the heated leaves were incubated at 22.5°C for 15 min [34,42]. In the present study, Rubisco following treatment at 40°C recovered more rapidly than when treated at 45°C.

The decrease of $P_a$ under heat stress and increase of $P_a$ during recovery was also associated with electron transport capacity. Figs. 2, 3, 4, and 5 show that the PSII and PSI were damaged. In addition, the relationship between $P_a$ and electron transport chain was dependent on temperature. Sage and Kubien [45] thought that it has been difficult to pinpoint specific limiting steps that control the temperature response of electron transport chain. However, the OJIP test may be used to demonstrate the limiting steps of electron transport of photosynthesis [44]. At present, the mechanism causing the decline in the electron transport rate above the thermal optimum remains uncertain. Inactivation of the oxygen-evolving complex (OEC) is implicated as a cause of heat–induced reduction in electron transport capacity, particularly at high temperatures (above 38°C in potato and above 40°C in spinach) [3,6]. However, at moderately warm temperatures, this lesion is probably not significant, as leaves can readily alter PSII properties to reduce heat sensitivity of the OEC [3]. The present results showed that 35°C did not result in damage to OEC. Heat stress can influence the PSII reaction center, and the density of RCQA may reflect the density of QA-reducing PSII reaction centers [45]. In the present study, during the heat stress at 40 or 45°C and the following recoveries, changing trends of $W_K$ and RCQA values almost corresponded to that of $P_a$ (Fig. 2). This indicated that heat stress and recovery influenced $P_a$ partially via the donor side (the oxygen-evolving complex) and reaction center of PSI. Moreover, the higher stress temperature led to a slower recovery of $P_a$.

In these experiments, $\varphi_{Po}$ declined and $\varphi_{Di,0}$ increased at 40°C or 45°C. However, after 1 d of recovery, they returned to control levels. Interestingly, $\psi_{Eo}$ and $\varphi_{Eo}$ significantly ($P<0.05$) decreased in grape leaves at 45°C but 40°C had almost no influence on $\psi_{Eo}$ and $\varphi_{Eo}$ (Fig. 3). $\varphi_{Di,0}$ demonstrates the quantum yield for dissipated energy. In this study, heat stress at 40 or 45°C increased $\varphi_{Di,0}$. However, $\varphi_{Di,0}$ recovered to control levels after some time. In
Figure 5. PSII efficiency and excitation energy dissipation in grape leaves under different heat treatments and following recovery. 25 °C: normal growth and recovery temperature; 35, 40 and 45 °C: high temperature treatments. Each value represents the mean ± S.E. of four replicates. At the same time point, the numerical values with different letters are significantly different (P<0.05) according to Duncan’s multiple comparison. doi:10.1371/journal.pone.0023033.g005
addition, \( \delta_{\text{Ro}} \) represents the efficiency with which an electron can move from PQ through PSI to the PSI end electron acceptor. The differential response of the \( \delta_{\text{Ro}} \) suggests that the redox state of PQ-pool was affected by the heat stress at 45°C but not 40°C (Fig. 4).

The efficiency of PSII under steady-state irradiance (\( \Phi_{\text{PSII}} \)) was closely related to the \( P_{\text{n}} \) [46]. In this study, under heat stress at 40°C or 45°C \( \Phi_{\text{PSII}} \) and \( g_{\text{s}} \) decreased while NPQ increased. This suggests more energy was partitioned to heat dissipation and less energy was used in CO₂ fixation under heat stress at both temperatures. However, the influence at 40°C was less than that at 45°C. After the second recovery, \( \Phi_{\text{PSII}} \) at 40°C increased more rapidly accompanied by an increase of \( g_{\text{s}} \) and a decline of NPQ than that at 45°C. A NPQ increase of PSII is widely observed at temperatures where electron transport capacity slows with rising temperature [34,36], which corresponds to the change in \( Q_{\text{D}} \).

Conclusions

Heat treatment at 35°C did not significantly (\( P \leq 0.05 \)) influence grapevine photosynthesis. The decrease of \( P_{\text{n}} \) in grape leaves exposed to heat stress (40 or 45°C) was mainly attributed to the activation state of Rubisco and the donor side and the reaction center of PSII. However, the increase of \( P_{\text{n}} \) in grape leaves following heat stress was also associated with a stomatal response. The acceptor side of PSII in grape leaves was responsive but less sensitive to heat stress.

Materials and Methods

Plant materials and treatments

One-year old ‘Zuoyouhong’ grapevines (\( Vitis amurensis \) L.) were planted in pots, then grown in a greenhouse at 70–80% relative humidity; 25/18°C day/night cycle, with the maximum photosynthetically active radiation at about 1,000 µmol photons m\(^{-2}\) s\(^{-1}\).

The progress of the experiment is shown in Table 1. Grapevines with identical growth (10 leaves) were acclimated for two days in a controlled environment room (70–80% relative humidity, 25/18°C day/night cycle and 800 µmol m\(^{-2}\) s\(^{-1}\)) and divided into four groups. On the following day (the first day of the experiment, Day 1), chlorophyll fluorescence and gas exchange parameters were analyzed at 9:30 h for all plants. Then, one group of grapevines was kept at 25°C in this controlled environment room. The other three groups were treated at 35, 40 or 45°C, respectively, in controlled environment rooms (except for temperature, the other conditions were the same as the 25°C room) until 14:30 h, when the relative photosynthesis parameters were then rapidly measured. The stressed grapevines were then allowed to recover at 25°C, with the other conditions the same as before heat treatments. On day 2, the same parameters were measured at 9:30 h, then the grapevines were stressed a second time until 14:30 h, when the relative photosynthesis parameters were then rapidly measured. The treated plants were again allowed to recover at 25°C as above. Chlorophyll florescence and gas exchange parameters were measured at 9:30 h on Day 3 and Day 6 during the following four days of recovery. All of the above measurements were made on the sixth leaf from the top of each plant. The experiment process is in Table 1. Four replications were made with leaves from different grape plants.

Analysis of photosynthetic gas exchange parameters

Photosynthetic gas exchange was analyzed with a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) which can control photosynthesis by means of photon flux density (PPFD), leaf temperature and CO₂ concentration in the cuvette. Net photosynthetic rate (\( P_{\text{n}} \)), stomatal conductance (\( g_{\text{s}} \)) and substomatal CO₂ concentration (\( C_{i} \)) were determined at a concentration of ambient CO₂ (360 µmol mol\(^{-1}\)) , a PPFD of...
800 μmol photons m⁻² s⁻¹, a 6 cm² leaf area, a 500 μmol s⁻¹ flow speed and at the treatment temperature.

**Chlorophyll fluorescence quenching analysis**

Chlorophyll fluorescence was measured with a FM-2 Pulse-modulated Fluorometer (Hansatech Instruments Ltd., King’s Lynn, Norfolk, UK). The maximal fluorescence level in the dark-adapted state (Fₘ₀) were measured by a 0.8 s saturating pulse at 8000 μmol m⁻² s⁻¹ after 15 min of dark adaptation. When measuring the induction, the actinic light (610 μmol photons m⁻² s⁻¹) was provided for 20 s by the FMS-2 light source. The steady-state fluorescence (Fₛ) was thereafter recorded and a second 0.8 s saturating light of 8000 μmol photons m⁻² s⁻¹ was provided to determine the maximum fluorescence in the light-adapted state (Fₘ). The actinic light was then turned off and the minimal fluorescence in the light-adapted state (F₀) was determined by illumination with 3 s of far red light. The following parameters were then calculated: (1) efficiency of excitation energy captured by open PSII reaction centers, Fₛ/Fₘ = (Fₘ₀ − F₀)/Fₘ₀; (2) the photochemical quenching coefficient, qₑ = (Fₘ₀ − Fₛ)/Fₘₐₓ − Fₛ; (3) the actual PSII efficiency, ΦPSII = (Fₘ₀ − Fₛ)/Fₘₐₓ; and (4) non-photochemical quenching, NPQ = Fₘ/Fₘₐₓ − 1 [47].

### Table 1. Sequence of experimental treatments.

| Sequence | Actions |
|----------|---------|
| Day 1 9:30 h | Measure photosynthesis parameters, start heat treatment |
| Day 1 9:30–14:30 h | Heat treatment |
| Day 1 14:30 h | Measure photosynthesis parameters, end the heat treatment, then start recovery |
| Day 2 9:30 h | Measure photosynthesis parameters, start heat treatment |
| Day 2 9:30–14:30 h | Heat treatment |
| Day 2 14:30 h | Measure photosynthesis parameters, end heat treatment, then start recovery |
| Day 3 and 6 9:30 h | Measure photosynthesis parameters |

**Table 2.** Summary of parameters, formulae and their description using data extracted from chlorophyll a fluorescence (OJIP) transient.

| Parameter | Description |
|-----------|-------------|
| Fₛ | Fluorescence intensity at time t after onset of actinic illumination |
| Fₘ₀ | Minimum reliable recorded fluorescence at 50 μs with the Handy PEA fluorimeter |
| Fₘₐₓ | Fluorescence intensity at 300 μs |
| Fₘₐₓ | Maximum recorded (= maximum possible) fluorescence at P-step |
| Area | Total complementary area between fluorescence induction curve and F = Fₘ |
| Fₘ = Fₘₐₓ | Minimum fluorescence, when all PSII RCs are closed |
| V₁ | Relative variable fluorescence at the J-step (2 ms) |
| V₂ | Relative variable fluorescence at the I-step (30 ms) |
| M₀ = 4 (F₃00 _µs−Fₙ)/Fₘₐₓ − Fₙ | Represent the damage to oxygen evolving complex (OEC) |
| Yields or flux ratios | Approximated initial slope (in ms⁻¹) of the fluorescence transient V = f(t) |

| Parameter | Description |
|-----------|-------------|
| φₚₛ = TRₚₛ/ABS | Maximum quantum yield of primary photochemistry at t = 0 |
| φₑₚₛ = ETₑₚₛ/ABS | Quantum yield for electron transport at t = 0 |
| φₑₒ = ETₑₒ/TRₑₒ = 1 − V₁ | Probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Qa⁻ |
| φₑₒ = Dₑₒ/ABS = 1 − φₑₒ | Quantum yield at t = 0 for energy dissipation |
| φₑₑₑ = REₑₑₑ/ETO = (1 − V₁)/(1 − V₂) | Efficiency with which an electron can move from the PQ through PSI to the PSI end electron acceptors |
| Density of reaction centers. RCₙₐₓ = φₑₑₑ × (ABS/CSₙₐₓ) × (V/M₀) | Amount of active PSII RCs (Qₑ-reducing PSII reaction centers) per CS at t = m |

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Measurement of the polyphasic rise of chlorophyll a fluorescence (O-J-I-P)

The so-called OJIP-test was employed to analyze each chlorophyll a fluorescence transient by a Plant Efficiency Analyzer (Hansatech Instruments Ltd., King’s Lynn, Norfolk, UK) which could provide information on photochemical activity of PSII and status of the plastoquinone pool [48]. The transients were induced by red light of about 3000 μmol photons m−2 s−1 provided by an array of six light emitting diodes (peak 630 nm). The fluorescence signals were recorded within a time span from 10 μs to 1 s with a data acquisition rate of 10 μs for the first 2 ms and every 1 ms thereafter. The fluorescence signal at 50 μs was considered as a true F0. The following data from the original measurements were used: maximal fluorescence intensity (Fm), fluorescence intensity at 300 μs (F0) [required for calculation of the initial slope (ΔM), the relative variable fluorescence (V) kinetics and W1]; and the fluorescence intensity at 2 ms (the J-step) denoted as Fv. Terms and formulae are as follows: relative variable fluorescence intensity, Fv = Fm - F0/Fm - F0; a parameter which represent the damage to oxygen evolving complex (OEC), W1 = (F0 - Fv)/ (Fm-F0) approximated initial slope of the fluorescence transient, ΔM = 4(F0-Fv)/ Fm-F0; probability that a trapped excitation moves an electron into the electron transport chain beyond QA−, φPSI = ET/PSII = TR0/ABS = Fm/F0; quantum yield of primary photochemistry at t = 0, φPSI = TR0/ABS = Eo/F0; quantum yield for electron transport (at t = 0), φET/ABS = (Fm-F0)/ F0; quantum yield at t = 0 for energy dissipation, φD10/ABS = D10/ABS = Eo/φET/ABS; the density of QA-reducing reaction centers, RCQA = φET/ABS/X/ABS/CS; and the efficiency with which an electron can move from PQ through PSI to the PSI end electron acceptors, δRo = (1 - V2/1 - V0). From OJIP transients, the extracted parameters led to the calculation and derivation of a range of new parameters (Table 2).

Extraction and assay of Ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco, EC4.1.1.39)

Leaves disks (1 cm2 each) were taken, then frozen in liquid nitrogen, and stored at −80°C until assay. Rubisco was extracted according to Chen and Cheng [49]. Three frozen leaves disks were ground with a pre-cooled mortar with 1.5 ml extraction buffer containing 50 mM Hepes-KOH (pH 7.5), 10 mM MgCl2, 2 mM EDTA, 10 mM dithiothreitol (DTT), 1% (v/v) Triton X-100, 1% (v/v) bovine serum albumin (BSA), 10% (v/v) glycerol, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 5% (v/v) insoluble polyvinypyrrolidone (PVPP). The extract was centrifuged at 13,000×g for 5 min in an Eppendorf microcentrifuge at 2°C, and the supernatant was used immediately for enzyme assays.

For Rubisco initial activity, a 50 μl sample extract was added to a semimicrowe gear containing 900 μl of an assay solution, immediately followed by adding 50 μl 0.5 mM RuBP, mixing well. The change of absorbance at 340 nm was monitored for 40 s. For Rubisco total activity, 50 μl 0.5 mM RuBP was added 15 min after a sample extract was combined with assay solution to activate all the Rubisco. Rubisco activation state was calculated as the ratio of initial activity to total activity [49,50].

Statistical analyses

Data were processed with SPSS 13.0 for Windows, and each value of the means and standard errors in the figures represents four replications. Differences were considered significant at a probability level of P<0.05 by Duncan’s multiple comparison.

Author Contributions

Conceived and designed the experiments: LJW SHL. Performed the experiments: HBL LM HEX JFJF. Analyzed the data: LJW WD. Contributed reagents/materials/analysis tools: SHL LJW. Wrote the paper: LJW HBL WL.

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