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Photosystem 2 is more tolerant to high temperature in apple (*Malus domestica* Borkh.) leaves than in fruit peel

L.-S. CHEN[*,**,+] and L. CHENG[***]

College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, China[*]
Institute of Horticultural Plant Physiology, Biochemistry and Molecular Biology, Fujian Agriculture and Forestry University, Fuzhou 350002, China[**]
Department of Horticulture, Cornell University, Ithaca, NY 14853, USA[***]

Abstract

Tolerance of photosystem 2 (PS2) to high temperature in apple (*Malus domestica* Borkh. cv. Cortland) leaves and peel was investigated by chlorophyll *a* fluorescence (OJIP) transient after exposure to 25 (control), 40, 42, 44, and 46 ºC in the dark for 30 min. The positive L-step was more pronounced in a peel than in leaves when exposed to 44 °C. Heat-induced K-step became less pronounced in leaves than in peel when exposed to 42 °C or higher temperature. Leaves had negative L- and K-steps relative to the peel. The decrease of oxygen-evolving complex (OEC) by heat stress was higher in the peel than in the leaves. OJIP transient from the 46 ºC treated peel could not reach the maximum fluorescence (F_m). The striking thermoeffect was the big decrease in the relative variable fluorescence at 30 ms (V_I), especially in the leaves. Compared with the peel, the leaves had less decreased maximum PS2 quantum efficiency (F_v/F_m), photochemical rate constant (K_P), F_m and performance index (PI) on absorption basis (PI_{abs}) and less increased minimum fluorescence (F_0) and non-photochemical rate constant (K_N), but more increased reduction of end acceptors at PS1 electron acceptor side per cross section (RE_0/CS_0) and per reaction center (RE_0/RC_0), quantum yield of electron transport from Q_A^- to the end acceptors (φ_{R0}) and total PI ($PI_{abs,total}$) when exposed to 44 °C. In conclusion, PS2 is more thermally labile than PS1. The reduction of PS2 activity by heat stress primarily results from an inactivation of OEC. PS2 was more tolerant to high temperature in the leaves than in the peel.

Additional key words: apple; chlorophyll *a* fluorescence (OJIP) transient; fruit peel; high temperature; K-step; L-step; leaves; *Malus domestica* Borkh.; photosystem 2; tolerance.

Introduction

With global warming, global temperature will continue to increase in the future. Hence, plants growing in tropical, subtropical, and temperate regions will be more often exposed to heat stress conditions. This will have both ecological and agricultural consequences. Photosynthesis is among the plant functions that are highly sensitive to heat stress and it is often inhibited before other cell functions are impaired (Berry and Björkman 1980). Heat stress brings damage to various aspects of photosynthetic functions, including both the photochemical reactions related to photosystem 1 (PS1) and PS2 and the dark reaction. However, PS2 has been shown to be the most thermally labile component of the photosynthetic apparatus (Berry and Björkman 1980, Srivastava *et al*. 1997).

Chlorophyll *a* fluorescence (OJIP) measurement is one of the oldest approaches to probe photosynthesis. All oxygenic photosynthetic materials investigated so far using direct, time-resolved fluorescence measurement show the polyphasic rise with the basic steps of O, J, I, P (Strasser *et al*. 1995, 2000, 2004). The O-step reflects the minimum fluorescence when all the primary quinone electron acceptor (Q_A) is oxidized. The P-step corresponds to the state in which all Q_A is reduced. The rise from phase O to phase J reflects a reduction of Q_A and is associated with the primary photochemical reactions of PS2. The intermediate step I and the final step P reflect the existence of fast and slow reducing plastoquinone (PQ) centers as well as different redox states of reaction center (RC) complex (Strasser *et al*. 1995). The OJIP transient is a rich and complex signal and it has been proved to be a sensitive and reliable method for the detection and quantification of heat-induced changes

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[+] Author for correspondence; fax: +86-591-83727618, e-mail: lisongchen2002@hotmail.com

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in the PS2 of plant leaves (Srivastava *et al*. 1997, Tóth *et al*. 2005, Wen *et al*. 2005). It is noteworthy that most studies of the effect of heat stress on PS2 were conducted in leaves. To our knowledge, little is known about the effects of heat stress on PS2 in fruits. Fruit sunburn is reported to result in a large yield and revenue losses (Gindaba and Wand 2005). Although the relative contribution of heat and light stresses to fruit sunburn is not yet fully understood, sunburn is caused by the interaction of high temperature and light (Schrader *et al*. 2003). Because fruits have less effective mechanisms of using and/or dissipating of solar radiation, under direct solar irradiation peel temperature of the sun-exposed side usually is higher than leaf temperature (Cheng and Ma 2004). Therefore, fruits are considered to be more susceptible to sunburn compared to leaves (Gindaba and Wand 2005). In some apple producing regions of the world, summer air temperatures normally rise above 40 °C (Gindaba and Wand 2005) and under conditions with high solar irradiance the leaf temperature increased several degrees. Centigrade above air temperature (Lombardini *et al*. 2005), however sunburn infrequently occurs in apple leaves even under such conditions. Hence, it is reasonable to believe that PS2 may be more tolerant to high temperature in apple leaves than in fruit peel.

In this paper, we investigated the changes in OJIP transient and related parameters under heat stress. This objective was to test the hypothesis that PS2 is more tolerant to high temperature in leaves than in fruit peel.

Materials and methods

Plants: Twenty-two-year-old apple (*Malus domestica* Borkh. cv. Cortland) trees were grown in the field at Cornell Orchards, in Ithaca, New York, USA. They received standard horticultural practices, and diseases and pest control. On 22 August 2006, sun fruits (approximately 100 days after full bloom/30 days before harvesting) and ca. 3-month-old leaves from the same trees were chosen for heat stress treatments. Both leaves and fruits received similar irradiance during their development. Experiments were performed with 7–9 replicates (one leaf or fruit from different plants per replicate).

Heat stress treatments: Sun fruits and leaves were taken right after sunset. Fruit peel (ca. 0.5 mm thick, 1 cm^2) was cut from the sun-exposed side of these fruits after being dark-adapted overnight. Similarly, leaf discs (1 cm^2 in size) were taken from the leaves after being dark-adapted overnight. Peel and leaf discs were directly placed into the smooth bottom of a small vessel (1.5 cm in height × 6.2 cm in diameter), which was made from aluminum foil. Beneath them there was a layer of wet paper and above them a layer of wet paper and a layer of aluminum foil. Then, the vessel was directly floated on water. Water temperature was regulated by a refrigerated bath (*NESLAB RTE-10*, *Thermo Electron Corp*., Newington, NH, USA). Heat equilibrium between peel and water was reached quickly. The peel and leaf discs were exposed to different temperatures (25 (control), 40, 42, 44, and 46 °C) in the dark for 30 min.

Measurements of OJIP transient: OJIP transient was measured after heat-treated peel and leaf discs had been kept for 30 min in the dark at room temperature by a *Handy PEA* (*Hansatech Instruments Limited*, Norfolk, UK) according to Strasser *et al*. (1995). The transient was induced by the red light of about 3 400 μmol (photon) m^{-2} s^{-1} provided by an array of 3 light-emitting diodes (peak 650 nm), which focused on the fruit peel and leaf disc surfaces to give homogenous irradiation over the exposed area of the fruit peel and the leaf discs.

JIP test: OJIP transient was analyzed according to the JIP test. From the OJIP transient, the measured parameters (F_o, F_m, $F_{300\mu s}$, F_J, F_I, tF_m etc.) were used to the calculation and derivation of a range of new parameters according to Strasser *et al*. 2000, 2004, and Jiang *et al*. 2008 (Table 1).

Table 1. Summary of parameters, formulae atheir description using data extracted from OJIP transient.

Fluorescence parameters	Description
Extracted parameters	
F_t	Fluorescence at time t after onset of actinic illumination
$F_{50\mu s}$ or $F_{20\mu s}$	Minimum reliable recorded fluorescence at 50 μs with the PEA- or 20 μs with Handy-PEA-fluorimeter
$F_{300\mu s}$	Fluorescence intensity at K-step (at 300 μs)
F_J	Fluorescence intensity at J-step (at 2 ms)
F_I	Fluorescence intensity at I-step (at 30 ms)
F_P (= F_m)	Maximum recorded (= maximum possible) fluorescence at P-step

Table 1 (continued)

Fluorescence parameters	Description
Derived parameters Selected parameters	
$F_0 \cong F_{50\mu s}$ or $F_0 \cong F_{20\mu s}$	Minimum fluorescence
$F_m = F_P$	Maximum fluorescence
$V_J = (F_{2ms} - F_0)/(F_m - F_0)$	Relative variable fluorescence at 2 ms
$V_I = (F_{30ms} - F_0)/(F_m - F_0)$	Relative variable fluorescence at 30 ms
$W_t = (F_t - F_0)/(F_J - F_0)$	Relative variable fluorescence for the normalization between F_0 and F_J
$W_K = (F_{300\,\mu s} - F_0)/(F_J - F_0)$	Ratio of variable fluorescence at K-step to the amplitude $F_J - F_0$
$V_K = (F_{300\,\mu s} - F_0)/(F_m - F_0)$	Relative variable fluorescence at 300 μs
$M_0 = 4\,(F_{300\mu s} - F_0)/(F_m - F_0)$	Approximated initial slope (in ms^{-1}) of the fluorescence transient V = f(t); It is a measure of the rate of the primary photochemistry
$S_m = Area/(F_m - F_0)$	Normalized total complementary area above the OJIP transient (reflecting multiple-turnover Q_A reduction events)
Fraction of OEC = $[1 - (V_K/V_J)]_{treated\ sample}/[1 - (V_K/V_J)]_{control}$	The fraction of OEC in comparison with the control
Yields or flux ratios	
$\varphi_{P0} = TR_0/ABS = 1 - F_0/F_m = F_v/F_m$	Maximum quantum yield of primary photochemistry at t = 0
$\varphi_{E0} = ER_0/ABS = (F_v/F_m) \times (1 - V_J)$	Quantum yield for electron transport at t = 0
$\psi_0 = ET_0/TR_0 = 1 - V_J$	Probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\delta_{R0} = RE_0/ET_0 = (1-V_I)/(1-V_J)$	Efficiency with which an electron can move from the reduced intersystem electron acceptors to the PS1 end electron acceptors
$\varphi_{R0} = RE_0/ABS = \varphi_{P0} \times \psi_0 \times \delta_{R0}$	Quantum yield of electron transport from Q_A^- to the PS1 end electron acceptors
$\rho_{Ro} = RE_0/TR_0 = \psi_0 \times \delta_{R0}$	Efficiency with which a trapped exciton can move an electron into the electron transport chain from Q_A^- to the PS1 end electron acceptors
Specific fluxes or activities expressed per reaction center (RC)	
$ABS/RC = M_0 \times (1/V_J) \times (1/\varphi_{P0})$	Absorption flux per RC
$TR_0/RC = M_0/V_J$	Trapped energy flux per RC at t = 0
$ET_0/RC = (M_0/V_J) \times \psi_0 = (M_0/V_J) \times (1-V_J)$	Electron transport flux per RC at t = 0
$DI_0/RC = ABS/RC - TR_0/RC$	Dissipated energy flux per RC at t = 0
$RE_0/RC = (RE_0/ET_0) \times (ET_0/RC)$	Reduction of end acceptors at PS1 electron acceptor side per RC at t = 0
Phenomenological fluxes or activities expressed per excited cross section (CS)	
$ABS/CS_0 = F_0$	Absorption flux per CS at t = 0
$ET_0/CS_0 = (ABS/CS_0) \times \varphi_{E0}$	Electron transport flux per CS at t = 0
$TR_0/CS_0 = (ABS/CS_0) \times \varphi_{P0}$	Trapped energy 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
$RE_0/CS_0 = (RE_0/ET_0) \times (ET_0/CS_0)$	Reduction of end acceptors at PS1 electron acceptor side per CS at t = 0
K_F	The rate constant of fluorescence emission from PS2
$K_P = (ABS/CS_0) \times K_F \times [(1/F_0) - (1/F_m)]$	Photochemical rate constant
$K_N = (ABS/CS_0) \times K_F \times (1/F_m)$	Non-photochemical rate constant
Density of RCs	
$RC/CS_0 = \varphi_{P0} \times (ABS/CS_0) \times (V_J/M_0)$	Amount of active PS2 RCs per CS at t = 0
Performance index	
$PI_{abs} = (RC/ABS) \times (\varphi_{P0}/(1-\varphi_{P0}) \times (\psi_0/(1-\psi_0)$	Performance index (PI) on absorption basis
$PI_{abs,total} = (RC/ABS) \times (\varphi_{P0}/(1-\varphi_{P0}) \times (\psi_{E0}/(1-\psi_{E0}) \times (\delta_{R0}/(1-\delta_{R0})$	Total PI, measuring the performance up to the PSI end electron acceptors

Results

Both control leaves and peel showed a typical polyphasic rise with the basic steps O, J, I, P, described in detail by Strasser *et al*. (1995). After exposure to 40 and 42 °C, J-, I- and P-steps in both leaves and peel were decreaced (Fig. 1*A*,*B*). The major effects of strong heat stress (44 and 46 °C) on OJIP transient in peel were the appearance

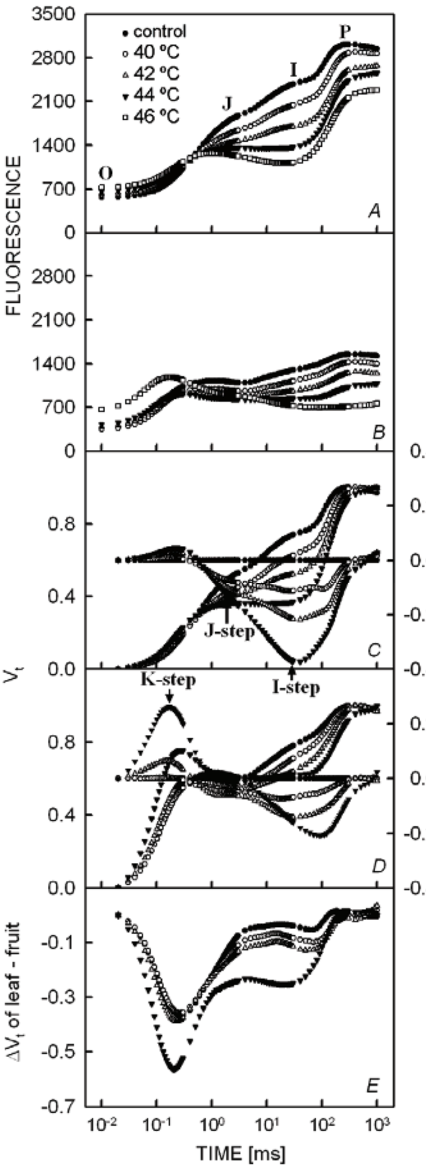

Fig. 1. Effects of temperature on the average OJIP transient (average of 7 - 9 samples) and relative variable fluorescence for the normalization between F_0 and F_m (V_t) in dark-adapted apple leaves (*A,C*) and peel (*B,D*) plotted on a logarithmic time scale (0.01 to 1 s): *A,B*: OJIP transient; *C,D*: between F_0 and F_m: $V_t = (F_t - F_0)/(F_m - F_0)$ and the differences (ΔV_t) of the four samples to the reference sample treated with 25 °C; *E*: the differences (ΔV_t) of leaves minus peel at each given temperature. For description of the parameters *see* Table 1.

of a clear K-step at around 200 - 300 µs, followed by a dip, a suppressed P-step and an increased F_0, while the heat-induced K-step became less pronounced in leaves (Fig. 1, 2*A-C*). This means that strong heat stress broke down the electron donation from the oxygen evolution complex (OEC) to a larger degree in peel than in leaves. If this lack of electrons is strong enough so that the reaction centers (RCs) cannot be fully closed, the sample will never reach F_m. T his happened in the peel after exposure to 46 °C (Fig. 1*B*). Because the signals for all RCs open and all RCs closed are preconditions for calculating most of the OJIP parameters, it is meaningless to calculate most of the OJIP parameters with this curve. Therefore, most of the OJIP parameters of 46 °C treated leaves and peel are not presented in Figs. 1-4.

The striking thermoeffect was the big decrease in $V_{I,}$ especially in leaves. Also, heat stress caused a decrease in V_J, which was less decreased in peel than in leaves (Fig. 1*C,D*). The difference (ΔV_t) of leaves minus peel revealed 3 negative steps: K, J and I (Fig. 1*E*).

By making the difference (ΔW_t) of normalized heat-treated transient minus the control transient, we saw a very nice positive K-step appearing as a function of temperature for heat-stressed leaves and peel. The differences (ΔW_t) of leaves minus peel revealed a clear negative K-step (Fig. 2*C*).

The differences (ΔW_K) showed a clear L-step in 120 - 150 µs range in heat-stressed leaves and peel (Fig. 2*D,E*). Compared with leaves, the L-step in peel was less pronounced after exposure to 40 °C, but became more pronounced after exposure to 44 °C. The difference (ΔW_K) of leaves minus peel revealed a negative L-step (Fig. 2*F*).

Under strong heat stress, F_0 was less increased in leaves than in peel (Figs. 1*A,B*, and 3*A*). Both F_J and F_I of leaves and peel decreased similarly as the temperature rose from 25 to 44 °C, whereas the two parameters decreased to a larger degree in peel than in leaves when exposed to 46 °C. F_m was less decreased in leaves than in peel with increasing temperature (Fig. 3*D*), whereas both area and tF_m increased to a larger degree in leaves than in peel (Fig. 3*E,F*).

As shown in Fig. 3*G-O*, RE_0/CS_0, ABS/RC, TR_0/RC, ET_0/RC, RE_0/RC, δ_{R0}, φ_{R0}, ρ_{R0} and S_m were less increased in peel than in leaves as the temperature rose from 25 to 44 °C except that ABS/RC increased to a larger degree in peel than in leaves after exposure to 44 °C. $PI_{abs,total}$ was less increased in leaves than in peel when exposed to 40 °C, but was more increased in leaves than in peel when exposed to 42 and 44 °C (Fig. 3*P*).

The relative effect of temperature on M_0 was similar for leaves and peel up to 42 °C. After exposure to 44 °C, M_0 was less increased in leaves than in peel. Heat treatments caused a large decrease in OEC, especially in peel (Fig. 4).

Maximum PS2 quantum efficiency (φ_{P0} or F_v/F_m) for leaves and peel remained relative stable after exposure to 40 °C, and then dropped with further increasing temperature, especially in peel (Fig. 4). ψ_0 was less increased in peel than in leaves as the temperature rose from 25 to 44 °C. φ_{Eo} for leaves and peel slightly increased as the temperature increased from 25 to 44 °C except a decrease in 44 °C treated peel (Fig. 4).

As shown in Fig. 4, K_P of both leaves and peel decreased as the temperature rose from 25 to 44 °C, K_P was less decreased in leaves than in peel when exposed to

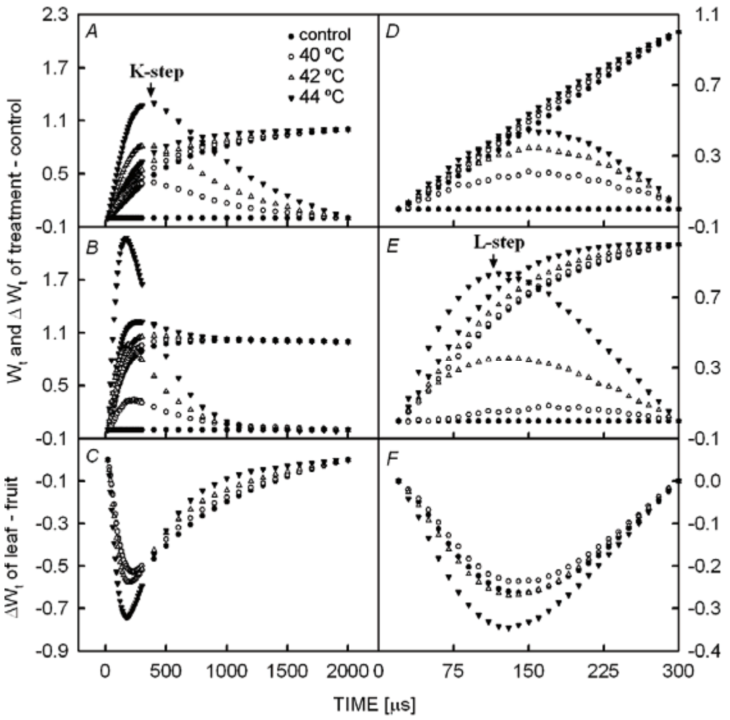

Fig. 2. The OJIP transient of Fig. 1*A,B* are presented as kinetics of different expressions of relative variable fluorescence: (*A*: leaf) and (*B*: peel) between F_0 and F_J: $W_t = (F_t - F_0)/(F_J - F_0)$ and the differences (ΔW_t) of the four samples to the reference sample treated with 25°C; *C*: the differences (ΔW_t) of leaves minus peel at each given temperature; (*D*: leaf) and (*E*: peel) between F_0 and $F_{300\mu s}$: $W_K = (F_t - F_0)/(F_{300\mu s} - F_0)$ and the differences (ΔW_K) of the four samples to the reference sample treated with 25°C; (F) the differences (ΔW_K) of leaves minus peel at each given temperature. For description of the parameters *see* Table 1.

44 °C. K_N was less increased in leaves than in peel with increasing temperature. Since the extents of K_N increase and K_P decrease were similar, $K_P + K_N$ showed a little change as the temperature rose from 25 to 44°C.

Trapped energy flux per CS (TR_0/CS_0) of leaves slightly increased with the increasing temperature, whereas TR_0/CS_0 of peel decreased. ET_0/CS_0 for leaves increased with the increasing temperature, whereas ET_0/CS_0 for peel slightly increased after exposure to 40 and 42 °C and decreased after exposure to 44 °C. The relative effect of temperature on RC/CS_0 is similar for leaves and peel up to 42 °C, but RC/CS_0 was less decreased in leaves than in peel when exposed to 44°C (Fig. 4).

Performance index on absorption basis (PI_{abs}) showed little change after exposure to 40 °C, then decreased with further increasing temperature. It was less decreased in leaves than in peel after exposure to 42 and 44 °C (Fig. 4).

Discussion

The finding that heat-treated leaves and peel had positive L-steps (Fig. 2*D,E*) means that the PS2 units in heat-treated samples are less grouped or less energy was being exchanged between independent PS 2 units. Loosing cooperativity implies the PS2 units of heat-treated samples lost stability and became more fragile (Strasser 1978). Our results showed that the positive L-step was more pronounced in peel than in leaves when exposed to 44 °C (Fig. 2*D,E*) and that leaves had a negative L-step relative to peel (Fig. 2*F*), indicating that leaves had a higher stability under heat stress.

Our finding that the decrease of F_v/F_m (φ_{P0}) by heat stress in leaves and peel due to an increase in F_0 and a decrease in F_m (Figs. 1*A,B*; 3*A,D*, and 4) is similar to those observed in the leaves of barley (*Hordeum vulgare* L.) (Tóth *et al*. 2005), spinach (*Spinacia oleracea* L.) (Tang *et al*. 2007), pea (*Pisum sativum* L.) (Srivastava *et al*. 1997), *Artemisia anethifolia* L. (Wen *et al*. 2005), and rice (*Oryza sativa* L.) (Yamane *et al*. 1997) and peel of apple fruit (Chen *et al*. 2008). The decrease in F_v/F_m ratio under heat stress is considered to reflect the damage to PS2 (Berry and Björkman 1980, Srivastava *et al*. 1997). Our results showed that F_v/F_m was less reduced in leaves than in peel when exposed to 42 and 44 °C (Fig. 4), indicating that PS2 was more tolerant to high temperature in leaves. This is in agreement with the above view that leaves have a higher stability under heat stress. As shown in Fig. 4, K_P was less decreased in leaves than in peel when exposed to 44 °C (Fig. 4). This also indicates that PS2 is more tolerant to high temperature in leaves. The increase of F_0 by heat stress may be attributed to a combination of several processes: (*1*) dissociation of light-harvesting complex 2 (LHC2) from the PS2 complex and accumulation of inactive RCs of PS2 (Yamane *et al*. 1997, 2000), (*2*) reduction of Q_A in the dark (Yamane *et al*. 2000), (*3*) enhanced back electron transfer from Q_B to Q_A (Kouřil *et al*. 2004), and heat-induced monomerization of LHC2 trimers (Garab *et al*. 2002). The temperature-dependent increase in F_0 has been routinely used for evaluating PS2 thermotolerance

(Knight and Ackerly 2002). In our study, F_0 increased to a larger extent in peel than in leaves after exposure to 44 and 46 °C (Figs. 1*A,B* and 3*A*). This also indicates that PS2 is more tolerant to high temperature in leaves than in peel. The lower fluorescence intensity at P-step (F_P or F_m) under heat stress (Figs. 1*A,B*, and 3*D*) may be due to a blockage of electron donation from the PS2 donor side, since the decrease of P-step can be restored by the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (Srivastava *et al.* 1997). Our results showed that F_m decreased to a larger extent in peel than in leaves when exposed to 40 °C or higher (Figs. 1*A,B* and 3*D*). This further demonstrates that PS2 is more tolerant to high temperature in leaves than in peel.

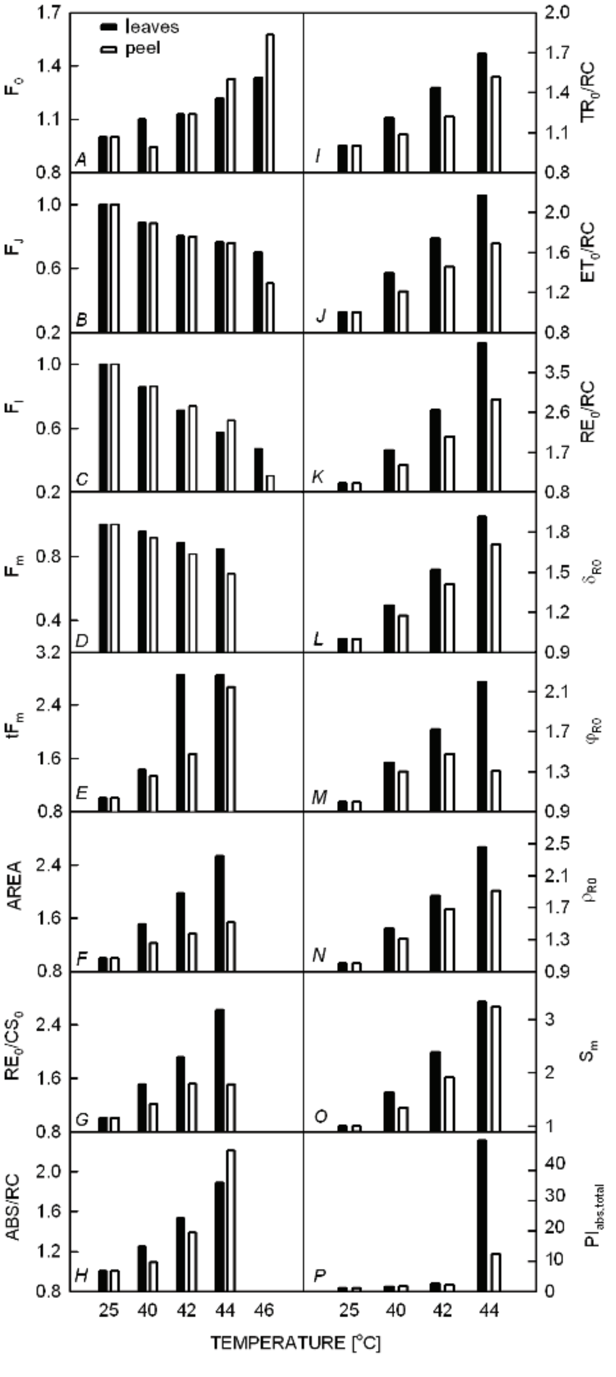

Fig. 3. F_0 (A), F_J (B), F_I (C) F_m (D), tF_m (E), Area (F), RE_0/CS_0 (G), ABS/RC (H), TR_0/RC (I), ET_0/RC (J), and RE_0/RC (K), δ_{R0} (L), φ_{R0} (M), ρ_{R0} (N), S_m (O) and $PI_{abs,total}$ (P) of dark-adapted apple leaves and peel in response to temperature. The parameters are the averages of 7 - 9 samples in each case. In the plot each parameter was normalized on that of the control treated with 25 °C (set as 1). For description of the parameters *see* Table 1.

We found that after exposure to 44 and 46 °C, OJIP transient for leaves and peel showed a K-step at around 200-300 μs, followed by a dip, a suppressed P-step and an increased F_o, especially in peel (Fig. 1 and 2*A-C*), which agrees with the earlier results obtained on tobacco (*Nicotiana tabacum* L.) (Yang *et al.* 2007), pea (*Pisum sativum* L.) (Srivastava *et al.*, 1997), wheat (*Tritium aestivum* L.) (Lu *et al.* 1999) and barley (Tóth *et al.* 2005) leaves, and apple fruit peel (Chen *et al.* 2008). K-step has been considered to result from an inhibition of electron donation from water to the secondary electron donor of PS2 (Y_Z) due to the inactivation of OEC resulting from the release of Mn from OEC and the amplitude of the K-step can be used as a specific indicator of damage to the OEC (Srivastava *et al.* 1997). The appearance of K-step may also reflect the changes in the energetic connectivity between photosynthetic units (Srivastava *et al.* 1997). The finding that heat-treated samples had less energy exchange between independent PS 2 units, as indicated by the positive L-step (Fig. 2*D,E*) agrees with the above inference. It has been proposed that the dip after K-step results from the oxidation of Q_A^-, which either can not be re-reduced due to a deficient electron donor or which is reduced but with a concomitant accumulation of P_{680}^+ (Srivastava *et al.* 1997). However, Lazár *et al.* (1999) suggested that the dip after K-step was caused by some kind of the recombination between P_{680}^+ and a negative charge. Our results showed that the K-step increased to a far larger extent in peel than in leaves and the dip after K-step was larger in peel than in leaves when exposed to 42 °C or higher temperature (Figs. 1 and 2*A,B*), and that leaves had a negative K-step relative to peel (Fig. 2*C*), meaning that the OEC is less damaged in leaves than in peel and PS2 is more tolerant to high temperature in leaves than in peel. This is also supported by our data that OEC was less decreased in leaves than in peel with the increasing temperature (Fig. 4).

Our results showed that heat stress caused a significant increase in the net rate of PS 2 closure, as indicated by the increase in M_o (Fig. 4). This is similar to previous report that M_0 increased for pea leaves after exposure to 40 and 44 °C (Strasser *et al.* 2000). As shown in Fig. 4, M_0 increased to a larger degree in peel than in leaves when exposed to 44 °C, meaning that the physiological fractional reduction of Q_A to Q_A^- (per total Q_A) is higher in peel than in leaves. Our data showed that heat stress

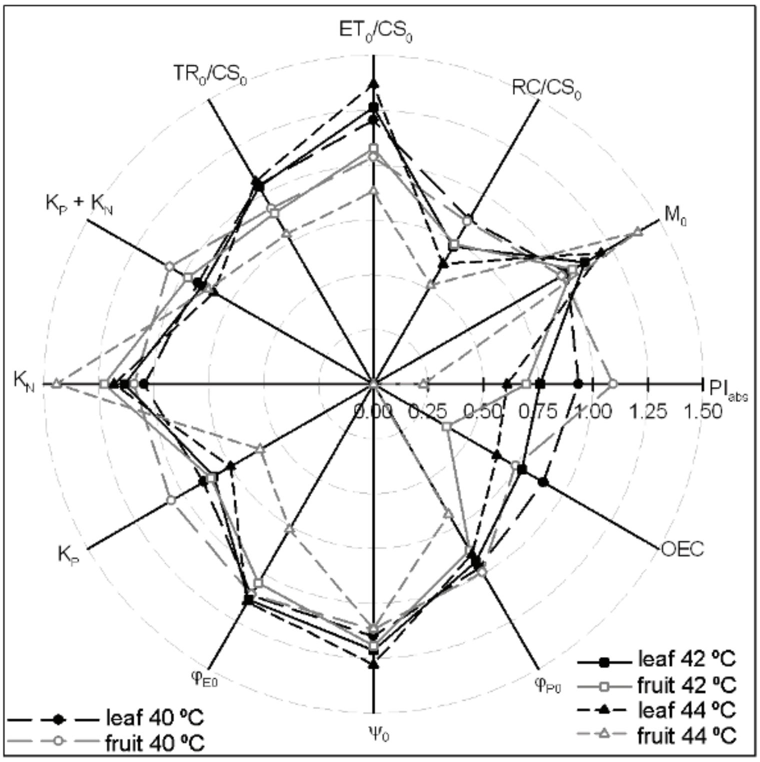

Fig. 4. Radar plot of 12 parameters derived by the JIP-test from OJIP transient of Figures 1*A,B*. In each case the parameters are derived from the corresponding average transient. All the values are relative to the control treated with 25 °C (set as 1). For description of the parameters *see* Table 1.

induced a decrease in V_J, which was less decreased in peel than in leaves (Fig. 1*C,D*), indicating that heat-treated leaves have decreased closure of PS2 RCs at 2 ms, especially in leaves. This is inconsistent with the previous report that V_J slightly increased after pea leaves were heated at 40°C and 44°C for 10 min (Strasser *et al*. 2000). The striking thermoeffect was the big decrease in V_I, especially in leaves (Fig. 1*C,D*). This means that plastochinone gets less reduced by heat stress up to 44 °C, especially in leaves. However, Jiang *et al*. (2006) reported that V_I in elm (*Ulmus pumila* L.) leaves increased as the temperature rose from 30 to 45 °C. As shown in Fig. 2*F*, S_m was less increased in peel than in leaves, meaning that less energy is needed to close all RCs in peel than in leaves (Strasser *et al*. 2000). Heat-induced increase in S_m is in agreement with the results obtained for pea leaves (Strasser *et al*. 2000). The fraction of electrons from the RCs at the acceptor side is not only related to the capacity of electron donation to the RCs, but also related to the capacity of the electron transport from the RCs to electron acceptors. Based on the lower V_I and V_J in heat-treated leaves and peel (Fig. 1*C,D*), we conclude that the donor side (OEC) of PS2 was damaged more severely than the acceptor side under heat stress. This is in agreement with the view that the reduction of PS2 activity by heat stress primarily results from an inactivation of OEC (Enami *et al*. 1994).

The finding that leaf TR_0/CS_0 slightly increased with the increasing temperature (Fig. 4) agrees with earlier report that TR_0/CS_0 in pea leaves slightly increased after exposure to 40 and 44 °C (Strasser *et al*. 2000). However, peel TR_0/CS_0 decreased with the increasing temperature (Fig. 4). This is similar to earlier study that TR_0/CS_0 in *Artemisia anethifolia* L. leaves decreased when exposed to 45°C (Wen *et al*. 2005). Leaf ET_0/CS_0 increased with the increasing temperature, whereas peel ET_0/CS_0 slightly increased after exposure to 40 and 42 °C and decreased after exposure to 44 °C (Fig. 4). In pea leaves, ET_0/CS_0 remained stable when exposed to 40 and 44 °C (Strasser *et al*. 2000), but in *Artemisia anethifolia* L. leaves, ET_0/CS_0 decreased when exposed to 45 °C (Wen *et al*. 2005). Thus, it appears that the influence of heat stress on TR_0/CS_0 and ET_0/CS_0 depends on plants and temperature. The relative effect of temperature on RC/CS_o was similar for leaves and for peel up to 42 °C, but RC/CS_0 was less decreased in leaves than in peel when exposed to 44 °C (Fig. 4). This means that the tolerance of RCs to high temperature is bigger in leaves than in peel. Heat-induced decrease in RC/CS_0 is consistent with earlier results obtained for *Artemisia anethifolia* L. (Wen *et al*. 2005) and pea (Strasser *et al*. 2000) leaves. The observed higher ABS/RC, TR_0/RC, ET_0/RC and RE_0/RC in leaves and peel (Fig. 3*H-K*) are at least partly due to their lower amount of active PS2 RCs, as indicated by lower RC/CS_0 (Fig. 4).

Performance index on absorption basis (PI_{abs}) is considered to be a very sensitive index for stress and is used widely to compare the whole primary photochemical reactions (Strasser *et al*. 2000) because it combines three main functional steps taking place in PS2, namely light energy absorption, trapping of excitation energy, and conversion of excitation energy to electron transport. Our results showed that PI_{abs} was less decreased in leaves than in peel when exposed to 42 and 44 °C (Fig. 4), indicating that the whole primary photochemical reactions are less damaged in leaves than in peel. This also supports the above view that PS2 is more tolerant to high temperature in leaves than in peel.

Heat-induced increase in K_N (Fig. 4) is in agreement with the increased requirement for dissipating excess excitation energy due to decreased photosynthetic capacity under heat stress. Because PS2 was less tolerant to high temperature in peel than in leaves, photosynthetic capacity may decrease to a larger extent in peel than in leaves under heat stress. As a result, excess excitation energy may increase to a larger extent in peel than in leaves under heat stress. Correspondingly, K_N increased to a larger extent in peel than in leaves under heat stress (Fig. 4).

Our results showed that the heat-treated leaves and peel had increased δ_{R0} (Fig. 3*L*), φ_{R0} (Fig. 3*M*), ρ_{R0} (Fig. 3*N*), RE_0/CS_0 (Fig. 3*G*), RE_0/RC (Fig. 3*K*) and $PI_{abs,total}$ (Fig. 3*P*), especially in leaves, meaning that PS1 was less damaged than PS2. This is in agreement with previous view that PS2 is the most thermally labile component of the photosynthetic apparatus (Berry and Björkman 1980, Srivastava *et al*. 1997), whereas PS1 is comparatively heat resistant (Havaux *et al*. 1991).

In conclusion, our finding supports the hypothesis that PS2 is more tolerant to high temperature in leaves than in peel. It's worth mentioning that PS2 was less tolerant to high temperature in approximately 40- and 60-day-old fruits (peel) than in the leaves of the similar age (data not shown). Although heat stress may result in apple fruit sunburn and yield loss, the leaves can keep normal physiological functions. Thus, the difference in tolerance to high temperature between leaves and fruits may be advantageous to apple tree survival under heat stress.

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