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Induction of cyclic electron flow around photosystem I during heat stress in grape leaves

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ABSTRACT

Photosystem II (PSII) in plants is susceptible to high temperatures. The cyclic electron flow (CEF) around PSI is thought to protect both PSII and PSI from photodamage. However, the underlying physiological mechanisms of the photosynthetic electron transport process and the role of CEF in grape at high temperatures remain unclear. To investigate this issue, we examined the responses of PSII energy distribution, the P700 redox state and CEF to high temperatures in grape leaves. After exposing 'Cabernet Sauvignon' leaves to various temperatures (25, 30, 35, 40 and 45 °C) in the light (600 μmol photons $m^{-2} s^{-1}$) for 4 h, the maximum quantum yield of PSII (*Fv*/*Fm*) significantly decreased at high temperatures (40 and 45 °C), while the maximum photo-oxidizable P700 (*Pm*) was not affected. As the temperature increased, higher initial rates of increase in post-illumination Chl fluorescence were detected, which were accompanied by an increase in high energy state quenching (qE). The chloroplast NAD(P)H dehydrogenase-dependent CEF (NDH-dependent CEF) activities were different among grape cultivators. 'Gold Finger' with greater susceptibility to photoinhibition, exhibited lower NDH-dependent CEF activities under acute heat stress than a more heat tolerant 'Cabernet Sauvignon'. These results suggest that overclosure of PSII reaction centers at high temperature resulted in the photoinhibition of PSII, while the stimulation of CEF in grape played an important role in the photoprotection of PSII and PSI at high temperatures through contributing to the generation of a proton gradient.

1. Introduction

A decrease in photosynthetic efficiency often occurs under excessive light, which is regarded as photoinhibition [1,2]. Photosystem (PS) II and PSI work coordinately to transport photosynthetic electrons efficiently. Photosynthetic process in plants provides at least two routes of electron flows. In the first route, the linear electron flow (LEF) is driven by PS II, cytochrome *b6f* and PSI [3]. In the second route, cyclic electron flow (CEF) is driven by PSI, electrons can be recycled either from a PGR5–PGRL1 protein-dependent pathway, or from NADPH by NAD(P)H dehydrogenase (NDH) pathway [4–6].

Heat stress inhibits photosynthetic efficiency, leading to the production of excess light energy [7,8]. PSII is sensitive to heat stress [9–11]; heat stress reduces the activity of the donor side [12,13], the acceptor side of PSII [9] and inactivates the PSII reaction centers [14,15]. Some studies have indicated that PSI is relatively resistant to heat [9,16], but Oukarroum et al. [17] found that heat stress can inhibit PSI in pea leaves. These studies were performed using plant materials grown in the dark [17,18] or under low levels of light (0–200 μmol photons $m^{-2} s^{-1}$) [16,19]. It is reported that fluctuating light induce PSI photoinhibition in plants even at optimum temperature [20,21], and a PGR5-dependent CEF plays an important role to protect PSI from photodamage [22]. Since light intensity affects CEF and non-photochemical quenching (NPQ) [16,23–25], it is important to study preferential PSI photoinhibition and the role of CEF in plants under moderate light intensity to reflect the actual effects of heat stress on PSI and PSII.

The absorption of light energy that exceeds a plant's capacity for CO_2 fixation induces the generation of ROS, which damage PSII reaction centers [26,27]. Plants have evolved a way to dissipate excess light energy through NPQ. According to relaxation kinetics in darkness following a period of illumination, the pH-dependent component (qE) is the major and most rapid component of NPQ in plants [28]. The qE requires the build-up of thylakoid ΔpH, which in the chloroplast is mainly induced by LEF and CEF [29–31].

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Reports have suggested that CEF mediated by NDH is responsible for enhanced ΔpH when CO_2 availability reduced [32,33]. Although previous studies have indicated that CEF is essential for protecting PSII and PSI against excess light energy, the role of CEF in protecting photosystems in grape under heat stress is not yet well understood.

In this study, we examined the effects of increased temperatures on CEF in grape leaves. The following questions were addressed: (i) Is CEF enhanced by increasing temperature in grape under a moderate light intensity? (ii) Whether there are some relationships between heat sensitivities and the extent of the CEF induction in different grape cultivators?

2. Materials and methods

2.1. Plant material and growth conditions

One-year-old grape cultivator, 'Cabernet Sauvignon' (CS) (*Vitis vinifera* L.), was grown in 25 cm-diameter plastic pots containing garden soil, sand and matrix soil (2:1:1) at a 26/22 °C day/night temperature in a growth chamber with a photon flux density (PFD) of 600 ± 100 μmol photons m^{-2} s^{-1}, a relative humidity of 60 ± 10% and a photoperiod of 14/10 h light/dark. Sufficient water was supplied to avoid drought stress. Another grape cultivator grown under same growth conditions, 'Gold Finger' (GF) (*Vitis vinifera* × *V. labrusca*), was used to compare the photoinhibition sensitivity with CS.

2.2. Heat treatments

Leaves (4/5) in the middle nodes of CS seedlings at the 10-leaf stage were used in the experiments. The leaves were exposed to various temperatures (25, 30, 35, 40 and 45 °C) for 4 h in the light (600 μmol photons m^{-2} s^{-1}) in a temperature-controlled chamber. Light was provided by light emitting diodes (LED; Plant System, China). Leaves of GF were exposed to normal temperature (25 °C) or high temperatures (40 and 45 °C) for 4 h in the same light. For each treatment, four to six replications were performed in a completely randomized block design. The measurements were taken immediately after heat stress treatments.

2.3. Measurement of Chl a fluorescence transients (O-J-I-P)

A Handy Plant Efficiency Analyzer (Hansatech, UK) was used to measure chlorophyll (Chl) a fluorescence transients (OJIP). Measurements were carried out on leaves that had been dark adapted for 30 min. Chl a fluorescence intensity rose rapidly from an initial minimal level, *Fo* (the O step), to the maximal level, *Fm* (P step). The maximum quantum yield of PSII (*Fv*/*Fm*) was calculated as (*Fm-Fo*)/Fm.

2.4. Measurement of PSI and PSII

Rapid light curves of Chl fluorescence and P700 were measured with a Dual PAM-100 (Walz, Germany) according to Pfündel et al. [34]. Leaves exposed to different temperatures (25–45 °C) were measured with the 60 s illumination periods gradually increasing in the sequence 10, 23, 41, 113, 212, 287, 565, 780 and 1215 μmol photons m^{-2} s^{-1}. The photochemical quenching coefficient, $qP = (Fm' - Fs)/(Fm' - Fo')$; non-photochemical quenching, $NPQ = (Fm - Fm')/Fm'$; actual PSII efficiency, $Y(II) = (Fm' - Fs)/Fm'$; the light-adapted maximum quantum yield of PSII, $Fv'/Fm' = (Fm' - Fo')/Fm'$; electron flow through PSII, $ETR(II) = 0.84 \times 0.5 \times Y(II) \times PPFD$; the quantum yield of non-regulated energy dissipation of PSII, $Y(NO) = Fs/Fm$; the quantum yield of regulated energy dissipation of PSII $Y(NPQ) = 1\text{-}Y(II)\text{-}Y(NO)$; rapidly relaxing quenching, $qE = (Fm/Fm')\text{-}(Fm/Fm^R)$ [24]; the quantum yield of PSI, $Y(I) = (Pm'\text{-}P)/Pm$; the donor side limitation of PSI, $Y(ND) = P/Pm$; the acceptor side limitation of PSI, $Y(NA) = (Pm\text{-}Pm')/Pm$; the electron flow through PSI, $ETR(I) = 0.84 \times 0.5 \times Y(I) \times PPFD$. *Fm*, the maximum fluorescence after overnight dark adaptation; *Fm′*, the maximal level of fluorescence under light; *Fs*, the level of steady-state fluorescence under light; Fm^R, the maximal level of fluorescence after the recovery of 30 min; *P*, the level of P700 signal under light; *Pm*, the maximum level of oxidizable P700; *Pm′*, the maximum level of oxidizable P700 under light.

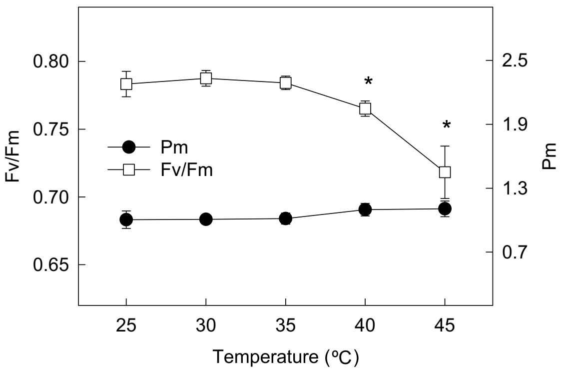

Fig. 1. Effects of heat stress on *Fv*/*Fm* and *Pm* in grape leaves. Leaves of 'Cabernet Sauvignon' (CS) grown at 25 °C in a greenhouse were exposed to temperatures of 25, 30, 35, 37.5, 40 or 45 °C in a growth chamber for 4 h under 600 μmol photons m^{-2} s^{-1} light. Each value was obtained after the leaves were dark-adapted for 30 min. Significant differences between leaves subjected to different temperatures were examined ($P < 0.05$). Asterisks indicate significant differences in leaves compared to the control. The means ± SE were calculated from 6 to 8 plants.

2.5. Statistical analyses

All experiments were repeated a minimum of three times. Data are reported as the means ± standard error (SE). The data were analyzed by performing one-way analysis of variance (ANOVA) and Duncan's multiple range tests for the independent samples using SPSS version 13.0. The confidence coefficient was set at 95%.

3. Results

3.1. Changes in energy distribution in PSII and P700 redox state

PSII was sensitive to high temperatures of 40 and 45 °C under a PPFD of 600 μmol photons m^{-2} s^{-1}, whereas PSI activity was stable. After exposure to a temperature of 40 or 45 °C for 4 h, the maximal photochemistry efficiency of PSII photochemistry (*Fv*/*Fm*) in CS leaves decreased significantly (Fig. 1), but the maximum photo-oxidizable P700 (*Pm*) remained stable. Light response curves indicated that the photochemical quenching coefficient (qP) and the light-adapted maximum quantum yield of PSII (*Fv'/Fm'*) were lower under heat stress (40 and 45 °C) than at 25 °C at all light dosages (Fig. 2). However, qP decreased more rapidly with increases of light, as indicated in the light response curves, compared to *Fv'/Fm'*. Since the effective quantum yield of PSII is determined by *Fv'/Fm'* and qP simultaneously, our results indicate that the temperature sensitivity of PSII photochemistry was significantly greater at temperatures above 35 °C and that the decrease in the effective quantum yield of PSII at high temperatures was mainly due to the decrease in qP.

The energy distribution in PSII in CS leaves varied between 25 and 45 °C. At high temperatures, the quantum yield of regulated energy dissipation in PSII [Y(NPQ)] was much higher than that at 25 °C, especially when light conditions exceeded 200 μmol photons m^{-2} s^{-1} (Fig. 3A). By contrast, the quantum yield of non-regulated