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Correlative Changes between Photosynthetic Activities and Chlorophyll Fluorescence in Wheat Chloroplasts Exposed to High Temperature

Park, Youn-Il, Min Chul Park[1] and Young-Nam Hong*
Department of Biology, Seoul National University, Seoul; and
[1]*Department of Biology, Songsim University, Puchon*

Correlative changes between photosynthetic O_2 exchange rates and room temperature Chl fluorescence were investigated in wheat (*Triticum aestivum* L.) chloroplasts treated with high temperature for 5 min. With increaseing treatment temperature, photosynthetic O_2 evolution rate mediated by PSII was decreased, showing 50% inhibition at 38℃ (I_{50}). But PSI activity measured by O_2 uptake rates was stimulated as a function of increasing temperature. Dark level fluorescence (Fo)-temperature (T) analysis showed that fluorescence rising temperature (T_r), critical temperature (Tc), and peak temperature (T_p) was 38, 43, and 52℃, respectively. Quenching analysis of Chl fluorescnece showed that both the oxidized fraction of plastoquinone (qQ) and the degree of thylakoid membrane energization (qNP) increased up to 40℃ and then declined dramatically. These results suggest that T_r is correlated with temperature showing a 50% inhibition of photosynthesis and under mild high temperature stress, qNP is worth regarding as indicator for heat-induced damage of photosynthesis.

Key words : Chl fluorescence quenching, chloroplast, high temperature stress, O_2 exchange rate, *Triticum aestivum* L.

Thermally fluctuating environment has various adverse effects on photosynthetic performance in aspects of function and structure. CO_2 fixation capacity, O_2 evolution rate, and photophosphorylation decline usually in leaves, protoplasts, and chloroplasts subjected to high temperature (Berry and Björkman, 1980; Quinn and Williams, 1985).

Chlorophyll fluorescence has been widely used for studing effects of various environmental stresses on photosynthesis (Briantias *et al.*, 1986). Many attempts have been tried to select the heat tolerant plants, showing usually high productivity. Analysis of Chl fluorescence has been known as a powerful tool for them due to its fast and simple way to select the heat tolerant plants and to investigate the heat-damaged symptom of photosynthetic apparatus. Various reports have shown that dark level fluorescence (Fo)-temperature (T) analysis was highly correlated with quantum yield of CO_2 fixation (Schreiber and Berry, 1977), photosynthetic electron transport rate (Armond *et al.*, 1978), and heat-induced leaf necrosis (Bilger *et al.*, 1984). Critical (T_c) and peak temperature (T_p) obtained from Fo-T plot have a good correlation with the relative sensitivity of photosynthesis in a variety of plants (Havaux *et al.*, 1988). In spite of these generalization, however, there were certain exceptions among plants grown under different environmental conditions (Monson and Williams III, 1982; Terzaghi *et al.*, 1989). Futhermore, this method has hardly revealed the photosynthetic induction process in heat-stressed photosynthetic apparatus. Recently quenching analysis of Chl fluorescence by the saturation pulse method has provided many significant information upon this process (Krause and

*Corresponding author : Fax 82-2-872-6881

Weis, 1991).

Therefore, in this study using high temperature-treated wheat chloroplasts, Fo-T curve was reinvestigated in terms of heat damage marker. And quenching analyses were also conducted to investigate the photosynthetic induction process.

MATERIALS AND METHODS

Plant materials

Wheat (*Triticum aestivum* L.) seeds were germinated in a rectangular polypropylene container (11.5 ×11.5×10 cm) containing 10 g of vermiculite and 60 mL of Hoagland solution (1/8 strength). Primary leaves from 7-8 day old wheat seedlings grown in a growth chamber at 25± 1℃ under continuous white light (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were used for experimental materials.

Preparations of protoplasts and chloroplasts

The middle portion of primary leaf, excluding the upper 1 cm and basal 3 cm, was used as experimental materials. Protoplasts and chloroplasts were isolated according to Edwards *et al* (1979). Approximately 5 g of excised leaves were incubated for 3 h under the dim light at 25℃ with 20 mL of cell wall digestion medium containing 5 mM Mes-KOH (pH 5.5), 0.5 M mannitol, 2 mM $CaCl_2$, 0.05% BSA, 1 mM arginine, 2% (w/v) Cellulase R-10 (Yakult, Japan), and 1% (w/v) macerozyme (Yakult, Japan). Protoplasts were collected and then resuspended with 3 mL of medium containing 50 mM Hepes-KOH (pH 7.6), 0.4 M mannitol, 2.4 mM $NaHCO_3$, and 0.5 mM KH_2PO_4. To prepare chloroplasts the protoplasts were broken by sucking into the syringe with a 20 μm nylon net and ejecting two times. The protoplast extracts were centrifuged at 250 *g* for 90s and then the supernatant discarded. The chloroplast pellet was suspended in the medium containing 50 mM Hepes-KOH buffer (pH 7.6), 0.33 M mannitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2.5 mM $NaHCO_3$, and 0.5 mM KH_2PO_4. The intactness of chloroplasts was more than 90% monitored by FeCN assay (Lilley *et al.*, 1975). Isolated chloroplast and protoplasts were kept on ice in the dark until use.

High temperature treatment

Aliquots (each 1 mL) of protoplasts and intact chloroplasts were incubated at the indicated temperature for 5 min in the dark and were rapidly cooled back at 25℃ for 3 min.

Measurements of O_2 exchange rates

O_2 exchange rates of broken chloroplasts were determined polarographically at 25℃ in a twin Clark type oxygen electrode system (YSI 5300 model, Yellow Spring Co, USA). Light was provided by a 300 W tungsten lamp in a slide projector with a blue filter at the intensity of about 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$. CO_2-dependent O_2 evolution by protoplasts was measured in a 2 mL of 50 mM Hepes-KOH buffer (pH 7.6) containing 0.33 M mannitol, 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 6.5 mM $NaHCO_3$ and 0.5 mM KH_2PO_4 adding protoplasts equivalent to 25 μg Chl/mL. O_2 evolution rate mediated by uncoupled noncyclic electron transport was measured in the resupending medium containing 5 mM $K_3Fe(CN)_6$ and 5 mM NH_4Cl. O_2 evolution rate by PSII activities was determined by adding 1 mM dichlolophenolindophenol (DCPIP). PSI-dependent O_2 uptake rate was measured in the presence of 1 mM DCMU, 0.1 mM NaN_3, 1 mM $DCPIPH_2$, 10 mM ascorbic acid, and 0.5 mM methyl viologen. The total volume of the assay media was adjusted to 2 mL including broken chloroplasts containing 25-30 μg Chl. Chlorophyll content was determined according to Arnon (1949).

Measurement of room temperature Chl fluorescence

Modulated Chl fluorescence from temperature-treated intact chloroplasts was monitored by a pulse amplitude modulation Chl fluorimeter (PAM 101, 102 and 103, H. Walz, Effeltrich, FRG) described by Schreiber *et al* (1986). The dark level fluorescence (Fo) was elicited with a very weak red light (0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) modulated at 1.6 kHz. Variable fluorescence (Fv) was induced by a nonmodulated white light (200 $\mu mol \cdot m^{-2} \cdot s^{-1}$) supplied by a Schott KL1500 light source. The superimposition of short pulse (900 ms) of intense saturating light (3700

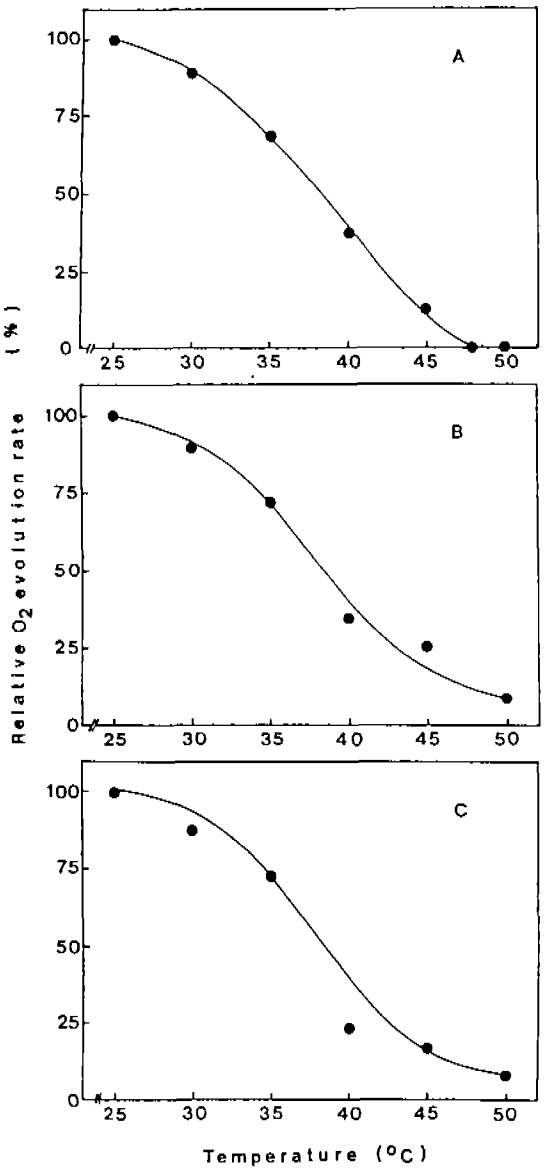

Fig. 1. Changes of photosynthetic O_2 exchange rates as a function of increasing high temperature treatments. a, relative CO_2-dependent O_2 evolution rate of wheat protoplasts, O_2 evolution rate at 25℃ was 76.4± 6.2 μmol O_2 mg Chl h; b, relative O_2 evolution rate mediated by uncoupled whole chain electron transport, O_2 evolution rate at 25℃ was 105.3± 6.5 μmol O_2 mg Chl h; c, relative O_2 evolution rate by PSII, O_2 evolution rate at 25℃ was 60.5± 6.5 μmol O_2 mg Chl h. After heat treatmetn at given temperature for 5 min, chloroplasts were osmotically broken and then O_2 exchange rates were measured.

$\mu mol \cdot m^{-2} \cdot s^{-1}$) applied every 20 s allowed to continuous determination of the maximal variable fluorescences (Fv)m during fluorescence induction. Four-armed fiberoptics were used to transmit the different modulated and nonmodulated lights onto the chloroplasts in a sample chamber (KS 101 suspension cuvette) and to transmit Chl fluorescence to the light detector as in Park *et al* (1993). Intact chloroplasts containing 25 μg Chl were used for each measurement. Coefficients of qQ and qNP were calculated according to Schreiber *et al* (1986): qQ=1−Fv/(Fv)s, qNP=1−(Fv)s/(Fv)m. Occurring Fo quenching (Fo', qO), quenching coefficients were calculated according to Bilger and Schreiber (1986): qQ=1−Fv'/(Fv)s', qNP=1−(Fv)s'/(Fv)m·(1−qO), qO=1−Fo'/Fo.

RESULTS AND DISCUSSION

Effects on photosynthesis

Relative CO_2-dependent O_2 evolution rates in wheat protoplasts were declined as the treated temperature increased and fully inactivated at 48℃ (Fig. 1a). Calvin cycle enzymes were not thermally inactivated by high temperatures at 40-50℃ (Thebud and Santarius, 1982). Hence we checked whether such inhibitory effect of high temperature on photosynthesis was related to photosynthetic electron transport. Relative O_2 evolution rate mediated by either uncoupled noncyclic electron trasport or PSII-water oxidizing activities was decreased (Fig. 1b and 1c). It was shown that 50% inhibition of O_2 exchange rates (I_{50}) occurred at near 38℃ in high temperature treated protoplasts and chloroplasts. However, PSI-mediated electron transport was stimulated by high temperature treatment (Fig. 2). These results are very similar with in various plant leaves and chloroplasts exposed to high temperature (Berry and Bj rkman, 1980; Quinn and Williams, 1985; Sabat and Mohanty, 1989; Stidham *et al*., 1982, Thomas *et al*., 1986; Williams *et al*., 1986).

Effects on dark level fluorescence

Fig. 3 shows the Fo-T curve of intact wheat chloroplasts heated slowly at a rate of 1℃/min. Fo began to rise at 38℃ (T_r) and reached to peak level (T_p) at 52℃ with the critical temperature (T_c) at 43℃. Analysis of Fo-T curve has been used to investigate correlation between the structural change of thylakoid membranes and the photosynthetic activity (Armond *et al*., 1978; Bilger *et al*., 1984; Schreiber

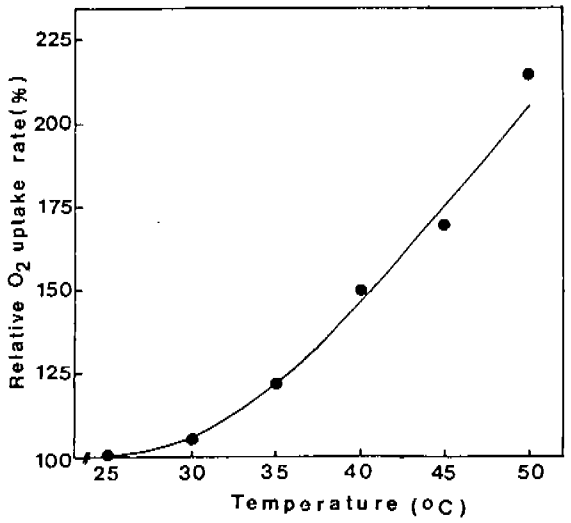

Fig. 2. Changes of relative O_2 uptake rates mediated by PSI. O_2 uptake rate at 25℃ was 72.3± 13.5 μmol O_2 mg Chl h.

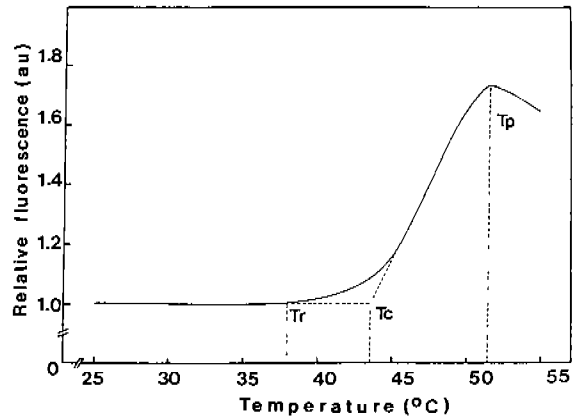

Fig. 3. Fo-T plot of temperature-treated chloroplasts. Modulated fluorescence was measured at Fo conditions (light intensity, 0.1 μmol·m^{-2}·s^{-1}) in the presence of 3 mM $NaHCO_3$ using intact chloroplasts. Temperature was increased at the rate of about 1℃/min. T_r, T_c and T_p were 38, 43, and 52℃, respectively.

and Berry, 1977). Numerous previous studies have suggested that T_c has highly correlated with temperature at which photosynthetic activity begins to decline (Monson and Williams, 1982). Furthermore, T_p was used to estimate the relative heat tolerance of various higher plants (Havaux *et al.*, 1988). In contrast, in this study, T_r has highly correlated with I_{50} of photosynthetic electron transport activity. In addition, T_c corresponds to 70-80% inhibitory temperature of PSII activities and T_p to the full inhibition temperatures of photosynthetic electron transport rate, respectively (Fig. 1).

Gounaris *et al* (1983; 1984) showed, using bean chloroplast subjected to heat-stress, that high temperature at 35-45℃ leaded to a progressive destacking of thylakoid membranes, with replacement of the normal granal arrangement by modified thylakoid attachment sites. They also reported that chloroplasts exposed in higher temperature range at 45-55℃ showed the phase-separated aggregates of non-bilayer-forming lipids. Hence, we might assume that T_r in this study corresponds to the temperature of grana destacking and T_c to that of formation of cylindrical inverted micelles, respectively.

Effect on Chl fluorescence induction

Photosynthetic performance after heat-pretreatment can be analyzed by Chl fluorescence quenching analysis (Briantais *et al.*, 1986; Krause and Weis, 1991). The effect of high temperature pretreatment on the saturation pulse induction curves of wheat intact chloroplasts are shown in Fig. 4. Fo level was slightly increased with increasing treatment temperature as shown in Fig. 3. Maximal fluorescence level (Fm), peak fluorescence level (Fp) and variable fluorescence level (Fv) at the photosynthetic steady state were declined with increasing temperature, but increased in 45℃-pretreated chloroplasts. These decreases in fluorescence parameters (Fm, Fp, and Fv) by heat pretreatment below 40℃ can be explained by donor-side related quenching of fluorescence yield through charge recombination at PSII reaction centers (Krause and Weis, 1991) or inhibition of PSII reduction (Park *et al.*, 1993). It is important to note the occurrence of dark level fluorescence quenching (Fo-quenching, Fo') only in 35℃- and 40℃-pretreated chloroplasts. Fo' occurs under condition of high energy state of thylakoid membranes and corresponds to the signal for the heat induced detachment of antennae pigments from PSII reaction centers (Bilger and Schreiber, 1986; Weis, 1982).

Fig. 5 depicts the differences of photochemical quenching (qQ) and nonphotochemical quenching coefficient (qNP) between control and temperature-treated chloroplasts during steady state photosynthesis. With increasing temperature, qQ increased slightly upto 40℃ and then decreased rapidly below control via transact temperature at 41℃. Also, qNP ma-

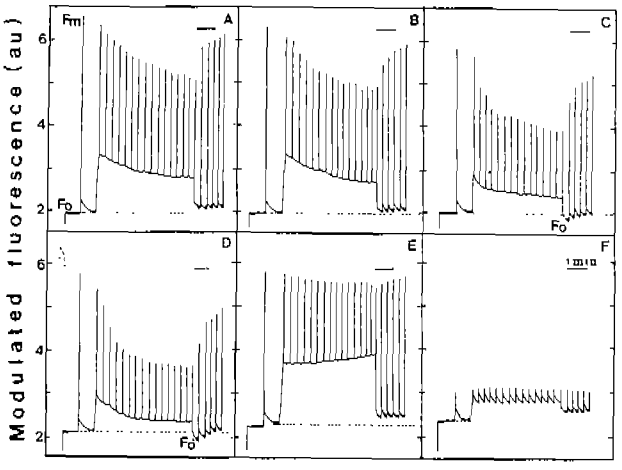

Fig. 4. The saturation pulse induction kinetics of high temperature-treated chloroplasts in the presence of 2.5 mM $NaHCO_3$. Prior to measurement, chloroplasts treated for 5 min at given temperature were cooled for 3 min at 25℃ in the dark. Intensities of measuring beam, actinic white light, saturation pulse light were 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 3700 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Pulse length was 900 ms and was applied repetitively every 20 s. A, 25℃-treated; B, 30℃-treated; C, 35℃-treated; D, 40 ℃-treated; E, 45℃-treated; F, 50℃-treated.

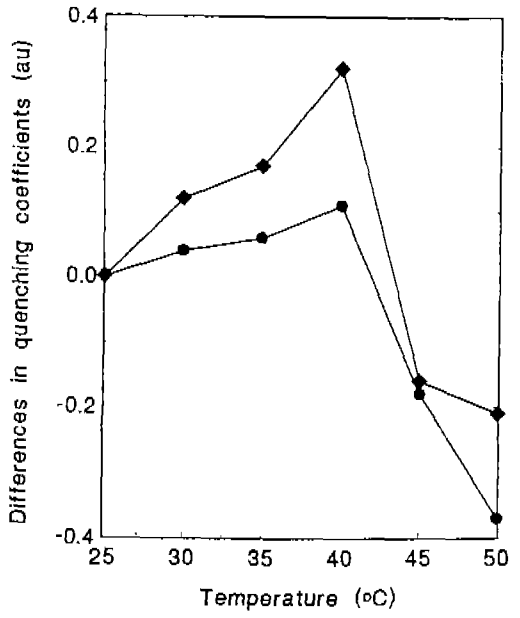

Fig. 5. Differences in two fluorescence quenching coefficents (−●−, Δ qQ; −◆−, Δ qNP) of control and temperatue-treated chloroplasts at the photosynthetic steady state. Each coefficient was calculated at 5 min after acitinc light illumination. The qQ and qNP for the control calculated from Fig. 4 was 0.76 and 0.25, respectively.

rkedly increased to 40℃ and then dramatically fell below control via transact temperature at 43℃.

Increase of qQ may be interpreted as the increase of oxidized plastoquinone (PQ) pool. This increase in oxidized PQ may be resulted from the malfunction of PSII by high temperature, considering the result that high temperature induced the inhibiton of PSII activity by limiting electron flow from water to PQ at PSII complexes (Park *et al.*, 1993). Increases of qNP might be mainly arised from the pH gradient formation and /or thermal deacay of photochemical energy (Krause and Weis, 1991). Judging from Fig. 1, the pH gradient across thylakoid membrane was hardly formed in high temperature-treated chloroplasts. Thus, qNP increase may reveal the increased thermal decay of photochemical energy. In fact increase of qNP has usually been regarded as an indicator for heat-induced stress and a dissipative protective mechanism of thylakoid membranes against adverse environment (Krause and Weis, 1991). However, this view may not be adaptable for stronger high temperature stress, in this case above 40℃, where there were little nonphotochemical quenchings. This higher temperature corresponded almost to that of full inhibition of photosynthetic electron transport rates (Fig. 1), probably caused by formation of the phase-separated aggregates of non-bilayer-forming lipids (Gounaris *et al.*, 1983; 1984).

Conclusionally, T_r from Fo-T curve and qNP under mild heat stress condition from quenching analyses can be used as markers for heat-induced damage of photosynthetic apparatus in heat-sensitive wheat.

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高溫 스트레스를 받은 밀 葉綠體에서 光合成能과 葉綠素 螢光의 關係

朴 淵 一 · 朴 敏 哲[1] · 洪 英 男*

서울大學校 自然科學大學 生物學科, [1]聖心女子大學校 自然科學大學 生物學科

적 요

고온 처리된 밀 엽록체에서 광합성 산소교환율과 실온 엽록소 형광의 변화 관계를 조사하였다. 고온에 의해 전사슬 전자전달능과 광계 II의 전자전달능은 감소하였으나 광계 I의 전자전달능은 촉진되었다. 이때 광합성능이 50% 억제되는 온도(I_{50})는 38℃ 였다. 일정형광-온도 상관 분석에서 형광 증가온도(T_f)는 38℃, 임계온도(T_c)는 43℃, 피이크 온도(T_p)는 52℃ 였다. 엽록소 螢光消散 분석결과, 산화된 플라스토퀴논 풀(qQ)과 틸라코이드막 에너지화(qNP)는 40℃까지 증가한 후 급격히 감소하였다. 이상의 결과로부터 T_c는 고온에 의한 광합성능이 50% 감소하는 온도를 의미하며 qNP는 비교적 온화한 고온스트레스에 대한 지표로 사용할 수 있는 것으로 나타났다.

주요어 : 엽록소 螢光消散, 엽록체, 산소교환율, 고온스트레스, 밀

*교신저자 : Fax (02) 872-6881