Bulg. J. Plant Physiol., Special Issue 2003, 304–313

THE VARIOUS MECHANISMS OF PHOTOSYNTHESIS LIMITATION IN HEAT-TREATED BARLEY SEEDLINGS OF DIFFERENT AGES

N. L. Pshybytko*, L. N. Kalituho, L. F. Kabashnikova

Institute of Photobiology, the National Academy of Sciences of Belarus, 27 Akademicheskaya str., 220072 Minsk, Belarus

Summary. The effect of heat shock (40°C, 3 h) on the photosynthetic activity of 4-, 7- and 11-day-old barley seedlings was studied. The rate of CO_2 gas exchange in young leaves was not changed under heat shock while in 11-day-old seedlings the high temperature affected both the average quantum yield and the shape of light response curves of photosynthesis. Nevertheless, in chloroplasts of young heated leaves the rate of potassium ferricyanide recovery was reduced. The impairment of PSII activity and diminution of oxidizing ability of the Cyt b_6/f complex caused the inhibition of photosynthetic electron transport. The preheating of 4-day-old leaves at 40°C led to a marked decrease of the nonphotochemical quenching of Chl fluorescence. It was related mainly to the lowering of ΔpH-dependent quenching mechanisms. Thus high temperature inhibited the photosynthetic reactions in young seedlings by the decrease of both electron transport rate and the transthylakoid proton gradient. The suppression of photosynthesis by heat shock in old leaves was caused mainly by a reduction of CO_2 uptake and by a decrease of carboxylation activity. These effects led to the impairment of ATP and NADPH consumption by the Calvin cycle and an increase in the transthylakoid proton gradient. As a results, the level of plastoquinone pool reduction was also increased and electron flow was decreased. According to the data obtained, the heating of young leaves inhibited the photosynthesis by reduction of the electron transport rate while in old leaves the activity of Calvin cycle enzymes was mainly limited. The suppression of photosynthetic activity by heat shock in old leaves was more compared to the young leaves.

---

* Corresponding author, e-mail: pshybytko@rambler.ru; ipb@biobel.bas-net.by

Key words: Photosynthesis, photosystem II, plastoquinone, cytochrome *f*, transtylakoid proton gradient, fluorescence.

Abbreviations: Chl – chlorophyll; Cyt b_6/f complex – cytochrome b_6/f complex; Cyt f_{HP} – high potential form of cytochrome *f;* F_m – maximum chlorophyll fluorescence yield; F_V – variable Chl fluorescence yield; LHCII – light harvesting complex PS II; NPQ – nonphotochemical quenching of Chl fluorescence; PFD – photon flux density; PS II – photosystem II; Q_A, Q_B – primary and secondary quinone acceptors; qE, qT qI – nonphotochemical quenching related to high energy state, state transition, photoinhibition, correspondently; Rubisco – ribulose-1,5-bisphosphate carboxylase/ oxygenase; $\Phi_{PS\ II}$ – effective quantum yield of PS II photochemistry.

Introduction

High temperature is considered to be one of the most important environmental factors influencing photosynthetic reactions in plants (Bukhov and Mohanty, 1999). Heat shock alters photosynthetic activity owing to both suppression of chloroplast electron transport and inhibition of the Calvin cycle activity (Feller et al., 1998; Pastenes and Horton, 1996b). It is considered that PS II is the most sensitive component of the photosynthetic chain (Pastenes and Horton, 1996a; Bukhov and Mohanty, 1999; Yamane et al., 1998; Yamane et al., 2000). Other sites that are sensitive to heat stress damage include the Cyt b_6/f complex and formation of the pH transthylakoid gradient linked to linear electron flow (Bukhov and Mohanty, 1999). Although water soluble Calvin cycle enzymes were found to be heat stable (McCain et al., 1989), light activation of Rubisco is one of the most heat-sensitive functions (Feller et al., 1998; Weis, 1981). In 1981 Weis reported that light-dependent activation of Rubisco in spinach chloroplasts was inhibited by moderately elevated temperatures and that inhibition was closely correlated with reversible inhibition of CO_2 fixation.

The seedlings of different ages are characterized by various rates of the photosynthetic reactions. Photosynthetic activity decreases with aging of the leaves (Sestak, 1985; Tobias et al., 1995), which is conditioned by the destruction of electron transport chain in chloroplasts and by the inactivation of Calvin cycle enzymes. Therefore it is possible to suppose that seedlings of different ages could possess variable stability under stress treatment.

In the present study the effect of heat treatment at 40°C during 3 hours on the photosynthetic activity of barley seedlings was examined and the response of young and old leaves to high temperature was compared. It was shown that the response of barley leaves to high temperature as well as targets of heat shock action depend on their age.

Materials and Methods

Experiments were carried out on primary leaves of 4-, 7- and 10-day-old *Hordeum vulgare L.* cv. Honar seedlings. Barley plants were grown on tap water at 120 µmol photons $m^{-2}.s^{-1}$, 16-h light/8-h dark photoperiod, 70% humidity, and a thermal regime of 24/22°C day/night. Heat treatment of intact plants was performed in an air thermostat for 3 hours at 40°C at 120 µmol photons $m^{-2}.s^{-1}$.

CO_2 gas exchange rate was measured by infra-red gas analyzer "Infralyt-3" (Junkalor, Germany) under 0.03% CO_2 (Bukhov et al., 1992). Chl fluorescence of intact primary barley leaves was measured at room temperature using Teaching-PAM Chl fluorometer (Walz, Germany). The basic Chl fluorescence terms were calculated according to Rohacek and Bartak (1999). The dark reoxidation kinetics of Q_A^- were measured and decayed into four kinetically distinct components as was described in Bukhov at al. (2001). The dark kinetic curves of NPQ were deconvoluted according to Horton and Hague (1988) and Walters and Horton (1991). The electron transport rate (Hill reaction) was detected in chloroplast suspension (Hill, 1951). The quantification of the amount of Cyt f_{HP} was performed by the method of difference spectroscopy (Farineau, 1976) using "Shumadzu YB-300" (Shumadzu, Japan) spectrophotometer. The total Rubisco assay was conducted as described by Salvucci and Anderson (1987).

Results

The light response curves of photosynthesis of 4-, 7- and 11-day-old leaves at 0.03% CO_2 in control and experimental (3 h, 40°C) variants is shown in Fig. 1. High temperature significantly influenced the light response curve of photosynthesis in 11-day-old leaves and almost did not change the CO_2 gas exchange of young plants. In old barley leaves heat shock led to a decrease of photosynthesis quantum yield by 25%, to the lowering of steady-state level of CO_2 fixation by 75% and the photosynthesis saturation point of stressed leaves was shifted from 750 µmol $m^{-2}.s^{-1}$ to 167 µmol $m^{-2}.s^{-1}$.

The activity of electron transport in the chloroplasts and efficiency of the dark reactions of CO_2 assimilation were studied to evaluate a reason for inhibition of photosynthesis. Heat stress suppressed the chloroplast linear electron flow measured by means of the Hill reaction (Fig. 2). The most essential changes were observed in young leaves. Rubisco activity was reduced in old leaves under high temperature, but in young seedlings the heating did not affect carboxylation activity (Fig. 2).

The state of the some electron carriers was studied for identification of the origin of inhibition of chloroplast photochemical activity. Potential quantum yield of PSII photochemistry did not change in heat stressed leaves in comparison with controls while $\Phi_{PS\,II}$ was reduced in heated young leaves (Table 1). Photochemical quenching (Table 1) characterizing the fraction of oxidized reaction centers of PSII was not altered

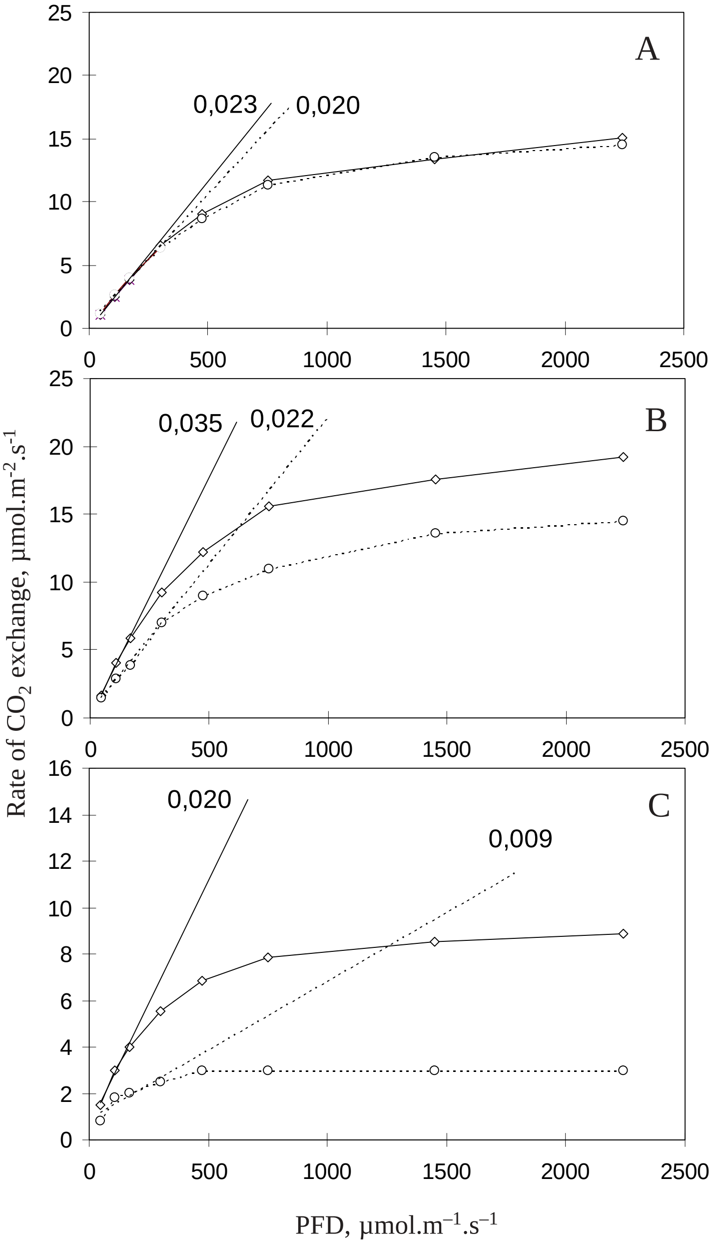

Fig. 1. Light response curves of the rate of CO_2 gas exchange for primary leaves of 4- (A), 7- (B) and 11-day-old control (open circles) and heat stressed (closed circles) barley seedlings. Numbers at the linear fits indicate the values of maximum quantum yield of photosynthesis. Each data point is the average of 3 measurements done with different leaves.

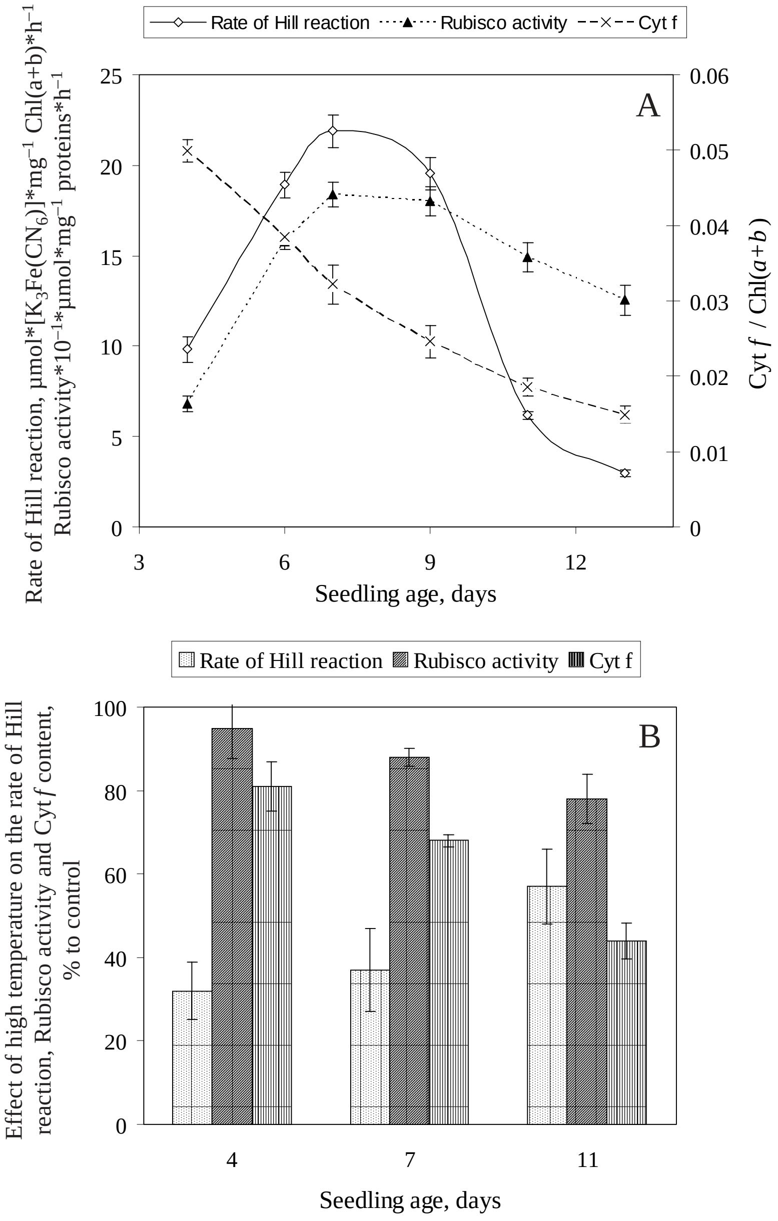

Fig. 2. Changes in the photochemical activity of chloroplasts, Rubisco activity and Cyt *f* content during leaf ontogenesis (A) and under high temperature (B).

under heat stress. The electron flow to the secondary quinone acceptor, Q_B, was estimated by the means of dark reoxidation of Q_A^-. According to Bukhov et al. (2001) in the dark kinetics of F_v relaxation it is possible to distinguish the four components delayed with half-times of several seconds (“slowest” component), 650–680 ms (“slow” component), 60–80 ms (“middle” component) and 6–8 ms (“fast” component). The slowest component is suggested to represent a minor population of inactive PS II centers (Cao and Govindjee, 1990, Bukhov et al., 2001) that was almost unchanged after high temperature impact (Table 1). The other three components reflect electron flows from Q_A^- with different rates. The heating decreased the fast and middle components and increased the amplitude of the slow phase in 4- and 7-day-old seedlings (Table 1). In old leaves high temperature did not affect the middle phase but reduced the fast component due to an increase in the slow phase (Table 1). The oxidation ability of Cyt b_6/f complex was determined by the amount of Cyt f_{HP}. Fig. 2 shows that heat shock decreased the content of Cyt f_{HP} especially in old leaves.

The efficiency of the light stage of photosynthesis was estimated by the parameters of Chl fluorescence quenching. Nonphotochemical quenching of Chl fluorescence (NPQ) decreased in 4- and 7-day-old leaves and increased in old stressed seedlings (Table 1). It is postulated that NPQ may be caused by three major mechanisms: energy-dependent quenching (qE), quenching related to transition from state 1 to state 2 (qT), and photoinhibitory quenching (qI) (Krause and Weis, 1991). The contribution of each component to total nonphotochemical quenching was estimated by means of NPQ relaxation kinetics. The changes of NPQ induced by heat were mainly caused by the alteration of the energy-dependent quenching mechanism. High temperature decreased qE in young seedlings and increased this parameter in old leaves (Table 1). The middle component of the dark F_v relaxation (qT) also decreased in 4- and 7-day-old seedlings under heat stress. In old leaves qT was absent. The contribution of component related to photoinhibitory quenching was almost unchanged under heating (Table 1).

Discussion

According to our data obtained the thermostability of the light and dark reactions of photosynthesis was strongly dependent on leaf age. The CO_2 gas exchange analysis indicated essential suppression of photosynthesis in old leaves under heat stress while the efficiency of both light and dark photosynthetic reactions in young leaves was not changed (Fig. 1). In order to identify the sites of photosynthesis inhibition the activity of certain carriers of the chloroplast electron transport chain and Rubisco activity were studied.

In young leaves heat shock was found to inhibit the photochemical activity of chloroplasts (Fig. 2). The suppression of the linear electron flow could be connected to the heat-induced damage of electron carriers. PS II is considered as the most heat sensitive component of the electron transport chain (Pastenes and Horton, 1996a; Bukhov and Mohanty, 1999; Yamane et al., 1998; Yamane et al., 2000). However ac-

Table 1. Effect of high temperature on the parameters of chlorophyll fluorescence

Age, days	Variant	F_V/F_m	$\Phi_{FS\ II}$	Phases of reoxidation Q_A, %				qP	NPQ	qE	qT	qI
				Slowest	Slow	Middle	Fast					
4	Control	0.79	0.67	9.4	38.4	37.9	14.3	0,79	0.74	0.22	0.33	0.19
	Stressed	0.76	0.57	9.9	53.3	26.4	10.4	0,78	0.57	0.15	0.25	0.17
	% to control	97	86	105	139	70	73	98	77	68	76	89
7	Control	0.82	0.63	9.1	45.7	28.7	16.5	0.82	0.92	0.39	0.28	0.25
	Stressed	0.80	0.59	9.1	57.0	23.3	10.6	0.80	0.68	0.23	0.21	0.24
	% to control	98	93	100	125	81	64	98	74	59	75	96
11	Control	0.80	0.60	9.9	50.3	20.0	19.8	0.81	1.09	0.91	-	0.18
	Stressed	0.78	0.59	10.4	58.7	19.3	11.6	0.79	1.45	1.25	-	0.20
	% to control	98	97	105	117	97	59	98	133	137	-	111

cording to our data the primary reactions in PS II reaction centers, which was described by potential quantum yield of PS II photochemistry, was not changed under heat stress (Table 1). The decrease of $\Phi_{\mathrm{PS\ II}}$ (Table 1) showed inhibition of linear electron flow on the acceptor side of PS II in heated young leaves. Since the photochemical quenching of Chl fluorescence was not changed by heat shock (Table 1) the high temperature did not affect the state of the primary qiunone acceptor Q_A. The analysis of dark reoxidation of Q_A demonstrated that linear electron transport was particularly suppressed between Q_A and the plastoquinone pool because the heat shock decreased the fast and middle components of the nonphotochemical quenching and raised the amplitude of the slow phase (Table1). According to Bukhov et al. (2001) and Krause and Weis (1991) the fast component shows rapid reoxidation of Q_A^- during electron flow to the plastoquinone pool. The middle component of F_v dark relaxation indicates a restricted electron transfer from Q_A^- to a mostly reduced plastoquinone pool. The formation of the slow component occurs only after the plastoquinone pool became fully reduced. It may be a result of the damage of the Q_B-binding site of D1 protein (Krause and Weis, 1991). The slow component is caused by recombination between Q_A^- and the S_2 state of the water-splitting complex. Thus, heat shock inhibited the linear electron flow in young leaves by means of the excessive reduction of the plastoquinone pool. The increase of the reduced plastoquinone amount under high temperature could be caused by the diminution of oxidizing ability of Cyt b_6/f complex because a decrease of Cyt f_{HP} was observed (Fig. 2).

The analysis of nonphotochemical quenching of Chl fluorescence showed that heat stress decreased the efficiency of the light stage of photosynthesis. NPQ is known to include several mechanisms of dissipative quenching related to high energy state (qE), state 1–2 transition (qT) and photoinhibition (qI) (Horton and Hague, 1988, Walters and Horton, 1991, Krause and Weis, 1991). The reduction of NPQ in young leaves under high temperature was caused by the decrease of qE and qT. Since qT is related to the transfer of absorbed energy from PS II to weakly fluorescent PSI due to the phospho-LHCII migration, the decrease of the middle phase of NPQ dark relaxation kinetics can be caused by reduction of the LHCII quantity or inactivation of phospho-LHCII phosphatase. The decrease of qE showed a reduction of the ability of chloroplasts to form a proton gradient across the thylakoid membrane. Thus high temperature suppressed the linear electron flows in chloroplasts of young leaves by means of the excessive reduction of the plastoquinone pool and decrease of the amount of active Cyt *f*. The stress-induced delay of the electron transport rate caused a decrease of the transthylakoid proton gradient and thus reduced the efficiency of the light photosynthesis stages without marked influence on the dark carboxylation reactions (Fig. 2) and CO_2 assimilation (Fig. 1).

The reaction of old leaves on the heat stress was different compared to the young seedlings. High temperature depressed both electron transport in chloroplasts and the dark the reactions of Calvin cycle in old leaves while in young seedlings only photochemical activity of chloroplasts was changed by heat stress. In addition, the linear electron transport was decreased in old leaves less than in young seedlings (Fig. 2). The proton transthylakoid gradient in old leaves was increased under heat stress (Table1). These data indicated that in old leaves the ability of chloroplasts to the formation of transthylakoid ΔpH was unchanged, but the reactions of the photosynthesis dark phase dissipating the proton gradient were partially inhibited. This conclusion was confirmed by the data that Rubisco activity (Fig. 2) and reactions of CO_2 assimilation (Fig. 1) in old leaves was strongly suppressed by heating. Apparently, an inhibition of the dark photosynthetic reactions by heat shock caused the reduction of ATP and NADPH consumption by Calvin cycle and feedback depression of linear electron transport. The inhibition of linear electron flow may be a result of overreduction of the electron carriers. Analysis of the dark kinetics of reoxidation of Q_A showed that decrease of the fast component under heating was due to the rise of the slow component (Table). Thus, heat-induced suppression of the chloroplast linear electron transport may be caused by the overreduction of the carriers of the electron transport chain.

The results obtained led to conclusion that there are various mechanisms of heat damage to photosynthesic processes in seedlings of different ages. According to the data obtained the heating of young leaves inhibited the photosynthesis by limitation of the electron transport rate while in old leaves the enzymes of Calvin cycle were the most vulnerable.

References

Bukhov, N., E. Egorova, T. Krendeleva, A Rubin, C. Wiese, U. Heber, 2001. Relaxation of variable chlorophyll fluorescence after illimination of dark-adapted barley leaves as influenced by the redox states of electron carriers. Photosynth. Res., 70, 155–166.

Bukhov, N. G., I.S. Drozdova, V. V. Bondar, A. T. Mokronosov, 1992. Blue, red and blue plus red light control of chlorophyll content and CO_2 gas exchange in barley leaves: quantitative description of the effects of light quality and fluence rate. Physiol. Plant., 85, 632–638.

Bukhov, N. G., P. Mohanty, 1999. Elevated temperature stress effect on photosystems: Characterization and evaluation of the nature of heat induced impairments. In: Concepts in hotobiology: Photosynthesis and Photomorphogenesis, Ed. G. S. Singhal, G. Renger, S.K. Sopory, K-D. Irrgang, Govindjee. Narosa Publishing House, New Delhi, India, 617–648.

Farineau, J., 1976. Simultaneous appearance of C-550 and cytochrome b559 (high potential form) in barley leaves greened under intermittent light. Physiol. Plant., 36, 393–398.

Feller, U., S. J. Crafts-Brandner, M. E. Salvucci, 1998. Moderately high temperatures inhibit ribulose-1,5-bisphospate carboxylase/oxygenase (Rubisco). Activase-mediated activation of Rubisco. Plant Physiol., 116, 539–546.

Hill, R., 1951. Oxidoreduction in chloroplasts. In: Enzymology, 12.

Horton, P., A. Hague, 1988. Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV. Resolution of non-photochemical quenching. Biochim. Biophys. Acta, 931, 107–115.

Krause G. H., E. Weis, 1991. Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant. Physiol. Mol. Biol., 42, 313–349.

McCain, D. C., J. Croxdale, J. L. Markley, 1989. Thermal damage of chloroplasts envelope membranes. Plant Physiol., 90, 606–609.

Pastenes, C., P. Horton, 1996. Effect of high temperature on photosynthesis in beans. I. Oxygen evolution and chlorophyll fluorescence. Plant Physiol., 112, 1245–1251.

Pastenes, C., P. Horton, 1996. Effect of high temperature on photosynthesis in beans. II. CO_2 assimilation and metabolite contents. Plant Physiol., 112, 1245–1260.

Rohacek, K., M. Bartak, 1999. Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. Photosynthetica. 37, 339–363.

Salvucci, M. E., J. C. Anderson, 1987. Factors affecting the activation state and the level of total activity of ribulose bisphosphate carboxylase in tobacco protoplasts. Plant Physiol., 85, 66–75.

Sestak, Z., 1985. Photosynthesis during Leaf Development. Academia, Praha.

Tobias, D. J., A. Ikemoto, T. Nishimura, 1995. Leaf senescence patterns and photosynthesis in four leaf flushes of two deciduous oak (quercus) species. Photosynthetica, 31, 231–239.

Walters, R. G., P. Horton, 1991. Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. Photosynth. Res., 27, 121–133.

Weis, E., 1981. The temperature sensitivity of dark-inactivation and light-activation of the ribulose-1,5-bisphosphate carboxylase in spinach chloroplasts. FEBS Lett., 129, 197–200.

Yamane, Y., T. Shikanai, Y. Kashino, H. Koike, K. Satoh, 2000. Reduction of Q_A in the dark: Another cause of fluorescence F_o increases by high temperature in higher plants, Photosynthesis Research., 63, 23–34.

Yamane, Y., Y. Kashino, H. Koike, K. Satoh, 1998. Effect of high temperatures on the photosynthetic systems in spinach: Oxygen-evolution activity, fluorescence characteristics and the denaturation process. Photosynthesis Research., 57, 51–59.