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Function of plastoquinone in heat stress reactions of plants

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ABSTRACT

The effect of high temperature treatment (40 °C, 3 h, illumination at 100 μmol m^{-2} s^{-1}) on the photosynthetic electron flow in barley seedlings of different age was investigated. Thermoinduced inhibition of the liner electron flow due to partial impairment of the water oxidizing complex (WOC) and the increase in the extent of Q_A^- reoxidation by Tyr_Z^{ox} in thylakoids isolated from 4-day-old leaves was shown by measurements of oxygen evolution using benzoquinone or potassium ferricyanide as electron acceptors, as well as by following Q_A^- reoxidation kinetics in the absence and presence of exogenous electron acceptors, DCBQ and DMBQ. Using HPLC analysis, an increase in the oxidation of the photoactive plastoquinone pool in young leaves under heating was shown. In older, 11-day-old leaves, heat treatment limited both photosynthetic electron flow and oxygen evolution. The same effects of heat shock on oxygen evolution caused an inhibition of electron flow on the donor side of PSII only. However, a rise in the proportion of PSII with Q_A^- reoxidized through recombination with the S_2/S_3 state of the WOC was observed. The addition of exogenous electron acceptors (DCBQ and DMBQ) and a donor (DPC) showed that the thermoinduced decrease in the electron transport rate was caused by an impediment of electron flow from Q_A^- to acceptor pool. The decrease in size of the photoactive PQ-pool and a change in the proportions of oxidized and reduced PQ in older leaves under heat treatment were shown. It was suggested that a thermoinduced change of the redox state of the PQ-pool and a redistribution of plastoquinone molecules between photoactive and non-photoactive pools are the mechanisms which reflect and regulate the response of the photosynthetic apparatus under heat stress conditions.

1. Introduction

The photosynthetic activity of plants is known to be inhibited by the activity of stressors such as high light, drought, high and low temperature, which are the source of oxidative stress [1,2]. Heat stress induces significant modifications in the composition of chloroplast membrane lipids and proteins together with structural changes of the thylakoid membrane [3,4]. These changes, along with the direct inactivation of photosystems, greatly affect the photochemical activity of chloroplasts [5,6].

Photosystem II (PSII) is considered to be the most thermosensitive component of thylakoid membranes [1,5]. It has been shown that the water oxidizing complex (WOC) is the most susceptible component of PSII to heat stress, although both the PSII reaction center and the light-harvesting complexes can also be disrupted by high temperature [7]. The thermal inactivation of PSII is due to the extraction of divalent Ca^{2+} and Mn^{2+} cations and Cl^- anion from the PSII pigment–protein complex and the release of extrinsic 18, 24 and 33 kDa polypeptides from thylakoid membranes [8–11]. The sensitivity of PSII to stress depends on the organization level of the system in question and there is also variation in the extent of the acclimation of PSII to heat stress [12]. In intact plants under heat stress, PSII is inactivated by higher temperatures than is the case with BBY-particles or thylakoids. In many cases the activation of protective mechanisms determines the thermotolerance of PSII. A more complicated system has more adaptive mechanisms which protects the thermosensitive elements of the photosynthetic apparatus. The primary causes of thermoinactivation of PSII *in vitro* and *in vivo* could be different.

An important component in the regulation of photosynthetic reactions is the redox state of the plastoquinone (PQ) pool. It has been implicated in the mediation of a number of photosynthetic responses, including regulation of state-transition, chlorophyll biosynthesis, light-harvesting complex polypeptide accumulation, rate of photosystem protein synthesis and the balance of photosystem stoichiometry [13–18]. There is also a consensus that the PQ-pool acts as a sensor of any imbalance in electron transport [12,19]. On the other hand, the redox state of the PQ-pool and the thylakoid ΔpH are a reflection of cellular capacity to utilize absorbed light energy and the

Abbreviations: Chl, chlorophyll; DCBQ, 2,5-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DPC, 1,5-diphenylcarbazide; DMBQ, 2,6-dimethylbenzoquinone; DPH, 1,6-diphenyl-1,3,5-hexatriene; HPLC, high-performance liquid chromatography; qP, photochemical quenching of chlorophyll fluorescence; PQ, plastoquinone; PQH_2, reduced plastoquinone, plastoquinol; PQ-pool, photoactive plastoquinone pool; PFD, photon flux density; PS, photosystem

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products of photosynthesis [20,21]. In the case of increased plastoquinone reduction, the limitation of Calvin cycle enzymes and the slowdown in ATP consumption, an increase in thylakoid ΔpH level and suppression of intersystem electron flow have been observed [22]. An increased level of plastoquinone pool reduction causes the double reduction of Q_A (Q_A^{2-}) in the reaction center of PSII, a recombination of the ion-radical pair [P_{680}^{+}Pheo^{-}] and triplet $^3P_{680}$ formation [23]. Oxidized plastoquinone molecules are known non-photochemical quenchers of chlorophyll excited states of both PSII and PSI antennas due to thermal dissipation [24].

In the present research, we have studied the mechanism of PSII thermoinactivation *in vivo*. The possibility of thermoinactivation both on the acceptor and donor sides of PSII was investigated. The results obtained using different methods allowed us to show a specific role of the redox state of plastoquinone pool in the limitation of the photosynthetic electron flow under heat treatment.

2. Materials and methods

Barley (*Hordeum vulgare* L.) plants were grown on tap water at 100 μmol photons m^{-2} s^{-1}, 16-h light/8-h dark period, 70% humidity, and a thermal regime of 24/22 °C day/night. Heat stress was induced by the heating of intact 4-, 7- and 11-day-old seedlings for 3 h at 40 °C, under continuous illumination (100 μmol m^{-2} s^{-1}).

The oxygen evolution activity of thylakoids isolated according to [25] was measured in a closed 3 ml cell using a Clark-type electrode (Hansatech, UK) under continuous illumination with white light (600 μmol photons m^{-2} s^{-1}) at 20 °C and in the presence of 1 mM *p*-benzoquinone or 0.5 mM $K_3[Fe(CN)_6]$. The activity of the Mehler reaction was determined by measurement of the oxygen consumption of thylakoid membranes in the presence of 0.5 mM 2,3,5,6-tetramethyl-*p*-phenylenediamine, 1 mM ascorbate, 25 μM methyl viologen, 2 U/ml superoxide dismutase and 1 mM NaN_3.

The chlorophyll (Chl) fluorescence of intact primary barley leaves was measured at room temperature with a PAM 201 chlorophyll fluorometer (Walz, Germany). The basic chlorophyll fluorescence parameters were calculated according to [26,27]. The dark reoxidation kinetics of Q_A^- were measured after 1 s irradiation of dark-adapted barley seedlings by red actinic light PFD of 480 μmol m^{-2} s^{-1}. The magnitude and half-time of different kinetic components of fluorescence relaxation were obtained from plots of log (F_v) as a function of relaxation time. A best linear fit was calculated for each kinetic component providing its half-time as the time at which F_{vi} is 50% of the maximum magnitude which is obtained from the intersection of the linear fit with the ordinate.

The redox state of PQ in leaves and the photoactive PQ pool (PQ-pool) was determined using HPLC method as described in Ref. [28]. Extracts from liquid nitrogen-frozen leaves were prepared in freezer-cold ethyl acetate and immediately evaporated to dryness under a stream of nitrogen. The concentration of both oxidized PQ and reduced PQ (PQH_2) in the sample were determined from the area of the corresponding peaks in the chromatograms and the area of the peaks of PQ and PQH_2 standards. The HPLC measurements were performed using a Jasco pump and a UV–VIS detector, a Shimadzu RF10 AXL fluorescence detector, a Teknokroma C18 reverse-phase column (250×4 mm, 5 μm), an isocratic solvent system (methanol/hexane, 340/20, vol/vol), flow rate of 1.5 ml/min, absorption detection wavelength at 255 nm, fluorescence excitation/emission detection at 290/330 nm and injection volume of 100 μl.

The PQ-pool was determined in separate experiments using conditions of a totally reduced and oxidized PQ-pool. The PQ-pool was completely reduced by a 15 s illumination of leaves by light with PPFD of 2000 μmol quanta m^{-2} s^{-1} at 22 °C. The conditions for the total oxidation of the PQ-pool were obtained by infiltration of the leaves with 50 μM DCMU solution in water and after 5 min of incubation by illumination at 500 μmol quanta m^{-2} s^{-1} light at 22 °C.

Changes in the viscosity of the thylakoid membranes were estimated by measurements of the fluorescence anisotropy (r) of the lipophilic fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) [29]. The incorporation of DPH into the thylakoids membranes was performed by 40 min incubation of thylakoids (at 10 μg/ml Chl concentration) in a medium containing 10 mM Tris/HCl (pH 7.9), 350 mM KCl and 1.5 μM DPH in darkness under continuous mixing. Unincorporated DPH was removed by washing the thylakoids with a buffer. The steady-state fluorescence anisotropy was measured at 20 °C with a Solar LSF 222 fluorometer (Solar, Belarus) at λ_{ex} = 360 ± 5 nm and λ_{em} = 460 ± 10 nm following the procedure described in ref. [29].

3. Results

The dependence of the oxygen evolution activity of thylakoid membranes on the age of seedlings in the presence of *p*-benzoquinone or ferricyanide shows the maximum for the 7-day-old plants (Fig. 1A). The activity in the presence of ferricyanide was lower than in the presence of *p*-benzoquinone for 7- and 11-day-old seedlings. Since *p*-benzoquinone accepts electrons from Q_B [30] and ferricyanide from the Fe–S proteins of PSI [31], the obtained data indicated a slowing down of the electron transport rate between the photosystems in older leaves. Heat treatment did not affect oxygen evolution activity measured in the presence of *p*-benzoquinone in the youngest leaves but limited essentially this process in older leaves (Fig. 1B). On the other hand, a thermoinduced decrease in the activity measured in the presence of ferricyanide was observed in all cases. The presented

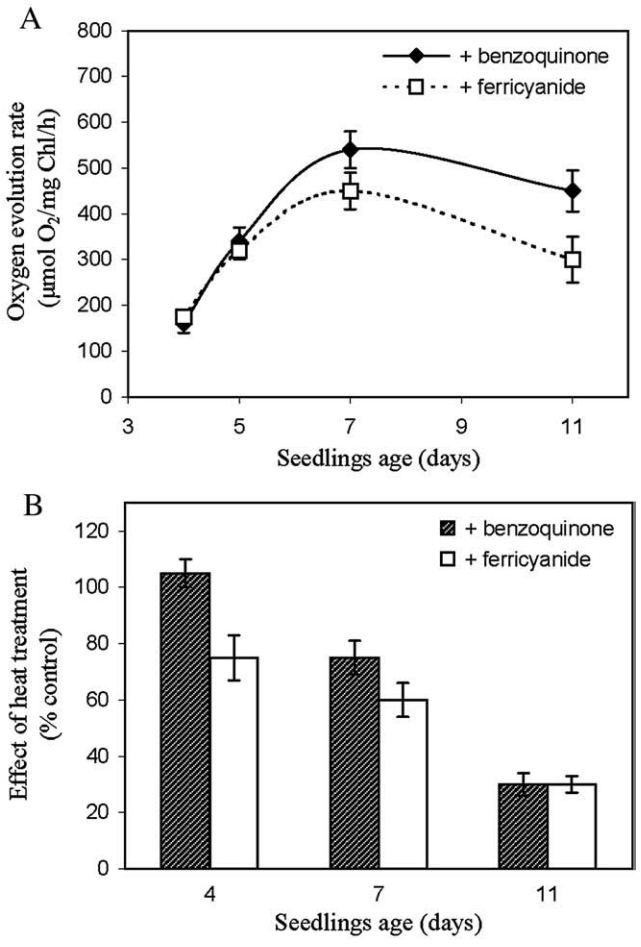

Fig. 1. Oxygen evolving activity of thylakoid membranes in control (A) and heated (B) seedlings of different age in the presence of 2.5 mM $[K_3Fe(CN)_6]$ or 1 mM *p*-benzoquinone. Each value represents the average with standard errors of results from four independent experiments.

oxygen evolving rates were normalized to the total chlorophyll content. Chlorophylls content per dry weight or leaf area remained practically unchanged under the applied treatment (not shown). This fact excludes a change in antenna size, which can lead to a change in the rate even under conditions of unchanged number and activity of functional intact PSII complexes. Thus, the data obtained indicate that inhibition of the oxygen evolving in older leaves is related to the limitation of the electron transport rate in thylakoids under heat treatment. In young 4-day-old leaves, heating did not affect the activity of the PSII donor side and the electron flow was apparently suppressed on the acceptor side of PSII.

Using PAM fluorometry, it was shown that heat treatment did not affect the potential quantum yield (F_v/F_m) of PSII photochemistry (Table 1). The effective quantum yield of PSII photochemistry (Φ_{PSII}) and photochemical quenching of chlorophyll fluorescence (qP) and the F_v/F_o level were not significantly changed under heat treatment. Thus, the applied heat treatment did not affect the electron flow within PSII.

The kinetics of Chl fluorescence dark relaxation was measured after 1 s of light pulse (480 μmol quanta m^{-2} s^{-1}) for 10 s with a sampling rate of 0.5 point/ms (Fig. 2A). The data obtained were analyzed by semi-logarithmic plots and the signals were deconvoluted into kinetically distinct components by means of the consecutive extrapolation of linear plots (Fig. 2B,C). Three kinetically distinct components were observed. The fast component was characterized by a half-life time ($\tau_{1/2}$) of 0.5–5 ms and an amplitude of about 40% of the total Chl fluorescence level. According to [32, 33] a PQ molecule bound to the Q_B-site accepts electrons from Q_A^- with a half-life time of 150–250 μs and semiquinone, Q_B^- is reduced with a half-life time of 400–600 μs. Longer Q_A^- reoxidation (half-life time of 5 ms) includes also processes of PQ-pool reoxidation by an electron acceptor pool which consists of PSI acceptor side, cytochromes, and plastocyanin [34]. A reoxidation of the PQ-pool leads to Q_A^- reoxidation. The middle fraction accounting for 30–45% of the total PSII population was characterized by decay kinetics with $\tau_{1/2}$ of 50–100 ms. This time is typical of a recombination between Q_A^- and Tyr_Z^{ox} in PSII complexes that are lacking a functionally competent water oxidizing complex (WOC) [35,36]. A small subpopulation of PSII complexes (15–25%) was characterized by F_v decay with a half-life time of 500–700 ms. In PSII centers lacking the capability of Q_A^- reoxidation via Q_B-site, the donor site acts as an oxidant of Q_A^-. Slow Q_A^- reoxidation is caused by a recombination between Q_A^- and the S_2 (S_3) state of the WOC [33]. After a 1 s illumination of leaves a stationary S_i-distribution of S_0:S_1:S_2:S_3 of 0.25:0.25:0.25:0.25 is expected [37]. Accordingly, the subpopulation of PSII complexes with slow decay component can amount to 25%.

During development of the first barley leaf, an increase in the proportion of PSII complexes where Q_A^- was reoxidized by the electron acceptor pool and recombination with the S_2/S_3 state of the WOC was observed (Fig. 3). Heat treatment led to a decreased amplitude of the fast component and a concomitant increase in the amplitude of the middle component of F_v dark relaxation in 4-day-old seedlings (Fig. 3). Thus, the heating caused primarily an impairment of the WOC in young leaves and the extent of Q_A^- reoxidation by Tyr_Z^{ox} is increased. In older leaves, a thermoinduced increase in amplitude of the slow component and a reduction of PSII subpopulation with fast decay was observed. These data indicate that the heating induced suppression of liner electron flow and the recombination of Q_A^- with the S_2/S_3 state of the WOC. Under these conditions, the WOC was kept functionally competent and donated electrons to PSII. However PQ-pool reoxidation was slowed down. The limitation of electron transfer to PSI could be a reason for the suppression of chloroplast electron flow.

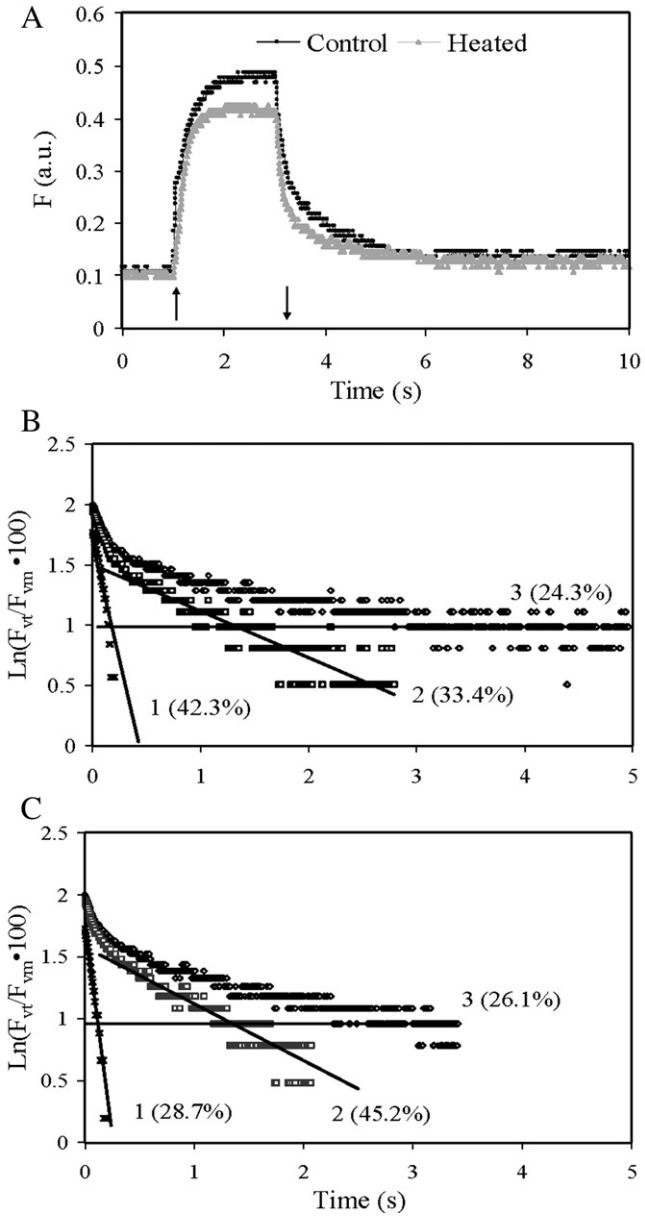

Fig. 2. A. Light-dependent increase of F_v and subsequent dark relaxation in dark-adapted 7-day-old barley leaves. Arrows indicate onset and termination of red actinic illumination (PPFD 480 μmol m^{-2} s^{-1}). Each curve is the average of 6 measurements performed with different leaves. B, C. Semi-logarithmic plots of dark relaxation of F_v after 1-s irradiation of dark-adapted control (B) and heated (C) 7-day-old barley leaves by red actinic light PPFD of 480 μmol m^{-2} s^{-1}. Curve 3 is a semi-log plot of the time course of F_v with slow components of F_v. Curves 1 and 2 are the deconvoluted fast and middle kinetic components of F_v. F_v values were normalized to maximum F_v at PPFD of 3500 μmol m^{-2} s^{-1}.

Table 1
Parameters of chlorophyll fluorescence induction during leaf development and the effect of heat shock (40 °C, 3 h)

Age (days)		F_v/F_m	Φ_{PSII}	qP	F_v/F_o
4	Control	0.791	0.671	0.79	3.78
	Heated	0.767	0.577	0.77	3.29
	Heated/control	97%	86%	98%	87%
7	Control	0.822	0.632	0.82	4.62
	Heated	0.864	0.588	0.80	4.16
	Heated/control	98%	93%	98%	90%
11	Control	0.800	0.604	0.81	4.00
	Heated	0.784	0.586	0.79	3.63
	Heated/control	98%	97%	98%	91%

The data shown is the average of 9 measurements performed with different leaves. Standard errors did not exceed 5–10% of the mean values.

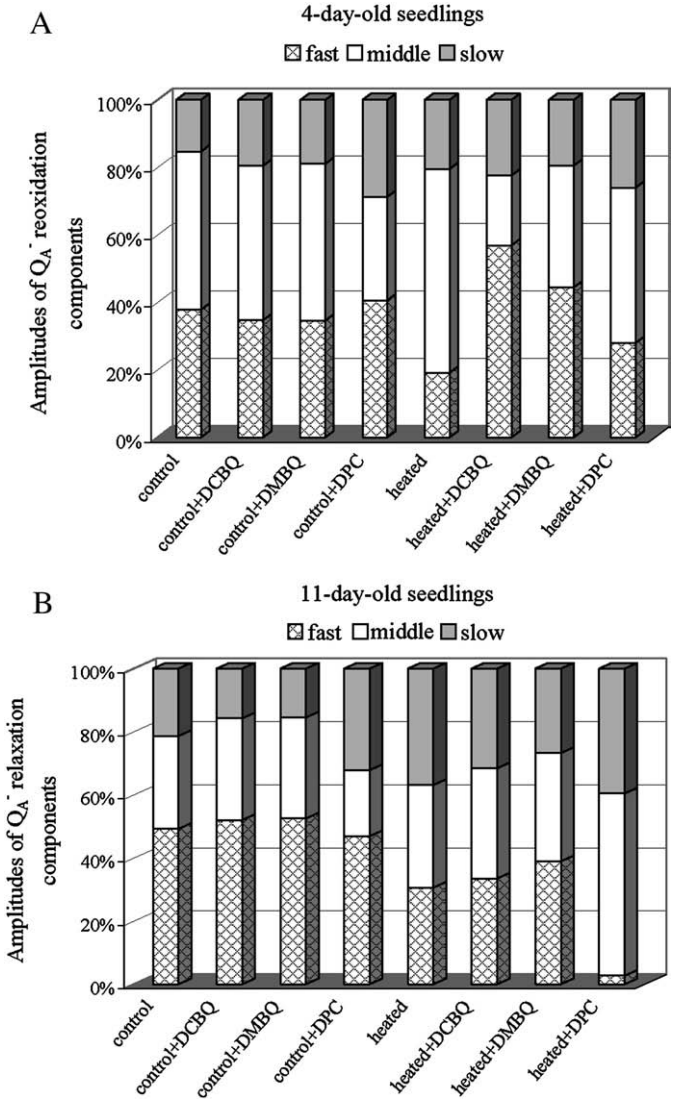

Fig. 3. Effect of high temperature treatment and exogenous electron acceptors and donor on amplitudes of Q_A^- relaxation components in chloroplast of 4- (A) and 11-days-old (B) leaves. The given values are the average of 6 measurements. Standard errors did not exceed 5–10% of the average.

The addition of exogenous 2,5-dichloro-*p*-benzoquinone (DCBQ), which accepts electrons from Q_A^-, diminished the effect of heat shock (Fig. 3). Moreover, in thylakoids from young heated leaves, the addition of DCBQ accelerated Q_A^- reoxidation more efficiently than in thylakoids from control leaves. The addition of exogenous 2,6-dimethylbenzoquinone (DMBQ), accepting electrons from Q_B^-, also restored the electron flow from Q_A^- to the acceptor pool. The effect of DCBQ and DMBQ on decay kinetics reflects their efficiency in oxidizing the reduced plastoquinone pool, probably including PQH_2 exchange at the Q_B-site and subsequent turnover of the exogenous quinones. An enhanced quinol replacement at the Q_B pocket caused faster reoxidation. After the addition of an exogenous electron donor to Tyr_z, diphenylcarbazide (DPC), a tendency to increase in fast and slow phases of decay kinetics was observed (Fig. 3). Since the addition of exogenous quinones accelerated Q_A^- reoxidation in heated seedlings to a higher extent than in control and an exogenous donor did not rescue the heat treatment effect, a thermoinduced limitation of electron flow by the acceptor pool of PSII could occur.

In older leaves, the addition of DCBQ and DMBQ decreased the amplitude of the slow component and accelerated the electron flow both for control and heated seedlings (Fig. 3). However, the effect of heat stress on Q_A^- reoxidation kinetics was preserved. The addition of DPC increased the amplitude of the slow component due to a diminution of the middle phase component. Exogenous donation of electrons to Tyr_z increased population of PSII, which is characterized by decay kinetics with $\tau_{1/2}$ of 500 ms. In the thylakoid membranes of heated leaves, the addition of DPC increased the amplitude of the middle component. At the same time the fast component of F_v decay kinetics nearly disappeared. Possibly, in untreated leaves, DPC accelerated oxidation of Tyr_z by P_{680}^+ and increased the recombination of Q_A^- and the S_2/S_3 state of the WOC. The addition of an electron donor to the heat damaged system induced the aggravation of thermo-inactivation on the PSII acceptor side due to the impossibility of Q_A^- recombination with broken WOC. Thus, the obtained result could indicate a high temperature-induced limitation of electron flow both on the donor and acceptor side of PSII.

During the leaf development of control plants, the total plastoquinone level increased. The size of the photoactive PQ-pool did not change significantly, although the PQ-pool was oxidized to the highest extent in the oldest leaves (Table 2). Heat treatment did not affect the total plastoquinone level or its redox state. However, thermoinduced changes in photoactive and non-photoactive PQ fractions were observed. The contribution of the photoactive PQ-pool decreased after the heat treatment of barley seedlings (Table 2). Moreover, the PQ-pool became more oxidized after heating. The heat response of the redox state of the PQ-pool was observed to depend on the age of the leaves. In older leaves, the heat treatment decreased the size of the photoactive plastoquinone pool more than in young seedlings. The size of the photoactive fraction in relation to total PQ in 11-day-old heated leaves was twice as small than in non-treated plants (Table 2). The ratio of photoactive PQ molecules per reaction center of PSII decreased from 3.5 to 1.5 in old leaves under heating. The obtained data suggest that the decrease in the electron flow rate on the acceptor side could be caused by a decrease in the number of photoactive plastoquinone molecules.

Plastoquinone is known to share electron transfer between linear and cyclic chains [29,30]. Therefore, the cyclic electron flow around PSI could affect PQ-pool reduction. PSI activity was measured as a rate of the Mehler reaction. Oxygen consumption increased twofold after heating of 4-day-old seedlings (Fig. 4). In older leaves this effect was observed to a smaller extent. Thus, heat treatment increased PSI activity and could induce an acceleration of the cyclic electron transport.

Table 2
The effect of high temperature treatment (40 °C, 3 h) on the content and redox state of PQ in barley seedlings (PQ_{total} = PQ oxidized + PQ reduced); $N = 3 \pm SE$

Plant age (days)	Treatment	PQ_{total}/1000 Chl (mol/mol)	PQ_{ox}/PQ_{total} (%)	Photoactive PQ (PQ-pool)		Non-photoactive PQ	
				PQ-pool/PQ_{total}, (%)	PQ_{ox}/PQ-pool, (%)	PQ_{nonph}/PQ_{total}, (%)	PQ_{ox}/PQ_{nonph}, (%)
4	Control	24.9±1.4	45.0±3.5	35.4±5.7	36.4±12.5	64.4±5.7	50.0±10.3
	Heated	25.1±1.0	50.7±1.4	30.5±6.0	50.5±6.7	69.5±6.0	50.7±9.7
7	Control	27.2±0.3	61.7±2.4	31.5±7.8	28.6±7.2	68.5±7.8	76.9±10.6
	Heated	26.5±0.0	61.1±0.8	23.2±2.9	45.0±3.0	76.8±2.9	65.9±4.3
11	Control	38.6±3.2	43.4±2.3	30.3±2.2	56.0±3.9	69.7±2.2	37.9±2.8
	Heated	37.6±5.7	45.2±6.9	13.1±12.3	67.2±10.6	86.9±12.3	41.9±17.5

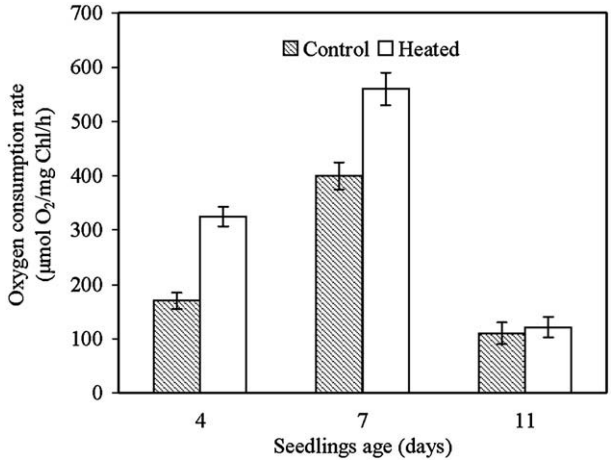

Fig. 4. Oxygen consumption of thylakoid membranes from control and heated seedlings of different ages in presence of 0.5 mM 2,3,5,6-tetramethyl-*p*-phenylenediamine, 1 mM Na-ascorbate, 25 μM methyl viologen, superoxide dismutase (2 U/ml) and 1 mM NaN_3. Each value represents the average with standard errors of results from four independent experiments.

In order to find an explanation for the decrease in the electron transport rate on the PSII acceptor side, the microviscosity of thylakoid membranes was estimated using measurements of DPH anisotropy. The microviscosity of thylakoid membranes increased during the ontogenesis of the first leaf (Fig. 5). Heat treatment did not change this parameter in young leaves but decreased the level of *r* in heated 11-day-old leaves. This fact could be connected with thermoinduced structural changes in the thylakoid membranes of older leaves.

4. Discussion

In the present study, seedlings of different ages were used as models for the investigation of various thermoinactivation mechanisms of the photosynthetic apparatus. The obtained data have shown the varying functional activity of thylakoid membranes during development of the first barley leaf. The young leaves showed lower oxygen evolution activity probably due to an incomplete assembling of the PSII components and a high PSI/PSII ratio [38]. The lower microviscosity of the lipid bilayer and the decreased level of thylakoid membrane stacking are characteristic of the thylakoid membranes of young leaves, which provides higher mobility of plastoquinone. During development of the first leaf, a decrease in the mobility of thylakoid membrane lipids and a rise in plastoquinone amount were observed. Moreover, 7-day-old leaves showed the highest rate of intersystem electron flow and oxygen evolving activity. In line with the literature, our data support the conclusion that 7-day-old barley leaves have a photosynthetic apparatus which is completely formed and maximally active. In 11-day-old seedlings, the formation of the second leaf was observed. This process was associated with a flow of assimilates from the first leaf to the second one and the exhaustion of the first one. Thus, 4-, 7- and 11-day-old barley leaves were characterized by different states and activities of photosynthetic membranes. The heat treatment of seedlings at different stages of leaf development induced various responses and adaptation mechanisms.

In young leaves, heat treatment neither changed the oxygen evolution rate nor the PSII photochemistry. Only a suppression of electron flow between the photosystems was observed. Q_A^- reoxidation kinetics both with and without electron acceptors and donor showed that electron flow through PSII was limited by partial impairment of the WOC. The modification of the plastoquinone redox state could be considered as a regulator of the response of the photosynthetic apparatus. In our case, the change in the redox state of the PQ-pool could control the activation of protective mechanisms, for example, cyclic electron transport around PSII [39,40].

In older leaves, heat treatment induced more pronounced changes in photosynthetic activity. Oxygen evolution was suppressed significantly in 11-day-old heated leaves. A similar degree of thermoinduced inhibition of oxygen evolution in the presence of *p*-benzoquinone and ferricyanide indicates that the limitation of oxygen evolution was not due to an inhibition of the PSII acceptor side but rather to damage to the oxygen evolving complex or to the disturbance of the PSII donor side. However, an analysis of Q_A^- reoxidation kinetics showed thermoinduced limitation of electron flow and reoxidation of Q_A^- by the S_2/S_3 state of the WOC. Since the addition of DCBQ or DMBQ did not compensate for the effect of heat shock and the addition of DPC aggravated it, the obtained data indicate a possible thermoinduced inactivation of PSII both on the donor and acceptor side. Under PSII inactivation on the donor side an increase in the lifetime of highly oxidizing cation-radicals, Chl_Z^+ and P_{680}^+ is observed [41]. Under the acceptor side mechanism of inhibition, the over-reduction of the acceptor side of PSII induces a charge recombination between the P_{680}^+ and $Pheo^-$ leading to the formation of a P_{680} triplet state. The P_{680} in triplet state subsequently reacts with molecular oxygen to form a singlet oxygen [41]. Inhibition of the PSII usually causes a degradation of both D1 and D2 proteins and induces lipid peroxidation [42]. The thermoinduced decrease in the microviscosity of thylakoid membranes could be caused by the latter process.

According to the HPLC data the proportion of oxidized PQ-pool increased after the heat treatment of leaves. These data can be explained by an impairment of the donor site capacity due to inactivation of the WOC. On the other hand, a decreased PQ-pool reduction in heat stressed leaves can be reconciled with the limitation of the electron transport rate on the acceptor side of PSII if the activation of PSI cyclic electron transport is taken into account. A cyclic flow is known to be essential for the maintenance of the correct ratio of ATP/NADPH production and the prevention of stroma over-reduction [24,43]. In green plants, the electron is recycled from the stroma-exposed side of PSI to the PQ-pool by the NAD(P)H dehydrogenase complex or the ferredoxin-plastoquinone oxidoreductase (FNR) [44]. Reduced PQ induces the increase in the photosynthetic capacity of PSI which is required to activate cyclic electron transport [24]. The regulation of the correlation between liner and FNR-related cyclic electron flow is suggested to occur through the release of FNR from PSI [45]. According to the model of lateral organization of thylakoid membranes proposed by Joliot and Joliot [45,46], linear and cyclic electron flows pass through different types of PSI and PSI_{linear} and PSI_{cyclic} are localized in different compartments of thylakoid membranes. PSI_{linear} associates with FNR and transfers

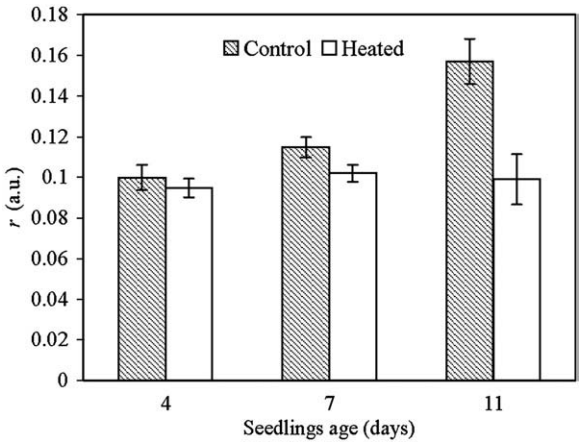

Fig. 5. Fluorescence anisotropy of DPH incorporated into thylakoid membranes of control and heated barley seedlings. Each value represents the average with standard errors of results from three independent experiments.

electrons to $NADP^+$ thought FNR. PSI_{cyclic} not associated with FNR are only able to transfer electrons to the soluble pool of ferredoxin. In this case, FNR is able to form a complex with cytochrome b_6f, which could provide a binding site for ferredoxin on the stromal side. Therefore, electron transfer via cyclic and linear pathways is fulfilled by different pools of plastoquinone molecules. Under the conditions of the activation of PSI cyclic electron transport, the release of FNR from PSI occurs and ratio PSI_{cyclic}/PSI_{total} is increased (maximum to 0.5 [46]) and the amount of plastoquinone molecules participating in the cyclic electron transport is increased accordingly. Then the proportion of oxidized PQ is lowered on the acceptor side of PSII, while the total oxidized fraction of PQ-pool is not changed or is even made higher.

Moreover, reduced PQ molecules are known to be efficient scavengers of the singlet oxygen [48] that is generated in PSII [7,42,47]. Under heat stress a production of reactive oxygen species occurs which, in turn, inhibits protein synthesis [49]. Plastoquinol, as a phenolic compound, is oxidized during the scavenging of singlet oxygen formed in PSII [48]. Oxidation of plastoquinol by the superoxide anion radical generated in PSI is also known to be effective scavenging mechanism [50]. Thus, the decrease in level of reduced plastoquinone molecules could be caused by the scavenging function of plastoquinol.

A thermoinduced redistribution of plastoquinone molecules between photoactive and non-photoactive pools was observed in older leaves. That could be considered as a mechanism for adapting to a decreased level of active PSII reaction centers and as a limiting factor for photosynthetic electron transport. The decreased level of the reduced photoactive PQ confirms the first assumption. It is known that plastoquinone is localized in thylakoid membranes, as well as in the envelope and plastoglobuli [51]. In thylakoid membranes, plastoquinone molecules mediate electron transfer and provide antioxidant protection [48,50]. Biosynthesis of plastoquinone takes place in the chloroplast envelope [52], while plastoglobuli have a storage function [51]. With increasing age of chloroplast and leaves, the number and size of plastoglobuli in chloroplast stroma increases and an accumulation of plastoquinone-9 is observed [51]. Thus, the decrease in the proportion of the photoactive and the increase in the size of the non-photoactive plastoquinone pool in old leaves under heat treatment could be connected with the segregation of plastoquinone molecules from thylakoid membranes to plastoglobuli or photochemically non-active aggregates within thylakoid membranes.

This redistribution of plastoquinone molecules from the photoactive to the non-photoactive pool could be considered as a protective mechanism for the prevention of PSII inactivation on the acceptor side. In plastoglobuli, PQ molecules are known to be deposited predominantly in a reduced form [51]. We observed an increase in amount of as reduced (42% of total non-photochemical PQ molecules increase) as oxidized (58%) non-photochemical PQ molecules. In the envelope, PQ molecules are localized in the oxidized form only [53]. Under high light, for example, an acceleration of the plastoquinone turnover rate has been shown [48]. Thus, the stress could induce a decomposition of plastoquinols following the scavenging of singlet oxygen in thylakoid membranes could be compensated by the fast biosynthesis of PQ molecules in the envelope.

The presented results indicate that the heat stress induced response is determined by the physiological state of the photosynthetic apparatus. Primarily, the components of the photosynthetic electron transport chain that are not fully developed are subject to inactivation. Plastoquinone could perform a regulatory function during changes of the redox state of the PQ-pool or in a redistribution between photoactive and non-photoactive pools.

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