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PHOTOSYNTHETIC APPARATUS AND HIGH TEMPERATURE: ROLE OF LIGHT

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Summary. The rate of oxygen evolution/consumption, *in vivo* electron transport activity, overall photosynthetic capacity and accumulation of the chloroplast 30 kDa heat shock proteins were studied after treatment of intact barley seedlings at 40 °C for 3 hours either in presence of low white light (100 µmol $m^{-2}.s^{-1}$) or in the dark. High temperature impact in the dark resulted in lowering of water-splitting capacity, photosynthetic electron transport rate, suppression of non-photochemical energy dissipation and membrane energization (qE), caused the photoinhibition of PS2 at high PFD and indicate impairment of CO_2-assimilation rate in old (11 d-old) leaves. The observed data revealed a positive influence of low light in the resistance of the photosynthetic apparatus of barley leaves to high temperature including primary photosynthetic reaction and carbon metabolism. The heat-induced damaging processes increased considerably with leaf senescence whereas the young seedlings were the most adaptive and flexible to the environment. Heat shock chloroplast 30 kDa proteins were found to be accumulated after plant heating under the both conditions, light and the dark. It was suggested that light-dependent resistance of intact plants to high temperatures could depend not only on transthylakoid proton gradient but also on the leaf transpiration rate, intracellular antioxidant balance and accumulation of low molecular weight organic compounds.

Key words: barley, heat shock, light and dark, photosynthetic apparatus, proteins

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Abbreviations: F_V/F_M – maximal quantum yields of PS2 photochemistry, HS – heat shock, NPQ – non-photochemical quenching of chlorophyll fluorescence, PS2 – photosystem 2, PS1 – photosystem 1, PFD - photon flux density, Q_A – primary quinone-type electron acceptor of PS2, qE – energy-dependent quenching, qI – photoinhibitory quenching, qP – photochemical quenching of chlorophyll fluorescence, Rubisco – ribuloso-1,5-phosphate carboxylase-oxygenase, EC 4.1.1.39.

Introduction

Temperature is one of the most essential regulatory factors for plant photosynthesis (Bukhov and Mohanty, 1999). At present contradictory information is available relating to the interactive effect of temperature and irradiance on the activity of the photosynthetic apparatus. The increase in plant tolerance to heat was explained by the light-dependent formation of the transthylakoid proton gradient (Weis, 1982; Mühlbauer and Eichacker, 1998). It is postulated also that light is an important factor for regulation of chloroplast gene expression (Mühlbauer, Eichacker, 1998), chloroplast ATP-ase activity (Gilmore and Bjorkman, 1995), CO_2 assimilation (Buchanan, 1991) and antioxidant level (Alscher et al., 1997), accumulation of anti-stress organic compounds etc. (Bohnert and Sheveleva, 1998; Gill et al., 2001). According to other data light may also destabilize PS2 at elevated temperatures. A hypothesis has been developed that under unfavorable conditions an excess energy that has not been used for photosynthesis can produce the large amount of reactive oxygen species and their products that induce damage to cell structures (Schreiber and Armond, 1978; Singh, Singhal, 2001).

The opposite data about the role of light in reaction of photosynthetic apparatus to heat could be explained by difference in duration of treatment, in level of irradiance and plant integrity, etc. In the frame of our topic isolated systems were investigated more intensively (Weis, 1982; Singh and Singhal, 2001).

The question of the mechanisms of the interaction between thermo- and light-regulated processes in intact plants is still open and of both scientific and applied interest. In the present work the rate of oxygen evolution/consumption, *in vivo* electron transport activity, overall photosynthetic capacity, and accumulation of chloroplast HS proteins in primary barley leaves were studied after heating of intact seedlings in light or in the dark. The data obtained testify to a heat-protective role of low light on the photosynthetic apparatus in the primary barley leaf including primary photosynthetic reactions and the dark steps of carbon metabolism. Heating in the dark and plant senescence resulted in a decrease in the heat tolerance of barley seedlings. The difference between light- and dark-treated leaves was not caused by differential accumulation of HS 30 kDa chloroplast proteins.

Materials and Methods

Barley plants (*Hordeum vulgare* L.) were grown in the soil "Floraton" (pH 5,0–6,5; salts, 1.5 g/l) at 100 µmol quanta $m^{-2}.s^{-1}$, 14 $h.d^{-1}$ and temperature 28–26ºC (day/night) for 5–11 days. Afterwards the intact seedlings were kept at 40ºC for 3 hours in total darkness or under low light (100 µmol quanta $m^{-2}.s^{-1}$). Control plants for dark-heated variants were kept in the dark for 3 hours at 28ºC.

Chlorophyll fluorescence was measured in vivo at 20ºC using a pulse-amplitude-modulated fluorometer (PAM 101, Walz, Effeltrich, Germany). Parameters of chlorophyll fluorescence were calculated as described in (Färber et al., 1997, Rohachek and Bartak, 1999). O_2-evolution/consumption ability of thylakoid membranes was measured with a Clark-type oxygen electrode at 20ºC in presence of 1 mM 1-4-Benzoquinone (PS2 activity), or 0.5 mM $K_3Fe(CN)_6$ (PS2+I activity), or 0.5 mM 2,3,5,6-Tetramethyl-p-phenylenediamine, 1 mM Na-ascorbate, 25 µM Methyl viologen, Superoxide dismutase (100 U/ml) and 1 mM NaN_3 (PS1 activity). The infrared gas analyzer "Infralit-3" (Junkalor, Germany) was used for measurement of the CO_2-gas exchange rate (Bukhov et al., 1992) at 0.03% CO_2, 21% O_2, and PFD from 2240 to 48 µmol quanta $m^{-2}.s^{-1}$.

Thylakoid membrane isolation, SDS-polyacrylamide gel electrophoresis and immunoblotting were performed according to Pötter et al. (1996). Blots were incubated with a primary antibody raised against barley 26–30 kDa HS proteins (Kruse et al., 1993). Goat anti-rabbit IgG coupled to alkaline phosphatase (Sigma) was used as a secondary antibody. The immunoreactive bands were visualized using the color reaction with 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrasodium.

Results

Heat treatment of intact barley seedlings of different ages at 40ºC during 3 hours under low light did not significantly affect the fluorescent parameters of the primary leaves (Table 1). Photochemical quenching of chlorophyll fluorescence (qP) increased slightly only in old (11 d-old) stressed leaves. A decrease of non-photochemical fluorescence quenching (NPQ) in plants of all ages was in parallel to the delay of thylakoid membrane energization (qE). Under the same conditions the photoinhibitory quenching (qI) decreased only in 6 d-old plants (Table 1).

High temperature treatment in the dark resulted in lowering of potential quantum yield of PS2 photochemistry (F_V/F_M) by 10–20% depending on the plant age (Table 1). Stress in the dark led also to an increase of qP in 8- and 11 d-old leaves, suppression of non-photochemical quenching (NPQ) and membrane energization (qE) and caused photoinhibition of PS2 (qI) at high PFD (Table1).

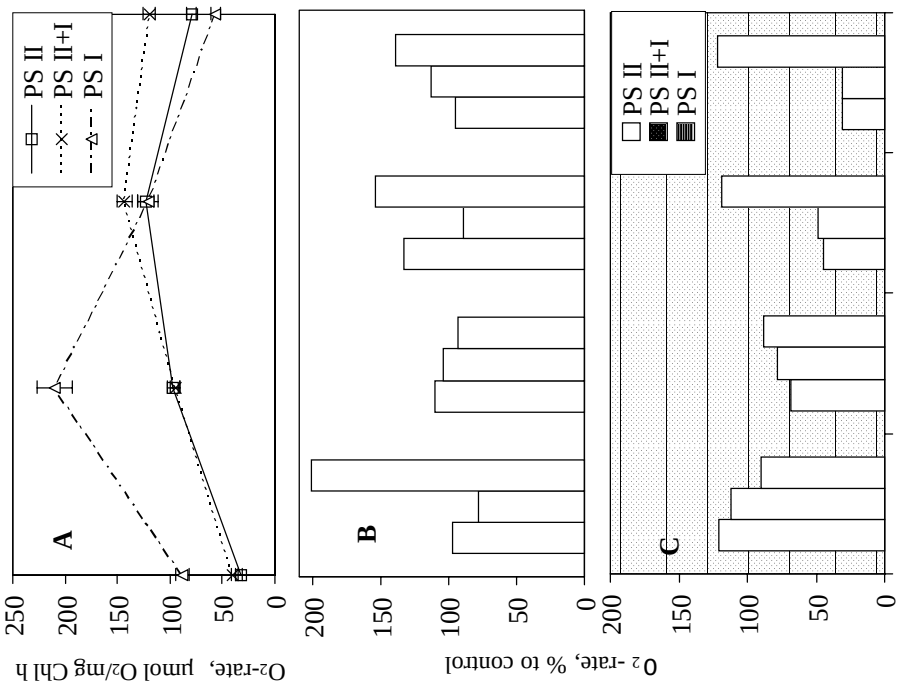
Table 1. Fluorescence parameters of 6-, 8- and 11 d-old primary barley leaves of after treatment of the intact seedlings with high temperature (at 40 °C for 3 h) in light (+) or darkness (-). The values of photochemical quenching of chlorophyll fluorescence (qP), non-photochemical fluorescence quenching (NPQ), energy-dependent (qE) and photoinhibitory (qI) quenching were calculated after 15 min action of actinic light at PFD 1200 µmol quanta $m^{-2}.s^{-1}$ and subsequent (for the case of qE and qI) dark incubation of the leaves for 10 min. Mean values of two to four independent experiments are shown. SE does not exceed 7%.

Temperature and light conditions	F_V/F_M			qP			NPQ		
	6-d	8-d	11-d	6-d	8-d	11-d	6-d	8-d	11-d
Control +	0,82	0,85	0,85	0,62	0,50	0,48	2,4	2,51	2,0
40°C +	0,81	0,82	0,86	0,68	0,52	0,58	1,93	2,25	1,86
Control –	0,83	0,85	0,84	0,59	0,55	0,47	2,84	2,75	2,59
40°C -	0,75	0,70	0,65	0,53	0,63	0,72	1,18	1,2	0,79
	qE			qI					
	6-d	8-d	11-d	6-d	8-d	11-d			
Control +	2,02	2,18	1,80	0,39	0,33	0,20			
40°C +	1,71	1,95	1,66	0,27	0,30	0,20			
Control –	2,61	2,53	2,36	0,23	0,22	0,23			
40°C –	0,81	0,71	0,34	0,37	0,49	0,44			

A combination of high temperature with low light did not affect the O_2-evolution rate induced by PS2 or linear electron transport but increased the activity of PS1 in young 5 d-old leaves (Fig. 1B). After heating in the dark PS1 activity was the same as in appropriate control plants whereas PS2 activity and linear electron transport decreased by 20–70% (Fig. 1C).

The overall photosynthetic capacity of the primary control leaves decreased significantly with aging, especially at high PFD (Fig. 2A,B). The assimilation rate of 5 d-old leaves measured at different PFD was insensitive to elevated temperature both in light and in the dark (Fig. 2A). Old barley leaves displayed strong inhibition of the photosynthetic rate after heating in light (Fig. 2B). The slope of the CO_2-uptake curve at low PFD that indirectly characterizes the electron transport rate (Ögrin, 1993) decreased to 70% of the control plants. At high PFD the steady-state level of light-stressed leaves was approximately 40% to the control plants meaning that activity of enzymes of carbon metabolism was more sensitive to heat than electron transport. After the plant heating in light and darkness the photosynthetic electron transport rate and activity of Calvin cycle enzymes decreased to 35% and 20% of the respective control plants (Fig. 2B).

Immunoblot analysis of the thylakoid membranes showed that 30 kDa HS proteins begun to appear after heating both in light and the dark and their levels was the greatest in the young stressed plants (Fig. 1D,E).



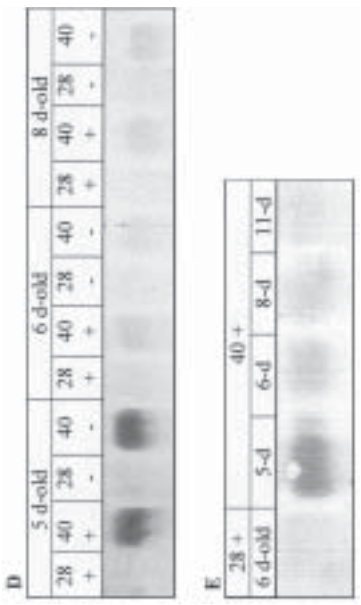


Fig. 1. Age-dependent dynamics of O_2-evolution/consumption rate of thylakoid membranes of barley primary leaves under control conditions (A) and after plant treatment at 40°C for 3 hours in light (B) or in the dark (C). O_2-rate was measured with a Clark-type oxygen electrode at 20°C in presence of 1mM 1-4-Benzoquinone (PS2 activity), or 0.5 mM $K_3Fe(CN)_6$ (PS2+I activity), or 0.5 mM 2,3,5,6-Tetramethyl-p-phenylenediamine, 1 mM Na-ascorbate, 25 μM Methyl viologen, Superoxide dismutase (100 U/ml) and 1 mM NaN_3 (PS1 activity). Values are expressed as mean ± SE.

Accumulation of heat shock proteins in thylakoid membranes of the primary barley leaves of different ages (D, E) after treatment of intact seedlings with high temperature (3 hours at 40°C) in the presence of low light (+) or in darkness (—). Thylakoid membrane isolation, SDS-polyacrylamide gel electrophoresis and immunoblotting were performed according to Pötter et al. (1996). Blots were incubated with a primary antibody raised against barley 26–30 kDa HS proteins (Kruse et al., 1993). Goat anti-rabbit IgG coupled to alkaline phosphatase (Sigma) was used as a secondary antibody. The immunoreactive bands were visualized using the color reaction with the 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrasodium. Typical data from three experiments are presented.

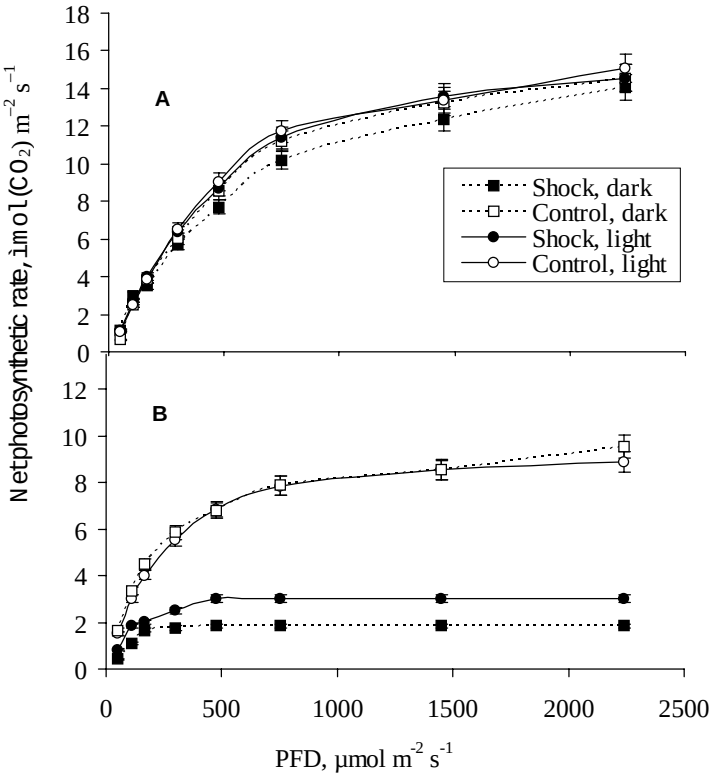


Fig. 2. Light response curves of the CO_2-gas exchange rate in primary barley leaves of the 5 d-old (A) and 11 d-old (B) barley seedlings heated at 40°C for 3 hours in the light (circles) or in the dark (squares). The infrared gas analyzer "Infralit-3" (Junkalor, Germany) was used for measurement of the CO_2 gas exchange rate at 0.03% CO_2, 21% O_2, and PFD from 2240 to 48 μmol quanta $m^{-2}.s^{-1}$. Vertical bars represent standard deviations of means.

Discussion

In the present work several experimental approaches were used to analyze the age- and light-dependent reactions of the photosynthetic apparatus in the primary leaves of barley seedlings to high temperature. The activity of the photosynthetic apparatus was studied *in vivo* using PAM-fluorometry and CO_2-uptake measurement whereas the thylakoid membranes were isolated from the control and stressed leaves to evaluate the rate of oxygen evolution/consumption and accumulation of chloroplast HS proteins.

The age-dependent curves of the O_2-rate measured by Clark-type electrode showed that maximal activity of PS2 as well as the rate of the linear electron transport from PS2 to PS1 were found after 6–8 days of plant growth when the primary leaves became

mature enough and destructive processes had not yet developed (Fig. 1A). Oxygen uptake by PS1 as a result of activation of the Mehler reaction also rose up to the 6th day followed by a decrease in the electron transport rate (Fig. 1A). As the primary leaves senescenced the overall photosynthetic rate (Fig. 2), and the efficiency of absorbed light utilization for photochemical and non-photochemical reactions (Table 1) were found out to also decrease.

According to the data obtained, the photosynthetic apparatus of intact barley seedlings displayed high thermal stability in the presence of low light. The maximal quantum yield of PS2 photochemistry (Table 1, F_V/F_M), the linear electron transport, oxygen-evolving capacity of PS2 (Fig. 1B) and assimilation rate of 5 d-old leaves were not affected significantly by elevated temperature in light. Oxygen uptake by PS1 increased in light, especially in the young (5 d-old) leaves (Fig. 1B). Activation of electron transport through PS1 could be caused by mobilization of the electron donation from stromal reductants at the level of cytochrome b_6/f instead of the electron uptake from PS2. According to the literature the higher activity of PS1 at elevated temperature could be explained by the migration of phosphorylated light-harvesting complex of PS2 toward PS1 that led to protection of the more sensitive PS2 against excess of light energy (Bukhov and Mohanty, 1999). With plant aging a clear infringement of the process of CO_2 fixation at elevated temperature is displayed (Fig. 2B). 11 d-old light-stressed leaves showed a smoller closure of PS2 reaction centers (Table 1, qP). Thus, the age-dependence of the plant reaction to elevated temperature in the light was expressed in a higher heat resistance of the younger seedlings.

A combination of high temperature and darkness led to dramatic impairment of the photosynthetic apparatus functioning, excluding the oxygen uptake by PS1 (Fig. 1C) and CO_2-uptake of the young leaves (Fig. 2A). A decrease of the steady-state fraction of closed (reduced) reaction centers in the 8- and 11 d-old leaves (Table 1, qP) could indicate the age-dependent limitation of electron donation to PS2 centers due to the heat-induced damage of the water-splitting apparatus (Fig. 1C) and (or) photoinhibition of the PS2 reaction centers that follows from the increase of qI in the leaves of all ages (Table 1). At the same time the thylakoid membrane energization (qE) dropped 3 and 7 times for the young and old plants respectively (Table 1). After heating in light the decrease of NPQ as well as qE was not that noticeable (Table 1). The data obtained suggest infringement of the photosynthetic electron transport chain, uncoupling of electron transport with ATP synthesis and enhancement of passive permeability of thylakoid membranes in dark-stressed leaves. All these negative reactions tended to increase with plant aging.

Analyzing the CO_2-fixation rate of the old plants (Fig. 2B), it is easy to calculate that light absence during plant treatment with high temperature led to a decrease of plant tolerance to heat by half. The data obtained using intact barley leaves are in accordance with the literature data concerning isolated spinach Rubisco (Weis, 1982; Buchanan, 1991). The authors postulated that Rubisco as well as several other Calvin

cycle enzymes are highly heat-sensitive and activated in light by means of thioredoxin that is reduced by the end product of photosynthetic electron transport, namely ferredoxin (Buchanan, 1991). Taking into account a different sensitivity of CO_2-assimilation to heat depending on the plant age it is possible to assign also a less stomata aperture in the dark followed by impairment of the leaf transpiration rate and a decrease of CO_2 uptake. The above reactions were more pronounced in the case of the old leaves due to the fact that their stomata remain more closed during heating relatively to the young leaves (data not present).

It was suggested that one of the reasons of the light-induced heat stability of the photosynthetic apparatus could be the different level of chloroplast HS proteins that began to accumulate after stress impact and were not present originally. Light-dependent synthesis of HS proteins localized both in cytosol and cell organelles (chloroplasts and mitochondria) was discovered earlier (Otto et al., 1992; Kruse et al., 1993; Nover and Scharf, 1997). On the other hand, under both conditions, light and dark, the amount of chloroplast 30 kDa HS proteins was found to be practically the same (Fig. 1D). According to our results this coincides with the data of Kruse et al. (1993), the higher amount of 30 kDa HS proteins accumulated in thylakoid membranes of the young stressed leaves (Fig. 1D,E) which might be a reason for their flexibility to changes in the environment.

Evidently a protective role of light is not limited to the light-dependent formation of the photosynthetic proton gradient as was published previously (Weis, 1982; Mühlbauer and Eichacker, 1998), at least in the case of heating of intact plants. From our point of view and some preliminary experiments the light-dependent resistance of intact plants to elevated temperatures could depend also on the leaf transpiration rate, intracellular antioxidant balance, accumulation of low molecular weight organic compounds etc. The possible light-dependent mechanisms that may provide a protective effect of low light against negative action of high temperature will be revealed during further investigations.

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References

Alscher, R. G., H. L. Donahue, C. L. Cramer, 1997. Reactive oxygen species and antioxidants: relationships in green cells. Physiol. Plant., 100, 224–233.

Bohnert, H. J., E. Sheveleva, 1998. Plant stress adaptations – making metabolism move. Curr. Opin. Plant Biol., 1, 267–274.

Buchanan, B. B. 1991. Regulation of CO_2 assimilation in oxygenic photosynthesis. Arch. Biochem. Biophys., 288, 1–9.

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 fluency rate. Physiol. Plant., 85, 632–638.

Bukhov, N. G., P. Mohanty, 1999. Elevated temperature stress effects on photosystems: characterization and evaluation of nature of heat induced impairments. In: Concepts in photobiology: photosynthesis and photomîrphogenesis, Eds. G.S. Singhal, G. Renger, S.K. Sopory, K-D. Irrang, Govindjee, New Delhi, 617–648.

Färber, A., A. J. Young, A. V. Ruban, P. Horton, P. Jahns. 1997. Dynamics of xantophyll-cycle activity in different antenna subcomplexes in the photosynthetic membranes of higher plants. Plant Physiol., 115, 1609–1618.

Gill, P. K., A. D. Sharma, P. Singh, S. S. Bhullar, 2001. Effect of various abiotic stresses on the growth, soluble sugars and water relation of Sorgum seedlings growth in light and darkness. Bulg. J. Plant Physiol., 27, 72–84.

Gilmore, A. M., O. Bjorkman, 1995. Temperature-sensitive coupling and uncoupling of ATPase-mediated, nonradiative energy dissipation: Similarities between chloroplasts and leaves. Planta, 197, 646–654.

Kruse, E., Z. Liu, K. Kloppstech, 1993. Expression of heat shock proteins during development of barley. Plant Mol. Biol., 23, 111–122.

Mühlbauer, S. K., L. A. Eichacker, 1998. Light-dependent formation of the photosynthetic proton gradient regulates translation elongation in chloroplasts. J. Biol. Chem., 273, 20935–20940.

Nover, L., K.-D. Scharf, 1997. Heat stress protein and transcription factors. Cell. Mol. Life Sci., 53, 80–103.

Ögrin, E., 1993. Convexity of the photosynthetic light-response curve in relation to intensity and direction of light during growth. Plant Physiol., 101, 1013–1019.

Otto, B., I. Ohad, K. Kloppstech, 1992. Temperature treatments of dark-grown pea seedlings cause an accelerated greening in the light at different levels of gene expression. Plant Mol. Biol., 18, 887–896.

Pötter, E., K. Kloppstech, 1996. Expression of early light-inducible proteins in the barley developmental leaf gradient under light stress. Eur J Biochem., 214, 779–786.

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

Singh, A. K., G. S. Singhal, 2001. Effect of irradiance on the thermal stability of thylakoid membrane isolated from acclimated wheat leaves. Photosynthetica, 39, 23–27.

Shreiber U., P. A. Armond, 1978. Heat-induced changes of chlorophyll fluorescence in isolated chloroplasts and related heat damage at the pigment level. Biochim. Biophys. Acta, 502, 138–151.

Weis, E., 1982. Influence of light on the heat sensitivity of the photosynthetic apparatus in isolated spinach chloroplasts. Plant. Physiol., 70, 1530–1534.