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Effects of long-term action of high temperature and high light on the activity and energy interaction of both photosystems in tomato plants

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Abstract

The acclimation to high light, elevated temperature, and combination of both factors was evaluated in tomato (*Solanum lycopersicum* cv. M82) by determination of photochemical activities of PSI and PSII and by analyzing 77 K fluorescence of isolated thylakoid membranes. Developed plants were exposed for six days to different combinations of temperature and light intensity followed by five days of a recovery period. Photochemical activities of both photosystems showed different sensitivity towards the heat treatment in dependence on light intensity. Elevated temperature exhibited more negative impact on PSII activity, while PSI was slightly stimulated. Analysis of 77 K fluorescence emission and excitation spectra showed alterations in the energy distribution between both photosystems indicating alterations in light-harvesting complexes. Light intensity affected the antenna complexes of both photosystems stronger than temperature. Our results demonstrated that simultaneous action of high-light intensity and high temperature promoted the acclimation of tomato plants regarding the activity of both photosystems in thylakoid membranes.

Additional key words: antenna complexes; electron transport rate; fluorescence; pigment-protein complexes; spillover.

Introduction

The structure and organization of pigment-protein complexes within thylakoid membranes are dynamic and flexible, thus allowing mutual reorganization of complexes within thylakoid membranes in response to changing environment and facilitating plant acclimation. (Anderson *et al.* 2012). Although PSI, PSII, and their antenna complexes and the main light-harvesting complex LHCII are separately located in grana and stroma regions of thylakoid membranes, they can rearrange under different light conditions in order to balance the energy distribution and to maintain the optimal photosynthetic activity. In respect to changing environmental conditions and duration of treatments, a response of plants includes short- and long-term acclimations (Tikkanen *et al.* 2012a, Wientjes *et al.* 2013). The dynamics of thylakoid membrane structure and abilities of its components to rearrange allow plants to balance the energy supply of both photosystems and to control the electron transport rate when the changes of irradiance lead to an imbalance in reducing efficiencies of PSI and PSII (Tikkanen *et al.* 2012b). It is well-known that PSII is more susceptible to high-light damage than PSI. In order to protect PSII from light-induced injury, plants have developed different strategies – to decrease excitation energy narrowed to PSII and to increase photochemical de-excitation of reaction centers' chlorophylls (Chl) by enhancing electron transport. In terms of short-time acclimation (seconds to minutes), higher plants regulate the photosynthetic process by decreasing energy supply of PSII by process of a phosphorylation-dephosphorylation cycle of LHCII and PSII core proteins. Under low light, parts of LHCII and PSII core undergo phosphorylation and migrate to stroma thylakoids, which is accompanied by a decrease of thylakoid stacking (Tikkanen *et al.* 2008, Tikkanen *et al.* 2012a). The changes in response to light intensity seem to concern mainly PSII and its antenna, while PSI and LHCI are not considerably affected (Ballotari *et al.* 2007).

While the effects of long-term (hours, days) treatment

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Abbreviations: 1,4 BQ – 1,4 benzoquinone; Car – carotenoids; Chl – chlorophyll; DCPIP – 2, 6- dichlorophenol-indophenol; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; HL – high-light intensity; HT – high temperature; MES – 2-(N-morpholino) ethanesulfonic acid; NT – normal temperature; NL – normal light intensity; R – recovery; Tricine – N-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine.
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with either high light or high temperature alone are well documented, the influence of simultaneous action of both factors have been studied only partially and for individual plant species. Response of plants to short or prolonged actions of high temperature is expressed in morphological and physiological changes as well as in alterations of primary photosynthetic reactions and of biochemical processes (Wahid *et al.* 2007 and references herein). The effect of elevated temperatures on photosynthetic apparatus in isolated thylakoid membranes and in chloroplasts is well documented and reviewed (Bukhov and Mohanty 1999). Early works on heat-induced changes in energy distribution between both photosystems indicated an increase in energy delivery to PSI at expense of PSII (Weis 1985). It has been shown that a mild-heat treatment of detached leaves significantly stimulated cyclic electron flow around PSI (Havaux 1996, Bukhov *et al.* 2000). A significant analogy between the response of photosynthetic apparatus of leaves to mild heating and to strong illumination has been observed and a possibility that similar phenomena underlie the short-time acclimation of photosynthesis to heat and to high light has been proposed (Havaux and Tardy 1996). It has been reported that the heat treatment resulted in a twice increase of the extent of LHCII phosphorylation and in a decrease of the amount of LHCII-related polypeptides of grana membranes in pea plants subjected to high temperature in dark for 15 h (Mohanty *et al.* 2002).

Plants differ in respect to their heat tolerance and different threshold temperature has been determined by different authors, although a correct estimation is complicated due to the influence of additional environmental factors (Wahid *et al.* 2007). It has been reported that short-time temperature treatment (2 h at 45°C) affected the functional activity of photosynthetic apparatus by different manner in respect to the thermotolerance of studied tomato cultivars (Camejo *et al.* 2005). The high temperature-induced inactivation of photosynthesis has been related to membrane damage and to changes of Chl and carotenoids (Car) contents (Camejo *et al.* 2005).

In recent years, few papers have been published reporting on the positive effect of light on the heat tolerance of different plants. A small but significant increase have been reported (Krause *et al.* 2015) in heat tolerance of illuminated leaves in comparison with those heated in dark in two neotropical tree species – *Ficus insipida* Willd and *Calophyllum longifolium*, determined by the maximal quantum efficiency (F_v/F_m). Heat treatment in light increased the critical temperature T_{50}, causing a 50% decline of F_v/F_m. Similar results but for three high-mountain species, *Rhododendron ferrugineum*, *Senecioincanus*, and *Ranunculus glacialis*, have been published recently (Buchner *et al.* 2015). The published results indicate that heat treatment applied in the dark reversibly reduce photosynthetic performance and the F_v/F_m ratio. In contrast, plants exposed to heat stress under natural irradiation were able to tolerate and recover from heat stress more readily and the critical threshold temperature for basic Chl fluorescence (F_0 and F_s) was higher under illumination than that in dark. Marutani *et al.* (2014) have reported similar observations that combination of heat treatment (40^0C) with light contributed to heat tolerance of photosystems in wheat *via* regulation of photochemical energy transfer. These results, concerning the effect of two main environmental stress factors – high temperature and high light, arise the question about the mechanisms of interplay between heat and light tolerance.

Under natural conditions, the high temperature stress is accompanied with high-light intensities and it is often complicated to differentiate the impact of both factors. The experimental design of our investigation allows to obtain information about the action of both factors acting separately or simultaneously and to throw light on the mechanisms of plants response to heat and light stress.

The aim of present study was to evaluate the effect of long-term (several days) simultaneous action of high temperature and high light on primary processes of photosynthesis – electron transport and energy distribution, taking place in thylakoid membranes. We followed the effect of both factors, separately and in combination, and observed that after an initial decline on the second day the treated plants seemed to be well acclimated to the respective treatment on the sixth day. We studied the changes of photochemical activity of PSI and PSII and energy distribution between both photosystems in thylakoid membranes, isolated from control (non-treated) and treated for two and six days with moderate high temperature, high light, or with combination of both factors and after five days of recovery at optimal conditions. Our results showed that the tomato plants, exposed to higher than optimal temperature, were less affected in respect to activities of both photosystems when the high temperature treatment was performed at high-light illumination. The observed changes in the 77 K fluorescence emission and excitation spectra pointed out that a possible mechanism of this light-induced tolerance to high temperature could be the rearrangement of pigment-protein complexes and balance of excitation pressure on PSI and PSII.

Materials and methods

Conditions for plant growth: Tomato plants (*Solanum lycopersicum* cv. M82) were grown in growth chambers (*Fytoscope 130, PSI,* Brno, Czech Republic) under optimal conditions [16 h dark/8 h light, 250 μmol(photon) m^{-2} s^{-1} PAR, temperature of 22/20°C, and humidity of 70%]. At the stage of 5–6 leaves, the plants were separated into four batches:

Treatment	Temperature [°C]	Light intensity [μmol(photon) m^{-2} s^{-1}]
NT-NL	22/20	250
NT-HL	22/20	800
HT-NL	38/29	250
HT-HL	38/29	800

After 6 d of the treatment the plants were allowed to recover (R) for 5 d under the normal conditions, NT-NL. Leaves from the control plants and plants grown at different conditions were collected at the beginning of every experiment (0 d), at 2 and 6 d of the respective treatment (DAT) and after 5 d of recovery.

Isolation of thylakoid membranes: Thylakoid membranes were isolated from tomato plants grown under control and different light and temperature treatments as described by Velitchkova and Popova (2005). The final pellet was resuspended in a buffer containing 0.33 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl, and 20 mM Tricine (pH 7.5).

Determination of pigment content: Chl *a*, Chl *b*, and Car contents of isolated thylakoids were determined spectrophotometrically in 80% acetone according to Lichtenthaler (1987) using a spectrophotometer *Specord 210 Plus* (*Analityk-Jena AG,* Jena*,* Germany). The procedure was carried out at 4°C in dark.

Photochemical activities of PSI and PSII: Photochemical activity was measured polarographically by a Clark-type electrode (*DW1, Hansatech Instruments Ltd.,* King's Lynn*, Norfolk,* UK) in a temperature-controlled vessel and at saturating white light. Activity of PSII (steady-state oxygen evolution) was determined by the rate of oxygen evolution in the presence of an exogenous electron acceptor, 1,4-benzoquinone (0.4 mM 1,4 BQ), in a reaction medium containing 0.33 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl, 20 mM MES (pH 6.5). PSI-mediated electron transport was determined by the degree of oxygen uptake in a medium containing 0.33 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl, 20 mM Tricine (pH 7.5), 0.4 μM 3-(3,4- dichlorophenyl)-1,1-dimethylurea (DCMU), 0.5 mM NH_4Cl, 5 mM NaN_3, and artificial electron donors and acceptor as follows: 0.1 mM 2, 6-dichlorophenol-indophenol (DCPIP), 4 mM Na ascorbate, and 0.1 mM methyl viologen. Both photochemical activities were measured at 22°C and at Chl(*a*+*b*) concentration of 25 μg cm^{-3}.

Low-temperature (77 K) fluorescence measurements: Samples from isolated thylakoid membranes were transferred into a tube for fluorescence measurement and immediately frozen in liquid nitrogen. Low-temperature fluorescence emission and excitation spectra were registered by a spectrofluorometer *Jobin Yvon JY3* (*Division d'Instruments S.A.,* Longjumeau*,* France) equipped with a red-sensitive photomultiplier (*Hamamatsu R928, Hamamatsu Photonics,* Japan) and a low-temperature device. The spectral bandwidth of emission and excitation monochromator was 4 nm. Chl concentration was 10 μg cm^{-3} in order to avoid reabsorption. Data were digitised by an in-built A/D converter and transferred to an online *IBM*-compatible computer for further retrieval and analysis. The spectra were analyzed by *Origin 6.0* (*Microcal Corp*., *OriginLab Corporation*, Northampton, MA, USA). The emission spectra were recorded under excitation with 436 nm (Chl *a*) and 472 nm (Chl *b*). Excitation spectra were recorded for emission at 685 nm (PSII) and 735 nm (PSI) in the red region (710–600 nm) and in the Soret region (400–510 nm).

Statistics: All data were presented as means ± SE. Control and treated samples were statistically compared. Comparison of means from three separate experiments, each in three replications, was done by the *Student*'s *t*-test.

Results

Photochemical activity of PSI and PSII: Data from oxygen evolution (PSII) and oxygen uptake (PSI) for thylakoid membranes isolated from the control tomato plants (NT-NL) and grown under combination of temperature and light intensities as NT-HL, HT-NL, and HT-HL are summarized in Fig. 1. The PSII activity was measured on 2 and 6 DAT and compared with the activity of the control plants (before light/temperature treatment) and the activity after recovery of plants for 5 d under optimal NT-NL conditions.

The activity of PSI was not inhibited but even stimulated after 2 DAT by HT-NL (Fig. 1*A*). This stimulation was more pronounced after 6 DAT at 38/29°C. The combined action of HT-HL resulted in a more considerable increase of the PSI-mediated electron transport rate. At the same time, after 2 DAT of HT-NL, a 20% inhibition of the PSII-mediated electron transport rate was observed and this inhibition was kept at the same level during prolonged treatment for 6 DAT (Fig. 1*B*). However, when HT-HL was applied, the photochemical activity of PSII was maintained higher than that at HT after 2 and 6 DAT. Exposure to NT-HL for 2 DAT led to a decrease of the PSII activity, but after 6 DAT, the activity was restored to values even higher than that of the control plants.

Low-temperature (77 K) fluorescence: In order to determine any possible temperature- and light- induced changes in the population of both photosystems and alterations in energy distribution between PSII and PSI, 77 K fluorescence emission and excitation spectra of isolated

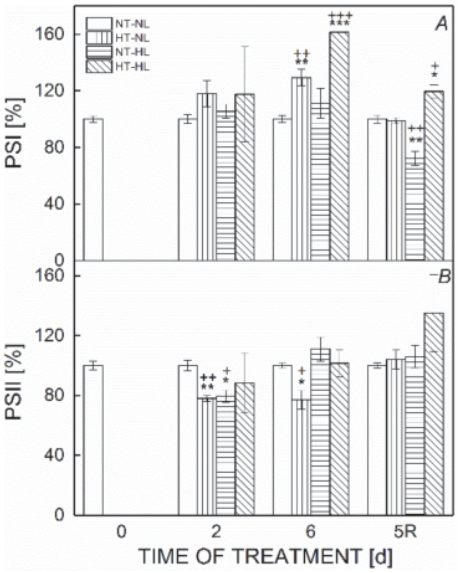

Fig. 1. Effect of high temperature and high light on the activity of PSI and PSII. (*A*) Photochemical activity of photosystem I of thylakoid membranes isolated from control plants and plants treated with different combinations of temperature and light. Data are presented as percentage from activity of thylakoid from control plants. 100% corresponds to 556.584 $\mu molO_2\ mg^{-1}(Chl)\ h^{-1}$. (*B*) Photochemical activity of photosystem II of thylakoid membranes isolated from control and treated tomato plants. 100% corresponds to 95.214 $\mu molO_2\ mg^{-1}(Chl)\ h^{-1}$. Mean values ± SE were calculated from three independent experiments. Statistically significant differences of every sample to the control (0 days) are marked by asterisks (* – $P<0.05$; ** – $P<0.01$; *** – $P<0.001$). Statistically significant differences of every sample to the control at corresponding day of treatment are marked by crosses ($^{+}$– $P<0.05$; $^{++}$ – $P<0.01$; $^{+++}$ – $P<0.001$).

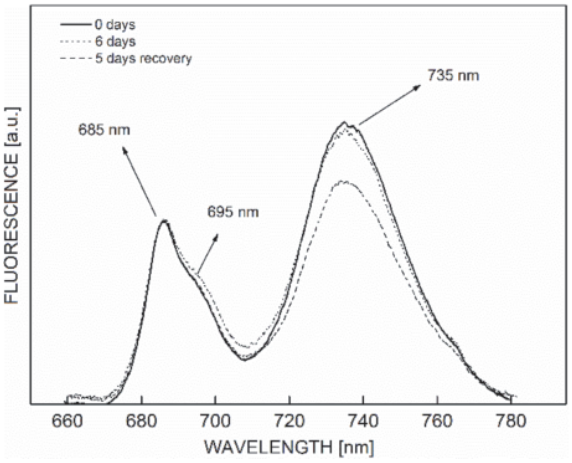

Fig. 2. 77K fluorescence emission spectra of thylakoid membranes isolated from plants grown at NT–NL. *Solid* line – 0 day; *dotted line* – 6 days, *dashed line* – after 5 days of recovery. Excitation wavelength – 436 nm. Resuspending medium and Chl concentration as described in Materials and methods section.

membranes were analyzed. The emission spectra of thylakoid membranes from tomato plants exhibited two maxima, at 685 nm and 735 nm, associated with the emission from PSII and PSI, respectively, and a shoulder at 695 nm, related to proximal antenna of PSII - CP47 (Fig. 2). The emission intensities of these bands correspond to: (*1*) the population of both photosystems; (*2*) the energy delivered to them that depends on the mutual organization of pigment-protein complexes within thylakoid membranes. Under optimal NT-NL conditions, the shape and relative intensity of the maxima in spectra did not change during 2 and 6 DAT (Fig. 2). After 11 DAT (including 5 d of recovery), a decrease of the intensity at 735 nm was observed probably due to an age-related decrease in the population of PSI and/or some separation of complexes of PSII and PSI leading to a decrease of energy transfer from PSII-LHCII to PSI (so called spillover) (Fig. 2).

The representative emission spectra of thylakoid membranes from plants grown at different temperature-light conditions at excitation with 436 nm (exciting preferably Chl *a*) are presented in Fig. 3. All spectra were normalized

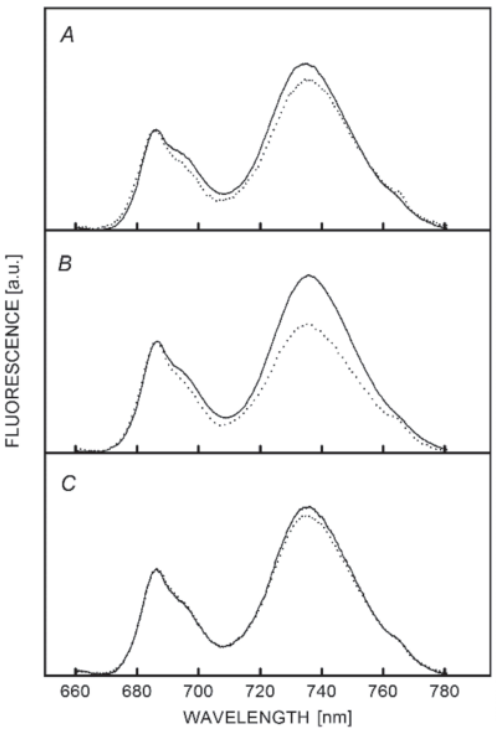

Fig. 3. Fluorescence emission spectra of isolated thylakoid membranes at 77K. (*A*) – spectra of thylakoid membranes isolated from tomato control plants (*solid line*) and treated with high temperature for 6 days (*dotted line*); (*B*) – spectra of thylakoid membranes isolated from control plants (*solid line*) and treated with high light for 6 day (*dotted line*); (*C*) – spectra of thylakoid membranes isolated from control plants (*solid line*) and grown at high temperature and high light for 6 day (*dotted line*). Excitation wavelength was 436 nm. Resuspending medium and Chl concentration as described in Materials and methods section.

at 685 nm. The spectra of thylakoid membranes isolated from plants grown for 0 and 6 DAT at HT-NL are shown in Fig. 3*A*. As can be seen, treatment for 6 DAT with HT resulted in a small decrease of emission at 735 nm and in the region 695 nm, more pronounced for the band at 735 nm that was not restored during the recovery period (data not shown). These spectra were compared with the fluorescence emission spectra at 77K of thylakoid membranes under excitation with 436 nm from plants grown at NT-HL and HT-HL (Fig. 3*B,C*). The HL treatment induced considerable decrease of the band intensity at 735 nm after 6 DAT and almost no restoration was observed after recovery for 5 d. Combination of HT and HL for 6 DAT led to a smaller decrease of 735 nm emission and of the shoulder at 695 nm, which was restored to higher extent after 5 d of recovery. For quantitative determination of the changes in intensities of fluorescence emission from different pigment-protein complexes, the ratios F_{735}/F_{685} under excitation with 436 nm (Chl *a*) and 472 nm (Chl *b*) were calculated (Fig. 4). The NT-HL treatment exhibited the most pronounced effect on F_{735}/F_{685} – the ratio decreased after 2 and 6 DAT under these conditions. Under simultaneous action of HL and HT, the values of F_{735}/F_{685} were close to the values obtained for plants grown under NT-NL. The same behavior was observed under excitation with 472 nm,

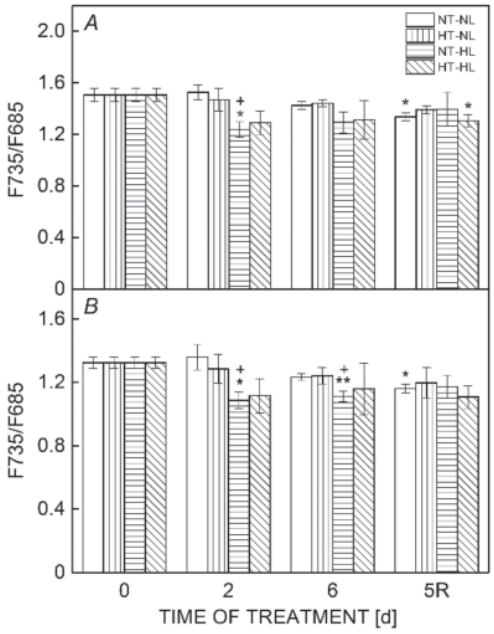

Fig. 4. Effect of different combinations of temperature and light intensity on the values of fluorescence ratio F735/F685 of thylakoid membranes isolated from control and treated tomato plants. (*A*) Excitation wavelength 436 nm; (*B*) excitation wavelength 472 nm. Statistically significant differences of every sample to the control (0 days) are marked by asterisks (*– P<0.05; ** – P<0.01; *** – P<0.001). Statistically significant differences of every sample to the control at corresponding days are marked by crosses ($^{+}$– P <0.05; $^{++}$ – P <0.01; $^{+++}$ – P <0.001).

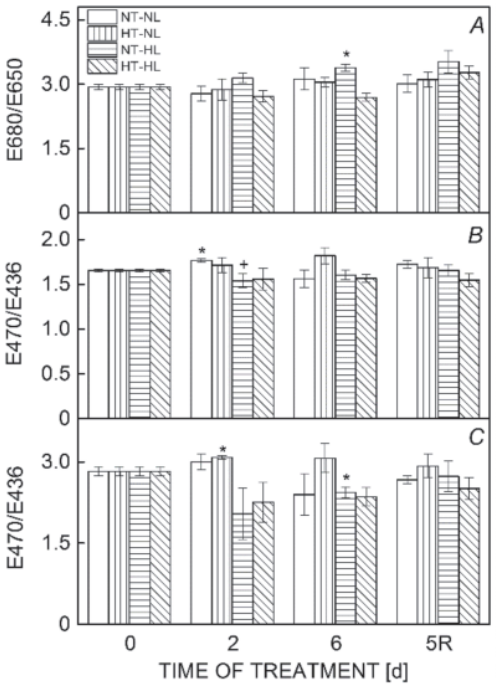

Fig. 5. Effect of different treatment on fluorescence ratios E680/650 and E470/436 calculated from excitation spectra of fluorescence emitted at 735 nm (PSI) in the "red" region (*A*) and in "blue" region (*B*) and at 685 nm (PSII) in "blue" region (*C*). Means and calculated standard errors (SE) are reported. Statistically significant differences of every sample to the control (0 days) are marked by asterisks (*– P<0.05; ** – P<0.01; *** – P<0.001). Statistically significant differences of every sample to the control at corresponding days are marked by crosses ($^{+}$– P<0.05; $^{++}$ – P<0.01; $^{+++}$ – P<0.001).

exciting preferably Chl *b*. After 5 d of recovery, the values for F_{735}/F_{685} were almost the same for all variants.

In order to determine the involvement of different Chl molecules in energy supply of both photosystems, which could occur due to changes in the size and/or rearrangements of antenna complexes, the excitation spectra of fluorescence emitted from PSII (685 nm) and PSI (735 nm) were analyzed. The excitation spectra of emission at 735 nm were recorded in the "blue" (500–410 nm) and in "red" region (700–610 nm), and for emission at 685 nm – in the "blue" region. Analysis of these excitation spectra gives the possibility to obtain information about the rearrangement of pigment-protein complexes, changes in antenna complexes and energy interaction between complexes.

In Fig. 5*A* data concerning the ratio E_{680}/E_{650} for emission of PSI at 735 nm were presented. The band at 680 nm is associated with Chl *a* molecules and that at 650 nm – with Chl *b*. The most pronounced effect on E_{680}/E_{650} was observed at HL during growing of tomato plants. After 2 and 6 DAT under HL of 800 μmol(photon) m^{-2} s^{-1}, the relative involvement of Chl *a* in energy supply of PSI increased in comparison with Chl *b*. On the other hand, HT-NL for 2 and 6 DAT did not affect considerably this

ratio. The content of Chl *b* is related to the light-harvesting complexes – LHCII and LHCI. These data were compared with the analysis of excitation spectra in the "blue" region of the emission at 735 nm (Fig. 5*B*). The decrease of the ratio E_{470}/E_{436} under NT-HL was in line with that observed in the "red" region – the relative decrease of Chl *b* participation in energy delivery to PSI. The combined action of HL and HT resulted in alleviation of the changes of E_{680}/E_{650} observed for HL (NT-HL).

Excitation spectra of emission at 685 nm give information on the energy supply of PSII complex. The ratio of E_{470}/E_{436} for emission at 685 nm was presented in Fig. 5*C*. The ratio was considerably affected by a 35% reduction after the first two DAT under NT-HL and by HT-HL growth conditions. Some restoration of this ratio was observed after 6 DAT under these conditions.

Data on the ratio of F_{685}/F_{695} of band intensities at 685 and 695 nm under excitation with 436 nm and 472 nm showed a statistically significant difference between control and plants grown at different temperature-light conditions (Table 1). After 2 DAT, this ratio decreased in thylakoid membranes isolated from plants treated at HT-NL and NT-HL, stronger expressed for the former one. This decrease was observed under excitation with 436 nm and 472 nm. After 6 DAT at HT-NL, NT-HL, and HT-HL, the ratio was close to that calculated for the control plants.

Table 1. Effect of different combination of elevated temperature and high light on the fluorescence ratio F685/F695 of thylakoid membranes isolated from control and treated plants at excitation wavelength 436 and 472 nm. Statistically significant differences of every sample to the control at corresponding days are marked by crosses ($^{+}$ – P<0.05; $^{++}$ – P<0.01; $^{+++}$ – P<0.001). HL – high light intensity; HT – high temperature; NL – normal light intensity; NT – normal temperature.

Parameter	Treatment	Time of treatment [d] 0	2	6	5 d recovery
F685/F695 exc. 436	NT-NL	1.42 ± 0.04	1.49 ± 0.02	1.38 ± 0.07	1.47 ± 0.06
	HT-NL	1.42 ± 0.04	1.43 ± 0.03	1.46 ± 0.02	1.37 ± 0.05
	NT-HL	1.42 ± 0.04	1.41 ± 0.02^{+}	1.46 ± 0.02	1.41 ± 0.03
	HT-HL	1.42 ± 0.04	1.46 ± 0.03	1.42 ± 0.04	1.36 ± 0.05
F685/F695 exc. 472	NT-NL	1.41 ± 0.02	1.51 ± 0.03	1.35 ± 0.04	1.43 ± 0.04
	HT-NL	1.41 ± 0.02	1.44 ± 0.03	1.46 ± 0.04	1.34 ± 0.05
	NT-HL	1.41 ± 0.02	1.41 ± 0.03	1.41 ± 0.04	1.41 ± 0.03
	HT-HL	1.41 ± 0.02	1.46 ± 0.04	1.42 ± 0.04	1.32 ± 0.04

Table 2. Values of the ratio Chl *a*/*b* and Chl(*a*+*b*)/Car in isolated thylakoid membranes from control plants and plants treated for 2 and 6 d at different combination of high temperature (HT) and high light intensity (HL) and after 5 d of recovery at normal temperature and normal light intensity (NT-NL). Mean values ± SE were calculated from three independent experiments each in three replications. Statistically significant differences of every sample to the control (0 d) are marked by asterisks (* – P<0.05; ** – P<0.01; *** – P<0.001). Statistically significant differences of every sample to the control at corresponding day of treatment are marked by crosses ($^{+}$– P<0.05; $^{++}$ – P<0.01; $^{+++}$ – P<0.001).

Parameter	Treatment	Time of treatment [d] 0	2	6	5 d recovery
Chl *a*/*b*	NT-NL	3.63 ± 0.02	3.64 ± 0.03	3.69 ± 0.01	3.56 ± 0.15
	HT-NL	3.63 ± 0.02	3.37 ± 0.08$^{*,+}$	3.56 ± 0.19	3.43 ± 0.10
	NT-HL	3.63 ± 0.02	3.72 ± 0.13	4.11 ± 0.09$^{**,++}$	3.64 ± 0.10
	HT-HL	3.63 ± 0.02	3.45 ± 0.23	3.37 ± 0.38	3.55 ± 0.31
Chl(*a*+*b*)/Car	NT-NL	4.46 ± 0.04	4.51 ± 0.14	4.63 ± 0.20	4.56 ± 0.06
	HT-NL	4.46 ± 0.04	4.17 ± 0.12	4.14 ± 0.10*	4.68 ± 0.03*
	NT-HL	4.46 ± 0.04	4.11 ± 0.07*	4.11 ± 0.08*	4.44 ± 0.07
	HT-HL	4.46 ± 0.04	3.76 ± 0.15$^{*,+}$	3.51 ± 0.09$^{***,++}$	4.43 ± 0.16

Pigment content: We followed the changes in the Chl *a*, Chl *b* and Car contents in isolated thylakoid membranes from the plants grown under different conditions for 2 and 6 DAT. The ratios of Chl *a*/*b* and Chl (*a*+*b*)/Car are presented in Table 2. The ratios did not change in membranes isolated from the NT-NL plants. Under the NT-HL conditions, the ratio was higher and indicated a relative decrease of the Chl *b* content in comparison with Chl *a*. The ratio decreased when HT was applied – at HT-NL and HT-HL.

Discussion

Under conditions of changing environment the plants are subjected to different stress factors, including extreme temperatures, high-light illumination, drought, increased UV-radiation, which impact the functional activity and productivity of plants (Ashraf and Harris 2013). In nature, plants are typically exposed not to a single but to the simultaneous action of multiple stress factors. The effect of high temperature on plant growth and productivity was intensively investigated both for high temperature-tolerant and sensitive plants species (Wahid *et al.* 2007, Mathur *et al.* 2014). The impact of high-light intensities on photosynthetic processes and on the structure and efficiency of photosynthetic apparatus has been widely studied and reported (Aro *et al.* 1993, Powles 1984). In recent years, an increasing number of studies focused on the simultaneous action of two or more stress factors on the development of plants and efficiency of photosynthesis – drought and salt stress, temperature and drought, *etc*., but these studies are still scarce and include limited plant species. Recently, data about the effect of combined action of high light and high temperature have been published and devoted to the response of very diverse plant species ranging from alpine plants (Buchner *et al.* 2015) to neotropical tree species *Ficus insipida* and *Calophyllum longifolium* (Krause *et al.* 2015). In the present work, we used tomato plants (*Solanium lycopersicum*) of cv. M82 that were grown at optimal conditions and then they were subjected to combinations of high light and/or high temperature for six days.

Tomato plants are known as temperate climate crops and the optimal growth temperature during the light period is 25–30°C. Exposure to higher temperatures – up to 38/29°C during a light/dark period could be considered as moderately high for plants; thus, we traced their ability to acclimate to the changed environment. Our results showed that photosynthetic response after two and six days of development at higher temperature included the decrease of PSII-mediated electron transport rate, while the PSI-mediated electron transport was stimulated. After 6 days, the PSII activity for NT-HL and HT-HL plants were similar to that of the nontreated plants. However, the plants at HT-NL did not adapt their PSII photochemical activity – it was still inhibited as during the first two days. This could occur not only due to the damage of D1 by high temperature, but also due to heat-induced changes of the oxygen evolving complex, known to be the most heat-sensitive thylakoid component (Murata *et al.* 2007). Effect of high temperature on the repair cycle of D1 (D1 resynthesis and incorporation into thylakoid membranes), which has been recently proposed to be one of the reasons for the D1 damage during photoinhibition, could not be excluded (Murata *et al.* 2012, Nishiyama and Murata 2014).

Our data showed that growing the tomato plants at higher temperature than optimal had more negative impact on PSII, whereas the PSI activity seemed to be slightly stimulated (Fig. 1*B*). This is in line with the lower sensitivity of PSI to high temperature reported by some authors (Allakhverdiev *et al.* 2008, Marthur *et al.* 2014 and references herein). After 6 days, the rate of PSI-mediated electron transport, measured by oxygen uptake in the presence of artificial electron acceptor and donor, was stimulated, thus compensating the decreased PSII activity in respect to maintaining the proton gradient. This increase could be due either to a higher population of PSI centers and/or to a stimulated activity of the existing centers. Since we did not observe higher ratio F_{735}/F_{685} (Fig. 4*A*) an increase of population of PSI is unlikely, rahter it is stimulated activity of existing centers. It is quite possible that under these conditions the cyclic electron transport around PSI was activated as reported earlier (Havaux *et al.* 1991).

Recently it has been reported that the microstructure of leaves and ultrastructure of tomato leaves were changed at elevated temperature up to 35°C (Zhang *et al.* 2014). Taking in mind that the structural alterations at a chloroplast level and in organization of grana reflect the mutual organization of pigment-protein complexes, the changes of energy interaction between both photosystems and their antenna complexes could be expected as a result of high-temperature treatment. Comparing 77 K data on PSI and PSII, it is evident that the most noticeable changes occurred in PSII and its antenna (Fig.5*C*). Analysis of excitation spectra permits to evaluate involvement of Chl *a* and Chl *b* molecules in energy supply of both photosystems and any changes in light-harvesting antennas of both photosystems, respectively. Comparison of the effect of both factors – HL and HT revealed that light intensity affected the antenna complexes of both photosystems more significantly than temperature did as we expected. At HL, the involvement of Chl *b* in energy supply of PSI decreased in comparison with Chl *a* – the ratio E_{680}/E_{650} was higher and the ratio E_{470}/E_{436} decreased, indicating a decrease in Chl *b* molecules which deliver excitation energy to PSI. This could be due to the decrease of LHCI and/or to a decrease of LHCII that is bound to PSI, as it has been supposed to occur when growth light intensity increases (Wientjes *et al.* 2013). Probably, the latter is more probable as it has been reported the light growth conditions do not influence the antenna of PSI in *Arabidopsis thaliana* plants (Ballottari *et al.* 2007). However, a probable decrease of light-harvesting complexes at HL could not be excluded as the ratio Chl *a*/*b* increased in thylakoid membranes (Table 2). Similar alterations in the excitation spectra were observed for PSII – the ratio E_{470}/E_{436} declined considerably during the first two days of treatment with HL. As Chl *b* belongs to the light-harvesting complexes, it could be supposed that one of the first responses of tomato plants to HL, concerning the structure and organization of photosynthetic apparatus, includes a decrease of light-harvesting capacity. These

observed changes for PSII were recovered to some extent after 6 days and it could be assumed that the acclimation of plants to this environment proceeded through balancing of excitation energy delivered to both photosystems.

The mechanism that underlies the damaging effect of HT and HL includes generation of various reactive oxygen species (Allahkverdiev *et al.* 2008). As a protective response, plants have evolved different defense systems including activation of antioxidant enzymes and synthesis of protective compounds. Heat treatment induces synthesis and accumulation of heat-shock proteins that could exert protective effect against photoinhibition and protect PSII from oxidative stress (Stapel *et al.* 1993, Neta-Sharir *et al.* 2005). HL induces activation of the xanthophyll cycle, thus increasing the quenching capacity against damaging oxygen forms (Yin *et al.* 2010, Dongsansuk *et al.* 2012). The application of higher light intensity during treatment of tomato plants with high temperature promoted their more rapid acclimation regarding the activity of both photosystems in isolated thylakoid membranes. The mechanism by which the light promoted this acclimation is not clearly understood but we suppose that this mechanism involves alterations in the energy supply and interaction of the two photosystems through light-related changes of their antenna complexes.

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