Heat-induced impairments and recovery of photosynthetic machinery in wheat seedlings. Role of light and prooxidant-antioxidant balance

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ABSTRACT

The extent of damage caused to the photosynthetic machinery of 10-d-old wheat seedlings by short-term exposure to mild heat, their capacity to recover from it and the possible roles of H₂O₂, SOD, catalase and ascorbate peroxidase on the recovery process were investigated. Seedlings were subjected to heat treatments at 40/42/44 °C for 20 min in the dark and allowed to grow for 72 h in light of different irradiances (40–800 μE m⁻² s⁻¹) at 20 °C for recovery from heat induced damage. Complete or partial recovery of photosynthetic activities was observed in the seedlings treated at 40 °C and 42 °C, but not at 44 °C. Our data suggest that the balance between (pro)oxidant and antioxidant levels poised by heat stress subsequent light is the crucial factor for the extent of recovery from heat induced damage. [*Physiol. Mol. Biol. Plants* **2009**; **15**(2) : 115–122] *E-mail* : vkreslav@mail.ru

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Abbreviations : APx – ascorbate peroxidase, Chl – chlorophyll, PM – photosynthetic machinery, PSI – photosystem I, PSII – photosystem II, ROS – reactive oxygen species, SOD – superoxide dismutase, TBARs – tiobarbituric acid reactive substances.

INTRODUCTION

Photosynthesis is one of the most heat sensitive cellular-functions (Berry and Björkman, 1980; Yordanov *et al.*, 1986). The subsequent recovery of photosynthetic activity in the presence of light from heat injuries caused due to as sudden rise in environmental temperature has been well documented (Kadir S and Von Weihe, 2007). However, only few studies have been reported on the exact nature and mechanism of injury and its paths of subsequent recovery from heat treatments under different light conditions. One of the key factors that influence balance between damage of photosynthetic machinery (PM) and the restoration of its activity is the relationship between the strength of oxidative stress and activity of antioxidant system. The latter can be important to decrease the photooxidative damage to heat-stressed seedlings (Dash and Mohanty, 2002).

A number of studies have demonstrated that ROS-scavenging mechanisms play an important role in protecting plants against high temperature stress and a combination of high light and heat stress (Larkindale and Knight 2002; Asada, 2006; Suzuki and Mittler, 2006). The extent of damage of PM depends on the pool of H₂O₂ and other ROS, activities of antioxidant enzymes and content of low-molecular antioxidants (Asada, 2006). Generation of H₂O₂ and several other ROS seems to be one of the early links in stress signal transduction, which are involved in stress protection and acclimation (Mittler *et al.*, 2004). Both decline in CO₂ assimilation and O₂ evolution due to heat stress are assumed to enhance ROS production and consequent loss in the light-dependent repair processes (Sairam *et al.*, 2000).

The *de novo* synthesis of photosynthetic proteins demanding energy in the form of ATP and light, is likely to play a crucial role in repair of heat-induced photoinhibition of PM (Adir *et al.*, 2003; Allakhverdiev *et al.*, 2008). Additionally, exposure to low or moderate light during short-time heat treatment is also shown to be beneficial to plants due to its diminishing effect on

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the extent of thermal damage to PM, especially PSII (Havaux *et al.*, 1991; Havaux, 1994). Strong light exposure, however, increases the magnitude of inhibition of PM induced by heat stress (Al-Khatib and Paulsen, 1989; Kreslavski and Khristin, 2003).

The aim of this study was to investigate heat-induced impairments and light-induced recovery of photosynthetic machinery and leaf growth depending on strength of heat stress, light intensity and leaf prooxidant-antioxidant balance.

MATERIALS AND METHODS

Plant cultivation and treatments

The 10-day-old wheat seedlings (*Triticum aestivum* L., cv. Moskovskaya-35) were grown under controlled conditions at 20 ± 0.1 °C and 16 h photoperiod ($I = 200 \mu\text{E m}^{-2}\text{s}^{-1}$ of PAR). The seedlings were carefully taken out from the pots, kept in dark for 30 min and then heated in dark in a thermo stated water bath at 40, 42, 44 °C (± 0.1 °C) for 20 min. After this, the plants were placed in water bath and incubated under three different light intensities (40, 240 and $800 \mu\text{mol m}^{-2}\text{s}^{-1}$ - low, moderate and strong light, respectively) at 20 ± 1 °C for 72 h. 60 W white light luminescence tubes and 1000 W incandescence lamp provided with infra-red absorbing filter were used to study the effect of light in heat-stress recovery.

Determination of chlorophyll (*Chl*) fluorescence parameters, CO₂ assimilation and chlorophyll contents

Prompt chlorophyll *a* fluorescence (PF) emitted from intact leaves was detected by a lab-built phosphoroscope. *Chl a* fluorescence was excited by the low-intensity measuring light ($\lambda_m = 480$ nm; $0.6 \mu\text{E}$ at the leaf surface) and emission was recorded at $\lambda_m = 685$ nm by a high-speed recorder Endim 322-01M (VEB MS, FRG). Different fluorescence parameters F_o , F_v , and the F_v/F_m were recorded as described by Maxwell and Johnson, 2000.

The photosynthetic CO₂ assimilation in the leaves was monitored by a Infralit-4 gas analyser. Leaf segments were homogenized using a mortar and pestle in 80 % (v/v) acetone and centrifuged for 15 min at 23,000 g. Chlorophyll content was determined spectrophotometrically according to Lichtenthaler and Wellburn (1987).

Numbers reported in table and graphs are the means from 3-6 replicates. In each individual measurement

leaves from 5-10 plants were analyzed to minimize probable ontogenetic differences between plants. Experimental data were analyzed statistically by *t*-test.

Determination of TBARs, H₂O₂ and assay of enzyme activities

For enzyme and thiobarbituric acid reactive substances (TBARs) analysis small portion (0.5 g) of freshly cut leaves was homogenized in a pre-cooled mortar in 4.5 ml of phosphate buffer (pH 7.4) containing 0.2 mM EDTA and 2 % polyvinylpyrrolidone. Homogenate was filtered through several layers of nylon. Part of the homogenate was used for assay of TBARs, whereas the remainder was centrifuged for 20 min at 15000 g. The supernatant (enzyme extract) was used for enzyme analysis. All operations were carried out at 4 °C. SOD activity was assayed spectrophotometrically by photochemical method (Giannopolitis and Ries, 1977). One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the rate of p-nitro-blue tetrazolium chloride reduction at 560 nm. Activity of Ascorbate peroxidase (APx) was assayed as the decrease in absorbance at 310 nm due to ascorbic acid oxidation (Nakano and Asada, 1981). The level of lipid peroxidation in the leaves was assayed as contents of TBARs (Dhindsa and Matove, 1981). The concentration of TBARs was calculated based on measurements of absorption at 532 and 600 nm. Catalase activity was measured in total protein extract (50mM potassium-phosphate buffer, pH 7.0) as a result of decrease in *D* at 240 nm (extinction coefficient $E = 43.6 \text{ l}^{-1} \text{ cm}^{-1}$) at an initial concentration of H₂O₂ -10 mM. Stationary content of H₂O₂ was evaluated in leaf extracts by standard method of luminol bioluminescence (Warm and Laties, 1982).

RESULTS AND DISCUSSION

Photosynthesis and fluorescence characteristics

Heating can inhibit plant growth and cause crop yield reduction. One of the main reasons of such effect of elevated temperatures is high sensitivity of PM to heat stress (Berry and Björkman, 1980). Recovery of photosynthetic response from heat injury under different light conditions plays an important role in acclimation of photosynthetic machinery to stress conditions. However, the paths and nature of recovery of photosynthetic responses after heat treatment, especially, role of antioxidant/oxidant balance are little investigated (Dash and Mohanty, 2001, 2002; Kreslavski and Khristin, 2003; Kadir and Von Weihe, 2007).

Damage and recovery of PM were evaluated on two parameters i.e. the fluorescence ratio F_v/F_m , characterizing activity of PSII and the P_N values, characterizing CO_2 assimilation. These parameters are often used for evaluation of stress-induced changes in the state of PM during heat stress (Bukhov and Mohanty, 1999). Heat treatment led to decrease in both parameters – net photosynthesis (P_N) and the F_v/F_m -ratio. The F_v/F_m ratio dropped after heat treatments from 0.78 to 0.67 and to 0.53 at 40 and 42 °C, respectively (Fig. 1A,B), while P_N decreased from 9.8 to 1.2 and 2 $nmol\ CO_2\ gm^{-2}\ s^{-1}$ at 40 and 42 °C, respectively (Fig. 2A,B). The reduction in F_v/F_m ratio was mainly due to the reduced F_v magnitude, whereas F_o level was slightly affected by heat-stress at 40/42 °C for 20 min (data not shown).

Our studies indicated two phases of changes in photosynthetic responses during post-stress seedling growth. The first stage was characterized by either slow increase or even a gradual post-stress decrease of the PSII activity as evaluated by the F_v/F_m ratios and P_N values in the seedlings treated at 40 °C or 42 °C for 20 min, and then exposed to weak, moderate or strong light in comparison with the corresponding values in the seedlings tested immediately after heating (Fig. 1) The value of maximal decline in the ratios depended on light intensity and temperature. At 42 °C, and exposure to

strong light, the decline in F_v/F_m was significant (Fig. 1B), whereas at 40 °C, upon exposure to weak light the extent of decrease was minimal (Fig. 1A). Changes in P_N values (Fig. 2, A, B) were in agreement with the alterations in the fluorescence ratios, however, CO_2 assimilation was more sensitive to elevated temperature than the fluorescence parameters.

After the first stage of reduction in activities, we observed a relatively rapid partial or complete recovery of photosynthesis as well as the activity of PSII. Upon heating at 40 °C and subsequent recovery of the seedlings in low light, the F_v/F_m ratio (Fig. 1) and P_N (Fig. 2) increased after 24 h in comparison with the non-treated control seedlings. The recovery after 72 h was complete in low light but partial in presence of strong light. Heat treatment at 42 °C led to impaired recovery of photosynthesis and Chl *a* fluorescence than those at 40 °C at similar light intensities (Figs. 1 and 2). The repair of PSII and CO_2 assimilation system in seedlings treated either at 42 °C and then exposed to strong or moderate light was always slow and partial. Similar trends were observed in seedlings treated at 40 °C before exposure to strong light. In contrast, in low light (40 $\mu E\ m^{-2}s^{-1}$), the recovery of both PSII activity (Fig. 1) and P_N (Fig. 2) were rapid (at least at 40 °C) and complete. The major measurements were conducted at 40 °C and 42 °C

Fig. 1. Changes of the F_v/F_m in the leaves of the seedlings exposed (dash lines, white symbols) and non-exposed (solid lines, black symbols) to heating at 40 °C (A) and 42 °C (B) for 20 min and then grown at 20 °C for 72 h in low (○,●), moderate (△) and strong light (■, □). Means $\pm$ SE ($n = 10$).

Fig. 2. Changes of CO_2 assimilation-rates in the leaves of the seedlings exposed (dash red lines) and non-exposed (solid dark lines) to heating at 40 °C (A) and 42 °C (B) for 20 min and then grown at 20 °C for 72 h in low (○,●) and strong light (□). Means $\pm$ SE ($n = 10$). Light and growth conditions are the same as in Fig. 1.

since at 38 °C the changes of photosynthetic activities were small and at 44 °C we did not observe any recovery independent of light intensity. (Fig. 3). Heat pre-treatment of the seedlings at 40 °C (20 min) and then growing in low light (40 $\mu\text{E m}^{-2}\text{s}^{-1}$) for 24 h improved the recovery of photosynthesis from heat exposure at 44 °C. We have also examined the contribution of dark recovery after both heat treatments at 40 °C and 42 °C. There was significant dark recovery at both temperatures (Fig. 4). However, this recovery was smaller in extent than the light recovery.

Chl (*a+b*) content is known to decline at high temperature and contribute to decrease in photosynthesis. Indeed, after heat treatment at 42 °C

Table 1. Effect of heat pretreatment on chlorophyll (*a+b*) contents [mg g^{-1} (f.m.)] and the level of TBARs [$\mu\text{M kg}^{-1}$ (f.m.)] in first leaves of wheat seedlings. 10-d-old seedlings were exposed to short-time heating at 40 °C, 42 °C or 44 °C, then grown for recovery in low ($I = 40 \mu\text{E m}^{-2}\text{s}^{-1}$) for 0, 6 and 48 h or in very low ($10 \mu\text{E m}^{-2}\text{s}^{-1}$, I_2) for 48 h and in moderate light ($I = 240 \mu\text{E m}^{-2}\text{s}^{-1}$, I_3) for 48 h. Values are mean $\pm$ S.E.

T, °C	Time after heat treatment, h	Chl (<i>a+b</i>)	Level of TBARs
20	—	1.25 $\pm$ 0.02	166 $\pm$ 6
—	48	1.22 $\pm$ 0.02	168 $\pm$ 9
40	0	1.22 $\pm$ 0.045	168 $\pm$ 10
—	6	1.21 $\pm$ 0.04	169 $\pm$ 9
—	48	1.23 $\pm$ 0.02	161 $\pm$ 8
—	48 (I_2)	1.25 $\pm$ 0.03	163 $\pm$ 7
—	48 (I_3)	1.22 $\pm$ 0.03	162 $\pm$ 6
42	0	1.21 $\pm$ 0.045	171 $\pm$ 8
—	6	1.16 $\pm$ 0.02*	175 $\pm$ 9
—	48	1.28 $\pm$ 0.03	171 $\pm$ 7
—	48 (I_3)	1.21 $\pm$ 0.025	170 $\pm$ 9
44	0	1.19 $\pm$ 0.025	190 $\pm$ 7
—	6	1.12 $\pm$ 0.03*	206 $\pm$ 10*
—	48	1.03 $\pm$ 0.03*	201 $\pm$ 10*

*significant difference between control and experiment ($p < 0.05$)

and subsequent exposure of seedlings to light for 6 h as well as after heat treatment at 44 °C and subsequent exposure of seedlings to light ($I = 40 \mu\text{E m}^{-2}\text{s}^{-1}$) Chl (*a+b*) content decreased (Table 1) from 1.25 in the control (no heating) to 1.16 ($p < 0.1$) and 1.12 ($p < 0.05$) mg g^{-1} , at 42 °C and 44 °C respectively. However, at 40 °C changes in Chl were insignificant.

Since the *de novo* synthesis of photosynthetic proteins is important for photosynthetic recovery, we have investigated the action of protein inhibitor lincomycin. The addition of lincomycin (final concentration 2 mM) to the nutrient medium immediately after heat stress and subsequent growth of the seedlings at light led to enhancement of photoinhibition though PSII repair was not observed (Fig. 5). Conversely, control plants cultivated in the water without lincomycin, under the same conditions, showed complete recovery of the PSII after heat treatment. Our observation that inhibition of protein synthesis by lincomycin had significant effects on the recovery, suggests that protein synthesis was involved in the same. The rates of photodamage in the presence of lincomycin were approximately equal in heat treated and non-treated seedlings, whereas without addition of this protein inhibitor they were different.

Contents of H_2O_2 , level of TBARs and activities of antioxidant enzymes

Level of H_2O_2 as well as activities of catalase, SOD and APx were measured to determine whether heat treatment and heat-induced photoinhibition caused oxidative

Fig. 3. Effect of heat hardening on recovery of P_n after heat stress at 44 °C. The seedlings were exposed to heat pre-treatment at 40 °C for 20 min (1) or not exposed (2) and incubated in low light ($40 \mu\text{E m}^{-2}\text{s}^{-1}$) for 24 h. Then a part of the seedlings (1, 2) was exposed to second heat stress at 44 °C for 20 min and grown at 20 °C for 72 h ($I = 20 \mu\text{E m}^{-2}\text{s}^{-1}$). Other seedlings (3) were grown under the same light conditions however, not exposed to heat treatments. $\pm$ SD ($n = 6$).

Fig. 4. Changes of the F_v/F_m (A) and CO_2 assimilation rates (B) in the leaves of the seedlings exposed (dash lines, white symbols) and non-exposed (solid lines, black symbols) to heating at 40 °C (○) and 42 °C (□) for 20 min and then grown at 20 °C for 72 h in dark. Means $\pm$ SE ($n = 10$). Growth conditions are the same as in Fig. 1.

Fig. 5. Influence of lincomycin (L) added to nutrient medium on the F_v/F_m ratio during the seedling recovery at $I = 40 \mu E m^{-2} s^{-1}$ after the seedlings were subjected to a heat exposure at 42 °C for 20 min. A. Non-treated (solid lines) and treated by heating (dash lines) seedlings were exposed to the light for 72 h without (●, ○) and in the presence (△, ▲) of 2 mM L at +20 °C. Control, no lincomycin application. Means $\pm$ SE ($n = 4$).

stress in this system. Subjecting wheat seedlings to 40 °C for 20 min in the dark did not significantly affect the level of endogenous H_2O_2 , in comparison to control plants grown at 20 °C; whereas pre-treatment at 42 °C caused significant increase ($P < 0.01$) (Fig. 6A). During subsequent light exposure, we observed the decrease in the level of H_2O_2 and subsequent increase in the level.

Fig. 6. Effect of heat pretreatment of on level of H_2O_2 (A), activities of superoxide dismutase (SOD) (B), catalase (C) and ascorbate peroxidase (APx) (D) in leaf extracts during post-stress seedling recovery at 20 °C and $I = 40 \mu E m^{-2} s^{-1}$. Initial activities of SOD, catalase and APx were 430 ± 40 unit g^{-1} (FW), $2500 \mu mol H_2O_2 g^{-1}$ (FW.) min^{-1} and $6.3 \pm 1 \mu mol min^{-1} 1g^{-1}$ (FW.), respectively. ○ - control; ● - 40 °C; ▼ - 42 °C

The heat pre-treatment at 44 °C increased the level of H_2O_2 more than two-fold (data not shown). Maximum activity of SOD was observed immediately after heat treatments that were restored to control levels after 24 h (Fig. 6B). Catalase activity (Fig. 6C) did not change significantly after heat shock both at 40 and 42 °C. After 6 h of light exposure it increased by 13 % at 42 °C ($P < 0.05$) and decreased after 48 h in both variants. At 40 °C, the level dropped by approximately 9 % ($P < 0.05$). It was noticed that the maximum level of H_2O_2 preceded the maximal catalase activity. Peroxidase activity was significantly increased at least for 6 h after heating before declining to levels close to the control (Fig. 6D).

Since there was no recovery at 44 °C we did not determine the activities of COD, AsP and catalase during heat-induced photoinhibition of plants. Heating at 40/42 °C had insignificant effect on level of TBARs (Table 1) while heat stress at 44 °C and subsequent exposure to light for 6 and 48 h, led to significant increase in lipid peroxidation. This is in agreement with data on irreversibility of photosynthetic activity in seedlings grown for recovery after this heat treatment.

Leaf growth

The rate of leaf growth in seedlings exposed to 40 °C was same as in the control without heating, whereas the leaf growth of seedlings exposed to heat stress at 42 °C was retarded and did not recover to initial levels even after 3 d of light exposure (Fig. 7). Retarding effect of heat treatment at 44 °C was higher than at 40 and 42 °C. The growth rate recovered after heating at 42 °C, but

Fig. 7. Effect of heat pretreatments on growth of first leaves of 10-d-old wheat seedlings, exposed and non-exposed (control) to heating 40 °C, 42 °C and 44 °C for 20 min, then grown at 20 °C for 72 h ($I = 40 \mu\text{E m}^{-2} \text{s}^{-1}$). $\pm$ SD ($n = 6$).

not at 44 °C. One of the main reasons of such response could be recovery of photosynthesis at 42 °C, which was absent at 44 °C.

The significant decline both in the PSII efficiency and CO_2 assimilation rate observed immediately after heat treatments and during heat-induced photoinhibition (Figs. 2-6) can be associated with damages to donor and/or acceptor sides of PSII and loss in activity of Rubisco as well as with losses in contents of Chl (*a+b*) (Kreslavski and Khristin, 2003; El-Shitnawy *et al.*, 2004; Sharkey, 2005; Allakhverdiev *et al.*, 2008). However, our data allows definite conclusions about insignificant or small changes in Chl contents resulting in moderate heat stress at 40 °C and 42 °C and subsequent growth of heat-treated seedlings in light (Table 1).

Our results indicate that heat-enhanced photoinhibition can be due to higher rate of formation of ROS, which was indicated by an increase in H_2O_2 and/or TBARs (Fig. 7A, Table 1), in leaves of heat-treated plants. It has been shown earlier that the generation of ROS can inhibit the protein synthesis required for repair of PSII (Nishiyama *et al.*, 2001; Murata *et al.*, 2007), which in turn can enhance photoinhibition (Takahashi *et al.*, 2004; Allakhverdiev *et al.*, 2008).

Wheat is sensitive to heat injury and the crop is often grown in areas where high temperatures together with high light intensity limit plant productivity (Paulsen, 1994). Indeed, the growth of wheat seedlings indicated by the fresh weight was significantly retarded in pre-treated at 42 °C and 44 °C seedlings (Fig. 1). Both stressful factors i.e. heating and strong light can retard leaf growth (Laing *et al.*, 1995; Dash and Mohanty, 2001). This effect can be due to decline in photosynthesis and Chl content. Indeed, reduced growth rate is in line with decreased photosynthesis and activity of PSII, accompanied by an increased accumulation of H_2O_2 at 42 °C and TBARS at 44 °C (Figs. 1-7, table 1). Conversely, photosynthetic recovery at 42 °C was accompanied by recovery in growth.

Extreme stress under certain conditions can be considered as a form of oxidative stress resulting in the enhancement of ROS in chloroplasts (Gong *et al.*, 2001; El-Shitnawy *et al.*, 2004). Our data (Fig. 7) demonstrated that moderate heat stress enhanced the production of H_2O_2 , likely, by inhibiting protein synthesis and hence, the repairs of PSII, as demonstrated for the CO_2 limitation-induced inhibition of PSII repair (Takahashi and Murata, 2005). Our data suggests that strong and even moderate light can enhance the generation of ROS

in chloroplasts of heat-damaged seedlings exposed to light for recovery. The formation of ROS and finally TBARs (Table 1) was presumably highest after heat treatment at 44 °C and subsequent light exposure. This could explain lack of significant recovery of photosynthetic activity in this case. The losses in Chl (*a+b*) and an increase in level of TBARs, observed after heat treatment at 44 °C and subsequent growth of seedlings in light are in agreement with this suggestion.

The *de novo* synthesis of photosynthetic proteins demanding light and energy, plays, likely, crucial role in repair of heat-induced photoinhibition of PM (Adir *et al.*, 2003). Indeed, there was no recovery of PS II in the presence of protein inhibitor lincomycin (Fig. 6). Light might be required for the photophosphorylation of photosynthetic proteins and activation of many light-regulated enzymes such as Rubisco (Sharkey, 2005; Allakhverdiev *et al.*, 2008). However, the partial recovery in dark is also possible due to likely enhancement of dark respiration resulting due to heat treatment (Fig. 5). Light in our case might also be important for activation of low-light dependent reactions of protein synthesis but not for photosynthesis; since a significant recovery (F_v/F_m ratio to 0.75 and P_n to 10 nmol CO₂ gm⁻¹ s⁻¹) was observed even after 48-h exposure of the seedlings in very low light (10 µE m⁻² s⁻¹). Prooxidant-antioxidant balance is also involved in restoration of photosynthetic activities. For example, at 44 °C the recovery of heat-damaged PM was not observed due to its strong oxidative damage (Fig. 3).

The development of oxidative processes in leaves indicated by level of H₂O₂, was accompanied with activation of the key antioxidative enzymes in chloroplasts – APx and in cytoplasm - catalase (Fig. 6), which play an important role for recovery by neutralization of ROS inhibiting the repair of photodamaged PSII and whole photosynthesis. H₂O₂ can be directly decomposed through catalase. On the other hand, by combining APx with GR, H₂O₂ can also be removed via recurrent oxidation-reduction reactions promoted by reduced glutathione, hence, preventing cell damage. The activation of both enzymes is in line with the formation of H₂O₂ in leaves at heat treatments and subsequent light exposure. Time course studies of H₂O₂ indicate that changes in levels of ROS can precede changes in antioxidant enzyme activities. In this case, H₂O₂ can play a signaling role and act via signal transduction chain, enhancing synthesis of antioxidative enzymes. Thus, enhancement of activities of SOD, APx and catalase can contribute to overcoming the oxidative stress and repairing PM.

Dash and Mohanty (2002) observed a correlation of heat-tolerance potential of the cultivars with the potential of APx and catalase to dissipate H₂O₂ co-operatively and decline photo-oxidative damage to heat-stressed seedlings. This is in line with our data that preliminary heat hardening of wheat seedlings improves the recovery of photosynthesis (Fig. 4). This is in line with our observations that heat hardening can enhance the resistance of PM in wheat seedlings to photoinhibition (Kreslavski *et al.*, 2008). One of the reasons of the positive effect of heat hardening could be the generation of high antioxidant potential in chloroplasts isolated from leaves of hardened plants.

It may be concluded that there is a close relationship between mode of recovery of PM and oxidant/antioxidant balance in seedlings exposed to heating and then to light for recovery. High oxidant level seems to be one of the basic reasons of heat-induced photoinhibition, which retards recovery from heat stress, whereas light support and prooxidant-antioxidant balance are crucial for the complete recovery of PM.

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