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The influence of antimycin A on pigment composition and functional activity of photosynthetic apparatus in *Triticum aestivum* L. under high temperature

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Abstract

The purpose of the current investigation was to evaluate the influence of antimycin A (AA) as an activator of the alternative respiratory pathway (AP) on photosynthetic pigment composition and functional activity of the photosynthetic apparatus of wheat seedlings (*Triticum aestivum* L.) under exposure to high temperature as well as their acclimation. Our results indicated that a significant decrease (44–74%) of photosynthetic pigment contents was caused by a long-term exposure to high temperature (42°C), while the short-term exposure resulted in 20–46% decline. However, a combined effect of AA and long-term high temperature reduced the total pigment contents by 28–41%. Our results demonstrated that the reduction of the chlorophyll *a*/*b* ratio was less significant under the combined effect of AA and high temperature than that under the stressful condition without AA. We observed that short-term and long-term high temperature modified PSII functionality of the first leaves in wheat seedlings, which was manifested by the low maximal quantum yield of PSII photochemistry, maximum fluorescence yield in the dark-adapted state, and by high minimum fluorescence yield in the dark-adapted state. The quantum yield of PSII photochemistry decreased rapidly by 16–24% under the combination of AA and high temperature. Overall, these results suggest that the activation of the alternative pathway, induced by AA, contributed to the stabilization of the photosynthetic apparatus in wheat seedlings under high temperature.

Additional key words: alternative respiratory pathway; chlorophyll *a* fluorescence; pigment content.

Introduction

A wide range of various adverse environmental stressors, such as high and low temperature, soil salinity, and high light intensity, adversely affects plant growth, development, productivity and may limit species distribution. Heat stress is a widespread problem around the world that affects thylakoid membrane reactions, leads to deactivation of Rubisco, and may reduce the rate of photosynthesis (Sharkey 2005). This environmental stressor induces oxidative stress causing oxidation of proteins, peroxidation of lipids, inhibition of enzymes, and damage of nucleic acids, which leads to mutations (Sharma *et al*. 2012).

The ranges of optimum temperatures may differ not only for different organisms but also for various organs of organism; *e.g*. high temperature (35ºC) decreases wheat root growth and accelerates its senescence, a cyclic photophosphorylation is inhibited at 42ºC, and lethal effects on active tissues of shoots usually occur in the range of 50 to 60ºC (Mavi and Tupper 2004).

Plant's acclimation to heat stress leads to adaptive changes of physiological and biochemical processes, such as changes in plant structure, growth rates, adjustment of metabolic pathways, stomatal conductance, an osmotic potential, and activities of antioxidative enzymes (Hasanuzzaman *et al*. 2013).

Changes in the photosynthetic apparatus, in particular, the content of photosynthetic pigments, their ratios, and analysis of chlorophyll (Chl) *a* fluorescence parameters, *e.g.* minimal fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m), and maximal quantum yield of PSII photochemistry

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Abbreviations: AA – antimycin A; AOX – alternative oxidase; AP – alternative pathway; Car – carotenoids; Chl *a*(*b*) – chlorophyll *a(b)*; DAD – days of development; ETC – electron transport chain; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; FM – fresh mass; FQR – ferredoxin plastoquinone oxidoreductase; F_v – variable fluorescence, F_v/F_m – maximal quantum yield of PSII photochemistry; LT – long-term high temperature; NDH – NADH-dehydrogenase; PGR – proton gradient regulation; ROS – reactive oxygen species; ST – short-term high temperature.

(F_v/F_m), are important ecological indicators of the physiological state and productivity of plants.

Plant mitochondria play an important role in a response to different biotic and abiotic stressors. Plant responses are directed to restructuring of metabolic reactions, the final stage of which is protection against stress factors. There are various mitochondrial energy dissipation mechanisms, *e.g.* alternative oxidase (AOX), NADPH dehydrogenases, uncoupling proteins (UCPs), free fatty acids (FFA), ADP/ATP translocator, which dissipate energy as heat, decreasing oxidative phosphorylation efficiencies and allowing to maintain energy balance in plant cells under stress conditions (Grabelnych *et al*. 2014). The possible role of AP in optimizing of photosynthesis process raises much of interest and attracted attention because AOX activity may optimize photosynthesis by dissipating chloroplastic reducing equivalents in coordination with cytochrome pathway and thus protects photosynthetic apparatus against photooxidation by means of energy dissipation (Strodkötter *et al*. 2009, Vanlerberghe 2013). Recently many researchers have reported that AP inhibition by *n*-propyl gallate (nPG) or salicylhydroxamic acid (SHAM) decreased the rate of photosynthesis (Bartoli *et al*. 2005, Yoshida *et al*. 2006). Chloroplasts accumulate an excess of redox equivalents which cause overreduction of electron transport chain (ETC), an increase of ROS production, and damages of the photosynthetic apparatus. AP effectively oxidizes redox equivalents across the inner mitochondrial membrane without restriction of proton gradients and has a special role in relieving the overreduction of chloroplasts (Yoshida *et al*. 2007). This respiration is induced in response to different environmental factors, *e.g.* high temperature, low temperature, drought, osmotic stress, oxidative stress, pathogen attack (Grabelnych 2005); AOX mRNA transcription is stimulated by AA, an inhibitor of cytochrome pathway (Gilliland *et al*. 2003).

AA is an inhibitor of the ferredoxin-dependent pathway of cyclic electron transport reactions around PSI in chloroplasts (Munekage and Shikanai 2005) and is also a fairly potent mitochondrial respiratory chain inhibitor that binds sites for the quinine reduction (Q_i site) of the cytochrome bc_1 complex (Huang *et al*. 2006). There are two different independent cyclic electron transport pathways operating around PSI in chloroplasts: one pathway is sensitive to AA and the other is insensitive to AA and involves a plastid NADH-dehydrogenase (NDH) complex. The AA-sensitive pathway provides extra ATP under photorespiration conditions, whereas the NDH-dependent pathway limits CO_2 assimilation rate (Joët *et al*. 2001).

The aim of the present study was to evaluate the influence of AA as an activator of the AP on the content of photosynthetic pigments, their ratios, and Chl *a* fluorescence parameters in different organs of wheat seedlings under exposure to short-term and long-term high temperature, as well as their acclimation.

Materials and methods

Plant material and growth conditions: The first leaves were used as a model of developing organs and coleoptiles as senescent organs of winter wheat seedlings (*Triticum aestivum* L., cv. Harmony). The grains were germinated for 24 h in the plastic cuvette (19 × 12 cm) containing humid filter paper in the thermostat (25 ± 1ºC). After germination, wheat seedlings were placed in other plastic cuvettes supplemented with solution of AA (1 mg l^{-1}) and in distilled water (control) for 3 d in the climate chamber [16/8 h of light/dark cycle; PAR of 150 µmol(photon) m^{-2} s^{-1}; temperature of 25–26ºC; relative humidity (RH) of 75%]. The seedlings were transferred to short-term (1 h, ST) and long-term (24 h, LT) high temperature (42ºC) after 3 and 5 d of development (DAD) and treated with AA (1 mg l^{-1}) and without AA. Wheat seedlings subjected to high-temperature exposure were transferred to the optimal conditions for 24 h in order to study the processes of recovery.

Determination of photosynthetic pigment concentrations: The quantitative determination of the photosynthetic pigment contents [Chl *a*, Chl *b*, and carotenoids (Car)] in developing and senescent organs was carried out spectrophotometrically in the acetone extract according to the method described by Gavrilenko and Zhigalova (2003). The samples of leaves and coleoptiles (0.2 g) were homogenized using mortar and pestle and extracted in 80% chilled acetone (4 ml) with the addition of $MgCO_3$ (0.25 g). Homogenized sample mixture was centrifuged at 4,500 × *g* for 15 min at 4ºC. Extracts were then kept at 4ºC in the refrigerator for 24 h. The supernatants were separated from each sample after 10 min of high-speed centrifugation (2,000 × *g*). The absorbance of pigment extracts was measured at 663 nm (Chl *a*), 646 nm (Chl *b*), and 470 nm (Car) using a single-beam spectrophotometer (*Cary 50 Scan UV/VIS, Varian*, Pittsburgh, USA). The concentrations of Chl *a*, Chl *b*, and Car [mg l^{-1}] were calculated according to Gavrilenko and Zhigalova (2003):

$$\text{Chl } a = 12.21\ A_{663} - 2.81\ A_{646};$$
$$\text{Chl } b = 20.13\ A_{646} - 5.03\ A_{663};$$
$$\text{Car} = \frac{1{,}000\ A_{470} - 3.27\ \text{Chl } a - 100\ \text{Chl } b}{229}$$

A_{663}, A_{646}, A_{470} – absorbance at an appropriate wavelength.

Pigment content (Chl *a*, Chl *b*, Car) per 1 g of fresh mass (FM) was calculated by the following formula. The content of pigments was expressed as mg g^{-1}(FM).

$$A = C \times V/(P \times 1{,}000)$$

where C is pigment concentration [mg l^{-1}], V is volume of extract [ml], P is plant fresh mass [g], A is photosynthetic pigments content [mg g^{-1}].

Chl *a* fluorescence measurements were performed at 20°C using modulated Chl fluorometer (*OS-30 Chlorophyll fluorimeter*, *Opti-Sciences*, USA) with special leaf-clip holder with the developing organs of wheat seedlings. F_0 was measured using a weak modulated light [0.2 μmol(photon) m^{-2} s^{-1}] for 2 s following a 30-min dark adaptation. A saturating light [5,000 μmol(photon) m^{-2} s^{-1}] was used to obtain F_m parameter in the dark-adapted state. The photochemical efficiency (F_v/F_m) of dark-adapted plants was calculated according to the formula:

$F_v/F_m = (F_m - F_0)/F_m$ (Murchie and Lawson 2013).

Statistical analysis: All measurements were analysed statistically and presented as means and standard deviations (SD). Statistical variance analysis of the independent data with three replicates (n = 3) for concentration of photosynthetic pigments and five replicates (n = 5) for Chl *a* fluorescence parameters was analysed using the program *Statistica 2010* and compared with least significant differences at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Results and discussion

Photosynthetic pigment analysis demonstrated that the concentration of Chl *a* was significantly reduced by 34% and Chl *b* by 14% under exposure to ST in the developing organs after 3 d DAD compared with control (Fig. 1*A*). It should be noted that the content of Chl *a* decreased by 56% and Chl *b* by 27% in the developing organs under prolonged exposure to elevated temperature after 3 DAD compared with unstressed seedlings (Fig. 1*B*). It could be

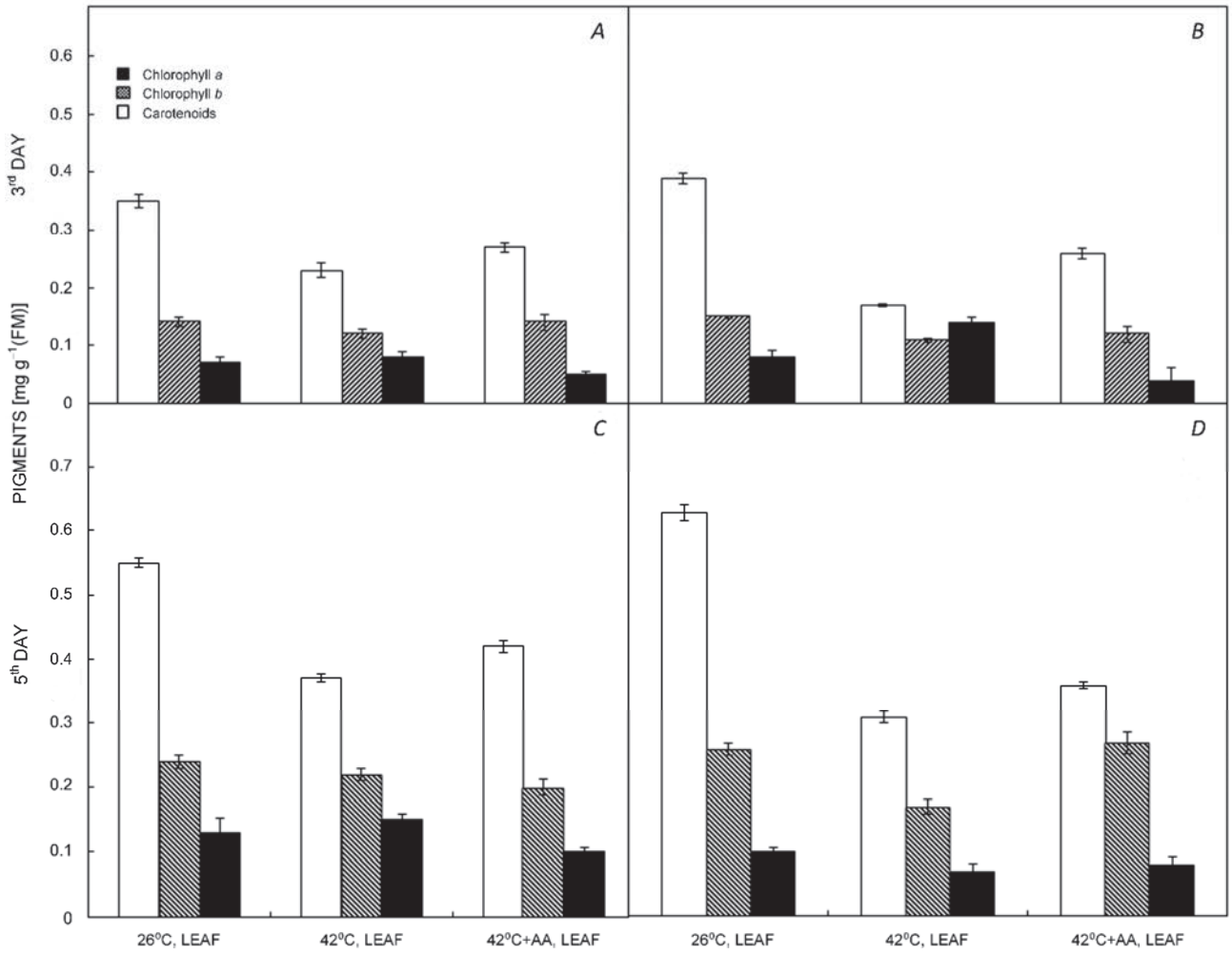

Fig. 1. Effect of antimycin A on the photosynthetic pigment contents in developing organs under short-term (*A*) and long-term (*B*) exposure to high temperature after 3 d of development and under short-term (*C*) and long-term (*D*) exposure to high temperature after 5 d of development. Data are presented as means ± SE of three replicates.

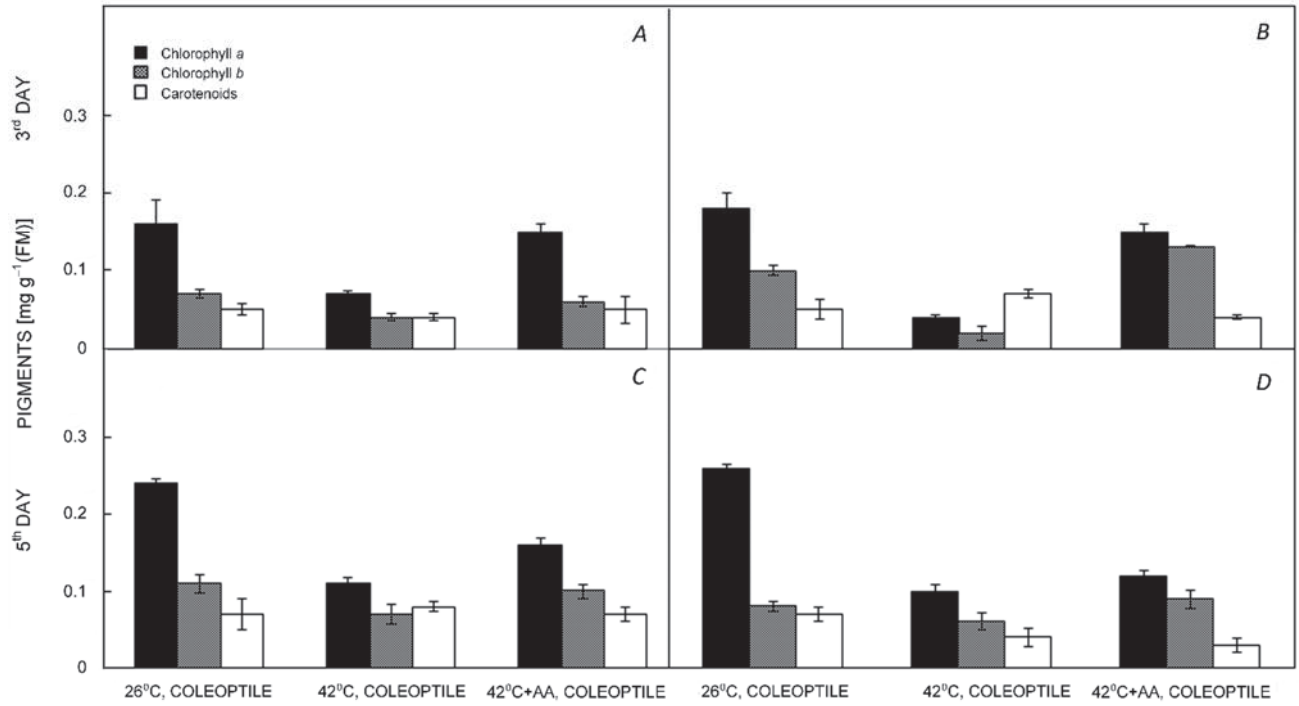

Fig. 2. Effect of antimycin A on the photosynthetic pigment contents in the senescent organs under short-term (*A*) and long-term (*B*) exposure to high temperature after 3 d of development and under short-term (*C*) and long-term (*D*) exposure to high temperature after 5 d of development. Data are presented as means ± standard errors of three biological replicates.

argued that Chl *b* was more resistant than Chl *a* under conditions of high temperature. Our results agree with the earlier investigations that declare a significant decline in photosynthetic pigment contents under heat stress in Karacadag and Firat wheat cultivars (Efeoglu and Terzioglu 2009). The considerable degradation of different photosynthetic pigments under high temperature occurs due to the inhibition of pigment biosynthesis (Ashraf and Harris 2013) and also disorganization of chloroplast structure (Camejo *et al*. 2006). The enhanced chlorophyllase activity and disruptions of the thylakoid membranes are in agreement with the decrease of pigment concentrations under heat stress (Hussain *et al*. 2014). Altogether, our results indicated that suppression of respiratory metabolism under exposure to high temperature disrupted the synthesis of Chl. It can be assumed that the rate of photosynthesis is suppressed in wheat seedlings due to structural changes of protein complexes in thylakoid membranes during the primary stress response.

Our results indicated significant decreases in the contents of Chl *a* (56%) and Chl *b* (43%) in the senescent organs under ST (Fig. 2*A*). It should be emphasized that the concentrations of Chl *a* and Chl *b* decreased by 78 and 80%, respectively, under LT in the senescent organs (Fig. 2*B*). Senescent organs of wheat seedlings were more sensitive to high temperature than the young and photosynthetically active leaves. This could be result of senescence – Chl degradation is a typical symptom of senescence. Previous findings suggested that upon exposure to 40°C, a substantial decrease of Chl content and rate of photosynthesis occurred, but the growth was absolutely stopped in coleoptiles at 45°C (Akman 2009).

The concentration of Chl *a* was reduced by 33–51% in the developing organs (Fig. 1*C*,D) and by 54–62% in the senescent organs (Fig. 2*C*,D) under ST and LT after 5 DAD compared with the control group. Our results are in agreement with Cui *et al*. (2006) who reported that leaves of *Festuca arundinaceae* L. under conditions of high temperature showed a decrease of Chl *a* and Chl *b*. The obtained results suggest that wheat seedlings were more resistant to high temperature at later stages of development. The inhibition of Chl biosynthesis under exposure to high temperature might occur due to a destruction of enzymes which participate in Chl synthesis (Ashraf and Harris 2013). Different photochemical reactions in thylakoid lamellae and carbon metabolism in the stroma of plastids are the main sites of damage under exposure to high temperature (Wise *et al*. 2004).

Car are a large group of red, yellow, and orange lipophilic molecules that are produced in all photosynthetic organisms and are integral constituents of the thylakoid membrane in chloroplasts. These nonenzymatic low-molecular-mass metabolites play an effective role in photoprotection. Our findings indicated that Car concentration increased by 12% during ST in the developing organs as compared with control seedlings (Fig. 1*A*). It should be noticed that the concentration of Car significantly increased (43%) in the developing organs under LT after

3 DAD (Fig. 1*B*). The data showed that the concentration of Car increased by 13% in the developing organs under ST after 5 DAD (Fig. 1*C*). Given the importance of these findings, which indicated a considerable increase in the Car content in the developing organs of wheat seedlings, we assumed that Car protected cell membranes against oxidative damage during the exposure to high temperature. Previous studies reported that high temperature (40°C and 60°C) caused an increase in the Car concentrations in *Daucus carota* L. (Fikselová *et al*. 2008). Based on the results described above, Car accumulation protected reaction centers of photosystems in wheat seedlings against temperature-induced damages, such as photooxidative degradation of pigments and ETC (Demnig-Adams and Adams 1992). Interestingly, the most significant concentration of Car was under LT exposure in the senescent organs (29%) (Fig. 2*B*). This could be probably explained in a way that Car protected the active site of photosystems against photooxidation, membranes against lipid peroxidation, and delayed the process of senescence in coleoptiles. Our data confirmed the findings presented in Camejo *et al*. (2005) and Lefsrud *et al*. (2005). These nonenzymatic low-molecular-mass metabolites protect photosynthetic apparatus against oxidative stress (Roy and Sengupta 2014). It can be assumed that during high temperature sharply increases energy consumption for maintaining the photosynthetic apparatus and activation of the adaptive responses.

It is noteworthy that wheat seedlings subjected to the AA treatment, an activator of AP significantly affected photosynthetic metabolism in the organs of wheat seedlings under high temperature. During the present experiment, we observed that wheat seedlings subjected to AA under exposure to ST showed reduction of Chl *a* (23%) in the developing organs compared with control seedlings after 3 DAD (Fig. 1*A*), suggesting the inhibition of nonphotochemical quenching by interferring with the aggregation of LHCII (Johnson 2011). As shown in Fig. 1*C*, AA and ST reduced Chl *a* (24%) and Chl *b* (17%) contents in the developing organs after the 5 DAD compared with controls indicating that throughout the development of wheat seedlings the degree of suppression did not significantly change under the influence of AA and high temperature. We found important to emphasize that the reduction of photosynthetic metabolites under the influence of high temperature and AA was not as significant as under the stressful condition without AA in the developing organs. It could be assumed that AA activated AP which supported redox balance in photosynthetic cells and prevented a destruction of the photosynthetic apparatus in wheat seedlings.

Meanwhile, we also observed that the reduction of photosynthetic pigments was not as significant in the presence of AA under high temperature as under stressful conditions without AA in the senescent organs. It was observed that the concentration of Chl *a* was reduced by 6% and Chl *b* by 14% in the senescent organs under combined exposure of AA and ST after 3 DAD as compared with control seedlings (Fig. 2*A*). AA also caused a decrease of Chl *a* (17%) in the senescent organs of wheat seedlings under LT exposure after 3 DAD (Fig. 2*B*). Thylakoids support two parallel pathways of cyclic electron transport with differential sensitivity to AA (Scheller 1996). The existence of an AA-insensitive pathway around PSI dominates in the presence of $NADP^+$ and involves NAD(P)H dehydrogenase activity (Joët *et al*. 2001). The results above demonstrated that AA alleviated the harmful effect of high temperature in the organs of wheat seedlings indicating an induction of AP that prevented overreduction of ETC in chloroplasts. Thus, our results indicated that AA protected the structure of chloroplast and minimized Chl loss. The present findings suggested that AA-insensitive pathway was activated by the exposure to high temperature in the senescent organs. AA-insensitive cyclic electron transport of ferredoxin-catalyzed electron transport involves the activity of ferredoxin-NADP reductase (Hosler and Yocum 2003).

We demonstrated that AA decreased the concentration of Car by 29% in the developing organs under exposure to ST after 3 DAD (Fig. 1*A*) and by 23% after 5 DAD compared with controls (Fig. 1*C*). Our present study showed that high temperature increased Car accumulation in the wheat seedlings but under the combined action of AA and high temperature, the Car content decreased. The results are in agreement with the earlier study that reported that AA did not induce carotenogenesis in *Verticillium agaricinum* (Mummery and Valadon 1973).

In the developing and senescent organs under the influence of AA and LT exposure, the synthesis of Car decreased by 20–50% (Figs. 1*B*, 2*B*) and by 20–57% after 3 and 5 DAD, respectively, relative to unstressed control (Figs. 1*D*, 2*D*). Throughout the development of wheat seedlings, the degree of suppression did not significantly change. These data indicated that NDH- and PGR-dependent pathways of cyclic electron transport of PSI were inhibited by AA (Shikanai 2007) and therefore the synthesis of Car decreased under combined exposure to AA and high temperature.

Overall, our results demonstrated that AA-activated AP was involved in the protection of wheat seedlings under stress conditions. According to Borovik *et al*. (2013), an increased activity of the alternative respiration in mitochondria of wheat leaves contributes probably to proper functioning of the photosynthetic apparatus of chloroplasts and prevents the oxidative stress and accompanying accumulation of carbohydrates under conditions of cold-hardening.

Thus, the involvement of AP reduced the production of ROS, inhibition of photosynthesis, and contributed to stabilization of the functional state of the photosynthetic apparatus in wheat seedlings under heat stress.

The ratios of photosynthetic pigments: The changes in the ratio of photosynthetic pigments include important

information about the structural changes of the photosynthetic apparatus under unfavorable environments and are important indicators of plant injury (Brito *et al*. 2011). A higher ratio of Chl *a*/*b* indicates an increased ratio of PSII to PSI in thylakoid membranes (Biswal *et al*. 2012). It is known that values of the Chl *a*/*b* ratio are in the range ~ 1.5– 4.2, which are typical for vascular land plants and algae, irrespective of light environments they inhabite (Beneragama and Goto 2010). The ratio of Chl/Car plays an equally important role in the characterization of the activity of photosynthetic apparatus and is a sensitive indicator of physiological state of plant during development, senescence, acclimation, and adaptation to different environments (Gitelson *et al*. 2002).

Our results showed that the ratio of Chl *a*/*b* decreased by average 24% in the developing and senescent organs under exposure to ST after 3 DAD (Table 1). This is in agreement with the previous results (Song *et al*. 2010); Chl *a*/*b* ratio decreased in *Wedelia* species under 40/35°C and indicated the sensitivity of Chl *a* to high temperature. It should be pointed out that the Chl *a*/*b* ratio significantly decreased by 23 and 8%, respectively, in the developing organs under combined effect of ST and AA after 3 and 5 DAD as compared with control (Table 1). The recent study by Joët *et al*. (2001) observed a sensitivity of photosynthesis to AA in tobacco leaves; they concluded that inhibition by AA of cyclic electron flow around PSI led to a reduced ability to use reducing power on the acceptor side of PSI.

Likewise, Chl *a*/*b* decreased by 40% in the developing organs after 3 DAD and by 25% and by 49% after 5 DAD in the developing and senescent organs, respectively, under LT which was the result of a concomitant decrease of Chl *a* and Chl *b* (Table 1). As pointed out by Kura-Hotta (1987), Chl *a* disappears more rapidly than Chl *b* during senescence, resulting in a reduction of the Chl *a*/*b* ratio. The decline of Chl *a/b* ratio under high temperature was reported by other researchers (Armond *et al*. 1978). It is noteworthy that Chl *a*/*b* ratio was reduced in the developing (17%) and senescent (36%) organs under LT and AA after 3 DAD. It is evident from the present study that ST (42°C, 1 h) strongly decreased the ratio of Chl *a/b* by 28% and the ratio of Chl/Car by 55% in the senescent organs after 5 DAD compared with the control (Table 1). The decrease of Chl *a*/*b* ratio usually results in increased grana stacking that is usually associated with a modulation of protein content (Biswal *et al*. 2012). The results presented here showed that Chl *a/b* ratio was higher in the developing organs under ST than that in the developing organs subjected to LT after 3 DAD (Table 1). The result indicated that the reduction of Chl *a*/*b* ratio in the developing organs under LT could be the result of decrease in LHCII antenna pigments (Montane *et al*. 1998). According to our data, Chl *a/b* ratio decreased by 28% in the senescent organs after 5 DAD under ST (Table 1). Reduction of Chl *a*/*b* ratio might be related to the low content of Chl *a* compared to Chl *b* in the senescent organs of wheat seedlings under exposure of high temperature. A decline in Chl *a*/*b* ratio in the senescent organs was caused by a rapid decline in the reaction center and core Chl (Hidema *et al*. 1991). Other researchers suggested that changes occuring in the ultrastructure of thylakoid membranes above 40^0C cause dissociation of LHCII Chl *a*/*b* proteins from the PSII core complex (Ashraf and Harris 2013).

Table 1. Effect of antimycin A on the ratios of photosynthetic pigments after 3 and 5 days of development in *Triticum aestivum* L. under high temperature (42°C, ST – short-term (1 h), LT – long-term (24 h). Each value represents the mean of three replicates ± SE.

Treatments	Total pigment [mg g^{-1}(FM)]	Chl (*a*+*b*)/Car	Chl *a*/*b*	Treatment	Total pigment [mg g^{-1}(FM)]	Chl (*a*+*b*)/Car	Chl *a*/*b*
First leaves				First leaves			
26°C, 3 d	0.56	7.00 ± 0.016	2.50 ± 0.019	26°C, 5 d	0.92	6.08 ± 0.017	2.29 ± 0.016
42°C, ST, 3 d	0.43	4.38 ± 0.020	1.92 ± 0.014	42°C, ST, 5 d	0.74	3.93 ± 0.012	1.68 ± 0.020
42°C, ST+AA, 3 d	0.46	8.20 ± 0.018	1.93 ± 0.024	42°C, ST+AA,5 d	0.72	6.20 ± 0.018	2.10 ± 0.018
Coleoptiles				Coleoptiles			
26°C, 3 d	0.28	4.60 ± 0.013	2.29 ± 0.018	26°C, 5 d	0.42	5.00 ± 0.012	2.18 ± 0.014
42°C, ST, 3 d	0.15	2.75 ± 0.020	1.75 ± 0.020	42°C, ST,5 d	0.26	2.25 ± 0.022	1.57 ± 0.016
42°C, ST+AA, 3 d	0.26	4.20 ± 0.019	2.50 ± 0.016	42°C, ST+AA,5 d	0.33	3.71 ± 0.017	1.60 ±0.015
First leaves				First leaves			
26°C, 3 d	0.62	6.75 ± 0.018	2.60 ± 0.017	26°C, 5 d	0.99	8.90 ± 0.014	2.42 ± 0.016
42°C, LT, 3 d	0.42	2.00 ± 0.022	1.55 ± 0.017	42°C, LT, 5 d	0.55	6.86 ± 0.015	1.82 ± 0.020
42°C, LT+AA, 3 d	0.42	9.50 ± 0.020	2.17 ± 0.018	42°C, LT+AA,5 d	0.71	7.88 ± 0.023	1.33 ± 0.016
Coleoptiles				Coleoptiles			
26°C, 3 d	0.33	5.60 ± 0.022	1.80 ± 0.016	26°C Control),5 d	0.41	4.86 ± 0.019	3.25 ± 0.019
42°C, LT, 3 d	0.13	1.43 ± 0.013	2.00 ± 0.013	42°C, 24h, 5 d	0.20	4.00 ± 0.017	1.67 ± 0.014
42°C, LT+AA, 3 d	0.32	7.00 ± 0.019	1.15 ± 0.010	42°C, T+AA,5 d	0.24	7.00 ± 0.018	1.33 ± 0.016

We also observed that the ratios of Chl *a/b* decreased by 23% and Chl/Car decreased by 37% in the developing organs of wheat seedlings under the influence of ST (Table 1). Georgieva and Lichtenhaler (2006) reported decreased Chl *a*/*b* in the *Pisum sativum* L. under high temperature conditions. It was noted a considerable reduction (48%) in the Chl/Car ratio in the senescent organs of wheat seedlings upon exposure to ST compared to the developing organs (Table 1) that was most likely due to the loss of Chl content. The strong decrease of this ratio is accompanied by a significant reduction in Chl content (Henriques 2008). Moreover, the ratio of Chl *a/b* was significantly reduced by 25% and Chl/Car ratio by 36% in the developing organs compared with the control leaves under exposure to ST (Table 1), indicating a decrease of the rate of photosynthesis and disturbances in chloroplast ultrastructure. In agreement with earlier report in wheat plants (Sarieva *et al*. 2010), LT brought about considerable decrease of the Chl/Car ratio by 47% likely due to the disruption of thylakoid membranes, damage of PSII reaction-center proteins, and Chl degradation (Warner *et al*. 1999).

It was observed that LT (24 h) and AA led to a decrease of the ratio of Chl *a*/*b* by 17% in the developing organs after 3 DAD (Table 1). AA inhibited the ratio of Chl *a*/*b* by 36 and 59%, respectively, in senescent organs under LT after 3 and 5 DAD compared with the control (Table 1). A decrease in the Chl *a/b* ratio might be a result of reduction in LHC or a decrease of the ratio of PSII relative to PSI (Montane *et al*. 1998).

The Chl/Car ratio also decreased by 35% in the developing organs and by 55% in the senescent organs after 5 DAD under ST. A significant decrease of Chl/Car ratio (23%) was observed in the developing organs under LT after 5 DAD (Table 1). We observed that Chl/Car ratio under LT and AA increased by 29% in the developing organs and by 20% in the senescent organs after 3 DAD compared with controls (Table 1). This result supports the previous studies which indicated that AA at low concentration did not affect photosynthesis directly and activated AP, which played a significant part in preventing the overreduction of photosynthetic apparatus (Padmasree *et al*. 2002). Thus, under the combination of AA under ST and LT, the ratio of photosynthetic pigments recovered to its previous level in the developing organs and was significantly reduced in the senescent organs due to tissue senescence.

However, the Chl/Car ratio was significantly reduced by 26% under combined effect of ST and AA in the senescent organs after 5 DAD. The decrease of Chl/Car ratios was also observed in tomato genotypes under high temperature (Camejo *et al*. 2005). The low Chl/Car ratio might occur due to the oxidative stress that is a marker of senescence in plant tissue under exposure to high temperature and also relates to a decline of LHC pigment complex under stress conditions.

Chl *a* fluorescence: Changes in the structural state of photosynthetic apparatus in the developing organs of wheat seedlings under exposure to ST and LT were accompanied by a considerable suppression of the photochemical activity of PSII, which was estimated by Chl *a* fluorescence.

The F_v/F_m ratio is a very stable parameter and its decrease is reliable evidence that plants are subjected to stress. Our results demonstrated that the value of F_v/F_m was 0.771 in the developing organs after 3 DAD and 0.778 after 5 DAD (Table 2). A slight decrease (3–5%) of this indicator was observed at the end of ST (Table 2). Insignificant changes in F_v/F_m showed that ST did not lead to plant injury, which was in agreement with Tsonev *et al*. (1999). However, LT declined the F_v/F_m ratio by 21 and 19% after 3 and 5 DAD, respectively, due to inhibition of biochemical processes. The significant reduction of the F_vF_m ratio under LT reflected the effect of high temperature on efficiency of the photochemical process in PSII and indicated a decline in the efficiency of primary photochemistry (Mathur *et al*. 2013, Song *et al*. 2014). Furthermore, this decrease occurred due to the greater extent of F_m reduction and a gradual increase in F_0 under conditions of high temperature.

Table 2. Chlorophyll fluorescence analysis. Minimal chlorophyll fluorescence (F_0), maximal fluorescence (F_m), and quantum yield efficiency of PSII (F_v/F_m) of *Triticum aestivum* L. ST – short-term (1 h), LT – long-term (24 h). Each data is presented as means ± SE (n = 5).

Treatment	F_0	F_m	F_t	F_v/F_m
26ºC, 3 d	170 ± 13	904 ± 52	221 ± 14	0.771 ± 0.003
42ºC, ST, 3 d	194 ± 14	833 ± 30	192 ± 10	0.731 ± 0.05
42ºC,ST +AA, 3 d	174 ± 17	272 ± 20	70 ± 20	0.636 ± 0.04
26°C, 4 d	177 ± 11	925 ± 39	227 ± 13	0.775 ± 0.03
42ºC, LT, 3 d	262 ± 16	700 ± 36	168 ± 72	0.611 ± 0.002
42ºC, LT+AA, 3 d	204 ± 16	622 ± 66	155 ± 16	0.607 ± 0.004
26ºC, 5 d	199 ± 16	963 ± 33	120 ± 35	0.778 ± 0.001
42ºC, ST, 5 d	203 ± 12	921 ± 21	113 ± 30	0.757 ± 0.004
42ºC, ST+AA, 5 d	264 ± 13	710 ± 18	108 ± 30	0.653 ± 0.004
26°C, 6 d	207 ± 15	977 ± 13	127 ± 31	0.786 ± 0.001
42ºC, LT, 6 d	231 ± 12	654 ± 21	108 ± 25	0.637 ± 0.004
42ºC, LT+AA, 6 d	286 ± 12	322 ± 24	76 ± 13	0.596 ± 0.005

We demonstrated that combined effect of AA and ST sharply decreased F_v/F_m by 18% after 3 DAD (Table 2), resulting in the decrease of functional activity of wheat seedlings which was in agreement with Tang *et al*. (2007). A decrease in the F_v/F_m in the developing organs suggested that high temperature and AA induced structural and functional damages at the level of reaction centers of PSII and pigment antenna complexes. At the same time, reduction of the F_v/F_m ratio from 0.775 to 0.607 indicated the decrease of PSII activity in the developing organs of wheat seedlings after 3 DAD under LT and AA (Table 2). The data on the sensitivity of PSII to high temperature and AA, obtained in the developing organs of wheat seedlings, agreed well with prior results obtained earlier in studies on high temperature and AA effects in *Spinacia oleracea* (Tang *et al*. 2007) and *Nicotiana tabacum* (Joët *et al*. 2001).

In our experiment, F_v/F_m decreased by 3% under ST after 5 DAD (Table 2), which was in agreement with Kadir and Weihe (2007). On the other hand, F_v/F_m declined by 19% under LT after 5 DAD (Table 2) which was in agreement with Kadir (2006). The obtained data indicated the structural and functional disorders of the photosynthetic apparatus and damage to the reaction centers of PSII under exposure to high temperature (Cui *et al*. 2006).

Minimal and maximal fluorescence are known to reflect the state of Chl *a* in the LHC and reaction centers of PSII (Lichtenthaler *et al*. 2005). It is noteworthy that F_0 significantly increased by 12% under ST and by 32% under LT in the developing organs after 3 DAD (Table 2), which was in agreement with Rodriguez *et al*. (2015) in *Brassica oleracea* L. Rising F_0 occurs when plants are exposed to a number of biotic and abiotic factors, which contributes to the metabolic disorders and structural alterations of PSII (Ashraf and Harris 2013). The increase of F_0 is a consequence of a shift of the redox equilibrium of PSII, thus this parameter increased by a closure of the reaction centers (Havaux *et al*. 1988). The increase of F_0 in the developing organs could be associated with a dissociation of a part of the outer antenna from the rest of the PSII (Rodríguez *et al*. 2015).

It was observed that F_m parameter significantly decreased under exposure to high temperature in the developing organs after 3 and 5 DAD (Table 2). The F_m decrease under high temperature in the developing organs might be related to the inhibition of the oxygen-evolving complex (Tóth *et al*. 2005) and structural alterations, which cause a decrease of PSII photochemistry, an increase in the decay of excitation energy as fluorescence, an increase in the radiationless decay, and the transport of dissipation energy in favor of PSI (Mishra and Singhal 1992).

Recovery period: In our experiment, wheat seedlings were subjected to ST and LT and then they returned to the optimum temperature (26°C). Results of photosynthetic pigment contents and their ratios were shown also after the recovery period (Figs. 3, 4; Tables 3, 4).

Our results demonstrated that during the recovery the concentration of Chl *a* recovered up to 54–63% and Chl *b* up to 55–66% of control values in the developing organs after LT (Fig. 3*B*,*D*). At the same time, the concentration of Chl *b* and Chl *a* recovered up to 48–50% of its control in the senescent organs after LT (Fig. 4*B*,*D*). Car content recovered to 65% of the original content in the developing organs (Fig. 3*B*,*D*) and to 75% in the senescent organs under LT (Fig. 4*B*,*D*). Car are nonenzymatic antioxidants that protect the photosynthetic apparatus of plants against oxidative damage by ROS. Chl *a* and Chl *b* concentrations had progressively recovered (90%) in the developing organs and to 77% of in the senescent organs after ST. Thus, wheat seedlings fully recovered after exposure to ST. Based on these results, we suggested that wheat seedlings were not strongly damaged under ST because of their fast acclimation. According to these results, one of the probable causes of the ability to recover after ST might be a synthesis of heat-shock proteins (Allakhverdiev *et al*. 2008). However, photosynthetic pigment contents did not recover absolutely in the wheat seedlings under LT, suggesting that irreversible disturbances were caused by this exposure.

The results of our study demonstrated that the concentration of Chl *a* and Chl *b* recovered to 76 and 86% of control contents, respectively, in the presence of AA and ST in the developing organs (Fig. 3*A*,*C*). Our results also demonstrated that the concentration of Car recovered to 36% of control in the senescent organs in the presence of AA and ST (Fig. 4*A*,*C*). By contrast, the synthesis of Car recovered to 75 and 81% of control group, respectively in the senescent and developing organs under the influence of AA and LT (Fig. 3*B*,*D*; 4*B*,*D*). On the basis of obtained experimental results we suggest that AA was able to induce AP that represents an important acclimation response, supports homeostasis, and energy metabolism.

Results revealed that in the developing and senescent organs, the Chl *a/b* ratio recovered to 72 and 80%, respectively, after LT. The Chl *a/b* ratio recovered to 90–96% in the senescent organs under ST (Table 3). The Chl/Car ratio recovered to 76 and 87% of control group under ST and AA in developing organs. One of the important adaptive responses under stress conditions is the induction of saturated and monounsaturated fatty acids that keeps membrane fluidity.

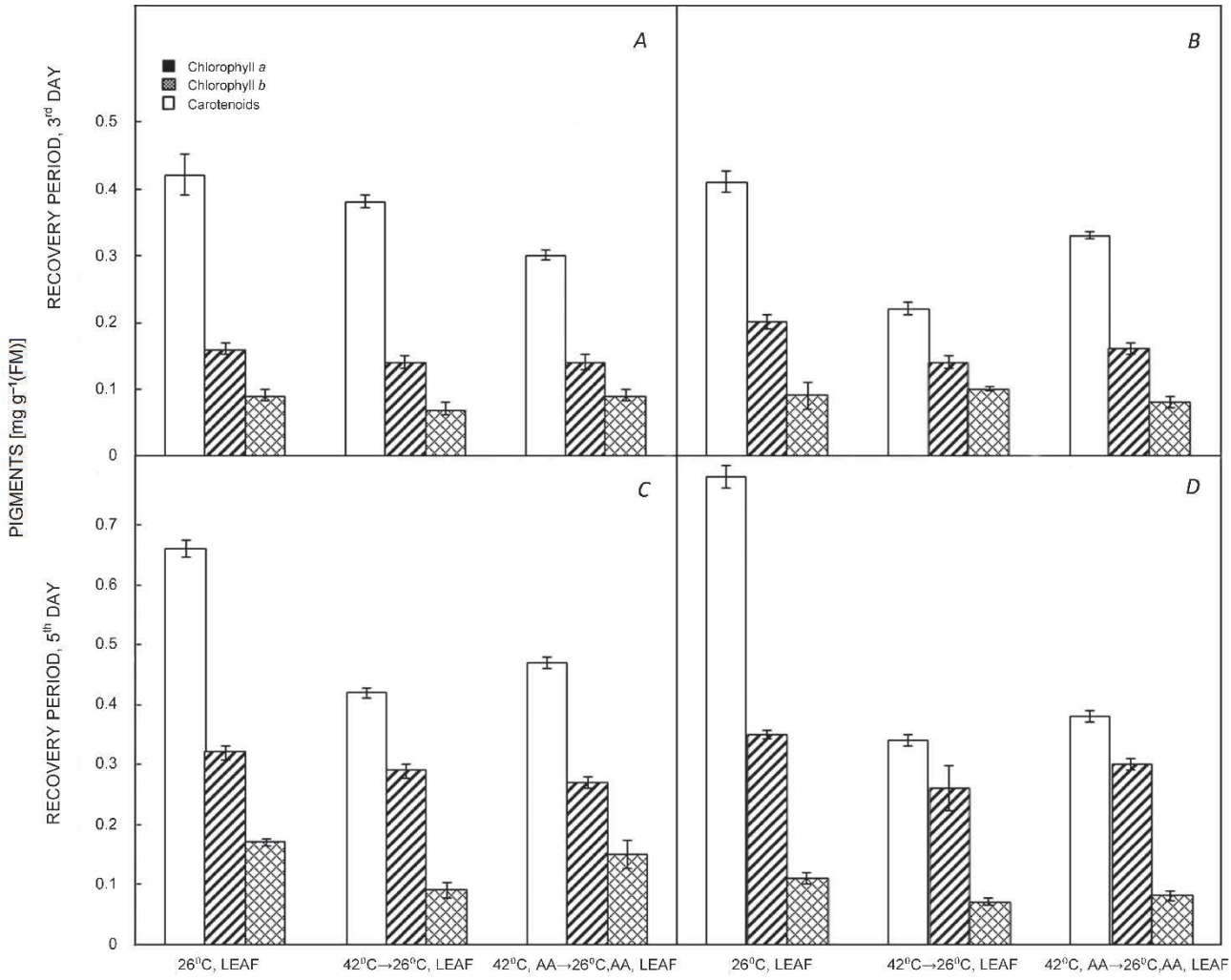

Fig. 3. Effect of antimycin A on the photosynthetic pigment contents in the developing organs during the recovery period after short-term (*A*) and long-term (*B*) high temperature, 3 d of development and short-term (C) and long-term (D) exposure to high temperature, 5 d of development. Data are presented as means ± standard errors of three biological replicates.

The capacity to recover PSII from ST and LT is presented in Table 4. In general, the efficiency of PSII was recovered, although developing organs differed in their ability to recover under ST and LT. F_m recovered to almost 96% of the control after 1 d of recovery after ST compared with 66% after LT. One day of recovery after ST resulted in almost total recovery of the quantum yield of PSII (98%) in the developing organs, whereas after LT, F_v/F_m recovered to 75% of the control. F_v/F_m recovery in the presence of AA and ST was more rapid than the recovery after LT and AA. F_v/F_m recovered to 87% of the control in the developing organs after ST and AA. The shorter was the stress period, the closer the F_v/F_m value was to the control in the developing organs. Our results demonstrated that F_v/F_m recovered (83%) after LT and AA in the developing organs. Our results indicated that ST was less damaging to PSII and the significant decline in F_v/F_m was in the developing organs under LT. Short-term acclimation to heat stress improves thermal PSII stability and preserves the function of photosynthetic apparatus at high temperature (Crafts-Brandner and Salvucci 2002).

Overall, the results demonstrated that the changes of induced Chl *a* fluorescence depended on the duration of the stress. It can be assumed that the stability of pigment systems, the activity of electron transport, and noncyclic photophosphorylation play an important role in acclimation of wheat seedlings to high temperature.

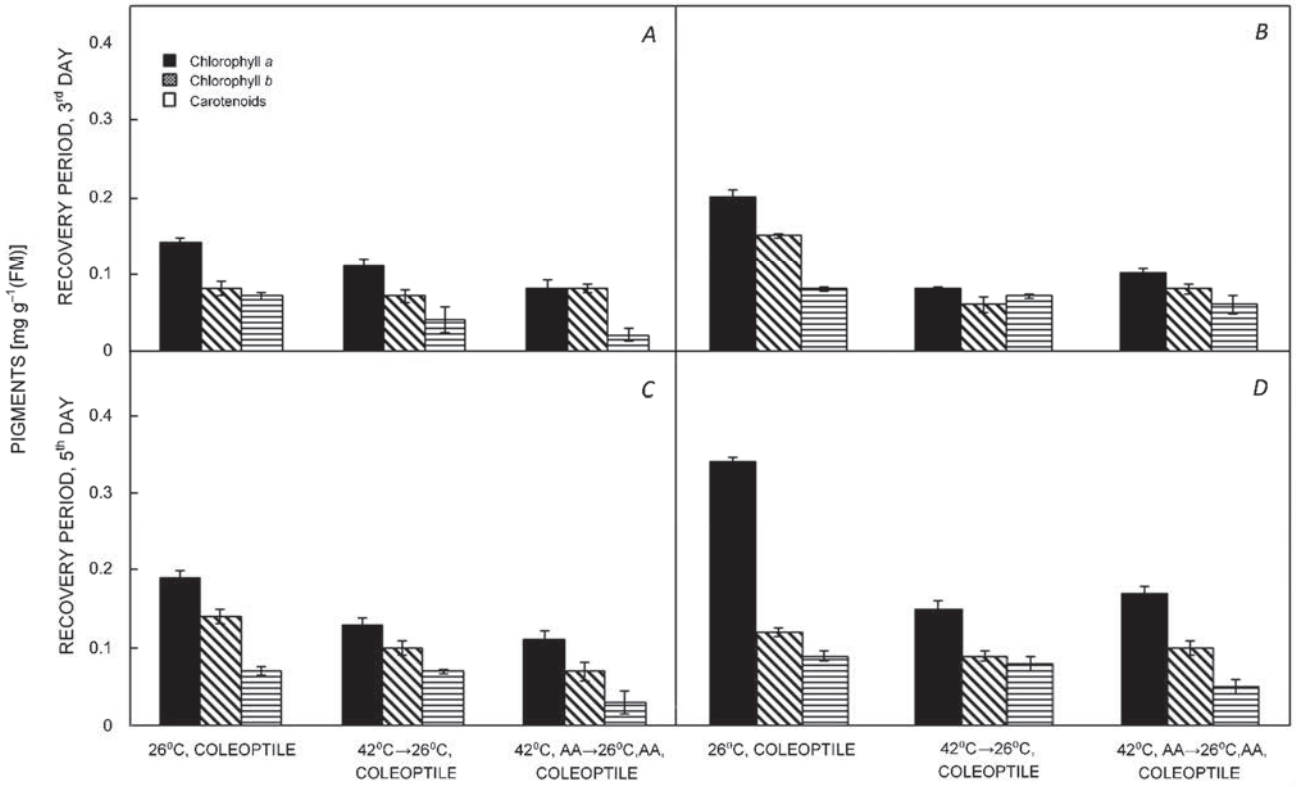

Fig. 4. Effect of antimycin A on the photosynthetic pigments content in the senescent organs during the recovery period after short-term (*A*) and long-term (*B*) high temperature, 3 d of development, and short-term (C) and long-term (D) exposure to high temperature, 5 d of development. Data are presented as means ± standard errors of three biological replicates.

Table 3. Effect of antimycin A on the ratio of photosynthetic pigments after 3 and 5 d of development during the recovery period after short-term (ST) (42ºC, 1 h → 26ºC, 24 h) and long-term (LT) (42ºC, 24 h → 26ºC, 24 h) high temperature. Each value represents as means of three replicates ± SE.

Recovery period (24 h)	Total pigment [mg g^{-1}(FM)]	Chl (*a*+b)/Car	Chl *a*/*b*	Treatment	Total pigment [mg g^{-1}(FM)]	Chl (*a*+b)/Car	Chl *a*/*b*
First leaves				First leaves			
26°C, 4 d	0.67	6.44	2.63	26°C, 6 d	1.13	5.65	2.00
42°C,ST→26ºC, 4 d	0.59	7.43	2.71	42°C, ST→26ºC, 6 d	0.80	7.89	1.45
42°C, ST→26ºC, AA, 4 d	0.53	4.89	2.14	42°C,ST→26ºC, AA, 6 d	0.89	4.93	1.74
Coleoptiles				Coleoptiles			
26°C, 4 d	0.29	3.14	1.75	26°C, 5 d	0.40	4.71	1.36
42°C,ST→26ºC, 4 d	0.22	4.50	1.57	42°C,ST→26ºC, 5 d	0.30	3.29	1.30
42°C, ST→26ºC, AA, 4 d	0.18	8.00	1.00	42°C,ST→26ºC, AA,5 d	0.21	6.00	1.57
First leaves				First leaves			
26°C, 5 d	0.70	6.78	2.05	26°C, 7 d	1.24	10.27	2.23
42°C, LT→26ºC, 5 d	0.46	6.60	1.57	42°C,LT→26ºC, 7 d	0.64	8.14	1.48
42°C, LT→26ºC, AA, 5 d	0.57	6.13	2.06	42°C, LT→26ºC, AA,7 d	0.76	8.50	1.27
Coleoptiles				Coleoptiles			
26°C, 5 d	0.43	4.38	1.33	26°C (Control), 5 d	0.55	5.11	2.83
42°C, LT→26ºC, 5 d	0.21	2.00	1.33	42°C, LT→26ºC, 5 d	0.32	3.00	1.67
42°C, LT→26ºC, AA, 5 d	0.24	3.00	1.25	42°C, LT→26ºC, AA, 5 d	0.32	5.40	1.70

Table 4. Chlorophyll fluorescence analysis during the recovery period. Minimal chlorophyll fluorescence (F_0), maximal fluorescence (F_m), and quantum yield efficiency of PSII (F_v/F_m) of *Triticum aestivum* L. ST – short-term (42ºC, 1 h → 26ºC, 24 h) and long–term (42ºC, 24 h → 26ºC, 24 h). Each data is presented as means ± SE (n = 5).

Treatments	F_0	F_m	F_t	F_v/F_m
26ºC, 4 d	262 ± 38	956 ± 33	266 ± 27	0.802 ± 0.003
42°C, ST→26ºC, 4 d	257 ± 62	921 ± 21	262 ± 10	0.795 ± 0.013
42°C, ST→26ºC, AA, 4 d	154 ± 24	566 ± 39	90 ± 12	0.675 ± 0.006
26ºC, 5 d	267 ± 37	1,004 ± 18	275 ± 14	0.819 ± 0.001
42°C, LT→26ºC, 5 d	263 ± 10	799 ± 20	174 ± 81	0.627 ± 0.002
42°C, LT→26ºC, AA, 5 d	140 ± 17	588 ± 57	204 ± 76	0.713 ± 0.028
26ºC, 6 d	211 ± 19	997 ± 33	502 ± 33	0.808 ± 0.006
42°C, ST→26ºC, 5 d	215 ± 32	959 ± 29	120 ± 54	0.790 ± 0.005
42°C, ST→26ºC, AA, 5 d	208 ± 27	476 ± 31	136 ± 26	0.734 ± 0.004
26°C, 7 d	275 ± 14	1,271 ± 25	327 ± 14	0.878 ± 0.001
42°C, LT→26ºC, 5 d	217 ± 17	664 ± 41	171 ± 12	0.647 ± 0.004
42°C, LT→26ºC, AA, 5 d	184 ± 44	382 ± 11	93 ± 33	0.696 ± 0.003

Conclusion: Present study demonstrated that parameters of Chl *a* fluorescence, concentrations of photosynthetic pigments and their ratios decreased under exposure to short-term and long-term high-temperature stress in the developing and senescent organs. However, AA reduced the negative effect of high temperature, suggesting that the activation of AP might prevent over-reduction of the ETC, a suppression of the production of ROS, and thus contributed to the stabilization of the photosynthetic apparatus of wheat seedlings under exposure to high temperature.

References

Akman Z.: Comparison of high temperature tolerance in maize, rice and sorghum seeds by plant growth regulators. – J. Anim. Vet. Adv. **8**: 358-361, 2009.

Allakhverdiev S.I., Kreslavski V.D., Klimov V.V. *et al.*: Heat stress: an overview of molecular responses in photosynthesis. – Photosynth Res. **98**: 541-550, 2008.

Armond P.A., Schreiber U., Björkman O.: Photosynthetic acclimation to temperature in the desert shrub, *Larrea divaricata*. – Plant Physiol. **61**: 411-415, 1978.

Ashraf M., Harris P.J.C.: Photosynthesis under stressful environments: An overview. – Photosynthetica **51**: 163-190, 2013.

Bartoli C.G., Gomez F., Gergoff G. *et al.*: Up-regulation of the mitochondrial alternative oxidase pathway enhances photosynthetic electron transport under drought conditions. – J. Exp. Bot. **56**: 1269-1276, 2005.

Beneragama C.K., Goto K.: Chlorophyll *a*:*b* ratio increases under low-light in "shade-tolerant" *Euglena gracilis*. – Trop. Agr. Res. **22**: 12-25, 2010.

Biswal A.K., Pattanayak G.K., Pandey S.S. *et al.*: Light intensity-dependent modulation of chlorophyll *b* biosynthesis and photosynthesis by overexpression of chlorophyllidae a oxygenase in tobacco. – Plant Physiol. **159**: 433-449, 2012.

Borovik O.A., Grabelnych O.I., Koroleva N.A. *et al.*: The relationships among an activity of the alternative pathway respiratory flux, a content of carbohydrates and a frost-resistance of winter wheat. – J. Stress Physiol. Bioch. **9**: 241-250, 2013.

Brito G., Sofiatti V., Brandão Z.N. *et al.*: Non-destructive analysis of photosynthetic pigments in cotton plants. – Acta Sci. Agron. **33**: 671-678, 2011.

Camejo D., Jiménez A., Alarcón J.J. *et al.*: Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants. – Funct. Plant Biol. **33**: 177-187, 2006.

Camejo D., Rodríguez P., Morales M.A. *et al.*: High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. – J. Plant Physiol. **162**: 281-289, 2005.

Crafts-Brandner S.J., Salvucci M.E.: Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. – Plant Physiol. **129**: 1773-1780, 2002.

Cui L., Li J., Fan Y. *et al.*: High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility. – Bot. Stud. **47**: 63-69, 2006.

Demmig-Adams B., Adams W.W.: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Phys. **43**: 599-626, 1992.

Efeoğlu B., Terzioğlu S.: Photosynthetic responses of two wheat varieties to high temperature. – Eurasia J. Biosci. **3**: 97-106, 2009.

Fikselová M., Šilhár S., Maraček J., Frančáková H.: Extraction of carot (*Daucus carota* L.) carotenes under different conditions. – Czech J. Food Sci. **26**: 268-271, 2008.

Gavrilenko V.F., Zhigalova T.V.: Large Workshop on Photosynthesis. Pp. 256. The Academy, Moscow 2003.

Georgieva K., Lichtenthaler H.K.: Photosynthetic response of different pea cultivars to low and high temperature treatments. – Photosynthetica **44**: 569-578, 2006.

Gilliland A., Singh D.P., Hayward J.M. *et al.*: Genetic modification of alternative respiration has differential effects on antimycin A-induced versus salicylic acid-induced resistance to *Tobaco mosaic virus*. – Plant Physiol. **132**: 1518-1528, 2003.

Gitelson A.A., Zur Y., Chivkunova O.B., Merzlyak M.N.: Assessing carotenoid content in plant leaves with reflectance

spectroscopy. – Photochem. Photobiol. **75**: 272-281, 2002.
Grabelnych O.I., Borovik O.A., Tauson E.L. *et al*.: Mitochondrial energy-dissipating systems (alternative oxidase, uncoupling proteins, and external NADH dehydrogenase) are involved in development of frost-resistance of winter wheat seedlings. – Biochemistry-Moscow+ **79**: 506-519, 2014.
Grabelnych O.I.: The energetic functions of plant mitochondria under stress. – J. Stress Physiol. Bioch. **1**: 37-54, 2005.
Hasanuzzaman M., Nahar K., Alam M. *et al*.: Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. – Int. J. Mol. Sci. **14**: 9643-9684, 2013.
Havaux M., Ernez M, Lannoye R.: Tolerance of poplar (*Populus* sp.) to environmental stresses: I. Comparative study of poplar clones using the *in vivo* chlorophyll fluorescence method. – Acta Oecol. **9**: 161-172, 1988.
Henriques F.S.: Photosynthetic characteristics of light-sensitive, chlorophyll-deficient leaves from sectorially chimeric stinging-nettle. – Bot. Stud. **49**: 235-241, 2008.
Hidema J., Makino A., Mae T., Ojima K.: Photosynthetic characteristics irradiances from full expansion through senescence. – Plant Physiol. **97**: 1287-1293, 1991.
Hosler J.P., Yocum C.F.: Evidence for two cyclic photophosphorylation reactions concurrent with ferredoxin-catalyzed noncyclic electron transport. – BBA-Bioenergetics **808**: 21-31, 1985.
Huang L., Cobessi D., Tung E.Y., Berry E.A.: Binding of the respiratory chain inhibitor antimycin to the mitochondrial bc1 complex: a new crystal structure reveals an altered intramolecular hydrogen-bonding pattern. – J. Mol. Biol. **351**: 573-597, 2005.
Hussain I., Wahid A., Rasheed R., Akram H.M.: Seasonal differences in growth, photosynthetic pigments and gas exchange properties in two greenhouse grown maize (*Zea mays* L.) cultivars. – Acta Bot. Croat. **73**: 333-345, 2014.
Joët T., Cournac L., Horvath E. M. *et al*.: Increased sensitivity of photosynthesis to antimycin A induced by inactivation of the chloroplast *ndhB* gene. Evidence for a participation of the NADH dehydrogenase complex to cyclic electron flow around photosystem I. – Plant Physiol. **125**: 1919-1929, 2001.
Johnson G.N.: Physiology of PSI cyclic electron transport in higher plants. – Biochim. Biophys. Acta **1807**: 384-389, 2011.
Kadir S.: Thermostability of photosynthesis of *Vitis aestivalis* and *V. vinifera.* – J. Am. Soc. Hortic. Sci. **131**: 476-483, 2006.
Kadir S., von Weihe M.: Photochemical efficiency and recovery of photosystem II in grapes after exposure to sudden and gradual heat stress. – J. Am. Soc. Hortic. Sci. **132**: 764-769, 2007.
Kura-Hotta M., Satoh K., Katoh S. Relationship between photosynthesis and chlorophyll content during leaf senescence of rice seedlings. – Plant Cell Physiol. **28**: 1321-1329, 1987.
Lefsrud M.G., Kopsell D.A., Kopsell D.E., Curran-Celentano J.: Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. – HortScience **40**: 2026-2030, 2005.
Lichtenthaler H.K., Buschmann C., Knapp M.: How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R_{Fd} of leaves with the PAM fluorometer. – Photosynthetica **43**: 379-393, 2005.
Mathur S., Mehta P., Jajoo A.: Effects of dual stress (high salt and high temperature) on the photochemical efficiency of wheat leaves (*Triticum aestivum*). – Physiol. Mol. Biol. Plants **19**:179-188, 2013.
Mavi H.S., Tupper G.T.: Agrometeorology: Principles and Applications of Climate Studies in Agriculture. Pp. 43-68. Haworth Press, Inc., New York, London, Oxford 2004.
Mishra R.K., Singhal G.S.: Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoid lipids. – Plant Physiol. **98**: 1-6, 1992.
Montané M.H., Tardy F., Kloppstech K., Havaux M.: Differential control of xanthophylls and light-induced stress proteins, as opposed to light-harvesting chlorophyll *a/b* proteins, during photosynthetic acclimation of barley leaves to light irradiance. – Plant Physiol. **118**: 227-235, 1998.
Mummery R.S., Valadon L.R.G.: The effect of antimycin A on carotenogenesis in *Verticillium agaricinum*. – Planta **109**: 353-356, 1973.
Munekage Y., Shikanai T.: Cyclic electron transport through photosystem I. – J. Plant Biotech. **22**: 361-369, 2005.
Murchie E.H., Lawson T.: Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. – J. Exp. Bot. **64**: 3983-3998, 2013.
Padmasree K., Padmavathi L., Raghavendra A.S.: Essentiality of mitochondrial oxidative metabolism for photosynthesis: optimization of carbon assimilation and protection against photoinhibition. – Crit. Rev. Biochem. Mol. **37**: 71-119, 2002.
Rodríguez V.M., Soengas P., Alonso-Villaverde V. *et al*.: Effect of temperature stress on the early vegetative development of *Brassica oleracea* L. – BMC Plant Biol. **15**: 1-9, 2015.
Roy C., Sengupta D.N.: Effect of short term NaCl stress on cultivars of *S. lycopersicum*: a comparative biochemical approach. – J. Stress Physiol. Bioch. **10**: 59-81, 2014.
Sarieva G.E., Kenzhebaeva S.S., Lichtenthaler H.K.: Adaptation potential of photosynthesis in wheat cultivars with a capability of leaf rolling under high temperature conditions. – Russ. J. Plant Physl+ **57**: 28-36, 2010.
Scheller H.V.: *In vitro* cyclic electron transport in barley thylakoids follows two independent pathways. – Plant Physiol. **110**: 187-194, 1996.
Sharkey T.D.: Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. – Plant Cell Environ. **28**: 269-277, 2005.
Sharma P., Jha A.B., Dubey R.S., Pessarakli M.: Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. – J. Bot. **2012**: 217037, 2012.
Shikanai T.: Cyclic electron transport around photosystem I: genetic approaches. – Annu. Rev. Plant Biol. **58**: 199-217, 2007.
Song L., Chow W.S., Sun L. *et al*.: Acclimation of photosystem II to high temperature in two *Wedelia* species from different geographical origins: implications for biological invasions upon global warming. – J. Exp. Bot. **61**: 4087-4096, 2010.
Song Y., Chen Q., Ci D. *et al*.: Effects of high temperature on photosynthesis and related gene expression in poplar. – BMC Plant Biol. **14**: 1-20, 2014.
Strodkötter J., Padmasree K., Dinakar C. *et al*.: Induction of the AOX1D isoform of alternative oxidase in A. thaliana T-DNA insertion lines lacking isoform AOX1A is insufficient to optimize photosynthesis when treated with antimycin A. – Mol. Plant. **2**: 284-297, 2009.
Tang Y., Wen X., Lu Q. *et al*.: Heat stress induces an aggregation of the light-harvesting complex of photosystem II in spinach plants. – Plant Physiol. **143**: 629-638, 2007.
Tóth S.Z., Schansker G., Kissimon J. *et al*.: Biophysical studies

of photosystem II-related recovery processes after a heat pulse in barley seedlings (*Hordeum vulgare* L.) – J. Plant Physiol. **162** :181-194, 2005.

Tsonev T., Velikova V., Lambreva M., Stefanov D.: Recovery of the photosynthetic apparatus in bean plants after high- and low-temperature induced photoinhibition. – Bulg. J. Plant Physiol. **25**: 45-53, 1999.

Vanlerberghe G.C.: Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. – Int. J. Mol. Sci. **14**: 6805-6847, 2013.

Warner M.E., Fitt W.K., Schmidt G.W.: Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. – P. Natl. Acad. Sci. USA **96**: 8007-8012, 1999.

Wise R.R., Olson A.J., Schrader S.M., Sharkey T.D.: Electron transport is the functional limitation of photosynthesis in field grown cotton plants at high temperature. – Plant Cell Environ. **27**: 717-724, 2004.

Yoshida K., Terashima I., Noguchi K.: Distinct roles of the cytochrome pathway and alternative oxidase in leaf photosynthesis. – Plant Cell Physiol. **47**: 22-31, 2006.

Yoshida K., Terashima I., Noguchi K.: Up-regulation of mitochondrial alternative oxidase concomitant with chloroplast over-reduction by excess light. – Plant Cell Physiol. **48**: 606-614, 2007.