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The effect of antimycin A on the intensity of oxidative stress, the level of lipid peroxidation and antioxidant enzyme activities in different organs of wheat (*Triticum aestivum* L.) seedlings subjected to high temperature

Anna Batjuka* and Nataļja Škute

Laboratory of Molecular Biology and Genetics, Ecology Department, Institute of Life Sciences and Technology, Daugavpils University, Parades Str.1a, Daugavpils, Latvia

***Corresponding author**: anna.batjuka@du.lv

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Abstract: The objective of the present investigation was to identify the effect of antimycin A (AA) as an activator of the alternative pathway (AP) of respiration, on oxidative stress intensity, the level of lipid peroxidation (LPO) and activities of H_2O_2 scavenging enzymes in functionally different organs of *Triticum aestivum* L. subjected to short- and long-term exposure to high temperature (HT). The level of LPO was assessed in terms of malondialdehyde (MDA), an indicator of oxidative injury. The results demonstrated increases in the total content of reactive oxygen species (ROS) and MDA production in developing and senescent organs of wheat seedlings, and significant augmentation of the activities of the antioxidant enzymes, catalase and ascorbate peroxidase, in different organs in response to exposure to HT. The activation of the AP by AA restrained ROS production in the mitochondrial electron transport chain (mETC) under exposure to HT.

Key words: antimycin A; antioxidant enzymes, high temperature, lipid peroxidation, reactive oxygen species

INTRODUCTION

Plants, as all other living organisms, are exposed to the influence of various environmental factors during development. Most agricultural lands are located in zones of potentially hazardous farming because of exposure severe climatic conditions. Wheat (*Triticum aestivum* L.) is a major crop whose yield and quality of grains are affected by climate change. Unfavorable environmental factors such as high light intensities, temperature extremes, excessive moisture, water deficiency and increased salinity, can negatively affect wheat growth, development and cause significant reduction of their productivity. At the same time, degraded soils and other resource limitations represent an increasingly serious threat and can also reduce agricultural production [1]. Many abiotic stressors disrupt normal physiological processes, disturb cellular homeostasis and induce excessive production of reactive oxygen species (ROS) in plant tissues [2,3]. Therefore, studying the mechanisms of plant resistance to the damaging effects of abiotic factors is essential for the conservation of productivity in agroecosystems and is one of the fundamental problems of modern agroecology and biology.

Air temperatures above an optimum are sensed as heat stress by all living organisms and represent one of the most common environmental challenges for plants. Elevated temperatures interfere with plant growth, metabolism and produce agricultural losses. The range of optimal temperatures can differ not only for different organisms but also for different organs of an organism. Thus, different elevated temperatures exert dissimilar effects; they decrease wheat root growth and accelerate senescence (35°C), compromise leaf functioning (42°C) and exert lethal effects on active tissues of shoots (50°C) [4].

Increased production of ROS, such as the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), singlet oxygen molecule ($^1O_2$) and hydroxyl radicals ($OH^{\cdot}$), is a threat to cells as it leads to the oxidation of proteins, enzyme inhibition, LPO, nucleic acid damage, ultimately

causing programmed cell death (PCD) [5]. However, apart from the many harmful effects on plant growth and metabolic processes, low concentrations of ROS are signaling intermediates in response to abiotic and biotic stressors [6-7]. A major source of ROS in the majority cell types, including plant cells is mETC [8].

Intracellular ROS levels are regulated by a wide range of enzymatic and non-enzymatic antioxidative defense mechanisms in stressful conditions which eliminate the generated ROS, playing an important protective role against oxidative damage of cellular components. Antioxidant components include the enzymes guaiacol peroxidases, catalases, superoxide dismutases, ascorbate peroxidases, polyphenol oxidases, glutathione peroxidases, glutathione S-transferases, glutathione reductases, dehydroascorbate reductases and non-enzymatic antioxidants such as ascorbic acid, glutathione, carotenoids, α-tocopherols, anthocyanins, prolines, polyamines, which are the first line of defense and are involved in a detoxification of excess ROS [9-10].

The inner mitochondrial membrane of plants contains different energy dissipating systems, including the AP, internal and external alternative NAD(P)H dehydrogenases, uncoupling proteins (UCPs), free fatty acids, ATP/ADP antiporters that decrease the efficiency of oxidative phosphorylation [11]. The AP is induced not only in response to variable environmental conditions, including high or low temperatures, oxidative stress, limited availability of nutrients, various infections, as well as inhibition of the respiratory chain [12]. The AP, through the oxidation of ubiquinol and UCPs and a decrease in a $\Delta\mu H^+$, acts as a regulator of ROS levels in cells [13] and participates in the process of plant thermoregulation in low-temperature stress [14]. Therefore, upregulation of energy dissipating systems prevents mitochondrial ROS production, promotes normal functioning of the photosynthetic apparatus of chloroplasts, preventing oxidative stress [15] and maintains cell homeostasis under changing environmental conditions [16]. Our previous research showed that the AP contributes to the stabilization of the functional state of the photosynthetic apparatus in wheat seedlings under heat stress [17].

Antimycin A (AA) is a potent inhibitor of ferredoxin-plastoquinone oxidoreductase (FQR) in photosystem I (PSI) in chloroplasts, which suppresses the FQR-mediated cyclic flow and NADH-dependent flow [18]. AA is also a fairly potent mETC inhibitor that disrupts the interaction of the electron flow between cytochrome *b* and cytochrome *c* in complex III. AA covers the proper binding site for ubiquinone which is the primary electron carrier in the ETC and prevents any interaction between ubiquinone and cytochrome *b* [19]. Expression of the alternative oxidase (AOX) protein increases when the cytochrome pathway is inhibited by AA [20].

The aim of this research was to investigate the influence of AA as an activator of the AP on the total ROS content, concentrations of MDA, and the activities of H_2O_2 scavenging enzymes in the functionally different organs of wheat seedlings under exposure to short-term and long-term HT.

MATERIALS AND METHODS

Plant material and growth conditions

As the objects for the study we selected the first leaves, which served as an experimental model of a developing organ, coleoptiles as a senescent organs and roots of wheat seedlings (*Triticum aestivum* L., cv. Harmony), because the processes that occur in these organs at HT-induced stress differ. The grains were germinated in plastic pots (19×12 cm) containing moistened filter paper, for 24 h in a thermostat (25-26°C). After germination, the seedlings were placed in plastic pots supplemented with AA (1 mg/L) in distilled water (control medium) for three days in a climatized chamber (temperature: 25-26°C; light regime: 16 h light/8 h darkness; irradiance: 150 μM m^{-2} s^{-1}, relative humidity (RH) 75%). Seedlings were covered with a sheet of glass to prevent them from drying out. On the fourth day of development the wheat seedlings were submitted to short-term (1 h) and prolonged (24 h) HT (42°C, photoperiod 16/8 h, light intensity150 mM m^{-2} s^{-1}, RH 75%) in the presence of AA (1 mg/L) and without AA. The different organs of wheat seedlings (first leaves, coleoptiles, roots) were harvested and their biochemical oxidative parameters were measured after exposure to short-term (1 h) and long-term (24 h) HT in the presence of (and without) AA.

Determination of the total ROS content

The total intracellular ROS content in the first leaves, coleoptiles and roots of wheat was investigated using the fluorescent dye 2',7'-dichlorofluorescein diacetate (H_2DCF-DA) [21]. The oxidation of nonfluorescent molecule H_2DCF-DA to the highly fluorescent dichlorofluorescein product (DCF) is rapidly oxidized by intracellular H_2O_2 [22]. Plant tissues were incubated with a solution of H_2DCF-DA (1 μM) in a thermostat at 26°C for 20 min. The fluorescence intensity of 2',7'-dichlorofluorescein (DCF) in the solution was determined using a fluorescence spectrometer FLS 920 (Edinburgh Instrument, UK); excitation λ=480 nm, emission λ=524 nm.

Quantification of lipid peroxidation (LPO)

The levels of LPO in the different organs of *Triticum aestivum* L. were expressed as accumulated MDA using the method based on the ability of MDA to react with 2-thiobarbituric acid (TBA) by heating [23]. Fresh plant material (0.15 g) was hand-homogenized using an ice-chilled mortar, pestle and the mixture was extracted with 1.5 mL of 0.1% (w/v) trichloroacetic acid (TCA) at 4°C. The homogenate was then centrifuged (Hermle Z216MK) at 4500×g for 15 min at 4°C. One mL of the extracted material was stirred with 2.5 ml of 20% TCA containing 0.5% TBA. After incubation for 30 min in a boiling water bath (95°C), the mixture was immediately cooled on ice and the samples were centrifuged for 15 min at 4500×g at 4°C. The concentration of MDA was determined spectrophotometrically (Cary 50 ScanUV/VISVarian spectrophotometer), and the value of the absorbance at λ=600 nm was calculated by subtracting from the absorbance of the supernatant at λ=532 nm. The concentration of MDA in the different organs of wheat seedlings was expressed as μM g^{-1}, using a molar absorption coefficient (ε=155 mM^{-1} cm^{-1}).

Measurement of catalase (CAT; EC 1.11.1.6) activity

An optimized process of extraction was performed at 4°C. Fresh samples (0.25 g) of wheat seedlings were homogenized in an ice-cold mortar in 3 ml of 50 mM sodium phosphate buffer (pH 7.8) that contained 2 mM ethylenediaminetetraacetic acid (EDTA), 5 mM β-mercaptoethanol and 4% (w/v) polyvinylpyrrolidine-40 (PVP-40). The homogenized samples were filtered and centrifuged for 20 min at 20000×g at 4°C. The activity of the enzyme was detected in supernatants following the method of Aebi [24]. The reaction mix (3 mL) contained 50 μL of the enzyme extract, 50 mM sodium phosphate buffer (pH 7) and 10 mM H_2O_2. The enzyme activity was determined by a UV-visible spectrophotometer following the consumption of H_2O_2 in 1 min at λ=240 nm at 25°C. The molar extinction coefficient of H_2O_2 was 39.4 mM^{-1} cm^{-1}.

Measurement of ascorbate peroxidase (APX; EC 1.11.1.1) activity

APX activity was estimated spectrophotometrically according to the modified method of Johnson et al. [25] in different organs of wheat seedlings. Fresh tissue material (0.25 g) was ground in cold sodium phosphate extraction buffer (pH 7, 50 mM) that contained 0.5 mM ascorbate, using a precooled mortar and pestle and then centrifuged at 20000×g for 20 min at 4°C. The reaction mixture included cold phosphate buffer (pH 7, 50 mM), 0.5 mM ascorbate, 1 mM H_2O_2. The reaction was initiated by adding 50 μL of enzyme extract in a total volume of 3 mL. The activity of the H_2O_2 scavenging enzyme was monitored with a UV-visible light spectrophotometer by measuring the change in absorbance at λ=290 nm at 25°C during 2 min at 20-s intervals due to ascorbate oxidation; enzyme activity was determined using an extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Statistical analysis

Statistical processing of the results was performed using the Statistical SPSS package (Version 13.0). The results presented in the figures are mean values±standard errors (SEs). All data were subjected to one-way analysis of variance (ANOVA), and a difference was considered to be statistically significant when $p<0.05$. Each experiment was repeated independently with three replicates ($n = 3$) for MDA concentration and total ROS production, and four times ($n=4$) for CAT and APX activities.

RESULTS AND DISCUSSION

HT causes alterations in plant growth and development. One of the main consequences of HT is a change in redox processes in plant tissues, accompanied by ROS generation which can be destructive to cells as a result of reaction with polyunsaturated fatty acids (PUFAs) of membranes, promotion of LPO, protein oxidation, nucleic acid damage, enzyme inhibition and activation of PCD [26]. In plant cells, particularly in non-photosynthetic cells, the mitochondria are a major source of ROS [27]. The study of oxidative processes in different organs of wheat seedlings served to evaluate plant stress status under exposure to short- and long-term HT. The coleoptile is a particularly short-lived organ that ages rapidly and is degraded during the early stage of development, in contrast to the photosynthetic leaf that is relatively long-lived, ranging from weeks to months [28].

Changes in total ROS content in different organs of wheat seedlings

The total ROS content increased by 16% ($p<0.05$) under exposure to prolonged HT in the developing organs of wheat seedlings at the early stage of development when compared to the control (Fig. 1A). The increase in the total ROS content was accompanied by an increase in MDA concentration, a product of membrane LPO (Fig. 2B) and antioxidant activities (Fig. 3A and B) in the developing organs. Such an enhanced accumulation of ROS in the developing organs of wheat seedlings under exposure to prolonged HT is associated with oxidative stress that can produce various physiological effects, such as membrane destruction, decrease in protein synthesis, damage of transport proteins, receptors, ion channels, leading to extensive cellular dysfunction. Our results are corroborated by findings based on studies carried out in wheat cells in winter when ROS production increases under exposure to HT, resulting in oxidative stress [29]. Increased ROS production is required for elevated expression of different heat shock proteins (HSPs) which are induced as a mechanism of cellular defense [30]. Our study also shows that the total ROS content increased by 8% ($p<0.05$) under short-term exposure (1 h) to HT in the developing organs at the early stage of seedling development in comparison

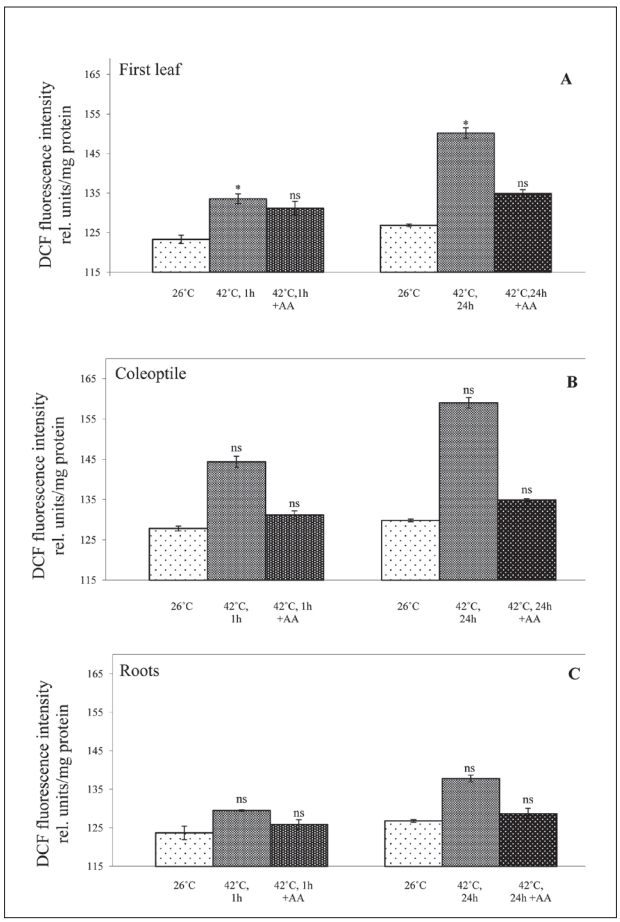

Fig. 1. The effect of antimycin A (AA) on the total ROS content in first leaves (**A**), coleoptiles (**B**) and roots (**C**) of wheat seedlings after exposure to elevated temperature (42^0C). Values are presented as means±SE for n=3. The values were subjected to ANOVA and the means were compared with the LSD test with P≥0.05.

with the control, maintained at 26°C (Fig. 1A). Previous studies also reported an increase in ROS production during short-term exposure to HT in leaves of highbush blueberry cultivars (*Vaccinium corymbosum* L.) [31]. Therefore, it is critical that the plants' antioxidant defense mechanisms are well-timed to neutralize ROS. Plants' mitochondria display three different strategies against oxidative stress. The avoidance of ROS production is the first line of defense, and is achieved by maintaining the components of ETC in a sufficiently oxidized state, while the second and the third mechanisms include detoxification of ROS and repair of the ROS-induced injury, respectively [32].

The mETC of plants has two pathways of electron transport: the Cyt respiratory pathway with cytochrome *c* oxidase (COX) as a terminal oxidase,

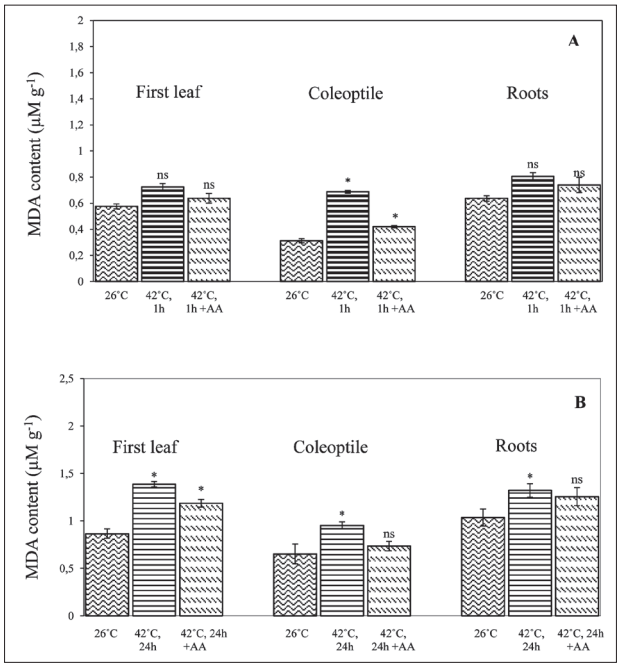

Fig. 2. The effect of antimycin A (AA) on MDA concentrations in the first leaves, coleoptiles and roots of wheat seedlings after (**A**) short-term (1 h) and (**B**) long-term (24 h) exposure to elevated temperature. Values are presented as means±SE for n=3. The values were subjected to ANOVA and the means were compared with the LSD test with P≥0.05.

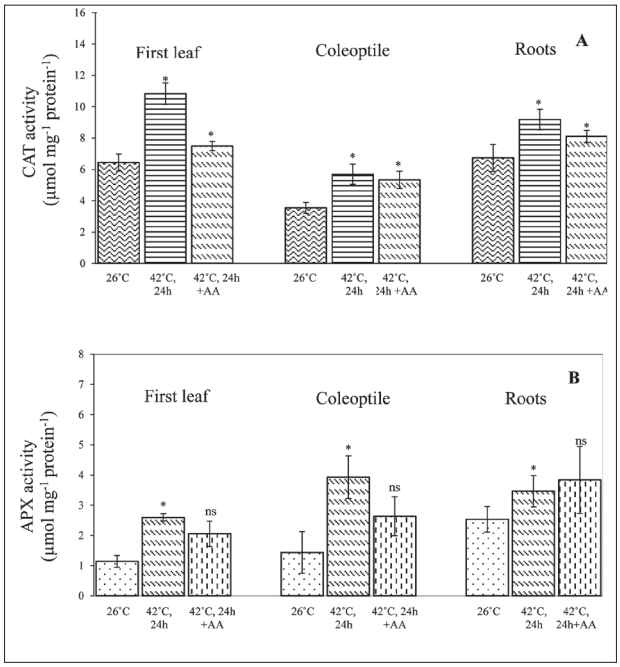

Fig. 3. Changes of catalase (CAT) (**A**) and ascorbate peroxidase (APX) (**B**) activities in the first leaves, coleoptiles and roots of *Triticum aestivum* L. after prolonged exposure (24 h, 42°C) to elevated temperature and antimycin A (AA). Values are presented as means±SE for n=4. The values were subjected to ANOVA and the means were compared with the LSD test with P≥0.05.

and the AP pathway with AOX. In contrast to the Cyt pathway, non-phosphorylating AP bypasses two proton translocation sites at complexes III and IV, transfers electrons directly from the ubiquinone pool to O_2 and does not lead to ATP synthesis [13]. To test the hypothesis that the AP in plant mitochondria functions in a mechanism that decrease the accumulation of ROS under exposure to environmental stressors, we investigated the influence of AA on the production of ROS in different organs of wheat seedlings subjected to HT. We observed that the total ROS content was slightly reduced, by 2% ($p>0.05$), in the developing organs under combined exposure to AA and short-term HT. The combined effect of AA and prolonged HT lowered the total ROS content by 10% ($p>0.05$) in comparison to samples exposed to stressful conditions without AA (Fig. 1A). Control of excessive accumulation of ROS as a result of AP functioning is the first line of protection against oxidative stress [33]. The results are in agreement with a study that showed that the activation of AP by AA application effectively eliminated overproduced ROS in *Arabidopsis thaliana* [34]. It was also demonstrated that AOX overexpression caused a decline in intracellular ROS production in a suspension of tobacco cells after AA treatment [21]. It can be assumed that the addition of AA caused a redistribution of the electron flow, leading to the activation of the AP, thus efficiently protecting the developing organs of wheat seedlings from ROS.

Coleoptiles are senescent organs of cereal plants which protect the first leaves against injuries during the process of seed germination and complete their physiological function by promoting a specific type of PCD [35]. In coleoptiles of wheat seedlings, apoptosis occurs on the 5th to 6th days of development at 26°C. In contrast with the results obtained for developing organs, the senescent organs generated more ROS than the developing organs under prolonged exposure to HT as compared to the control (Fig. 1B). This result could indicate that at this stage of development, gradual damage of structures and disruption of functions in coleoptiles of wheat seedlings take place. Meanwhile, in the senescent organs of wheat seedlings exposed to HT, the activity of the antioxi-

dant system decreased and therefore, they could not effectively combat the HT which led to oxidative damage. Internucleosomal fragmentation and degradation of coleoptile nuclear DNA was observed in 5-day-old wheat seedlings, and increased plant sensitivity to apoptosis induction was associated with the natural cycle of ROS production in wheat seedlings [36]. In our experiment, the ROS level increased by 21% ($p>0.05$) in the senescent organs after prolonged exposure to HT (Fig. 1B). ROS accumulation in the senescent organs was associated with the interaction of unsaturated fatty acids and activation of LPO that resulted in the destruction of cellular organelles, accelerating PCD in coleoptiles exposed to prolonged HT. Thus, senescent organs of wheat seedling exposed to prolonged HT showed significant oxidative processes at the early stage of development and were therefore incapable of protecting themselves against oxidative stress. Similar to the present finding, a significant increase in the rate of O_2^- generation was also reported in coleoptile cells at the late stages of development under exposure to HT (42°C) [37].

The AP influences cellular processes such as nuclear gene expression and PCD, providing an important link between functions of mitochondria, signal transduction, and acclimation to stress [38]. The results of this study revealed that AA caused a decrease in ROS generation by 12% ($p>0.05$) in the senescent organ as compared to the stressful condition without AA. This result supports findings that showed that the addition of AA generally lowered H_2O_2 production [39]. AA triggered the induction of gene expression of components associated with the AP that slowed down the processes of senescence, PCD and enhanced tolerance to HT. AA induced the AP in transgenic tobacco cells, which maintained respiration and cell viability and prevented the activation of PCD [40].

ROS at relatively low concentrations are required for cell expansion during morphogenesis of roots where they control the activity of calcium channels required for polar growth [41]. The effect of prolonged and short-term HT on the total ROS content in root tissues is shown in Fig. 1C. We found that the total ROS content_increased by 8% ($p>0.05$) after exposure to prolonged HT. The increase in the total ROS content in root tissue during HT indicates that HT disrupted root processes, accelerated senescence and caused cessation of grain development. We observed that the total content of ROS increased by 4% ($p>0.05$) in root tissue of wheat seedlings exposed to the short-term H (Fig.1C). Kolupaev et al. [42] reported that the ROS content markedly diminished under exposure to short-term HT in root tissue of wheat seedlings.

The ROS content remained at the control level in roots in the presence of AA during HT (Fig. 1C). Previous studies suggested that AA had virtually no significant effect on the rate of O_2^- generation in *Pisum sativum* L. roots [43]. The decrease in ROS production by the respiratory chain after AA addition pointed to the antioxidant function of AP and effective protection against ROS in roots of wheat seedlings. In studies performed by Grabel'nykh et al. [44] it was reported that addition of AA did not increase ROS generation in isolated mitochondria of wheat seedlings subjected to the first phase of cold hardening, indicating an antioxidant function of AP.-This study implies that the induction of AP by AA could dampen the excessive mitochondrial ROS content and mitigate its damage in functionally different organs of wheat seedlings under short- and long-term exposure to HT.

Changes in MDA concentration

The level of oxidative damage was assessed by examining the accumulation of the final product of PUFA peroxidation (MDA). The results presented in Fig. 2A and B show the changes in MDA concentration in the developing senescent organ and roots, which increased by 19% ($p>0.05$) and 37% ($p<0.05$) after short- (Fig. 2A) and long-term (Fig. 2B) exposure to HT, respectively. A sharp increase in MDA content was accompanied by an increased ROS content. MDA accumulation increased by 55% ($p<0.05$) and 32% ($p<0.05$) under exposure to short- (Fig. 2A) and long-term exposure to HT (Fig. 2B), respectively. The intensity of the oxidative stress i.e. the MDA content in the senescent organ was higher than in the developing organs during HT. We assumed that coleoptiles protected the first leaves against oxidative injury during development. The elevated temperature led to membrane damage, disturbed metabolism and caused increased senescence of coleoptiles. This result is consistent with Zhao et al. [45] who reported that an increase of LPO was observed in senescent

tissues of *Cucumis sativus* L. With an increase in the MDA content, more cell membranes are damaged [46]. We observed that the MDA content increased by 20% ($p>0.05$) and 21% ($p<0.05$) in roots exposed to short- (Fig 2A) and long-term (Fig 2B) HT, respectively. This is in agreement with a study in which the level of MDA increased during HT in roots of creeping bentgrass [47]. These findings point to increased vulnerability of the root system to the oxidative stress and point to significant damage of cell membranes as a result of exposure to HT. HT increased oxidative stress in different organs through an increase in ROS that initiated MDA accumulation.

The concentration of MDA was reduced by 11% ($p>0.05$) and 14% ($p<0.05$) in the developing organs in samples subjected to AA and short- and long-term HT (Fig 2A and B), respectively. Our results are corroborated by findings based on studies carried out on *Nicotiana tabacum* where overexpression of AOX triggered a decrease in MDA and less oxidative damage [48]. In contrast with the results observed in developing organs, MDA was reduced by 39% ($p<0.05$) in the senescent organs in samples exposed to AA and short-term HT, and by 22% ($p>0.05$) in samples exposed to AA under prolonged HT. It can be assumed that AA, as an activator of AP, decreased oxidative damages and prevented apoptosis in coleoptile cells. AA also reduced lipid peroxidation by 7% ($p>0.05$) and 5% ($p>0.05$) in roots of wheat seedlings exposed to short-term and prolonged HT, respectively. We assumed that AA caused a redistribution of the electron flow, activated the non-phosphorylating AP, which represents an important mechanism in the process of regulation of cell homeostasis during metabolic fluctuation. It thus eliminated ROS generation and reduced LPO processes.

Changes in catalase and ascorbate peroxidase activities

The main enzymatic components of the antioxidative defense system, including CAT and enzymes of the ascorbate-glutathione (AsA-GSH) cycle such as APX, are important components in plants, and are involved in the detoxification of intracellular H_2O_2. They can provide important information about the physiological condition of wheat seedlings under HT.

Analysis of enzymatic antioxidant systems revealed that CAT activity increased by 41% ($p<0.05$) under exposure to long-term HT in the developing organs (Fig. 3A). It is evident that the level of APX activity rapidly increased to 56% ($p<0.05$) under prolonged exposure to HT (Fig. 3B). It could be argued that prolonged HT directly or indirectly caused significant ROS generation, substantial intensification of LPO in cell membranes and increased antioxidant activity. These results are in agreement with an earlier study that demonstrated a significant increase in the activities of antioxidant enzymes in the first fully expanded leaves of wheat exposed to HT [49]. Also, the activities of free radical scavenging enzymes was increased in wheat (*Triticum aestivum* L.) genotypes under elevated temperatures [50]. These findings indicate that the increase in CAT despite its restricted location in peroxisomes and glyoxysomes, and in APX, which is distributed in chloroplasts, cytosol, mitochondria and peroxisomes, plays a significant role in defenses against oxidative stress in wheat seedlings.

The activity of CAT was also stimulated and increased by 38% ($p<0.05$) in senescent organs under exposure to long-term HT (Fig. 3A). APX activity significantly increased by 63% ($p<0.05$). APX activity was significantly higher than CAT activity in the senescent organs in stress. The AsA-GSH cycle operated more efficiently in the senescent organs, mitigating the accumulation of H_2O_2 under HT. Degradative processes during senescence of coleoptiles are accompanied by increased ROS production, LPO and membrane leakage [51]. Senescent organs of wheat seedlings were more sensitive to HT than the young and photosynthetically active leaves. It can be assumed that the antioxidant systems functioned more effectively in the senescent organs, protecting developing organs against oxidative damage. It was reported [52] that CAT and APX activities significantly increased in cucumber seedlings (*Cucumis sativus* L.) under heat stress.

CAT and APX activities increased by 27% ($p<0.05$) in root tissue under prolonged exposure to HT (Fig. 3A, B). The activity of CAT is higher in organs of wheat seedlings in which a large amount of ROS are produced. CAT activity in root tissue decreased in comparison to the developing organs,

pointing to lower ROS production during stress. The results are in agreement with an earlier study in which a significant increase in antioxidant enzyme (APX, CAT, SOD, POX, GR) activities in five wheat genotypes under HT was reported [53].

Given the importance of the different localization of the examined enzymes, we investigated the effect of AA on the electron transport in chloroplasts which inhibits cyclic electron flow around PS I. our results show that the activity of CAT decreased by 31% ($p<0.05$) and of APX 21% ($p>0.05$) under the influence of AA and HT (Fig. 3A, B), indicating that AA efficiently suppressed electron flow between the plastoquinone pool and PSI, e.g. at the cytochrome b_6f complex or plastocyanin [54]. The results suggest that NADH dehydrogenase (NDH) and the proton gradient regulation (PGR) dependent pathway of cyclic electron transport of PSI were inhibited by AA [55]. Therefore, the activity of APX decreased in the developing organ under combined exposure to AA and HT.

CAT activity decreased by 6% ($p<0.05$) and APX activity decreased by 33% ($p>0.05$) in senescent organs after exposure to AA and HT (Fig. 3A and B), suggesting that AA can suppress oxidation of the PSI acceptor side in chloroplasts. A decrease in the content of ascorbate as the preferential electron donor in the AsA-GSH cycle is often considered as an indicator of senescence in many annual plants [56]. Findings suggest that APX activity does not increase in senescent tissues of *Ramonda serbica* in drought [57].

As shown in Fig. 3A and B, the combined effect of AA and long-term HT reduced CAT activity by 12% ($p<0.05$) whereas APX activity increased by 10% ($p>0.05$) in roots of wheat seedlings. Chloroplasts support two independent pathways of cyclic electron transport around PSI, with differential sensitivity to AA [58]. One pathway cycles electrons from ferredoxin to the plastoquinone (PQ) pool and is sensitive to AA, while the second pathway includes the NDH complex and is insensitive to AA [59]. We suggest that the AA-insensitive pathway was activated under exposure to HT in roots. The results are in agreement with the earlier study that reported that AA increased the activity of the enzymes of the Asa-GSH cycle in cultured alfalfa roots [60].

CONCLUSION

The data presented here indicate that short- and long-term HT accelerated ROS production, the intensity of the LPO and increased the activities of CAT and APX in different organs of wheat seedlings. HT contributed to the disruption of physiological processes in tissues, which in turn led to the oxidative stress. Different organs displayed different tolerance to HT, which was attributed to the enhanced activities of antioxidant enzymes. The highest concentrations of MDA and ROS and antioxidant enzyme activities were detected under exposure to HT without AA. The AP prevented excessive ROS production, lowered the destructive processes caused by HT, serving to reestablish redox homeostasis in wheat seedlings.

Conflict of interest disclosure: The authors declare that they have no competing interests.

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