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THE INFLUENCE OF THERMAL PREADAPTATION ON SOME OXIDATIVE PROCESSES IN THE FIRST LEAVES OF WHEAT SEEDLINGS (*TRITICUM AESTIVUM* L.) UNDER HEAT STRESS

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Heat stress is a widespread problem that seriously influences crop production and quality worldwide. Preadaptation is the first stage of adaptation that ensures the survival of the organisms under changing environmental conditions. The purpose of this study was to investigate the effect of thermal preadaptation (32°C) on some biochemical changes such as the rate of superoxide ($O2^{\cdot-}$) production and activity of catalase (CAT) (E.C. 1.11.1.6) under heat stress (42°C) in the first leaves of wheat seedlings at the early and late stages of development. Spectrophotometrically it was shown that the rate of $O2^{\cdot-}$ production and activity of the antioxidant enzyme significantly increased during heat stress in the first leaves of wheat seedlings. However, our results demonstrated that conditions of preadaptation (32°C) decreased the rate of $O2^{\cdot-}$ production and CAT activity in the first leaves of etiolated wheat seedlings at the early and late stages of development that prevented oxidative stress. It can be assumed that the effect of thermal preadaptation differs for $O2^{\cdot-}$ production and H_2O_2 accumulation at the early and late stages of development.

Key words: heat stress, preadaptation, catalase, superoxide production, *Triticum aestivum* L.

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INTRODUCTION

Plants during their development are continuously exposed to a wide range of abiotic stressors in natural conditions of habitation. Such unfavorable environmental factors as low and high temperatures, drought, salinity, excessive moisture adversely affect crops productivity by causing multiple disturbances of the metabolic processes. Wheat (*Triticum aestivum* L.) is produced worldwide under largely temperate but also under tropical and subtropical temperatures. This distribution implies that the crop is versatile and adapted to various growing conditions (Dakhim et al. 2012).

Many abiotic stressors disturb cellular homeostasis and enhance accumulation of reactive oxygen species (ROS) such as the superoxide ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydrogen

peroxide (H_2O_2), hydroxyl radicals (OH^-) in plants tissues (Suzuki and Mittler 2006). The sources of ROS in a majority of cell types, including plant cells, are the chloroplasts, mitochondria, peroxisomes, cytosol, NADPH-oxidases of the plasma membrane, cell wall peroxidases and some other enzyme systems (Blokhina and Fagerstedt 2010; Kreslavski et al. 2012). Plants protect cells and subcellular systems against the cytotoxic effects of ROS using various non-enzymatic antioxidants (carotenoids, ascorbate, glutathione, tocopherols, phenolic compounds) and enzymatic antioxidant systems (superoxide dismutase, ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase, monodehydroascorbate reductase, guaiacol peroxidase, catalase, ascorbate peroxidase) (Gill and Tuteja 2010).

IPCC has projected a temperature increase of 1.8 – 4°C by 2100 (IPCC 2007). Elevated temperatures lead to a decline of physiological activity in plants and as consequence to inactivation of enzymes and other proteins. Different elevated temperatures exert dissimilar effects; they decrease wheat root growth and accelerate senescence (35°C) and exert lethal effects on active tissues of shoots (50°C) (Mavi, Tupper 2004). It is known, that high temperature effects on metabolism and structure of plants, especially cell membranes, resulting in the desiccation of plants, disturbances of many physiological processes such as respiration, photosynthesis, water relations (Wahid et al. 2007). Our previous research indicated that high temperature (42°C) led to a significant decrease of photosynthetic pigment contents and modified PSII functionality in wheat seedlings (Batjuka et al. 2016).

Preadaptation is the first stage of adaptation that ensures the survival of the organism under changing environmental conditions (Ketola et al. 2013). At the subcellular level, this phenomenon is defined as a genetically determined qualitative composition of molecules and its quantitative ratio that allows maintaining a functional metabolic stability in cells. The study of mechanisms of adaptation, as well as preadaptation in response to environmental stimuli, is an important research topic in plant ecology.

A threshold of around 30°C is broadly consistent for the critical temperature of grain set at anthesis across a number of environments (Semenov et al. 2014). Results of earlier studies shown the effect of day temperature during 3 day transfers to controlled environment cabinets during anthesis on grain yield, grains per spikelet, mean grain weight of wheat and showed that 30-32°C temperature is initial stress temperature for South Europe wheat (Semenov et al. 2014). Therefore, these thermal conditions (32°C) may be used as a preadaptation to heat stress.

Thereby the aim of this study was to investigate the effect of a thermal preadaptation (32°C) on some biochemical parameters such as the rate of $O_2^{\cdot -}$ production and CAT activity under heat stress (42°C) in the first leaves of wheat seedling at the early and late stages of development.

MATERIAL AND METHODS

Plant material and growth conditions

The object of the investigation were etiolated first leaves of wheat seedlings (*Triticum aestivum* L., cv. Harmony) at the early (from 5th to 6th days) and late (from 7th to 8th days) stages of development. Wheat seeds were germinated on moistened filter paper in darkness at 26°C for 24 h. On the 3rd day of development, the germinated seeds were divided into the control and experimental groups. A control group was further grown on moistened filter paper in a thermostat at 26°C, but the experimental group was grown in the preadaptation conditions (32°C) for 24 h. On the 4th day of development plant material (first leaves) was sampled for analysis from two groups. The remaining wheat seedlings continued to grow. On the same day from the control and experimental groups which were grown in conditions of preadaptation (32°C) was taken a part of the seedlings and was transformed to another experimental group that was moved for growing under stressful conditions (42°C)

for 24 h. The control group was left for further growth. Samples were taken and analyzed for all three groups on the 5th day of development. The same experiment was made at the late stages of development: 7th and 8th days.

Determination of the rate of superoxide ($O_2^{\cdot-}$) production

Plant material (first leaves) was incubated with or without 1µl/ml superoxide dismutase (SOD) at 26°C for 1h in an incubation buffer (4 ml) (with 0.05% nitroblue tetrazolium (NBT)). The incubation buffer contained 10µl EDTA, 10mM K_2HPO_4, 1mg/ml Triton X-100 and 0.05% NBT. The rate of $O_2^{\cdot-}$ production was determined by the NBT reduction assay. After 1h incubation, NBT reduction was monitored spectrophotometrically (*Cary 50 Scan UV/VIS Varian*) at a wavelength of 530 nm (Shorning et al. 2000).

Extraction and determination of catalase (CAT) activity

First leaves (250 mg) were homogenized in 1.5 ml of 50mM phosphate buffer at pH 7.0 including 2mM ethylene-diamine-tetra-acetic acid (EDTA) using an ice-chilled pestle and mortar. Homogenates were then centrifuged at 4°C in an Eppendorf centrifuge at 15000 rpm for 20 min. The rate of decomposition of H_2O_2 was followed by the absorbance decline at 240 nm in a reaction mixture containing 50mM K-phosphate buffer (pH 7.0), H_2O_2 and enzyme extract. The enzyme activity based on absorbance was calculated using the extinction coefficient (39.4 mM^{-1} cm^{-1}) and expressed as units (1µmol of H_2O_2 decomposed per minute) per mg protein (Aebi 1984).

Statistical analysis

All measurements were analyzed statistically and presented as mean arithmetic values and their standard errors of the independent experiments with three replicates. Statistical analyses were performed using *Statistica 2010* software and significant difference between the groups was determined ($p < 0.05$).

RESULTS AND DISCUSSION

The effect of thermal preadaptation on the rate of superoxide ($O_2^{\cdot-}$) production in the first leaves of wheat seedlings during heat stress

An elevated production of ROS is a universal reaction of an organism in response to practically all environmental stressors which at high concentrations pose a threat to cells by causing oxidation of proteins, lipid peroxidation (LPO), damage to nucleic acids, enzyme inhibition and activation of programmed cell death (PCD) (Sharma 2012). $O_2^{\cdot-}$ is a product of the one-electron reduction of oxygen that is potentially dangerous because is the precursor of most other ROS and a mediator in oxidative chain reactions.

Our data (Fig. 1) showed that the rate of $O_2^{\cdot-}$ production changes from 4th to 8th day of development in the first leaves of wheat seedlings. It was increased from 1.07±0.01 µmol/h to 1.67±0.08 µmol/h on the 4th and 5th days respectively, and it was decreased by 0.05±0 µmol/h on the 7th day and by 0.53±0.04 µmol/h on the 8th day of development. This can be explained by the fact, that the development of the etiolated wheat seedlings is accompanied by the cyclic production of $O_2^{\cdot-}$, which at low concentrations are essential for plant growth and development (Shorning et al. 2000, Batjuka et al. 2013). ROS

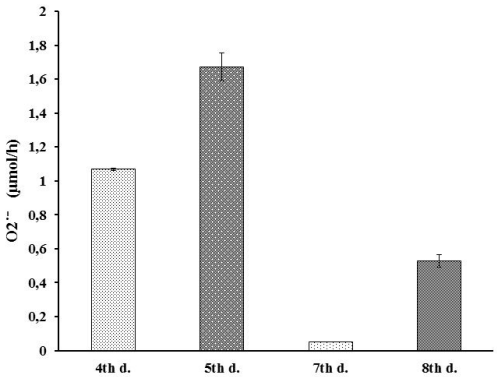

Fig. 1. The rate of superoxide ($O_2^{\cdot-}$) production (µmol/h) in the etiolated first leaves at the early (from 4th to 5th days) and late (from 7th to 8th days) stages of seedlings development.

play a central role in the activation of PCD in plants (Zamyatnina et al. 2003). An induction and progress of PCD in plants is tissue-specific and depends on the intracellular redox conditions during various developmental stages.

Our results indicated that elevated temperature (32°C) enhanced the rate of $O_2^{\cdot-}$ production by 28% (Δ1.49 ±0.15 μmol/h) at the early stage of development (Fig. 3A) and by 96% (Δ1.19 ±0.15) at the late stage of development compared to control (Fig. 3B). It was corroborated by findings based on studies carried out in wheat cells and first leaves (*Triticum aestivum* L.), when ROS production increased under exposure to high temperature (Fedyaeva et al. 2014, Savicka & Škute 2010). In addition, our recent results showed an increase of the total ROS production under stressful conditions in the developing organs of wheat seedlings that initiated oxidative stress, induced membrane biochemical and functional alterations (Batjuka and Škute 2016). The results of this study demonstrated, that the rate of $O_2^{\cdot-}$ production significantly increased by 50% (Δ1.66±0.04) and by 81% (Δ2.26±0.09), respectively at the early and late stages of development under exposure to heat stress (42°C) in the first leaves of wheat seedlings compared to control (Fig. 3A, B). Such an enhanced rate of $O_2^{\cdot-}$ production in the first leaves of wheat seedlings under exposure to heat stress is associated with strong oxidative stress which subsequently leads to extensive cellular dysfunctions.

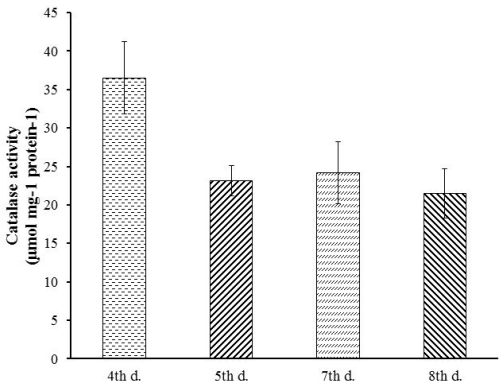

Fig. 2. Catalase activity in the etiolated first leaves at the early (from 4th to 5th days) and late (from 7th to 8th days) stages of seedling development.

Changes of the rate of $O_2^{\cdot-}$ production in the first leaves of wheat seedlings in conditions of preadaptation (32°C) at the early and late stages of development were investigated (Fig. 3A, B). We found that the rate of $O_2^{\cdot-}$ production reduced by 17% (Δ0.57±0.11) at the early stage of development and by 47% (Δ1.3±0.35) at the late stage of development in the first etiolated leaves under conditions of preadaptation (32°C→42°C) compared to heat stress without preadaptation (26°C→42°C) (Fig. 3A, B). Thermal preadaptation (32°C) prevented the development of oxidative stress decreasing the influence of heat stress (42°C) and most

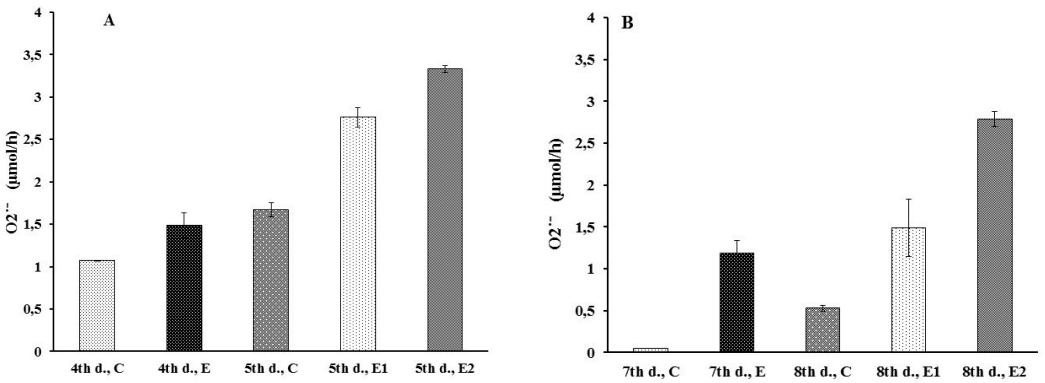

Fig. 3. The rate of superoxide ($O_2^{\cdot-}$) production (μmol/h) in the etiolated first leaves at the (A) early (from 4th to 5th days) and (B) late (from 7th to 8th days) stages of seedlings development (C – control 26°C; E – 26°C → 32°C; E1 – 32°→42°C; E2 – 26°C → 42°C).

effectively this effect was observed at the late stage of development (Fig. 3B). It was suggested that preadaptation conditions (32°C) can effectively reduce the risk of membranes damages in mitochondria and alleviate the negative effect induced by heat stress.

The effect of thermal preadaptation on catalase (CAT) activity in the first leaves of wheat seedlings during heat stress

Plant cells possess a majority of defense strategies against oxidative damages caused by heat stress. Such strategies involve specific detoxifying antioxidant enzymatic systems, as well as various low molecular weight antioxidants that eliminate hazardous free radicals. CAT plays a significant role in defense against oxidative stress which catalyzes the decomposition of H_2O_2 to water and oxygen (Mhamdi et al. 2010).

We studied the changes of CAT activity in the first leaves from 4th to 8th days of their development (Fig. 2). It was shown, that CAT activity was changed in this period: CAT activity decreased by 34% (from 36.5 μmol/mg^{-1} to 24.16 μmol/mg^{-1}) from the 4th to 5th days of development in the first leaves and after it does not change. This can be explained by the fact that accumulation of H_2O_2 occurs more significantly in the first leaves at the early stages of development. However, our previous study showed an increase of electrophoretic activity of CAT in apical parts of the first leaves at the late stage of development compared to basal parts in which cells are divided (Batjuka et al. 2013).

Changes of the CAT activity in the first leaves of wheat seedlings under high temperature were investigated (Fig. 4A, B). These data demonstrated, that CAT activity decreased by 21% (Δ 7.6 μmol/mg^{-1}) at the early stage of development (5th day) and increased by 27% (Δ8.87±1.7 μmol/mg^{-1}) at the late stage of development (8th days) compared to control. However, it was shown, that CAT activity increased by 60% at the early stage of development (5th day) and increased by 54% at the late stage of development (8th day) under heat stress (42°C). Our results are in accordance with some other studies showing a significant increase of the antioxidant enzyme activities in the fully expanded leaves of plants under heat stress (Sairam et al. 2000; Fariduddin et al. 2014). It was assumed that the changes of H_2O_2 accumulation differ at the early and late stages of development under high temperature and heat stress.

The effect of thermal preadaptation on CAT activity in the first leaves of wheat seedlings during heat stress is shown in Fig. 4. It was shown that the conditions of thermal preadaptation (32°C) significantly reduced CAT activity under heat stress (42°C) in the first leaves of wheat seedlings at the early and late stages of

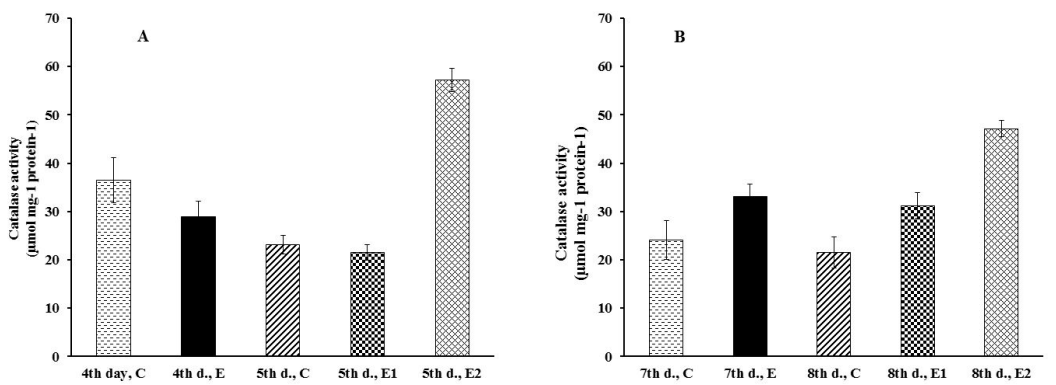

Fig. 4. Catalase activity in the etiolated first leaves at the (A) early (from 4th to 5th days) and (B) late (from 7th to 8th days) stages of seedling development (C – control 26°C; E – 26°C → 32°C; E1 – 32°→42°C; E2 – 26°C → 42°C).

development. Our data (Fig. 4A, B) indicated that CAT activity reduced by 62% (Δ35.65±1.6) and 34% (Δ15.9±2.63), respectively in the first leaves at the early and late stages of development under conditions of preadaptation (32°C) compared to heat stress without preadaptation (42°C). It was shown that thermal preadaptation acts more effective on H_2O_2 accumulation at the early stage of development in the first leaves of wheat seedlings than that at the late stage of development. It was assumed that H_2O_2 was accumulated under high temperature (32°C) and H_2O_2 as second messenger induced the gene expression which control antioxidative ferments before heat stress. Therefore, antioxidant system functioned more effectively in the first leaves of wheat seedlings at the early stage of development under exposure to heat stress. Thus, the conditions of preadaptation provide better protection against oxidative damages in the first leaves of wheat seedlings under heat stress.

CONCLUSIONS

High temperatures lead to a decline of physiological activity in plants and it is a widespread problem in plant ecology. In this study, it was shown that $O_2^{\cdot -}$ accumulation differs during the development of first leaves of wheat seedlings and under the high temperature, its accumulation increased. Preadaptation is very important stage of thermal adaptation of plants. This study demonstrated that thermal preadaptation decreased the $O_2^{\cdot -}$ production under the heat stress, especially at the late stages of development of wheat seedlings. CAT activity, which reflects H_2O_2 accumulation, differs at the early and late stages of development also and its activity increased under the high temperature. Thermal preadaptation (32°C) decreased the CAT activity and also H_2O_2, but in this case, it was shown especially in the first leaves during early stage of development. Summarizing the data the obtained during research, it can be assumed that the effect of thermal preadaptation differs for $O_2^{\cdot -}$ production and H_2O_2 accumulation at the early and late stages of development. The obtained data suggest that conditions of preadaptation promoted attenuation of damaging action of heat stress preventing oxidative stress in the etiolated first leaves of wheat seedlings.

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