

# SOME CHANGES IN OXIDATIVE PROCESSES IN THE ORGANS OF WHEAT SEEDLINGS (*TRITICUM AESTIVUM* L.) IN THE PRESENCE OF ANTIMYCIN A

Anna Batjuka, Aleksandrs Petjukevičs, Natalja Škute

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The effect of an inhibitor of mitochondrial electron transport chain antimycin A on the rate of production of superoxide and electrophoretic activity of enzymatic antioxidative systems (superoxide dismutase and catalase) in the apical parts of the first leaves and coleoptiles of wheat seedlings was investigated. In the apical parts of the first leaves and coleoptiles were observed cyclicality in the production of superoxide and its increasing in the presence of antimycin A.

In the apical parts of the first leaves and coleoptiles antimycin A treatment also caused an increase in the electrophoretic activity of superoxide dismutase and catalase.

Key words: *Triticum aestivum* L., the first leaf, coleoptile, antimycin A, superoxide dismutase, catalase, superoxide.

Anna Batjuka, Aleksandrs Petjukevičs, Natalja Škute. *Daugavpils University, Institute of Ecology, Vienibas Street 13, Daugavpils, Latvia, LV-5401, e-mail: anna.batjuka@du.lv, aleksandrs.petjukevics@du.lv, natalja.skute@du.lv.*

## INTRODUCTION

Reactive oxygen species (ROS) are bioproducts of normal cellular metabolism and play a role of secondary messengers in the regulation of cellular processes (Дмитриева et al. 2007). ROS include superoxide ( $O_2^{\cdot-}$ ), hydroxyl radical ( $OH^{\cdot}$ ), superoxide radicals ( $RO_2^{\cdot-}$  etc.) and neutral molecules, such as hydrogen peroxide ( $H_2O_2$ ), singlet oxygen ( $^1O_2$ ), ozone ( $O_3$ ) (Maxwell et al. 1999). ROS can damage cell structure mainly exert destructive influence on the membranes, cause destructive changes of proteins and nucleic acids (Blokhina et al. 2003), but also they lead to the programmed death of the cells (Korsukova et al. 2013).

Antimycin A (AA) is a potent electron transport chain (ETC) inhibitor, that blocks the flow of electrons through complex III of the ETC by blocking the passage of electrons from cytochrome *b* to cytochrome *c* in mitochondria (Joet et al. 2001). The presence of AA has an impact on the redox potentials of the various  $e^-$  carriers taking part in the Q cycle (Staniek et al. 2000). AA addition to tobacco cell cultures resulted in an increase, in intracellular ROS (Maxwell et al. 1999). When mitochondria uncoupled with FCCP (carbonyl cyanide p-(trifluoromethoxy) phenyl hydrazone), AA stimulates Q-cycle ROS generation to an even greater extent (Votyakova 2001).

However, in the absence of AA the ubisemiquinone  $Q_0$  site is not stabilized, and  $O_2^{\cdot-}$  production by complex III is low (Morel et al. 2009). The mitochondrial respiratory chain inhibitor AA could stimulate superoxide and hydrogen peroxide formation on submitochondrial particles (Li et al. 2003). ROS generation at complex III increases, when AA inhibits electron flux downstream and while succinate uses to supply electrons into it (Chandel et al. 2000).

Mitochondria are the main source of ROS in non-photosynthetic tissues (Grabelnych et al. 2011). One of the factors in protecting cells from oxidative damage to mitochondria is the transition to an alternative way (AOX) of respiration. AOX activation reduces the degree of reduction of ubiquinone's pool due to rapid reset the of electrons to oxygen to form water (Garmash 2010). AA causes the induction of AOX gene expression (Juszczuk et al. 2003).

However, in all organisms exists enzymatic and non-enzymatic antioxidative mechanisms that protect cells from oxygen toxicity (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX)) (Maxwell et al. 1999). Under the influence of exogenous and endogenous factors or due to insufficient antioxidative defence systems may be excessive accumulation of ROS and oxidative stress (Дмитриева et al. 2007).

The apical parts of wheat seedlings are a convenient model for the study of oxidative processes in the senescent cell populations and the role of various cell organelles in these processes. With AA it possible to block the ETC of the mitochondria and chloroplasts and to evaluate the role of each of them.

Therefore, the aim of this study in the first step was investigating the changes of oxidative processes (rate of superoxide ( $O_2^{\cdot-}$ ) production and electrophoretic activity of enzymatic antioxidative systems superoxide dismutase (SOD) and catalase (CAT) in apical parts of the first leaves and coleoptiles of wheat seedlings in

the presence of AA.

## MATERIAL AND METHODS

### Plant material and growth conditions

Experiments were performed with apical parts of the first leaves and coleoptiles of etiolated seedlings of winter wheat (*Triticum aestivum* L., cv. Harmony) that were germinated in the thermostat at  $26 \pm 1$  °C and in the presence of 1 mg/l AA. On 3 - rd, 4 - th, 5 - th, 6 - th and 7-th days from seedlings separated apical parts of the first leaves and coleoptiles length of 7 mm. AA concentration and treatment time of the first leaves and coleoptiles were chosen based on the results of preliminary experiments.

### The rate of superoxide ( $O_2^{\cdot-}$ ) production

The rate of  $O_2^{\cdot-}$  production was evaluated by differential spectra of reduced tetranitro blue tetrazolium chloride (the spectrum of a sample incubated without SOD minus the spectrum of a sample in the presence of 1 µg/ml SOD added) (Shorning et al. 2000).

The apical parts of wheat seedlings were placed in cuvettes with 10 ml 0,1 M phosphate buffer (pH 7,6), contained 0,05% tetranitro blue tetrazolium chloride, 10 µl EDTA, 0,1% Triton X-100 for 1 hour at 25 °C, and determined optical density of solution at 530 nm.

### The electrophoretic activity of enzymatic antioxidative systems

Electrophoretic activity of superoxide dismutase and catalase was determined by the method of Paulauskas and Tubelyte – Kirdiene (Paulauskas & Tubelyte – Kirdiene 2002).

Samples homogenized in 0,1 M potassium phosphate buffer (pH 7,8). The homogenate centrifuged for 15 min at 14 500 g. SOD and CAT isoforms in the first leaves and coleoptiles separated by PAGE in 15% and 7, 5% (30% AKA, 10×Tris HCl, TEMED, 10% PSA) polyacrylamide gels. Electrophoretic activity

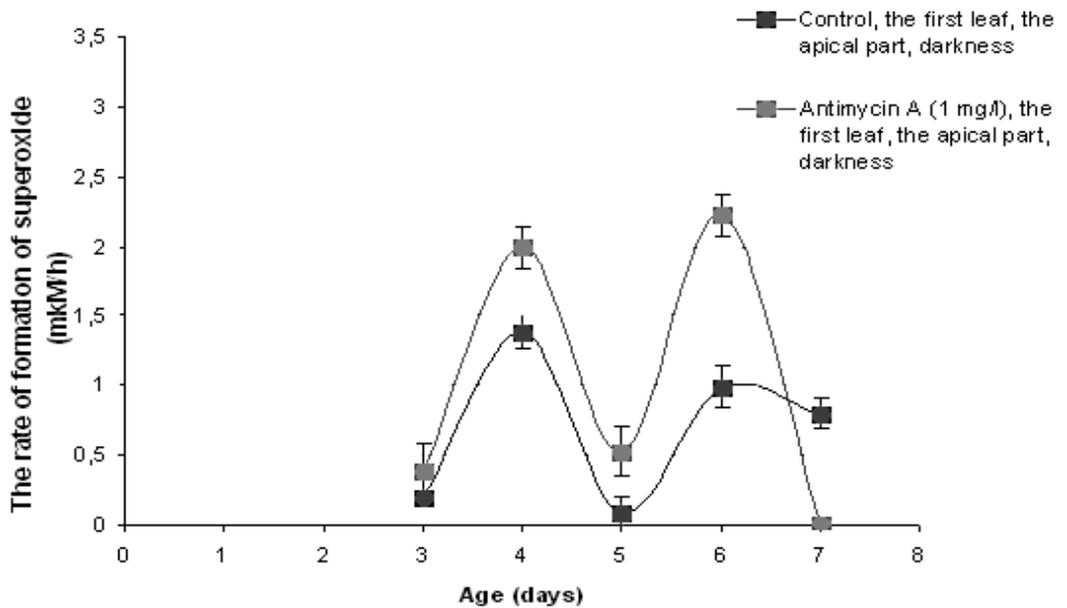


Fig. 1. The effect of antimycin A on the rate of superoxide production (mkM/h) in the apical parts of the first leaves.

for SOD and CAT determined with a vertical electrophoresis. The resulting gels visualized: for SOD - 50 mM potassium phosphate buffer, (pH=7,8), NBT, PMS, MgCl<sub>2</sub> (incubate 1:00 hour); for CAT - solution A (pH=7,0-7,2), containing 0,01% H<sub>2</sub>O<sub>2</sub> (incubate 10 min), solution B (1% FeCl<sub>3</sub>\*6H<sub>2</sub>O, 1% K<sub>3</sub>Fe (CN)<sub>6</sub>.

## RESULTS AND DISCUSSION

### The rate of superoxide (O<sub>2</sub><sup>•-</sup>) production in the presence of antimycin A

To research the rate of O<sub>2</sub><sup>•-</sup> production in apical parts of the first leaves and coleoptiles wheat seedlings grown in water (control) and in the presence of AA (thermostat 26 ± 1 ° C). In the apical parts the cells are senescent and started the programmed cell death. We have analysed the effect of AA on the rate of O<sub>2</sub><sup>•-</sup> production in the apical parts of the first leaves. The experimental results are shown in Fig. 1. As it can be seen from the results, in the apical parts the rate of O<sub>2</sub><sup>•-</sup> is cyclical and is possible to observe two peaks on the fourth

and the sixth days of the seedlings development. A similar cyclic rate of O<sub>2</sub><sup>•-</sup> production in the whole first leaves observed by other authors only at later developmental stages (from 5 to 10 days) (Shorning et al. 2000). At these stages the authors also observed two peaks on the sixth and the tenth days of the seedlings development. Thus, on the obtained results of experiments can be assumed that the rate of cyclic recurrence of O<sub>2</sub><sup>•-</sup> is typical for the whole period of the first leaf development. It is assumed that the observed peaks of O<sub>2</sub><sup>•-</sup> associated with active cell division in this period (Полесская 2007).

We assume that each cell division accompanied by a simultaneous increase in the rate of O<sub>2</sub><sup>•-</sup> production. The second peak of rate of O<sub>2</sub><sup>•-</sup> production, which is formed on the 6th day was by 28% less than the first peak, which can be observed on the 4th day of development. We can assume that the process of development in the apical parts of the rate of cyclical recurrence remains of O<sub>2</sub><sup>•-</sup> production, but the amplitude of the oscillation rate of O<sub>2</sub><sup>•-</sup> production decreases during the development of wheat seedlings.

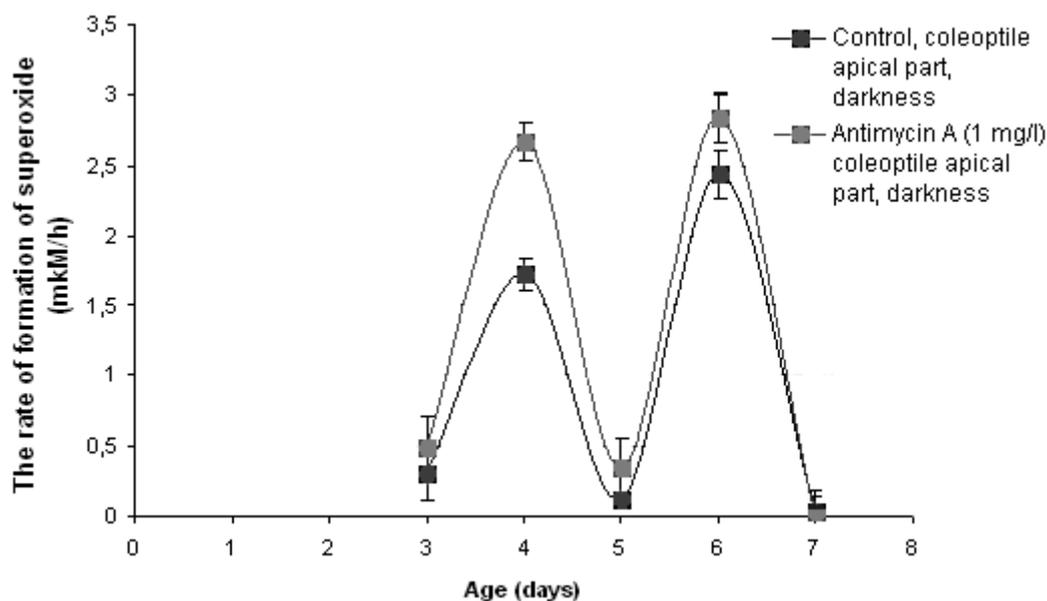


Fig. 2. The effect of antimycin A on the rate of superoxide production (mkM/h) in the apical parts of the coleoptiles.

AA applies in animal and plant cells to study the effects of respiratory inhibition on cellular processes (Maxwell et al. 1999). AA causes a disturbance of ETC in mitochondria, which increase the yield of  $O_2^{\cdot-}$  to cytosol.

Generation of  $O_2^{\cdot-}$  increases in the presence of AA as inhibitor bc<sub>1</sub> segment (complex III) of the mitochondrial respiratory chain, that block the transfer of electrons from cytochrome b<sub>566</sub> to oxidized coenzyme Q (Шумаев et al. 2006).

We have investigated the effects of AA on the rate of  $O_2^{\cdot-}$  production in the apical parts of the first leaves grown in the darkness. As seen from the results, in the presence of AA the rate of  $O_2^{\cdot-}$  production in the apical parts of the first leaves increased (Fig. 1). In particular, it can be seen in later stages of development (the sixth day). AA increases the rate of  $O_2^{\cdot-}$  production in the apical parts of the first leaves by 31% on the fourth day and by 55% on the sixth day of development. It is known that at these stages in the apical parts of the first leaves begin to decrease the quantity of DNA and begin a process of programmed

cell death (Ванюшин 2001). Perhaps under the influence of AA increases the rate of  $O_2^{\cdot-}$  production by the termination of ETC in mitochondria.

The effect of AA on the rate of  $O_2^{\cdot-}$  in apical parts of coleoptiles was investigated. The results are shown in Fig. 2. Coleoptile cereal seedlings are juvenile organs and are characterized by shortness of the period of development and the completion of the physiological function of the programmed cell death. It is known that in etiolated seedlings of wheat coleoptile apoptosis occurs on the 6th or 8th days (Korsukova et al. 2013).

According to the obtained results it can be seen that in the apical parts of etiolated coleoptiles rate of  $O_2^{\cdot-}$  production gradually increases and reaches a peak on the 4th and 6th day of development (Fig. 2). On the 4th day the amount of DNA and the protein begin to decline (Ванюшин 2001).

Similar results were obtained by other authors,

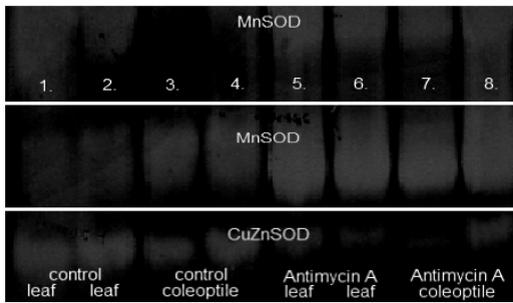


Fig. 3. The electrophoretic activity of superoxide dismutase in different parts of the coleoptiles and the first leaves (1, 3, 5, 7 - apical parts, 2, 4, 6, 8 – basal parts).

but they studied the whole coleoptile, and the rate of production of ROS began with the fifth day and reached a peak on the 6th day of development (Shorning et al. 2000). During this time in etiolated coleoptiles decrease the amount of DNA and observed nucleosomal DNA fragmentation (Кирнос et al. 1999). This is probably the fact that ROS act as signalling molecules in various gene activation processes, including processes related with aging and apoptosis (Mittler 2002).

The effect of AA on the rate of  $O_2^{\cdot -}$  production in the apical parts of the coleoptiles was investigated which have grown in the darkness. AA increases the rate of  $O_2^{\cdot -}$  production in the apical parts of etiolated coleoptiles. The peaks observed on the 4th and 6th days of development. On the 4th day AA increased the rate of  $O_2^{\cdot -}$  production in the coleoptiles apical parts grown in the darkness by 35%, but on the sixth day of AA increased by 14% (Fig. 2). The effect of AA on switching to an alternative cytochrome of respiration is not so actually because mitochondria are effectively degraded. Perhaps in etiolated coleoptiles AOX does not function, and AA damages the ETC and increases the rate production of  $O_2^{\cdot -}$ .

### Electrophoretic activity of superoxide dismutase and catalase in the presence of antimycin A

In plant cells are a strong enzymatic antioxidative systems which protect the plant

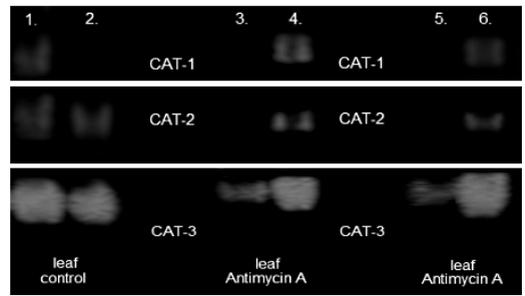


Fig. 4. The electrophoretic activity of catalase in different parts of the first leaves (2, 4, 6 - the apical parts, 1, 3, 5 – basal parts).

cells from the ROS.  $O_2^{\cdot -}$ , which is rapidly converted to  $H_2O_2$  through the action of SOD, produced during respiration primarily by the autoxidation of reduced mitochondrial electron – transport components (Maxwell et al. 1999). It is known that in the mitochondrial matrix  $O_2^{\cdot -}$  quickly neutralized with MnSOD, whereas in the intermembrane space of mitochondria and cytosol ROS neutralized CuZnSOD. Electrophoretic activity of SOD was investigated. The results are shown in Fig. 3. As can be seen from the electropherogram in the basal and apical parts of coleoptiles and the first leaves of wheat seedlings found two isoforms of SOD: MnSOD and CuZnSOD1.

The data obtained in the presence of AA in the apical parts of the first leaves is increasing all isoforms of SOD, especially MnSOD1 and MnSOD2. In the apical parts of the coleoptiles, in the presence of AA, appears MnSOD1 area that is not in control seedlings. It is known that the MnSOD is mainly localized in mitochondria and chloroplasts (Rio et al. 2003), where in the presence of AA increased ROS. Perhaps this is due to the decrease in  $O_2^{\cdot -}$  production, in the intermembrane space of mitochondria and the cytosol. Given the multi-directional influence on AA different forms of SOD, it can be assumed that the disturbance of ETC increases  $O_2^{\cdot -}$  production in mitochondria and chloroplasts, but a decrease in the cytosol.

We have analysed also the electrophoretic

activity of catalase in the apical and basal parts of the first leaves, which shown in Fig. 4. It is known that catalase neutralizes such ROS as  $H_2O_2$ . As can be seen from the data, the effect of AA on catalase isoforms in developing and senescent cells is not equal. Thus in the basal parts of the first leaves in the presence of AA, electrophoretic activity decreases, but in the apical - increases. Perhaps the decrease in the activity of catalase is associated with the effect of AOX in developing cells (in basal parts) and the lack of it in senescent cells (in apical parts).

## CONCLUSIONS

AA is ETC inhibitor that blocks the flow of electrons through complex III. ROS generation at complex III increases, when AA inhibits ETC. ROS are bio-products of normal cellular metabolism and play a role of secondary messengers in the regulation of cellular processes. The results showed cyclical rate of  $O_2^{\cdot-}$  production in apical parts of different organs of wheat seedlings during their development. In apical parts occurs senescence of cell. The treatment of wheat seedlings leads to increase the rate of production of  $O_2^{\cdot-}$ . The changes of the rate of  $O_2^{\cdot-}$  production in the presence of AA in apical parts of etiolated seedlings occurs due to the mitochondria. In apical parts of coleoptiles, in this stage, begins the programmed cell death, mitochondria are destroyed and the influence of AA decreased. The changes in the rate of  $O_2^{\cdot-}$  production also leads to changes in the electrophoretic activity of enzymatic antioxidants SOD and CAT in the apical parts of the first leaves and coleoptiles.

The results suggest that increasing the rate of  $O_2^{\cdot-}$  production in the apical parts of the first leaves of wheat seedlings attribute to electrophoretic activity of SOD increasing.

Thus we can assume that mitochondria play a central role in the natural production of cyclic  $O_2^{\cdot-}$  in senescent parts of the plant cells.

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