EFFECT OF CYTOKININS ON THE ACTIVITY OF SUPEROXIDE DISMUTASE IN NITROGEN DEFICIENT WHEAT

ABSTRACT: Reactive oxygen species (ROS), such as O$_2$•⁻, are formed by electron transfer to a molecule with stable electron configuration, in electron transport chains in the cell. ROS are very reactive molecules which are formed at higher rates under stress, such as drought, high insolation, heath, inadequate mineral nutrition, and such conditions lead to impairment of various physiological and biochemical processes in the cell. To reduce production of ROS, and their detrimental effect, plants developed various enzymatic and non-enzymatic protective mechanisms. Superoxide dismutase (SOD) is one of the most important antioxidative enzymes, which removes superoxiden anion radical (O$_2$•⁻), whose rate of production is the highest under unfavorable environmental conditions. Plant tissues that exhibit delayed senescence often have higher cytokinin content, which is accompanied by reduced amount of ROS. The focus of this paper is to examine whether foliar application of cytokinins to young wheat plants insufficiently supplied with nitrogen affects the activity of SOD and amount of O$_2$•⁻. Application of trans-zeatine (CK) reduced the activity of SOD, but this reduction was not accompanied by an increase in the amount of O$_2$•⁻. Application of benzyl adenine (BA) also reduced the activity of SOD, with concomitant increase in the amount of O$_2$•⁻ in wheat leaves.

KEY WORDS: wheat, nitrogen supply, cytokinin, trans-zeatine, benzyl adenine, superoksid-dismutase, superoxid anion radical

INTRODUCTION

Reactive oxygen species (ROS) are extremely reactive molecules because they have unpaired electron in their structure. ROS formation takes place in all parts of the cell, in which electron transport takes place (Elsasser, 1991). Superoxide anion radical (O$_2$•⁻), one of the ROS, is formed by electron transfer to molecular oxygen (O$_2$) (Mehler, 1951): • (e) + O$_2$ → O$_2$•⁻. This reaction takes place during the electron transport processes in a number of cell com-
partments. The highest amount of $O_2^{•−}$ in plant cells are formed in chloroplasts, mitochondria and peroxisomes (Fridovich, 1986).

Superoxide anion radical ($O_2^{•−}$) is very reactive molecule which acts as progenitor of destructive chain reactions that finally result in the damaging of the molecules close to the site of $O_2^{•−}$ production (Berrett and Stadtmann, 1997). Therefore, keeping the low cellular level of $O_2^{•−}$ is of crucial importance for cell metabolism. To do that, plant cells developed a number of efficient biochemical mechanisms during the evolution. The most efficient mechanism for removal of $O_2^{•−}$ from the cell is “ascorbate-glutathione” cycle, which functions in chloroplasts, mitochondria and peroxisomes, where the highest amount of $O_2^{•−}$ is formed (Asada, 1999).

The first reaction in the “ascorbate-glutathione” cycle is transformation of two molecules of superoxide anion radical ($O_2^{•−}$), with two protons ($H^+$), into one molecule of hydrogen peroxide ($H_2O_2$) and one molecule of oxygen ($O_2$). The enzyme that catalyses this reaction is superoxide dismutase (SOD):

$$O_2^{•−} + O_2^{•−} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

The importance of SOD in the removal of $O_2^{•−}$ is thoroughly proven (Perl et al., 1993; Yu et al., 1999). Plants with increased synthesis of SOD in their chloroplasts had higher tolerance to stress-inducing factors such as drought, low or high temperatures, imbalanced mineral nutrition, etc. Under stress, in plants with higher SOD activity the growth was less reduced than in plants in which SOD activity did not increase upon the induction of stress (Štajner et al., 2004). Therefore, the activity of SOD may be used as a parameter to evaluate the level of plant tolerance to particular stress-inducing factor.

Cytokinins can also participate in removal of ROS from the cell. After Leshem et al. (1979) the molecular structure of cytokinins allows them to react directly with superoxide anion, but also with the other ROS, thus removing them from the cell metabolism.

As the possible direct antioxidant activity effect of cytokinins can also be considered their effect on the activity of antioxidant enzymes, including SOD. While studying the effects of cytokinins on senescence, several authors (Grossman and Leshem, 1978; Liu et al., 1996) found that cytokinins, such as zeatin and benzyl adenine, can increase the activity of some antioxidative enzymes (SOD, catalase), and therefore delay senescence of the plant tissue. Moreover, Leshem et al. (1981) showed that cytokinins inhibit the activity of xanthine oxidase, an enzyme that is one of the generators of ROS in the cell. The senescence was delayed also by reduction of lipoxygenase activity induced by cytokinins, which contributed to preserve the integrity of cell membranes.

Although it is known that cytokinins have the potential to reduce oxidative stress in plants, this was not tested under nitrogen starvation in wheat. Therefore, the aim of this study was to analyze the effect of foliar application of cytokinins (zeatin and benzyl adenine) on the activity of SOD and amount of $O_2^{•−}$ in the leaves of young nitrogen-deficient wheat plants.
MATERIALS AND METHODS

The effect of cytokinins on the activity of SOD and amount of O$_2$·¯ was analyzed in bread wheat (Triticum vulgare), cultivar Renesansa, differently supplied with nitrogen. Young wheat plants were grown in water cultures and foliary treated with solutions of trans-zeatine (CK) and benzyl adenine (BA), under semi-controlled conditions.

Plant growth

Germination was done in an incubator, in the dark, at 26°C. Seedlings were divided into three groups at planting. One was supplied with the complete ½ strength Hoagland nutrient solution (Hoagland and Arnon, 1950) (control, N1, nitrogen supplied as [2.5 mM KNO$_3$ and 2.5 mM Ca(NO$_3$)$_2$]), another onto the same solution but with the N concentration reduced to ½ (N1/2, nitrogen supplied as [1.25 mM KNO$_3$ and 1.25 mM Ca(NO$_3$)$_2$]), and for the third group N concentration was reduced to ¼ of the full dose (N1/4, [0.625 mM KNO$_3$ and 0.625 mM Ca(NO$_3$)$_2$]). Nutrient solutions were aerated each day and replaced every 3—4 days. Fifteen days after planting, each group was divided into 3 sub-groups of 6 pots and 8 plants per pot each, and treated with solutions of trans-zeatine (CK) and benzyl adenine (BA).

Treatment with cytokinins

Plants were treated with water solution of trans-zeatine (CK) of the following concentrations: 0 mg/dm$^3$ (deionized water-control, CK 0), 2.5 mg/dm$^3$ (CK 2.5), 5.0 mg/dm$^3$ (CK 5) and 10 mg/dm$^3$ (CK 10). Benzyl adenine (BA) was applied at following concentrations: 0 mg/dm$^3$ (deionized water-control, CK 0), 10 mg/dm$^3$ (BA 10), 40 mg/dm$^3$ (BA 40). Foliar treatments with both CK and BA were done twice during the experiment by spraying 0.1 dm$^3$ of each solution of 144 plants. The first treatment was done 7 days after planting and the second 14 days after planting. Plants were analyzed 7 days after the second treatment.

Extraction of O$_2$·¯ and SOD

O$_2$·¯ and SOD were extracted from 1 g of fresh leaf tissue, in phosphate buffer pH 7.0, as described by Quy Hai et al. (1975).

Determination of the amount of O$_2$·¯ and the activity of SOD

Superoxide radical (O$_2$·¯). The amount of O$_2$·¯ was determined by the method of Misra and Fridovich (1972), based on the reaction of inhibition of the auto oxidation of adrenaline.
Superoxide dismutase (SOD). The total activity of SOD was determined on the basis of the inhibition of transformation of adrenaline into adrenochrome in the presence of the air (Misra and Fridovich, 1972).

Statistical analysis

The results were statistically processed by the analysis of variance, and calculation of the least statistically significant differences (LSD) between the control and cytokinin treatment, for each level of nitrogen nutrition and for probability of 5%, using the computer program STATISTICA 7.

In the figures, in the Results section, numerical values above the bars represent relative (in %) increase or decrease of the measured value of each treatment with respect to the control (control = 0). The control plants are those grown on complete ½ strength Hoagland solution and untreated with cytokinins (N1, CK 0, BA 0).

Following the ranking of mean values by the Duncan test, the significance of differences between the means is marked by letters inside the figure bars. Two means differ significantly (for p < 0.05) if they do not share any letters.

RESULTS

The effect of cytokinins on the activity of SOD

The activity of SOD ranged from 33.57 U/g FW (in treatments N1/2 and N 1/4, CK 10) to 205.52 U/g FW (in the control). Such a high span between measured values suggests that both nitrogen nutrition and treatment with cytokinins affected the activity of SOD (Fig. 1).

An increase in N concentration in the nutrient solution increased the activity of SOD, whereas increase in CK concentration significantly reduced activity of SOD in young wheat leaves. Analysis of variance permits to conclude

![Fig. 1 — Activity of superoxide dismutase (SOD) in leaves of young wheat plants grown in the presence of different N concentrations and treated with different concentrations of CK](image-url)
that there is an interaction between applied N and CK concentrations on the activity of SOD. The effect of N concentration on the activity of SOD significantly varied with the increase in the concentration of CK.

In the experiment with BA, the highest activity of SOD was found in the control (N1 and BA0 — 186.05 U/g FW). The lowest activity was found in plants supplied with the lowest N and the highest BA concentration (N1/4 and BA40 — 26.00 U/g FW) (Fig. 2).

With the reduction of N concentration in the nutrient solution the activity of SOD declined as well. On the contrary, an increase in the BA concentration reduced the activity of SOD. However, analysis of variance showed that there was no statistically significant interaction between N and BA concentration on the activity of SOD.

The effect of cytokinins on the amount of superoxide radical ($O_2^{-}$)

In the experiment with CK, the highest amount of $O_2^{-}$ (4.35 mmol/g FW) was found in plants supplied with $\frac{1}{4}$ of full dose of N and treated with 2.5 mg CK/dm³ solution, whereas the lowest amount of $O_2^{-}$ (2.64 mmol/g FW) was found in plants supplied with full dose of N and treated also with 2.5 mg CK/dm³ solution (Fig. 3).

The concentration of N in the nutrient solution exhibited statistically significant effect on the reduction of the amount of $O_2^{-}$ in wheat leaves. This reduction was the most outstanding in plants grown in the presence of full N dose (N1) (Fig. 3). The effect of treatment with CK on the amount of $O_2^{-}$ in wheat leaves was not statistically significant.

Treatment with BA significantly increased the production of $O_2^{-}$ in leaves. The highest amount of $O_2^{-}$ (4.68 mmol/g FW) was found in leaves of wheat
grown on nutrient solution containing ¼ of full dose of N and treated with solution containing 40 mg BA/dm³. At the same time, in control plants only 2.14 mmol O₂⁻/g FW were detected (Fig. 4).

Both alteration of N and BA concentration significantly affected the production of O₂⁻ in wheat leaves. Analysis of variance showed that an increase in N concentration, contrary to BA, reduced the amount of O₂⁻. Moreover, significant interaction between effects of N and BA was present.

**DISCUSSION**

There is a negative correlation between measured activities of SOD and the determined amounts of O₂⁻ in plants treated with different concentrations of BA (Fig. 2, Fig. 4), which is in accordance with the results of Aschler.
et al. (2002). However, in the plants treated with CK, there is an absence of the correlation between the activity of SOD and the amount of \( \text{O}_2^{-} \) (Fig. 1 and Fig. 3). In other words, in the plants treated with CK significant reduction in SOD activity was not accompanied by significant increase in the amount of \( \text{O}_2^{-} \). This may be explained by the role of trans-zeatine as the signal molecule in the plants. For example, Takei et al. (2002) found that the synthesis of cytokinins in root tips and their concentration in the plant tissue increased with an increase in the concentration of nitrate N in the nutrient substrate. High concentrations of nitrate in the soil substrate are typical for conditions of sufficient content of organic matter in the soil, optimal temperature and moisture in the soil and in such conditions the synthesis of cytokinins is stimulated. Concurrently, the transport of cytokinins through xylem is also accelerated and this represents the signal to a plant that nutritive and moisture conditions are favorable for growth.

Because the biosynthesis of cytokinins is stimulated when environmental conditions are favorable for plant growth and development, it is highly probable that binding of cytokinins for specific receptors in the cell membranes induces the production of a signal that triggers the synthesis of enzymes that allow plants to utilize as much as possible favorable environmental conditions. This assumption is supported by experimental results of Chen and Leisner (1985), Chen (1989) and Andersen et al. (1996), who, after application of cytokinins, found an increase in activity of Rubisco, fructose-1,6-diphosphatase, glyceraldehyde-3-phosphate dehydrogenase, NADP-dependant malate dehydrogenase, hydroxypyruvate reductase and nitrate reductase in plant tissues.

In addition, under favorable environmental conditions, accompanied by higher cytokinin content, the need for antioxidant enzymes is reduced. Therefore, it is probable that under the experimental conditions described in this paper treatment with CK induced transient increase in cytokinin concentration in leaf tissues, which resulted in the reduced synthesis of antioxidant enzymes, including SOD. Similar result was described by Crowell and Amasino (1991). In cell culture of soybean cells, isolated from seedlings, and grown on a medium without addition of cytokinins the synthesis of FeSOD increased, while addition of cytokinins reduced the level of mRNA corresponding to gene encoding FeSOD. On the contrary, He et al. (2005) found that in hybrid corn exhibiting delayed senescence an increase in cytokinin content is accompanied by an increase in SOD activity. Moreover, Liu and Huang (2002) found an increase in SOD activity following addition of zeatin-riboside to the rhizosphere of the grass Agrostis palustris, which was exposed to combined stress provoked by high temperatures of the soil and the air. Durmus and Kadiglu (2005) treated maize leaves with 2.5 and 25 mg BA/dm³ and recorded an increase in the activity of SOD if plants were treated with paraquat 8 h after the BA treatment, but if paraquat was applied 12 or 24 h after BA there was no increase in the activity of SOD.

So variable effects of different cytokinins on the activity of SOD can be explained by the fact that the activity of SOD depends, among the other factors, on the plant and tissue age, complexity of environmental factors, partitio-
ning between enzymatic and non-enzymatic antioxidants in the plant, and on
the nature and intensity of the eventual stress factor(s).

CONCLUSIONS

The effects of foliar treatment of nitrogen-deficient young wheat plants
with CK and BA on activity of SOD and amount of $O_2^{-}$ were studied under
semi-controlled conditions. On the basis of the experimental results, the fol-
lowing conclusions can be drawn out:

Foliar application of CK and BA significantly reduced the activity of
SOD in the leaves of nitrogen-deficient young wheat plants.

Foliar application of BA increased the amount of $O_2^{-}$ in leaves of nitro-
gen-deficient young wheat plants while treatment with CK did not affect the
amount of $O_2^{-}$.

ACKNOWLEDGEMENTS

Goran Stoparić would like to express his gratitude to AD “Mlekara” Su-
botica, Tolminska 10, 24000 Subotica, Serbia, for financial support.

REFERENCES

Aschler, R. G., Neval, E., Lenwood, S. H. (2002): Role of superoxide dismu-
tases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 372: 1331—
1341.

scriptional regulation of hydroxypyruvate reductase gene expression by cytokinin

Asada, K. (1999): The water-water cycle in chloroplasts: Scavenging of active oxy-
Biol. 50: 601—639.


Chen, C. M. (1989): Cytokinin-modulated macromolecular synthesis and gene expres-
p. 245—256.


Soybean cells during cytokinin or auxin starvation. Plant Physiol. 95: 711—715.

Durmus, N., Kadioğlu, A. (2005): Reduction of paraquat toxicity in maize leaves

plant cells. In: Active oxygen/oxidative stress and plant metabolism. Eds. Pell E.


УТИЦАЈ ЦИТОКИНИНА НА АКТИВНОСТ СУПЕРОКSID-ДИСМУТАЗЕ ПШЕНИЦЕ У УСЛОВИМА НЕДОСТАТКА АЗОТА

Горан З. Стопарић¹, Ивана В. Максимовић²

¹ Општина Суботица, Општинско веће за заштиту животне средине и здравство, Трг Слободе 1, 24000 Суботица, Србија
² Пољопривredni факултет, Трг Д. Обрадовића 8, Нови Сад и Институт за ратарство и повртарство, М. Горког 30, Нови Сад, (E-mail: ivanam@ib.ns.ac.yu)

Резиме

Слободни кисеонични радикали (ROS) настају принудним преносом електрона са неког од многобројних електрон-транспортних ланца у ћелији, на молекулу са стабилном конфигурацијом електронског омотача. Настали радикали су веома реакцијни молекули који се у већој количини стварају у стресним условима (висока осветљеност, суша, недовољна исхрана), који доводе до поремећаја фiziолошко-биохемијских процеса у ћелији. Да би се смањило настајање радикала и умањило њихово шtetно дејство, у биљкама су се током еволуције развили ензимски и неензимски системи заштите. Супероксид дисмутаза (SOD) је један од најважнијих антиоксидантних ензима који уклања настали супероксид андон радикал (O2⁻·), чије је настајање најинтензивније у неповољним условима спољашње средине. Уочено је да је једна од карактеристика биљних ткива, чије је старење одложено, да је повећан садржај цитокинина у биљном ткиву праћен смањењем количине кисеоничних радикала. На основу ове чињенице одређен је и циљ овог рада, да се, у условима недостатка азота, утврди утицај третирања младих биљака пшенице растворима једињења са цитокининском активношћу на активност SOD и количину O2⁻·. Третирање пшенице растворима транс-зеатина (CK) смањило је активност SOD, али то смањење није праћено повећањем количине O2⁻·. Примена раствора бензил аденина (BA) је такође довела до смањења активности SOD, али је ово смањење активности праћено повећањем количине O2⁻·.