

EFFECT OF Cu^{2+} IONS ON NITROGEN ASSIMILATION AND METABOLISM ENZYMES IN SELECTED FIELD CROPS

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Abstract

The effect of Cu^{2+} ions on nitrogen assimilation and metabolism enzymes (nitrate reductase, glutamine synthetase and glutamate dehydrogenase) in leaves of young plants of wheat and maize has been investigated. Cu^{2+} concentrations used were 10^{-8} (control), 10^{-7} , 10^{-5} and 10^{-3} mol dm^{-3} . Enzymology of nitrogen assimilation and metabolism was significantly different between examined genotypes: in wheat, NR activity decreased with the increased Cu^{2+} concentrations while the GS activity increased (except for the 10^{-3} mol dm^{-3} Cu^{2+} concentration). In maize, NR activity was at its minimum while GS activity was not detected. GDH activity varied in both species examined. The obtained results also indicate that the investigated maize hybrid is more Cu-sensitive in the sense of nitrogen assimilation and metabolism, compared to wheat. In wheat, two nitrogen assimilation pathways were established (GS/GOGAT- and GDH-pathway). In maize, only GDH-pathway was present.

Introduction

Plant world is becoming more endangered due to increasing contamination of the environment. Among other pollutants, heavy metals (HM) in soils very often affect cultivated plants. The assimilation, transport, distribution, accumulation and physiological properties of those elements are very important for high yield and quality of cultivated plants. Beside that, HM are of great importance from the ecological aspect because they enter the food chain through plants. Knowing the ecology and mechanisms of uptake, accumulation, distribution and effects of HM on life processes of plants is of great importance, both from physiological and ecological aspect [1].

Increased concentrations of HM, where copper (Cu) is classified, cause phytotoxicity. One of the primary effects of HM on plants is changing the structure and function of cell membranes and organelles. HM at higher concentrations could also cause increased reduction of molecular oxygen (O_2), and therefore produce toxic oxygen species [1]. In this study, Cu was chosen because of its double effect on the plant metabolism processes. It belongs to the group of microelements - essential for plants in low concentrations, but when found in excess it may become phytotoxic. Primary effects of Cu toxicity are the inhibition of enzyme activity and damaging the membrane system. This can result in depressed photosynthesis, direct inhibition of growth processes and decreased nutrient uptake and transport which can ultimately cause the death of plants.

The assimilation and metabolism of inorganic nitrogen in plants is a complex process involving a series of enzymes. Nitrate is reduced to NH_4^+ by the reaction of nitrate reductase (NR) and nitrite reductase (NiR) (Fig. 1). The cytosolic NR is the first enzyme in the pathway of nitrate assimilation, and its activity is highly regulated. Sufficient NR activity is a prerequisite for optimal utilization of soil N. NR has a major role in incorporation of N for plant yields under field conditions and it is widely known to be substrate inducible [2].

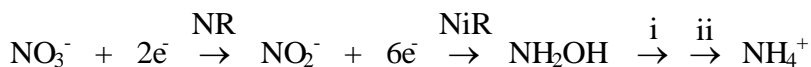


Figure 1. Reduction of NO_3^- to NH_4^+ by the reaction of nitrate reductase (NR) and nitrite reductase (NiR)

Glutamine synthetase (GS) is the key enzyme responsible for the assimilation and reassimilation of ammonia (Fig. 2). In higher plants GS is one of the major enzymes responsible for the assimilation of ammonium absorbed from the growth medium, generated by nitrate reduction or reassimilated after release of endogenous NH_4^+ by ammonium-evolving processes such as photorespiration [3, 4].

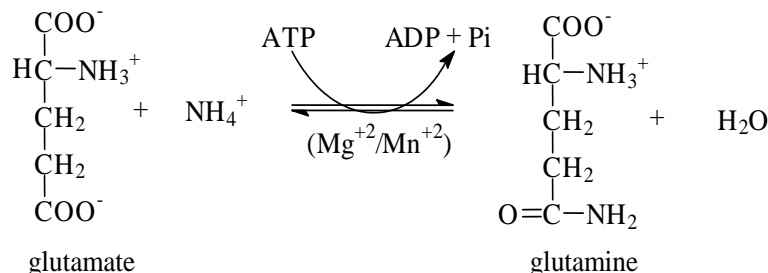


Figure 2. Reductive amination of glutamate (Glu) to glutamine (Gln)

The conversion of NH_4^+ into glutamate (Glu) proceeds *via* two pathways. In the GS/GOGAT pathway, NH_4^+ is incorporated into glutamine (Gln) by glutamine synthetase (GS) (Fig. 2), which is then converted with 2-oxoglutarate (2-OG) to Glu by glutamate synthase (GOGAT). Glutamate dehydrogenase (GDH) catalyzes the incorporation of NH_4^+ into Glu by reversible reductive amination of 2-OG [5,6].

The aim of this study was to investigate and determine NR, GS and GDH activities in two major field crops in the regions of Central Europe - wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.), affected by different concentrations of copper ions (Cu_2^+).

Experimental

One cultivar of wheat (*Evropa 90*) and maize hybrid (*NSSC 640*) were chosen for the experiment. Material for the investigations were leaves of young plants. Seeds were germinated for 48 h in thermostat at 25-27°C. Seedlings were grown on nutrient solution Reid and York, pH 5.5 (controlled every second day), under conditions of glass-house. There were eight plants of wheat, and six plants of maize in the pots, respectively. After 25 days, plants were treated with three concentrations of Cu (10^{-7} , 10^{-5} and 10^{-3} mol dm⁻³), for six days. Cu^{2+} was supplied in the form of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

The enzyme extract was prepared by homogenizing 1.0 g of fresh leaves in 10 cm³ of extraction buffer containing 5 mM imidazole, 5 mM 2-mercaptoethanol and 0.5 mM EDTA, pH 7.2, followed by centrifugation at 10000 g for 10 minutes. After centrifugation, the supernatant aliquots were used to measure enzyme activities. The NADH-dependent NR activity was determined *in vivo* according to Inokuchi et al. [7], in which the formation of nitrite from nitrate was measured spectrophotometrically at 540 nm. The GS activity was assayed using the reverse-glutamyl transferase reaction, which measured the formation of glutamyl hydroxamate [5]. Measurement of GDH activity was based on decrease of absorbance at 340 nm, caused by NADH oxydation. Calculation of activity was done

according to changes in NADH concentration in presence and absence of ammonium acetate. NADH concentration was established according to NADH absorbance at 340 nm [8]. Statistical evaluation was performed using software Statistica, Version 10.0. The experiments were repeated four times, and differences between treatments were determined using LSD test for 0.05 significance level.

Results and discussion

The initial step of NO_3^- reduction is catalyzed by the NR enzyme, which is considered a cytoplasmic enzyme in higher plants. This enzyme plays an important role in the regulation of nitrogen metabolism, and it is labile *in vivo* under the environmental stress. Our results for NR activities under conditions of excess of Cu showed, both in wheat and maize, a decrease in the enzyme activity, with the increased Cu^{2+} concentrations (Fig. 3). In maize they were much lower. Although the NR is substrate-induced enzyme, its activity may be suppressed by different ecological factors, such as HM, herbicides, UV-radiation etc. [1]. Inhibition of the activity could be promoted by 1) non-competitive enzyme inhibition, 2) Cu^{2+} reaction with -SH groups of the NR protein, and 3) Cu^{2+} reaction with O_2 where oxygen free radicals are produced [9]. It seems that the main nitrogen source for maize is not NO_3^- , but NH_4^+ , which could be seen from the GDH activities as well.

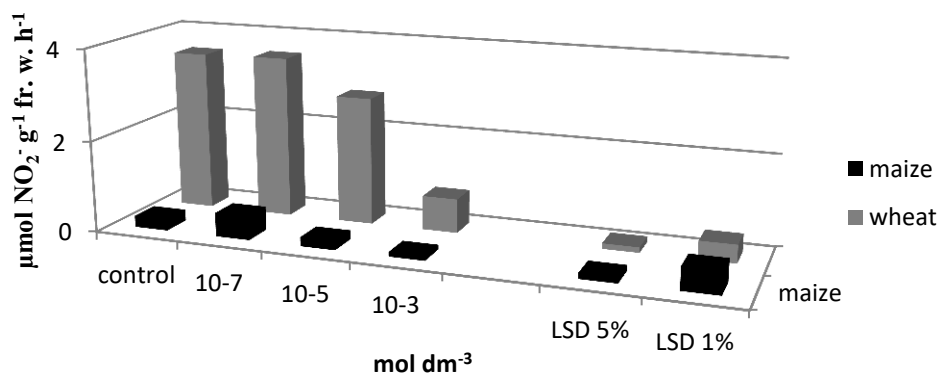


Figure 3. NR activity ($\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ fr. w. h}^{-1}$) in leaves of wheat and maize treated with different Cu^{2+} concentrations

GS activities in wheat increased with the increasing Cu^{2+} concentrations in the range of control - $10^{-5} \text{ mol dm}^{-3}$, but not at the highest concentration ($10^{-3} \text{ mol dm}^{-3}$; Fig. 4). This could be reaction on nitrogen translocation from older to younger leaves in water stress conditions [10]. HM in excess can cause this as a result of decrease in root cells permeability [11]). In maize there was no GS activity. Absence of GS activity in maize point out that the nitrogen was not utilized to amino acids by GS/GOGAT-pathway, but the other pathway - GDH. GDH activity was inhibited in wheat by the 10^{-7} and $10^{-3} \text{ mol dm}^{-3} \text{ Cu}^{2+}$ probably due to SH-groups oxidation and in the non-competitive manner, but not by the $10^{-5} \text{ mol dm}^{-3} \text{ Cu}^{2+}$ (Fig. 5). In maize, GDH activity was highest at the $10^{-5} \text{ mol dm}^{-3} \text{ Cu}^{2+}$. Induction of GDH at $10^{-5} \text{ mol dm}^{-3} \text{ Cu}^{2+}$ in both genotypes could be result of metal-induced stress. In both examined genotypes GDH-pathway of nitrogen assimilation was present. In wheat it represented a side way but in the maize it was a fundamental/major metabolic way, especially because the NR activity was minimal while there was no GS activity.

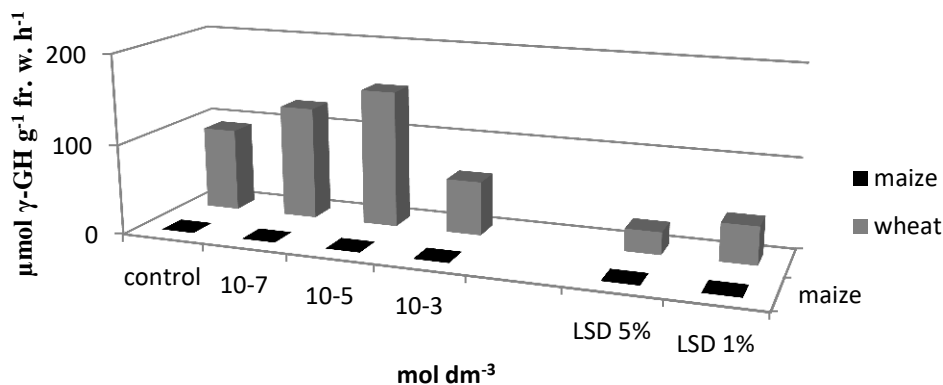


Figure 4. GS activity ($\mu\text{mol } \gamma\text{-glutamyl hydroxamate g}^{-1} \text{ fr. w. h}^{-1}$) in leaves of wheat and maize treated with different Cu^{2+} concentrations

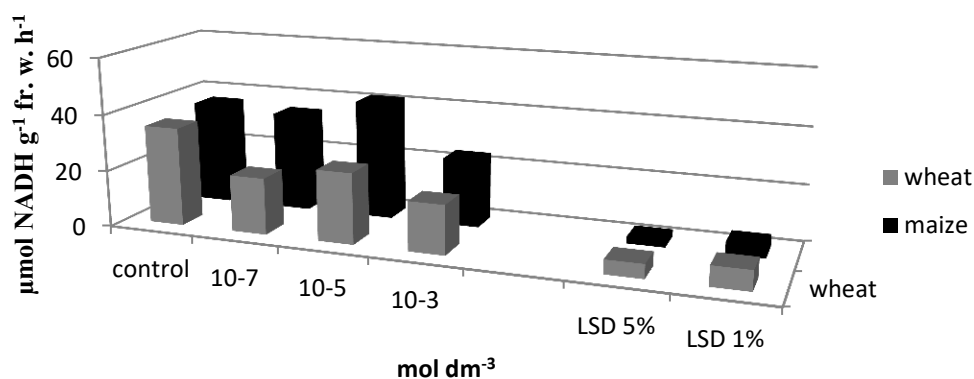


Figure 5. GDH activity ($\mu\text{mol NADH g}^{-1} \text{ fr. w. h}^{-1}$) in leaves of wheat and maize treated with different Cu^{2+} concentrations

Conclusion

Enzymology of nitrogen assimilation and metabolism was significantly different between examined genotypes: in wheat, NR activity decreased with the increased Cu_2^+ concentrations while the GS activity increased (except for the $10^{-3} \text{ mol dm}^{-3} \text{ Cu}_2^+$ concentration). In maize, NR activity was at its minimum while GS activity was not detected. GDH activity varied in both species examined. The obtained results also indicate that the investigated maize hybrid is more Cu-sensitive in the sense of nitrogen assimilation and metabolism, compared to wheat. Also, the dominant nitrogen assimilation pathway in wheat was GS/GOGAT-pathway, while in the maize that was GDH-pathway.

Acknowledgements

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