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# GLUTATHIONE AND ASCORBATE ACCUMULATION OF RAPE PLANTS (*BRASSICA NAPUS* L.) UNDER INFLUENCE OF HEAVY METALS AND TREPTOLEM

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In this study, the joint effect of heavy metals zinc and copper compatible with the new Ukrainian plant growth regulator Treptolem on non-enzymatic antioxidants were investigated on *Brassica napus* plants. In particular, the study focuses on the effect of these metals on the content of glutathione and ascorbate. We establish the protective qualities of the growth regulator Treptolem on growth plants under influence of zinc and copper.

*Keywords: Brassica napus* L., glutathione, ascorbic acid, copper, zinc, Treptolem.

Heavy metals are important environmental pollutants, particularly in areas with high anthropogenic pressure. Their presence in atmosphere, soil and water – even in trace concentrations – can cause serious problems to all organisms, and heavy metal bioaccumulation in the food chain can be highly dangerous [9, 16].

Heavy metals are defined as metals with a density higher than 5g/cm$^3$. Heavy metal pollution is posing several problems to mankind and these elements are increasing in the environment due to industrialization, mining and urban activity. Metals such as Hg, Pb, Cd, Al, Co, Mn, Ni, Cu and Zn are extremely important metals and their phytotoxicity has been well documented [4].

Metal toxicity symptoms include chlorosis, necrosis, leaf rolls, inhibition of root growth, stunted growth of plant, altered stomatal action, decreased water potential, efflux of cations, alterations in membrane functions, inhibition of photosynthesis, altered metabolism, altered activities of several key enzymes, etc. [8, 16, 17].

The important heavy metal tolerance mechanisms include metal binding by cell wall, reduced transport across the cell membrane, active efflux of metals, compartmentalization, chelation and sequestration of heavy metals by particular ligands such as phytochelatins and metallothioneins [6, 7]. The antioxidant system includes low-molecular compounds – ascorbic acid (AA), glutathione (GSH), α-tocopherol and several enzymes – peroxidase, superoxide dismutase and catalase [15].

All metals directly or indirectly generate ROS in plants [14]. Plant protection against metal toxicity occurs, besides other mechanisms, through an elevated level of non-enzymatic and enzymatic components of antioxidative defense system [9]. Therefore, the induction of AA, GSH and related enzymes is an important protective mechanism to minimize oxidative damage in plants exposed to heavy metals [5].

The current object of this research is finding means to increase tolerance of plants to high concentrations of heavy metals. Growth regulators increase plants resistance to adverse factors. As a result of this activity, they accelerated accumulation of biomass and root system, and therefore they more widely used nutrients, increasing the protective properties of plants [3]. Today much attention is paid to the development of new growth regulators. Ukrainian regulator Treptolem – an effective composition of plant growth regulators which increase the productivity of sunflower and rape plants [1]. However, little research has been undertaken to study the problem of influence of heavy metal together with the growth regulator on plants.

The aim of the present research was to determine the impact of zinc and copper with the new growth regulator Treptolem on rape plants (*Brassica napus* L. cv. Mykytynetskyy). In particular, the study focused on the effect of these metals on the content of glutathione and ascorbate.

**Materials and methods**

Rape seeds were sterilized with $KMnO_4$ for 15 min and washed with distilled water. Then seeds were germinated in darkness for 3 days at 25±1°C on a filter paper humidified with distilled water or Treptolem solution (1 ml/l). Afterwards seedlings were transferred into Hoagland-Arnone nutrient solution with zinc ($10^{-3}$ M $ZnSO_4$) or copper ($10^{-5}$ M $CuSO_4$). The control plants were grown on Hoagland-Arnone nutrient solution. Plants were grown in a greenhouse. On 7, 14 and 21 days of growth, the content of reduced and oxidized glutathione [13] and ascorbate [2] were determined.

**Results and discussion**

AA and GSH are the two most important soluble reducing compounds in living cells and are integral in maintaining a net reducing environment.

Ascorbate is generally the most abundant small molecule antioxidant in plants – particularly in leaves, being about 5–10 times more concentrated than glutathione [11]. AA is known to play a prominent role in scavenging free oxy-radicals [12, 20].

In 7-day old seedlings, the ascorbic acid content decreased in leaves relatively to control on medium with zinc and copper (Fig. 1, *A*). After 14 and 21 days, the increasing AA content in plants that grown with copper ions was observed (Fig. 1, *B*, *C*). In leaves AA content was considerably decreased.

In the leaves of plants treated with heavy metals and Treptolem, there was a significant decrease of ascorbate without a significant variation in control, which may be attributed to high consumption of ascorbate as an antioxidant to combat oxidative stress.

The content of ascorbic acid decreased in variants with Treptolem in comparison with plants grown at various concentrations of zinc and copper.

Glutathione is a tripeptide with the sequence γ-l-glutamyl-l-cysteinyl-glycine that plays an important role in a wide range of organisms. Glutathione can exist either in the reduced state (GSH) or in an oxidized state (GSSG), in which two glutathione molecules are linked via a disulfide bond. GSH has physiological roles in reactive oxygen species detoxification, heavy metal detoxification, xenobiotic conjugation, and also in a variety of cellular and tissular functions [18, 19].

In a 7-days rape plants, the level of reduced glutathione insignificantly differed from plants of the control group (Table). In other variants with addition of growth regulator the content of GSH slightly increased as compare with the control and variants with metals. In 14- and 21-days plants the reduced glutathione content increased in almost all variants in comparison with control ones.

The changes in the glutathione pool were observed mainly due to increase of the reduced form, i.e. GSH. The glutathione content also increased in the roots under Zn and Cu exposure.

Oxidized glutathione concentrations increased during plant development. The concentration of GSSG increased from 56,2 μM/g FW in 7-day-old plants to a high of 83,6 μM/g FW in 21-day-old plants. GSSG also increased in the roots under heavy metals exposure.

The ratio of GSH/GSSG plays an important role in indicating cellular redox status [10]. A high GSH/GSSG ratio is necessary to keep glutathione in its reduced form as an antioxidant. In the present study, when rape plants were exposed to heavy metals, the GSH/GSSG ratio in

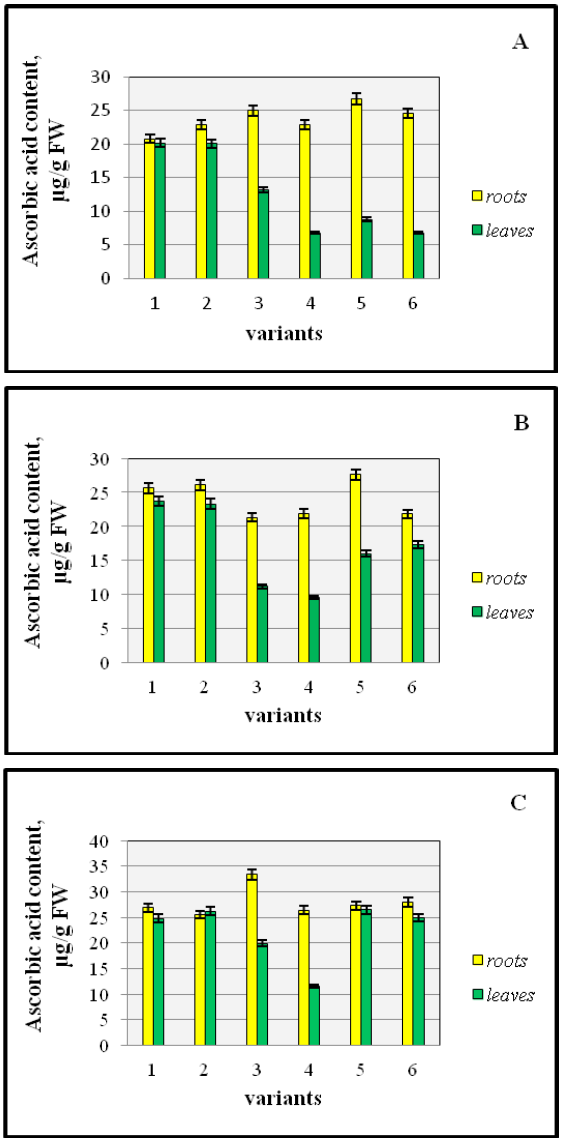


Fig. 1. Ascorbic acid content in rape plants (*Brassica napus* L. cv. Mykytynetskyy) under influence of heavy metals ($10^{-3}$ $ZnSO_4$, $10^{-5}$ $CuSO_4$) and Treptolem (1 ml/l) (*A* – 7-day; *B* – 14-day; *C* – 21-day): 1 – control (Hoagland-Arnone solution); 2 – Treptolem; 3 – $ZnSO_4$; 4 – $ZnSO_4$ + Treptolem; 5 – $CuSO_4$; 6 – $CuSO_4$ + Treptolem.

the leaves had a more obvious change than it did in the roots. The result suggests that the GSH-involved defensive mechanism in different parts of the plants possibly play different roles in the detoxification of heavy metals.

The initial response of the cellular glutathione pool to stresses such as chilling, salinity and drought is an increase in the GSH/GSSG ratio, followed by a marked increase in total glutathione concentration [19].

The changes in reduced glutathione (GSH) (μM/g FW), oxidized glutathione (GSSG) (μM/g FW) content and the GSH/GSSG ratio in rape plants (*Brassica napus* L. cv. Mykytynetskyy) under influence of heavy metals and Treptolem (1 ml/l)

| | Variants | ***Roots*** | | | ***Leaves*** | | |
|---|---|---|---|---|---|---|---|
| | | **GSH** | **GSSG** | **GSH/GSSG** | **GSH** | **GSSG** | **GSH/GSSG** |
| 7 day | Control | 418,2±3,2 | 56,2±0,8 | 7,44 | 424,7±2,3 | 53,9±1,6 | 7,88 |
| | Treptolem | 428,8±1,8 | 58,0±1,7 | 7,39 | 431,7±2,8 | 58,6±1,5 | 7,37 |
| | $ZnSO_4$ | 368,8±1,5 | 112,4±1,4 | 3,28 | 352,5±3,2 | 47,1±1,1 | 6,91 |
| | $ZnSO_4$ + Treptolem | 460,0±3,1 | 109,5±1,9 | 4,20 | 384,7±1,9 | 43,4±0,7 | 8,86 |
| | $CuSO_4$ | 385,8±2,6 | 86,9±0,6 | 4,13 | 378,2±2,7 | 27,5±1,2 | 13,75 |
| | $CuSO_4$ +Treptolem | 472,9±4,1 | 84,5±1,8 | 5,60 | 488,2±4,3 | 21,8±1,1 | 22,39 |
| 14 day | Control | 339,4±2,4 | 68,7±1,3 | 4,94 | 303,5±2,5 | 69,3±2,2 | 4,38 |
| | Treptolem | 328,8±1,9 | 69,0±0,5 | 4,76 | 334,1±3,7 | 66,7±0,9 | 5,01 |
| | $ZnSO_4$ | 381,1±1,7 | 85,1±0,9 | 4,48 | 361,1±1,3 | 47,5±1,3 | 7,60 |
| | $ZnSO_4$ + Treptolem | 352,9±2,2 | 88,7±2,1 | 3,98 | 369,4±2,4 | 44,1±1,0 | 8,38 |
| | $CuSO_4$ | 331,7±3,6 | 80,7±1,5 | 4,11 | 348,2±3,0 | 49,7±1,6 | 7,01 |
| | $CuSO_4$ +Treptolem | 322,9±2.6 | 78,2±1,1 | 4,13 | 330,5±2,2 | 52,7±0,8 | 6,27 |
| 21 day | Control | 285,8±3,1 | 83,6±2,0 | 3,42 | 261,7±2,9 | 67,1±0,9 | 3,90 |
| | Treptolem | 268,8±1,7 | 80,6±0,7 | 3,33 | 255,8±2,7 | 73,4±1,6 | 3,49 |
| | $ZnSO_4$ | 210,5±1,3 | 91,6±0,9 | 2,30 | 294,7±1,3 | 78,4±1,2 | 3,76 |
| | $ZnSO_4$ + Treptolem | 224,7±2,6 | 92,2±1,3 | 2,44 | 300,0±2,8 | 82,9±2,4 | 3,62 |
| | $CuSO_4$ | 270,0±2,9 | 91,8±1,8 | 2,94 | 281,7±3,1 | 73,6±1,9 | 3,83 |
| | $CuSO_4$ +Treptolem | 314,1±3,4 | 113,4±1,2 | 2,77 | 277,6±1,8 | 68,9±0,7 | 4,03 |

Since virtually all environmental impacts cause photooxidative stress in plants, responses of the plant antioxidative systems in general, and glutathione-related in particular, were observed upon exposure to, for example, natural abiotic stresses, biotic stresses (pathogens), or pollutant impacts. However, although numerous recent publications deal with the role of glutathione in plant resistance, acclimation, and adaptation to photo-oxidative stress, the emerging picture is an increasingly complex one. According to different studies, glutathione levels may or may not increase or may even decrease upon stress exposure. The GSH/GSSG redox state may change towards being more oxidized, more reduced, or not at all. Glutathione-related enzyme activities may be related to higher resistance or higher susceptibility. Among others, there are extensive reviews on the response of glutathione to environmental stress impacts [15, 18, 19].

We observed decreases plant growth under the influence of heavy metals and increases under the growth regulator Treptolem, and is normalized at their compatible action. Thus, the adaptive possibilities of plants are increased.

Both AA and GSH pools were larger in the roots, than in the leaves, probably as a consequence of a higher steady-state rate of ROS production in the non-photosynthetic tissues than in the photosynthetic tissues.

Our results demonstrated that ascorbic acid and reduced glutathione levels decreased in 7-day old plants of all variants, whereas on the 14 and 21 days of growth levels of ascorbate and reduced glutathione increased, particularly under the treatment of zinc and copper.

In general, it was shown that the influence zinc and copper compatible with the new Ukrainian growth regulator Treptolem on the rape plants activate an adaptive processes, making them resistant to adverse effects of heavy metal ions.

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## АКУМУЛЯЦІЯ ГЛУТАТІОНУ Й АСКОРБІНОВОЇ КИСЛОТИ У РОСЛИН РІПАКУ (*BRASSICA NAPUS* L.) ЗА ДІЇ ВАЖКИХ МЕТАЛІВ І ТРЕПТОЛЕМУ

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Досліджено сумісну дію іонів важких металів, таких як цинк і мідь, разом із новим українським регулятором росту на вміст неферментативних антиоксидантів у листках і коренях *Brassica napus*. Зокрема, дослідження фокусується на впливі цих металів на вміст глутатіону й аскорбінової кислоти. Виявлено захисні властивості регулятора росту трептолему на ріст рослин за дії іонів цинку і міді.

*Ключові слова*: *Brassica napus* L., глутатіон, аскорбінова кислота, мідь, цинк, трептолем.

## АККУМУЛЯЦИЯ ГЛУТАТИОНА И АСКОРБИНОВОЙ КИСЛОТЫ У РАСТЕНИЙ РАПСА (*BRASSICA NAPUS* L.) ПРИ ВОЗДЕЙСТВИИ ТЯЖЕЛЫХ МЕТАЛЛОВ И ТРЕПТОЛЕМА

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Исследовано совместное действие ионов тяжелых металлов, таких как цинк и медь, вместе с новым украинским регулятором роста на содержание неферментативных антиоксидантов в листьях и корнях *Brassica napus*. В частности, исследование фокусируется на воздействии этих металлов на содержание глутатиона и аскорбиновой кислоты. Установлены защитные свойства регулятора роста трептолема на рост растений под влиянием ионов цинка и меди.

*Ключевые слова: Brassica napus* L., глутатион, аскорбиновая кислота, медь, цинк, трептолем.