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Biochemical and growth effects of silver in wheat plants

Research article

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Abstract
Our experiments on the cultivation of wheat plants in the laboratory, on nutrient solutions supplemented with $AgNO_3$, showed that the exposure to Ag^+ at concentrations of 10 μmol·L^{-1} and 100 μmol·L^{-1} for 7 days led to growth inhibition and modification of certain biochemical indices, compared to control plants. The observed differences concerned the activity of soluble peroxidases and the degree of lipid peroxidation in the roots of wheat plants, as well as the quantity of chlorophylls in their leaves. These results may reflect both a toxic effect and a regulatory role of silver in plants.

Keywords: wheat plants, silver nitrate, peroxidase activity, lipids peroxidation, glucose and protein concentration, chlorophylls and carotenoids.

1. INTRODUCTION

In natural environments, as a result of various anthropogenic activities and inadequate waste management, the plants are often exposed to heavy metals, whose presence in water and soil can directly or indirectly affect their growth and development. In recent years, due

to the diversification and expansion of its uses, silver has also received special attention as a pollutant. Interaction of the plants with silver is two facetted, concerning toxicity and tolerance, both in the case of silver salts and silver nanoparticles, that nowadays are among the most commonly used nanomaterials [1]. In addition to pollution, the intentional, human-mediated relationship between plants and Ag derives from its various uses: as a fungicide in agriculture [2], for the fine-tuning of phytohormone signaling, especially of ethylene [3], and delaying senescence by modulating the antioxidant response of the plants [4], for managing aflatoxin-induced biotic stress [5, 6], and the list can go on.

The aim of our study, the results of which will be presented below, was to investigate certain physiological and biochemical responses of wheat plants exposed to silver nitrate, pursuing the following objectives:

- Cultivation of the plants on nutrient solutions supplemented with Ag^+ as $AgNO_3$, at different concentrations, for 7 days, and monitoring the evolution of their *growth parameters*;
- Assay of some *biochemical parameters* regarding the metabolic and redox status of the plants (glucose, soluble proteins, photosynthetic pigments, secondary products of lipid peroxidation, peroxidase activity);
- Correlation of the plants' biochemical and growth data with the level of exposure to silver nitrate.

2. MATERIALS AND METHODS

2.1. Biological material and experimental conditions

Wheat caryopses (*Triticum aestivum* L. cv. PB-1) were obtained from the collection of the Agricultural Research and Development Station Simnic-Craiova. Surface sterilized caryopses, as previously described [7], were placed in Petri dishes, on filter paper moistened with distilled water, and were kept in the dark for 24 hours. The uniformly germinated caryopses were then transplanted into Petri dishes Φ 90

mm, containing 50 mL of nutrient solution [8] solidified with 0.5% agar. Part of the nutrient solutions were supplemented with the appropriated volumes of a stock solution (1 mmol·L^{-1}) of $AgNO_3$, obtaining final concentrations of the tested substance of 10 and 100 μmol·L^{-1}, respectively (Table 1). Growth media without the addition of silver nitrate were considered as control.

Table 1. The significance of the experimental variants

Experimental variant	$AgNO_3$ stock solution/ mL	Final volume/ mL	Ag^+ concentration in the growth media/		
			μmol L^{-1}	mg·L^{-1}	μg·probe^{-1}
1 (CONTROL)	0	50	0	0	0
2	0.5	50	10	1.0786	53
3	5	50	100	10.786	530

The Petri dishes were kept under conditions of natural lighting (photoperiod of 16/8 hours), and temperature of 25/20ºC. After 7 days, the plants were harvested, and the length of their roots and aerial parts were registered.

2.2. Biochemical assays

Fresh root samples weighing about 0.2 g, collected from each of the experimental variants, were ground with quartz sand and 3 mL of 0.1 mol·L^{-1} Tris-HCl buffer, pH 7.2, using a mortar and pestle. The obtained tissue homogenates were centrifuged at 10,000 rpm, for 10 minutes at 4°C, using a Sigma 2-16K refrigerated centrifuge, and the supernatants were used for the biochemical assays. Indexes of the antioxidant (redox) and metabolic state of the roots were assessed, as it follows: the total soluble peroxidase activity (EC 1.11.1.7) was measured using guaiacol as reducing substrate [9], p-nitrophenyl phosphate was used as substrate for the assay of acid phosphatase activity (EC 3.1.3.2), at pH 4.7 in acetate (CH_3COOH-CH_3COONa) buffer [10], and the secondary lipid peroxidation products were assayed by the method with thiobarbituric acid [11]. The total soluble protein concentration in the extracts was assayed by the Bradford method [12], and the coupled

enzymatic reaction of glucose oxidase and peroxidase was used for glucose concentration assay.

Acetone extracts were obtained from leaf samples (0.03 g/3 mL), in order to assess the chlorophylls and carotenoids content of the young wheat plants. The extracts were centrifuged for 10 min at 12,000 rpm, and the absorption spectra of the supernatants were recorded from 350 to 750 nm. Pigments content of the leaves was calculated according to Lichtentaler's equations [13], using the absorbance values inferred from the registered spectra of the leaf extracts. All the spectrophotometric measurements were done using a Varian Cary 50 UV-Vis spectrophotometer. The obtained biochemical data were expressed in appropriated units, and reported to the dry weight (DW) of the analysed samples. The average ratio of dry weight (g DW) to fresh weight (g FW) of the samples were 0.09 for leaf, and 0.11 for root, respectively.

The presented results are average values of three measurements ± standard deviations. The calculations and graphs were performed in Microsoft Excel. Bars with different letters are significantly different at $P<0.05$ (Anova Single Factor, $\alpha=0.05$).

3. RESULTS AND DISCUSSION

3.1. Enzyme activity of the soluble peroxidases

In the roots of wheat plants that weren't exposed to Ag^+, the activity of soluble peroxidases (POD, unspecific or guaiacol-type) was 740.38 $U \cdot g_{(DW)}^{-1}$. Compared to the control, the activity increased by about 20% in the plants grown on media with 10 $\mu mol \cdot L^{-1}$ Ag^+ but didn't differ from control at 100 $\mu mol \cdot L^{-1}$ Ag^+ (Figure 1). At low silver concentration, increase of peroxidase activity could be an adaptive response, as these enzymes are involved in cell wall remodeling and changing its permeability.

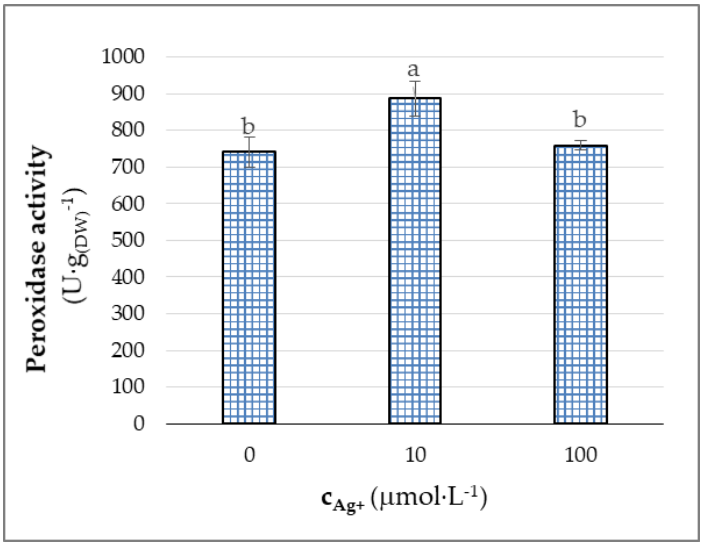

Figure 1. Activity of the unspecific peroxidases in the roots of wheat plants grown on media supplemented with $AgNO_3$.

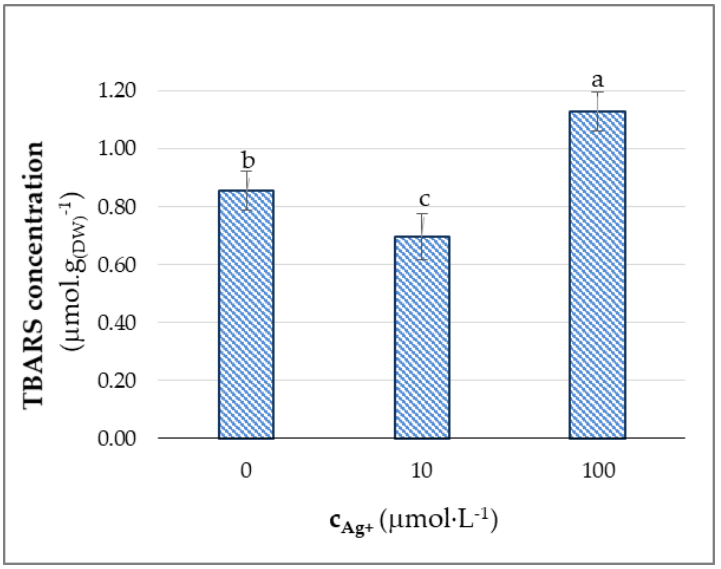

Figure 2. The concentration of the secondary products of lipid peroxidation in the roots of wheat plants grown on media supplemented with $AgNO_3$

3.2. Secondary products of lipid peroxidation (TBARS)

The acronym TBARS (thiobarbituric acid reactive substances) refers to the products of advanced peroxidation of the unsaturated lipids, especially malondialdehyde (MDA) and 4-hydroxynonenal

(HNE). Being more stable than the lipid hydroperoxides that are the primary products of lipid peroxidation, TBARS are often preferred for lipid peroxidation assay, with MDA as the reference compound [14].

MDA derives mainly from the peroxidation of polyunsaturated fatty acids in plant cell membranes [15]. Thus, a high cellular level of MDA is considered an indicator of the oxidative damage of plant cell membranes that can lead to proteins and nucleic acids deterioration. However, at low levels, MDA may act as a protective mean against oxidative stress by activating defense enzymes [15, 16].

Our data on TBARS concentration (Figure 2) pointed out a decrease of approximately 20% in the roots of the plants exposed to 10 $\mu mol \cdot L^{-1}$ Ag^+, and an increase with 32% at 100 $\mu mol \cdot L^{-1}$ Ag^+, compared to the control. It's worth to mention that soluble peroxidase activity (Figure 2) had an opposite trend of variation compared with TBARS.

3.3. Glucose concentration

The concentration of glucose in the roots of wheat plants grown on media without Ag^+ was of 13.42 $mg \cdot g_{(DW)}^{-1}$. A marked dose-dependent increase of glucose concentration was observed in the plants that have grown on media supplemented with Ag^+ (Figure 3): of 76% and 170% respectively, compared to the control, at the two tested concentrations. In addition to its energetic and structural role, glucose is also an important signal molecule involved in the developmental processes and stress responses of plants [17]. In this case, the increase of glucose concentration can be an adaptive response to the osmotic stress that accompanies heavy metal (*i.e.* silver) toxicity.

3.4. Total soluble proteins

Proteins are fundamental constituents of the cells, fulfilling numerous and diverse structural and functional roles. Most of the proteins in a cell are enzymes, catalysts of the biochemical reactions. Under the conditions of this experiment, the amount of soluble proteins in the roots of the plants that weren't exposed to Ag^+ (control) was 50.27±4.92 $mg \cdot g_{(DW)}^{-1}$ (Figure 4). Increased protein contents, with 26.5% and 21% respectively were observed in the plants exposed to Ag^+, at the

two tested concentrations. The soluble protein concentration is not linearly correlated with the Ag^+ dose, as was observed for glucose. Proteins may act as heavy metal chelators, diminishing metal exposure of the sensitive targets and the heavy metal induced oxidative stress.

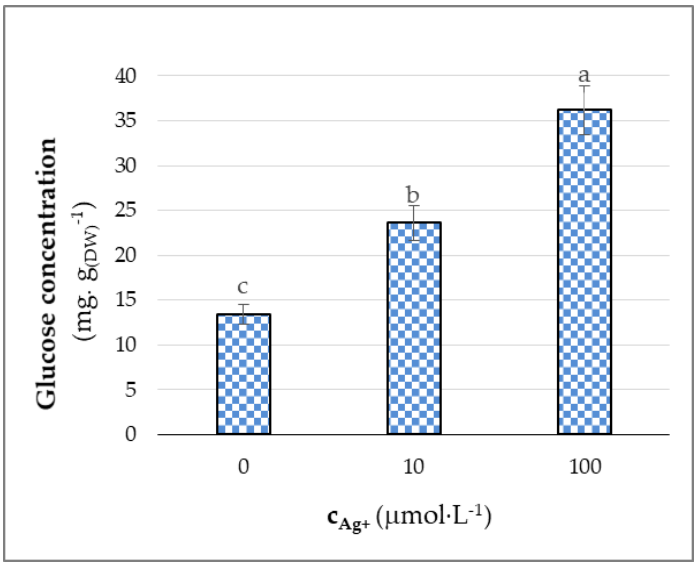

Figure 3. Glucose concentration in the roots of wheat plants grown on media with 10 and 100 µmol·L^{-1} Ag^+, compared to control plants.

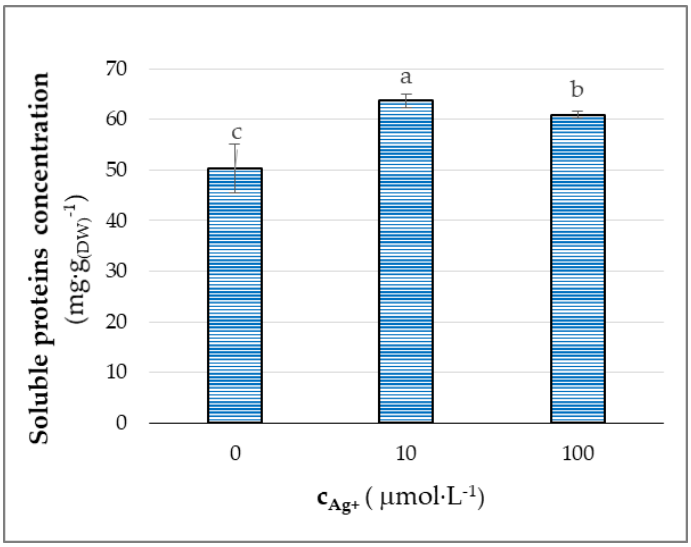

Figure 4. Soluble proteins concentration in the roots of wheat plants grown on media with 0, 10 and 100 µmol·L^{-1} Ag^+, respectively.

3.5. Acid phosphatase activity

In the roots of the wheat plants grown on media without Ag^+, acid phosphatase activity was of 3.6±0.41 $U \cdot g_{(DW)}^{-1}$ (Figure 5). Phosphatase activity increased by 55% compared to control at 10 $\mu mol \cdot L^{-1}$ Ag^+. The 10-fold increase of the exogenous concentration of Ag^+ restored enzyme activity to the value measured in control plants.

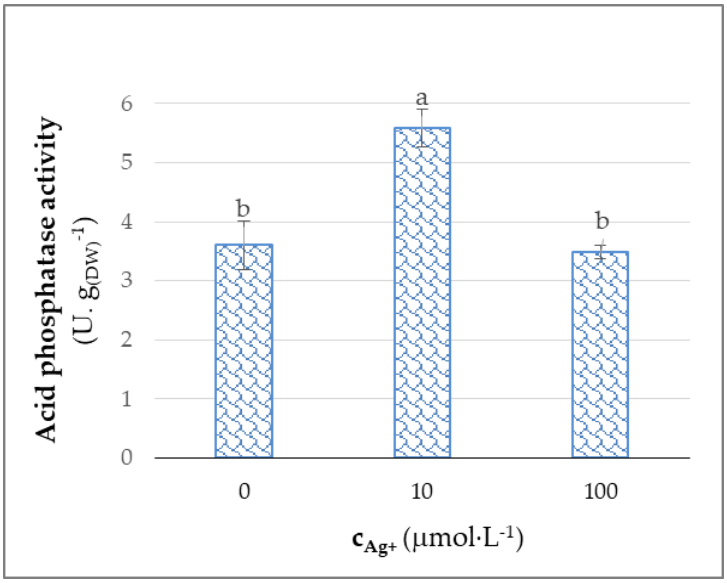

Figure 5. Acid phosphatase activity in the roots of wheat plants grown on media with 0, 10 and 100 $\mu mol \cdot L^{-1}$ Ag^+, respectively.

3.6. Chlorophylls and carotenoids

In photosynthesis, the role of chlorophylls is to capture light energy. Quantitative changes of these pigments contribute to the adaptation of the plants to the growing conditions, for the optimal use of the available light [12]. Carotenoid pigments are involved in photoprotection and optimization of the photosynthetic process by expanding the range of light radiation used in photosynthesis [18].

Data on chlorophylls and carotenoids concentrations in the leaves of wheat plants grown on media containing silver are presented in Figures 6 and 7. Compared to control plants, increased chlorophyll *a* levels were observed in the plants that were exposed to Ag^+, with 10.9% and 16.7% respectively, at the two tested concentrations. Chlorophyll *b*

concentration also increased compared to control, with about 15%, irrespective of the concentration of silver nitrate. Minor increases of the total carotenoids concentrations, that didn't exceed 10% from control value, were also observed in the leaves of silver exposed plants.

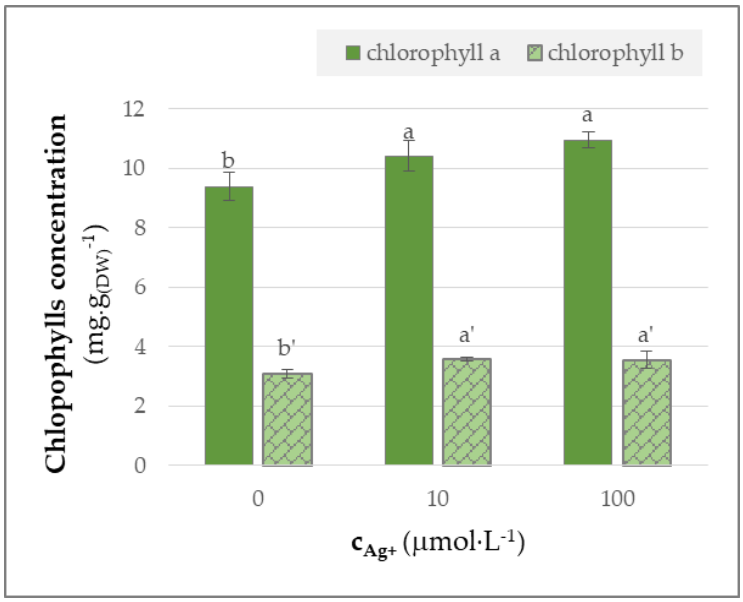

Figure 6. Chlorophylls concentration in the leaves of wheat plants grown on media with 0, 10 and 100 µmol·L^{-1} Ag^{+}, respectively.

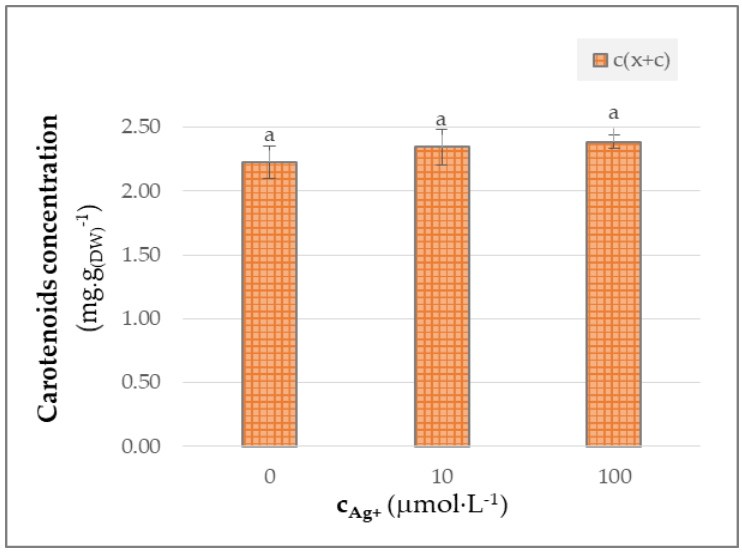

Figure 7. Total carotenoids ($c_{(x+c)}$) concentrations in the leaves of wheat plants grown on media with 0, 10 and 100 µmol·L^{-1} Ag^{+}, respectively

3.7. Growth parameters of the plants

Wheat plants grown on nutrient media without Ag^+ (control) had mean root and stem length values of 107.67±3.95 mm, and 148.49±2.52 mm, respectively. The plants grown on media containing Ag^+, at the two tested concentrations, presented a dose-dependent inhibition of root elongation, by 30% and 40%, respectively, compared to control (Figure 8). Shorter stems were also observed in the plants exposed to Ag^+, but growth inhibition was lower than in roots (with 5% and 10%, respectively, compared to control).

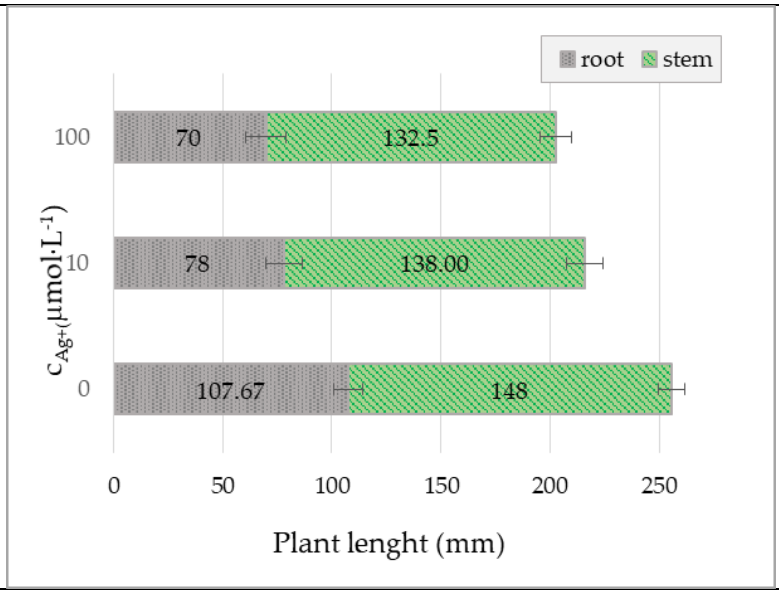

Figure 8. The length of wheat plants grown on media with 10 and 100 μmol·L^{-1} Ag^+, respectively, compared to control plants.

4. CONCLUSION

The exposure of the wheat plants to silver nitrate within their growth media, at concentrations of 10 μmol·L^{-1} and 100 μmol·L^{-1}, respectively, for 7 days, was followed by a dose-dependent inhibition of their growth and modification of some biochemical indices, compared to the unexposed ones.

Increased concentration of the leaf pigments confirms the functionality of the photosynthetic apparatus, and it is suggestive for the expression of an adaptive response, of Ag retention at the root level.

The observed inhibition of root growth is another adaptive response to limit the access of silver ions in the plant, with peroxidases playing an important role in remodeling the cell wall. However, in the absence of a specific mechanism for the retention or exclusion of silver ions, the decrease in the permeability of the root can affect the mineral nutrition of the plant in the long term.

The obtained results pointed out the phytotoxicity of silver but also its regulatory role in plants signaling.

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