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Effects of the short term exposure of wheat seedlings to silver nitrate

Research article

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Abstract

Wheat seedlings grown in the laboratory on liquid nutrient solutions were exposed to Ag^+ at concentrations of 100 μmol·L^{-1} and 200 μmol·L^{-1} by immersing theirs roots in $AgNO_3$ solutions for 90 min. 48 hours thereafter, certain biochemical parameters of the plants were assayed using appropriate analytical methods. Following the exposure to Ag^+, a marked increase of both glucose concentration and soluble peroxidases activity, along with a decrease in the concentration of soluble proteins were measured in the roots of wheat plants, compared to control plants. Leaf chlorophylls and carotenoids contents, as well as the analyzed biometric data did not vary significantly in the plants exposed to Ag^+ compared to control ones. However, significant changes in root branching were observed following the exposure of wheat plants to silver. A regulatory role of Ag^+ through the modulation of phytohormones' signaling is discussed.

Keywords: wheat seedlings, silver nitrate, soluble proteins and glucose concentration, peroxidase activity, photosynthetic pigments, phytohormones, root architecture.

1. INTRODUCTION

Our previous experiments carried out with wheat seedlings that had grown on nutrient solutions supplemented with Ag^+ as $AgNO_3$, at concentrations from the micro molar range, pointed out a dose-dependent inhibition of plants roots elongation, and the modification of certain biochemical parameters compared to the control plants [1]. As both the concentration and duration of plants' interaction with chemicals are important, here we set out to analyse the outcomes of the short term exposure of wheat seedlings to silver nitrate. In the experimental study, the results of which will be presented below, we pursued the following objectives:

- cultivation of wheat seedlings on liquid nutrient media for 7 days, followed by their exposure to $AgNO_3$, for 90 min, at concentrations of 0 (control), 100 and 200 $\mu mol \cdot L^{-1}$, respectively;
- analysis of some biochemical parameters of the plants, namely: the activity of soluble peroxidases, glucose and proteins concentration in the plants roots, and the concentration of photosynthetic pigments in theirs leaves;
- correlation of the obtained results with the level of plants' exposure to silver nitrate.

2. MATERIALS AND METHODS

2.1. Biological material and experimental conditions

Wheat caryopses (*Triticum aestivum* L.) were obtained from the Department of Agricultural and Forestry Technologies of the Faculty of Agronomy at the University of Craiova. Prior to germination, the caryopses were surface sterilized by immersion for 5 min in a 5% NaClO solution, then were washed repeatedly with distilled water. In order to germinate, the caryopses were placed in Petri dishes 90 mm in diameter, between two layers of filter paper moistened with distilled water. The dishes were closed and kept at room temperature, in the dark, for 24 h [2]. Uniformly germinated caryopses were transferred on half strength

Hoagland solutions [3], 20 caryopses per 500 mL, in plastic containers that were kept in the laboratory. 7 days thereafter, part of the plants were exposed to Ag^+ by placing them with their roots in $AgNO_3$ solutions, at two different concentrations: 100 $\mu mol \cdot L^{-1}$ and 200 $\mu mol \cdot L^{-1}$ respectively, while the control plants were put in distilled water (the significance of the experimental variants is presented in table 1). 90 min thereafter, plant roots were washed with distilled water, and the plants were returned on their original nutrient solutions. The plants were further kept under natural lighting conditions (photoperiod of 16/8 hours), and temperature of 25/20 °C. After a 48-hour recovery period, plants were sampled for determination of glucose [4] and soluble proteins concentrations [5], as well as peroxidase enzyme activity [6]. Leaf samples were also collected for the extraction and analysis of photosynthetic pigments [7].

Table 1. Significance of the experimental variants

Experimental variant	$AgNO_3$ 10 $mmol \cdot L^{-1}$/ mL	Final volume/ mL	Ag^+ quantity		
			$\mu mol \cdot L^{-1}$	$mg \cdot L^{-1}$	$mg \cdot probe^{-1}$
1 (CONTROL)	0	500	0	0	0
2	5	500	100	10.786	5.393
3	10	500	200	21.572	10.786

2.2. Sample preparation and biochemical assays

Fresh root samples, having masses of 0.35 g, were homogenized with 4 mL of 20 $mmol \cdot L^{-1}$ Tris-HCl buffer solution, pH 7.5. The obtained homogenates were centrifuged for 10 min, at 12000 rpm and 4°C, in a Sigma 2-16 K centrifuge, and the supernatants were used for the biochemical tests. Glucose from the root extracts was assayed using a glucose oxidase/peroxidase kit, the resulted quinoneimine being monitored at 500 nm [4]. The soluble proteins content was measured using the Bradford method, which is based on the interaction of Coomasie brilliant blue G-250 dye with certain functional groups within the protein. The blue form of the unbound dye turns in brown upon protein binding, and the absorption maxima shifts from 465 nm to 595

nm [5]. The absorbance readings taken at 595 nm for the samples of roots extracts were converted into protein concentration using a calibration curve obtained with bovine serum albumin as standard. Enzyme activity of the soluble peroxidases in plant roots samples was assayed using guaiacol as reducing substrate, the resulted tetraguaiacol being monitored at 436 nm [6]; absorbance values were converted into concentrations using the micro molar extinction coefficient of tetraguaiacol.

For chlorophylls and carotenoids extraction, samples of 0.03 g of fresh leaf tissue were ground using 3 mL of 100% acetone. The extracts were centrifuged for 10 min at 12000 rpm and 4°C, and the supernatants were decanted into clean test tubes; their absorption spectra from 350 to 750 nm were recorded with a Varian Cary 50 spectrophotometer equipped with the Scan Application Software, Version 3.00, at the scanning speed of 300 $nm \cdot min^{-1}$, in glass cuvettes with the optical path of 10 mm. Calculation of the pigments' concentration, as $\mu g \cdot mL^{-1}$ in the acetone extract, was performed using the equations of Lichtenthaler [7].

The obtained biochemical data were expressed in appropriate units and reported to the sample's dry weight (DW). The average values of *DW (g) to FW (g) ratio* were of 0.1 for the leaf samples and 0.0845 for the root samples, respectively. Each sample was performed in triplicate, three different extracts being prepared from each of the experimental variants; the presented results are the mean values ± standard deviations of the assayed parameters.

All the calculation and graphs were done with the Microsoft Excel software, the 2013 version. Within the graphs, bars with different letters are significantly different at $P<0.05$ (Anova, single factor).

3. RESULTS AND DISCUSSION

3.1. Activity of the soluble peroxidases

The activity of soluble peroxidases from the roots of wheat plants that weren't exposed to Ag^+ (control) was 617.42±8.33. $U \cdot g_{(DW)^{-1}}$. In the plants exposed to Ag^+, root peroxidase activity increased compared to

the control plants, by 8.64 % and 16.23% at variants 2 and 3, respectively (Figure 1). The soluble peroxidases' activity in wheat plant roots was positively correlated with the level of exposure to silver. (Pearson's linear correlation coefficient r=0.999).

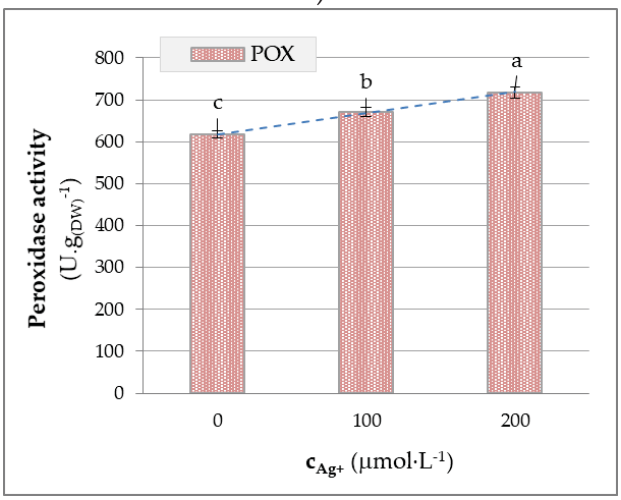

Figure 1. Activity of the soluble peroxidases in the roots of wheat plants, after short-term exposure to different concentrations of silver.

The so-called class III peroxidases or Prxs (EC 1.11.1.7; hydrogen donor: H_2O_2 oxidoreductase), are heme peroxidases, higher plants specific enzymes that exist as large multigene families [8]. Most of these peroxidases are secreted into the apoplast, being involved in modulation of the levels of reactive oxygen species [8-10]. Prxs have an important role in the remodeling of the plants cell wall, both in the sense of permeabilizing and stiffening it, as they can act bidirectionally, by consuming or generating hydrogen peroxide [11]. As a result, these enzymes bear physiological importance in various processes, like cell elongation, lignification, seed germination and plant defense under abiotic and biotic stress conditions. Thus, there are myriad reactions invoving Prxs, due to the spatio-temporal regulation of their gene expression and protein distribution, as well as to the differentiation of theirs oxidizing properties [8]. Therefore, it is difficult, if not impossible to assign a precise role or effect to the increase in peroxidase activity observed in our experiment.

In conditions of heavy metals toxicity, increased peroxidase activity may result in lignification and stiffening of the cell wall at the root level, to block the access of toxic cations. In this experiment, however, no such aspects of silver toxicity were visible, the plants' exposure being of a short duration, and the slightly increased values of the peroxidases' activity (compared to the control) should rather be associated with their regulatory role. It is known that peroxidases participate in the fine modulation of plants' responses to environmental challenges [10, 12, 13], which also involves hormonal adjustments by changes in gene expression. Recent advances in understanding the functions and regulation of Prxs at the molecular level, derived from transcriptomic and proteomic studies, pointed out these enzymes as candidates for crop plant improvement by genetic manipulation, as they are key players in plant responses to abiotic and biotic stress [9, 14, 15].

3.2. Total soluble protein concentration

In the roots of control plants, which were not exposed to Ag^+, the concentration of soluble proteins was 37.19±1.54 $mg \cdot g_{(DW)}^{-1}$. In the plants exposed to Ag^+, concentration of the soluble proteins in roots decreased compared to control by 10.51% and 35.19%, respectively, at the two tested concentrations (Figure 2).

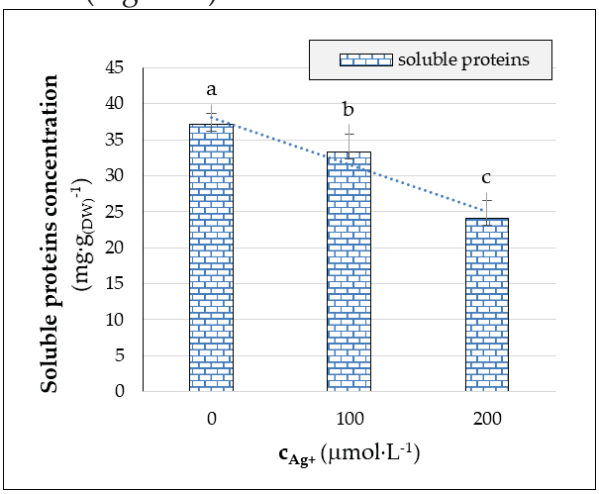

Figure 2. Soluble proteins concentration in the roots of wheat plants, after short-term exposure to silver.

The values of soluble protein concentration were negatively correlated with the exogenous concentration of silver cations (Pearson's linear correlation coefficient r=-0.974). Decreased protein concentration in the roots of the plants exposed to Ag^+ could be the consequence of increased proteolysis, leading to increased content of amino acids and small peptides that are important not only as nitrogen sources for plant growth, but also for the communication within the rhizosphere, as well as between root and shoot [16-19]. Decreased protein concentration in the plants from various species, which had been exposed to silver either as salt or nanoparticles, has been reported by different authors. Qualitative differences regarding the synthesized proteins (*i.e*. the expressed genes) between the silver treated plants and control ones also exist, as revealed by proteomic studies carried out with different plant species [20-22]. The dose-dependent variation of root protein concentration is suggestive for a modified protein turnover within Ag^+ exposed plants, compared to control plants. Protein degradation is important for the disposal of damaged protein, adaptation of the protein complement to the environmental conditions, recycling amino acids and ATP generation by root respiration [23].

3.4. The sugars in plant roots: glucose concentration

Plant sugars, the most common of which are glucose, fructose, and sucrose, play structural, energetic, regulatory, developmental, and adaptive roles [24]. Their distribution is spatio-temporally controlled with the involvement of specific enzymes and transport proteins, for the production of energy and biomass. *Also,* the expression of a large number of genes is transcriptionally regulated by variations in the concentration of soluble sugars [25]. Produced by photosynthesis in the green tissues of the plants and exported to the roots by phloem, sucrose is the major source of carbon for plant cells [26]. The long-distance transport of sugars in plants mainly as sucrose (a disaccharide) is more energetically advantageous than glucose transport. Moreover, since sucrose is a non-reducing sugar, oxidation reactions and glycosylation of proteins are avoided, both during transport through the phloem at high micromolar concentrations and at the target cells [27].

For the coordinated growth and development of a plant, either in times of plenty or during stress conditions, communication is essential. In this regard, sucrose is also a mobile signal that coordinates the development of above- and belowground parts of the plants. As evidenced by the studies carried out with *Arabidopsis*, sucrose is involved in both guiding plant's primary root elongation [28] and initiation of lateral roots [29].

In order for the plants to use sucrose, it is first hydrolyzed to glucose and fructose in equimolar amounts, a reaction catalyzed by a cell wall-bound invertase (EC 3.2.1.26, β-fructofuranosidase**)** [30]. Alternatively, in sink tissues like the roots, sucrose is split by a glycosyl transferase, *(*EC 2.4.1.13, sucrose synthase) providing fructose and UDP-glucose or ADP-glucose for diverse metabolic pathways [31].

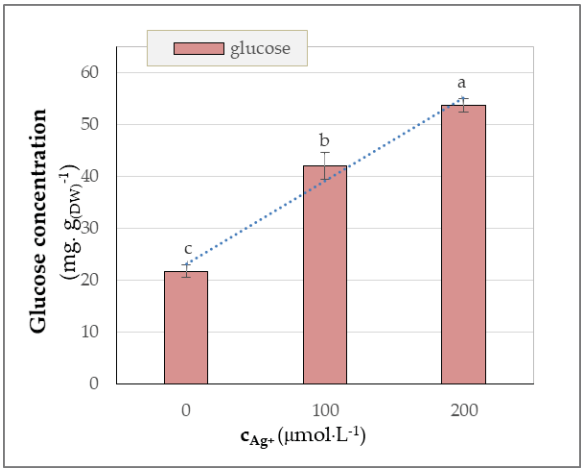

Figure 3. Glucose concentration in the roots of wheat plants, after short-term exposure to silver.

In our experiment, root glucose concentration was 21.74±1.23 $mg \cdot g_{(DW)}^{-1}$ in the roots of wheat plants that weren't exposed to Ag^+ (control). Glucose concentration increased over the control in the plants exposed to Ag^+, by 93.30% and 146.92%, respectively, at the two exogenous concentrations tested (Figure 3). The increase of glucose concentration was positively correlated with the exogenous concentration of silver cations (correlation coefficient r=0.988).

The marked differences regarding root glucose concentration among the experimental variants are suggestive for an increased glucose utilization that occurred in control plants compared to those exposed to

silver. Glucose is directed by a hexokinase to glycolysis, in the cytosol, then to the Krebs cycle, in the mitochondria, to obtain more energy for growth. It is also known that glucose has a hormone-like signaling role. Alongside with ethylene, auxin, cytokinin and other phytohormones, glucose is involved in signaling networks that integrates plant growth and development with environmental challenges [32]. Thus, accumulation of glucose might also have a regulatory significance related to root development, as it is known that high concentrations of glucose inhibit root growth [33].

3.5. Photosynthetic pigments: chlorophylls and carotenoids

In the leaves of wheat plants grown in this experiment, chlorophyll concentrations (Figures 4 and 5) didn't markedly differ from one variant to another, the average value for the three experimental variants being 11.02±0.41 $mg \cdot g_{(DW)}^{-1}$ for chlorophyll *a* and 3.41±0.18 $mg \cdot g_{(DW)}^{-1}$ for chlorophyll b.

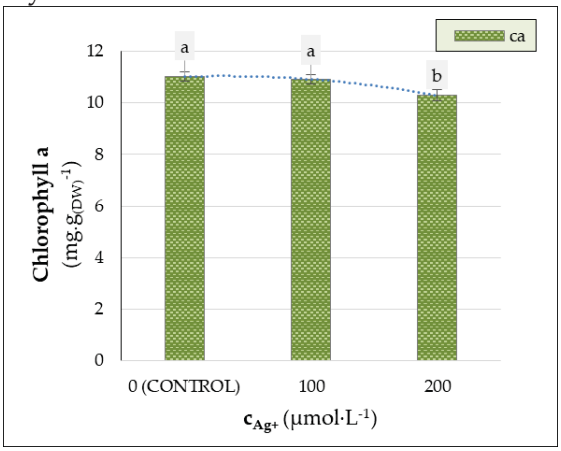

Figure 4. Chlorophyll *a* concentration in the leaves of wheat plants, after the short-term exposure to silver.

However, a downward trend in chlorophylls' concentration was observed as the exogenous concentration of Ag^+ increased, especially chlorophyll *a* (by approx. -7% from control value, at the maximum concentration of tested Ag^+.

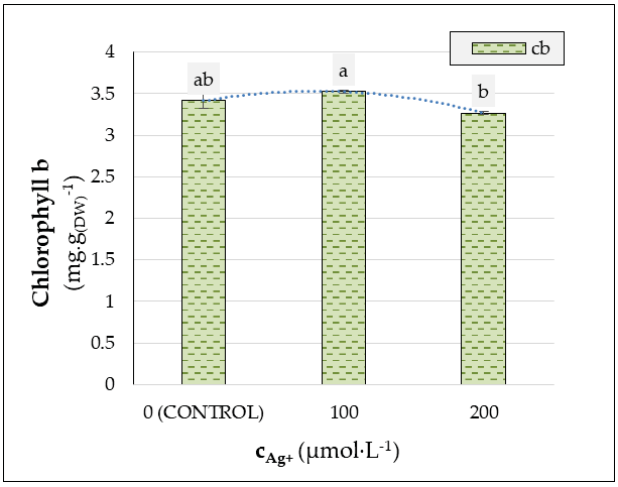

Figure 5. Chlorophyll *b* concentration in the leaves of wheat plants, after the short-term exposure to silver.

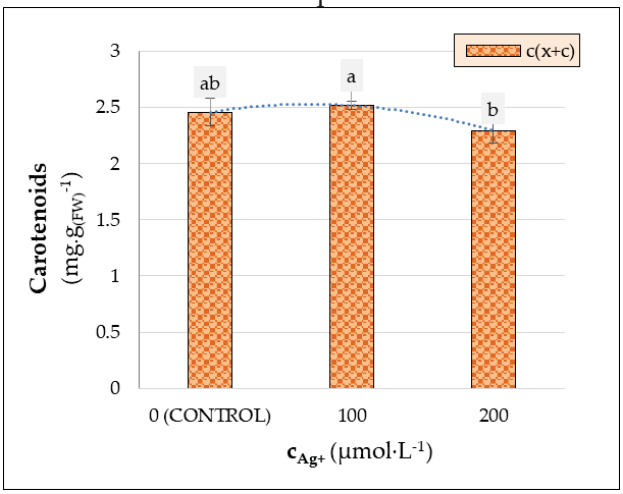

Figure 6. Total carotenoids concentration in the leaves of wheat plants, after the short-term exposure to silver.

Similarly, in the leaves of wheat plants that weren't exposed to Ag^+, the total carotenoids concentration (carotenes and xantophylls, c+x) was of 2.46±0.12 $mg \cdot g_{(DW)}^{-1}$ Carotenoids concentration didn't vary significantly among the experimental variants; a minor decrease, of about 7% was observed in plants exposed to 200 µmol L^{-1} of Ag^+.

3.6. Growth parameters of wheat plants

At the $AgNO_3$ concentrations tested and during the experiment, a marked inhibition of plant growth was not evident; the plants didn't present aspects suggesting Ag^+ toxicity. Thus, for a closer look, we have repeated the experiment, in order to measure the length of the roots and shoots and took photos of the plants. Before the exposure to silver nitrate and 3 days thereafter, the longest root and shoot length of the plants were measured and photographs of the roots were taken. The average values of the biometric data are presented in figure 7.

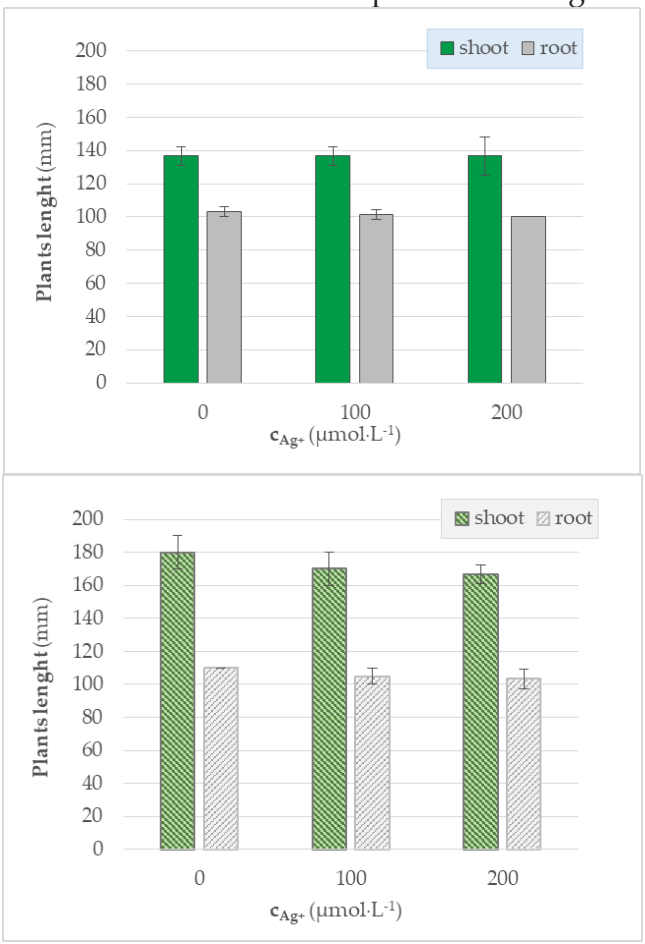

Figure 7. Data on root and shoot length for the plants grown in this experiment *up*: 7-days old wheat plants, before the 90 min exposure to $AgNO_3$; *down:* at 3 days after the exposure to $AgNO_3$.

Prior the exposure to silver, each of the seedlings in the experiment had five roots. From the data presented in Figure 7, it can be seen that after the exposure to Ag the growth rate of the plants' stems was higher than that of theirs roots. However, plant length values weren't significantly different between the groups, at $P<0.05$.

A macroscopic view of the root system of the 11-days old wheat plants is presented in figure 8. As can be seen, the roots of control plant had more and longer branches (lateral roots) than the plants exposed to silver. Moreover, the plants exposed to silver also had a pair of short (new) roots, of 4-6 mm in length.

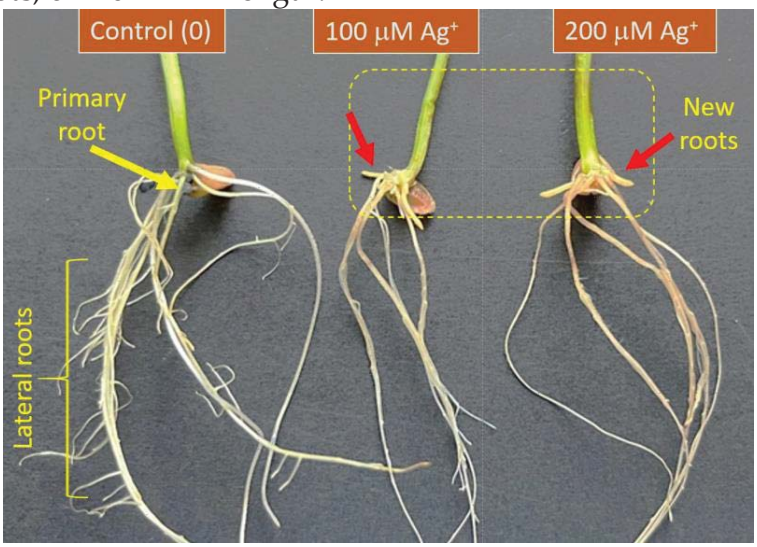

Figure 8. Comparative macroscopic image of the wheat plants' root system at four days from the exposure to silver, alongside the unexposed ones (control). Lateral roots appear as branches of the primary/seminal roots, being more numerous and longer in control plants. New roots of few mm in length were visible in the plants exposed to silver nitrate.

The roots of plants that were treated with Ag^+ had a light-brown color, which intensified during their exposure to ambient light and air. This is suggestive for a physico-chemical interaction of silver with plant roots.

3.7. Silver and phytohormones signaling

In our experiments, the observed differences regarding both biochemical and growth data on wheat plants may be related to a

regulatory role of silver in ethylene's signaling and crosstalk with other phytohormones. Also, our data emphasize the responsiveness of plant roots to environmental challenges that, undoubtedly, involves phytohormones. Ag^+ is well known as an inhibitor of ethylene signaling. As the ethylene receptor has a binding site for Cu^+, it was thought that silver inhibits ethylene signaling by binding at copper site, thus blocking the dimerization of the receptor and the activation of ethylene responses [34]. A noncompetitive inhibition exerted by Ag^+ on ethylene signaling is also considered [35].

Ethylene is a phytohormone whose role, in essence, is to balance the growth and development of plants with environmental challenges [36]. Although considered a stress hormone, ethylene is also a growth hormone: its difficult mission is carried out in crosstalk with the signaling pathways of many other phytohormones [37, 38]. Numerous proteins, with the role of kinases, transcriptional factors and others, alongside with different small molecules are also involved in the expression of its actions.

Ethylene inhibitors are popular for agricultural and horticultural purposes, as well as for the research on its actions at the molecular level. They may act either as ethylene synthesis inhibitors or as ethylene perception inhibitors [39, 35], among these, silver nitrate being probably the most used. Ethylene also is also a promising factor in the attempts to improve crop plants, where root architecture appears as an important target.

In terrestrial plants, roots serve for their anchoring in the soil and for the absorption of water and nutrients; these are essential roles for plant survival, growth and development in an ever-changing environment, and rely on complex, interconnected communication networks**.** As a staple food for humans, wheat is the source for approximately 20% of protein and calories [40]. Its importance for global food security is prominent as a result of both the growing demand due to increased human population [41] and the accelerate climate change [42]. Advanced knowledge on the 'hidden half' of the plants and the direct targeting of root architecture coud lead to crop improvement [43], to meet present and future challenges.

CONCLUSION

Following the short-term exposure to $AgNO_3$, increased soluble peroxidases activity and glucose concentration, as well as decreased concentrations of soluble proteins were observed in the roots of wheat plants, compared to control plants. The quantitative variations of the assayed biochemical parameters were well correlated, positively or negatively, with the exogenous concentration of Ag^+, this aspect being suggestive of a possible causal relationship. The content of leaf pigments (chlorophylls and carotenoids) didn't markedly differ in the plants exposed to Ag^+, compared to control plants.

The roots of the plants exposed to silver were less branched than the control ones, and the branching degree was negatively correlated to the silver concentration. In other words, an effect of silver on root system architecture was observed, that is probably mediated by phytohormone signaling modulation.

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