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# Impact of Silver Nitrate on the Antioxidant System of Rosa canina L.

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# Abstract

Dog rose is a plant species known for its strong antioxidant properties, primarily due to its high content of biologically active compounds. Silver nitrate (AgNO<sub>3</sub>) is widely used in plant biotechnology as an ethylene action inhibitor, capable of delaying senescence and promoting plant development, as well as an antibacterial agent. However, silver ions can also exert phytotoxic effects by inducing excessive production of reactive oxygen species, potentially disrupting cellular homeostasis and causing oxidative stress. In this study, we investigated the effect of silver nitrate on the antioxidant system of *Rosa canina* L. We measured the activity of ascorbate oxidase, ascorbate peroxidase, guaiacol peroxidase, ascorbic acid, vitamin P, and thiol-containing compounds to assess both enzymatic and non-enzymatic antioxidant responses. Guaiacol peroxidase activity increased at all concentrations of AgNO<sub>3</sub>, indicating activation of enzymatic defense mechanisms. The content of vitamin P increased at 10 and 50 mg/L, while ascorbic acid levels decreased at all concentrations of AgNO<sub>3</sub>. The concentration of high-molecular-weight thiols decreased at 50 mg/L of AgNO<sub>3</sub>. These findings provide valuable insights into the modulation of the antioxidant system in dog rose under silver-induced stress, highlighting the potential of *R. canina* as a model for studying plant responses to metal ions exposure. The obtained results may be useful for optimizing micropropagation protocols of *R. canina* and related species, as well as for using this system as a model to study metal-induced oxidative stress in plant tissues *in vitro*.

Keywords: micropropagation, Rosa canina, silver nitrate, antioxidant, reactive oxygen species

#### 1. INTRODUCTION

The dog rose (*Rosa canina* L.) is well known for its high content of biologically active substances, including vitamins, phenolic compounds, flavonoids, fatty acids, saponins, tannins, and other compounds (Roman et al., 2013). These bioactive compounds are extensively utilized in the pharmaceutical, food, and cosmetic industries. Rose hips are particularly valued for their antioxidant and anti-inflammatory properties and are used in the treatment of various diseases.

Some studies have shown that tiliroside, a flavonoid derivative found in dog rose, has anti-obesity effects (Ninomiya et al., 2007), while rose hip extract can reduce blood glucose levels during long-term use. Comparative studies have demonstrated that R. canina possesses one of the highest antioxidant capacities among medicinal plant species (Halvorsen et al., 2002). This activity is mainly attributed to ascorbic acid, carotenoids such as lycopene,  $\beta$ -carotene, rubixanthin, and zeaxanthin, as well as phenolic compounds including quercetin derivatives.

Silver nitrate (AgNO<sub>3</sub>) is extensively used in agriculture and plant biotechnology (Vishwakarma et al., 2017). AgNO<sub>3</sub> is known as an inhibitor of ethylene action, key phytohormone that affects aging, fruit ripening, and leaf fall. Thus, it has the potential to slow down plant aging. Silver ions (Ag<sup>+</sup>) are believed to interfere with ethylene perception by disrupting ethylene binding sites on the receptors (Rodriguez et al., 1999; Kumar et al., 2009). Although AgNO<sub>3</sub> does not inhibit ethylene biosynthesis, it prevents plant tissues from perceiving ethylene through the substitution of copper (Cu<sup>+</sup>) ions, which are essential for ethylene binding at its receptor. As a result, downstream ethylene signaling is effectively blocked.

In addition to its effects on plant physiology, AgNO<sub>3</sub> exhibits antimicrobial activity. Its action is associated with the production of reactive oxygen species (ROS), which damage nucleic acids, inhibit protein synthesis, destroy membranes, decrease chlorophyll contens, inhibit cell wall synthesis, and the activity of the electron transport chain, ultimately leading to cell death (Feng et al., 2000; Yin et al., 2020; Husak et al., 2024b). Consequently, AgNO<sub>3</sub> is extensively used to control microbial contamination and enhance the longevity of cultured plant tissues.

At the same time, excessive accumulation of silver ions may induce oxidative stress in plant cells. Studies indicate that Ag<sup>+</sup> and Ag-based nanoparticles can lead to the overproduction of ROS, lipid peroxidation, and disruption of antioxidant homeostasis (Yan et al., 2019). The release of Ag<sup>+</sup> from AgNO<sub>3</sub> has been identified as a major source of phytotoxicity in numerous plant species. For instance, in *Brassica* plants, AgNO<sub>3</sub> treatment triggers oxidative stress markers, lipid peroxidation, and damage to DNA and cell membranes (Vishwakarma et al., 2017). Thus, while AgNO<sub>3</sub> can serve as a valuable tool for modulating ethylene responses and controlling microbial contamination, it may also impair redox balance and enzyme activity in plant tissues.

Given the high antioxidant potential of *R. canina* and the dual role of AgNO<sub>3</sub> as both a growth regulator and a stress inducer, it is important to elucidate how silver ions affect the antioxidant defense system in this species. Unlike previous studies, this work simultaneously examines key antioxidant enzymes, vitamin P compounds and thiol-containing molecules in R. canina plants grown in vitro under silver nitrate treatment, thus providing an integrated view of silver-induced redox responses in this species. Therefore, the present study aimed to investigate the influence of AgNO<sub>3</sub> at concentrations of 1, 10, and 50 mg L<sup>-1</sup> on the enzymatic and non-enzymatic antioxidant components of *R. canina* cultivated *in vitro*. The activities of ascorbate oxidase, ascorbate peroxidase, and guaiacol peroxidase, as well as the contents of ascorbic acid, vitamin P, and thiol-containing compounds, were measured to evaluate the plant's physiological response to silver-induced oxidative stress.

#### 2. MATERIALS AND METHODS

#### 2.1. Reagents

All other reagents were received from local suppliers (Ukraine) and they were of analytical grade.

# 2.2. Plant growing conditions

The influence of varying silver nitrate concentrations on biochemical parameters was investigated in dog rose plantlets. Dog rose plantlets, previously established *in vitro* as microclonal cultures in our laboratory, were transferred to glass jars containing 25 mL of Murashige and Skoog (MS) medium, supplemented with 3% sucrose, 100 mg L<sup>-1</sup> myo-inositol, 6.0 g/L agar, 3 mg/L benzylaminopurine, and 0.1 mg/L indole-3-butyric acid (IBA) (Murashige and Skoog, 1962). The following concentrations of silver nitrate were added to the medium: 0 (control), 1, 10, and 50 mg L<sup>-1</sup>. Each biological replicate consisted of five plants, and eight biological replicates were performed for each treatment. The plantlets were maintained for 40 days at 24–26 °C under a photoperiod of 16 hours of light (50 µmol m<sup>-2</sup> s<sup>-1</sup>, cool white light, light-emitting diode) and 8 hours of darkness.

# 2.3. Determination of enzyme activities

Ascorbate peroxidase (APX) activity was determined spectrophotometrically (Chen et al., 1989). The decrease in OD was recorded for 90 seconds at a wavelength of 290 nm. For calculations, the molar extinction coefficient of ascorbate was used 2800 M<sup>-1</sup>cm<sup>-1</sup>. The protein concentration in the sample was determined separately.

Ascorbate oxidase (AO) activity was also determined spectrophotometrically by recording a decrease in optical density at 265 nm (Diallinas et al., 1997). The molar extinction coefficient of 14000  $\,\mathrm{M}^{-1}\mathrm{cm}^{-1}$  was used for calculations.

Guaiacol peroxidase (GPX) activity was assessed by measuring the change in absorbance resulting from the oxidation of guaiacol to tetraguaiacol at 412 nm. The molar extinction coefficient of tetraguaiacol ( $\varepsilon$  = 26,600 M<sup>-1</sup>·cm<sup>-1</sup>) was used for quantification.

Enzyme activities were expressed as U/mg protein or mU/mg protein, depending on the magnitude of the measured activity.

To determine the total protein content, the Bradford method was used, which is based on the ability of protein to bind to the Coomassie G-250 dye (Bradford, 1976). The optical absorbance was recorded at 595 nm.

## 2.4. Determination of thiol-containing compounds

Thiol-containing compounds were quantified using the thiol-disulfide exchange reaction with DTNB (Ellman's reagent) (Ellman, 1959). Low-molecular-weight thiols (LMW-thiols) were determined after protein precipitation with 30% trichloroacetic acid (TCA) added to the supernatant at a 2:1 ratio, followed by centrifugation (5 min, 13,000 rpm, 4 °C). The clarified supernatant was then reacted with 100 mM Tris-HCl and DTNB; a blank without supernatant was prepared in parallel. Total thiols were measured in non-precipitated samples by adding 100 mM potassium phosphate buffer, DTNB, and supernatant without TCA treatment. After incubation at room temperature for 30 min, absorbance was recorded at 412 nm. The content of high molecular weight thiols (HMW-thiols) was calculated as the difference between total thiols and LMW-thiols (HMW-thiols).

# 2.5. Determination of ascorbic acid content

Ascorbate content was determined using Tillman's method, which is based on the oxidation of ascorbic acid to dehydroascorbic acid by 2,6-dichlorophenolindophenol (DCPIP). The titration is carried out in an acidic environment to prevent degradation of ascorbic acid. For the analysis, 1 g of raw plant material was homogenized in a mortar with 2 mL of 1% hydrochloric acid. Then, 2 mL of oxalic acid was added, and the total volume was adjusted to 8 mL with distilled water. From this extract, 2 mL was taken and titrated with 0.001N DCPIP solution until a persistent pink colour was observed.

#### 2.6. Determination of vitamin P content

The vitamin P content was determined by titration with 0.1 N potassium permanganate solution in the presence of indigocarmine. A 0.5 g sample of the plant material was mixed with 20 mL of preheated boiling water and boiled for 5 minutes. The resulting extract was cooled, filtered, and the volume was adjusted to its original value. The leaf extract was diluted 10-fold. An aliquot of 0.4 mL of the diluted extract was combined with 12.5 mL of distilled water and 2.5 mL of indigo carmine solution. The mixture was titrated with 0.1 N potassium permanganate (KMnO<sub>4</sub>) solution until a yellow color appeared. A control titration was performed using 12.9 mL of distilled water to which 2.5 mL of indigo carmine solution was added.

## 2.7. Statistical Analysis

Data are presented as the mean ± standard error of the mean (SEM). Outliers were identified using the Chauvenet and Dixon tests. Statistical differences between the control and experimental groups were assessed using Dunnett's test (Python 3.13.10). A probability value of p<0.05 was considered statistically significant.

#### 3. RESULTS AND DISCUSSION

## 3.1. Effect of silver nitrate on enzyme activities

Guaiacol peroxidase is the main heme-containing peroxidase in plants, which removes excess hydrogen peroxide, playing a key role in protecting plants from stress and in the biosynthesis of lignin and other cell wall components (Sharma et al., 2012). Our experiment found that guaiacol peroxidase activity increased under the influence of all concentrations of silver nitrate. In plants treated with AgNO<sub>3</sub> at concentrations of 1, 10, and 50 mg/L, activity increased by 1.9, 2.3, and 2.3 times, respectively (Fig. 1).

Treatment with silver nitrate has been reported to induce the accumulation of phenolic compounds in plant tissues, which act as substrates for guaiacol peroxidase and may contribute to the observed increase in its activity. The activity of ascorbate peroxidase in leaves of dog rose was not statistically different between the control and experimental groups under the influence of various concentrations of silver nitrate (Table 1).

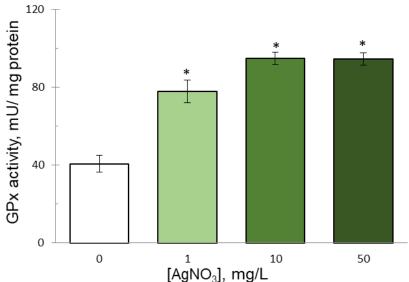


Fig. 1. The activity of GPX in dog rose leaves, exposed to different concentrations of AgNO<sub>3</sub>. Data are presented as means  $\pm$  SEM, n = 6-8. \*Significantly different from the control (without AgNO<sub>3</sub>) group of plants (p < 0.05) according to ANOVA followed by Dunnett's test.

Previous studies have demonstrated similar results when investigating the effect of different concentrations of silver nitrate (10  $\mu$ M, 20  $\mu$ M, and 50  $\mu$ M) on *Allium cepa* L. During the 6 h direct

treatment, GPX activity increased 0.5-fold in 50  $\mu$ M as compared to the control in shoots of *A. cepa* (Pradhan et al., 2017). Santos et al. (2001) reported that under KCl-stress, the accumulation of  $H_2O_2$  led to catalase inactivation in sunflowers. Increase in activity of GPX could be due to activation or new synthesis of GPX by the AgNO<sub>3</sub> treatment, which is associated with the deactivation of catalase due to overproduction of  $H_2O_2$ .

Gheisary and colleagues (2018) reported that treatment of *Hyoscyamus reticulatus* L. with different concentrations of silver nitrate (0, 0.5, 1, 1.5, and 2 mM) resulted in a marked increase in guaiacol peroxidase activity, indicating activation of the enzymatic antioxidant defense system in response to  $Ag^+$  exposure. The highest GPX activity was observed in hairy roots treated with 2 mM  $AgNO_3$  after 24h. To mitigate the harmful effects of ROS, plants enhance the activity of antioxidant enzymes such as guaiacol peroxidase, which catalyzes the reduction of  $H_2O_2$  to water using phenolic compounds as electron donors. This enzymatic response helps to prevent oxidative damage to lipids, proteins, and nucleic acids, thereby maintaining redox homeostasis and protecting cellular integrity. Moreover, the authors proposed that the activation of GPX is dose-dependent: low concentrations of silver ions stimulate antioxidant defenses as an adaptive mechanism, whereas higher concentrations may exceed the plant's detoxification capacity, resulting in oxidative injury.

In the study by Khan et al. (2019), the activity of guaiacol peroxidase (GPX) in *Pennisetum glaucum* L. seedlings decreased by 31% and 18% under 2 mM AgNO<sub>3</sub> and AgNPs treatments, respectively, while at 6 mM the reduction reached 61% and 51%, indicating a strong inhibitory effect of silver compounds on antioxidant enzyme activity. In the present study, *P. glaucum* seedlings treated with AgNPs exhibited a decrease in antioxidant enzyme activities compared with the control; however, this reduction was less pronounced than that observed in plants treated with AgNO<sub>3</sub>. This suggests that AgNPs may strongly interact with proteins located in the lipid bilayer and cytosol, leading to conformational changes that adversely affect the antioxidant defense system (McShan et al., 2014). Previous reports have indicated that the influence of AgNPs on antioxidant enzymes depends on plant species, concentration, and duration of exposure (Thuesombatet et al., 2016).

**Table 1.** AO and APx activity in rose hips grown under control conditions or exposed to 1, 10, and 50 mg/L  $AgNO_3$  for 40 days

Parameters	Plant group			
_	Control	1 mg/L	10 mg/L	50 mg/L
AO, U/mg protein	$438 \pm 42$	$466 \pm 25  \mathrm{ns}$	$484 \pm 15$ ns	$483 \pm 34$ ns
APX, U/mg protein	$5.29 \pm 0.13$	$5.03 \pm 0.22$ ns	$4.96\pm0.48~\mathrm{ns}$	$5.23 \pm 0.35$ ns

**Note:** Data are presented as means  $\pm$  SEM, n = 6-8; ns – not different from the control value (p<0.05)

Previous studies have shown that *Populus nigra* L. plants exhibited a significant increase in APX activity when treated with AgNPs at concentrations of 2.5 mg/L and 5 mg/L, but when treated with AgNO<sub>3</sub>, no statistical differences in APX activity were found at either 2.5 or 5 mg/L. Under conditions of moderate stress, the antioxidant defense system is activated more effectively, whereas at higher doses, prolonged exposure can lead to enzyme inhibition or depletion of antioxidant capacity. The authors also emphasized that the phytotoxicity of AgNPs is not only due to the release of Ag<sup>+</sup> ions, but is also closely related to the physicochemical properties of functionalized nanoparticles, which can enhance the formation of ROS and, as a result, cause stronger APX activation compared to AgNO<sub>3</sub> (Iori et al., 2023).

In contrast, the results of the effect of silver nitrate on *Paulownia* were previously obtained, where ascorbate peroxidase activity increased at 1 and 10 mg/L (Husak et al., 2024a). An increase in APX activity was also observed in Lemna species at different concentrations (0, 20 and 50 mg/L) of AgNPs. Although the functionalized silver nanoparticles caused less cellular damage than AgNO<sub>3</sub>, they still promoted the activation of APX. The authors suggested that even a limited release of Ag<sup>+</sup> ions from the nanoparticles, or the intrinsic physicochemical properties of the particles themselves (such as surface reactivity and interaction with cellular membranes), could trigger the antioxidant response. Therefore, the alteration in APX activity observed under both treatments can be interpreted as a compensatory response of duckweed cells to silver-induced oxidative stress, resulting from the accumulation of silver and/or the interaction of nanoparticles and ionic silver with cellular structures (Iannelli et al., 2022)

As presented in Table 1, AO activity in *R. canina* leaves remained statistically unchanged compared with the control under exposure to all tested concentrations of silver nitrate. In contrast, sunflower plants were observed to have increased AO activity with increasing silver nitrate levels. Increased AO activity was associated with enhanced oxidative stress and the need to regulate apoplastic redox homeostasis under exposure to Ag<sup>+</sup> and AgNPs (Saleeb et al., 2019).

Changes in the activity of antioxidant enzymes caused by the addition of silver nitrate during cultivation indicate the occurrence of oxidative stress and the action of active oxygen species. Increased enzyme activity indicates the mobilization of enzymatic antioxidant systems under these conditions (Kang et al., 2007).

# 3.2 Effect of silver nitrate on the content of thiol-containing compounds

Low- and high-molecular-weight thiol-containing compounds play an important role in antioxidant protection, acting as reducing agents and electron acceptors, thereby neutralizing reactive oxygen species. The most common low-molecular-weight thiol is glutathione. Existing in reduced (GSH) and oxidized (GSSG) forms, glutathione is involved in maintaining the redox balance of the cell. It is capable of reacting with metals and detoxifying xenobiotics. Glutathione reduces the binding of metals to the thiol groups of proteins, preventing a decrease in enzyme activity (Jozefczak, 2012). This tripeptide can also bind to protein cysteines, reducing protein damage (Kükürt et al., 2021). In this context, Ag+ ions can be bound by thiol groups of glutathione and related peptides, and GSH may participate in the formation of metal-thiol complexes and phytochelatins, thereby contributing to silver detoxification in plant cells. As shown in Figure 2a, the content of low molecular weight thiols did not change under the influence of any of the silver nitrate concentrations.

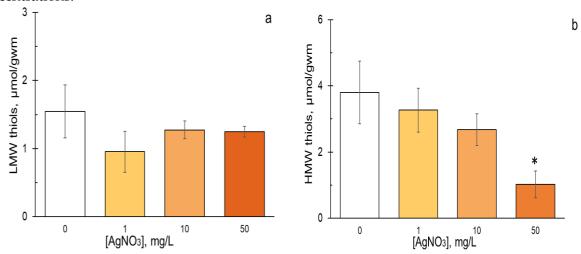


Fig. 2. The concentrations of low- (a) and high-molecular-weight thiols (b) ( $\mu$ mol/gwm) in plants, exposed to different concentrations of AgNO<sub>3</sub>. Data are presented as means  $\pm$  SEM, n = 6-8. \*Significantly

different from the control (without  $AgNO_3$ ) group of plants (p < 0.05) according to ANOVA followed by Dunnett's test.

The content of high molecular weight thiols was 3.7-fold lower compared to the control at a silver nitrate concentration of 50 mg/L (Fig. 2b). The same results were obtained by Dewez and colleagues (2018), who studied the effect of silver nanoparticles on *Lemna gibba* L. A decrease in high-molecular-mass thiols under exposure to silver nitrate or silver nanoparticles may result from several interconnected mechanisms. Silver ions (Ag+) can directly bind to sulfhydryl (–SH) groups of proteins, leading to thiol oxidation and the formation of Ag–S complexes that reduce the pool of protein-bound thiols. In addition, Ag-induced oxidative stress enhances the generation of ROS, which can oxidize thiol groups and promote disulfide bond formation, thereby altering the redox state of cellular proteins (Tripathi et al., 2017). Moreover, AgNPs may disrupt cellular membranes and protein folding, contributing to protein denaturation and aggregation, which further decreases the availability of reactive thiols (Vannini et al., 2014).

#### 3.3 Effect of silver nitrate on vitamin contents

Figure 3 illustrates that exposure to silver nitrate at concentrations of 1, 10, and 50 mg/L resulted a decrease to 85, 85 and 81% of the control, respectively. Similar results were demonstrated in the aquatic plant *Lemna gibba* L. (Varga et al., 2018), where treatment with colloidal silver led to a decrease in the content of ascorbic acid. Exposure to silver nitrate or silver nanoparticles can lead to a decrease in ascorbic acid (vitamin C) content in plants through multiple mechanisms. Silver ions and nanoparticles induce oxidative stress by generating reactive oxygen species, which are readily scavenged by ascorbate, leading to its depletion. Additionally, Ag<sup>+</sup> can interact with ascorbate directly, promoting its oxidation and conversion to dehydroascorbate, thereby reducing the pool of reduced ascorbic acid.

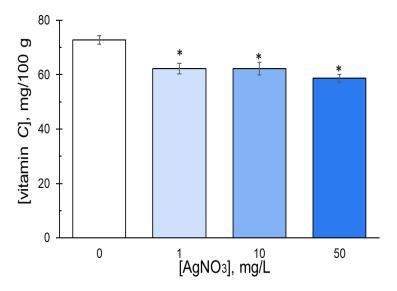


Fig. 3. The content of ascorbate (mg 100 g<sup>-1</sup> fresh weight) in plants, exposed to different concentrations of AgNO<sub>3</sub>. Data are presented as means  $\pm$  SEM, n = 6-8. \*Significantly different from the control (without AgNO<sub>3</sub>) group of plants (p < 0.05) according to ANOVA followed by Dunnett' test.

The observed decrease may be related to ascorbate's antioxidant properties, as it is known to be one of the most potent antioxidant molecules. Ascorbate can donate electrons to reactive oxygen species (Paciolla et al., 2016) and participates in the removal of lipid radicals, thereby contributing to the reduction of lipid peroxidation (Szarka et al., 2012). Its content in the apoplast is crucial for

plant stress perception (Horemans et al., 2000). In addition, ascorbate serves as an electron donor for antioxidant enzymes, including ascorbate oxidase and ascorbate peroxidase.

Vitamin P is a collective name for some flavonoids, such as rutin, quercetin, heme, which are not a plant vitamin but have anti-inflammatory, antihemorrhagic activity, and a strengthening effect on human capillaries (Kurisawa, M. et al., 2003). In the present study, the rutin content was measured. It is known about the antioxidant properties of rutin and the ability to chelate metal ions. At a silver nitrate concentration of 1 mg/L, the rutin content remained unchanged. At concentrations of 10 and 50 mg/L, it increased by 1.2- and 1.6-fold, respectively (Fig. 4).

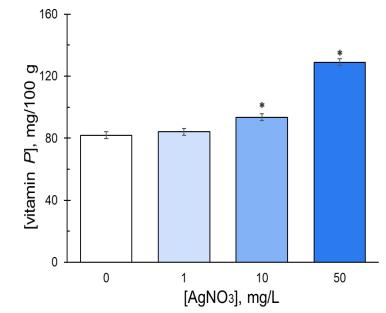


Fig. 4. The content of vitamin P (mg 100 g<sup>-1</sup> fresh weight) in plants, exposed to different concentrations of AgNO<sub>3</sub>. Data are presented as means  $\pm$  SEM, n = 6-8. \*Significantly different from the control (without AgNO<sub>3</sub>) group of plants (p < 0.05) according to ANOVA followed by Dunnett' test.

Similar results were described by Pedroso and colleagues (2019), in a study related to the effect of silver nitrate on the growth of *Hyptis marrubioides*. It was shown that the addition of AgNO<sub>3</sub> to the medium increased the rutin content by 1.17 times. The results of Salih et al. who studied the effect of silver nanoparticles on the content of bioactive compounds in *Juniperus procera* also showed an increase in rutin content under the influence of 50 mg/L AgNPs (Salih et al., 2022).

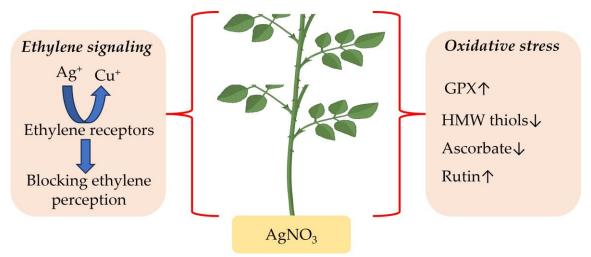


Fig. 5. Expanded hypothetical model of silver nitrate (AgNO<sub>3</sub>) action in *Rosa canina in vitro*. Ag<sup>+</sup>-mediated inhibition of ethylene perception and ROS-driven redox imbalance leading to increased lipid peroxidation, depletion of low- and high-molecular-weight antioxidants (ascorbate

and thiols), adaptive induction of guaiacol peroxidase, and stress-associated activation of phenylpropanoid metabolism with rutin accumulation (10−50 mg/L AgNO₃).

Considering the obtained changes in antioxidant parameters of R. canina, several mechanisms may underlie the action of silver nitrate in vitro. Silver ions released from AgNO<sub>3</sub> can substitute for copper in ethylene receptors, thereby blocking ethylene perception and modulating developmental and stress-related signalling pathways (Rodríguez et al., 1999; Kumar et al., 2009). At the same time, Ag+ and Ag-based particles are known to stimulate the generation of reactive oxygen species, which leads to enhanced lipid peroxidation and disturbance of redox homeostasis (Yan and Chen, 2019). Under such conditions, ascorbate and thiol-containing compounds are consumed as primary lowmolecular-weight antioxidants and redox buffers; their oxidation and participation in detoxification reactions may explain the decline in ascorbic acid content and the decrease in high-molecular-mass thiols at the highest AgNO<sub>3</sub> concentration (Fig. 5) (Jozefczak et al., 2012; Tripathi et al., 2017). Ascorbate is directly involved in ROS scavenging and in the functioning of the ascorbateglutathione cycle, so its depletion is a typical marker of oxidative stress in plant cells (Horemans et al., 2000; Paciolla et al., 2016; Szarka et al., 2012). In parallel, activation of guaiacol peroxidase likely reflects an adaptive reinforcement of the enzymatic antioxidant system, which uses phenolic substrates to reduce H<sub>2</sub>O<sub>2</sub> and limit oxidative damage. The observed increase in rutin content at 10 and 50 mg/L AgNO<sub>3</sub> may be associated with stimulation of the phenylpropanoid pathway under silver-induced stress and with the ability of flavonoids to chelate metal ions and scavenge ROS, thus contributing to the overall antioxidant capacity of dog rose tissues (Fig. 5) (Kurisawa et al., 2003; Pedroso et al., 2019; Salih et al., 2022).

#### 4. CONCLUSIONS

The results showed no statistically significant changes in ascorbate oxidase or ascorbate peroxidase activity. However, guaiacol peroxidase activity increased at tested concentrations of AgNO<sub>3</sub>. The content of vitamin P increased at 10 and 50 mg/L silver nitrate in the medium, while ascorbic acid levels decreased at all concentrations. The concentration of low-molecular-weight thiol-containing compounds remained unchanged, whereas high-molecular-weight thiols decreased at 50 mg/L of AgNO<sub>3</sub>. These findings may be useful for optimizing the use of silver nitrate in micropropagation protocols of *R. canina* and related species.

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**Author contributions:** VH: conceptualization, writing – review and editing. UK, VN: methodology, data collection, figures, tables, writing – original draft preparation. US: writing – review and editing.

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#### **Declarations**

**Conflict of interest**. The author declares no competing interests relevant to this article. **Research involving human participants and/or animals**. Not applicable.

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**Гусак В., Кільчицька У., Николишин В., Стамбульська У.** (2025) Вплив нітрату срібла на антиоксидантну систему *Rosa canina* L. Журнал Прикарпатського національного університету імені Василя Стефаника. Біологія 12: 34-45.

#### Анотація

Шипшина — це рослина, відома своїми сильними антиоксидантними властивостями, що зумовлено, насамперед, високим вмістом біологічно активних сполук. Нітрат срібла (AgNO<sub>3</sub>) широко використовується в рослинній біотехнології як інгібітор дії етилену, здатний уповільнювати старіння і сприяти розвитку рослин, а також як антибактеріальний засіб. Однак іони срібла можуть також чинити фітотоксичну дію, викликаючи надмірне вироблення активних форм кисню, що може порушувати клітинний гомеостаз і викликати окислювальний стрес. У цьому дослідженні ми вивчали вплив нітрату срібла на антиоксидантну систему Rosa canina L. Рослини культивували на середовищі Мурасіге і Скуга, доповненому AgNO<sub>3</sub> у концентраціях 1, 10 і 50 мг/л протягом 40 днів. Ми вимірювали активність аскорбатоксидази, аскорбатпероксидази, гваяколпероксидази, аскорбінової кислоти, вітаміну Р і тіольмісних сполук для оцінки як ферментативних, так і неферментативних антиоксидантних реакцій. Активність гваяколпероксидази збільшувалася при всіх концентраціях AgNO<sub>3</sub>, що вказує на активацію ферментативних захисних механізмів. Вміст вітаміну Р збільшувався при 10 і 50 мг/л, тоді як рівень аскорбінової кислоти зменшувався при всіх концентраціях. Концентрація високомолекулярних тіолів знизилася при 50 мг/л. Ці результати надають цінну інформацію про модуляцію антиоксидантної системи в шипшині під впливом стресу, спричиненого сріблом, підкреслюючи потенціал R. canina як моделі для вивчення реакцій рослин на вплив іонів металів.

**Ключові слова:** мікророзмноження, *Rosa canina*, нітрат срібла, антиоксидант, активні форми кисню