PEROXIDASE ACTIVITY AND ISOENZYME PATTERN IN TOBACCO PLANTS EXPOSED TO SILVER NANOPARTICLES AND SILVER NITRATE

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INTRODUCTION

With a rapidly growing implementation of nanomaterials in various consumer products, comes an even greater environmental concern. Silver nanoparticles (AgNPs) are the most commonly used nanomaterial in consumer products due to their antibacterial and antifungal properties. However, being released to water or soil, AgNPs are likely to interact with plants, which are a vital part of the ecosystem, and thereby enter the food chain. Although AgNPs are known to induce toxicity in prokaryotic and eukaryotic organisms, their effect on plants has not been fully elucidated.[1] In this study, tobacco (*Nicotiana tabacum*) plants were simultaneously exposed to the same concentrations (25, 50, 75, 100 and 500 µM) of AgNPs and ionic silver ($AgNO_3$), in order to determine AgNPs potential phytotoxic effects on this economically important crop plant. In terms of oxidative stress, changes in activities of antioxidant enzymes, pyrogallol (PPX) and ascorbate peroxidase (APX), were determined in both roots and leaves and compared to non-exposed, control plants. Furthermore, changes in PPX and APX isoforms expression in treated plants were observed in regard with Ag form and its concentration and compared to control.

MATERIALS AND METHODS

TREATMENTS:

TOBACCO SEEDS

Cultivation on solid Murashige Skoog (MS)[2] medium **60 DAYS**

ADULT PLANTS

$AgNO_3$ treatment | **AgNPs treatment** | **Control (Millie Q water)**

25, 50, 75, 100 or 500 µM

7 DAYS

ANALYSES:

1. Concentration of accumulated Ag measured by ICP MS (ELAN DRC-e)

2. Soluble proteins extraction

EXTRACTED PROTEINS

APX

a) **APX isoform pattern[3]**
b) **APX activity[4]**

PPX

a) **PPX isoform pattern[5]**
b) **PPX activity[4]**

RESULTS

Table 1. Concentration of Ag measured in roots and leaves of adult tobacco plants. The results represent the mean value of 6 replicates ± standard error. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test. Value < 0.0001 µg g^{-1} represents instrument quantification bound

Treatment	ADULT PLANTS	
	ROOT	LEAF
	Ag concentration (µg g^{-1} $_{fresh\ weight}$)	Ag concentration (µg g^{-1} $_{fresh\ weight}$)
Control	< 0.0001^{a}	< 0.0001^{a}
25 µM AgNPs	1247.4 ± 122.0^{b}	12.1 ± 2.6^{b}
50 µM AgNPs	1395.2 ± 351.5^{b}	13.9 ± 2.6^{b}
75 µM AgNPs	1712.1 ± 80.8^{bc}	19.0 ± 1.0^{b}
100 µM AgNPs	1742.2 ± 192.8^{bc}	36.3 ± 3.4^{c}
500 µM AgNPs	2480.1 ± 141.9^{d}	79.2 ± 5.2^{d}
25 µM $AgNO_3$	1121.5 ± 136.2^{b}	18.2 ± 4.0^{b}
50 µM $AgNO_3$	1450.9 ± 436.2^{b}	21.4 ± 7.3^{b}
75 µM $AgNO_3$	1741.7 ± 134.5^{bc}	23.0 ± 4.1^{b}
100 µM $AgNO_3$	1747.4 ± 150.0^{bc}	38.3 ± 5.4^{c}
500 µM $AgNO_3$	2399.1 ± 310.6^{cd}	82.2 ± 3.9^{d}

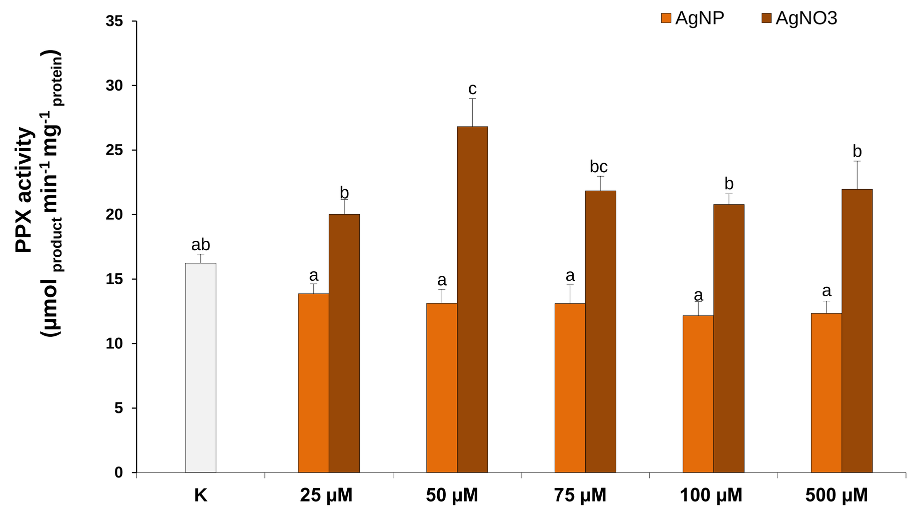

Figure 1. Activity of PPX in tobacco roots after exposure to 25, 50, 75, 100 and 500 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. K - control. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

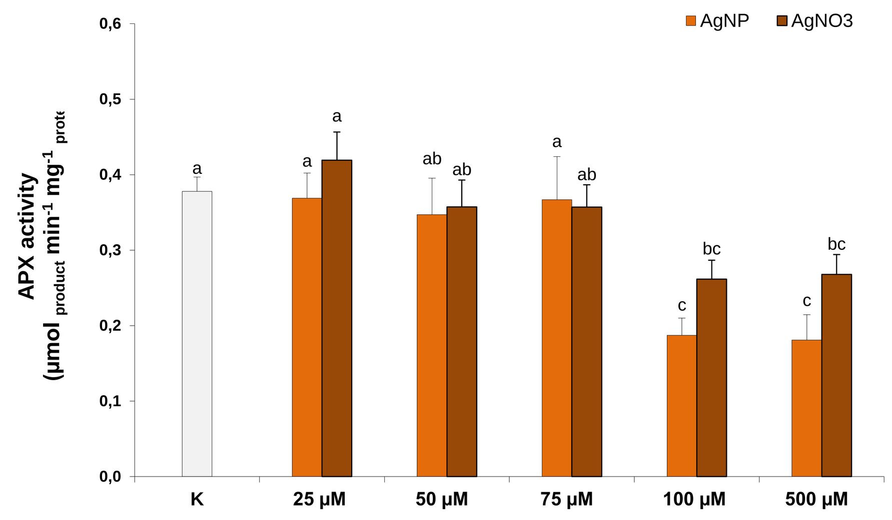

Figure 2. Activity of APX in tobacco roots after exposure to 25, 50, 75, 100 and 500 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. K - control. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

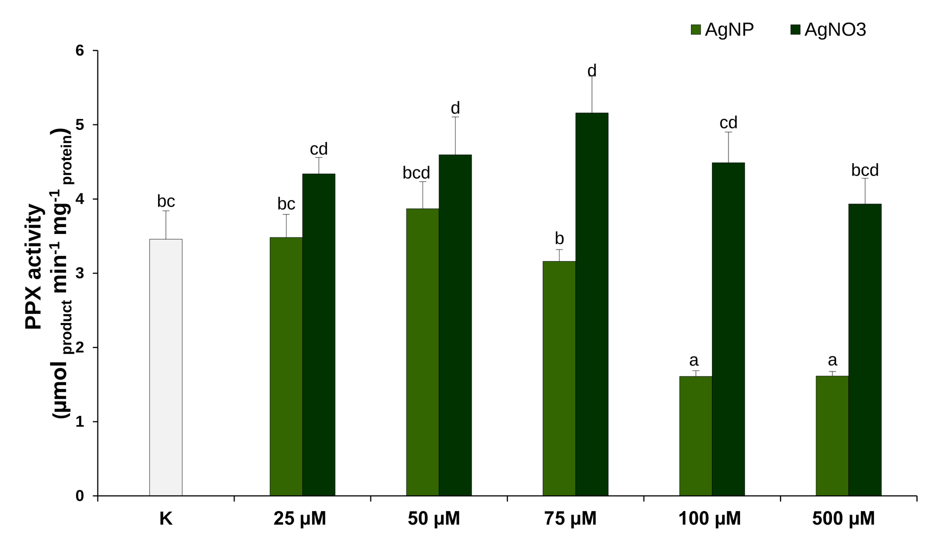

Figure 3. Activity of PPX in tobacco leaves after exposure to 25, 50, 75, 100 and 500 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. K - control. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

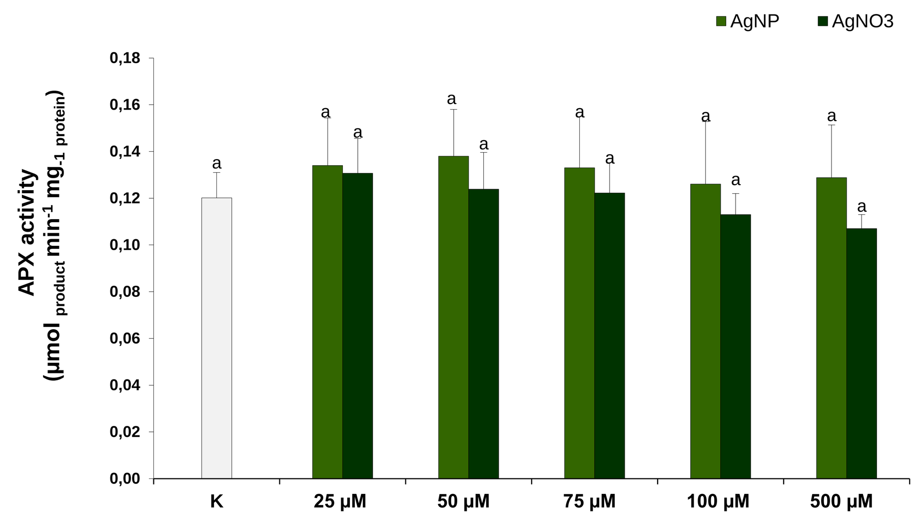

Figure 4. Activity of APX in tobacco leaves after exposure to 25, 50, 75, 100 and 500 µM AgNPs and $AgNO_3$. The presented results show mean values of 6 replicates ± standard error. K - control. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

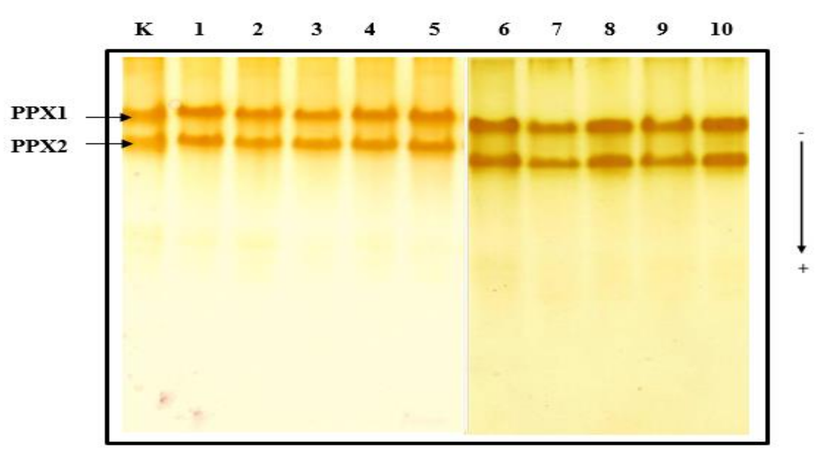

Figure 5. PPX isoforms of tobacco roots after conducted native PAGE. K - control; 1 - 25 µM, 2 - 50 µM , 3 - 75 µM, 4 - 100 µM and 5 - 500 µM AgNPs; 6 - 25 µM, 7 - 50 µM, 8 - 75 µM, 9 - 100 µM and 10 - 500 µM $AgNO_3$.

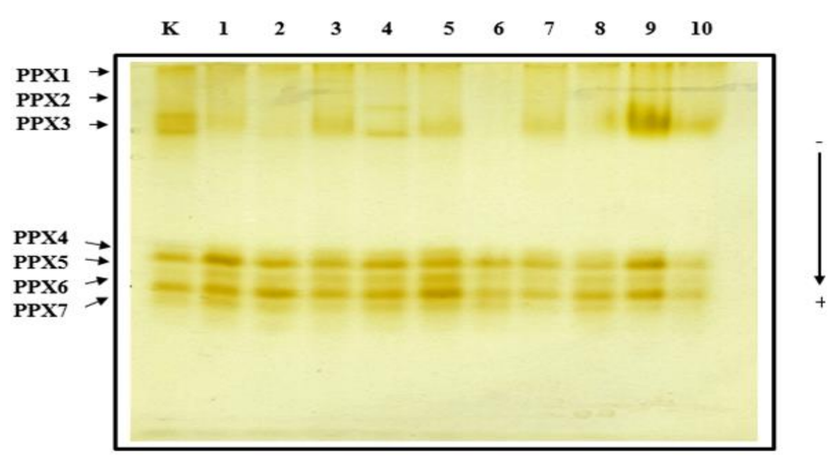

Figure 6. PPX isoforms of tobacco leaves after conducted native PAGE. K - control; 1 - 25 µM, 2 - 50 µM , 3 - 75 µM, 4 - 100 µM and 5 - 500 µM AgNPs; 6 - 25 µM, 7 - 50 µM, 8 - 75 µM, 9 - 100 µM and 10 - 500 µM $AgNO_3$.

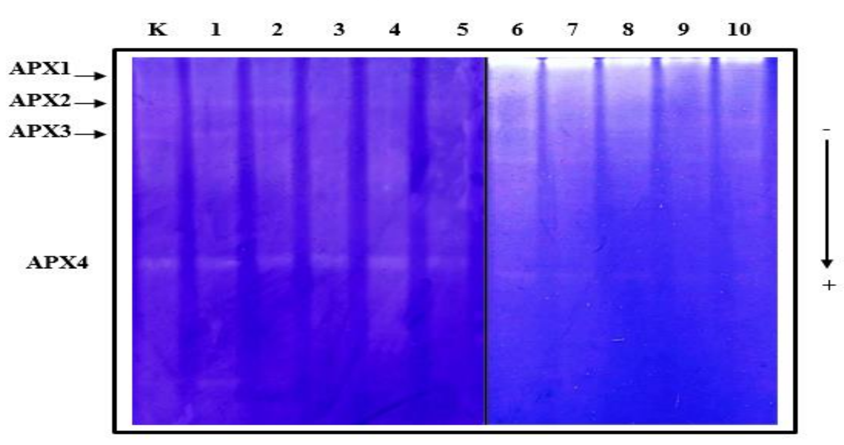

Figure 7. APX isoforms of tobacco roots after conducted native PAGE. K - control; 1 - 25 µM, 2 - 50 µM , 3 - 75 µM, 4 - 100 µM and 5 - 500 µM AgNPs; 6 - 25 µM, 7 - 50 µM, 8 - 75 µM, 9 - 100 µM and 10 - 500 µM $AgNO_3$.

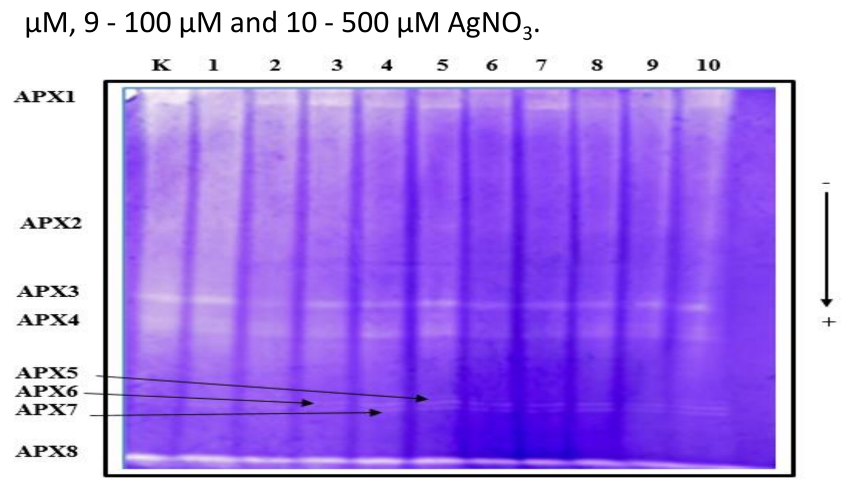

Figure 8. APX isoforms of tobacco leaves after conducted native PAGE. K - control; 1 - 25 µM, 2 - 50 µM , 3 - 75 µM, 4 - 100 µM and 5 - 500 µM AgNPs; 6 - 25 µM, 7 - 50 µM, 8- 75 µM, 9 - 100 µM and 10 - 500 µM $AgNO_3$.

CONCLUSION

- After both types of treatments similar accumulation of Ag was obtained, which was significantly higher in roots than in leaves.
- Concentrations and the form of applied Ag seemed to be correlated with activities of both APX and PPX since significant differences were obtained between AgNP- and $AgNO_3$-treatments in both roots and leaves.
- Peroxidase isoenzyme patterns exhibited that the Ag form and its concentration affect the peroxidase isoforms expression; namely, quantitative and qualitative changes were observed in PPX and APX isoforms in treated plants compared to control, but also in roots and leaves of plants exposed to different forms of Ag.
- Obtained results suggest that AgNPs induce different and specific response in plant's antioxidant enzymes compared to $AgNO_3$.

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