

CYSTEINE CAN ALLEVIATE SILVER NANOPARTICLE–INDUCED PHYTOTOXICITY IN TOBACCO SEEDLINGS

Biba R.¹, Cvjetko P.¹, Peharec Štefanić P.¹, Tkalec M.¹, Domijan A-M.², Šabarić J.³, Jukić M.³, Šikić S.³, Lyons D.M.⁴, Babić S.⁵, Balen B.¹

¹ Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

² Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovacica 1, 10000 Zagreb, Croatia

³ Department of Ecology, Andrija Stampar Teaching Institute of Public Health, 10000 Zagreb, Croatia

⁴ Center for Marine Research, Ruđer Bošković Institute, G. Paliaga 5, 52210 Rovinj, Croatia

⁵ Division of Materials Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia



e-mail: renata.biba@biol.pmf.hr

INTRODUCTION

Silver nanoparticles (AgNPs) are a commonly used nanomaterial because of their antibacterial and antifungal properties that are exploited in numerous consumer products and agriculture. Increased AgNPs utilisation results in their significant discharge into the environment and many studies have already revealed their toxic effects on living organisms.¹ Plants, with an important role in bioaccumulation and distribution of environmentally released substances, could serve as a potential route for AgNPs into the food chain.² In this study we compared the effects of two differently coated AgNPs [polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB)] on oxidative stress parameters of tobacco (*Nicotiana tabacum* L.) seedlings. To examine whether the toxic effects of AgNPs originate from dissolved Ag⁺ or nanoparticles themselves, cysteine, a strong silver ligand, has been applied.

MATERIALS AND METHODS

Two weeks old tobacco (*Nicotiana tabacum* L.) seedlings were treated with 25, 50 and 100 µM of AgNP-PVP or AgNP-CTAB. To estimate the contribution of dissolved Ag⁺ to the effects of AgNPs, 125, 250 and 500 µM of cysteine has been applied. Size distribution and zeta potential of the AgNPs were measured using dynamic light scattering (DLS, Malvern, UK). Silver uptake in the plant tissue was determined with inductively coupled plasma mass spectrometry (ICP-MS)³. Dihydroethidium (DHE) test was used to determine the ROS level.³ To examine the oxidative stress response the content of malondialdehyde (MDA)⁴ and protein carbonyls⁵, as well as the activity of antioxidant enzymes [pyrogallol peroxidase (PPX), ascorbate peroxidase (APX)⁶, catalase (CAT)⁷ and superoxide dismutase (SOD)⁸] was spectrophotometrically measured.

RESULTS

Table 1. Physico-chemical characteristics of AgNPs-PVP and AgNPs-CTAB stock solutions.

Characteristics		AgNP-PVP	AgNP-CTAB
Size peak I	dH, nm	16,03 ± 4.00	10.71 ± 1.79
	mean volume, %	94.63 ± 15.08	86.72 ± 2.03
Size peak II	dH, nm		50.77 ± 4.07
	mean volume, %		13.28 ± 2.03
ζ potential, mV		-22.63 ± 5.29	41.77 ± 3.08

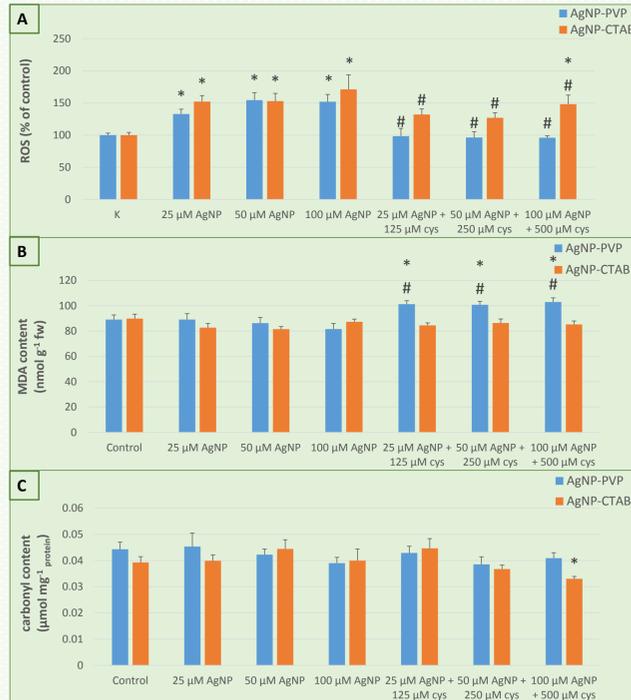


Figure 2. Content of ROS (A), MDA (B) and protein carbonyls (C) in tobacco seedlings treated with AgNPs. Values are means ± SE of two different experiments, each with six replicas. Among each Ag-treatment asterisks denote significant difference from control and hash sign denotes significant differences among treatments with and without cysteine.

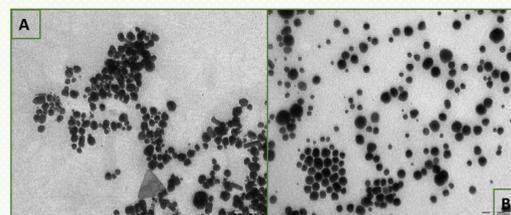


Figure 1. TEM images of AgNPs-PVP (A) and AgNPs-CTAB (B).

Table 2. Silver content in tobacco seedlings treated with AgNPs, alone and in combination with cysteine. Values are means ± SE of three replicas. Among each Ag-treatment asterisks denote significant difference from control and hash sign denotes significant differences among treatments with and without cysteine.

treatment	concentration	AgNP-PVP	AgNP-CTAB
control	0	0	0
AgNP	25 µM	39.57 ± 5.79*	33.30 ± 3.94*
	50 µM	42.92 ± 3.85*	48.24 ± 2.45*
	100 µM	45.30 ± 4.29*	80.62 ± 1.94*
AgNP + cysteine	25 µM + 125 µM	14.34 ± 1.49*#	20.68 ± 6.10*#
	50 µM + 250 µM	22.72 ± 0.89*#	37.92 ± 4.05*
	100 µM + 500 µM	21.14 ± 3.14*#	45.04 ± 2.57*#

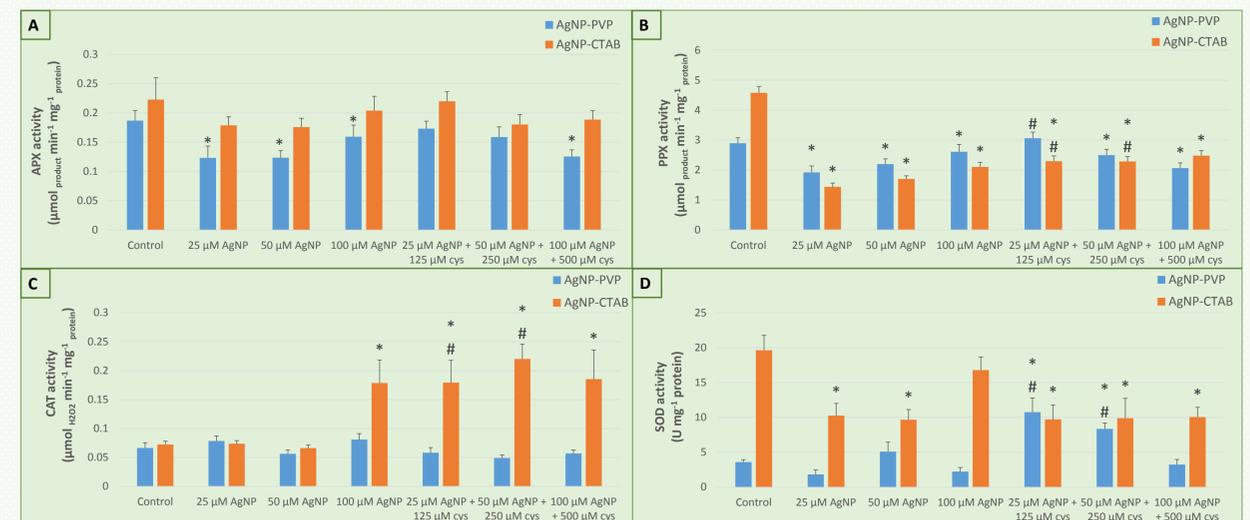


Figure 3. Specific activities of APX (A), PPX (B), CAT (C) and SOD (D) in tobacco seedlings treated with AgNPs. Values are means ± SE of two different experiments, each with six replicas. Among each Ag-treatment asterisks denote significant difference from control and hash sign denotes significant differences among treatments with and without cysteine.

CONCLUSION

- compared to control, AgNPs induced significant silver uptake and elevated ROS levels in tobacco seedlings
- no significant difference in content of MDA and protein carbonyls was detected in AgNP treatments
- AgNPs-PVP decreased APX and PPX activities, but had no significant effect on CAT and SOD activity
- AgNPs-CTAB increased CAT activity and decreased PPX activity, but no significant change in SOD and APX activities was detected
- cysteine significantly reduced silver uptake in the plant tissue, decreased ROS formation in both AgNP treatments, elevated MDA content and alleviated antioxidant enzyme activities in combined AgNP and cysteine treatments
- this results show that AgNPs phytotoxicity at least partially derives from dissolved silver



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