Physiological and proteomic responses of tobacco seedlings exposed to silver nanoparticles

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INTRODUCTION

Nanoparticles (NPs) with three dimensions between 1 and 100 nm show unique electrical, chemical and physical properties. Because of that they are often found in various consumer products.[1] Silver nanoparticles (AgNPs) are the most commonly used nanomaterial because of their antibacterial and antifungal properties that are used in production of medical applications and devices, textiles, food packaging and healthcare and household products.[2] Studies have shown detrimental effects of AgNPs on bacteria, algae, plants, animals and human cells, but the mechanisms of AgNP toxicity are not yet fully clarified.[3] To examine whether toxicity of AgNPs is nanoparticle-specific or comes as a result of ionic silver released from AgNPs, we investigated physiological and proteomic changes in seedlings of tobacco (*Nicotiana tabacum* L.) exposed to AgNPs and $AgNO_3$.

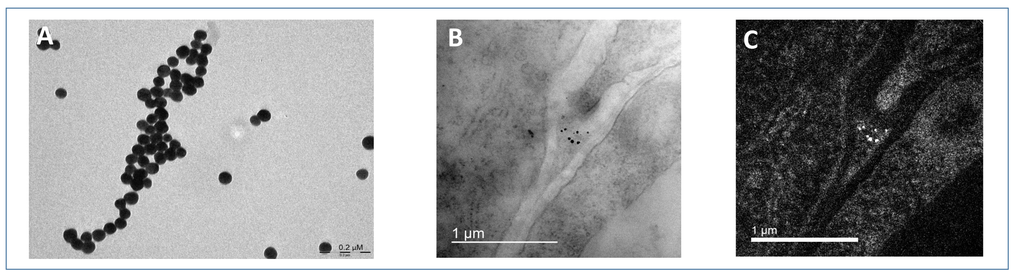

Figure 1. TEM image of AgNP suspension (A), AgNP localization in the root cells of the 100 uM AgNP-treated tobacco seedlings (B) and bright field image (C).

MATERIALS AND METHODS

Experiments were performed using commercial citrate-coated AgNPs (nanoComposix, San Diego, USA). Tobacco (*Nicotiana tabacum* L.) seedlings were grown in solid Murashige and Skoog[4] medium supplemented with AgNP or $AgNO_3$ stock solutions to obtain 25, 50, 75 and 100 µM concentrations. The exposure period lasted for 30 days. Silver uptake in plant tissue was determined with inductively coupled plasma mass spectrometry (ICP-MS).[5] To examine the oxidative stress response the content of malondialdehyde (MDA)[6] and protein carbonyls[7] as well as the activity of antioxidant enzymes [pyrogallol peroxidase (PPX), ascorbate peroxidase (APX)[8], catalase (CAT)[9] and superoxide dismutase (SOD)[10]] was spectrophotometrically measured. Dihydroethidium (DHE) test was used to determine the ROS level.[5] For the genotoxicity assessment, alkaline version of Comet assay was applied.[5] Tobacco seedlings treated with 100 µM AgNP and $AgNO_3$ were used to study morphological and ultrastructural changes and to detect the uptake of AgNPs in plant cells, using light and electron microscopy.[5] Same treatments were used to detect changes in protein expression. To separate the proteins two-dimensional (2-DE) electrophoresis was conducted; excised and digested peptides were analysed with matrix-assisted laser desorption/ionization-time of flight mass spectrometer (MALDI TOF/TOF) and proteins were identified using global protein server explorer software for Mascot search against National Center for Biotechnology Information protein database (NCBIprot).[11]

RESULTS

Table 1. Silver content in tobacco seedlings treated with AgNPs and $AgNO_3$. Values are means ± SE of three different experiments, each with three replicas. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

treatment (µM)	AgNP	$AgNO_3$
0	$<0.001^{a}$	$<0.001^{a}$
25	271.27 ± 14.71^{bc}	230.39 ± 28.10^{b}
50	333.31 ± 22.60^{de}	298.36 ± 18.33^{cd}
75	336.18 ± 18.59^{de}	299.20 ± 14.17^{cd}
100	375.63 ± 9.96^{e}	316.51 ± 11.51^{cd}

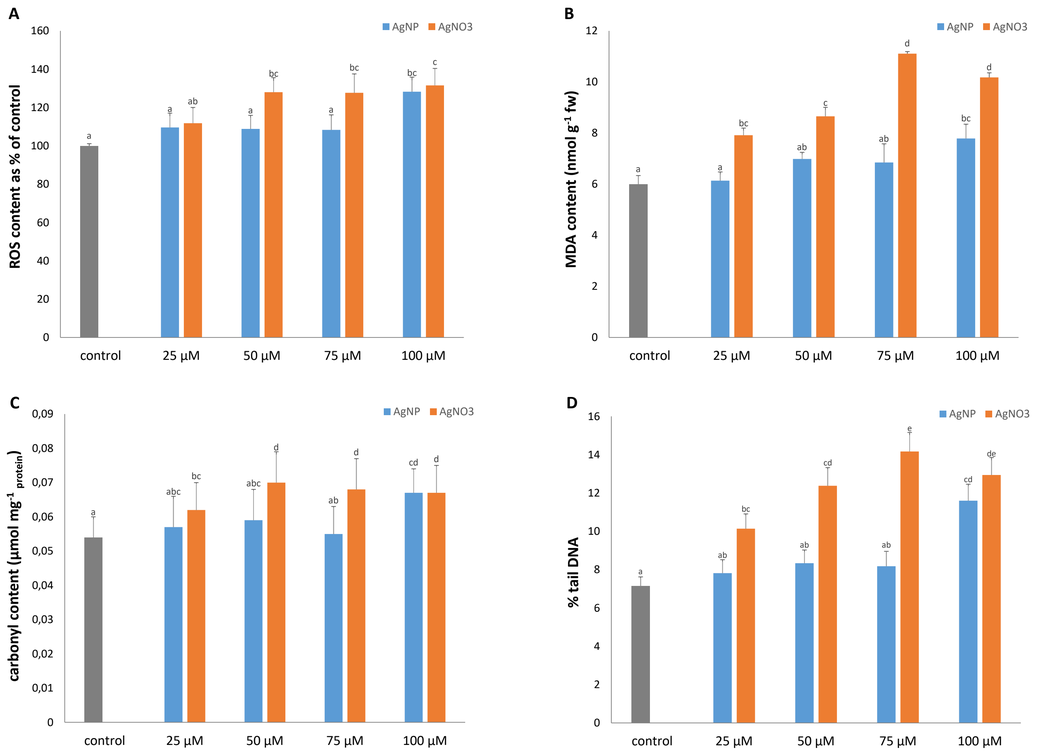

Figure 2. Content of ROS (A), MDA (B), protein carbonyl (C) and % tail DNA (D) in tobacco seedlings treated with AgNPs and $AgNO_3$. Values are means ± SE of three different experiments, each with three replicas. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

Table 2. Specific activities of SOD, PPX, APX and CAT in tobacco seedlings treated with AgNPs and $AgNO_3$. Values are means ± SE of three different experiments, each with three replicas. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

	concentration (µM)	SOD activity (U mg^{-1} protein)	PPX activity (µmol product min^{-1} mg^{-1} protein)	APX activity (µmol product min^{-1} mg^{-1} protein)	CAT activity (µmol H_2O_2 min^{-1} mg^{-1} protein)
control	0	1.93 ± 0.14^{a}	7.17 ± 1.03^{a}	0.063 ± 0.002^{a}	0.178 ± 0.01^{bc}
AgNP	25	3.23 ± 0.64^{bc}	3.72 ± 0.64^{b}	0.104 ± 0.009^{b}	0.217 ± 0.03^{c}
	50	3.42 ± 0.62^{c}	4.11 ± 0.65^{b}	0.109 ± 0.009^{b}	0.163 ± 0.02^{bc}
	75	3.23 ± 0.69^{bc}	3.93 ± 0.64^{b}	0.115 ± 0.009^{b}	0.159 ± 0.03^{abc}
	100	5.39 ± 0.65^{d}	3.83 ± 0.35^{b}	0.128 ± 0.000^{bc}	0.172 ± 0.02^{bc}
$AgNO_3$	25	5.64 ± 0.86^{d}	3.81 ± 0.91^{b}	0.070 ± 0.008^{a}	0.148 ± 0.02^{ab}
	50	2.99 ± 0.55^{bc}	3.43 ± 0.65^{b}	0.107 ± 0.006^{b}	0.125 ± 0.03^{ab}
	75	2.38 ± 0.34^{a}	3.37 ± 0.97^{b}	0.119 ± 0.008^{bc}	0.102 ± 0.02^{a}
	100	2.74 ± 0.27^{abc}	3.21 ± 0.47^{b}	0.114 ± 0.009^{c}	0.100 ± 0.02^{a}

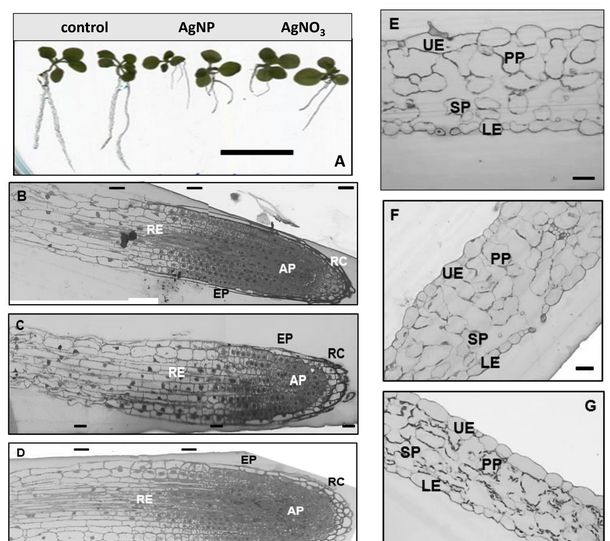

Figure 3. Root length of tobacco seedlings (A). Semithin sections of root from control (B), 100 µM AgNP-treated (C) and 100 µM $AgNO_3$-treated (D) tobacco seedlings (bar = 33.1 µm) and leaf from control (E), 100 µM AgNP-treated (F) and 100 µM $AgNO_3$-treated (F) tobacco seedlings (bar = 30.6 µm). RC – root cap, AP – apical meristem, RE – region of elongation, EP – epidermis, UE – upper epidermis, LE – lower epidermis, PP – palisade parenchyma, SP – spongy parenchyma.

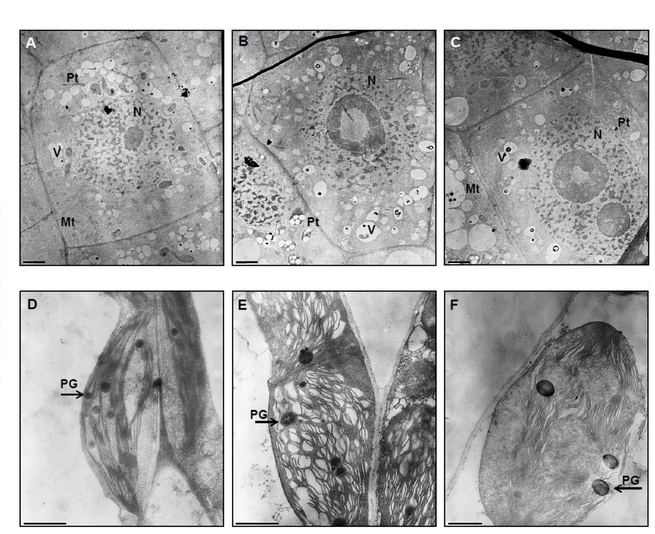

Figure 4. Ultrastructure of root cells and leaf chloroplasts. Root cells of control (A), 100 µM AgNP-treated (B) and 100 µM $AgNO_3$-treated (C) tobacco seedlings (bar = 2 µm). Chloroplasts in leaf cells of control (D), 100 µM AgNP-treated and 100 µM $AgNO_3$-treated tobacco seedlings (bar = 1 µm). N – nucleus, V – vacuole, Mt – mitochondrion, Pt – plastid, PG – plastoglobules.

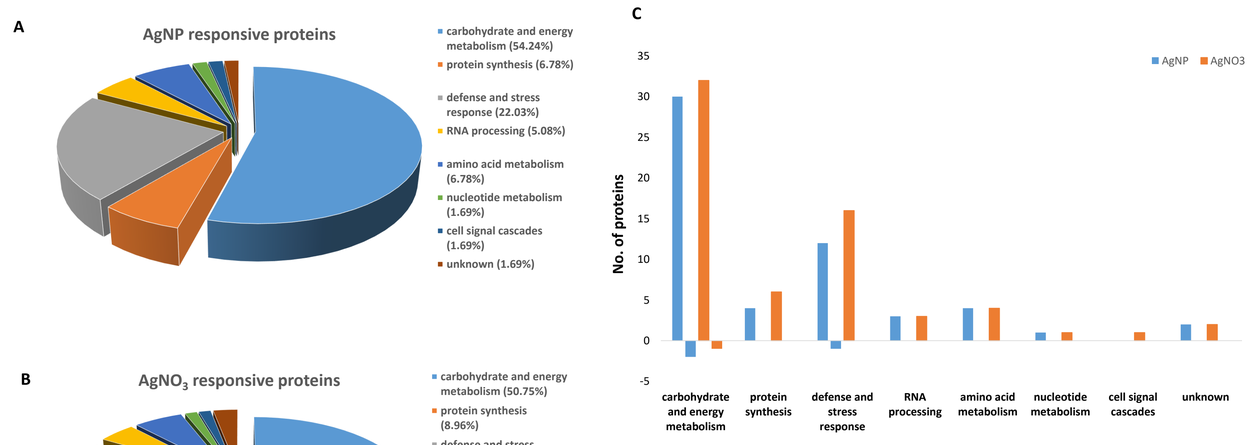

Figure 5. Functional categorization of the differentially expressed proteins of tobacco seedlings treated with 100 µM AgNPs (A) and 100 µM $AgNO_3$ (B). Differentially expressed proteins identified by MALDI-TOF/TOF MS/MS were classified according to their putative biological process reported in the Uniprot database. Analysis of responsiveness of differentially expressed proteins of tobacco seedlings exposed to AgNPs and $AgNO_3$ (C).

CONCLUSION

- higher Ag content was measured in seedlings exposed to AgNPs than to $AgNO_3$ of the same concentration
- obtained results on oxidative stress parameters revealed that in general higher toxicity was recorded in $AgNO_3$-treated seedlings compared to those exposed to nanosilver
- presence of silver in the form of nanoparticles was confirmed in the root cells, which may explain the lower toxicity of AgNPs
- proteomic study showed that both AgNPs and $AgNO_3$ can affect photosynthesis
- majority of the proteins involved in the primary metabolism were up-regulated after both types of treatments, indicating that enhanced energy production, which can be used to reinforce defensive mechanisms, enables plants to cope with silver-induced toxicity

References:
[1]Maynard et al. (2011), Toxicol Sci 120:109-129
[2]Zhang et al. (2016), Int J Mol Sci 17:1534
[3]Tripathi et al. (2017), Front Microbiol 8:07
[4]Murashige and Skoog (1962), Physiol Plant 15:473-497
[5]Cvjetko et al. (2018), Environ Sci Pollut Res Int 25:5590-5602
[6]Heath and Packer (1968), Arch Biochem Biophys 125:189-198
[7]Levine et al. (1990), Methods Enzym 186:464-478
[8]Nakano and Asada (1981), Plant Cell Physiol 22:867-880
[9]Aebi, H (1984), Methods Enzymol 105:121-126
[10]Beauchamp and Fridovich (1971), Anal Biochem 44:276-287
[11]Rogić et al. (2015), Plant Cell Tiss Organ Cult 122:127-146

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