Induction of oxidative stress in tobacco seedlings treated with differently coated silver nanoparticles

Renata Biba[1], Petra Cvjetko[1], Karla Košpić[1], Petra Peharec Štefanić[1], Mirta Tkalec[1], Daniel Lyons[2], Biljana Balen[1]

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

[2]Center for Marine Research, Ruđer Bošković Institute, Rovinj, Croatia

INTRODUCTION

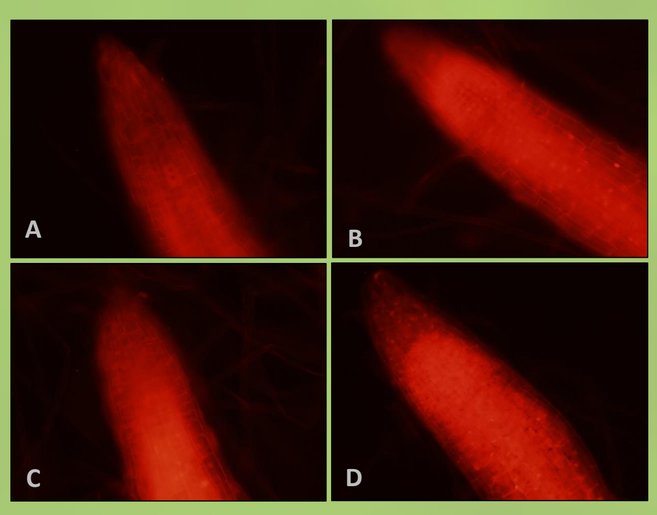
Among various nanomaterials, silver nanoparticles (AgNPs) stand out due to their enhanced antimicrobial effects that have been exploited in many industrial sectors and daily life. Increase in AgNP applications has led to greater potential for their release into the environment, where they can be absorbed by plants and enter the food chain, posing a threat to human health.[1] The main mechanism of AgNP toxicity lies in excessive formation of reactive oxygen species (ROS) and subsequent oxidative stress induction, but the degree of oxidative damage depends on the intrinsic properties of AgNPs (size, shape and coating), which determine their stability against aggregation and dissolution in the environment.[2] This study compared the effects of two differently coated AgNPs [polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB)] and ionic silver ($AgNO_3$) on oxidative stress parameters in tobacco (*Nicotiana tabacum* L.) seedlings.

MATERIALS AND METHODS

Three week old tobacco (*Nicotiana tabacum* L.) seedlings were treated with 100 µM AgNP-PVP, AgNP-CTAB and $AgNO_3$ for seven days. *In situ* detection of ROS[3] was performed using dihydroethidium for superoxide radical ($O_2^{\cdot -}$) and 2',7'-dichlorodihydrofluorescein diacetate for H_2O_2. Activity and changes in isoenzyme patterns for superoxide dismutase (SOD)[4], catalase (CAT)[5], ascorbate peroxidase (APX)[6] and pyrogallol peroxidase (PPX)[6] were analysed spectrophotometrically and in gel, respectively, while their expression was determined with immunoblotting.

RESULTS

ROS *in situ* detection



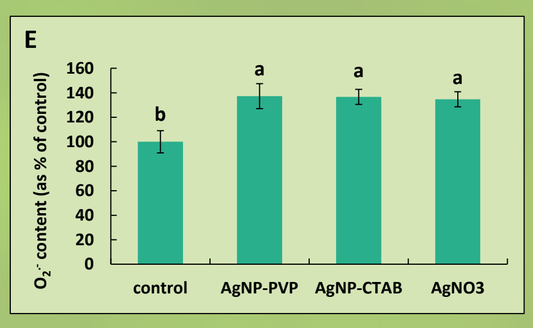
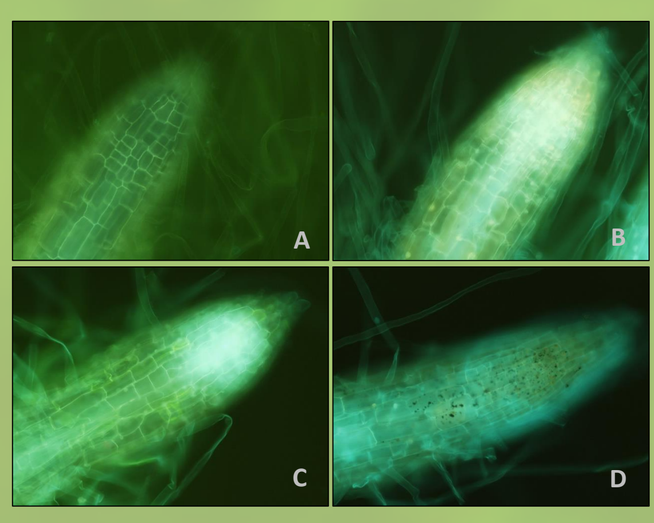


Figure 1. $O_2^{\cdot -}$ detection in roots of untreated tobacco seedlings (control, A), and seedlings treated with 100 µM of AgNP-PVP (B), AgNP-CTAB (C), $AgNO_3$ (D); 20x magnification. Total $O_2^{\cdot -}$ content (E) measured in 100 cells ± SE. Different letters denote significant difference among treatments according to Duncan test ($P \leq 0.05$).



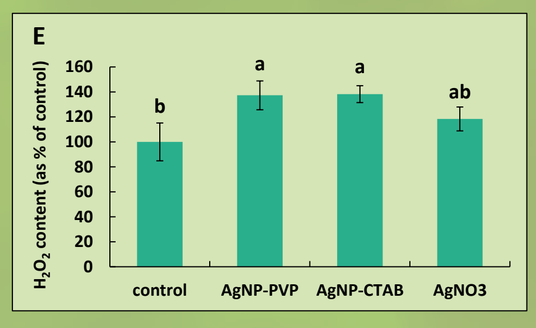


Figure 2. H_2O_2 detection in roots of untreated tobacco seedlings (control, A), and seedlings treated with 100 µM AgNP-PVP (B), AgNP-CTAB (C), $AgNO_3$ (D); 20x magnification. Total H_2O_2 content (E) measured in 100 cells ± SE. Different letters denote significant difference among treatments according to Duncan test ($P \leq 0.05$).

Activity and expression of antioxidant enzymes

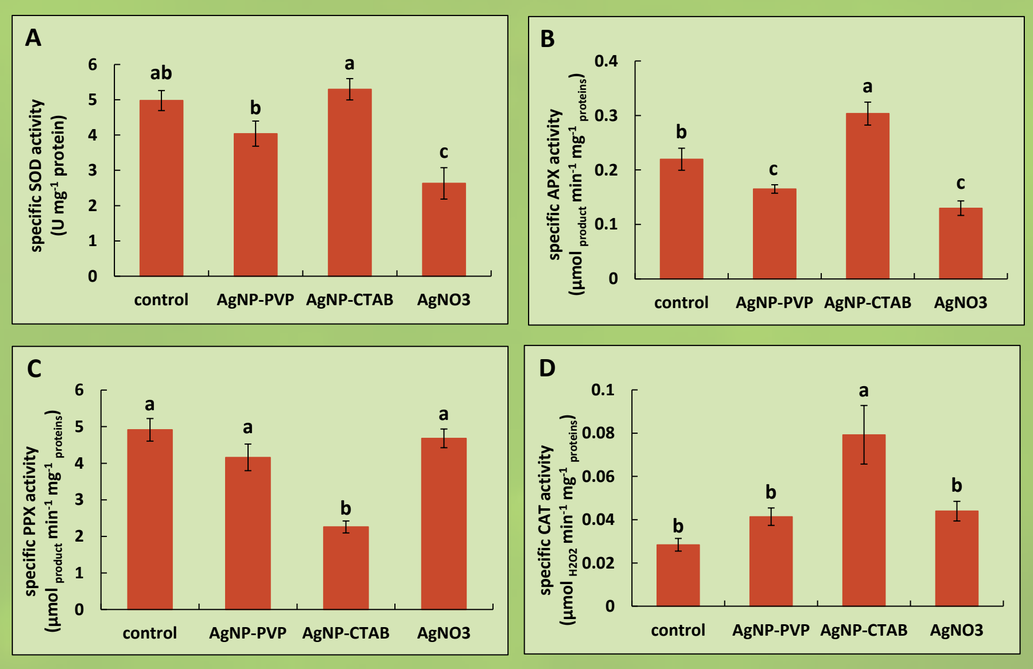


Figure 3. Specific activities of SOD (A), APX (B), PPX (C) and CAT (D) in tobacco seedlings treated with 100 µM solution of AgNP-PVP, AgNP-CTAB or $AgNO_3$. Values are means ± SE of two different experiments, each with six replicates. Different letters denote significant difference among treatments according to Duncan test ($P \leq 0.05$).

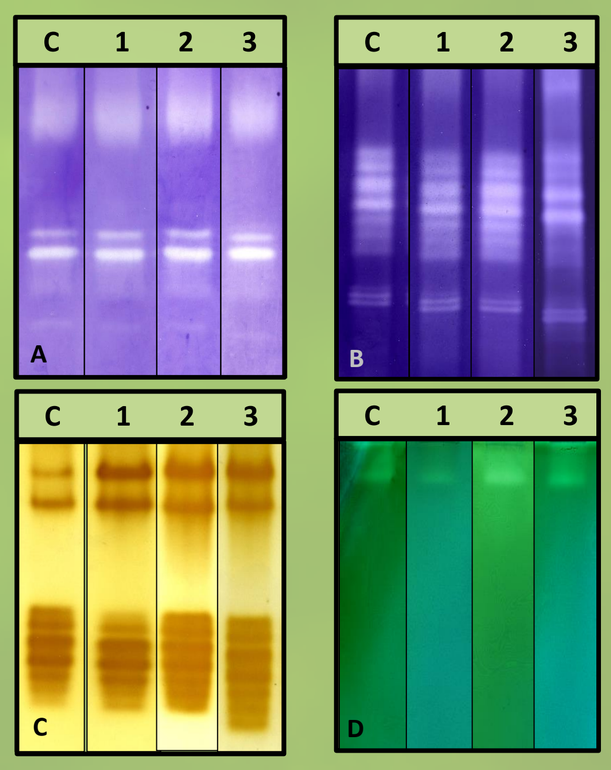


Figure 4. Isoenzyme patterns of SOD (A), APX (B), PPX (C) and CAT (D) in tobacco seedlings treated with 100 µM solution of AgNP-PVP (1), AgNP-CTAB (2) or $AgNO_3$ (3). C – control.

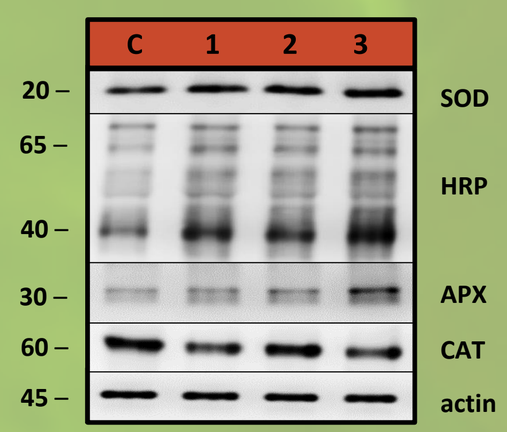


Figure 5. Expression of SOD, HRP, APX, CAT and actin (loading control) in tobacco seedlings treated with 100 µM solution of AgNP-PVP (1), AgNP-CTAB (2) or $AgNO_3$ (3). C – control.

CONCLUSION

- all Ag treatments significantly enhanced $O_2^{\cdot -}$ and H_2O_2 production in tobacco seedlings
- AgNP-CTAB treatment increased APX and CAT activities, but decreased PPX, while AgNP-PVP and $AgNO_3$ decreased SOD and APX; isoenzyme patterns also revealed differences in activities of certain isoforms of APX, PPX and CAT among treatments
- even though Western blots showed higher abundance of HRP in all treated seedlings, only $AgNO_3$ caused changes in expression of other enzymes as well
- **these results show that the coating used for AgNP stabilization plays an important role in AgNP toxicity, which cannot be ascribed only to the release of Ag^+ ions**

Literature

[1]Yan and Chen (2019), Int J Mol Sci 20: 1003

[2]Tejamaya et al. (2012), Environ Sci Technol 46:7011-7017

[3]Cvjetko et al. (2018), Environ Sci Pollut Res Int 25:5590-5602

[4]Beauchamp and Fridovich (1971) Anal Biochem 44:276-287

[5] Aebi, H (1984) Methods Enzym 105:121-126

[6] Nakano and Asada (1981) Plant Cell Physiol 22:867-880

This work was supported by the Croatian Science Foundation [grant number IP-2014-09-6488] and University of Zagreb by grant number 20281222.