Acute exposure to silver nanoparticles and ionic silver triggers oxidative stress in tobacco plants

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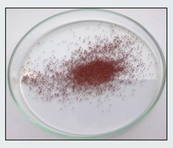
INTRODUCTION

Antimicrobial properties of silver and enhanced reactivity when applied in the nanoparticle form (AgNPs) has led to their increased utilisation in consumer products. However, their increasing release into water or soil represents a potential environmental hazard. AgNPs can impose detrimental effects on plants, mainly through excess generation of reactive oxygen species (ROS), leading to induction of oxidative stress[1]. In this work, detached roots of *in vitro* grown tobacco (*Nicotiana tabacum)* plants were exposed to AgNPs stabilised with cetyltrimethylammonium bromide (CTAB) or polyvinylpyrrolidone (PVP) coating or to ionic silver ($AgNO_3$), applied in the same concentration (100 µmol/L) for 24 h. The aim was to investigate *in situ* early physiological responses that are the first signs of stressful conditions. Generation of ROS in root cells was monitored by confocal laser scanning microscopy, using highly sensitive and specific fluorescent probes: dihydroethidium (DHE) to detect O_2^- and 2',7'-dichlorofluorescein-diacetate (H_2DCF-DA) to detect H_2O_2[2]. Propidium iodide was used as a counterstain to verify cell viability[3].

MATERIALS AND METHODS

Tobacco cultivation:

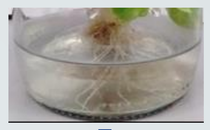
TOBACCO SEEDS



Cultivation on solid Murashige Skoog (MS)[4] medium **60 DAYS (23 °C, 90 µmol/photon, 18/6 h day/night)**

ADULT PLANTS

TREATMENTS:



24 h

Control (Ultrapure water)

100 µM AgNP-PVP; AgNP-CTAB or $AgNO_3$

-23 °C

-90 µmol/photon

INCUBATION IN FLUORESCENT DYES

-washing the roots in ultrapure water

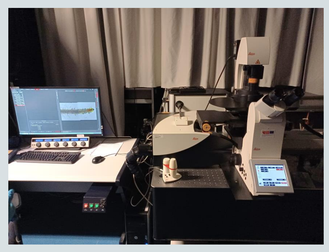
50 µM H_2DCF-DA

25 µM PI

10 µM DHE

-10 min incubation in the dark

SCANNING



Leica SP8 confocal laser scannig microscope

H_2DCF-DA- Ex: 504 nm, Em: 550 nm

PI Ex: 500 nm, Em: 580 nm

DHE Ex: 520 nm, Em: 610 nm

Reflection (476 nm)

Mean pixel density evaluation with Lucida software

RESULTS

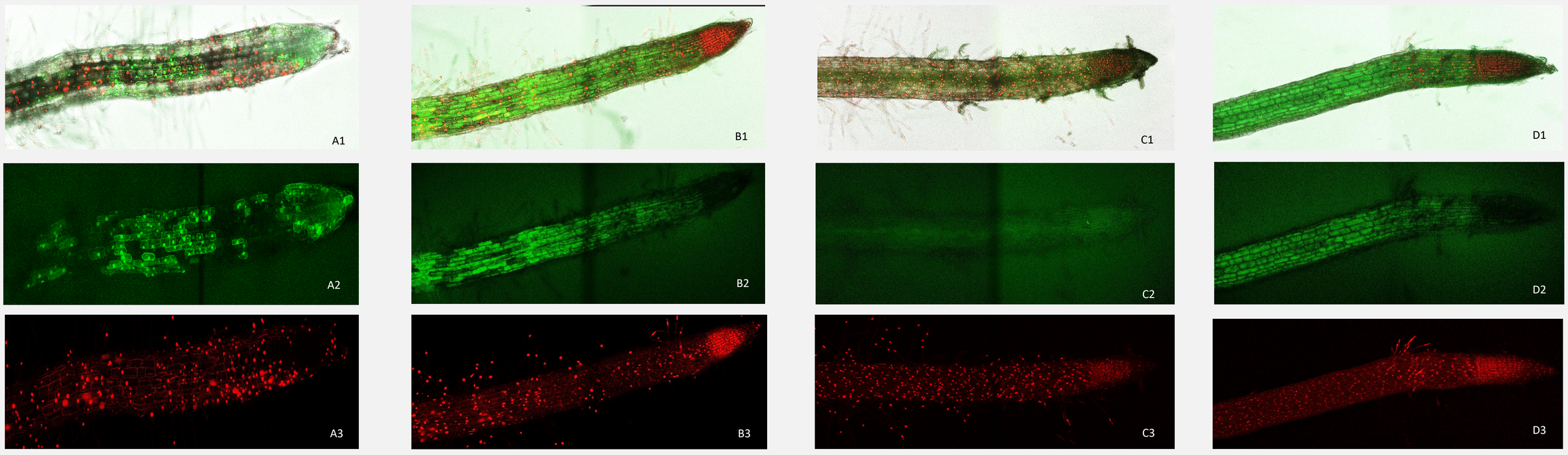


Figure 1. Confocal imaging of *N.tabacum* roots stained with H_2DCF-DA that labels H_2O_2 to give a green flourescent product, after a 24 h incubation with specified treatments: (A1-3) control, untreated cells; (B1-3) cells treated with 100 µM AgNP-PVP; (C1-3) 100 µM AgNP-CTAB; (D1-3) 100 µM $AgNO_3$ solution. Red fluorescence corresponds to PI staining that labels nuclei of dead cells. All images represent maximum intensity projection of Z-stack of between 30 to 40 images (Z-step: 0,03 µm, magnification: 20x). Images A1, B1, C1 and D1 represent an overlay of brightfield chanel, green and red fluoroescence channels; whereas A2, B2, C2 and D2 represent only the green fluorescent chanel (showing the levels of H_2O_2 in the cells) and A3, B3, C3 and D3 represent only the red fluorescent chanel (showing the amount of dead cells stained red).

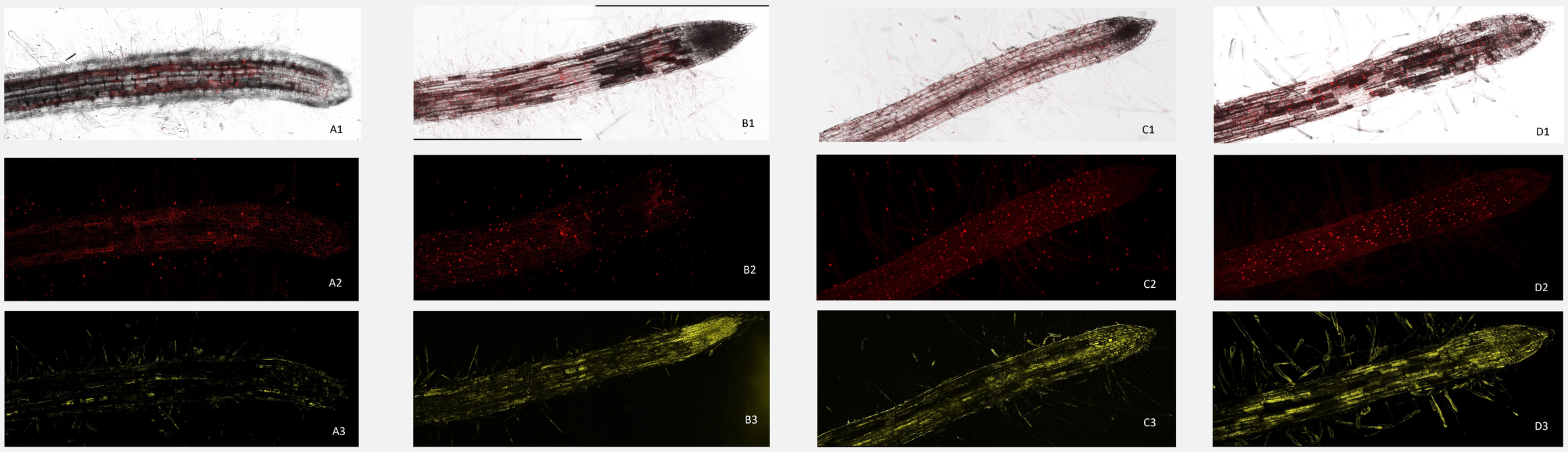


Figure 2. Confocal imaging of *N.tabacum* roots stained with DHE that labels O_2^- to give a red flourescent product, after a 24 h incubation with specified treatments: (A1-3) control, untreated cells; (B1-3) cells treated with 100 µM AgNP-PVP; (C1-3) 100 µM AgNP-CTAB; (D1-3) 100 µM $AgNO_3$ solution. All images represent maximum intensity projection of Z-stack of between 30 to 40 images (Z-step: 0,03 µm, magnification: 20x). Images A1, B1, C1 and D1 represent an overlay of brightfield chanel, red fluorescence channel and reflection (476 nm) chanel; whereas A2, B2, C2 and D2 represent only the red fluorescent chanel (showing the levels of O_2^- in the cells) and A3, B3, C3 and D3 represent only the reflection chanel (where the yellow coloured areas most likely represent AgNPs).

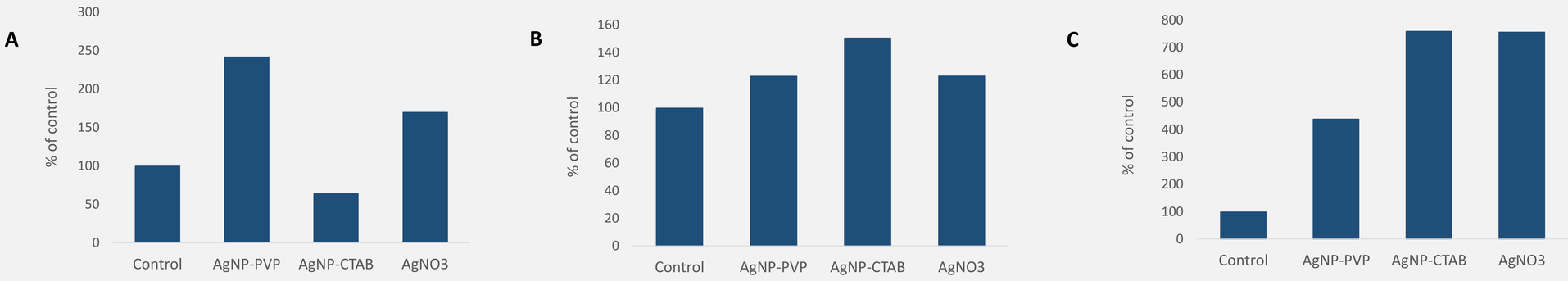


Figure 3. Graphs represent the mean pixel density evaluated from the maximum projection image of roots stained with (A) **H_2DCF-DA**; (B) **PI**; (C) **DHE**, after incubation with specified treatments. The results are expressed as % of control, where the control represents 100 %.

REFERENCES

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3. Ortega-Villasante C, Rellán-Alvarez R, Del Campo FF, Carpena-Ruiz RO, Hernández LE.. *J Exp Bot*. **2005**, 56, 418-22.
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CONCLUSIONS

- Increased amount of H_2O_2 and O_2^- was observed in roots exposed to either AgNPs or $AgNO_3$ comparing to non-exposed, control roots, which was correlated to an increased percentage of dead cells; while the changes were most prominent after AgNP-CTAB treatment. Tissue necrosis was predominantly observed in root tips.
- Additionally, AgNPs were visualized and were mostly found attached to the root surface and on roots hairs, and their accumulation could be linked to excess generation of ROS.
- The results show that AgNPs not only compromised cellular redox-homeostasis but possibly caused cell necrosis, while different level of impact was observed depending on the form of applied silver and possibly on the intrisic properties of AgNP coatings.