

Detection and localisation of silver nanoparticles in roots cells of *A. thaliana* seedlings

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Nanoparticles (NPs) research is currently an area of intense scientific surveys due to a variety of their potential applications. Silver NPs (AgNPs) are of particular interest because of their well-known antibacterial and antifungal properties. Therefore, they can be found in various consumer products. Since plants play a significant role in accumulation and biodistribution of many environmentally released substances, they could be influenced by AgNPs, serving as a potential pathway for AgNP-transport and bioaccumulation into food chains^{1,2,3}. In this study, we aimed to detect the localisation and accumulation sites of AgNPs in root cells of wild type Arabidopsis seedlings, after exposure to AgNPs stabilised with polyvinylpyrrolidone (AgNP-PVP). Four days old seedlings grown *in vitro* on a solid MS medium were transferred to a modified solid 1/2 strength MS medium supplemented with 100 µM AgNP-PVP. Arabidopsis roots were analysed after the 1st and 11th day of treatment and for detection and localization of AgNP-PVP in root cells, multiphoton confocal microscopy (Leica TCS SP8 X) and monochromated TF20 (FEI Tecnai G2) transmission electron microscope with EDX detector were used. Roots were placed on the microscopic slide and acquisition of reflection and transmission images was done with the excitation light source 476 nm argon laser. The emission signal was filtered using a system of acusto-optical beam splitter, prism-based dispersion and mirrors before the desired emission light reached the internal HyD detectors. Multiphoton and FLIM images were acquired using the excitation light source of 740 nm from multiphoton pulsed laser (Chameleon Discovery TPC, tunable output, 80 MHz, 140 ± 10 fs). The emission signal was filtered using SP680, dichroic mirror 560, band-pass filters 525/50 and 610/70. Non-descanned

Leica HyD detectors were used to acquire both multiphoton and FLIM images. FLIM images were acquired using Becker and Hickl SPC-150 using time correlated single photon counting method (TCSPC) from 300 sec. For TEM-EDX analyses, roots were fixed with 1% glutaraldehyde in 50 mM cacodylate buffer (pH 7.2) for 1 h at +4 °C, washed twice and post-fixed with 1% OsO₄ in the same buffer for 1 h at +4 °C followed by 10 min wash in ice-cold water. After dehydration in a graded series of ethanol, the tissue was embedded in Spurr's resin. The sections were examined using the TEM-EDX for confirmation of AgNPs localization in the tobacco cells. Multiphoton confocal images revealed that the reflective and luminescent particles with short fluorescence lifetime (120 ps) could be observed in the root cap of Arabidopsis seedlings grown on a solid nutrient medium supplemented with AgNPs already after the 1st day of treatment. After the 11th day of AgNP exposure, further accumulation of these particles could be seen in the root cap (Fig.1). TEM-EDX analyses confirmed that small black dots in the root cell wall contained silver (Fig. 2), which proves that AgNPs have been directly uptaken by root cells, in which they accumulated after the 1st and 11th day of exposure. The obtained results show that the AgNPs are uptaken by the roots cells already after the 1st day of exposure and that they accumulated in the root cap. Furthermore, it is shown that the AgNPs could be seen with the confocal microscope as reflective and luminescent particles with short fluorescent lifetime.

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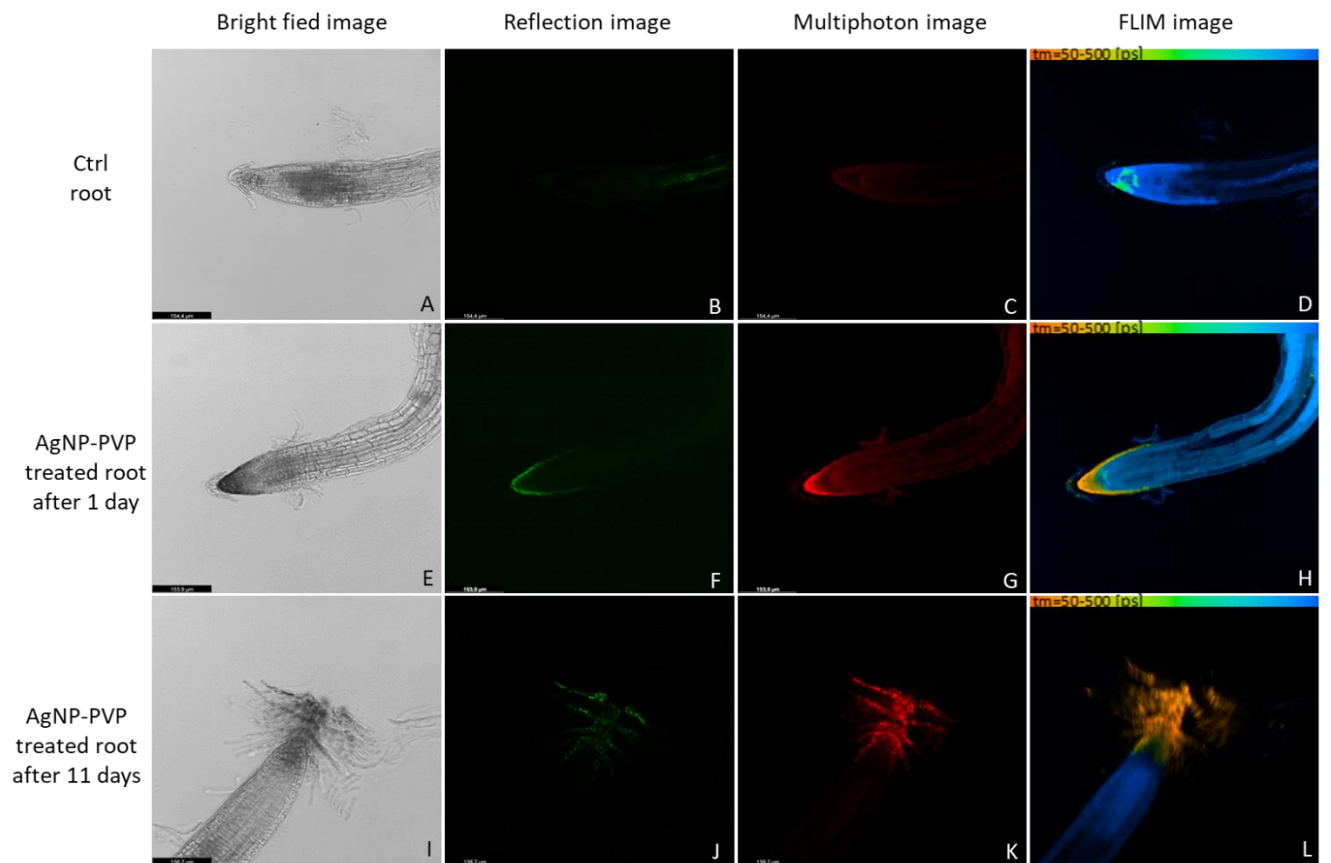


Figure 1. Confocal images of control root (A-D) and roots of 100 mM AgNP-treated *A. thaliana* seedlings after 1st (E-H) and 11th day (I-L) of treatment. Bar=154,4 μ m

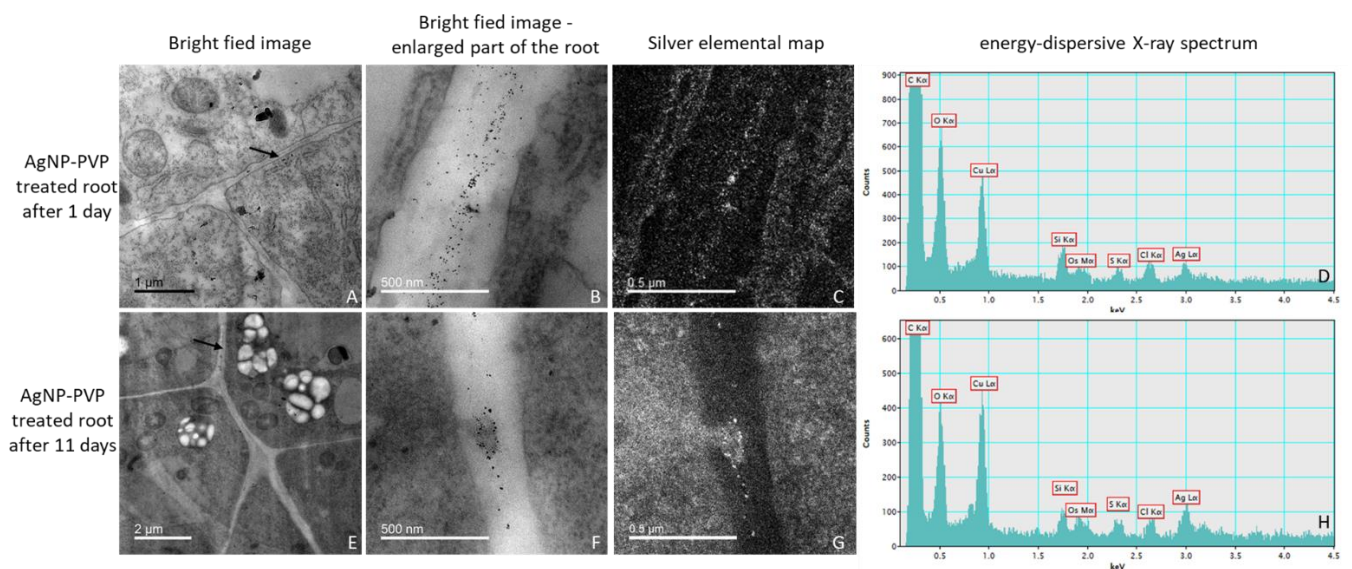


Figure 2. AgNP localization in the root cells of the 100 mM AgNP-treated *A. thaliana* seedlings after 1st and 11th day of treatment. TEM images of (A, E) a root cells, (B, F) silver nanoparticles in the cell wall, (C, G) silver elemental map of the AgNPs in the cell wall, and (D, H) energy-dispersive X-ray spectrum. Arrow points where AgNPs are localized.