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Engineered silver nanoparticles are sensed at the plasma membrane and dramatically modify the physiology of *Arabidopsis thaliana* plants

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SUMMARY

Silver nanoparticles (Ag NPs) are the world's most important nanomaterial and nanotoxicant. The aim of this study was to determine the early stages of interactions between Ag NPs and plant cells, and to investigate their physiological roles. We have shown that the addition of Ag NPs to cultivation medium, at levels above 300 mg L^{-1}, inhibited *Arabidopsis thaliana* root elongation and leaf expansion. This also resulted in decreased photosynthetic efficiency and the extreme accumulation of Ag in tissues. Acute application of Ag NPs induced a transient elevation of $[Ca^{2+}]_{cyt}$ and the accumulation of reactive oxygen species (ROS; partially generated by NADPH oxidase). Whole-cell patch-clamp measurements on root cell protoplasts demonstrated that Ag NPs slightly inhibited plasma membrane K^+ efflux and Ca^{2+} influx currents, or caused membrane breakdown; however, in excised outside-out patches, Ag NPs activated Gd^{3+}-sensitive Ca^{2+} influx channels with unitary conductance of approximately 56 pS. Bulk particles did not modify the plasma membrane currents. Tests with electron paramagnetic resonance spectroscopy showed that Ag NPs were not able to catalyse hydroxyl radical generation, but that they directly oxidized the major plant antioxidant, L-ascorbic acid. Overall, the data presented shed light on mechanisms of the impact of nanosilver on plant cells, and show that these include the induction of classical stress signalling reactions (mediated by $[Ca^{2+}]_{cyt}$ and ROS) and a specific effect on the plasma membrane conductance and the reduced ascorbate.

Keywords: silver nanoparticles, *Arabidopsis thaliana*, stress signalling, ion channels, reactive oxygen species, ascorbic acid, cell calcium.

INTRODUCTION

Silver nanoparticles (Ag NPs) have gained particular attention from industrialists because of their relatively low cost of production and tremendously enhanced physical/chemical characteristics (Nowack *et al.*, 2011). Since the 19th century, the unique antimicrobial and fungicidal properties of Ag have encouraged the very widespread use of Ag NPs in medical products, fabrics, antiseptics, food containers, cosmetics, paints, and even plush toys (Morones *et al.*, 2005; Kim *et al.*, 2009; Rai *et al.*, 2009; Nowack *et al.*, 2011). Nowadays, nearly 25% of all nanotechnology consumer products include Ag NPs (according to the *Inventory of Nanotechnology Consumer Products*, http://www.nanotechproject.org/cpi/). Between 320 and 480 tons (different estimates) of Ag NPs are industrially produced and consumed every year (Nowack *et al.*, 2011). The dramatic increase in the industrial use of Ag NPs has raised considerable concern about their potential release and effects on flora and ecosystems, as well as the possibility of Ag NPs entering the human food chain through plants (Monica and Cremonini, 2009; Gottschalk and Nowack, 2011).