

# PROTECTIVE EFFECTS OF CYSTEINE AGAINST SILVER NANOPARTICLES-INDUCED PHYTOTOXICITY

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## INTRODUCTION

Nanoparticles (NPs), with three dimensions between 1 and 100 nm, exhibit new characteristics compared to the same material without nanoscale features and are applied in the wide range of commercial products (Maynard et al. 2011). Therefore, they are likely to be released into the environment and affect plants. Since plants play a significant role in accumulation and biodistribution of environmentally released substances, they can serve as a route for NPs transportation and bioaccumulation into food chains (Rico et al. 2011). In this study we have examined the effects of silver nanoparticles (AgNPs) with two different coatings [polyvinylpyrrolidone (AgNP-PVP) and cetyltrimethylammonium bromide (AgNP-CTAB)] on photosynthesis and oxidative stress parameters of tobacco (*Nicotiana tabacum*) seedlings.

## MATERIALS AND METHODS

To determine the effects of AgNPs on photosynthesis, two weeks old seedlings were treated with 25, 50 and 100  $\mu\text{M}$  AgNP-PVP or AgNP-CTAB. Size distribution of around 50 nm of AgNPs was confirmed using dynamic light scattering (DLS, Malvern, UK) measurements. Zeta potential measurements showed that AgNP-PVP carry a negative surface charge ( $\zeta = -22.6 \pm 5.3$ ), whereas AgNP-CTAB appear to be positively charged ( $\zeta = 41.7 \pm 3.1$ ). After seven days of exposure, chlorophyll fluorescence was measured using pulse-modulated chlorophyll fluorometer (FluorPen, Czech Republic). To examine the oxidative stress response, the content of malondialdehyde (MDA) (Heath and Packer, 1968) and protein carbonyls (Levine et al. 1990) was spectrophotometrically measured. In order to estimate the contribution of dissolved  $\text{Ag}^+$  to the effects of AgNPs, 125, 250 and 500  $\mu\text{M}$  cysteine, a strong silver ligand, has been applied to complex  $\text{Ag}^+$ .

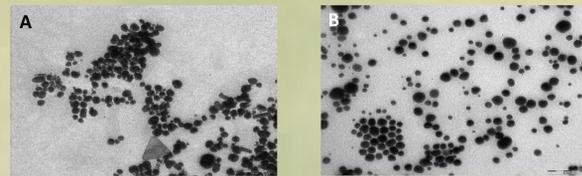


Figure 1. TEM images of (A) AgNP-PVP and (B) AgNP-CTAB.

## RESULTS

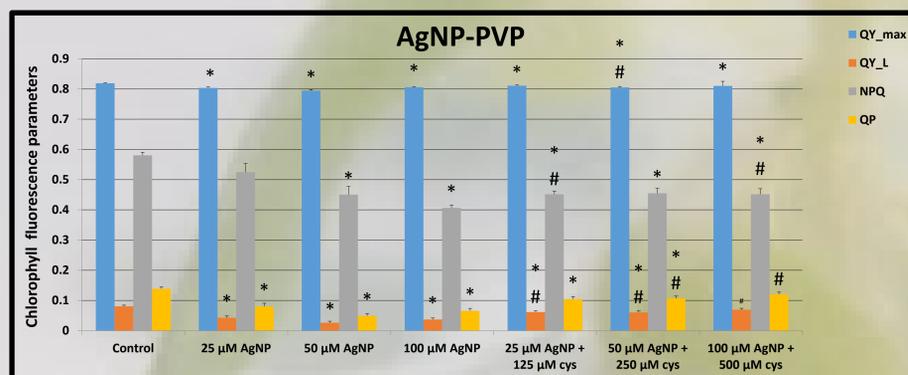


Figure 2. Chlorophyll fluorescence parameters in tobacco leaves treated with AgNP-PVP. Results are expressed as means of six replicates  $\pm$  SE. Among each Ag-treatment asterisks denote significant difference from control. Hash sign denotes significant differences among treatments with and without cysteine.

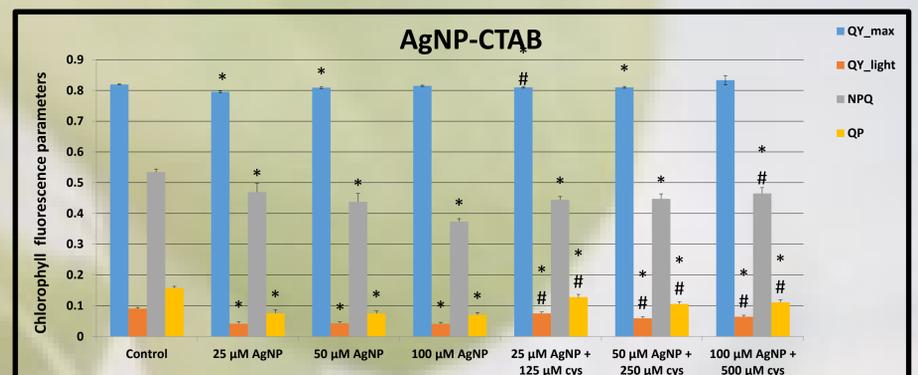


Figure 3. Chlorophyll fluorescence parameters in tobacco leaves treated with AgNP-CTAB. Results are expressed as means of six replicates  $\pm$  SE. Among each Ag-treatment asterisks denote significant difference from control. Hash sign denotes significant differences among treatments with and without cysteine.

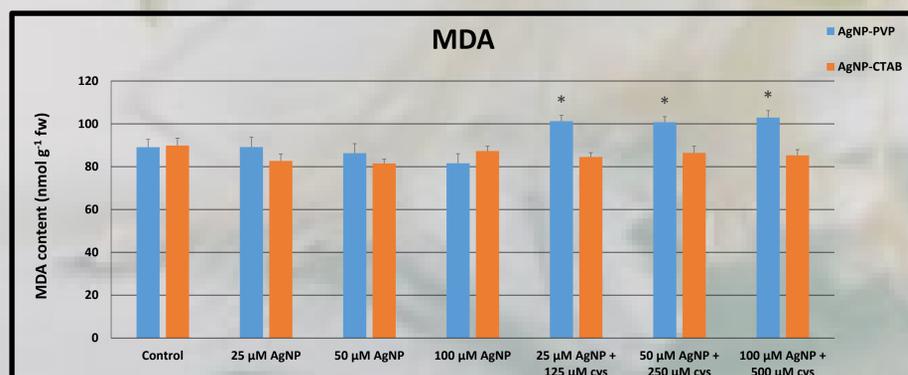


Figure 4. Content of MDA in tobacco seedlings treated with AgNPs. Values are means  $\pm$  SE of two different experiments, each with six replicates. Values marked with different asterisk represent significant difference ( $p \leq 0.05$ ) from control according to Duncan test.

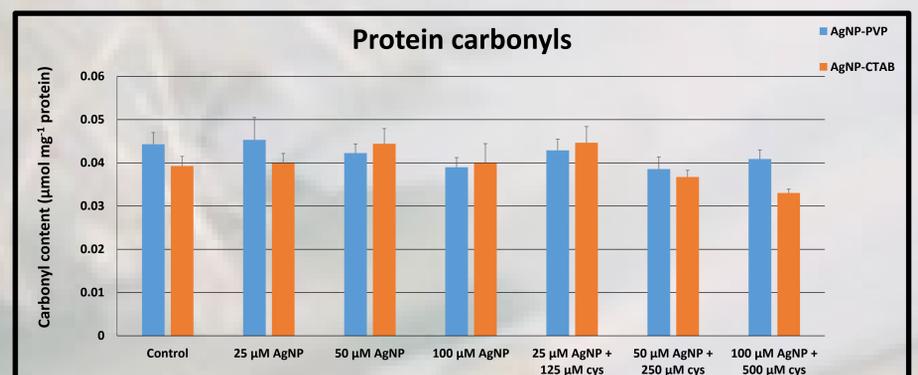


Figure 5. Content of protein carbonyls in tobacco seedlings treated with AgNPs. Values are means  $\pm$  SE of two different experiments, each with six replicates. Values marked with different asterisk represent significant difference ( $p \leq 0.05$ ) from control according to Duncan test.

## CONCLUSION

Results of chlorophyll fluorescence revealed that photosynthesis efficiency was decreased in AgNP treatments, indicating a negative impact on photosynthetic apparatus, which was, however, alleviated when cysteine was added to the medium. On the contrary, MDA content showed that addition of cysteine to AgNP-PVP-containing growth media has induced significant lipid peroxidation in tobacco seedlings. At the same time no such effect was obvious for seedlings treated with AgNP-CTAB medium enriched with cysteine. Moreover, in almost all AgNP treatments, regardless of nanoparticle coating, the level of oxidatively modified proteins was not significantly different from control, suggesting that oxidative stress was not induced during the seedlings growth.