Physiological and proteomic responses of tobacco seedlings exposed to silver nanoparticles

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INTRODUCTION

Nanoparticles (NPs)

- three dimensions between 1 and 100 nm
- advanced electrical, chemical and physical properties

Silver nanoparticles (AgNPs)

- great antibacterial and antifungal properties
- medical application and devices, textiles, food packaging, healthcare and household products
- toxic effects shown on bacteria, animal cells, algae and plants

Model organism

- tobacco (*Nicotiana tabacum* L.) seedlings

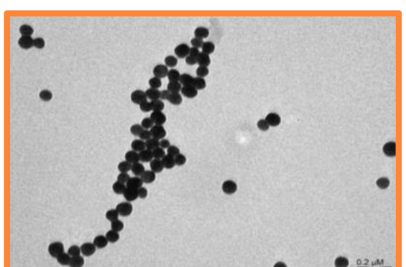

Figure 1. TEM image of AgNP suspension.

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Is AgNP toxicity related to nanoparticle-specific effects or is it result of ionic silver released from AgNPs?

Materials and methods

AgNPs/$AgNO_3$

25 µM
50 µM
75 µM
100 µM

Determination of silver content

- ICP - MS

Oxidative stress parameters

- ROS levels - DHE test
- lipid peroxidation
- protein carbonyl quantification
- DNA damage - Comet assay

Antioxidant enzymes activites

- SOD, PPX, APX, CAT

100 µM

Microscopy analyses

- AgNP localization
- morphological changes - light microscopy
- ultrastructural changes - electron microscopy

Proteome changes

- 2-D electrophoresis → MALDI TOF/TOF → protein identification using NCBIprot

Silver content

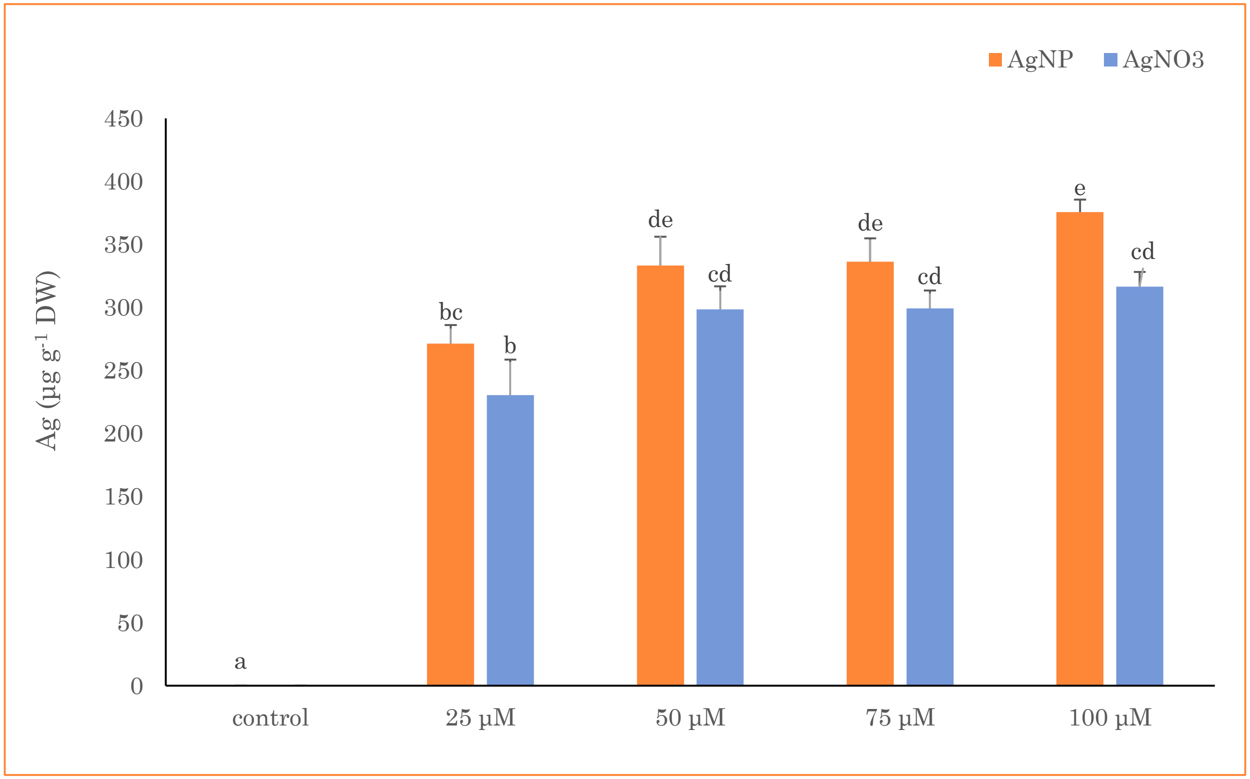


Figure 1. Silver content in tobacco seedlings treated with AgNPs and $AgNO_3$. Values are means ± SE of three different experiments, each with three replicas. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

Oxidative stress parameters

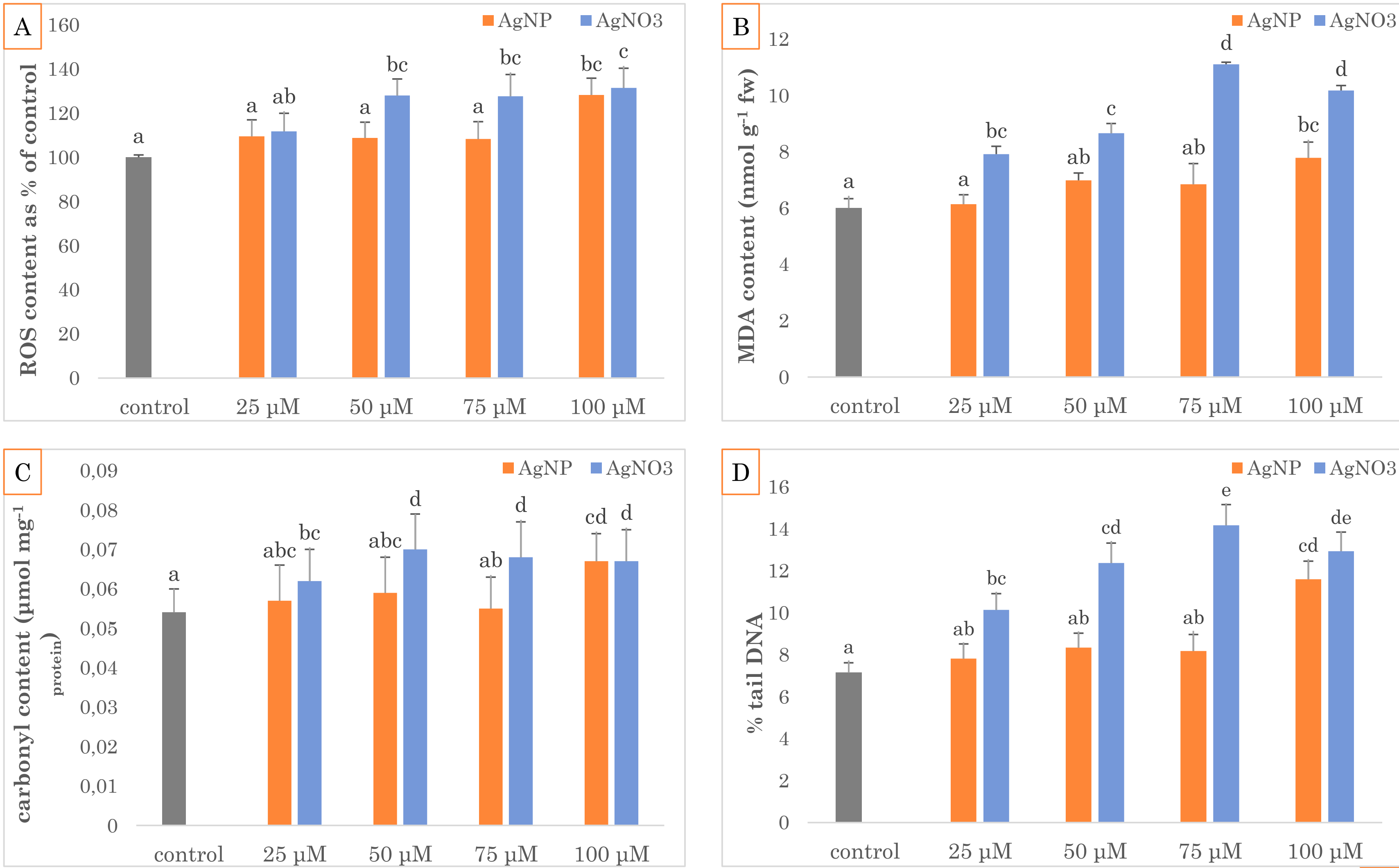


Figure 2. Content of ROS (A), MDA (B), protein carbonyl (C) and % tail DNA (D) in tobacco seedlings treated with AgNPs and $AgNO_3$. Values are means ± SE of three different experiments, each with three replicas. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

Antioxidant enzyme activity

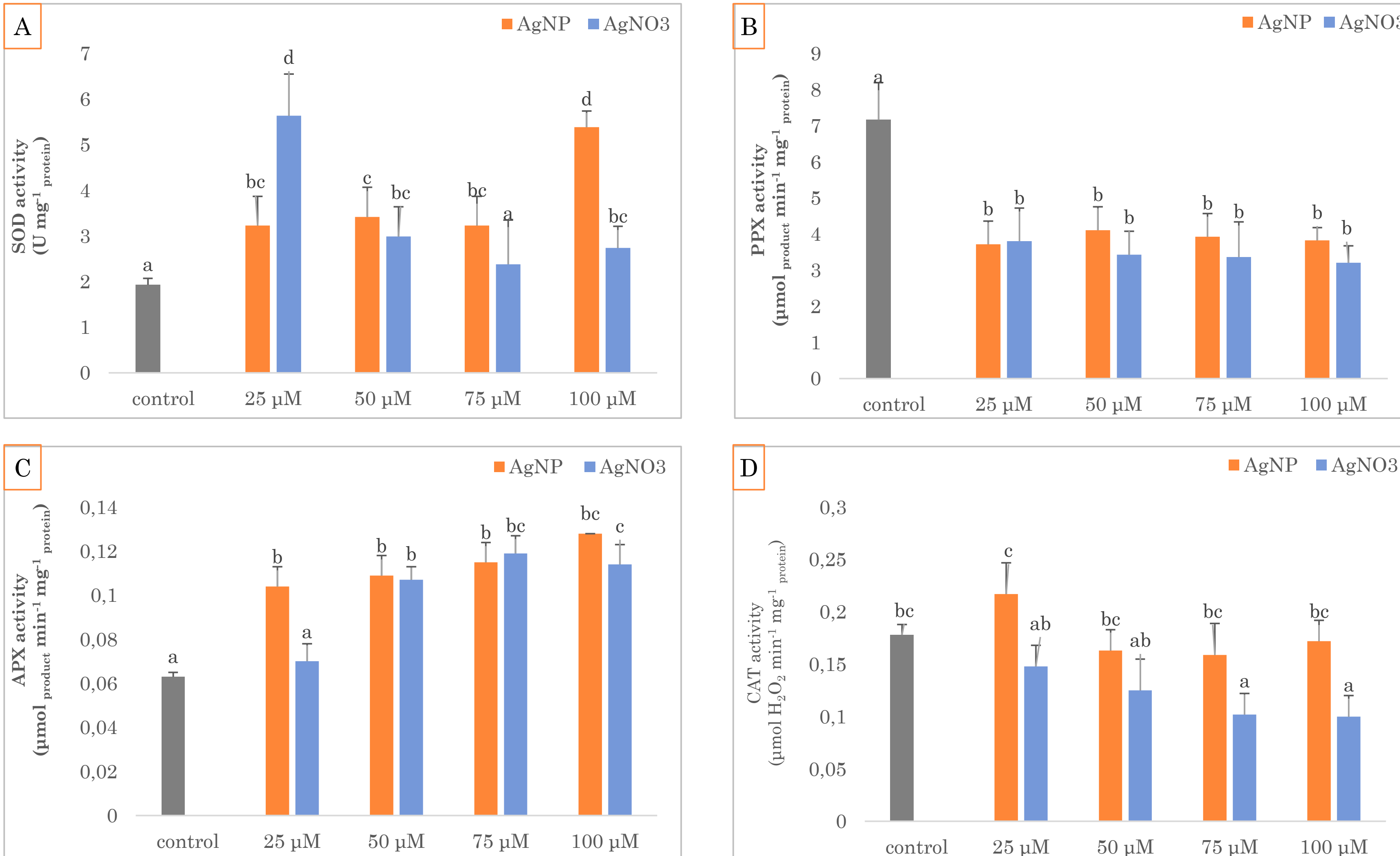


Figure 3. Specific activities of SOD, PPX, APX and CAT in tobacco seedlings treated with AgNPs and $AgNO_3$. Values are means ± SE of three different experiments, each with three replicas. Values marked with different letters represent significant difference ($p \leq 0.05$) according to Duncan test.

Microscopy analyses

AgNP localization

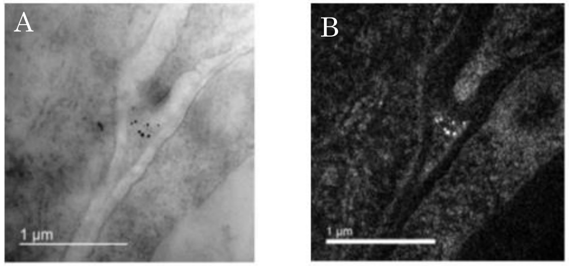
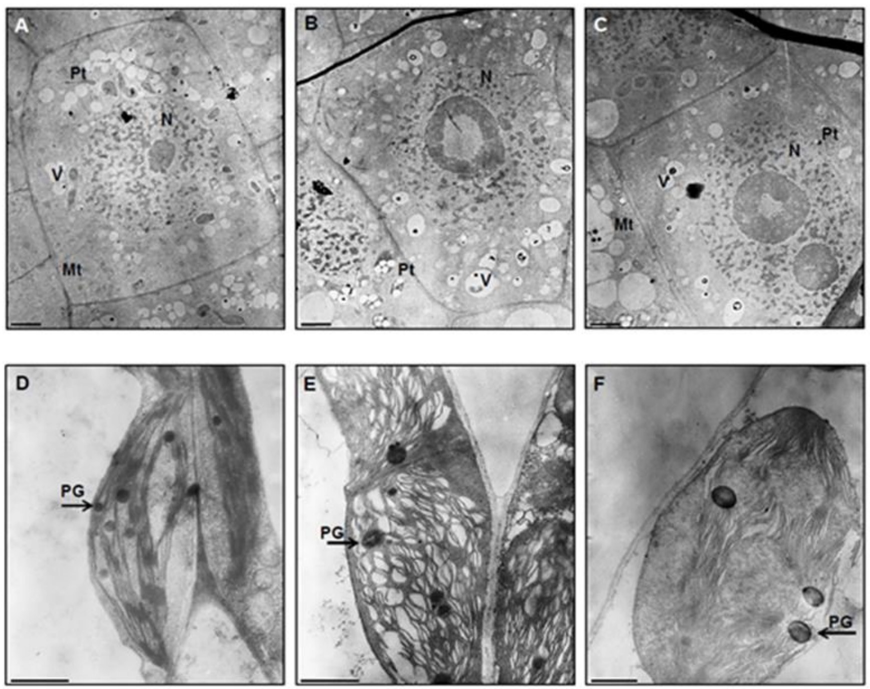


Figure 4. AgNP localization in the root cells of the 100 uM AgNP-treated tobacco seedlings (A) and bright field image (B).

Ultrastructural changes



Morphological changes

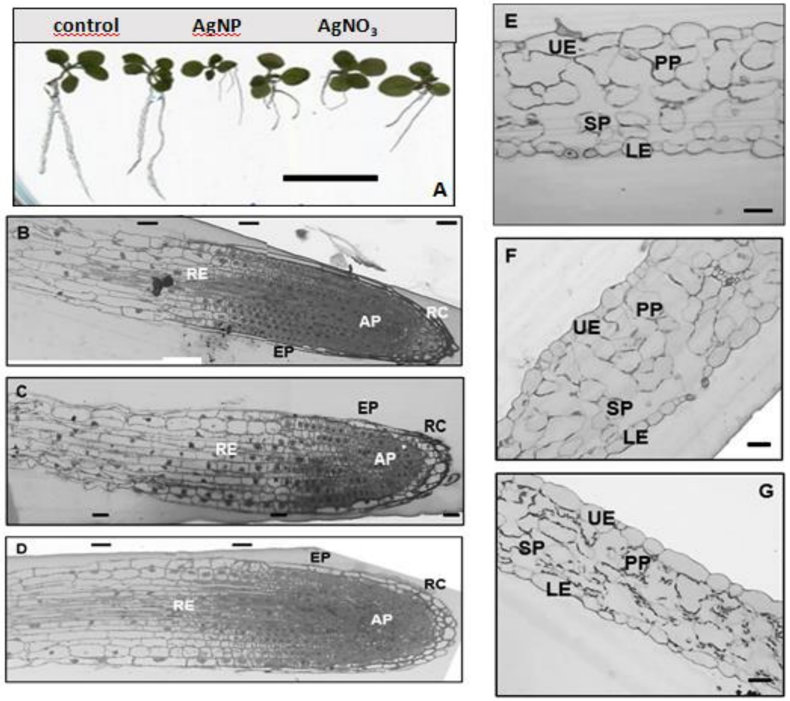


Figure 5. Root length of tobacco seedlings (A). Semithin sections of root from control (B), 100 µM AgNP-treated (C) and 100 µM $AgNO_3$-treated (D) tobacco seedlings (bar = 33.1 µm) and leaf from control (E), 100 µM AgNP-treated (F) and 100 µM $AgNO_3$-treated (F) tobacco seedlings (bar = 30.6 µm).

RC – root cap, AP – apical meristem, RE – region of elongation, EP – epidermis, UE – upper epidermis, LE – lower epidermis, PP – palisade parenchyma, SP – spongy parenchyma.

Figure 6. Ultrastructure of root cells and leaf chloroplasts. Root cells of control (A), 100 µM AgNP-treated (B) and 100 µM $AgNO_3$-treated (C) tobacco seedlings (bar = 2 µm). Chloroplasts in leaf cells of control (D), 100 µM AgNP-treated and 100 µM $AgNO_3$-treated tobacco seedlings (bar = 1 µm).

N – nucleus, V – vacuole, Mt – mitochondrion, Pt – plastid, PG – plastoglobules.

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Proteomic changes

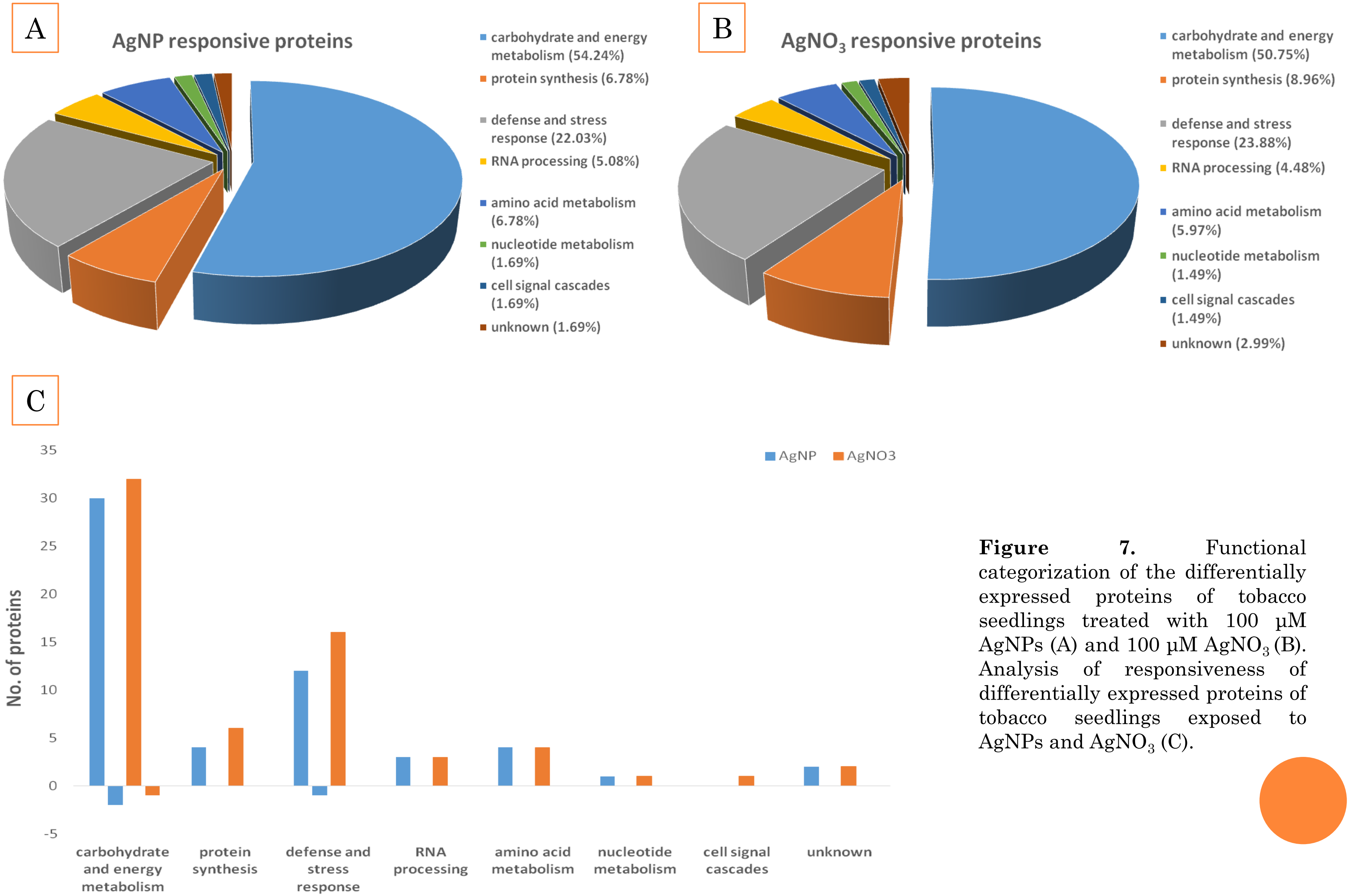

Figure 7. Functional categorization of the differentially expressed proteins of tobacco seedlings treated with 100 µM AgNPs (A) and 100 µM $AgNO_3$ (B). Analysis of responsiveness of differentially expressed proteins of tobacco seedlings exposed to AgNPs and $AgNO_3$ (C).

Conclusions

- higher Ag content was measured in seedlings exposed to AgNPs compared to $AgNO_3$ of the same concentration
- obtained results on oxidative stress parameters revealed that in general higher toxicity was recorded in $AgNO_3$-treated seedlings compared to those exposed to nanosilver
- presence of silver in the form of nanoparticles was confirmed in the root cells, which may explain the lower toxicity of AgNPs
- proteomic study showed that both AgNPs and $AgNO_3$ can affect photosynthesis, and that is in correlation with the observed ultrastructural changes of chloroplasts
- majority of the proteins involved in the primary metabolism were up-regulated after both types of treatments, indicating that enhanced energy production, which can be used to reinforce defensive mechanisms, enables plants to cope with silver-induced toxicity

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