Differently coated silver nanoparticles cause oxidative stress and induce cellular damage in tobacco (*Nicotiana tabacum*) seedlings

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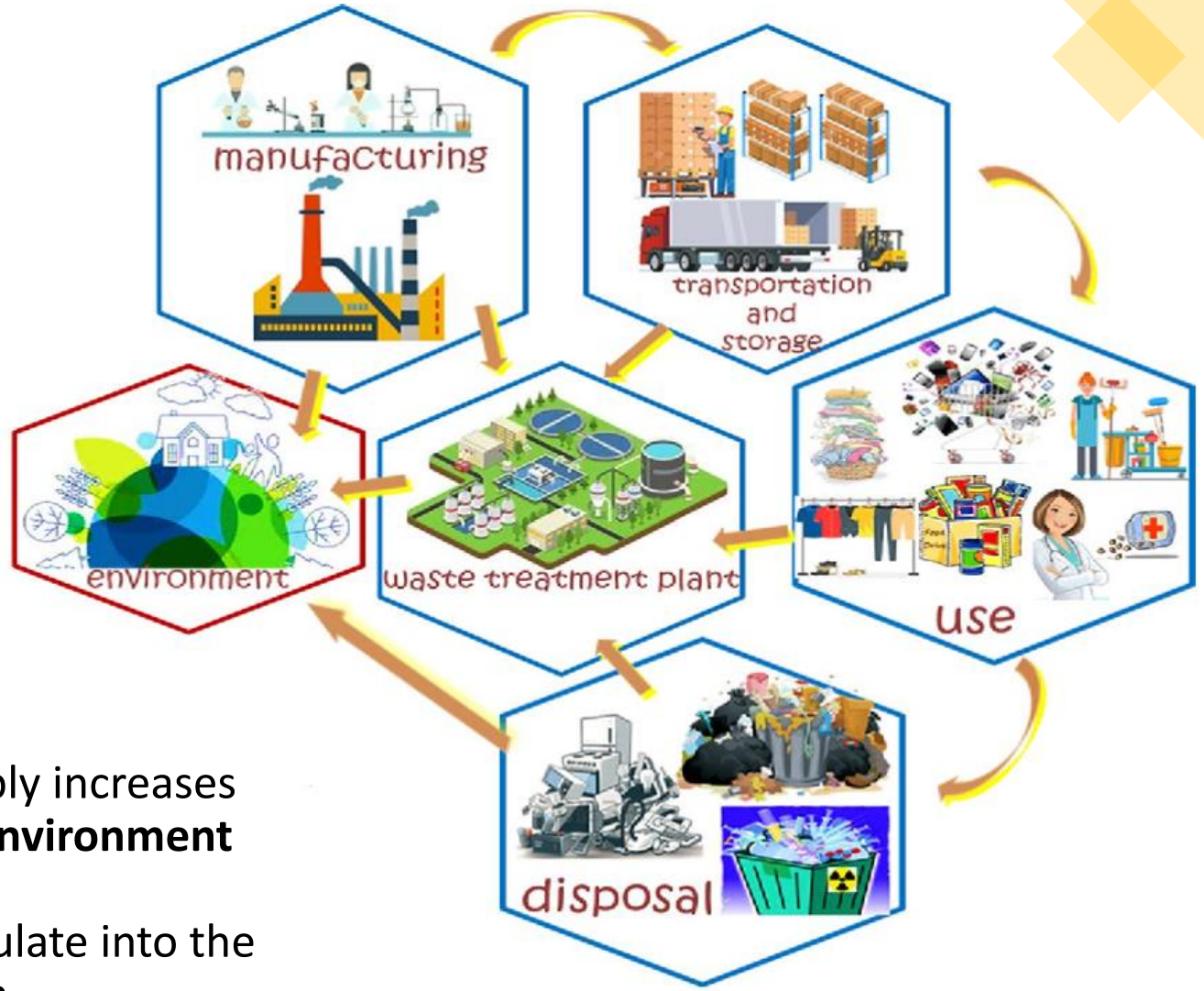
Silver nanoparticles (AgNPs)

- small size (1 – 100 nm) and unique physico-chemical characteristics

→ application in many industrial sectors and daily life

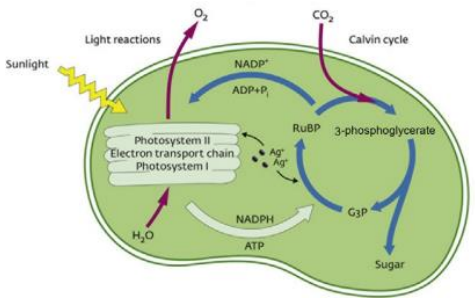
- antibacterial and antifungal properties – household products, food packaging, textiles, medical devices, antiseptics



- growing consuption of AgNPs inevitebly increases the chance of their **release into the environment**

  → through plants they can bioaccumulate into the food chain → **threat to human health**

Phytotoxic effects of AgNPs

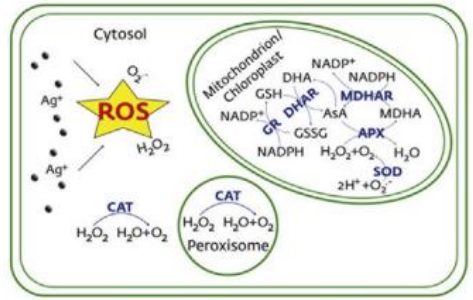


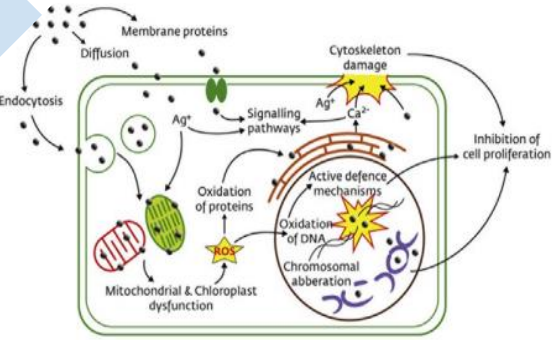
Damage of photosynthesis

- reduced photosynthetic activity
- decreased ATP and NADPH synthesis

Changes in antioxidant machinery

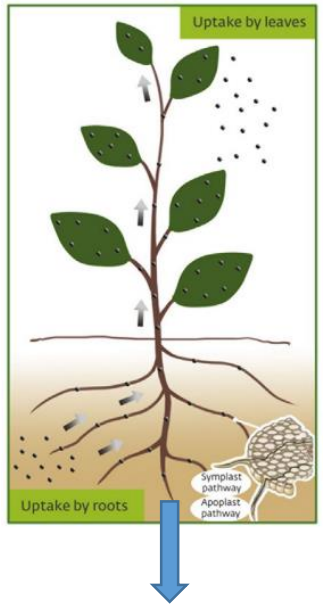
- enzymatic antioxidants (SOD, CAT, APX, GR…)
- non-enzymatic antioxidants (proline, GSH, ascorbate…)





Overall cellular effects

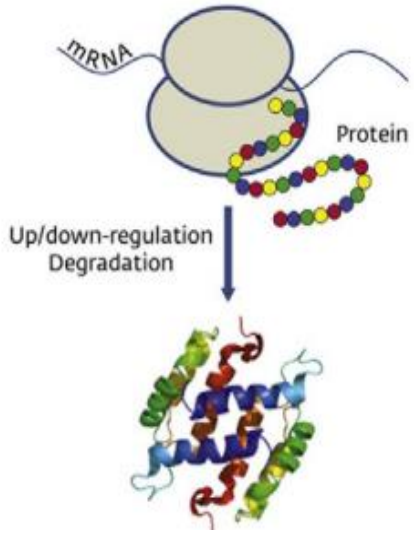
- excessive ROS formation
- disruption of cell membrane integrity
- damage to lipids, proteins and DNA



Changes in plant morphology

Changes in protein expression

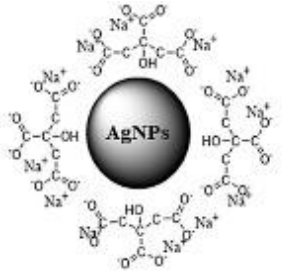
- glycolysis
- respiration
- protein biosynthesis, folding and assembly
- pathogenesis response
- antioxidant response

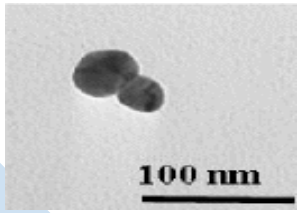


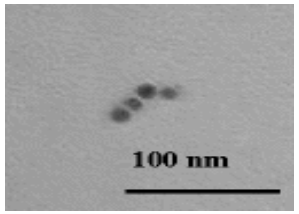
What affects AgNP phytotoxicity?

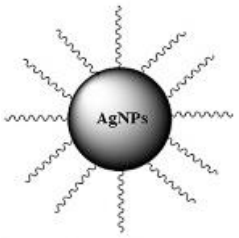
Physico-chemical properties

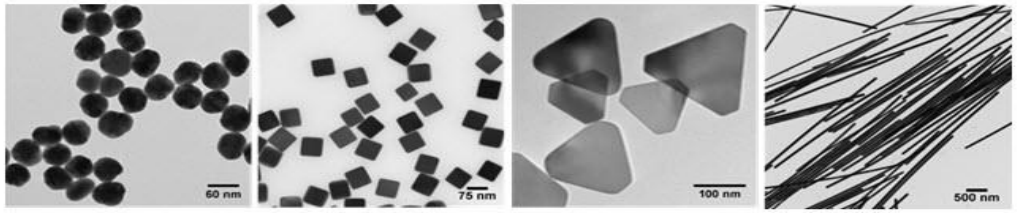
- size (1 – 100 nm)
- shape
- concentration
- surface coating





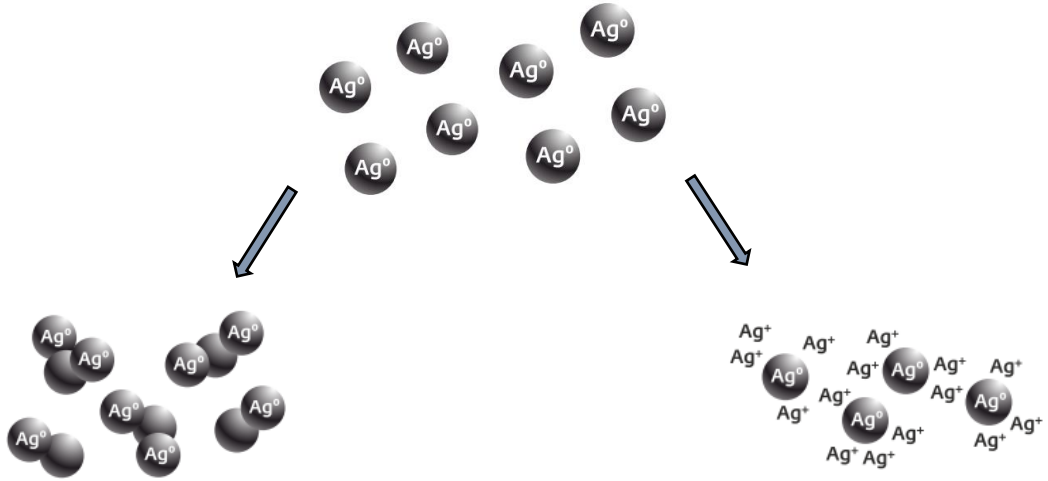






Experimental conditions

- plant species
- exposure period
- composition of nutrient medium, aqueous solution or soil

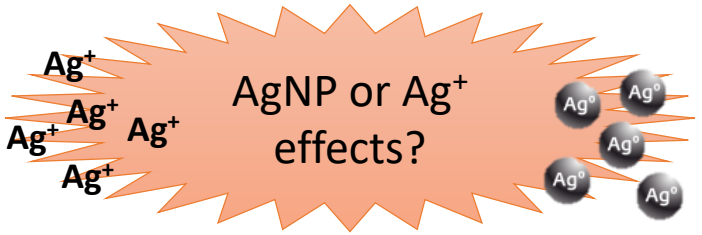


aggregation/agglomeration

dissolution

Aim

- determine the effects of two differently coated AgNPs [polyvinylpyrrolidone (AgNP-PVP) and cetyltrimethylammonium bromide (AgNP-CTAB)] on oxidative stress parameters of tobacco (*Nicotiana tabacum* L.) seedlings and compare them to the effects of $AgNO_3$
- distinguish differences in effects between silver applied in the form of nanoparticles and its ionic form by comparing the effects of AgNP-PVP and AgNP-CTAB to $AgNO_3$ applied at the same concentration



Materials and methods

AgNP-PVP, AgNP-CTAB and $AgNO_3$

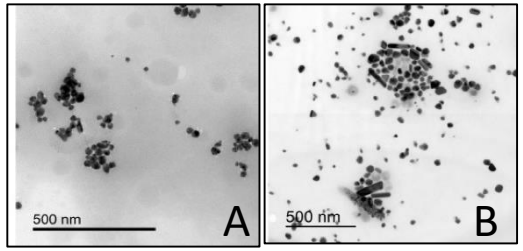
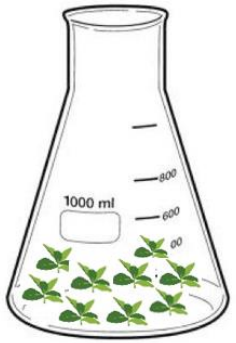


Figure 1. TEM images of AgNP-PVP (A) and AgNP-CTAB (B).

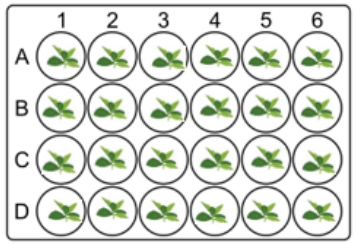
25 µM, 50 µM and 100 µM
in ½ MS nutrient medium



7 days

- ROS levels:
  - DHE test
  - H_2O_2 content
- non-enzymatic antioxidants:
  - proline content
  - glutathione assay

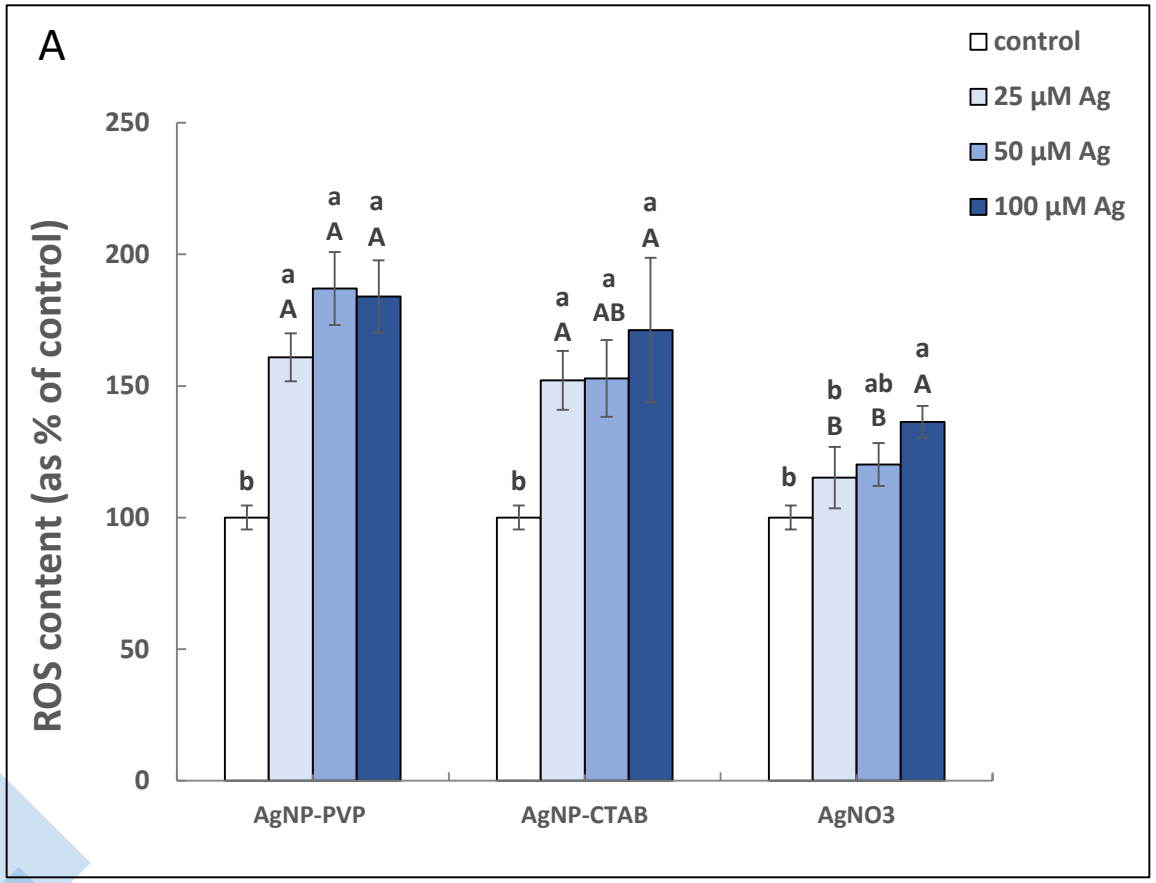
100 µM
in MilliQ H_2O



24 hours

- early stress response *in situ*
  - ROS formation (DHE for $O_2^{\bullet-}$ and H_2DCF for H_2O_2)
  - glutathione level (MCB)
  - cell death (PI)

Induction of ROS



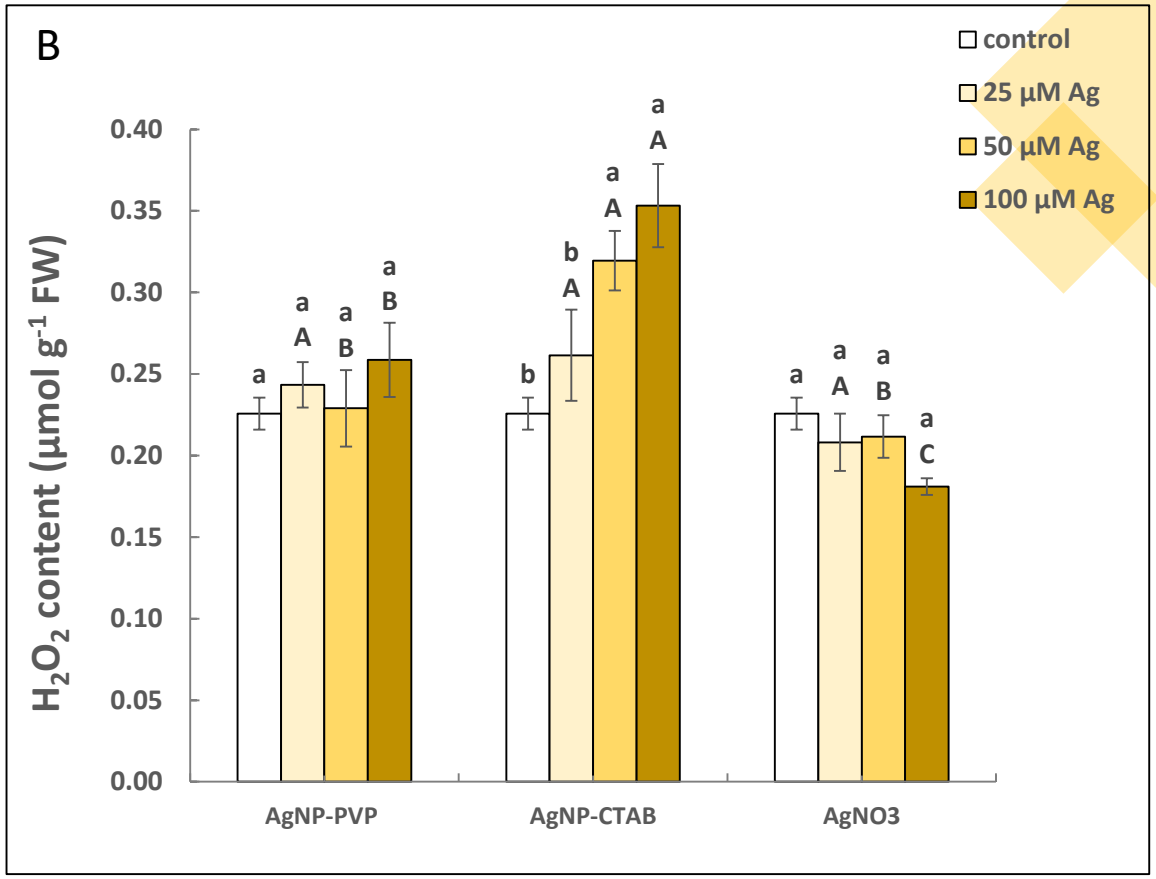
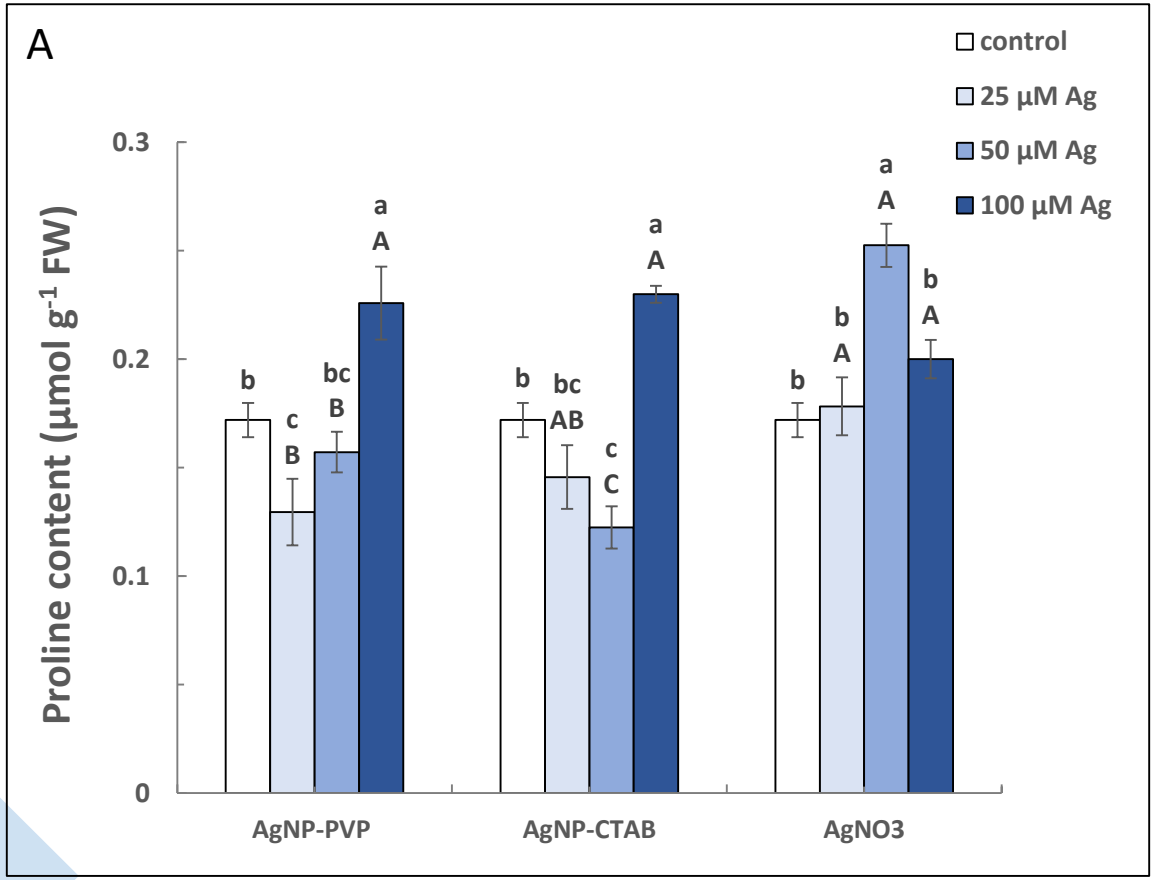


Figure 2. ROS (A) and H_2O_2 (B) content in tobacco seedlings treated with AgNP-PVP, AgNP-CTAB and $AgNO_3$. Values are means ± SE of six replicates. Small letters mark the differences among different concentrations of the same treatment type as well as control while capital letters mark the differences among different treatment types of the same concentration, according to Duncan test ($P \leq 0.05$).

Non-enzymatic antioxidants



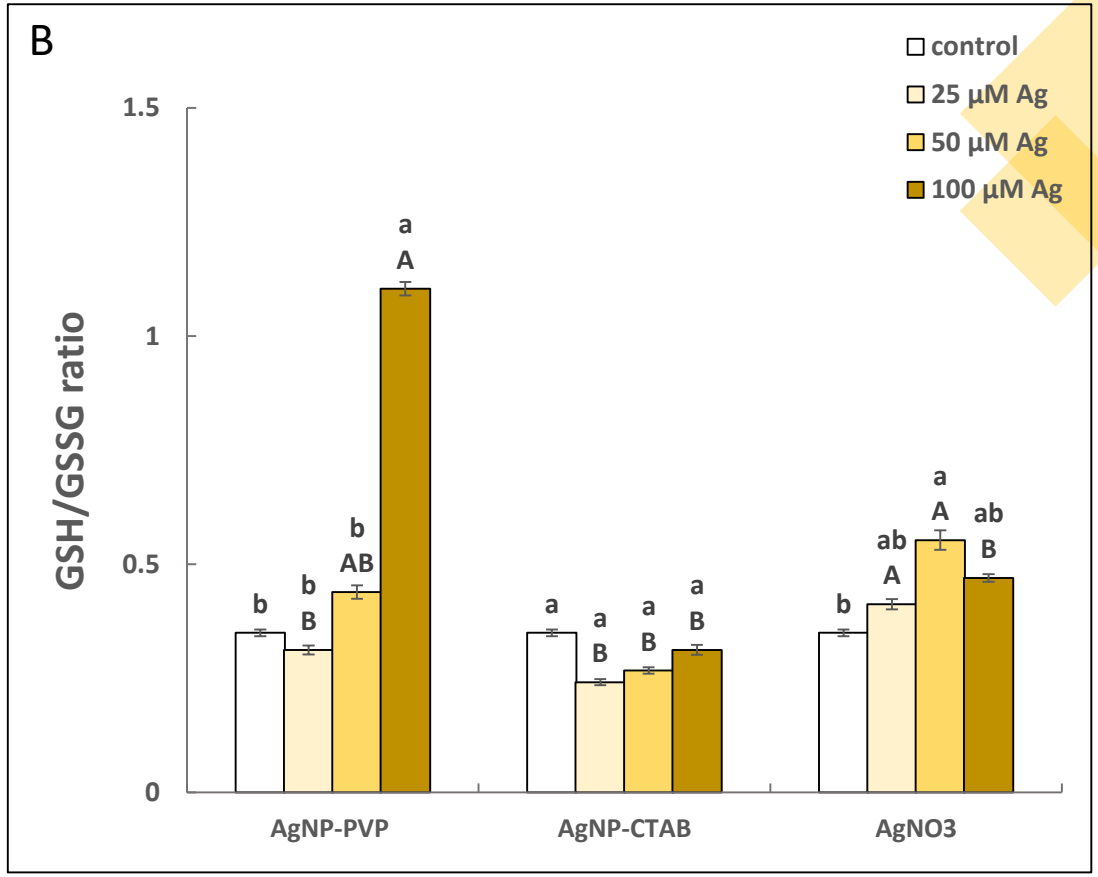
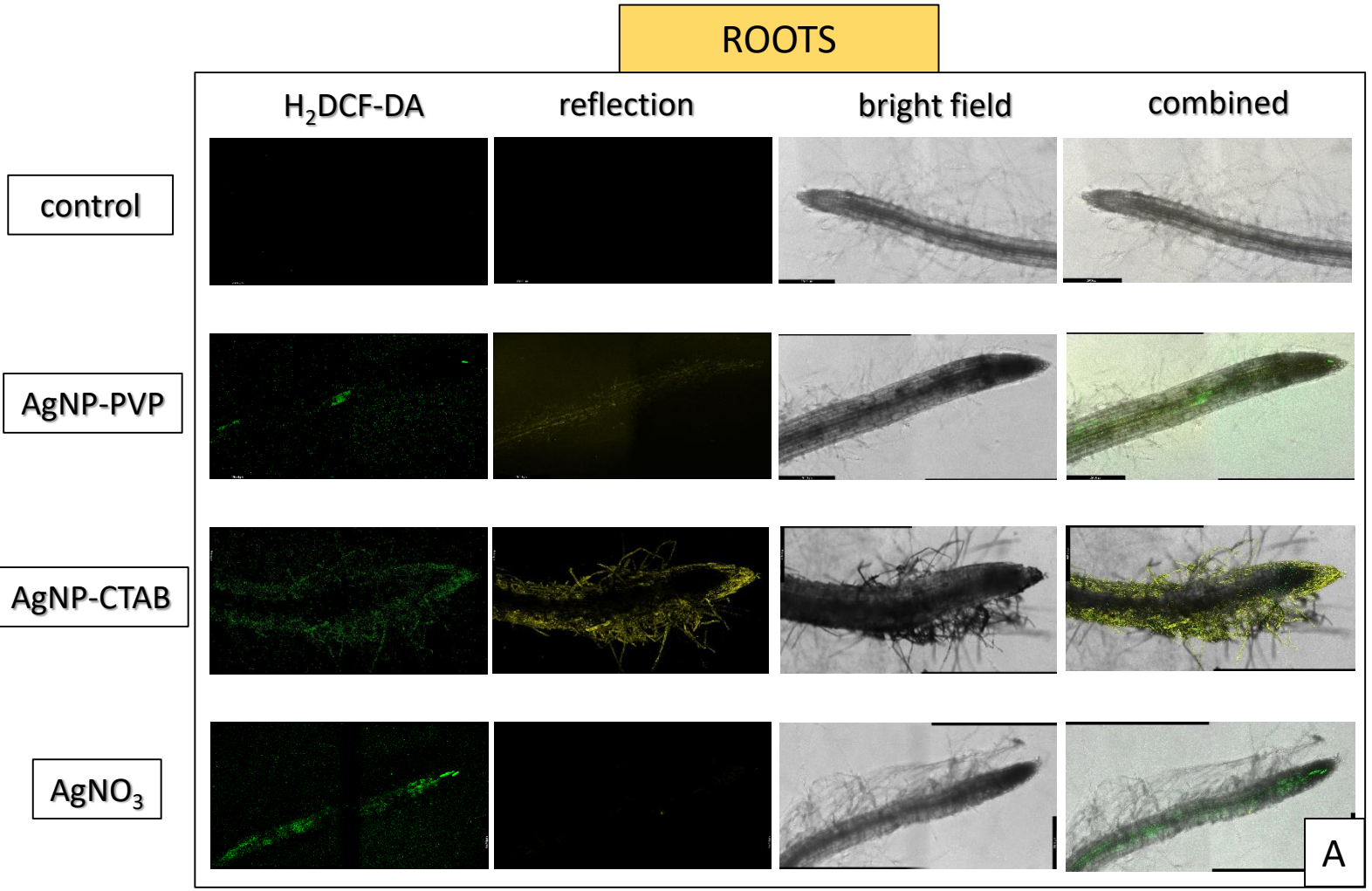


Figure 3. Proline (A) and glutathione (B) content in tobacco seedlings treated with AgNP-PVP, AgNP-CTAB and $AgNO_3$. Values are means ± SE of six replicates. Small letters mark the differences among different concentrations of the same treatment type as well as control while capital letters mark the differences among different treatment types of the same concentration, according to Duncan test ($P \leq 0.05$).

Detection of H_2O_2



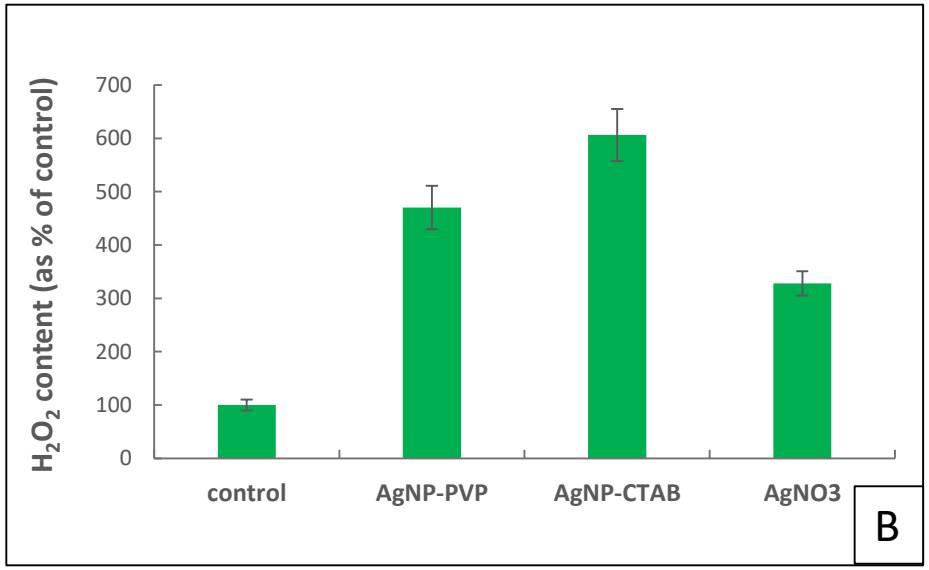
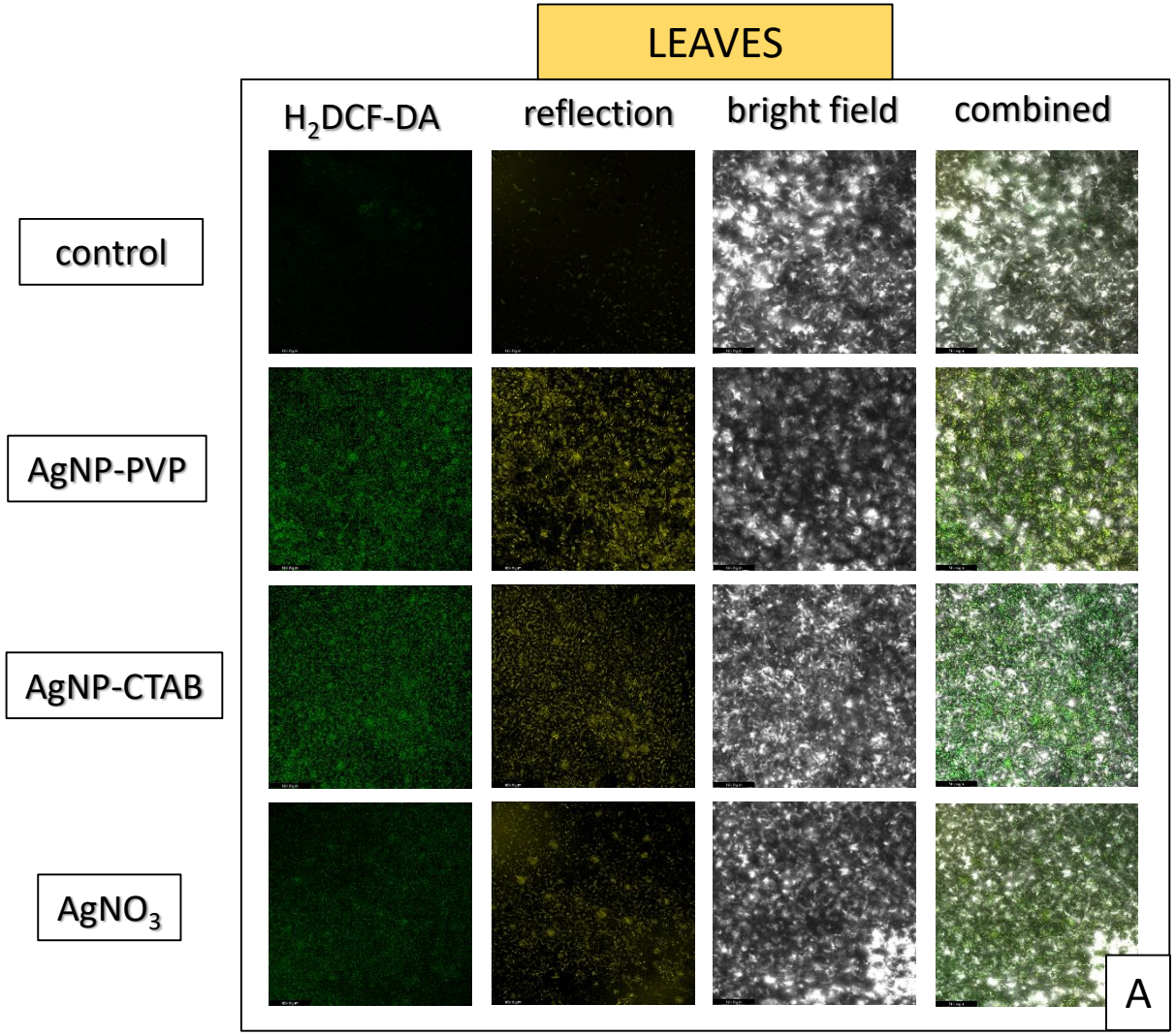


Figure 4. (A) H_2O_2 detection in control roots of tobacco seedlings and roots treated with 100 µM AgNP-PVP, AgNP-CTAB and $AgNO_3$; shown pictures represent maximum intensity projection over 30-40 scans in "Z" dimension; bar = 282.6 µm. (B) Total H_2O_2 content in roots measured as total intensity of Z scan ± SD.

Detection of H_2O_2



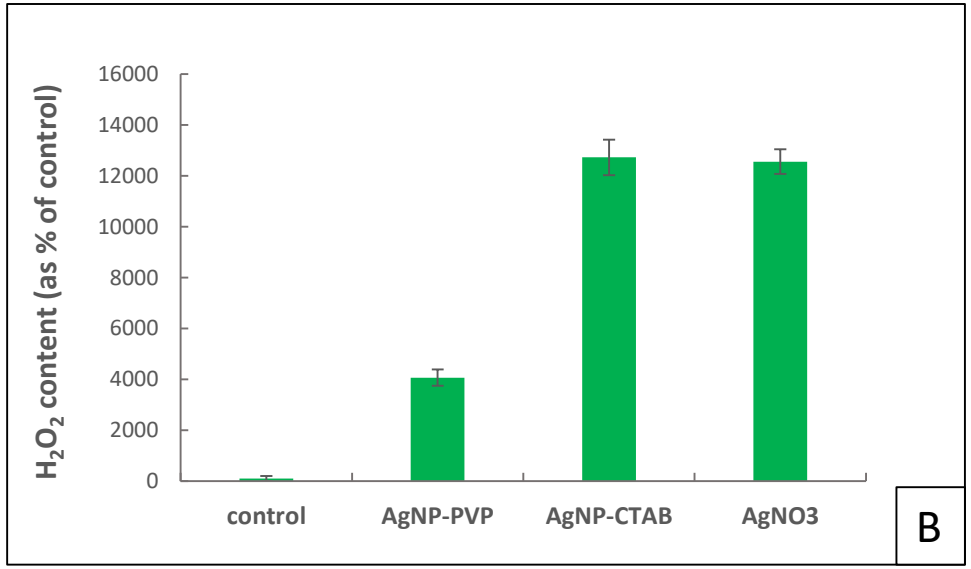
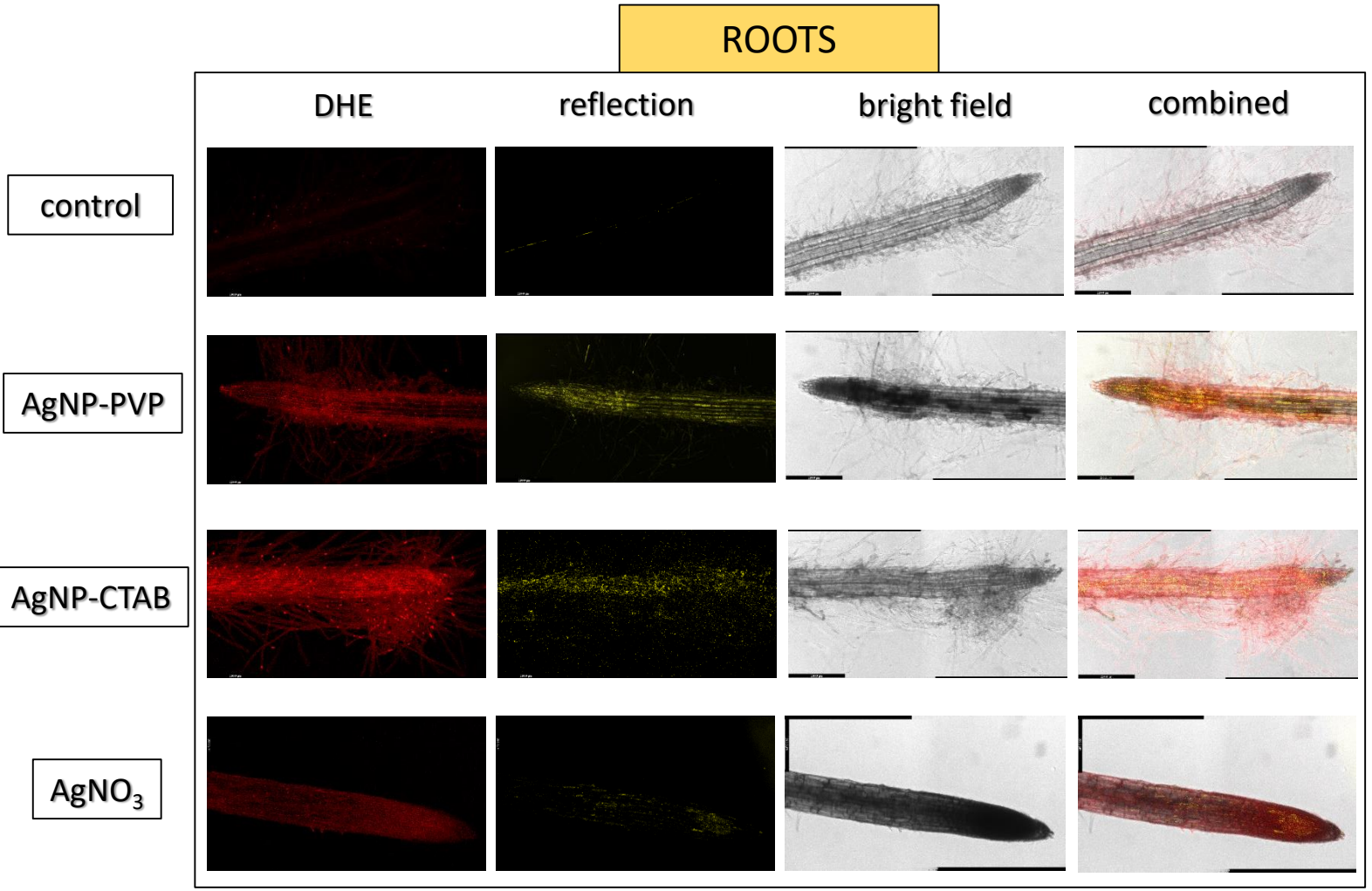


Figure 5. (A) H_2O_2 detection in control leaves of tobacco seedlings and leaves treated with 100 µM AgNP-PVP, AgNP-CTAB and $AgNO_3$; shown pictures represent maximum intensity projection over 30-40 scans in "Z" dimension bar = 154.5 µm. (B) Total H_2O_2 content in leaves measured as total intensity of Z scan $\pm$ SD.

Detection of $O_2^{\cdot -}$



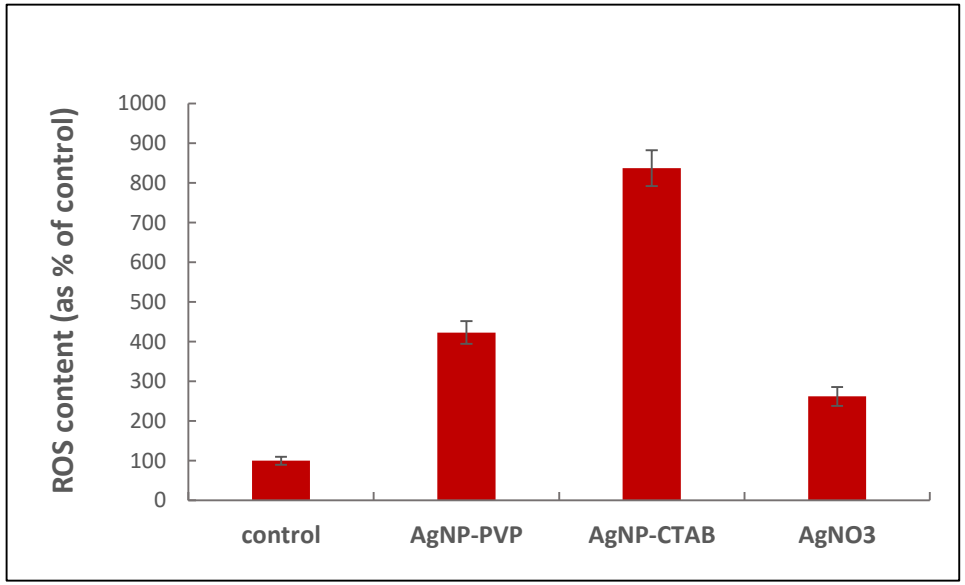
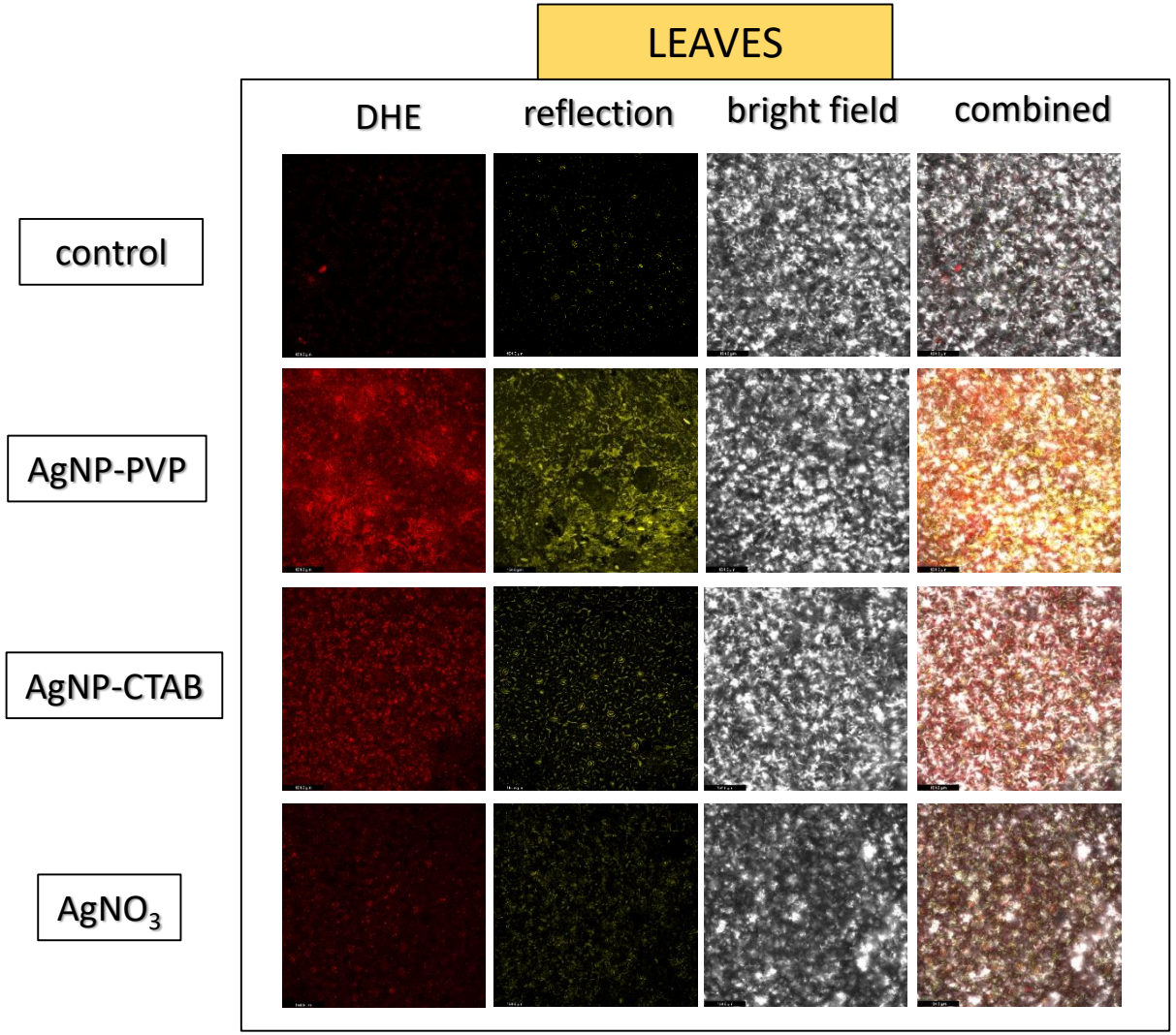


Figure 6. $O_2^{\cdot -}$ detection in control roots of tobacco seedlings and roots treated with 100 µM AgNP-PVP, AgNP-CTAB and $AgNO_3$; shown pictures represent maximum intensity projection over 30-40 scans in "Z" dimension; bar = 282.6 µm. (B) Total $O_2^{\cdot -}$ content in roots measured as total intensity of Z scan ± SD.

Detection of $O_2^{\cdot -}$



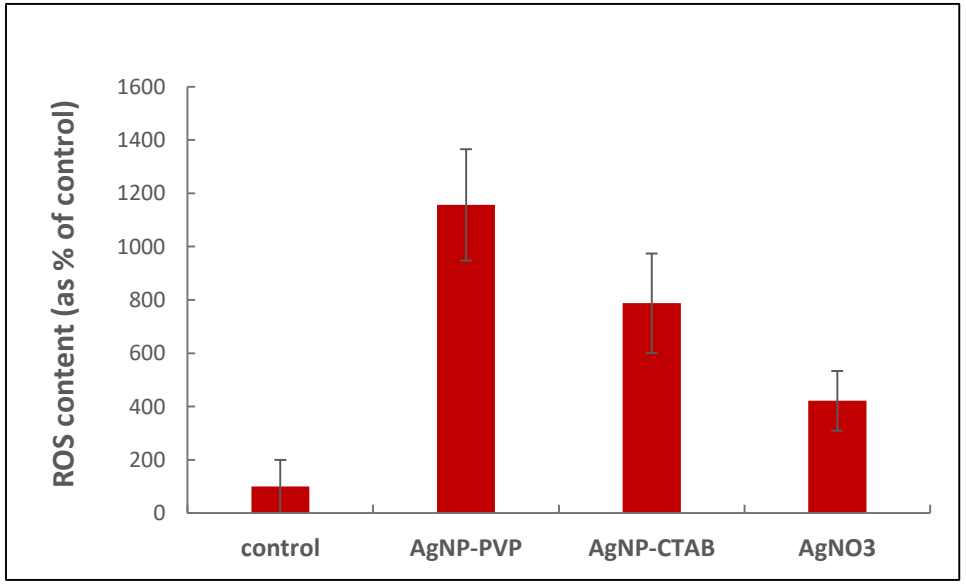


Figure 7. $O_2^{\cdot -}$ detection in detection in control leaves of tobacco seedlings and leaves treated with 100 µM AgNP-PVP, AgNP-CTAB and $AgNO_3$; shown pictures represent maximum intensity projection over 30-40 scans in "Z" dimension bar = 154.5 µm. (B) Total $O_2^{\cdot -}$ content in leaves measured as total intensity of Z scan ± SD.

Detection of glutathione and cell viability

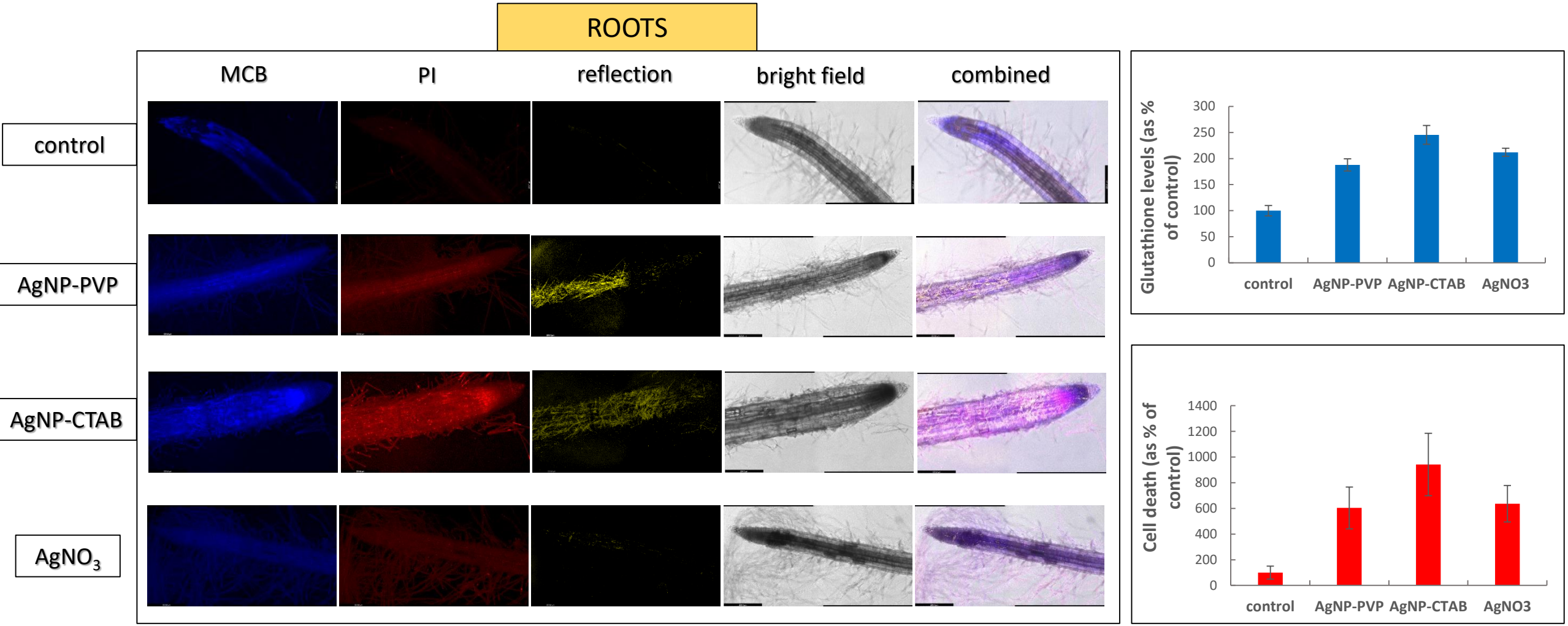
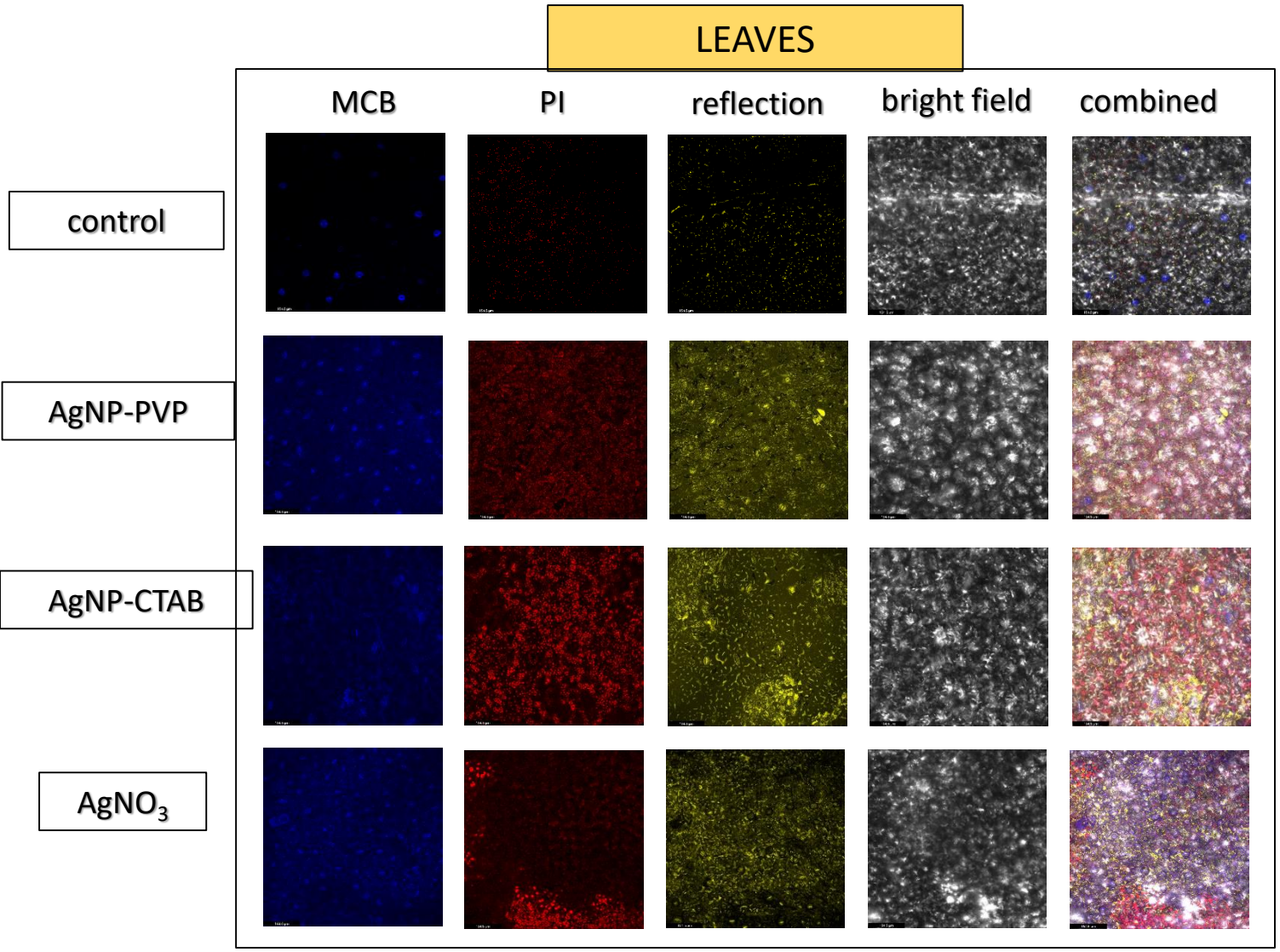
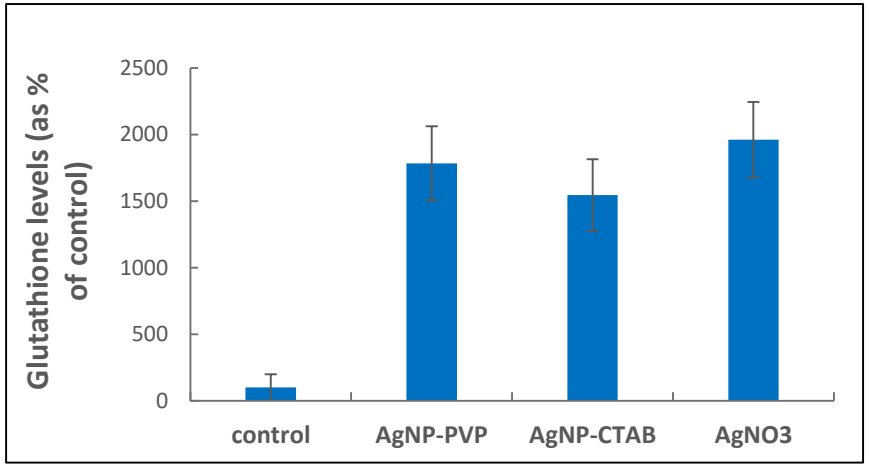


Figure 8. Glutathione detection and cell viability in control roots of tobacco seedlings and roots treated with 100 µM AgNP-PVP, AgNP-CTAB and $AgNO_3$; shown pictures represent maximum intensity projection over 30-40 scans in "Z" dimension bar = 282.6 µm. (B) Total glutathione content and cell death in roots measured as total intensity of Z scan ± SD.

Detection of glutathione and cell viability





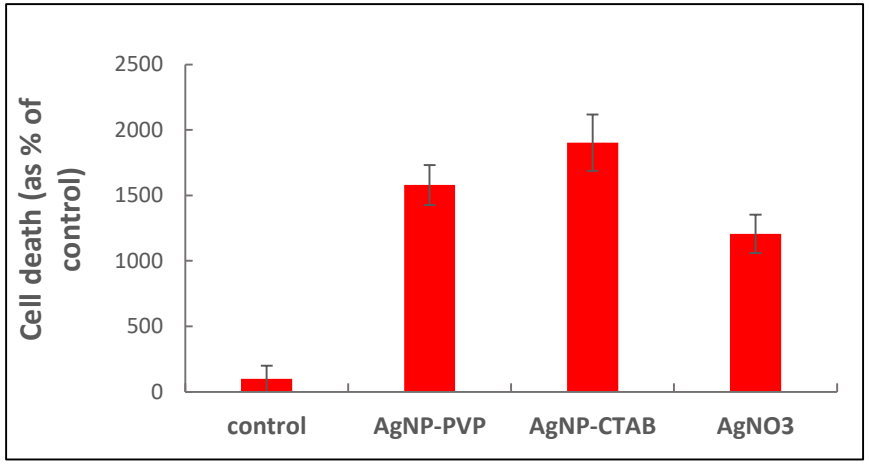


Figure 9. Glutathione detection and cell viability in control leaves of tobacco seedlings and leaves treated with 100 µM AgNP-PVP, AgNP-CTAB and $AgNO_3$; shown pictures represent maximum intensity projection over 30-40 scans in "Z" dimension bar = 154.5 µm. (B) Glutathione content and cell death in leaves measured as total intensity of Z scan ± SD.

Conclusion

- even though both types of AgNPs induce oxidative stress causing cellular damage, those effects were more pronounced in treatments with positively charged AgNP-CTAB
- since effects of AgNPs differ to those of $AgNO_3$, we can conclude that phytotoxicity of AgNPs goes through mechanisms that cannot be completely assigned to Ag^+ release

Literature

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Thank you! ☺