

PLANT EXTRACT REDUCED AND STABILIZED GOLD NANOPARTICLES AS MODEL PARTICLES FOR FOLIAR APPLICATION OF FERTILIZERS

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Abstract

Foliar application of nanofertilizers is currently regarded as a promising alternative to conventional soil fertilization. For successful development of nanofertilizers, firstly, a better understanding of interactions of nanoparticles with the leaf surface is needed. In the framework of this study a globally available cereal grain *Hordeum Vulgare* (barley) was chosen as model plant. Gold nanoparticles (Au NPs) were chosen as model particles for foliar application. Two synthesis methods were used: established photoreduction with citric acid and phytochemical synthesis using *Aegopodium Podagraria* (ground elder) extract. In both cases the reducing agent was acting also as surface stabilizer. Au NPs formation was confirmed by UV-VIS spectroscopy. To our knowledge it is the first time *A. Podagraria* extract was used for Au NPs synthesis. The morphology of the produced particles was characterized by transmission electron microscopy (TEM). The interaction of Au NPs with the adaxial side of barley leaves was studied by low-vacuum scanning electron microscopy (SEM). It was established that citrate stabilized Au NPs agglomerates evenly distribute in droplet-leaf contact areas avoiding stomates. Plant extract stabilized Au NPs form a film in the contact areas and accumulate on all leaf parts – stomates, trichomes and lamina. It was shown that Au NPs produced via green phytochemical synthesis have improved interaction with the leaf surface of barley.

Keywords: Au NPs, phytochemical synthesis, foliar fertilization

1. INTRODUCTION

One of the most important current challenges facing agriculture is to improve the efficiency and sustainability of fertilization procedures. Application of conventional fertilizers results in poor nutrient uptake due to low bioavailability and releasing rates overwhelming actual plant absorption rates. Furthermore, nutrients trapped in the soil are causing serious environmental constraints worldwide, including ground water pollution and eutrophication, soil quality degradation, and air pollution [1,2].

Foliar application of nanofertilizers is currently regarded as a promising alternative to conventional soil fertilization [3-5]. It was shown that NPs are capable of crossing plant mechanical barriers (e.g. cuticle and cell walls) with further translocation [6-8]. Moreover, NPs surfaces are easily modifiable with a variety of coatings and, similar to drug delivery, NP surface properties can theoretically be tuned to deliver them to specific leaf structures or tissues in plants [9,10]. This could also provide slow and controlled release of the nutrients in the desired locations of plants [8]. All this advantages combined with the application approach which bypasses soil have the potential to reduce unsustainable fertilization practices and increase yields through optimized nutrient management. However, despite the great potential of NPs as nanofertilizers, there remains limited understanding of how the physical and chemical properties (e.g. size, charge, solubility, coating, chemical composition) of NPs influence their interaction with the leaf surface. A better understanding of these NP-leaf interactions is needed for successful nanofertilizers development.

The leaf cuticle can be described as a composite membrane, which is mainly composed of cutin, an amorphous and non-soluble polyester formed by condensed polyhydroxylated fatty acids, which acts as a solid matrix for the deposition of the other components (e.g., polysaccharides and phenolic compounds). The leaf itself is covered with a layer of hydrophobic epicuticular waxes [11,12]. In general, there are three main natural pathways how NPs could penetrate cuticle layer – stomates, trichomes and pores [6,7,9].

In the framework of the study, a globally available cereal grain *Hordeum Vulgare* (barley) was chosen as model plant. Au NPs were chosen as model particles due their stability and absence of background signal within the plant. To evaluate the impact of surface modification on interaction with the leaf surface, two different agents were used – citric acid and *A. Podagraria* plant extract. Citrate reduced and stabilized Au NPs were synthesized by using an established photoreduction process [13]. Plant extract reduced and stabilized Au NPs were synthesized via phytochemical reduction also involving UV light. Phytochemical synthesis of NPs using plant extracts is a cost-effective and environmentally friendly approach [14,15]. The types of solvents used during extraction affect the type and amount of reducing agents extracted. According to [16] using water as solvent results in the extraction of the following compounds: tannins, terpenoids, saponins, starches, and polypeptides. Saponins are amphiphilic secondary plant compounds, which should improve interaction of metallic Au NPs with the hydrophobic surface of barley leaf.

The aim of the study is to investigate the interaction between these Au NPs as model particles for foliar application of fertilizers and the barley leaf surface. It is expected that the different nature of the reducing/stabilizing agents for Au NPs synthesis will result in different accumulation patterns on the barley leaf surface.

2. MATERIALS AND METHODS

2.1. *A. Podagraria* extract preparation

For plant extract preparation, the aerial parts of *A. Podagraria* plants were collected in May 2022 in Denmark, washed and air dried in the shadow for 2 weeks. The air-dried samples were ground until pieces with size around 0.2-0.5 mm were obtained. The extract was prepared via ultrasonification using DI water as extractant. For extraction, 1.0000 g of the ground material was weighted, and 30.00 ml of water was added. The sample was kept in an ultrasonic bath at room temperature for 20 min. The extraction process was repeated twice. The extract was filtered and stored at 2°C.

2.2. Synthesis and characterization of Au NPs

Photoreduction of the citrate reduced and stabilized Au NPs was performed as follows: a mixture of 0.039 mL of 0.052 M chloroauric acid trihydrate $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (Sigma-Aldrich, $\geq 99.9\%$ trace metal basis) and 1.0 mL of 0.10 M citric acid $\text{C}_6\text{H}_8\text{O}_7$ (anhydrous, Thermo Scientific, ACS, 99.5%) solutions was diluted with DI water until the total volume of the mixture was 10 mL. The final content of HAuCl_4 was 0.2 mM and the $\text{HAuCl}_4:\text{C}_6\text{H}_8\text{O}_7$ ratio was 1:50. Mixture was stirred at ambient conditions under 254 nm light irradiation (8 Watt) for 100 minutes.

For the plant extract reduced and stabilized Au NPs synthesis a mixture of 0.039 mL of 0.052 M HAuCl_4 solution and 1.0 mL of *A. Podagraria* plant extract (see 2.1. Section) was diluted with DI water until the total volume of the mixture was 10 mL. The final content of HAuCl_4 was 0.2 mM. The mixture was stirred at ambient conditions under 254 nm light irradiation for 100 minutes. Au NPs formation was confirmed by UV-VIS spectroscopy (VWR UV-3100PC). The produced NPs size and shape were characterized with TEM (Tecnai T20 G2 S-TEM, DTU National Centre for Nano Fabrication and Characterization) under 200 kV. For imaging, lacey carbon supported copper grids (grid size 300 mesh) were used. The particle size was calculated using the ImageJ software. The results are summarized in the Section 3.

2.3. Investigation of Au NPs and leaf surface interaction

Barley plants (*Hordeum vulgare* L. cv. KWS Irina) were cultivated in a greenhouse of the Department of Plant and Environmental Sciences, University of Copenhagen, Denmark with minimum day/night temperatures of 18 °C/15 °C and a day/night cycle of 16 h/8 h. Plants were provided with all required nutrients and 1.5 L water per week in the tray.

The Au NPs colloidal solutions were applied on two different undamaged, young, fully expanded leaves of barley crops. For that, few 10 µL droplets were directly applied on the adaxial side of the leaf and air-dried. The surface of the leaves was investigated with SEM (QFEG 200 Cryo ESEM, DTU National Centre for Nano Fabrication and Characterization) in low vacuum (60 Pa). Sample were prepared as follows: small pieces of leaves were secured on a sample holder with double-sided carbon tape. A leaf without applied Au NPs was used as control. For imaging secondary electron (SE) and back-scattered electron (BSE) detectors were used. The results are summarized in Section 3.

3. RESULTS AND DISCUSSION

Time-dependent UV-VIS spectra of the exposed solutions are shown in the **Figure 1** (A row – citrate reduction, B row – plant extract reduction). Au NPs formation was confirmed by the appearance of a gold corresponding LSPR absorption maximum with the intensity increasing with the irradiation time. For citrate reduced Au NPs after 100 min of irradiation, the LSPR absorption maximum was observed at 521 nm, whereas for plant reduced Au NPs at 534 nm. Both values correspond to in literature available data [13,14] and probably differ because of differences in surface properties.

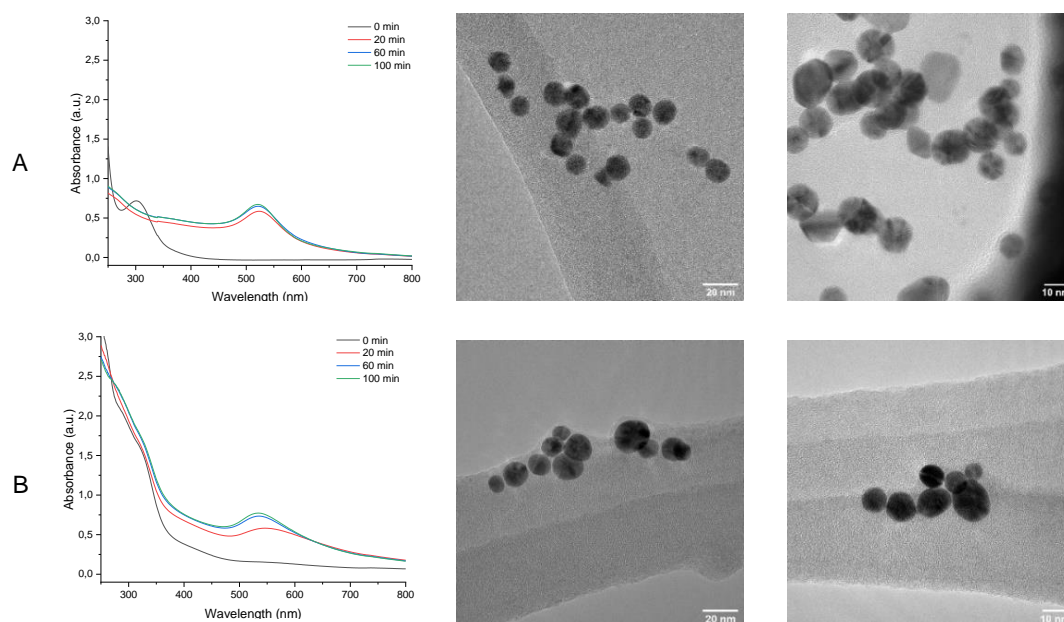


Figure 1 UV-VIS spectra of UV-exposed solutions and TEM images of resulting Au NPs particles: A – citrate reduced particles, B – plant extract reduced particles. Magnification: middle column – x285K, right column – x400K.

According to TEM images (see **Figure 1, row A**) the citrate reduced Au NPs are rounded-shaped and have diameters from 8 to 26 nm with 13.9 nm average size. Phytochemical reduction also resulted in the formation of rounded-shaped particles with size from 8 to 24 nm (see **Figure 1, row B**). The average size of the plant extract reduced Au NPs is 13.4 nm. For both samples, calculations were performed for 60 particles.

For investigating the interactions between Au NPs and the adaxial side of barley leaves, two different detectors were used: SE detector for surface characterization and BSE detector for getting NPs contrasting images. **Figure 2** shows SEM images of the control sample without Au NPs. The image in SE mode (**Figure 2, A**) shows the adaxial side of the barley leaf surface with all leaf features – trichomes, stomates and lamina. Examples of leaf structural features are highlighted with red for stomata, yellow for trichome, and green for lamina. The identical image of the same area but in the BSE mode resulted in a darker image with low contrast as the BSE detector is more sensitive to elements with higher atomic number. This confirms that there are no heavy elements on the leaf surface.

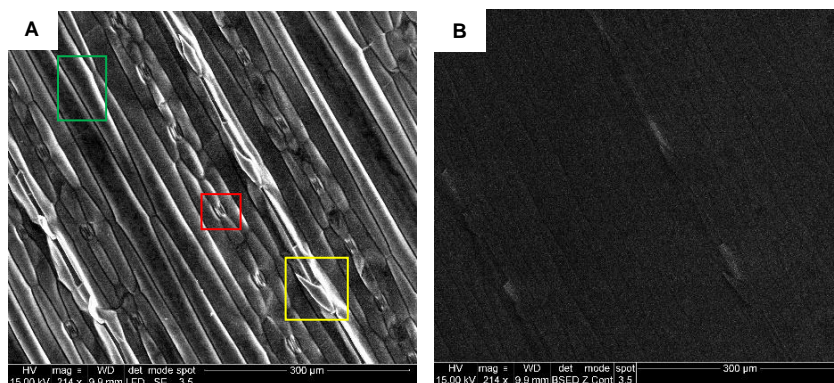


Figure 2 SEM images of control barley leaf adaxial side: A – in SE mode, B – in BSE mode. Examples of leaf structural features are highlighted with red for stomata, yellow for trichome, and green for lamina.

A SEM image of the adaxial side of barley leaf with applied citrate reduced NPs in SE mode is shown in the **Figure 3A**. It can be seen that citrate stabilized Au NPs agglomerates evenly distribute in the droplet-leaf contact area but avoiding stomates. The same area can be seen in BSE mode on the **Figure 3B**. There are no bright particles on and around stomates.

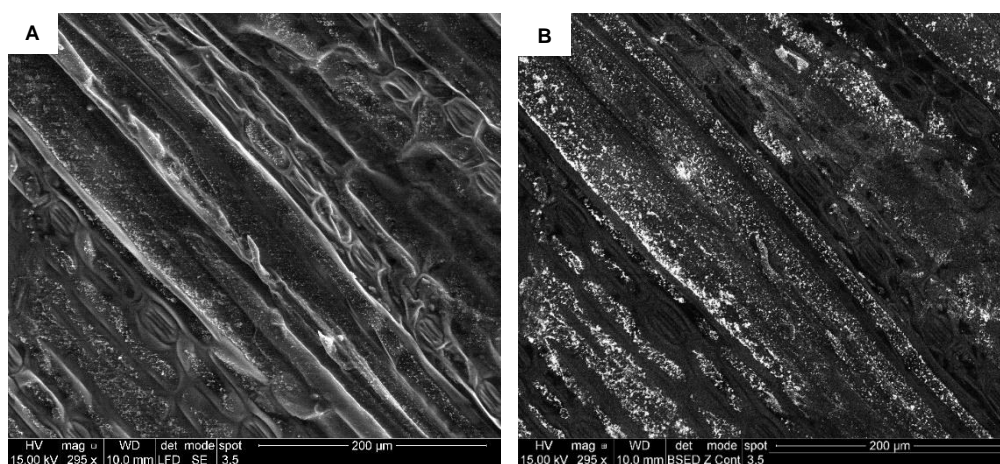


Figure 3 SEM images of citrate reduced Au NPs applied on the adaxial side of a barley leaf: A – SEM image in SE mode, B – SEM image in BSE mode.

SEM images of the adaxial side of a barley leaf with applied plant extract reduced NPs in SE mode are shown in **Figures 4A and 4C**. In the area where the applied droplet dried, the plant extract stabilized Au NPs formed a film. Contrasting SEM images in BSE mode confirm that Au NPs are distributed in this layer (see **Figures 4B and 4C**). It is shown that Au NPs are accumulating on all leaf parts: stomates, trichomes and lamina. An example of covered stomata is highlighted on the **Figure 4A**. It proves that Au NPs produced via phytochemical

synthesis have improved interaction with the leaf surface of the model plant, probably due to amphiphilic compounds contained in the plant extract [16].

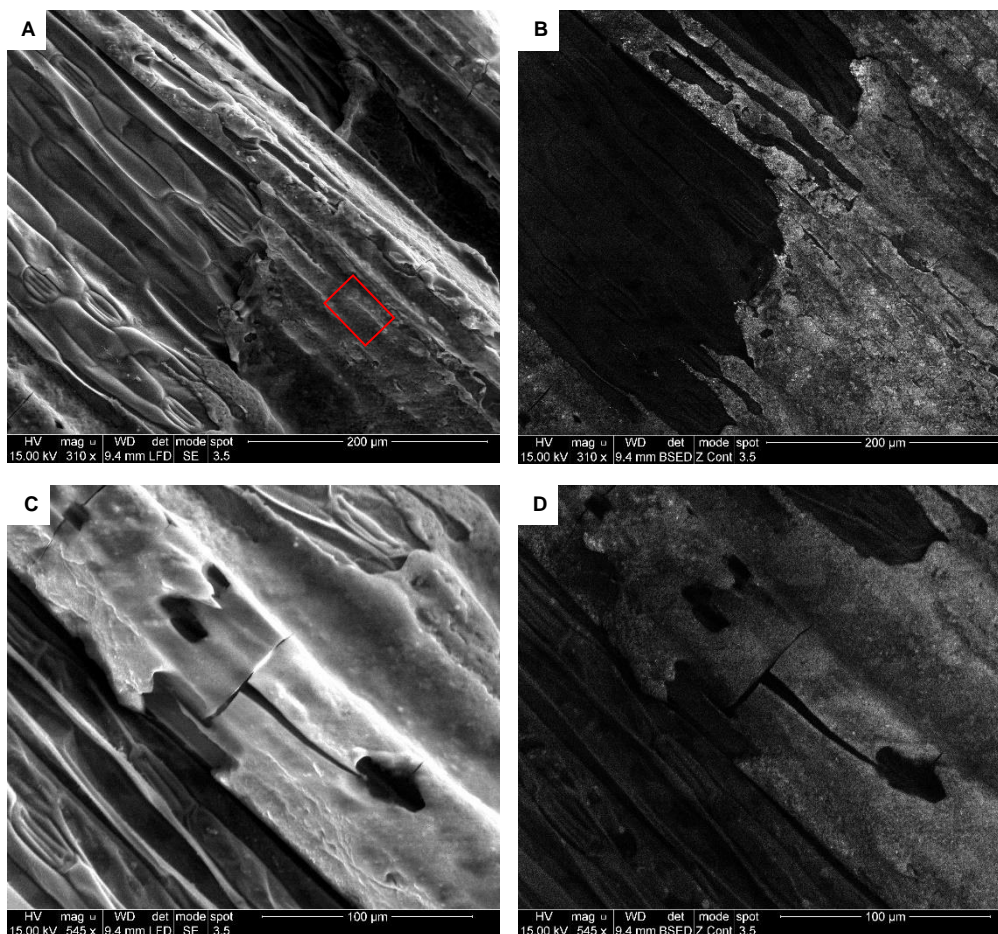


Figure 4 SEM images of plant extract reduced Au NPs applied on the adaxial side of a barley leaf: A, C – SEM image in SE mode; B, D – SEM image in BSE mode.

4. CONCLUSION

In this work, the interaction between Au NPs selected as model particles for foliar application of fertilizers and the barley leaf surface was studied. Au NPs were synthesized via reduction by UV light using two different reductants – citric acid and *A. Podagraria* leaf extract. The reductants performed also as NPs stabilizers. To our knowledge, it was the first time *A. Podagraria* extract was implemented for Au NPs synthesis. The formation of Au NPs was confirmed by UV-VIS spectroscopy. It was shown by TEM that in both cases rounded-shaped particles with around the same average size are formed: 13.9 and 13.4 nm for citrate and plant reduced Au NPs, respectively.

The different nature of the reducing/stabilizing agents resulted in different accumulation patterns on the barley leaf surface. It was established that citrate stabilized Au NPs agglomerates evenly distribute in the droplet-leaf contact area avoiding stomates. Plant extract stabilized Au NPs form a film in the contact area and accumulate on all leaf parts: stomates, trichomes and lamina. It was shown that Au NPs produced via green phytochemical synthesis using *A. Podagraria* extract as both reducing and stabilizing agent have an improved interaction with the leaf surface of the model plant.

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