Electronic Supplementary Material (ESI) for RSC Advances

Root system of live plants is powerful resource for green synthesis of Au-NPs

P. Pardha-Saradhi[a,*], G. Yamal[a,b], Tanuj Peddisetty[c], P. Sharmila[d], Jyoti Singh[e], Rajamani Nagarajan[e] and K.S. Rao[b]

[a]Department of Environmental Studies, University of Delhi, Delhi – 110007, India;

[b]Department of Botany, University of Delhi, Delhi – 110007, India;

[c]Department of Chemical Engineering, TKM College of Engineering, Kollam – 691005, Kerala, India;

[d]Department of Chemistry, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India

eDepartment of Chemistry, University of Delhi, Delhi – 110007, India;

*Corresponding Author email address: ppsaradhi@gmail.com

Methods

Plants belonging to diverse taxonomic groups were collected from distinct habitats/conditions, namely, natural conditions (i.e., growing in wild), maintained garden beds and controlled conditions. 16 plant species belonging to 11 distinct families of Angiosperms were collected from wild, natural, and maintained garden beds of North/Main campus of University of Delhi, details of which are given in Table 1. In all the cases utmost care was taken to cause least damage to the plants as well as to the root system at the time of collection.

Raising plants under controlled conditions

Seeds of *Brassica juncea* cv. Varuna (Brassicaceae), *Cicer arietinum* cv. PG-114 (Fabaceae), *Vigna mungo cv.* PS-1 (Fabaceae), and *Lycopersicon esculentum* (Solanaceae) were collected from IARI, New Delhi, and that of *Triticum aestivum* cv. H1-1544 (Poaceae) from IARI, Regional Station, Indore. Seeds were treated with 0.5% cetrimide for 5 min, washed with distilled water, surface sterilized with 0.1% (w/v) mercuric chloride for 3 min and washed thoroughly with sterile distilled water.

(i) Raising under sterile conditions: capped glass bottles of (2.5x5") dimensions containing glass beads (~75g) with 25 ml of mineral growth medium [consisting of 2500 mg L^{-1} KNO_3, 250 mg L^{-1} $MgSO_4$, 150 mg L^{-1} $CaCl_2$, 150 mg L^{-1} NaH_2PO_4, 134 mg L^{-1} $(NH_4)_2SO_4$, 10 mg L^{-1} $MnSO_4$, 3 mg L^{-1} H_3BO_3, 2 mg L^{-1} $ZnSO_4$, 0.75 mg L^{-1} KI, 0.25 mg L^{-1} Na_2MoO_4 and iron source (27.8 mg L^{-1} $FeSO_4$ and 37.3 mg L^{-1} EDTA)][1] were sterilized by autoclaving at a pressure of 1.06 Kg cm^{-2} and at temperature of 121^{o}C for 30 min. Surface sterilized seeds were sown in these bottles under sterile conditions, in laminar air flow.

(ii) Raising under non-sterile conditions: surface sterilized seeds of *B. juncea, C. arietinum, T. aestivum, V. mungo* and *L. esculentum* were sown in 7x2.5"earthen pots with sand and watered alternately with water and mineral growth medium.

All plants raised under controlled conditions were kept at room temperature (25±2°C) with 16/8h light/dark cycle. White fluorescent light (Phillips, India) with a light intensity of 120 µmol m^{-2} s^{-1} photon flux density was used for illuminating the plants.

Incubation of plants with salt solution

Different concentrations (viz. 0.01, 0.05, 0.1, 0.25, 0.5, 1 and 2 mM) of Hydrogen tetrachloroaurate trihydrate ($HAuCl_4.3H_2O$) was used for evaluating potential of plants to generate Au NPs. Solution was prepared in sterile double distilled water.

All the plants placed in various salt solutions were incubated at room temperature (25±2°C) with a 16/8h light/dark cycle. White fluorescent light (Phillips, India) with a light intensity of 120 µmol m^{-2} s^{-1} photon flux density was used for illuminating the plants.

Incubation of plants under strict sterile conditions with salt solutions

For experiments carried under strict sterile conditions the stock solutions of Au was filter sterilized under sterile conditions in laminar flow cabinet. Different concentrations were prepared from this stock using sterile double distilled water in laminar air flow. The glassware(s) used in these experiments were sterilized by autoclaving at a pressure of 1.06 Kg cm^{-2} and at temperature of 121°C for 30 min.

The plants raised under sterile conditions were carefully taken out and after washing their roots 2-3 times with sterile double distilled water these were transferred to 25x200 mm sterile Borosil tubes containing sterile salt solutions with their roots immersed in the salt solution, in laminar air flow. To confirm the absence of any microorganism in association with immersed roots of plants that were maintained under strict sterile conditions, roots as well as the test solutions at the time of termination of experiments were inoculated on Luria Bertani agar plates and incubated at 37 °C.

UV-Vis spectral studies

UV-Vis absorption spectra of various test solutions in which roots of intact plants were submerged for different time intervals were recorded between 190-1100 nm using Specord 200 Analytikjena UV-Vis spectrophotometer. The formation of Au-NPs was indicated by the appearance of characteristic absorption peak generally at 530-580 nm and ~410 nm, respectively[2,3,4].

TEM investigations

Solutions of different salts incubated with the roots of intact plants for duration of ~24 h were collected and subjected to sonication for 30 min to minimize primary particle agglomeration. 10 µl drop of resulting colloidal solution was deposited onto a 200-mesh copper TEM grid covered with an ultrathin continuous C film and allowed to evaporate in a desiccator.

The grids were viewed in transmission electron microscope of Technai G^2 T30 U-TWIN at a voltage of 300 kV. Hardware associated with the machine also allowed (i) Energy dispersive X-ray (EDX) spectra, and (ii) Selected Area Electron Diffraction (SAED) pattern measurements.

PXRD Studies

The solutions of different salts incubated with the roots of intact plants were drop coated on Silica surface and dried. The PXRD pattern was collected using Rigaku Rotaflex RAD-B or Bruker or Pan Analytika instruments using Cu target with monochromator which allowed CuK(α) at the rate of 0.020 step in 1.2 s in the 2 theta (θ) range 30-80°.

Dynamic Light Scattering (DLS) Studies

For comparing the NPs generation potential of sodium citrate (0.1mM) and roots of *V. mungo* and *T.aestivum*, different concentrations of Au salt solution were incubated under strict sterile conditions for 5h. For determining the size distribution of the particles, the resultant colored colloidal solutions were measured using a Nano ZS system (Malvern instruments) based on dynamic light scattering.

Determining root surface redox activity

To measure the redox activity associated with roots, a strong artificial electron acceptors, namely 2,6-dichlorophenol indo-phenol (DCPIP)[5]. In order to determine reduction potential associated with root surface, roots of intact plants were submerged in 0.1 and 0.2 mM DCPIP solutions. Reduction potential of roots of intact plants was determined by monitoring decrease in the optical density at 600 nm due to reduction of DCPIP to $DCPIPH_2$. Capacity of roots of intact plants to reduce DCPIP was expressed in terms of nmoles of DCPIP reduced $h^{-1}g^{-1}$ root fresh weight.

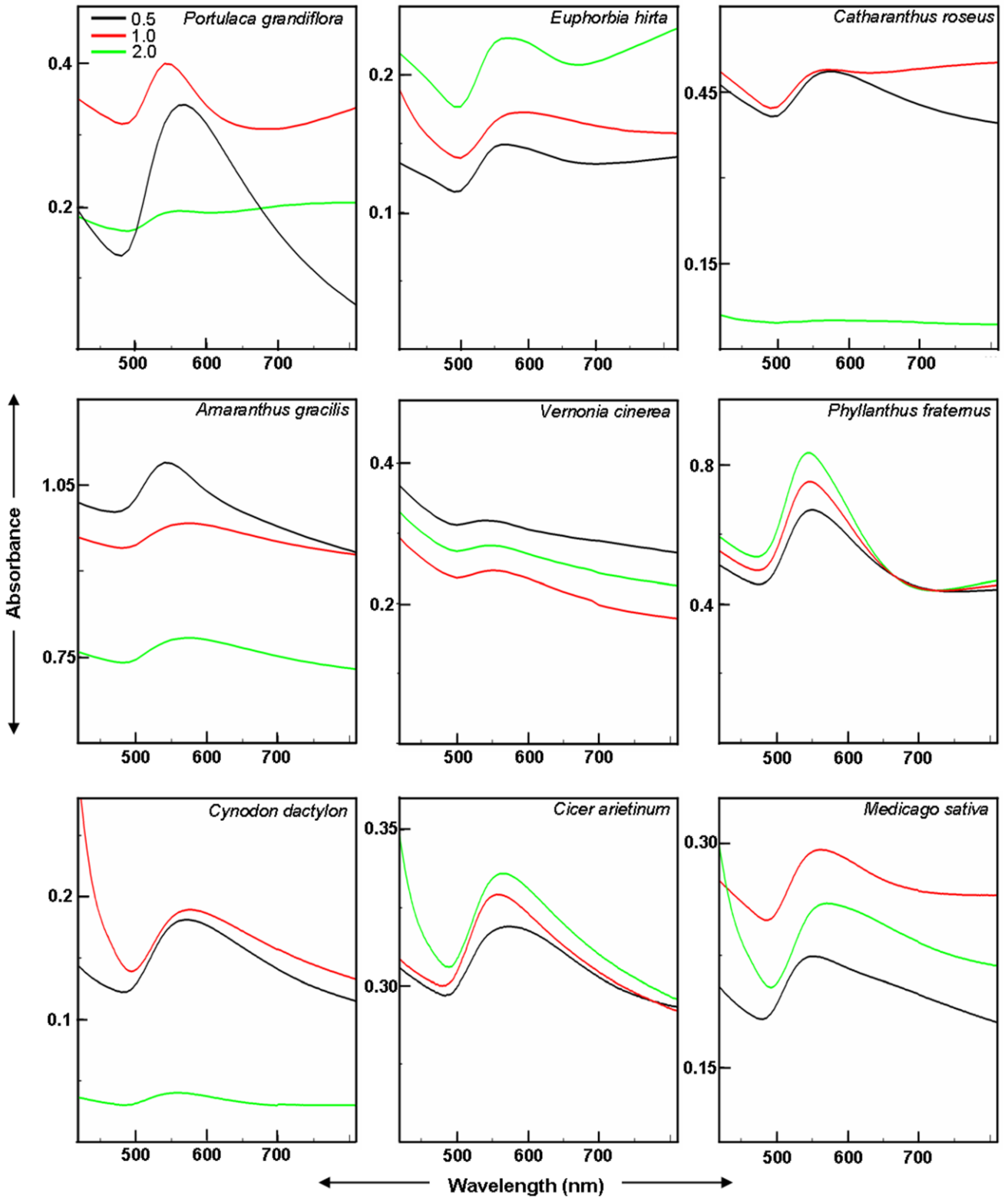

Figure S1 Supplementary Figure 1: UV-Vis absorption spectra of different concentrations of $HAuCl_4$ (0.5, 1.0 and 2 mM) exposed to roots of intact plants.

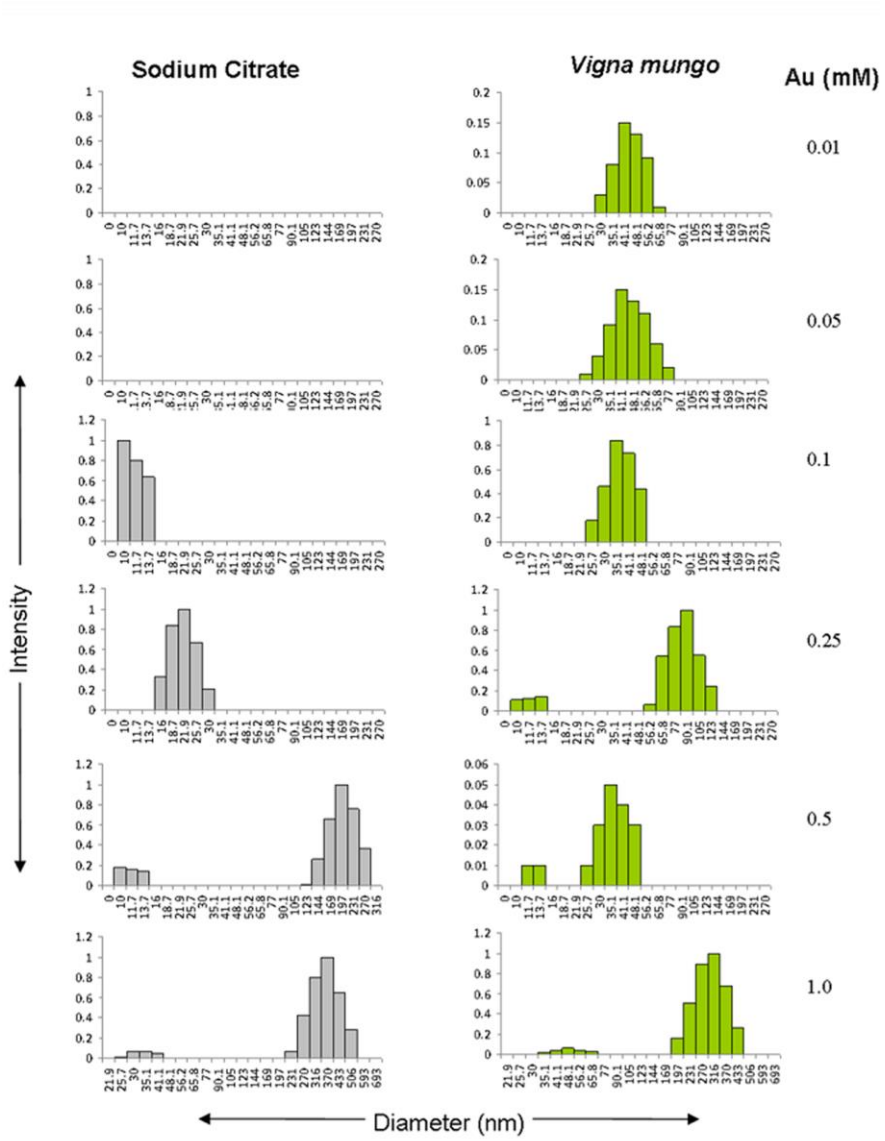

Figure S2 DLS analysis of Au-NPs generated with different concentrations (0.01 to 1mM) of $HAuCl_4$ on incubation with sodium citrate and roots of intact plants of *Vigna mungo* for 5 h.

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