



## Roots of *Pennisetum* sp. possess the competence to generate nanoparticles of noble metals

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Roots of plants have immense reducing potential. Ions of noble metals namely Au<sup>3+</sup> and Ag<sup>+</sup> get reduced easily to form Au and Ag nanoparticles (NPs), respectively. Therefore, we hypothesize that plant roots could have potential to form Au-NPs and Ag-NPs. For present investigations, plants of *Pennisetum glaucum* L. were used to evaluate if their roots possess capacity to generate metal NPs. The generation of Au-NPs and Ag-NPs was initially presumed based on colour change, and confirmed by UV-vis spectra, TEM and EDX investigations. Pale yellow Au<sup>3+</sup> and colourless Ag<sup>+</sup> solutions turned purple and brown, respectively, by roots of *Pennisetum* sp. within 8 h. Absorption spectra of respective solutions showed plasmon resonance band at 560 nm and 420 nm confirming the presence of Au-NPs and Ag-NPs. TEM coupled with SAED revealed the presence of crystalline spherical NPs in the size range of 5-50 nm in these solutions. EDX further confirmed the presence of Au and Ag as NPs of respective solutions. These results confirmed that the roots of *P. glaucum* possess ideal reducing strength to generate Au-NPs and Ag-NPs exogenously in the aqueous phase.

**Keywords:** Ag-NPs, Au-NPs, *Pennisetum glaucum* L., Reducing strength, Root system

Grasses possess capacity to tolerate various heavy metals due to immense phytoremediation potential<sup>1</sup>. In general, grasses have greater root surface area as compared to the dicot plants. Rubenstein and co-workers<sup>2</sup> demonstrated the redox activity of roots of intact plants of oat (*Avena sativa*, Poaceae). They further demonstrated that the electron transport complexes prevail in association with plasma membrane at the root surface that are responsible for the redox reactions.

It is well known that precious metal ions in particular that of gold and silver are prone to rapid reduction to form respective atoms which rapidly agglomerate to form nanoparticles<sup>3-5</sup>. Previously, our research team has also specifically demonstrated that Au-NPs and Ag-NPs can be formed with ease through redox reactions under the guidance of biomolecules as well as root surface of several terrestrial plants<sup>4-7</sup>.

Au-NPs made in aqueous solution have surface charge to stabilize them against aggregation via electrostatic repulsion and find utility in diverse fields such as drug-delivery, cancer therapy, gene transfer

and recognition, biological markers, *etc.*<sup>7-11</sup>. The antimicrobial properties of Ag-NPs make these the most widely used in daily commodities such as creams, lotions, upholstery, shoe insoles, handwash, shampoo, room sprays, air conditioners, washing machines, surface cleaners, in the field of medicine *viz.* artificial teeth, medical catheters, bone coating, wound dressings, surgical instruments and sensing applications such as biolabeling, optical imaging of cancer and detection of DNA sequences<sup>6,11-12</sup>.

Minor cereals such as *Pennisetum glaucum* L. (Pearl millet) can be grown under drought prone conditions due to their immense root system<sup>13</sup> besides other drought resistant features. Previously we demonstrated that the plant root surface has the capacity to reduce both Au as well Ag ions with ease under ambient conditions and Rubenstein and co-workers<sup>2</sup> has specifically demonstrated the immense reducing strength of the root system of one of the minor millets (Oat). During present investigations we evaluated if the root system of *P. glaucum* can be exploited for generation of Au-NPs as well as Ag-NPS with ease under ambient conditions. In this communication we are conveying for the first time that root system of intact plants of *P. glaucum*

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possess strong reducing strength and (ii) this reducing strength can be exploited for the generation of Au-NPs and Ag-NPs with ease under ambient conditions.

## Material and Methods

### Materials

Plants of *Pennisetum glaucum* L. were raised in earthen pots for 30 days, uprooted carefully from the pots without damaging the root system. The roots were thoroughly washed in tap water and then rinsed with distilled water.

### Determining root surface redox activity

The reduction potential of root surface of intact plants of *Pennisetum* was determined by using DCPIP and Potassium ferricyanide  $\{K_3[Fe(CN)_6]\}$  solutions. The roots of intact plants of *Pennisetum* were submerged in 100  $\mu$ M and 200  $\mu$ M DCPIP Solutions, and 0.5 mM and 1 mM Potassium ferricyanide  $\{K_3[Fe(CN)_6]\}$  solutions. The reduction potential of roots was determined by recording a decrease in the optical density at 600 nm due to reduction of DCPIP to DCPIPH<sub>2</sub>, and expressed as nano moles of DCPIP reduced  $h^{-1} g^{-1}$  root fresh weight. Reduction of ferricyanide to ferrocyanide was evaluated by measuring decrease in the optical density at 420 nm, and expressed as nano moles of ferricyanide reduced  $h^{-1} g^{-1}$  root fresh weight. The spectrophotometric observations were recorded on Specord 200 Analytik jena UV-vis spectrophotometer.

### Incubation of plants with HAuCl<sub>4</sub> and AgNO<sub>3</sub> salt solutions

To find out the potential of roots of intact plants to generate nanoparticles, root system of intact plants of *P. glaucum* were exposed to concentrations (*viz.* 0, 0.5, 1 and 2 mM) of Hydrogen tetrachloroaurate (III) (HAuCl<sub>4</sub>); and silver nitrate (AgNO<sub>3</sub>) solutions. The solutions were made using double distilled water. The roots of intact plants of *Pennisetum* were transferred to 15×150 mM Borosil tubes with their roots immersed in salt solutions of different concentrations. Plants were kept undisturbed at the room temperature (25±2°C) with a 16 h light with intensity of 120  $mmol m^{-2} s^{-1}$  photon flux density and 8 h dark cycle. The exogenously generated metal nanoparticles were characterized by UV-vis spectra, TEM coupled with EDX and SAED studies.

### UV-vis spectral studies

UV-vis absorption spectra of solutions of HAuCl<sub>4</sub> and AgNO<sub>3</sub> in which roots of intact plants were

submerged were recorded between 190-1100 nm using Specord 200 Analytik jena UV-vis spectrophotometer.

### Transmission electron microscopic investigations

Coloured colloidal solutions that resulted due to the incubation of roots of intact plants of *Pennisetum*, in HAuCl<sub>4</sub> and AgNO<sub>3</sub> salt solutions were observed under transmission electron microscope (TEM). 10  $\mu$ L of the colloidal solutions were separately drop coated on a C coated copper grid and viewed at Technai G2 T30 U-TWIN at a voltage of 300 kV. Selected Area Diffraction Pattern (SAED) and Energy Dispersive X-Ray (EDX) were also recorded from the same instrument.

## Results

### Reduction of ferricyanide and DCPIP

The reduction capacity of the roots of *Pennisetum glaucum* was estimated by incubating the roots of intact plants in two artificial electron acceptors *i.e.*, potassium ferricyanide and DCPIP. Roots of intact plants of *P. glaucum* reduced both these reductants within 12 h. As evident from (Fig. 1), the roots of intact plants of *P. glaucum* reduced blue coloured DCPIP to colourless DCPIPH<sub>2</sub>. In general, each gram of live roots of *P. glaucum* reduced  $\sim 127$  nmoles of DCPIP  $h^{-1}$ ; and  $\sim 790$  nmoles  $h^{-1}$  of ferricyanide.

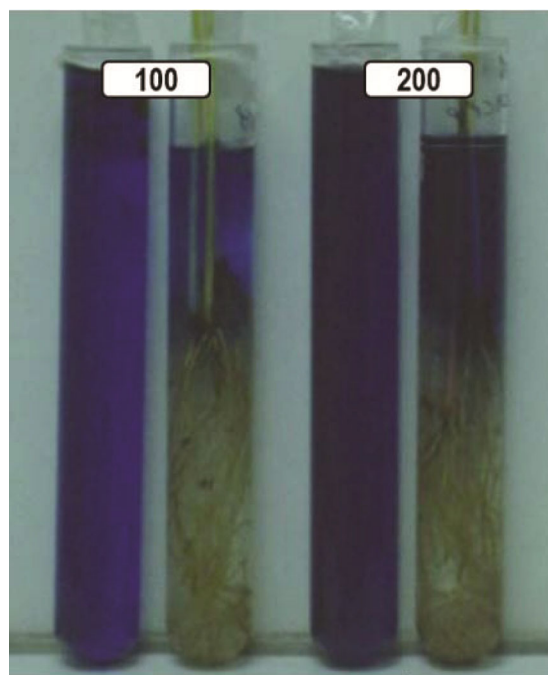


Fig. 1 — Efficacy of root system of intact plants of *Pennisetum glaucum* L. to reduce blue coloured DCPIP to colourless DCPIPH<sub>2</sub> under ambient conditions. Note that both 100  $\mu$ M and 200  $\mu$ M DCPIP around the roots turned completely colourless

### Generation and characterization of Au-NPs

The color of  $\text{HAuCl}_4$  changed from pale yellow to purple with 0.5 and goldenish with 1, 2 mM when incubated with roots of intact plants of *P. glaucum*, within 12 h (Fig. 2A), while the colour of the salt solution without plants remained unchanged. In fact, a closer view at the roots of *P. glaucum* reveal that these also turned purplish with 0.5 mM  $\text{HAuCl}_4$  and golden with of 1 and 2 mM  $\text{HAuCl}_4$  solutions.

The change in the colour of salt solution is associated with the change in the speciation state and the formation of metal NPs<sup>8</sup>. Therefore, the UV-vis absorption spectra of these purple and golden coloured colloidal solutions were recorded. The purple-coloured colloidal solution formed with 0.5 mM  $\text{HAuCl}_4$  incubated with roots of intact plants of *P. glaucum* showed absorption peak at  $\sim 550$  nm, which arise due to the surface plasmon resonance in Au-NPs<sup>14</sup>. However, the golden coloured colloidal solutions formed with higher concentrations of  $\text{HAuCl}_4$  viz. 1 and 2 mM did not show clear peak (Fig. 2B). Similar observations have been recorded by our group with some other terrestrial plant species, where some plants formed golden colloidal solution with higher levels of  $\text{HAuCl}_4$  and did not show peaks specific to Au-NPs<sup>4</sup>.

To determine the information, about the size and morphology of NPs, TEM measurements were made. The TEM images of the Au-NPs produced exogenously in  $\text{HAuCl}_4$  salt solutions in which the roots of intact plants of *P. glaucum* were incubated showed distinct spherical Au-NPs in the size range of

$\sim 5$ -10 nm (Fig. 3A & B). The Energy Dispersive X-ray (EDX) measurements (Fig. 3D) of NPs validated that these were composed of Au. The peaks situated at binding energies of 2.10 and 9.71 keV correspond to Au, while the 8.06 and 1 keV peaks correspond to Cu and C. The peaks for C and Cu arose due to the C coated copper grids, on which the samples were loaded. Further, the Selected Area Electron Diffraction (SAED) pattern (Fig. 3C) of these Au-NPs showed distinct rings and bright spots implying the crystalline nature of these NPs.

### Generation and characterization of Ag-NPs

The colour of the  $\text{AgNO}_3$  salt solution without plants remained unchanged while the salt solutions incubated with roots of intact plants of *P. glaucum* turned brownish, in a way similar to the  $\text{HAuCl}_4$  treatments in these studies (Fig. 4A). The roots of *P. glaucum* which were incubated in  $\text{AgNO}_3$  salt solutions not only changed the colour of the solution but also the colour of the roots became brown (whereas in case of  $\text{HAuCl}_4$ , the roots became purple or golden).

In case of  $\text{AgNO}_3$ , the UV-vis absorption spectra of the brown coloured colloidal solutions showed absorption maxima between 430 to 450 nm (Fig. 4B). Absorption peak in this range has been reported to arise due to surface plasmon resonance in Ag-NPs<sup>15</sup>. The intensity of peak increased with increasing concentration.

TEM investigations of brown colloidal solution (that resulted due to incubation of root system of intact plants

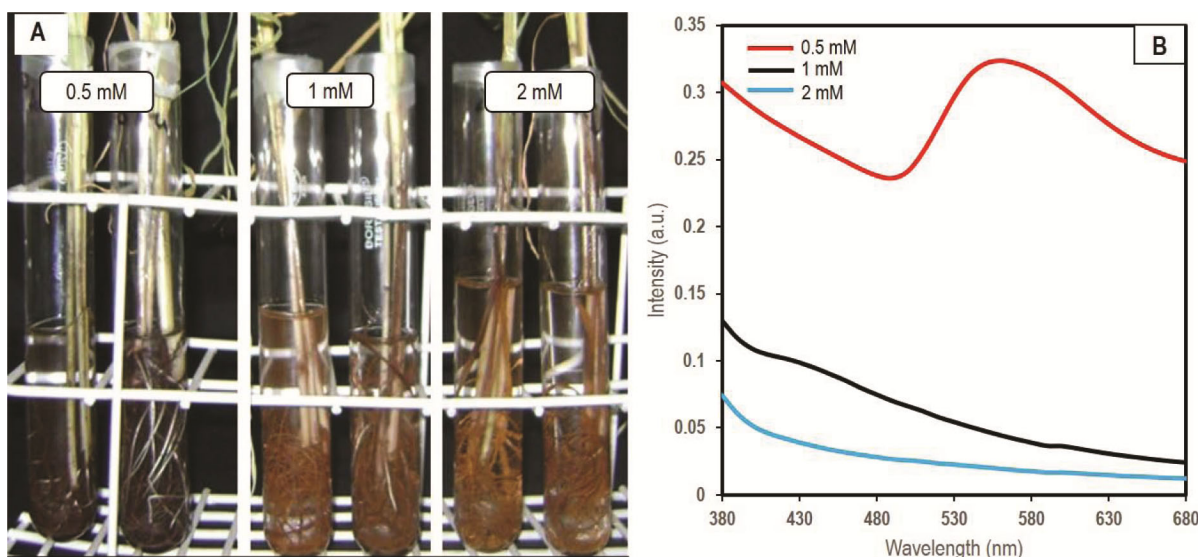


Fig. 2 — (A) The potential of roots of intact plants of *P. glaucum* to turn 0.5, 1 and 2 mM  $\text{HAuCl}_4$  solutions purple/goldenish on incubation for 12 h under ambient conditions; and (B) The UV-vis spectra of these coloured colloidal solutions

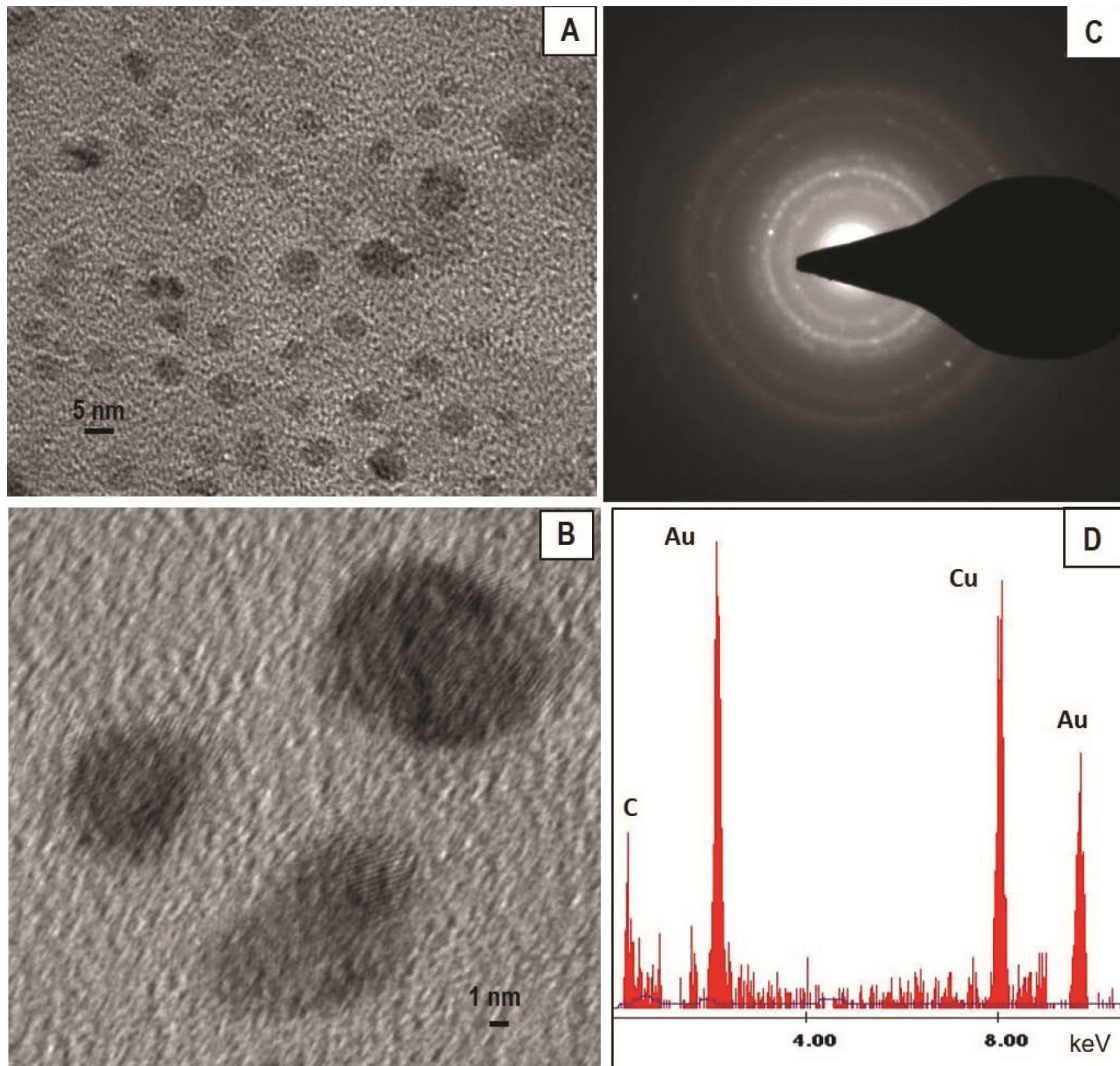


Fig. 3 — (A-D) Characterization of Au-NPs synthesized by the roots of *P. glaucum*. (A and B) Transmission electron micrographs of the purple-coloured colloidal solution showing distinct Au-NPs; (C) Energy Dispersive X-Ray (EDX) of these NPs showing distinct peaks for Au; and (D) Selected Area Diffraction Pattern (SAED) of Au-NPs revealing crystalline nature of Au-NPs

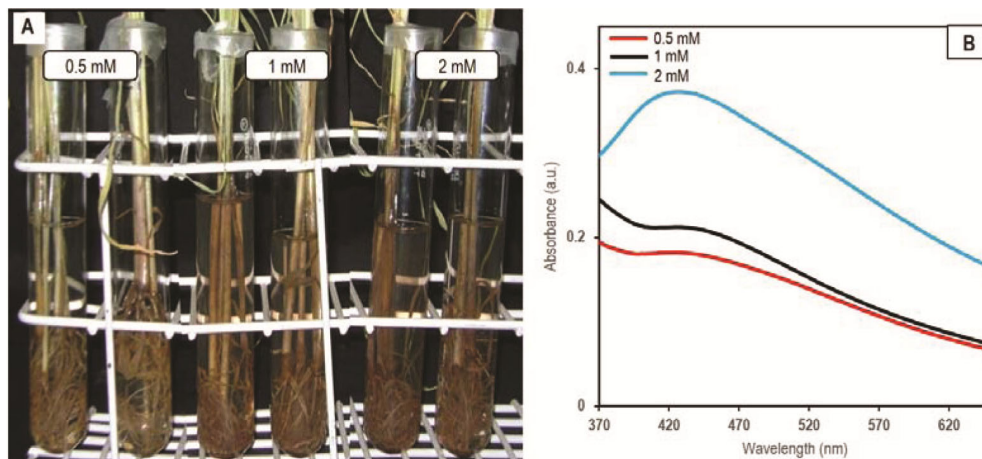


Fig. 4 — (A) Incubation of roots of intact plants of *P. glaucum* in (0.5, 1 and 2 mM)  $\text{AgNO}_3$  solutions for 12 h turned the solutions brown; and (B) The UV-vis spectra of these coloured colloidal solutions

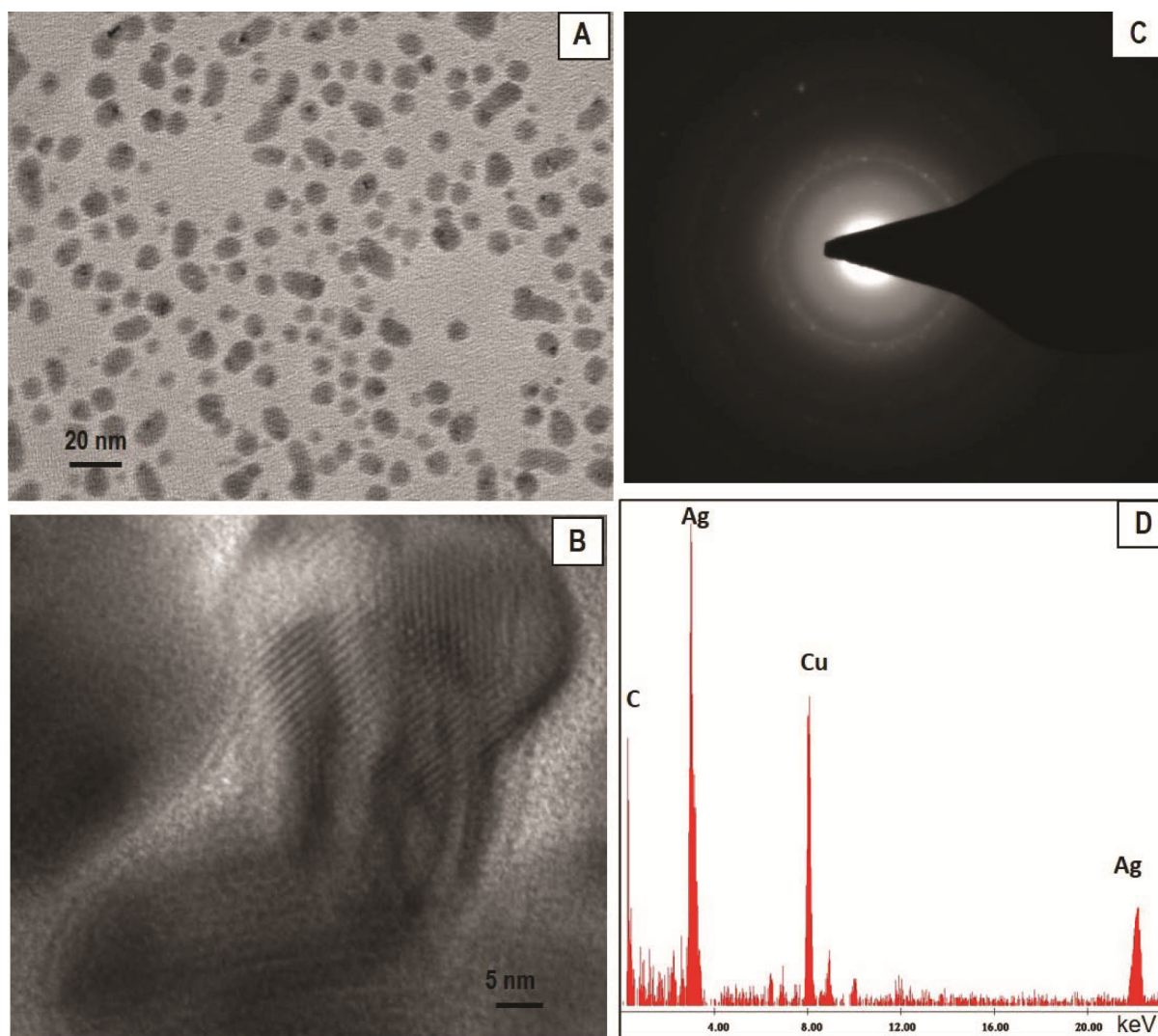


Fig. 5 — (A-D) Characterization of Ag-NPs synthesized by the roots of *P. glaucum*. (A and B) Transmission electron micrographs of the brown-coloured colloidal solution showing distinct Ag-NPs; (C) Energy Dispersive X-Ray (EDX) of NPs showing distinct peaks for Ag; (D) Selected Area Diffraction Pattern (SAED) of Ag-NPs revealing crystalline nature of Ag-NPs

of *P. glaucum* in  $\text{AgNO}_3$  solutions) confirmed the presence of distinct nearly spherical NPs in the size range of 25-50 nm (Fig. 5A & B). EDX studies confirmed the presence of Ag in these NPs (Fig. 5C). The EDX pattern showed distinct peaks at 3.40 keV and 22 keV corresponding to Ag, along with the peaks for C and Cu. The SAED pattern showed the presence of distinct bright spots and distinct rings, suggesting the crystalline nature of these Ag NPs (Fig. 5D).

### Discussion

The reduction of ferricyanide to ferrocyanide by the roots of intact plants of *Avena sativa* was recorded by Rubinsetin and his coworkers<sup>2</sup>. It is well known

that ferricyanide is membrane impermeable and hence gets reduced at root surface. During present investigations we recorded the potential of roots of *P. glaucum* to effectively reduce ferricyanide to ferrocyanide. In addition the roots of *P. glaucum* turned blue coloured DCPIP to colourless DCPIPH<sub>2</sub> which further corroborates strong reducing strength prevailing on the root surface. The presence of strong reducing strength at the root surface of few terrestrial plants was demonstrated earlier<sup>4,5</sup>.

Generation of noble metal NPs primarily involves reduction of its ions followed by rapid agglomeration of resultant atoms<sup>3</sup>. As root surface has strong reducing strength and ionic speciation state of noble metals are prone to reduction, we presumed that noble

metal ions can accept electrons with ease at the root surface similar to ferricyanide and DCPIP. As expected, roots of *P. glaucum* could reduce both  $\text{Au}^{3+}$  as well as  $\text{Ag}^+$  to form respective NPs, under ambient aqueous conditions. The same is evident from biotransformation of pale yellow/clear colourless solution to colloidal coloured solutions. Biotransformation of metal ions at the root surface was also recorded in few other terrestrial plants<sup>4,5,16</sup>.

Change in the colour of the salt solutions when incubated with the roots of intact plants of *P. glaucum* occurred. It has been established that alteration in the colour of the salt solutions is recorded due to the change in the reduction state of the metal ions. In case of  $\text{HAuCl}_4$ , the colour of the colloidal solution formed due to the reduction of  $\text{Au}^{3+}$  exhibit various shades ranging from pink to purple to blue to green to orange to red and even golden depending on the reductant used and the shape of the NPs<sup>8</sup>. During the present investigations we recorded alteration in the colour of  $\text{HAuCl}_4$  solution from clear pale yellow to colloidal purple or golden. In a similar manner clear, colourless  $\text{AgNO}_3$  solutions turned colloidal brown upon reduction. The colours may vary from the light yellow to dark yellow to light brown and to dark brown and even gray<sup>5,6,17</sup>.

The UV-vis absorption spectra of the coloured colloidal solutions showed distinct surface plasmon resonance peak for Au-NPs at ~550 nm with 0.5 mM  $\text{HAuCl}_4$ . However, the coloured colloidal solutions formed on incubation of roots of intact plants of *P. glaucum* with higher concentrations (*i.e.* 1 and 2 mM) of  $\text{HAuCl}_4$ , distinct peaks were not observed. In case of Au-NPs the absorption peak from 500 to 550 nm are size-relative, and arise due to the surface plasmon resonance and not observed when either the NPs are small ( $d < 2$  nm) or in the bulk material<sup>18</sup>. Further, TEM studies along with SAED and EDX corroborated the presence of crystalline Au-NPs in the coloured colloidal solutions.

In case of  $\text{AgNO}_3$ , brown coloured colloidal solutions were formed, which showed peak in 430-450 nm range which arises due to surface plasmon resonance for Ag-NPs and is specific for Ag. The absorption peak in this region is known to contain Ag-NPs in the size range of 20-50 nm<sup>19</sup>. The same was corroborated by the TEM studies carried out under present investigations, where distinct Ag-NPs in the size range of 25-50 nm were observed. The SAED pattern showed distinct rings indicating the crystalline nature of the NPS. Crystalline nature can also be

interpreted from the TEM image (Fig. 5B), where clear Bragg fringes are clearly visible<sup>20</sup>.

## Conclusion

The present investigations demonstrated that the root system of intact plants of *P. glaucum* possess potential to effectively (i) turn the blue coloured DCPIP to colourless DCPIPH<sub>2</sub>, and membrane impermeable ferricyanide to ferrocyanide, revealing the existence of strong reducing strength on root surface; and (ii) reduce  $\text{Au}^{3+}$  and  $\text{Ag}^+$  to generate respective nanoparticles under ambient conditions in aqueous medium. We strongly believe that the plasma membrane bound dehydrogenases associated with root surface epidermal cells are responsible of the reduction of  $\text{Au}^{3+}$  and  $\text{Ag}^+$  to generate respective nanoparticles using intracellular NAD(P)H as the source of electrons.

## Conflict of interest

All authors declare no conflict of interest.

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