5.1 INTRODUCTION

Owing to unique physical and chemical properties, gold (Au) nanoparticles find application in innumerable sectors of engineering including aerospace & defence, medicine, environment and agriculture (Iravani 2011; Dreadean et al., 2012). Au nanoparticles synthesized/grown in aqueous phase are of immense importance in biological applications such as drug-delivery, cancer therapies, gene transfer and recognition, biological markers etc. (Jain et al., 2008; Dreaden et al., 2012; Dykman and Khlebtsov 2012). Au nanoparticles of different sizes and shapes are routinely synthesized by various chemical and physical methods [Masala and Seshadri 2004; Iravani 2011]. Unfortunately, these synthetic approaches have limitations and negative impact on environment (Tapan and Andrey 2009; Iravani 2011; Zhang et al., 2011). Therefore, there has been constant urge across the globe to develop an ideal green protocol, alternate to physical or chemical method(s) for synthesis of Au nanoparticles. In the maneuver of developing an apt green method for synthesis of Au nanoparticles, researchers made efforts to use biological systems.

Amongst living systems, significant amount of research has been focused on use of microorganism(s), their biomass and cell free extract(s) (Narayanan and Sakthivel 2010; Iravani 2011; Gan and Li 2012). However use of microorganisms for generation of Au nanoparticles has several limitations such as (i) culture and maintenance of the microorganism(s) is tedious; (ii) use of pathogenic species viz. *Klebsiella pneumonia*, *Bacillus subtilis*, *Micrococcus luteus*, *Serratia marcescens*, *Aspergillus* etc. requires special safety measures (Narayanan and Sakthivel 2010; Honary et al., 2012; Arunkumar et al., 2012). Simultaneously, some research teams used extracts of various plant materials such as leaves, seeds, flowers, fruit, fruit peel,
bark, tuber etc. for the generation of Au-Nanoparticles (Gan and Li 2012). Although, researchers using plant extract proposed the involvement of certain biomolecules such as sugars, amino acids, phenolics etc. for generation of Au nanoparticles, it is very difficult to precisely pin-point, what all biomolecule(s) present in extract could be playing a role in generation of Nanoparticles. Moreover, in the majority of cases, Au nanoparticles could be generated following heating and stirring for different time intervals. Biomass of oat, wheat and maize has also been used for generation of Au nanoparticles (Armendariz et al., 2004; de la Rosa et al., 2009). However, extraction of nanoparticles from the biomass would be complicated. Another approach being advocated by few research teams is the intracellular synthesis of Au nanoparticles in different parts of live plants. So far, Sesbania drummondii, Brassica juncea, Arachis hypogea and Medicago sativa were shown to possess potential to synthesize Au nanoparticles intracellularly (Gardea-Torresdey et al., 2002; Sharma et al., 2007; Bali and Harris 2010; Beattie and Haverkamp 2011; Raju et al., 2012). However, extraction of intracellularly formed Au nanoparticles from plants for commercial application would be tedious.

In search for an alternate, ideal green method we felt it wise to exploit the root system of intact plants for generating Au nanoparticles, as plant biologists clearly established that the root system of plants possess immense reducing strength (Rubinstein et al., 1984; Qiu et al., 1985; Crane et al., 1991; Vuletic et al., 2005) and generation of Au nanoparticles from ions involves reduction (Xia et al., 2009). Therefore, present investigations were carried with the aim to evaluate the potential of plants to generate Au nanoparticles exogenously at the root surface. In this communication, using 16 plant species from 11 diverse taxonomic groups of angiosperms we are reporting for the first time that the root system of intact plants can be exploited for exogenous generations of Au nanoparticles. The present study has also elaborated on the advantages of using root system of intact plants for rapid bulk synthesis of Au nanoparticles.
5.2 RESULTS AND DISCUSSION

The root system of intact plants of all species tested turned clear pale yellow HAuCl₄ solutions colloidal purple/golden (Fig. 5.1 a-f). Such an alteration in color of HAuCl₄ is recorded when Au nanoparticles were generated using sodium citrate (Frens 1973).

![Figure 5.1: Exogenous fabrication of Au nanoparticles at root surface of live plants exposed to 0, 0.5, 1 and 2 mM HAuCl₄. Live plants of (a) Phyllanthus fraternus, (b) Portulaca grandiflora, (c) Cicer arietinum, (d) Medicago sativa, (e) Euphorbia hirta, (f) Amaranthus gracilis and (g) Vernonia cinerea with their root system dipped in Au salt solution showing alteration in color.]

The UV-Vis absorption spectra of purple colloidal solutions showed maximum absorption between 520-580 nm, which is known to arise due to surface plasmon resonance in Au nanoparticles (Daniel and Astrue 2004; Eustis and El-Sayed 2006) (Fig. 5.2). In general, the intensity of the absorption peak increased with
increase in concentration of HAuCl$_4$. However, wherever root system of intact plants turned HAUCl$_4$ solution goldenish, no clear peak could be recorded (Fig. 5.1 and 5.2). The intensity of the absorption peak varied between plant species, indicating variations in reducing the strength of the root system.

**Figure 5.2:** UV-Vis absorption spectra of different concentrations of HAUCl$_4$ (0.5, 1.0 and 2 mM) exposed to roots of intact plants.
TEM investigations revealed the presence of well dispersed distinct Au nanoparticles, by and large in the range of 5 to 50 nm in these colloidal solutions (Fig. 5.3 a-d, 5.4 a-d and 5.5 a-d). The variation in the size range of Nanoparticles generated exogenously by the root system of intact plants of different plant species is shown in Table 5.1. Irrespective of plant species the Nanoparticles generated by root system were composed of Au as indicated by Energy dispersive X-ray (EDX) measurements (Fig. 5.3 i-l, 5.4 i-l and 5.5 i-l). Further, selected area electron diffraction (SAED) pattern of Au nanoparticles generated by the root system of all plant species disclosed the presence of distinct rings corresponding to Bragg reflections, implying their crystalline nature (Figure 5.3 e-h, 5.4 e-h and 5e-h). The PXRD analysis of Au nanoparticles fabricated and released by the root system of intact plants into the surrounding solution confirmed their crystalline nature.

![Figure 5.3: TEM (a-d), SAED (e-h) and EDX (i-l) of Au nanoparticles synthesized by roots of intact plants of *Lycopersicon esculentum*, *Portulaca grandiflora*, *Medicago sativa* and *Catharanthus roseus* respectively, on exposure to HAuCl₄.](image-url)
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Figure 5.4: TEM (a-d), SAED (e-h) and EDX (i-l) of Au nanoparticles synthesized by roots of intact plants of *Vernonia cinerea*, *Euphorbia hirta*, *Amaranthus gracilis*, and *Phyllanthus fraternus* respectively, on exposure to HAuCl₄.

Figure 5.5: TEM (a-d), SAED (e-h) and EDX (i-l) of Au nanoparticles synthesized by roots of intact plants of *Cicer arietinum*, *Cynodon dactylon*, *Cannabis sativa* and *Brassica juncea* respectively, on exposure to HAuCl₄.
These Au nanoparticles showed prominent Bragg reflections that can be assigned to (111), (200), (220) and (311) planes, which matched with the standard diffraction pattern of the Joint Commission for Powder X-ray Diffraction Studies (JCPDS) File No. 04-0784 (Figure 7.6).

**Figure 5.6:** PXRD of Au nanoparticles synthesized exogenously by roots system of intact plants of various plant species.
Earlier, live plants of *Medicago sativa*, *Sesbania drummondii*, and *Brassica juncea*, were shown to generate Au nanoparticles in situ i.e. within their cells (Gardea-Torresdey et al., 2002; Sharma et al., 2007; Bali and Harris 2010; Beattie and Haverkamp 2011; Raju et al., 2012). While, Beattie and Haverkamp (2011) reported 2-100 nm Au nanoparticles in the cells of *B. juncea*, Bali and Harris (2010) recorded Nanoparticles of different shapes and sizes ranging between 2 nm to 2 µm and 2 nm to 1µm in cells of *B. juncea* and *M. sativa*, respectively. Such a broad range of Nanoparticles recorded by these researchers must be due to complex array of biomolecules present in the cells. Presence of complex array of biomolecules viz. phenolics, organic acids, amino acids, sugars with potent reduction capacity, (Bhargava et al., 2005; Wang et al., 2007) in plant cells is well documented. In contrast, as is evident from Table 5.1, during present investigations, irrespective of the plant species, the root system of plants generated Au nanoparticles in a narrow range, when incubated in HAuCl$_4$ solution for a duration of 12-24 hours.

5.2.1 Mechanism of generation of au nanoparticles by root system of intact plants

Earlier, Gardea-Torresdey et al. (2002) reported that Au$^0$ is actively taken up from the semi-solid agar medium by the roots of live alfalfa plants and Au nanoparticles are synthesized through nucleation and growth within plant cells. However, these researchers remained silent on the origin (i.e. mode of formation) of Au$^0$ in the agar medium. Sharma et al. (2007) observed Au nanoparticles in the cytoplasm of cells of *S. drummondii*. Although, these investigators hypothesized that “gold” is transported through symplasm following their entry through root cells, they remained silent on the oxidation state of “gold” in which it is transported. Beattie and Haverkamp (2011) reported that reducing sugars promote reduction of Au$^{3+}$ to form Au nanoparticles intracellularly in *Brassica juncea* plants exposed to Au salt solutions. However, Bali and Harris (2010) are of the opinion that secondary metabolites like glutathione and
phytochelatins play an important role in reduction of \(\text{Au}^{3+}\) and synthesis of Au nanoparticles.

Although the synthesis of Au nanoparticles in the cells of plants is certainly a clean and green method, it has two major limitations. While the first is the synthesis of a broad range (both in terms of size and shape) of Nanoparticles due to the wide array of biomolecules present in plant cells, the second is the unfeasibility of extraction of intracellularly formed Nanoparticles from these plants. Therefore, exogenous generation of Au nanoparticles by the root surface of intact plants discovered by us during present investigations would be superior. Generation of Au nanoparticles from ions essentially require reduction (Xia et al., 2009). Earlier, it has been shown that plant roots exude organic acids, phenolics, proteins etc. (Blaylock and James 1994; Dong et al., 2007) which are known to reduce \(\text{Au}^{3+}\) to generate Au nanoparticles (Bhargava et al., 2005; Wang et al., 2007). Bali and Harris (2010) and Raju et al. (2012) hypothesized the involvement of root exudates for the generation of Au nanoparticles. In order to evaluate if the capacity of the root system of plants to reduce \(\text{Au}^{3+}\) and generate Au nanoparticles exogenously, recorded during present investigations is due to biomolecules that are exuded by the roots, 2 sets of investigations were carried.

In the first set of experiments, this study tested if the solutions in which roots of intact plants were incubated for 12 and 24 hours in sterile double distilled water, contained any detectable levels of amino acids, phenolic compounds and proteins. No detectable levels of these biomolecules were detected in the incubation solution in which roots of intact plants were incubated. In the second set of experiments, this medium (distilled water) in which roots of intact plants were incubated was evaluated for its potential to generate Au nanoparticles. Surprisingly, the medium (distilled water) collected after incubating roots of intact plants for 12 or 24 hours failed to alter the color of \(\text{HAuCl}_4\) solution. These results ruled out the possible involvement of biomolecules released (if any), by the root system of intact plants used during present investigations, at least until 24 hours. These findings are in conformity with the observations of Rubinstein et al. (1984) and Qiu et al. (1985). These researchers reported that leachates from the roots of oat and maize plants do not possess the
capacity to reduce membrane impermeable ferricyanide. However, this study revealed that whenever, the root system is damaged (either intentionally or accidentally) this study did record that the distilled water in which such damaged roots of intact plants were incubated could turn clear Au solutions turbid with slight violet to purple coloration. Further, this study could detect phenolics in distill water in which such damaged roots of intact plants were incubated. However, during present investigations, care was taken to use plants with the undamaged root system.

Another likely possibility for the exogenous generation of Au nanoparticles could be due to root associated microorganisms. Several microorganisms are used to generate Au nanoparticles (Narayanan and Sakthivel 2010; Iravani 2011). Manceau et al. (2008) reported that wetland plants can form metallic Nanoparticles in and near roots with the assistance of endomycorrhizal fungi. As plants used during present investigations would invariably be associated with microorganisms, there is a probability that the Nanoparticles generated by plants during present investigations could be due to root associated microorganisms. In order to verify if root associated microorganisms are necessary for the generation and release of Au nanoparticles, plant species namely (i) *Triticum aestivum* (wheat) (b) *Vigna mungo* (black gram) (c) *Cicer arietinum* (chickpea) (d) *Brassica juncea* (mustard) and (e) *Lycopersicon esculentum* (tomato) grown under strict sterile conditions were tested for their potential to fabricate Au nanoparticles.

Roots of intact plants grown under sterile conditions similar to other plants altered color of HAuCl₄ solutions from pale yellow to purple/golden and turned them colloidal even under strict sterile conditions (Fig. 5.7 a-b). Analysis of these colloidal solutions with (i) TEM coupled with EDX, SAED and (ii) PXRD confirmed that these plant species generated crystalline Au nanoparticles even under sterile conditions (Fig. 5.8). These results clearly demonstrated that the root system of intact plants possess potential to generate Au nanoparticles even in absence of any associated microorganism(s). It has been established that roots of plant system have good reducing strength (Rubinstein et al., 1984; Qiu et al., 1985).
Figure 5.7: Potential of roots of intact plants of 4 day old *Vigna mungo* and *Triticum aestivum* plants and sodium citrate to generate Au nanoparticles upon exposure to different concentrations (mM) of HAuCl₄. Solutions of HAuCl₄ incubated for 5 h under ambient conditions with (a) roots of intact plants of *V. mungo* and (b) *T. aestivum* raised and (c) 0.1 mM sodium citrate maintained under sterile conditions. UV-Vis spectra of Au solution exposed to (d) *V. mungo*, (e) *T. aestivum* and (f) 0.1 mM citrate.
Using a membrane impermeable artificial electron acceptor, ferricyanide, Rubinstein and co-workers (1984) and Qiu et al. (1985) clearly established the prevalence of strong reducing strength at the root surface. Being impermeable, reduction of ferricyanide to ferrocyanide has to take place at the root surface or by the lechates from the roots. By demonstrating incapability of lechates to reduce ferricyanide, these authors established the presence of plasma membrane bound dehydrogenases in association with root surface cells, that promoted reduction of ferricyanide to ferrocyanide, with simultaneous oxidation of NAD(P)H to NAD(P)$^+$. 

**Figure 5.8:** TEM (a-b), SAED (c-d), EDX (e-f) and PXRD (g-h) of Au nanoparticles synthesized by roots of intact plants of *Vigna mungo* and *Triticum aestivum* respectively, on exposure to HAuCl$_4$. 

This reducing capacity of the root has subsequently been confirmed by other researchers, and the same has been reviewed (Vuletic et al., 2005). Even during present investigations using 2,6-dichlorophenolindophenol (DCPIP), another artificial electron acceptor, we had recorded potential of the root system of all the plant species tested by us to possess reducing strength, as they could reduce blue colored DCPIP to colorless DCPIPH$_2$ (Fig. 5.9).

Therefore, based on the results obtained with DCPIP supported by well accepted findings of earlier researchers (Rubinstein et al., 1984; Qiu et al., 1985; Crane et al., 1991; Vuletic et al., 2005) the present study propose that dehydrogenases/reductases associated with the plasma membrane of cells prevailing at root surface must be playing a vital role in the reduction of Au$^{3+}$ to Au$^{0}$, which nucleate to form Au nanoparticles.

Figure 5.9: Reduction of blue colored DCPIP to colorless DCPIPH$_2$ by root system of live plants of (a) Portulaca grandiflora and Triticum aestivum under (c) sterile and (d) non-sterile conditions.
5.2.2  The Superiority of the Root System of Intact Plants to Generate Au Nanoparticles over other Methods

Amongst various chemical methods, citrate method (Frens 1973) is considered to be superior. Therefore, to evaluate the effectiveness of the method demonstrated during present investigations, potential of the root system of intact plants of two plant species viz. *V. mungo* and *T. aestivum*, to generate Au nanoparticles was compared with that of sodium citrate under strict sterile conditions, but at room temperature. As is evident from figure 5.7, root system of both these plant species possesses superior strength to reduce Au$^{3+}$ and synthesis Au nanoparticles compared to sodium citrate at room temperature. The superiority of the root system of intact plants over sodium citrate was evident from the intensity of (i) purple color and (ii) Au nanoparticles specific absorption peak. Further, it is interesting to note that the root system of plants generated Nanoparticles even when Au$^{3+}$ was present at concentrations as low as 0.01 mM, but sodium citrate could generate notable Au nanoparticles when concentration was ≥0.1 mM. The same is also clear from dynamic light scattering (DLS) analysis (Fig. 5.10). Moreover, Au nanoparticles synthesized with 1 mM Au$^{3+}$ using sodium citrate were primarily in the range of ~270 to 370 nm while those synthesized by the root system of *V. mungo* were in range of ~25-50 nm. This shows that smaller Au nanoparticles are formed by the roots of intact plants compared to sodium citrate under similar ambient conditions.

However, the root system of intact plants of *V. mungo* was superior to *T. aestivum*. The variation in potential of these plant species to generate Au nanoparticles could be due to the type of root system and/or genetic make-up. Biological methods for synthesis of Nanoparticles are considered green and superior over various physical and chemical methods as it neither uses any toxic chemicals nor generate any toxic byproducts (Iravani 2011). Amongst biological approaches, the use of microorganisms and plant extracts for the generation of Au nanoparticles is more widely reported/popularized. Some of the microorganisms used by researchers included pathogenic species such as *Klebsiella pneumonia*, *Bacillus subtilis*,
Micrococcus luteus, Serratia marcescens, Aspergillus etc. (Narayanan and Sakthivel 2010; Honary et al., 2012; Arunkumar et al., 2013).

Figure 5.10: DLS analysis of Au nanoparticles generated with different concentrations (0.01 to 1mM) of HAuCl₄ on incubation with sodium citrate and roots of intact plants of Vigna mungo for 5 hours.
Generation of Nanoparticles through microorganisms not only require special facilities for maintenance of cultures, but also need to address essential biological safety measures (Gan and Li 2012). Therefore, Au nanoparticles synthesis using extracts of plant material is considered superior. However, as it is difficult to precisely identify the biomolecule(s) amongst the cocktail of molecules present in the plant extract, none of the reported methods could be appropriately exploited for commercial production of Au nanoparticles.

Intracellular generation of Au nanoparticles by live plants (Gardea-Torresdey et al., 2002; Sharma et al., 2007; Bali and Harris 2010; Beattie and Haverkamp 2011; Raju et al., 2012) seems to be advantageous as it is a green/environment-friendly method. However, this method has two major drawbacks namely (i) extraction of intracellularly generated Au nanoparticles would be tedious/unfeasible; and (ii) formation of a broad range (in terms of size and shape) of Au nanoparticles due to complex array of biomolecules present in plant cells. In contrast, during present investigations exogenous generation of Au nanoparticles by the roots of intact plants was recorded which is advantageous and cost-effective as these exogenously formed Nanoparticles would not require downstream processing and can be used for fabrication of nanomaterials.

In contrast the present investigations lead to the discovery of a novel and simple method for exogenous generation of Au nanoparticles exploiting reducing strength prevailing at the root surface of plant species. Further, the root system of plants could generate a relatively narrow range of Au nanoparticles within a duration of 12-24 hours under ambient and aqueous conditions. Relatively uniformly appearing Nanoparticles are generated as plasma membrane bound dehydrogenases of root surface cells could be playing a critical role in reducing $\text{Au}^{3+}$ to $\text{Au}^0$ and the generation of Au nanoparticles. In other words, unlike a complex array of factors or biomolecules that regulate intracellular synthesis of Au nanoparticles, synthesis of Au nanoparticles at the root surface is regulated relatively more precisely.
5.3 INFERENCES

The present study demonstrated that the roots of intact plants possess potential to exogenously generate Au nanoparticles. Through experimental evidences we propose that dehydrogenases/reductases present at the root surface are responsible for the reduction of $\text{Au}^{3+}$ to $\text{Au}^0$ and exogenous generation of Nanoparticles. Also, the results of this study clearly reveal that the roots of intact plants of certain species can be exploited for bulk synthesis of Au nanoparticles exogenously under ambient conditions in aqueous phase. This study paves a novel, simple, economically feasible and eco-friendly scheme for exogenous production of Au nanoparticles.
Table 5.1: Details of plant species used for exogenous generation of Au nanoparticles by root system of intact plants. Size of nanoparticles generated exogenously by root system of various plant species is also shown.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Common Name</th>
<th>Family</th>
<th>Size of Nanoparticles (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus gracilis</em></td>
<td>Slender Amaranth</td>
<td>Amaranthaceae</td>
<td>5-25</td>
</tr>
<tr>
<td><em>Brassica juncea</em></td>
<td>Indian Mustard</td>
<td>Brassicaceae</td>
<td>2-5</td>
</tr>
<tr>
<td><em>Cannabis sativa</em></td>
<td>Hemp</td>
<td>Cannabinaceae</td>
<td>20-30</td>
</tr>
<tr>
<td><em>Catharanthus roseous</em></td>
<td>Madagascar Periwinkle</td>
<td>Apocynaceae</td>
<td>5-25 nm</td>
</tr>
<tr>
<td><em>Cicer arietinum</em></td>
<td>Chickpea</td>
<td>Fabaceae</td>
<td>2-10</td>
</tr>
<tr>
<td><em>Cynodon dactylon</em></td>
<td>Couch Grass</td>
<td>Poaceae</td>
<td>20-30</td>
</tr>
<tr>
<td><em>Euphorbia hirta</em></td>
<td>Asthma Plant</td>
<td>Euphorbiaceae</td>
<td>5-20</td>
</tr>
<tr>
<td><em>Lycopersicon esculentum</em></td>
<td>Tomato</td>
<td>Solanaceae</td>
<td>5-15</td>
</tr>
<tr>
<td><em>Medicago sativa</em></td>
<td>Alfalfa</td>
<td>Fabaceae</td>
<td>5-15</td>
</tr>
<tr>
<td><em>Ocimum sanctum</em></td>
<td>Holy Basil</td>
<td>Lamiaceae</td>
<td>20-50</td>
</tr>
<tr>
<td><em>Phyllanthus fraternus</em></td>
<td>Gulf Leaf Flower</td>
<td>Euphorbiaceae</td>
<td>5-20</td>
</tr>
<tr>
<td><em>Portulaca grandiflora</em></td>
<td>Moss Rose</td>
<td>Portulacaceae</td>
<td>15-30</td>
</tr>
<tr>
<td><em>Tagetes erecta</em></td>
<td>Marigold</td>
<td>Asteraceae</td>
<td>20-50</td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
<td>Wheat</td>
<td>Poaceae</td>
<td>5-20</td>
</tr>
<tr>
<td><em>Vernonia cinerea</em></td>
<td>Little Ironweed</td>
<td>Asteraceae</td>
<td>10-30</td>
</tr>
<tr>
<td><em>Vigna mungo</em></td>
<td>Black Gram</td>
<td>Fabaceae</td>
<td>10-40</td>
</tr>
</tbody>
</table>