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Research article

Biogenic synthesis and spatial distribution of silver nanoparticles in the legume mungbean plant (*Vigna radiata* L.)☆

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ABSTRACT

The present investigation aimed to study the *in vivo* synthesis of silver nanoparticles (AgNPs) in the legume *Vigna radiata*. The level of plant metabolites such as total phenolics, lipid, terpenoids, alkaloids and amino acid increased by 65%, 133%, 19%, 67% and 35%, respectively, in $AgNO_3$ (100 mg L^{-1}) treated plants compared to control. Whereas protein and sugar contents in the treated plants were reduced by 38% and 27%, respectively. FTIR analysis of $AgNO_3$ (20–100 mg L^{-1}) treated plants exhibited changes in the IR regions between 3297 and 3363 cm^{-1}, 1635–1619 cm^{-1}, 1249–1266 cm^{-1} and that corresponded to alterations in O–H groups of carbohydrates, O–H and N–H groups of amide I and II regions of protein, when compared with the control. Transmission electron micrographs showed the spatial distribution of AgNPs in the chloroplast, cytoplasmic spaces, vacuolar and nucleolar plant regions. Metal quantification in different tissues of plants exposed to 20–100 mg L^{-1} $AgNO_3$ showed about a 22 fold accumulation of Ag in roots as compared to shoots. The phytotoxic parameters such as percent seed germination and shoot elongation remained almost unaltered at low $AgNO_3$ doses (20–50 mg L^{-1}). However, at higher levels of exposure (100 mg L^{-1}), the percent seed germination as well as root and shoot elongation exhibited concentration dependent decline. In conclusion, synthesis of AgNPs in *V. radiata* particularly at lower doses of $AgNO_3$, could be used as a sustainable and environmentally safe technology for large scale production of metal nanoparticles.

1. Introduction

Nanotechnology is an important field of modern research dealing with the synthesis, applications and manipulations of nanoparticles. Metallic nanoparticles are known to have multiple applications such as targeted drug delivery and safety control, cancer therapy, bio-sensing and antimicrobial applications (Salata, 2004). Silver nanoparticles are of great scientific interest because of their unique optical, electromagnetic and physicochemical properties (Firdhouse and Lalitha, 2015). The wide range of AgNPs applications include the production of antimicrobial products, biosensors, composite fibers, cryogenic superconductors, cosmetics and many electronic gadgets (Korbekandi and Iravani, 2012). Due to their antimicrobial characteristics, AgNPs have been widely used in the field of agriculture and production of miscellaneous industrial products (Chen and Schluesener, 2008). An increasing demand for AgNPs due to their vast applications, there is need for large scale production of AgNPs by using low cost and environmentally safe technology. Recently, efforts are being made to develop a synthesis process that is economical and environmentally friendly for the production of AgNPs through biogenic methods. This bio-mimetic approach utilizes microorganisms like bacteria, yeast and fungi (Salunke et al., 2011; Kowshik et al., 2003; Li et al., 2012) as well as plants (Makarov et al., 2014). Among all the bioresources, the plant-mediated synthesis of silver nanoparticles is being preferred over other methodologies due to its low cost and surplus availability of resources which can successfully meet the current market demand. It is also a green technology for the rapid production of silver nanoparticles that does not need any special, complex and multi-step procedure such as isolation, purification and culture preparation or culture maintenance (Iravani, 2011).

The biogenic synthesis of nanoparticles in living plants is an emerging field of nanotechnology, where the natural ability of plants to accumulate metals and transform them into desired nanocrystals can be easily exploited (Iravani, 2011). Gardea-Torresdey et al. (2002), for the first time, reported the *in vivo*

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synthesis of nanoparticles in *Medicago sativa* (alfalfa) and suggested that the plants have ability to synthesize metal nanoparticles of different shapes and sizes. Shah et al. (2015) suggested that an interaction between the plant metabolites and metal ions was responsible for the synthesis of stable metal nanoparticles. However, any *in vivo* synthesis of metal nanoparticles also necessitates a complete knowledge about the phytotoxic response of the selected plants against metal toxicity.

Plants can be used as a "bio-factory" for *in vivo* synthesis of metallic nanoparticles with no additional maintenance cost (Rupiasih et al., 2013). However, the *in vivo* synthesis of nanoparticles by plants has an added advantage over the *in vitro* processes as it can also prevent the spillover of toxic metals in the environment. There are limited references available on the *in vivo* synthesis and fate of silver nanoparticles by living plants (Marchiol et al., 2014), but there are no reports related to the *in vivo* synthesis of AgNPs by the leguminous crop plant like *V. radiata*. The present investigation aimed to study the *in vivo* synthesis of AgNPs by the legume crop *V. radiata* and the role of plant metabolites. In addition, the phytotoxic effect of AgNPs on legume plant *V. radiata* was also evaluated including the spatial distribution of silver nanoparticles in different plant tissues.

2. Materials and methods

2.1. Experimental plant

Vigna radiata L. is commonly known as green gram or mung bean and it is a major edible legume crop in Asia and Southern Europe. It is a rich source of minerals, vitamins and proteins (Deshpande, 1992). Seeds of the mung bean (*V. radiata* L.) [Wilczek] was purchased from Kisan Seeds Limited, Lucknow.

2.2. Chemicals

Silver as $AgNO_3$ was obtained from Labline traders, Lucknow, India. Different dilutions of $AgNO_3$ suspensions (0–100 mg L^{-1}) were prepared in deionized water.

2.3. $AgNO_3$ treatment to plant seedlings

Seeds of mung bean (*V. radiata*) were pretreated with 10% (v/v) sodium hypochlorite solution for 10 min and then rinsed thoroughly with distilled water to remove the dust particles. These seeds were used directly for further experiments. Different concentrations of $AgNO_3$ (0, 20, 50, 100 mg L^{-1}) were prepared in distilled water. The viable seeds were first pretreated with $AgNO_3$ solution for atleast 4 h. Ten seeds were kept for germination in Petri dishes (containing water soaked filter paper on bottom) and then 1.0 mL of different concentrations of $AgNO_3$ suspensions were added for treatments. Distilled water was used in control. Moisture in the petri dishes was maintained by adding distilled water as and when required. After seed germination in Petri-plates, seedlings were transferred in soil pot culture.

2.4. Plant metabolites assay

Plant metabolites i.e., carbohydrate, protein and amino acid, alkaloids, saponins, steroids, tannins, total phenolics, flavonoids, glycosides and tri-terpenoids were analyzed in the control as well as $AgNO_3$ treated plants by following standard biochemical methodologies (Horbone, 1984; Kokate et al., 1995).

2.4.1. Phytochemical screening

Phytochemical screening of active metabolites in *V. radiata* was carried out as given below:

2.4.1.1. Test for carbohydrates. In 2.0 mL of aqueous plant extracts, few drops of Molisch's reagent and 1.0 mL conc. H_2SO_4 were added slowly along the side of the test tube. The appearance of red ring at the junction of two layers indicated the presence of carbohydrate.

2.4.1.2. Test for protein and amino acids. A freshly prepared solution of 0.2% (w/v) ninhydrin reagent was added to 3.0 mL of aqueous plant extract and mixture was heated to boiling temperature. The appearance of pink or purple colour indicated the presence of proteins, peptides or amino acids.

2.4.1.3. Test for alkaloids (Mayer's test). About 0.2 g fresh leaf sample was boiled with 5 mL of 2% hydrochloric acid on a water bath for 5 min. In 3.0 mL of filtrate, 2–3 drops of Mayer's (potassium mercuric iodide solution) reagent was added. A creamy white colored precipitate after adding Mayer's reagent indicated the presence of alkaloid.

2.4.1.4. Test for saponin (Foam test). 0.5 mL of plant extract was diluted with 100 mL of distilled water and shaken well for 15 min. Formation of foam or no foam was considered indicator for the presence and absence of saponins, respectively.

2.4.1.5. Test for tannins. 3 mL of aqueous plant extract was mixed with 5–6 drops of 1% (w/v) solution of gelatin containing 10% of sodium chloride. Formation of white precipitates indicated the presence of tannins.

2.4.1.6. Test for phenols. In 3.0 mL of aqueous plant extract, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

2.4.1.7. Test for flavanoids. In 5.0 mL of aqueous plant extract, 2.0 mL of the 10% sodium hydroxide solution was added to produce a yellow colouration. A change in colour from yellow to colourless after adding few drops of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans, 2002).

2.4.1.8. Test for anthocyanin. 3.0 mL of methanolic plant extract was mixed with 5.0 mL of 1 N sodium hydroxide. Solution colour changed to blue that indicates the presence of anthocyanin.

2.4.1.9. Test for glycosides. Keller Killiani test for glycosides was carried out using 3.0 mL of plant extracts treated with 2.0 mL of glacial acetic acid containing one drop of ferric chloride solution. This was followed by addition of 1.0 mL of concentrated sulfuric acid. Formation of two distinctly colored layers, comprised of lower reddish brown layer and upper bluish green layer, indicated a positive test for glycosides.

2.4.1.10. Test for phytosterols. Salkowski's test: Plant extract mixed with 10 mL of chloroform was filtered and was supplemented with 5–6 drops of concentrated H_2SO_4. The mixture was shaken gently until it turned golden red in colour, which was indicative of positive test for the presence of phytosterol.

2.4.1.11. Test for terpenoids. Liebermann – Burchard test: 3.0 mL of plant extract was mixed with 3.0 mL of chloroform, 1.0 mL acetic anhydride and few drops of H_2SO_4. The mixture turned dark green in colour, which indicated the presence of terpenoids.

2.4.2. Quantitative determination of plant metabolites

Quantitative assay of plant metabolites in control and $AgNO_3$