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SHORT COMMUNICATION

Plant growth and diosgenin enhancement effect of silver nanoparticles in Fenugreek (*Trigonella foenum-graecum* L.)

B. Jasim, Roshmi Thomas, Jyothis Mathew, E.K. Radhakrishnan *

School of Biosciences, Mahatma Gandhi University, PD Hills (PO), Kottayam, Kerala 686 560, India

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Abstract Various methods have been used to enhance production of chemically diverse phytochemicals especially medicinal natural products. With the advancement in nanotechnology, nanoparticles have been reported to have varying impact in plant growth and inducibility of phytochemical composition. Major objective of the study was to study the secondary metabolite modulatory effect of silver nanoparticles. In the current study, treatment of fenugreek seedlings with biosynthesized silver nanoparticles (Ag-NPs) was found to have significant impact on its growth parameters such as leaf number, root length, shoot length and wet weight. On HPLC based analysis, Ag-NPs treated seedlings showed an enhancement in the production of major phytochemical diosgenin to a level of 214.06 ± 17.07 μg/mL. An untreated control gave an yield of only 164.44 ± 7.67 μg/mL of diosgenin, and the observed phytochemical enhancement effect induced by Ag-NP was very significant. Most remarkably, the Ag-NP used in the study was found to play dual role of enhancement of both plant growth and diosgenin synthesis. Hence the study is of immense application as it opens up development of new methods based on nanoelicitors to enhance the biosynthesis of medicinal natural products in plants.
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1. Introduction

Enhanced biosynthesis of phytochemicals is highly attractive because of their broad bioactivity properties. Because of this, various methods have been used to enhance the phytochemical composition of medicinal plants. This ranges from tissue culture based methods to the development of transgenic plants. But with the advancement in nanotechnology and various bio-interaction properties of nanoparticles, there lies much opportunities to explore nano-elicitor potential of NPs. The

* Corresponding author.
E-mail address: radhakrishnanek@mgu.ac.in (E.K. Radhakrishnan).
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nano-elicitive function of nanoparticle may likely to be determined by nature of nanoparticle and method used for synthesis. Various methods have been used for nanoparticles synthesis and among these, microbial synthesis is highly attractive as it is eco-friendly and the process occurs at normal temperature and pressure (Das et al., 2014). Among the various nanoparticles, one of the most widely studied one is silver nanoparticles (Ag-NP) because of its diverse antimicrobial applications, use as coating material for stainless steel, and also in treatment for water purification (Durán et al., 2007). Their remarkable physical and chemical features and the resulting activity are considered to be due to its small size which usually ranges from 1 to 100 nm (Nowack and Bucheli, 2007).

Nanoparticles have been reported to have both positive and negative effects on growth and development of various plant species. Ag-NPs have been shown to have its effect on activating the aminocyclopropane-1-carboxylic acid (ACC)-derived inhibition of root elongation in Arabidopsis seedlings. Enhanced seed germination and seedling growth of tree *Boswellia ovaliofoliolata* have also been reported upon treatment with Ag-NPs (Savithramma et al., 2012). Effect of Ag-NPs on plant growth enhancement parameters such as shoot and root length, leaf area and biochemical attributes such as chlorophyll, carbohydrate and protein contents and antioxidant enzymes were also reported on *Brassica juncea* (Sharma et al., 2012; Zea and Salama, 2012). However, the influence of NPs towards secondary metabolite enhancement in medicinal plants are least studied. This is very important as the positive modulatory effect of Ag-NPs on phytochemical composition can be exploited for the enhanced production of medicinally important natural products from plants. So we have selected Fenugreek as a model system to study the effect of Ag-NPs on both plant growth and secondary metabolite modulation.

Fenugreek (*Trigonella foenum-graecum* L.) is a well-known spice which is used commonly in most parts of the world. Its significance is due to the presence of sapogenins such as diosgenin which has both pharmaceutical and nutraceutical applications (Acharya et al., 2008; McAnuff et al., 2002). In addition, diosgenin has significant effect in plant physiology to control both biotic and abiotic stress responses as the elevated level of phytochemicals playing significant role in plant stress management. So the study used changes in diosgenin concentration as a metabolite marker to know nanoelicitor role of Ag-NPs. As diosgenin is used as medicines and is also a precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids and progesterone, its enhanced production is of significant applications. So the effect of biosynthesized Ag-NPs was analyzed on the plant growth and diosgenin variation in *Trigonella foenum-graecum* L.

2. Materials and methods

2.1. Biosynthesis of silver nanoparticles

Bacillus sp. (SJ 14), which was found to have highly efficient Ag-NPs synthesizing properties as confirmed by our previous study using UV–Vis spectroscopy, FTIR, SEM, HR-TEM and EDS was selected for the Ag-NPs synthesis (Thomas et al., 2015). For this, the bacterial isolate was inoculated into 100 mL nutrient broth and incubated at room temperature for 24 h in a rotating shaker (200 rpm). After incubation, the biomass was collected by centrifugation and about 2 g of bacterial wet biomass was resuspended in 100 mL aqueous solution of 1 mM $AgNO_3$ in a 250 mL Erlenmeyer flask. The mixture was then kept on a rotating shaker set at 200 rpm for a period of 24 h at room temperature under visible light. The formed nanoparticles were purified as per previous report (Thomas et al., 2014). Briefly, the whole bacterial mixture with AgNPs was centrifuged at 15,000 rpm for 15 min and the collected pellets were washed and resuspended in 50 mM Tris buffer (pH 7). The cells were disrupted by ultrasonication and the resulting solution was filtered through a 0.22 μM filter (Millipore) and the purified AgNPs thus obtained were further characterized by UV–Vis spectroscopy and HR-TEM.

2.2. Effect of Ag-NPs on plant growth promotion

For the study, Fenugreek (*Trigonella foenum*) seeds were surface sterilized using 2% sodium hypochlorite and 70% ethanol (Aravind et al., 2009) and were soaked in sterile distilled water for 24 h. After this, sprouting seedlings were placed on 2% agar in conical flasks and 200 μL of Ag-NPs (1 μg/mL (w/v)) was added to each seedling and were kept for 5 days along with control. The concentration of Ag-NPs for treatment was selected based on previous report on effect of Ag-NPs on plants (Harris and Bali, 2008). After 5 days of incubation on agar, the seedlings were taken and planted on conical flask containing 100 g sterile soil. Ag-NPs addition to the planted seedlings was repeated by adding the same concentration used earlier (200 μL of 1 μg/mL (w/v)) to each seedlings. The planted flasks were kept under regulated conditions for 5 more days. The experiment was conducted in triplicate in which each set contained 10 seedlings. After incubation the seedlings were collected and the root length, shoot length, number of leaves, and wet weight were measured.

2.3. Diosgenin quantification

For this, the seedlings were dried in a hot air oven at 65 °C for 5 h and powdered and equal quantity (0.8 g) from each treatment was extracted with 50 mL of hexane. The container with the mixture was sealed and was then incubated for 10 days at 50 °C under shaking condition. Finally, the extract was filtered using Whatman No. 1 filter paper and was evaporated. The residual powder was reconstituted in 1 mL of methanol. Diosgenin present in the methanolic extract of the sample was quantified by C18 reverse-phase column of analytical HPLC (Thermo Scientific) with elution using an isocratic binary system of acetonitrile/water (90:10), at flow rate of 1 mL min^{-1} (Jasim et al., 2015). Quantification of the diosgenin produced was calculated from the area of the peak using the equation.

$$\text{Conc} \cdot \text{of unknown} = \frac{\text{Area of unknown} \times \text{Conc} \cdot \text{of Standard}}{\text{Area of Standard}}$$

2.4. Statistical analysis

The results were analyzed using statistical program IBM SPSS Statistics 20. One-way analysis of variance was used for comparison among two treatments. Post hoc multiple comparison

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