Biosynthesis of Silver Nanoparticles by Callus Cultures of Vigna radiata

R. Indira Iyer1*, C. Selvaraju2 and S. T. Santhiya1

1Department of Genetics, Dr. ALMPGIBMS, University of Madras (Taramani Campus), Chennai - 600113, Tamil Nadu, India; riiyer@yahoo.co.in, v_santhiya@hotmail.com

2National Centre for Ultrafast Processes, Dr. ALMPGIBMS, University of Madras (Taramani Campus), Chennai- 600113, Tamil Nadu, India; selvaraj24@hotmail.com

Abstract

Objectives: To study the potential of callus cultures of Vigna radiata for the biosynthesis of silver nanoparticles. Methods: Callus cultures of Vigna radiata were established in MS media and the aqueous extract of callus cultures was used for biogenic synthesis of silver nanoparticles using 1 mM AgNO₃ at ambient temperature. The nanoparticles were characterized by UV-visible spectroscopy and scanning electron microscopy. Findings: This is the first report of use of callus cultures of Vigna radiata for the biogenesis of silver nanoparticles. The synthesis was rapid and completed in 2 hours. UV-visible spectrophotometry revealed an absorbance peak at 455 nm. Scanning electron microscopy revealed the presence of a high density of spherical or ellipsoidal silver nanoparticles. Phytochemical studies revealed the presence of phenolics in the extract which could have played a role in the formation of silver nanoparticles. The findings are important since this is a novel approach and there are very few reports of synthesis of silver nanoparticles using callus cultures. Application: Callus cultures are more suitable for biosynthesis of bio-compatible nanoparticles as compared to microbes or plant parts since these are generated axenically and are renewable resources that are easy to authenticate.

Keywords: Callus, Green Gram, Silver Nanoparticles, Vigna radiata

1. Introduction

The synthesis of silver nanoparticles is of great interest owing to their diverse range of applications in various fields including catalysis, electronics, optics1-3 and has biomedical applications for their anti-cancer4 and anti-microbial5-8 effects and plant growth-promoting effect9. Synthesis of silver nanoparticles by conventional physical and chemical means is often with toxic residues besides being not cost-effective and eco-friendly10,11 and hence not suitable for bio-medical applications. Synthesis of silver nanoparticles by biological means12 is an important aspect of nanotechnology. There are several reports of plant-mediated biogenesis13-17 of nanoparticles and use of plant extracts is considered to be more effective than microbes18 for biosynthesis of nanoparticles. Synthesis of nanoparticles by callus cultures seems to be a promising alternative to use of microbes or field-grown plant parts since callus cultures are readily renewable resources generated axenically and there are no hazardous by-products. Unlike microbial cultures, callus does not require frequent subculturing. So far, only in very few species19-23 biosynthesis of valuable nanoparticles through callus cultures has been achieved.

Vigna radiata of Fabaceae, commonly known as green gram, a protein rich staple food is one of the important pulses in India. It contains about 25 percent protein24,25 and is reported to have antioxidant properties as well as anti-cancer properties25,26. A wide range of important metabolites-1-triacontanol, spermidine, spermine, amino acids, peptides and flavonoids as well as phenolics with anti-microbial effect have been reported in this species25,27. In the present study, the potential of callus cultures of Vigna radiata for the biosynthesis of silver nanoparticles was

* Author for correspondence
investigated and the rapid synthesis of silver nanoparticles at ambient temperature and pH is being reported.

2. Materials and Methods

2.1 Plant Material
Seeds of *Vigna radiata* obtained locally in Chennai were used for the study.

2.2 Preparation of Culture Media
MS media supplemented with various combinations of growth regulators and solidified with 1% agar after adjustment of pH to 5.7 was used for culture of explants. Culture tubes (150 x 25 mm) containing the media, were sterilized in an autoclave at 121°C at 105 kPa for 15 min.

2.3 Establishment of Cultures
The seeds were washed with running tap water for a few min and surface-sterilized by immersing in 0.1% HgCl\(_2\) for 3 min followed by several rinses with sterile distilled water in a laminar flow cabinet. The seeds were germinated aseptically either on the surface of filter paper circles in 5 cm Petri plates or directly transferred to MS medium supplemented with combinations of the growth regulators-2,4-D (2,4-dichlorophenoxyacetic acid), NAA (naphthaleneacetic acid), BA (benzyl adenine) in culture tubes. After germination, explants from seeds germinated in Petri plates or those from *in vitro* seedlings - cotyledon pieces, stem segments, leaf segments etc. were cultured in MS media supplemented with various combinations of growth regulators. The cultures were maintained in continuous light (25 μmol m\(^{-2}\) s\(^{-1}\)) source provided by cool white fluorescent lamps (Philips, India) at 25±2°C and 60-65% relative humidity. Subcultures were done at regular intervals of 4 - 6 weeks. Cultures were examined regularly and photomicrographs were taken using an Euromex stereo-zoom microscope (Holland) with CCD camera and image processing system. A digital camera (Canon) was also used for taking photographs.

2.4 Phytochemical Analysis of the Callus
Phenolics were extracted from the callus after crushing in a mortar and pestle using hot 80% methanol, filtered, taken to dryness and detected by a simple colour test with Folin-phenol reagent\(^{29}\).

3. Biosynthesis of Silver Nanoparticles with the Extracts of Callus

A weighed amount of 7 week old callus (12.7 g) was used for preparation of silver nanoparticles. The callus was ground with 5 volumes of sterile double distilled water in a sterile mortar and pestle. The extract was filtered with Whatman No.1 filter paper. To 10 mL of the filtered extract 1 mM AgNO\(_3\) (90 mL) was added at a ratio 1:9. The mixture was incubated at room temperature. Appearance of reddish brown color indicated the presence of silver nanoparticles. Visual observations were taken at regular intervals and photographs of the colour changes at different time intervals were taken using a canon digital camera. The bioreduction of silver nitrate was analysed using Cary 100 Bio UV-Vis spectrophotometer, Agilent Technologies, USA.

3.1 SEM (Scanning Electron Microscopy) Analysis of Silver Nanoparticles
The prepared silver nanoparticles were characterized using high resolution SEM. SEM images were obtained by using a Hitachi S4800 field emission high resolution scanning electron microscope.

4. Results and Discussion

4.1 Formation of Callus
Callusing was observed in all media irrespective of the hormonal composition. Callusing was obtained on stem segments, root segments, cotyledon explants, leaf explants (Figure 1(a)) from the *in vitro* seedlings when cultured in MS medium with 5 mg/L NAA, 1 mg/L benzyl adenine and 15% coconut milk) medium after 2 weeks. Profuse callusing was observed on stem segments on culture in MS medium with (2,4-D at 5 mg/L, NAA at 1 mg/L and 15% coconut milk) medium (Figure 1(b) and (c)). These results indicate the potential of the explants taken from seedlings for formation of fast growing callus for the yield of biomass which can be used for catalysing silver nanoparticle formation. Eventually, the callus could be maintained by subculture at regular intervals in media of similar composition and can act as a stable, readily available source of biomass for the production of nanoparticles.
4.2 Phytochemical Analysis
Detection of Phenolics in the Callus Extract

Development of blue coloration on testing for the presence of phenolics in the extract of the callus with the Folin-Phenol reagent indicated the potential of the biomass for synthesis of phenolics which have a wide variety of medicinal uses including anti-oxidant and anti-microbial effect.

4.3 Synthesis of Silver Nanoparticles
When the aqueous extract of the callus was added to 1 mM Ag NO₃, it was observed that the reaction started in half an hour and the colour gradually deepened. The solution turned reddish brown. The reaction was almost completed in 2 hours (Figure 2 (a)-(d)). The results are significant since the synthesis was carried out at room temperature and at ambient pH and the reaction is quite rapid compared to many other species. The time taken for completion of reaction was 9 h with leaf extracts of Pongamia pinnata, 12 h with Syzygium fruit extract, 20 h in Pulicaria glutinosa plant extract and 32 h with Semecarpus anacardium leaf extract. This indicates the high potential of callus cultures of Vigna radiata for synthesis of AgNPs. It may therefore be possible to achieve an even faster rate of AgNP synthesis by increasing the concentration of callus extract and modifying the reaction parameters suitably. The callus consists of rapidly growing meristematic cells richly endowed with the capacity of biosynthesis of phytochemicals such as phenolics which may be crucial for the formation and stabilisation of nanoparticles. Our results are in agreement with the findings in Sesuvium that rapid synthesis of silver nanoparticles could be achieved with callus cultures rather than plant parts and this was attributed to the presence of rapidly dividing cells in the callus rich in phytochemicals.

Figure 1. (a) Callusing on in vitro produced leaf explants. (b), (c) Massive callusing on stem segments from in vitro seedlings.

Figure 2. Visual observations of nanoparticle production by aqueous extract of callus showing control (left) and treatment (right) at different intervals of time after start of reaction. (a) 30 min. (b) 60 min. (c) 2 h. (d) 4 h.

Figure 3 shows the UV-vis spectra recorded after the completion of the reaction using Cary 100 Bio UV-VIS spectrophotometer. There was an absorbance peak at 455 nm which indicates the presence of silver nanoparticles.
Biosynthesis of Silver Nanoparticles by Callus Cultures of Vigna radiata

Figure 4. SEM micrographs of silver nanoparticles recorded at different magnifications.

SEM images reveal the presence of a high density of well-dispersed silver nanoparticles which are spherical or ellipsoidal. Figure 4 shows representative SEM images recorded at different magnifications of the silver nanoparticles synthesized. The resulting silver nanoparticles were predominantly spherical or ellipsoidal with a size range of 10 to 50 nm.

This is the first report on the production of silver nanoparticles by callus cultures of this species. Hitherto production of silver nanoparticles employing callus cultures has been reported in very few species unlike synthesis of silver nanoparticles by extracts of field-grown plants which has been extensively reported.

The synthesis of nanoparticles by callus cultures is highly desirable as these are produced under axenic conditions and apt for bio-medical applications. In the present study rate of reduction has been observed to be relatively fast since visual observations revealed that the reaction was almost completed in 2 hours. The method of synthesis is facile and the reaction was accomplished at ambient temperature and pH and this indicates the immense potential of the callus tissue of Vigna radiata for the biosynthesis of the silver nanoparticles.

The synthesis of silver nanoparticles by the callus cultures of Vigna radiata can be attributed to the presence of various biomolecules including phenolics and the results are in agreement with other reports for the role of functional groups of phytochemicals in production and stabilisation of silver nanoparticles in plant-mediated synthesis of nanoparticles.

5. Conclusion

Long term callus cultures established from the explants of Vigna radiata have shown the potential to synthesize Ag NPs rapidly at ambient temperature and pH. Synthesis of silver nanoparticles has been documented for the first time with callus cultures of this particular species. This may be extended to the large scale production of biomass of Vigna radiata useful for generation of silver nanoparticles.

6. Acknowledgement

R. Indira Iyer thanks University of Madras for award of Teaching cum Research Fellowship. Prof. P. Rammurthy, Director, National Centre for Ultrafast Processes, University of Madras (Taramani Campus) is gratefully acknowledged for permission to use the spectrophotometric facilities for this work.

7. References

1. El-Nour KMA, Eftaiha AA, Al-Warthan A, Ammar RA. Synthesis and applications of silver nanoparticles. Arabian Journal of Chemistry. 2010; 3(3):135–40.
2. Daniel SGEK, Sironmani TA, Tharmaraj V, Pitchumani K. Synthesis and characterization of fluorophore attached silver nanoparticles. Bull Mater Sci. 2011; 34(4):639–43.
3. Sudhan EPJ, Meenakshi KS. Synthesis of silver nanofluid by a novel one pot method for heat transfer application. Indian Journal of Science and Technology. 2011 Apr; 4(4):417–21. DOI:10.17485/ijst/2011/v4i4/30013.
4. Firdhouse JM, Lalitha P. Apoptotic efficacy of biogenic silver nanoparticles on human breast cancer MCF-7 cell lines. Prog Biomater. 2015; 4(2):113–21.
5. Jain P, Pradeep T. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. Biotechnology and Bioengineering. 2005; 90(1):59–63.
6. Prabhoo S, Poulose EK. Silver nanoparticles: Mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letters. 2012 Dec; 2(32):1–10. DOI:10.1186/2228-5326-2-32. Available from: http://www.inl-journal.com/content/2/1/32
7. Naghsh N, Safari M, Hajmehrabi P. Comparison of nanosilver inhibitory effects growth between Aspergillus niger and E. coli. Indian Journal of Science and Technology. 2012 Mar; 5(S3):2448–50. DOI:10.17485/ijst/2012/v5iS3/30409.
8. Naghsh N, Ghyastiyann, Soleimani S, Torkan S. Comparison between alcoholic eucalyptus and nano-silver as a new nanocomposition in growth inhibition of Aspergillus niger. Indian Journal of Science and Technology. 2012 Mar; 5(S3):2445–7. DOI:10.17485/ijst/2012/v5iS3/30408.
9. Shyla KK, Natarajan N. Customizing zinc oxide, silver and
titanium dioxide nanoparticles for enhancing groundnut seed quality. Indian Journal of Science and Technology. 2014 Jan; 7(9):1376–81. DOI:10.17485/ijst/2014/v7i9/50905.

10. Vithiya K, Sen S. Biosynthesis of nanoparticles. Int J Pharm Sci Res. 2011; 2(11):2781–5.

11. Karnani RL, Chowdhary A. Biosynthesis of silver nanoparticle by eco-friendly method Indian Journal of Nano Science. 2013 Feb; 1(2):25–31.

12. Moharrer S, Mohammadi B, Gharamohammadi RA, Yargoli M. Biological synthesis of silver nanoparticles by Aspergillus flavus, isolated from soil of Ahar copper mine. Indian Journal of Science and Technology. 2012 Mar; 5(S3):2443–4. DOI:10.17485/ijst/2012/v5iS3/30407.

13. Iyer RI, Panda T. Biogenic Synthesis of gold and silver nanoparticles by seed plants. J Nanosci Nanotechnol. 2014; 14(2):2024–37.

14. Gopalakrishnan S, Lakshmi SYS, Banu F. Comparison of antimicrobial activities of silver nanoparticles synthesized from Dysoxylum parasiticum. Indian Journal of Medicine and Healthcare. 2015 Jul; 4(2):1–5.

15. Lakshmi SYS, Mala D, Gopalakrishnan S, Banu F, Brindha V, Gajendran N. Green synthesis and characterisation of silver nanoparticles from the medicinal plant Pithecellobium dulce. Indian Journal of Nano Science. 2014 Aug; 2(8):4–9.

16. Gopalakrishnan S, Lakshmi SYS, Banu F. Antimicrobial activity of synthesized silver nanoparticles and phytochemical screening of the aqueous extract of Mussaenda ferruginea. Indian Journal of Nano Science. 2015 Jul; 3(2):1–4.

17. Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences. 2016; 9(1):1–7.

18. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au and bimetallic Au core - Ag shell nanoparticles using neem (Azadirachta indica) leaf broth. J Colloid Interface Sci. 2004; 275(2):496–502.

19. Mude N, Ingle A, Gade A, Rai M. Synthesis of silver nanoparticles using callus extract of Carica papaya. A first report. J Plant Biochem and Biotechnol. 2009; 18(1):83–6.

20. Nabikhan A, Kandasamy K, Raj A, Alikunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from salt marsh plant Sesuvium portulacastrum L. Colloids Surf B: Biointerf. 2010; 79(2):488–93.

21. Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T. Biomedical potential of silver nanoparticles synthesized from calli of Citrullus colocynthis (L). Schrad. J Nanobiotechnol. 2011 Sep 26; 9(4):1–8. DOI:10.1186/1477-3155-9-43. Available from:http://www.jnanobiotechnology.com/content/9/4/43.

22. Iyer RI, Yashwanthi RP, SowmyaH, Selvaraju C, Santhiya ST. Bio-production of medicinal compounds and silver nanoparticles with cultured tissues of Nyctanthes arbor-tristis. JMAPS. 2013; 35(1-2):11–8.

23. Iyer RI, Yashwanthi RP, Sowmya H, Selvaraju C, Santhiya ST. Green synthesis of silver nanoparticles of bio-active phytochemicals with anti-bacterial activity from callus cultures of bitter gourd Momordica charantia L. JMAPS. 2013; 35(3-4):147–53.

24. RameshCK, Rehman A, Prabhakar BT, Avin BR, Rao SJ. Antioxidant potentials in Prabakar BT, Avin BR, Rao SJ. Antioxidant potentials in sprouts vs seeds of Vigna radiata and Macrotyloma uniflorum. Journal of Applied Pharmaceutical Science. 2011; 1(7):99–103.

25. Tang D, Dong Y, Ren H, Li L, He C. A review of the phytochemistry, metabolite changes and medicinal uses of the common food mung bean and its sprouts (Vigna radiata). Chemistry Central Journal. 2014 Jan 17; 18(1):4. Available from:http://journalchemistrycentral.com/content/8/1/4.

26. Wongekalak LO, Sakulsom P, Jirasiripongpuk K, Hongsprabhas P. Potential use of antioxidative mung bean protein hydrolysate as an anticancer asiatic acid carrier. Food Research International. 2011; 44(3):812–7.

27. Pattu G, Male A, Haripriya T, Malleswari VN, Reesha SK. Phytopharmacological review on Vigna species. Pharmacist-An Int J Adv Pharm Sci. 2011; 2(1):62–7.

28. Murashige T, Skoog F. A revised medium for rapid biosay and growth with tobacco tissue cultures. Physiol Plant. 1962; 15(3):473–97.

29. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. In: Glick D, editor. Methods of Biochemical Analysis. New York: Interscience Publishers; 1954; 1:27–52.

30. Maurya S, Singh D. Quantitative analysis of total phenolics content in Adhatoda vasica Nees extracts. Int J Pharm Tech Res. 2010; 2(4):2403–6.

31. Salawu SO, Ogundare AO, Ola-Salawu BB, Akindahunsi AA. Anti-microbial activities of phenolic containing extracts of some tropical vegetables. African J of Pharmacy and Pharmacology. 2011; 5(4):486–92.

32. Raut RW, Kolekar NS, Lakkakula JR, Mendiulkar VD, Kashid SB. Extra cellular synthesis of silver nanoparticles using dried leaves of Pongamia pinnata (L) pierre. Nano-Micro Lett. 2010; 2(2):106–13.

33. Mittal AK, Bhauunik K, Kumar S, Banerjee UC. Biosynthesis of silver nanoparticles. Elucidation of prospective mechanism and therapeutic potential. J Colloid Interface Sci. 2014 Feb 1; 415:93–97.

34. Khan M, Khan M, Adil SF, Tahir MN, Tremel W, Siddiqui MRH. Green synthesis of silver nanoparticles mediated by Bacillus subtilis. J Nanomaterials. 2014 Apr 17; 8(1):1507–16. Available from: http://dx.doi.org/10.1155/2014/743309.

35. Raju D, Hazra S, Mehta U. Phytosynthesis of silver nanoparticles by Sesamum indicum L. leaf extract. Mater Lett. 2013 Jul; 102–3:5–7.

36. Mahanty A, Mishra S, Bose R, Maurya UK, Netam SP, Sarkar B. Phytosynthesis of silver nanoparticles inhibit bacterial fish pathogen Aeromonas hydrophila. Indian J Microbiol. 2013 Dec; 53(4):438–46.

37. Kaviya S, Santhanalakshmi J, Viswanathan B. Green synthesis of silver nanoparticles using Coriandrum sativum leaf extract and their application in nonlinear optics. Adv. Science Lett. 2010; 3(1-6):138–43.