

***In vitro* generation of pharmaceutically important medicinal plants using Silver Nanoparticles: A concise Review**

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ABSTRACT

Plant tissue culture plays an extremely important role in contemporary plant biotechnology due to its potential for mass production of enhanced crop varieties and high yield of significant secondary metabolites. Utilizing biotic and abiotic elements, several attempts have been made to increase the efficiency and output of plant tissue culture. Due to its efficacy in microbial cleaning and the increase of secondary metabolites, the use of nanoparticles as elicitors has recently attracted interest on a global scale. Nanoparticles are objects with a nanometric dimension; they have distinct physico-chemical characteristics. Among all nanoparticles, silver nanoparticles (AgNPs) are well-known for their antibacterial and hormetic properties, which, in the right doses, improved plant biomass and promoted the accumulation of secondary metabolites. The assessment of the application of nanotechnology to plant tissue culture is the main objective of this review. The emphasis is placed mostly on the augmentation of secondary metabolites, their impacts on plant development and biomass accumulation, as well as their potential mechanisms of action.

Key words: Plants, Secondary metabolites, Silver Nanoparticles, Tissue culture

Introduction

In the 21st century, plant biotechnology has fulfilled various expectations of modern science to improve the quality of plants through tissue culture technique. In this technique plant cells, tissues or organs are grown in the artificial nutrition media under a controlled environment and aseptic condition for the generation of *in vitro* plants. This technique is very much useful to improve agriculturally produced crop varieties for enhancement of their crop quality, yield, resistant ability against environmental stress and pathogenic attack to develop disease free *in vitro* plants (Ali *et al.*, 2016; Singh, 2018;

Bidabadi and Jain, 2020). The enormous success behind the plant tissue culture is only because of 'Totipotency' i.e., ability of a plant cell to regenerate whole plant through cell division and cell differentiation, unlike animal cell (Ali *et al.*, 2016; Bidabadi and Jain, 2020). The presence of secondary metabolites like alkaloids, phenolics, steroids, volatile oils etc in plants have made them meridionally so important (Fouad *et al.*, 2021; Khan *et al.*, 2021). The quantity of secondary metabolites in plants has been enhanced through plant tissue culture and those *in vitro* derive plants are useful for pharmacological as well as industrial purposes (Rahmawati *et al.*, 2022). The quantity of secondary metabolites in plants can

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be increased by many factors like genotype of mother plant, type of explants, physiological status, intensity and period of the incident light, plant growth regulators and temperature (Abdi *et al.*, 2008; Bhoite and Palshikar, 2014). Normally, composition of tissue culture media plays the key role which strongly influence morphogenesis of explants. Different components present in any plant tissue culture medium have their own roles e.g., macro and micro salts as nutrients, sucrose as carbon source, agar as solidifying agent. Vitamins, plant hormones, iron salts, organic supplements also have their unique roles. Sometimes few selected elicitors i.e., molecular agents are added to culture media to induce a response in the organisms, such as enhancing phytochemical production in plants (Angelova *et al.*, 2006; Patel and Krishnamurthy, 2013). These elicitor molecules are of biological or non-biological origin and can recognize cytoplasmic membrane receptors on the plant cells. Recognition and binding led to signal elicitation, which stimulates the expression of genes related to secondary metabolite production. With respect to this, metallic nanoparticles conjugates show high potential as *in vitro* culture elicitors for their physico-chemical properties and fall within the field of nanotechnology (Patel and Krishnamurthy, 2013; Kokina *et al.*, 2017; Dabuwar *et al.*, 2019).

The term 'nano' is derived from a Greek word meaning 'the dwarf' and Nanotechnology is the science and technology of small things (<100 nm) with few changes in their physico-chemical structure with higher reactivity and solubility which leads to enhance the reaction rate to switching faster speed, increased surface area, higher productivity, variations in different cellular interactions, control of several molecular events etc. (Chakravarthi and Balaji 2010; Troncarelli *et al.*, 2013; Enamala *et al.*, 2019; Prasad, 2019; Luo, 2020; Paul, 2022). Nanomaterials exhibit unique properties such as low molecular weight, large surface area against volume, ability to engineer electron exchanges, special electronic and optical attributes, and surface reactive capability. It has also reported that metallic nanoparticles such as silver, gold and iron nanoparticles and metal oxide nanoparticles (nano-ZnO₂, TiO₂, and CuO₂) have positive impact on plant tissue culture by supporting morphological potential and propagation, as well as improving plant resistance to stress (Kim *et al.*, 2017; Bayda *et al.*, 2019; Salachna *et al.*, 2019).

Though nanobiotechnology is a new emerging field of study so more study should be required to understand the magical power of nanoparticles when they are incorporated in biological system which will be enlightening us to a marvellous area of nanobionics (Paul, 2022). As the secondary metabolites controlled *in vitro* generated plants will be more beneficial for the pharmaceutical industries to develop various lifesaving drugs, it has been focused on how nanotechnology is applied in plant tissue culture system for the enhancement of quantity as well as quality of various secondary metabolites of *in vitro* generated plants in this present article.

Synthesis of Silver nanoparticles

In general, the nanoparticles (NPs) have been synthesized by three different synthesis processes, such as physical, chemical and biological. Silver NPs were synthesized by using traditional physical techniques including pyrolysis and spark discharge (Pluym *et al.*, 1993; Tien *et al.*, 2008). Through evaporation-condensation in a furnace tube operating at atmospheric pressure, silver NPs are synthesized by utilizing physical processes (Gurav *et al.*, 1994; Magnusson *et al.*, 1999; Kruis *et al.*, 2000; Zhang *et al.*, 2016). Though physical processes are quick, employ radiation as reducing agents, and free from any hazardous chemicals, there are some limitations as poor yield and high energy consumption, solvent contamination and uneven distribution (Tsuji *et al.*, 2005; Shameli *et al.*, 2010; Elsupikhe *et al.*, 2015).

Silver NPs are chemically synthesized by using water or organic solvents. The three primary components of this procedure are typically metal precursors, reducing agents and stabilizing or capping agents. Essentially, there are two stages involved in the reduction of silver salts, i.e. initial nucleation followed by subsequent growth (Wiley *et al.*, 2005; Tao *et al.*, 2006).

The inadequacies of chemical techniques have given a way to viable alternatives in the form of biological techniques. Recently, it has been demonstrated that using various biological systems, such as bacteria, fungi, plant extracts and small biomolecules like vitamins and amino acids, biologically-mediated nanoparticle synthesis methods are straightforward, reliable, economical and environmentally friendly. Before this biological approach became widely used, the bio-sorption of metals by

Gram-positive and Gram-negative bacteria gave a clue for the synthesis of nanoparticles (Gurunathan *et al.*, 2013, 2015).

When microorganisms take target ions from their surroundings and convert the metal ions into the element metal using enzymes produced by cell activities, nanoparticles are said to be biosynthesized. Depending on the position of the nanoparticles developed, it can be divided into intracellular and extracellular production (Simkiss and Wilbur, 1989; Mann, 2001).

In this green chemistry method, various bacteria were used, including *Pseudomonas stutzeri* AG259, *Lactobacillus* strains, *Bacillus licheniformis*, *Escherichia coli*, *Brevibacterium casei*. The nanoparticles were extracted from fungi including *Fusarium oxysporum*, *Ganoderma neo-japonicum* etc. or even from various plant extracts (Zhang *et al.*, 2016). The major advantage of biological methods is the availability of amino acids, proteins or secondary metabolites present in the synthesis process and the use of biological molecules for the synthesis of silver nanoparticles is eco-friendly and pollution-free (Zhang *et al.*, 2016).

Silver nanoparticle as elicitor in plant tissue culture

Silver nanoparticles are increasingly used in various fields, including medical, food, health care, consumer and industrial purposes, due to their unique optical, electrical and thermal, high electrical conductivity and biological properties (Gurunathan *et al.*, 2015). The biological activity of silver nanoparticles depends on factors including surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration and dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type and the type of reducing agents used for the synthesis of silver nanoparticles are crucial factors for the determination of cytotoxicity (Rahmawati *et al.*, 2022).

In agrotechnology, plant tissue culture plays a crucial role for crop improvement. One of the best methods for crop enhancement is plant micropropagation through vegetative multiplication. This reduces the possibility of pathogen accumulation and the slow pace of generative multiplication, both of which result in a loss in crop quantity and quality. From numerous reports it was confirmed that the addition of silver nanoparticles in culture media exhibited positive response for callus

induction, shoot formation, and growth (Espinosa-Leal *et al.*, 2018; Bidabadi *et al.*, 2020; Chandran *et al.*, 2020).

The physio-chemical properties of nanoparticles enhance the bioavailability of therapeutic agents after both systematic and local administration and on the other hand it can affect cellular uptake, biological distribution, penetration into biological barriers and resultant therapeutic effects. Therefore, the development of silver nanoparticles with controlled structures that are uniform in size, morphology and functionality are essential for various biomedical applications (Ali *et al.*, 2019; Rahmawati *et al.*, 2022).

The callus biomass increment is positively correlated with the water content of explant and that was the vital parameter for metabolic and physiological status. Application of low concentration of silver nanoparticle in *Caralluma tuberculata* culture medium exhibited sudden increase of callus biomass. However, administration of silver nanoparticles above the estimated optimum level (60 µg/l) caused a significant reduction in callus biomass. This study also revealed that the accumulation of important nutrients in the medium, such as N, Mg and Fe was also increased (Ali *et al.*, 2019). Consequently, larger amounts of nutrients involved in the formation of chlorophyll may result in enhanced photosynthetic activity; on the other hand, higher levels of silver nanoparticle may result in phytotoxicity. These findings were consistent with earlier research on *Cucurbita pepo*, *Raphanus sativus* and *Stevia rebaudiana* B in Murashige and Skoog tissue culture medium (Stampoulis *et al.*, 2009; Zuverza-Mena *et al.*, 2016).

Silver nanoparticle in culture medium improved dramatically the number of shoots as well as the plant height and biomass and that nanoparticle also enhanced the accumulation of plant secondary metabolites (Chandran *et al.*, 2020; Rahmawati *et al.*, 2022). An unorganised or undifferentiated cell mass is known as callus, which is formed from plant organs cultivated *in vitro*, can be used as a potential plant biotechnology platform to produce considerable bioactive chemicals of pharmaceutical interest. Moreover, it has been suggested that callus cultivation combined with nano-elicitation is a promising method for increasing phenolic, flavonoid and alkaloid chemicals (Ali *et al.*, 2019; Fouad *et al.*, 2021). For example, it has been observed that silver nanoparticles in cell suspension culture increase the content of several flavonoids, including hydroxybenzoic and hydroxycinnamic acids in the

bitter gourd. Moreover, total phenolic and flavonoid content of *Caralluma tuberculata* and *Prunella vulgaris* callus cultures showed increased after being stimulated by silver nanoparticle and silver-gold nanoparticles, respectively (Ali *et al.*, 2019; La and Risuleo, 2021).

Enhancement of secondary metabolites of Plants through Silver nanoparticles

Secondary metabolites in plants are helpful for better survive against environmental stress. Furthermore, plant secondary metabolites are also well known as an important source of pharmacological or industrial means. It has been reported that *in vitro* plant cell and organ culture has beneficial effects for enhancement of plant secondary metabolite production. The application of metallic elicitors, such as silver nanoparticles, also has a positive response on secondary metabolite enhancement in several studies. Total phenolic compounds including flavonoid content, phenylalanine ammonia-lyase and DPPH free radical activity have been increased in *in-vitro* culture of *Caralluma tuberculata* callus in MS medium supplemented with silver nanoparticle alone as compares to MS culture medium supplemented with phytohormones and silver nanoparticles both.

In sugarcane, the total amount of phenolic compounds has been increased in *in-vitro* condition with media supplemented with silver nanoparticle in medium concentration. But the application of silver nanoparticle in higher concentration in culture medium occurs negative effects where the production of phenolic compound is decrease in higher concentration of silver nanoparticle due to antioxidant production and reactive oxygen species (ROS) continued to rise. Like the use of optimum concentration of silver nanoparticle, the proper size of the silver nanoparticle is equally effective for the enhancement of camalexin compound in *Arabidopsis thaliana*.

Silver nanoparticles actually increase sugar metabolism, TCA cycle, shikimate phenyl propanoid metabolism etc. in *A. thaliana* and those leads to give resistance to this plant against abiotic stress. Thus, the immunity power or resistant power of *A. thaliana* becomes enhanced against phytopathogen through the *in vitro* induction of higher generation of phytoalexins and other kind of secondary metabolites.

Total phenolic and flavonoid content in *Curcumis anguria* will be enhanced through the hairy root culture derived via *Agrobacterium*-mediated transformation by the direct uptake of the silver moiety in

the root surface.

Silver nanoparticle in cell suspension culture technique gives positive impacts for the *in vitro* enhancement of capsaicin in *Capsicum* sp. after few days of inoculation of culture. Same result has been achieved to enhance secondary metabolite in bitter gourd.

Mechanism of action of silver nanoparticles in plant cell

In the initial phase of exposure, silver nanoparticles were said to stick to the primary root's surface. In plants, there are two ways that particles are transported: either by vascular tissue or intercellular transport. Nanoparticles first enter the root's epidermal layer, then go through a sequence of processes that lead to the entry of plant vascular tissue. The transfer of silver nanoparticles in whole plants or explants is made possible by this process (Yan and Chen, 2019). After being ingested into plant cells, silver nanoparticles are said to stay in nanoparticle form and cause less severe effects than free Ag⁺ produced by silver nitrate (Stefanic *et al.*, 2018). Silver nanoparticles could enter cells through plasmodesmata, after direct contact with the cell membrane (Fig.1). In this adverse condition, plants produce ROS that causes damage to the biological membrane system and to macromolecules (Marslin *et al.*, 2017, Yan and Chen, 2019). As reported in *A. thaliana*, the initial recognition of silver nanoparticles by membrane-bound receptors triggers Ca²⁺ burst and ROS induction. It may be speculated that these particles may enhance the plant uptake inflicting damage to the cell wall.

Thus, cell wall malfunction may lead to higher uptake of nutrients and water; incidentally, the higher penetration of drugs consequent to cell membrane permeabilization has been observed in plant cells. The significant increase in some macronutrient contents and the accumulation of larger amounts of N, Mg and Fe in the shoots of sugarcane exposed to silver nanoparticles may contribute to the increased shoot number and length (Bello-Bello *et al.*, 2017; Castro-Gonzalez *et al.*, 2019).

Proteins, nucleic acids, hormones and chlorophyll are all made up of nitrogen. It is probable that this mechanism aids in enhancing growth in plants exposed to silver nanoparticles because photosynthesis causes the creation of biomass in plants.

The application of nanoparticles has an impact on the hormonal pool, as demonstrated by Vankova *et*

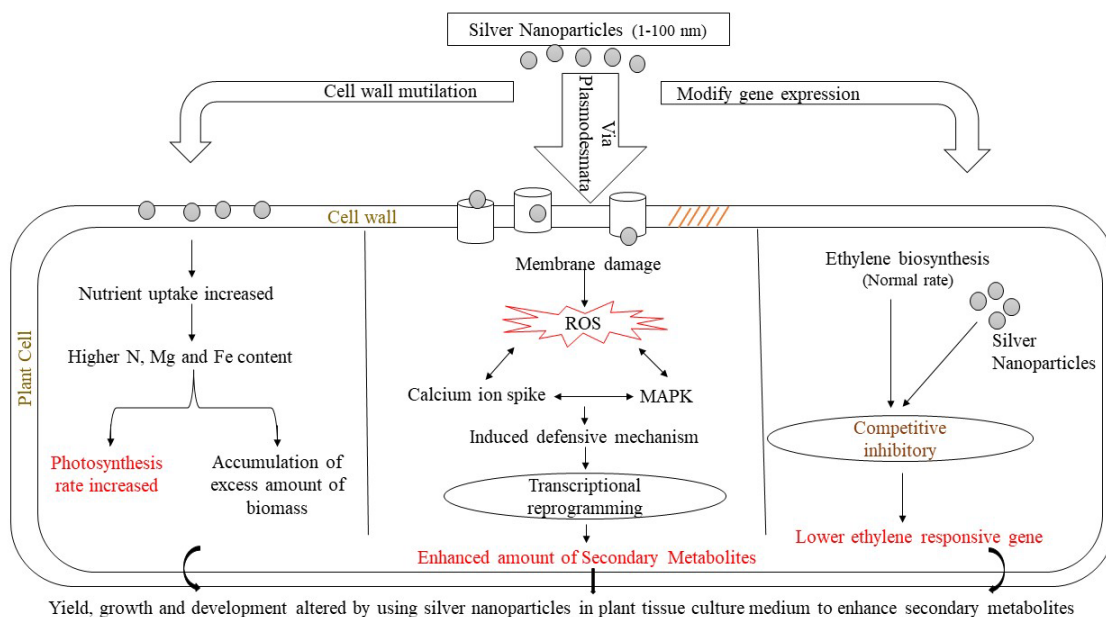


Fig. 1. Three possible mechanisms of silver nanoparticles to enhance the quantity of secondary metabolites of *in vitro* plants.

al. (2017). Different pools of PGRs may produce diverse plant growth and physiological responses in various plant species, according to some research. The shoot apical meristem's auxin and cytokinin production was inhibited by a high nanoparticle concentration (SAM).

Jasmonic acid was similarly reduced when being exposed to nanoparticles in a manner similar to that. In the meantime, exposure to nanoparticles caused a build-up of salicylic acid, cis-zeatin and abscisic acid in plant roots. This suggests that nanoparticles are a source of plant stressors. Nanoparticles reduced the expression of genes that are responsive to ethylene and ABA, which in turn reduced the rate of callus regeneration (Manickavasagam *et al.*, 2019). In closed container culture, self-produced gases, including ethylene, which may control senescence, will have a significant impact on plant development.

silver nanoparticles were introduced into olive cells and this resulted in improved growth vigour, decreased necrosis and good effects on cell growth (Hassan *et al.*, 2019). Plant cultures may experience rapid growth because of silver ions' inhibition of ethylene synthesis. By substituting the copper cofactor; silver ions from silver nanoparticles operate as ethylene perception inhibitors, which causes the downregulation of genes involved in ethylene synthesis (Zhang *et al.*, 2018; Manickavasagam *et al.*, 2019).

According to reports, silver ions and nanoparticles both induced changes in the secondary metabolite profile, which suggested that silver nanoparticles have an impact on plant metabolism through the release of ions into the medium. High concentrations of AgNPs have reportedly accumulated in plant cells and tissues of the stevia plant (*Stevia rebaudiana*) (Kruszka *et al.*, 2020). Through the circulatory system, these compounds were taken up by the plant and transported to nearby cells by apoplasts. Because nanoparticles must cross a membrane as part of the transfer route via apoplasts, nanoparticles uptake must be size-specific. It is also feasible to transport nanomaterials via plasmodesmata, which have a diameter of about 40 nm and can accommodate silver nanoparticles as small as 40 nm.

Conclusion

The application of silver nanoparticles to plant tissue culture could benefit plant development and the production of secondary metabolites. The reviewed was conducted using silver nanoparticles of various sizes and concentrations. It had a size range of 1-40 nm and a concentration range of 30 g/l to 200 mg/l. The effects of this treatment on plant growth include changes in callus development, shoot multiplication and elongation and root formation. Silver

nanoparticles also cause the build-up of plant secondary metabolites. These reactions are the consequence of intricate physiological processes that modify the functions of antioxidant enzymes, the expression of genes, the communication and control of plant hormones and the generation of ROS.

However, depending on the plant species and explant type, various reactions and hormetic effects were seen. Hence, each plant species' silver nanoparticle characteristics must be optimised in order to increase secondary metabolite enhancement. Silver nanoparticles, however, exhibit a strong potential for use in plants for those goals of improvement. Future secondary metabolite manufacturing techniques include large-scale methods like nano-integrated suspension culture.

The research on silver nanoparticles' effects on growth enhancement might also reveal the fundamentals of using this nanomaterial for plant propagation, which might aid attempts to both conserve and improve crops. To comprehend the mechanism underlying the impacts of silver nanoparticles in plant culture, additional examination and evaluation are necessary.

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