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(REVIEW ARTICLE)

Role of nanoparticles in plant tissue culture

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Abstract

There are many applications of plant tissue culture which is used in the production of somatic embryos, plant regeneration via organogenesis, germplasm conservation, synthetic seeds, protoplast culture for the production of somatic hybrids, anther culture for the production of haploids, synthesis of secondary metabolites of pharmaceutical interest, and plant cells used for the bio-energy. Nanomaterials have emerged as powerful tools in advancing plant tissue culture techniques, offering enhanced capabilities in enhancing growth, inducing secondary metabolite production, and facilitating genetic modifications. Nanoparticles improved micropropagation efficiency by reducing contaminations, improving callus induction and increasing yields of secondary metabolites through cell suspension cultures. Despite their promising benefits, the potential toxicity of nanoparticles (NPs) poses significant challenges and requires careful consideration. Due to their small size, detecting and studying their toxicity poses a challenge, and their health effects are not yet fully understood. However, the toxicity of nanoparticles (NPs) on *in vitro* cultures has not been extensively studied by researchers.

Keywords: Callus; Nanomaterials; Nanotechnology; Plant tissue culture; Silver nanoparticles; Toxicity

1. Introduction

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs on a synthetic nutrient medium under sterile conditions inside a vessel [1-25-229]. The resulting clones are true to type of selected genotypes and used for the large scale plant multiplication [1-35-228]. Plant *in vitro* propagation using tissue culture techniques have been exploited for the commercialization of ornamental plants (orchids), vegetable and fruit plants (papaya, mango, and grape), medicinal, woody plants, teak (*Tectona grandis* and sandalwood (*Santalum album*) and conifers with economically important products [1-40]. The main application of plant tissue culture is mass propagation and currently a major tool in crop improvement, disease elimination, secondary metabolites synthesis and plant conservation [1-45]. Plant tissue culture involves various stages, such as explant sterilization, micropropagation, and direct and indirect organogenesis through the calli, cells, protoplasts, and haploid culture, somatic hybridization and somatic embryogenesis [1-75]. There are many applications of plant tissue culture which is used in the production of somatic embryos, plant regeneration via organogenesis, germplasm conservation, synthetic seeds, protoplast culture for the production of somatic hybrids, anther culture for the production of haploids, synthesis of secondary metabolites of pharmaceutical interest, and plant cells used for the bio-energy [1-120]. Since plant tissue culture is simple, low-cost and environment friendly, it is imperative to employ this technique for the development of sustainable agriculture in order to meet the food demand of increasing human population [1-120]. However, the plant tissue culture technique also has limitations of somaclonal variation resulting in the chimeric plants with low yields[1-120-229]. Reprogramming of plant somatic cells under *in vitro* conditions towards somatic embryogenesis is also a challenging process since many factors and signaling molecules govern and controlled the process [1-120].

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The integration of nanotechnology into plant tissue culture represents a significant advancement in agricultural biotechnology [121-216-229]. Nanoparticles improved micropropagation efficiency by reducing contaminations, improving callus induction and increasing yields of secondary metabolites through cell suspension cultures [121-214-229]. Additionally, the positive effects of nanoparticles on organogenesis, somatic embryogenesis, protoplast cultures and somaclonal variations are reviewed [121-216]. Various types of nanoparticles, including silver, gold, zinc, cobalt, silica, and carbon-based nanoparticles, are analyzed for their specific applications and mechanisms of action [121-216-229]. Nanotechnology researches and studies have advanced rapidly worldwide and the applications of nanoparticles (NPs) in various fields including agriculture, biomedical applications, cell labeling, drug delivery, biomarkers, automobile industry and energy sector have become significant subjects of study in recent years [121-216-229].

2. Application of Nanotechnology in Plant Tissue Culture

Nanotechnology is applied in every aspect of life—medicine, food, or the environment—and nanomaterials are available in different forms, such as powder, particles, clusters, tubes or films, and sizes [121-190]. One of the latest ideas is the use of nanomaterials, which are substances of very small size (1–100 nm) and unique properties are used in plant tissue culture [121-140]. Recently, nanoparticles have been suggested as biostimulators that might improve plant propagation or enhance fruit ripening [121-214]. Nanoparticles have also been used in plant tissue culture to improve plant growth, increase yield, improve seed germination, enhance bioactive compounds production, and enable genetic transformations [121-216]. AgNPs have been proven to reduce microbial contaminations in various plants and may be used for surface disinfection of explants[121-216]. The antimicrobial property of silver NPs seems well studied. Its effectiveness depends on size, dimension, and type of nanoparticles [121-213]. Recently, there has been a different, green approach proposed. There are three possible means of nanoparticles synthesis: physical, chemical, and biological. The last one, concerning the biogenic synthesis of NPs, is described as nontoxic [121-216-228]. The "green biotechnology" methods include obtaining nanoparticles from plants' organs, algae, or even microbes [121-216]. It was possible to obtain AgNPs from the *Clitoria ternates* [123, 124], *Costus speciosus* [125], *Cathranthus roseus* [122], *Daucus carota* extract or wheat straw extracted lignin [121-216]. The "green" methods are promising, but there is still little information on the subject [121-216].

There are different nano-substances, metals, and metal oxides, including silver (Ag), gold (Au), zinc (ZnO), copper (Cu), titanium dioside (TiO_2), titanium dioxide[225], magnesium oxide (MgO), nickel (Ni), iron oxide (Fe_3O_4), Selenium[227], and silicon (Si) [121-214-228]. The best-known activity of nanoparticles (NPs) is its ability to eliminate different microorganisms [121-214]. Silver nanoparticles [224] are the most often used nanomaterials in different sectors, especially in agriculture[224]. In the plant production sector, silver nanoparticles (AgNPs) were developed as plant growth stimulators, or components of new fertilizers, as well as plant protection products and herbicides [121-214-228]. According to Sarmast et al., (2014) [170], AgNPs showed a high ability to eliminate microorganisms in *Tecomella undulata* without negative effects on plant growth and development, but many researchers have studied the influence of AgNPs on explant disinfection and plant regeneration, and they indicated both positive and negative effects in the case of plants [121-170]. Another study suggested that substances used for sterilization are not neutral for the environment, humans or animals [121-214-228]. One of the study indicated that the influence of NPs on explants excised from different plant species should be studied to determine a proper method for AgNPs' application in plant material disinfection and cultivation, especially given that plants are a basic element of the world and a source of food [121-214]. Knowledge of AgNPs' influence on plants is therefore important, especially because the common use of AgNPs increases the chance that they would be released into the environment [128–213-230]. Silver is the second most toxic metal to aquatic organisms after mercury, and, therefore, further studies are needed to clarify these contradictory observations concerning growth stimulation or inhibition of AgNP-treated plants [121-214-228]. Moving forward, integrating nanotechnology into plant tissue culture holds immense potential for addressing global agricultural challenges and improving crop productivity, provided that comprehensive risk assessments and regulatory frameworks are developed to mitigate potential adverse effects on plants and ecosystems[121-216-228-230].

3. Examples of use of Nanoparticles in Plant Tissue Culture

In one of the study, Parzymies (2021) [136] reported that *Aldrovanda vesiculosa* is a carnivorous water plant which is endangered by extinction worldwide [136]. One of the main problems is disinfection of the explants [136]. Therefore, Parzymies (2021) [136] treated the explants with nano silver particles [136]. The explants were shoot fragments which were disinfected with sodium hypochlorite and then placed in a liquid 1/5 MS medium, supplemented with silver nanoparticles (AgNPs) at a concentration of 5 $mg \cdot dm^{-3}$ [136]. It was observed that AgNPs reduced the number of contaminations but also led to necrosis of the shoots [136]. The shoots, which undertook regeneration in presence of AgNPs, were smaller and did not form traps[136]. However, after being moved to fresh media twice, they started to

develop normal leaves [136]. Taking into consideration both disinfection and regeneration rates, it might be advisable to disinfect aldrovanda shoots in sodium hypochlorite only, without AgNPs[136]. The results of the research might indicate a toxic activity of AgNPs towards water plants, which seems a big problem, as nanoparticles are commonly used in all the fields of life[136]. Nanomaterials have great potential to be used for plant tissue culture to disinfect the plant material, but their effect has to be tested on each plant species [136]. Undoubtedly, AgNPs reduce microbial contaminations during tissue culture initiation, making it possible to establish a healthy culture but, on the other hand, they may have a negative effect on further regeneration of explants[136]. In the case of *Aldrovanda vesiculosa*, it might be concluded that the inhibition of the regeneration capacity of explants outweighs the disinfection ability of the AgNPs used as a media supplementation[136]. Therefore, it is advisable to surface-sterilize explants with only sodium hypochlorite [136].

To obtain in vitro cultures free of contaminations, the plant material has to be surface disinfected [121-136]. It is extremely difficult to find a balance between the use of disinfection sub- stances strong enough to get rid of any microorganisms, and mild enough to not kill the plants [121-136-214]. The disinfection part is also highly important because remaining microorganisms usually grow fast in tissue culture121-136-214]. There are many sterilizing substances used for the surface disinfection of plant material. The most often used are calcium hypochlorite (CaOCl), sodium hypochlorite (NaOCl), ethanol (EtOH), hydrogen peroxide (H_2O_2), mercuric chloride ($HgCl_2$), silver nitrate ($AgNO_3$), fungicides, and antibiotics [121-136-214]. Some disinfecting substances are harmful to plant tissues and, therefore, they negatively affect organogenesis, or even lead to necrosis due to their phytotoxicity[121-136-214]. As such, the type, concentration and exposition time of disinfectants are important. Sometimes, it seems almost impossible to obtain pathogen-free cultures. Bacteria seem especially difficult to eliminate [121-136-214]. Therefore, researchers and plant producers look for new methods and substances.

Several researchers have reported the successful use of metal and metal oxide NPs as antibacterial and antifungal agents including gold (Au), silver (Ag), nickel (Ni), silicon (Si), Copper (Cu), copper oxide (CuO), magnesium oxide (MgO), zinc oxide (ZnO), iron oxide (Fe_3O_4), aluminum oxide (Al_2O_3) and titanium dioxide (TiO_2) [121-136-214]. Numerous studies have been conducted to explore the effectiveness of silver (Ag) nanoparticles in surface sterilization in plant tissue culture [121-136-214]. Their strong anti- microbial properties, coupled with minimal adverse effects on plant growth, position them as an effective alternative to conventional sterilization methods. Different concentrations of silver NPs and exposure times have been studied for various kind of plant explants in previous research [121-136-214-228].

Silver NPs obtained through green synthesis from *Alkanna tinctoria* rhizomes and *Pinus nigra* leaves were used to surface sterilize plant seeds with various shapes and sizes[121-136-214]. The results revealed that silver NPs were effective surface sterilant, especially for plant seeds with rough coats, outperforming NaOCl [121-136-214]. Pinus-NP exhibited greater efficacy (85.11 %) in surface sterilization compared to Alkanna-NP (74.77 %)[121-136-214]. The sterilization percentage gradually increased as the sterilization duration was prolonged [121-136-214]. Furthermore, there were no adverse effects of silver NPs on seed germination when compared to the control group[121-136-214]. Another study reported that the higher concentrations of 100 mg/L with 60 min exposure time of biosynthesized nanosilver on Ocimum seeds had no harmful effects[121-136-214]. Additionally, a 20 min exposure time was efficient for sterilization at all the tested concentrations (100, 50, 10 mg/L) of biosynthesized nanosilver ([121-136-214]. Silver NPs play a crucial role in sterilizing phenolic-rich plant explants by preventing browning [121-136-214]. In another study, no browning was observed during surface sterilization and the culture period when green synthesized silver NPs were used for *Rosmarinus officinalis* stem explants[121-136-214].

Chemically synthesized NPs were also used in surface sterilization, and they yielded similar results to biologically synthesized NPs [121-136-214]. A study reported that plant tissues were generally highly tolerant to silver NPs, but concentrations above 100 ppm caused toxicity and burn the grape leaf explants[121-136-214]. According to the results, silver NPs effectively controlled bacterial infections, especially internal bacterial infections better than the fungi infections [121-136-214]. However, several studies showed that concentrations above 100 mg/L silver NPs were more efficient at disinfecting plant explants[121-136-214]. A 200 mg/L nano-silver solution effectively controlled bacterial and fungal contamination without negatively impacting the regeneration of Gerbera plantlets[121-136-214].

Low concentration of silver NPs (50 mg/L) or short exposure times (5 min) were ineffective for surface sterilization of ex- plants due to high contamination rates[121-136-214]. Moreover, prolonged exposure (30 min) to silver NPs was also ineffective, as it caused the leaf explants to become necrotic[121-136-214]. However, treating Strawberry leaf explants with 200 mg/L silver NPs for 20 min was the most effective method for disinfection, proving to be more efficient than using 1 g/L $HgCl_2$ [121-136-214]. Silver NPs were effectively used to sterilized Chrysanthemum leaf explants, and treatment with 250 ppm silver NPs for 15–20 min of leaves proved optimal for controlling the contamination [121-136-214]. The use of nano silver on Valerian stem explants with internal contamination proved

ineffective for disinfection without proper surface sterilization and then with 100 mg/L of nano silver, the highest disinfection rates were achieved [161].

Lankarani er al., (2024) [130] investigated the effects of different levels of zinc oxide nanoparticles (ZnONPs) (0, 10, 20, and 30 mg/L) and iron sulfate (13.9, 27.8, and 55.6 mg/L) on morphological and physiological responses of *Stevia rebaudiana* Bertoni plant under *in vitro* conditions [130]. Results indicated that the combined application of ZnONPs at 10 mg and iron at 27.8 mg led to the highest increase in shoot number, height, and biomass, showing a respective rise of 17.37%, 39.66%, and 45.02% compared to control cultures [130]. The highest pigment content and tissue antioxidant activity (83.48%) was observed with the combined presence of 10 mg/L ZnONPs and 27.8 mg/L iron[130]. These findings suggest that ZnONPs could stimulate the growth and enhance the bioactive components of stevia plants, making them a viable option as elicitors in in vitro batch cultures [130]. Despite their promising benefits, the potential toxicity of NPs poses significant challenges and requires careful consideration[130-216].

In another study by Dasauni and Nailwal, (2025) [134] explores the impact of green synthesized sulphur nanoparticles (gSNPs) on *in vitro* regeneration of *C. sativa*[134]. Out of 50 axillary buds inoculated, approximately 40 buds showed shoot induction and proliferation on MS medium supplemented with 2.5 µM TDZ, 5.0 µM GA_3, and 50.0 mg L^{-1} gSNPs, significantly outperforming the control without gSNPs[134]. Rooting experiments with 50.0 mg L^{-1} gSNPs in ½ MS medium with up to 5.0 µM NAA and IBA revealed that 2.5 µM indole- 3-butyric acid (IBA) with 50.0 mg L^{-1} gSNPs yielded highest number of roots (17.7 ± 1.20) and root length (12.0 ± 1.52 cm) [134]. This standardized protocol presents a novel approach to *C. sativa* micropropagation using gSNPs with potential applications in both research and industrial applications of *Cannabis* [134].

One of the study conducted by Oluwasegun et al., (2025) [135] examined the effects of silver nitrate ($AgNO_3$), a known ethylene action blocker on the *in vitro* growth and development of four accessions of *D. rotundata* in a 7*6*4 factorial outline in a completely randomized design [135] Addition of 1.0 mg L^{-1} $AgNO_3$ to the culture medium significantly improved *in vitro* growth and development of the shoots, while 2.0 mg L^{-1} $AgNO_3$ significantly influenced root formation [228]. This study demonstrated the effectiveness of silver nitrate ($AgNO_3$) in enhancing the morphogenesis of *D. rotundata* [135].

The Vinh et al., (2024) [129] reported the effects of iron nanoparticles (FeNPs) on in vitro rooting, chlorophyll content, antioxidant and hydrolysis enzyme activities, acclimatization and subsequent growth of *Gerbera jamesonii* var. Revolution Yellow plantlets under greenhouse conditions [129]. The results showed that 2-cm shoots cultured in medium supplemented with 100 mM FeNPs induced in vitro rooting 2 days earlier than shoots cultured in MS0 medium [129]. In addition, plantlet height (6.83 cm), number of main roots and lateral roots (13.00 roots and 4 roots), root length (5.27 cm), leaf length and width (2.07 cm and 1.83 cm), fresh and dry weights (1188.67 and 155 mg), dry matter ratio (13.04%), and chlorophyll a, b, a + b content (25.25, 18.35 and 43.6 mg/g, respectively) of the shoot culture in 5.6 mg/L FeNPs treatment were also recorded higher than other amounts of FeNPs and control (MS0) treatments after 4 weeks of culture [129]. The plantlets derived from the 5.6 mg/L FeNPs treatment had the highest survival rate (93.00%) in comparison to those in MS0 medium (83.67%) and without FeNPs (73.33%) after 2 weeks under greenhouse conditions, and the early flower bud formation (10.17 weeks), increased flower diameter (7.87 cm), and peduncle length (22.17 cm) after 12 weeks [129].

Another study by Twaij et al., (2025) [214] investigated the optimization of callus induction using plant growth regulators (PGRs) in *Datura innoxia* and *Datura stramonium* and secondary metabolites (SMs) production from calli treated with aluminium oxide (Al_2O_3) and tungsten oxide (WO_3) nanoparticles [214]. Inducted calli were treated with varying concentrations of Al_2O_3 and WO_3 nanoparticles [214]. In terms of alkaloid production, both species showed height production (increment 263% and 283% respectively compared to the controls) with a supplantation of 60 mg/l tungsten oxide (WO_3)[214]. Around 80.8% increment of phenolic compounds was observed in *Datura stramonium* with 60 mg/g tungsten oxide (WO_3) supplementation [214]. Again, 81.81% higher flavonoids production was observed in *D. innoxia* in response to WO_3[214]. These findings suggest that aluminium oxide (Al_2O_3) and tungsten oxide (WO_3) nanoparticles can effectively enhance SMs production in *Datura* spp., offering a novel approach for maximizing the yield of valuable bioactive compounds [214]. This research provides a foundation for the large-scale production of pharmaceutically important compounds from *Datura* through biotechnological interventions using nanoparticle technology [214].

Türkoğlu et al., (2025) [181] reported that nanoparticles have proven effective in enhancing regeneration, morphological development, morpho physiology, and biochemical parameters of plantlets produced in vitro [181]. Biochemical analyses revealed increased antioxidant enzyme activity and malon di-aldehyde content under Ag treatments, suggesting oxidative stress, while hydrogen peroxide levels decreased, indicating enhanced reactive oxygen

species scavenging at higher concentrations (4–8 mg L−1) [181]. These experimental results by Türkoğlu et al., (2025) [181] showed the Ag treatments enhanced the uptake of phosphorus, potassium, and magnesium, while high concentrations resulted in decreased sodium and calcium levels, suggesting potential toxicity and impaired ion transport [181]. This study by Türkoğlu et al., (2025) [181] found that Ag-NPs significantly influenced the growth parameters and other traits of the plantlets [54]. Furthermore, results of this study by Türkoğlu et al., (2025) [181] suggested that the efficiency of quinoa tissue culture can be enhanced by increasing the application of Ag in the form of nanoparticles [181]. The findings demonstrated that both Ag-NO_3 and Ag-NPs influence essential plant growth parameters such as shoot length, callus formation, leaf number, and shoot weight, with Ag-NPs generally showing more pronounced positive effects[181].

In one of the study reported by Tamimi and Othman, (2023) [215] the influence of (80 nm–100 nm) AgNPs on the micropropagation of banana was examined by incorporating AgNPs into shoot multiplication and rooting media at concentrations of 3 mg/L–15 mg/L [215]. The results showed that all concentrations of AgNPs stimulated shoot growth and enhanced root development [215]. The highest response was observed in media supplemented with 12 mg/L AgNPs [215]. This optimal level of AgNPs caused a threefold increase in shoot growth parameter and a similar increase in root numbers/shoot and root length[215]. Treatment with AgNPs at 12 mg/L also increased chlorophyll and proline content of shoots by 25% and 120% over control, respectively[215]. Although the application of AgNPs increased the level of lipid peroxidation in shoots, it however, had a limited influence on membrane stability index[215]. These results suggested that the administration of AgNPs to culture media can be effectively utilised for the enhancement of banana micropropagation with minimal toxic effects [215]. The findings of the present investigation byTamimi and Othman, (2023) [215] suggested that the addition of 12 mg/L of (80 nm–100 nm) AgNPs as a component of culture media could be a low toxicity efficient strategy for the micropropagation of banana [215].

Rodríguez-Rojas et al., (2026) [224] reported that Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) is the worldwide symbol of Christmas, and its demand constantly grows [224]. The main challenge in the poinsettia *in vitro* propagation protocol is maintaining aseptic conditions in the culture, as the latex present in poinsettia stems significantly contributes to carbohydrates and other molecules that promote the development of endogenous phytopathogens in the culture medium after establishment[224]. Therefore, the primary objective of this work is to assess the efficacy in achieving an aseptic in vitro culture by the addition of AgNPs at six concentrations (0, 100, 200, 300, 400, 500, and 600 mg/L) to the MS culture medium[224]. Entirely aseptic nodal segments were obtained with the addition of 300 to 600 mg/L of AgNPs. The best shoot induction and growth promotion was obtained with the addition of 400 mg/L to the medium, without compromising explant viability[224]. It is essential to note that shoot induction and growth promotion were achieved without the addition of 6-benzylaminopurine (BA) or naphthalene acetic acid (NAA), which have previously been reported as the optimal conditions for poinsettia micropropagation [224]. These results highlight the potential of AgNPs to accomplish both tasks in the *in vitro* establishment of poinsettia cultures: eliminating bacterial and fungal contamination and promoting conditions for shoot induction and growth[224].

Nanomaterials have emerged as powerful tools in advancing plant tissue culture techniques, offering enhanced capabilities in enhancing growth, inducing secondary metabolite production, and facilitating genetic modifications [130-216]. Studies have demonstrated varied effects of NPs on plant morphology, gene expression, and cellular processes, underscoring the need for further research to optimize NP application methods and ensure environmental safety[130-216].

4. Conclusion

Silver nanoparticles (AgNPs) have numerous applications in plant biotechnology. The unique biological activities of AgNPs in reducing microbial contamination and promoting *in vitro* plant growth have encouraged their use in the development of novel culture systems for the in vitro cultivation of several plant species. Recently, nanoparticles have been suggested as biostimulators that might improve plant propagation or enhance fruit ripening. Nanoparticles have also been used in plant tissue culture to improve plant growth, increase yield, improve seed germination, enhance bioactive compounds production, and enable genetic transformations. However, the potential toxicity of nanoparticles and their impact on plant health and the environment are critical concerns that are also reviewed. The use of nanoparticles in tissue culture is still a new approach, which needs further study and research for proper understanding and implementation. For instance, it is reported that ionic silver released from AgNPs may induce phytotoxicity to cultured plants such as cell membrane damage and generation of reactive oxygen species (ROS). In addition, some studies suggested that the phytotoxicity of AgNPs is size and concentration dependent and that smaller AgNPs are more toxic to plants. Silver nanoparticles (AgNPs) are widely used in plant tissue culture for their antimicrobial properties to eliminate contamination. However, they can exert significant phytotoxic effects, particularly at high concentrations or

with small particle sizes. The disadvantages of AgNPs on *in vitro* plants include morphological, physiological, and cellular damage.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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