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Review Article: Role of Nanoparticles in Callus Induction and Enhancing Secondary Products in Vitro

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ABSTRACT: Plant tissue culture (PTC) is a fundamental, critical, and instrumental element of plant biology. PTC enables studies of morphogenesis, embryogenesis, clonal propagation, advances in agriculture, and production of secondary metabolites; thus, making it an essential platform. Many uses of nanoparticles (NPs) have shown promise, including microbial disinfection, somatic alterations, callus differentiation, cryopreservation, biotransformation, organogenesis, and enhanced production of bioactive compounds. There are countless uses of NPs across sectors such as biotechnology, electronics, energy, medicine, skincare, pharmaceuticals, and agriculture. This review compiles what is presently known about the use of NPs in plant tissue culture on the premise of favorable results. This review synthesizes current knowledge regarding the use of nanoparticles (NPs) in plant tissue culture by discussing the ways in which they work, the areas in which they are used, and the problems that exist when using them. A systematic search through peer-reviewed journal articles in databases such as Scopus, Web of Science, and PubMed was completed. This review only includes research published between 2010 and 2025, specifically studying the use of nanoparticles in vitro on plant systems.

Keywords: secondary products, nanoparticles, somaclonal variation, callus induction

1. INTRODUCTION

1.1 THE USE of NANOPARTICLES IN THE PROPAGATION OF PLANT TISSUE

The use of nanoparticles (NPs) is an extensive application across many sectors, including biotechnology, electronics, medicine, cosmetics, energy, and agriculture, that demonstrates promising results in areas such as enhanced synthesis of bioactive compounds, callus differentiation, organogenesis, somaclonal variation, biotransformation, cryopreservation, and microbial decontamination [1].

Nanomaterials have special qualities because of their minuscule size, which also increases their surface area, rendering them more engaging. Several size-dependent properties distinguish these nanoparticles from their bigger analogs. In this context various nanoparticles (NPs) such as copper, silver, cobalt, selenium, gold, zinc, iron, palladium, titanium, manganese, aluminum, silicon, nickel, barium, zirconium, are employed to improve the response of explants in plant tissue culture (PTC) to protection, genetic control, bulk cultivation, bioactive chemical production, and plant enhancement. Currently, the use of nanosystems in combination with PTC is essential for genetic modification, conservation, mass propagation, agro enhancement, and to obtain bioactive compounds [2]. Additionally, this improves protoplast isolation and cell wall modification to eliminate microbial contamination, enhance callus induction, organogenesis, metabolic alterations, production of secondary metabolites, somaclonal variation, cryopreservation, and genetic transformation.

This review aims to synthesize the most current knowledge concerning the use of nanoparticles in plant tissue culture, specifically in terms of callus induction, the enhancement of secondary metabolites, and the induction of somaclonal variation. It also presents an evaluation of the various mechanisms that underlie how NPs influence these

functions, particularly focusing on reactive oxygen species (ROS) signalling and gene regulation. The review will also highlight significant gaps in existing literature, including an understanding of how nanoparticles enter plant tissues, their stability once introduced into plant tissue, their potential toxicological effects, and their biosafety in in vitro systems, as well as recommend avenues for future research.

1.2 NPS FOR CALLUS INDUCTION, SHOOT MULTIPLICATION, AND ROOTING

Many nanomaterials have been used to induce calluses, shoot multiplication, and root in tissue culture in vitro (Table 1).For this objective, the growth effects of Ocimum basilicum L. callus were examined. As a result of this application, the stem segment had a higher callus production percentage and callus weight than the leaf as an explant source. Using 324 mg of leaf and 741 mg of stem in (75 mg/l Al2O3+MS) nutritional media, Dağlioğlu et al. [3] reported that the highest callus formation percentage was (100%). In the research of Kavianifar et al. [4], the elicitor treatments used were nano-ZnO, nano-SiO2, and nano-Al2O3 at concentrations of 5, 10, and 20 mg/L. The cultures were kept in growth conditions under a photoperiod regime of 16/8 h at 25 ± 1 °C. After two weeks of incubation, the fresh weight of the callus, mucilage yield, and percentage of mucilage were measured, finding that callus suppression and mucilage production were enhanced due to low concentrations of nanoelicitors. At four distinct doses (10, 25, 50, and 100 mg l-1), ZnO nanoparticles, ZnO bulk particles (BPs), or equivalent concentrations of Zn2+ were introduced to MS media containing the ideal hormonal combination. The findings showed that while the same quantities of other treatments were unable to stimulate callus and shoot regeneration, 10 mg/l ZnO NPs could [5]. Another research investigates the impact of green-synthesized Cu-NPs and varied plant growth regulators (PGR) concentration combinations to achieve the optimum outcomes for both plant regeneration and callus induction and documents new, effective in-vitro effects of Cu-NPs on Sorghum bicolor callus to increase phenolic contents, which are crucial substances in the pharmaceutical industry, particularly for antioxidant medications [6]. The impact of 25 mg/l of ZnO NPs on callus development and somatic embryogenesis was also positive when applied to C. arabica leaf explants in vitro [7]. When callus was cultured from the marine algae Kappaphycus striatus, Ag NPs at 500 mg/l resulted in approximately 80% survival rate, 54.4% callus induction, and somatic embryo formation in Provasoli's modified seawater (PES) medium [8] while callus was cultured in MPI (liquid) or MS (solid) medium separately. Khodakovskaya et al. [9] observed a 64% increase in callus growth on tobacco tissue samples, providing 100 µg/mL of multiwalled carbon nanotubes (MWCNTs) in solution with 1 mg/l (2,4-D). It was hypothesized that the enhanced expression of genes associated with the expansion of cell walls (NtLRX1), water transport (NtPIP1), and cell proliferation (CycB) was responsible for the increased callus growth. This demonstrates a direct cause-and-effect relationship between exposure to nanoparticles and the transcriptional control of significant morphogenic genes. In vitro culture of Prunella vulgaris L., a plant with self-healing properties, clearly demonstrated that naphthalene acetic acid (NAA) + AgNPs had a significant enhancing effect on the growth of calluses compared to the control, whether used alone or in combination with gold nanoparticles (AuNPs). Co-NPs and 2.5 mg L^{-1} IBA increased the rooting rate (up to 86.67%) and decreased the production of callus at the shoot base during the rooting stage [10].

While there is an abundance of studies documenting positive results from NPs, findings are not broadly applicable; consequently, each plant subject will have varying responses contingent upon the specific concentration/type of NP used and the explant source, as well as the plant species tested. In particular, lower concentrations of NPs typically foster growth while higher concentrations can be deleterious or cytotoxic. Currently, there is no evidence within the published literature to suggest a direct comparative analysis of efficacy between types/classes of NPs (i.e., metal vs. carbon-based NPs). Additionally, an understanding of the complex interactions exhibited between NPs and conventional PGRs remains uncertain due to a lack of systematic studies that evaluate the dose response of various combinations of NP and conventional PGR. According to the aforementioned research, adding NPs to the tissue medium alters antioxidant enzyme activity, ROS creation, gene expression, and suppresses production of ethylene, all of which affect callus formation, somatic embryogenesis, shoot development, and rooting [11]. NP activity involves the modulation of specific signaling pathways. For instance, in a controlled fashion, NPs may induce an increase in ROS levels that ultimately results in the induction of signaling cascades, with the concomitant consequences of enhanced cell division and differentiation. NPs also may interact directly with the cell wall or cell membrane to activate MAPK cascades, altering gene expression relating to the cell cycle, stress tolerance, or hormone metabolism [12].

Table 1. - NPs application for callus formation and shoot growth, and rooting

No.	plant	NPs	concentration	Function	reference
1	Ocimum basilicum L	Al2O3	25, 50, 75, as well as 100 mg/L	Callus formation, pigment content, destruction of cells, and enzyme activity	[3]
2	Linum usitatissimum L.	ZnO, SiO2 &Al2O3	5, 10, and 20 mg/L	enhancing callus and the formation of mucilage	[4]
3	Rapeseed	ZnO	10, 25, 50, 100 mg/L	inducing callus and/or shoot regeneration	[5]

4	Sorghum bicolor	Cu-NPs	(5, 10, and 20) mg/L	Callus induction to increase phenolic contents	[6]
5	Coffea Arabica L.	ZnO	25 mg/L	Induction of callus as well as somatic embryogenesis	[7]
6	Kappaphycus striatus	Ag	500 mg/L	Enhanced survival and the stimulation of somatic embryogenesis and callus	[8]
7	Nicotiana tabacum L.	Carbone nanotubes	5 to 500 µg/ml	Increased cell proliferation at very low levels	[9]
8	purple passion fruit (Passiflora edulis Sims)	Co-NPs	0.3 mg/ L	Decreased callus formation at the shoot base and improved rooting rates	[10]

1.3 NPS ON ENHANSING ACTIVE COMPOUNDS

Plants produce secondary metabolites to protect themselves from environmental stresses, which have important physiological and ecological ramifications. Terpenoids, phenolic compounds, alkaloids, and sulfur-containing substances are the four most common forms of secondary metabolites found in plants, and these phytochemicals can have antimicrobial, attractant/repellent, or herbivore-deterrent properties. Undifferentiated plant cells manufacture a wide spectrum of phytochemicals in the laboratory, which can be further induced by elicitors [13] and might be challenging to extract and purify depending on the environmental conditions. Furthermore, Secondary metabolites have played an important role in medicine for ages [14], but Plant cell culture systems have difficulty creating secondary metabolites due to low metabolite output [15], so elicitors are frequently used in cell cultures to stimulate the production of secondary metabolites.

Plant tissue culture is a prominent way of creating secondary metabolites, which are employed in both pharmacological and industrial purposes [16]. Much recent research indicates that nanoparticles can increase the formation of bioactive plant metabolites [17,18 ,19]. In addition, the results of Ahmad et al. [20] provide opportunities to use the beneficial properties of metallic oxide (ZnO and CuO) nanoparticles to improve the production of medicinal plants' bioactive metabolic components in in vitro batch cultures for the nutraceutical sector in Stevia rebaudiana. Moreover, other research on Arabidopsis thaliana found an increase in active compounds using Ag Nps [21].

For instance, Hegazi et al. examined how SiO2 and TiO2 nanoparticles can increase the accumulation of _-α-tocopherol in Argan (Argania spinosa) [22]. Zafar et al found that Ipomea turbinate and Convolvulus arvensis had higher phenol concentrations upon Ag Nps induction.[23]. Syringic acid, gallic acid, quercetin, ellagic acid, lutein, quercetagetin, and kaempferol—all of which are induced by SiO2 in Tagetes erecta L.—were among the most significant secondary metabolites detected in the callus of leaves, according to a different study [24]. Abiotic stressors and biostimulants are two important examples of Elicitors that improve plants' nutrition, production, etc.

Elicitors stimulate plants to produce various secondary metabolites when stressed; other than abiotic stressors (which include biostimulants) act as Elicitors and stimulate the so-called 'fine tuning' of plants' chemical defence systems and thus provide more products for plant breeding. Several approaches using NPs demonstrate how the use of NPs can be a strong elicitor of plants but work differently depending on the NP being tested. There is evidence for many nanoparticles (NPs) inducing oxidative stress in plants, causing imbalances within their redox state [25,26]. Several published studies indicate that the vascular tissue of the plant is involved in the production of reactive oxygen species (ROS). Many of these NPs such as Ag, ZnO and Al_2O_3 induce the production of ROS by different processes.

In addition to ROS, NPs activate plant NADPH oxidase at the level of Plasma Membrane which accumulates hydrogen peroxide (H_2O_2) and superoxide (O^{2-}), and the resulting oxidative burst activates mitogen-activated protein kinase (MAPK) pathways that cause the transcription of several genes related to defence and defence signalling pathways [12]. This ROS signalling mechanism increases the production of key metabolic genes associated with phenolic and terpenoid metabolism including genes encoding phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and other enzymes involved in phenolic and terpenoid metabolism [17].

For example, Arabidopsis thaliana relies on NADPH oxidase (RBOH), and as a result, two root hair-deficient mutants produced significantly fewer ROS due to AgNPs, supporting the functionality and roles of specific enzymatic production of ROS in elicitory roles for NPs [27]. According to Sosan et al. [28], the source of reactive oxygen species (ROS) forming within cells would be the enzymes found in the apoplast directly adjacent to the plasma membrane, which would produce ROS. The inhibition of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the photoprotective function of PSII in Spirodela polyrhiza following exposure to silver nanoparticles (AgNPs) would also increase chloroplastic ROS production in the plant. It is also noted that it is the ion released from AgNPs that acts as an ROS activator, not the AgNPs themselves [29]. Consequently, internalized silver ions and AgNPs are responsible for

generating ROS within S. polyrhiza. Similarly, other authors have cited that ZnO, CuO, and CeO_2 will enable the release of ions from their respective solutions (Zn^{2+}, Cu^{2+}, and Ce^{4+}) in order to increase ROS levels in a plant, as well as cause an ionic stress [30, 31] (Table 2).

Table 2. - (NPs) as an active chemical elicitor

No.	plant	NPs	concentration	function	References
1	Selfheal (Prunella vulgaris L.)	Ag, Au, as well as Naphthalene acetic Acid (NAA)	Each NP was used at a concentration of 30 _g/L with AgAu: 1:2, 1:3, 2:1, and 3:1 ratio with NAA.	Increased biomass, phenols, DPPH-radical scavenging activity, and flavonoids	[32]
2	Candyleaf (Stevia rebaudiana)	ZnO and CuO	0, 2, 20, 200 and 2000 mg/L	DPPH inhibition percentage, antioxidant activity, reducing power, phenolic and flavonoid content, stevioside content, and rebaudioside A. Weight and callus quality are decreased by increased focus.	[20]
3	T. vulgaris, or garden thyme, T. daenensis, T. Satar and Kotschyanus (Zataria multiflora)	ZnO	100 and 150 mg/L	Augmentation of thymol and carvacrol content	[20]
4	Isatis (Isatis constricta)	Ag	0, 0.25, 0.5, 1, 1.5, and 2 mg/L	Indigo and tryptanthrin levels increased after treatment, but declined after 10 and 15 days, while indirubin levels fell.	[19]
5	Mouseear cress (Arabidopsis thaliana)	Ag	0.5, 1.0, 5.0 mg/L	Elevated glutathione disulfide concentrations, Kaempferitrin, succinyl malate, 6-gluO-ICOOGlu, 4-glucosyloxy-benzoate, G(8-5)FA dihexoside, 6-MeO-ICOOH and sinapic acid, and 4-hydroxy glucobrassicin, coniferylaldehyde hexoside, and camalexin. decreased concentrations of kaempferol-3-O-glucoside, pinresinol hexoside, and 4-glucosyloxybenzoate.	[21]
6	Argan (Argania spinosa)	SiO2 and TiO2	5, 10, and 20 ppm following a 15-day incubation.	Enhance _-tocopherol accumulation	[22]
7	IPOMEA TURBINATA AND CONVOLVULUS ARVENSIS	Ag	25, 50, 75, 100, 150, and 200 mg/l	Increased the concentration of phenols and flavonoids in plantlets.	[23]
8	Tagetes erecta L	SiO2	50, 100,150,200 mg/l	The vast majority of secondary metabolites from leaf callus, such as ellagic acid, syringic acid, gallic acid, quercetagetin, quercetin, kaempferol, and lutein, were of great importance.	[24]

1.4 THE ROLE OF NPS IN SOMACLONAL VARIATION

Somaclonal variance is a major issue in all micropropagation systems. It refers to phenotypic and genotypic changes caused by tissue culture (Table 3). Nanomaterials have been used in many studies to induce somaclonal variation in tissue culture [33]. Studied the effect of Au and AgNPs on the somatic variation of Linum usitatissimum. Growing calli and regenerants on Au and AgNPs media resulted in more somaclonal variations. The usage of Ag and Au NPs in PTC has lately increased; however, the mechanism of DNA alteration remains unknown. The effect of NP-induced oxidative stress may contribute to genetic variability. When NPs generate ROS, they may produce oxidative damage to DNA, including strand breaks and base modifications, ultimately resulting in mutations. Additionally, it is possible that NPs affect the accuracy of DNA repair or replication mechanisms.

It has also been shown that the treatment of bitter melon with Se-NPs results in the modification of the DNA methylation patterns via epigenetic modification [34]. Therefore, the resulting modifications could lead to heritable phenotypic variation without altering the original DNA sequence. The new Lamprocapnos spectabilis cultivars could be produced as a result of metabolites and forcing genetic changes to affect species characteristics [35]. Supplementation with Nps in bitter melon results in an increase in genes such as 4-coumarate: CoA-ligase (4CL) and PAL, leading to an epigenetic response [34]. DNA changes and mutations in four different rice genotypes, as well as somaclonal variants using RAPD and SSR, were examined due to the alteration induced by SiO2NP.

The genotype had a significant effect on callus formation and plant regeneration. The greatest responsive endpoint was the genome template stability percentage (GTS%), a measure of variation in RAPD profiles. RAPD profiles provide a DNA-based method for evaluating variation at the sequence level. Two SSR markers indicated polymorphisms that were useful for developing drought-tolerant rice plants [36]. There were six plants that exhibited changes in phenotype with treatment with 10 ppm and 20 ppm AgNPs, including alterations in pigment (carotenoid and anthocyanins) concentration and/or types of inflorescences [37].

Results from sweet potatoes shown by GtNPs that GtNPs were shown to sterilize tissues and induce genetic diversity in the somaclonal aspect of tissue culture, and from GtNPs treatments showed how well graphite nanoparticles (GtNPs) sterilize tissues and contribute to genetic variety. When combined, GtNPs and somaclonal variants can raise the frequency of induced mutations. By altering one or a few particular cultivar qualities, mutation can be utilized to select desired traits in sweet potatoes and increase yield. Additional testing may be carried out to determine the commercial biosafety of the resulting modifications [38]. Although NPs represent a method of enhancing genetic diversity for crop improvement, they also pose a potential threat to the clonal fidelity of commercial micropropagation. Due to the unpredictable nature of NP-induced variation, all regenerative plants intended for conservation or mass-producing should be subjected to both genomic and phenotypic evaluations.

Table 3. - NPs enhancing somaclonal variations

No.	*Plant*	*Nanoparticles*	*concentration*	*function*	*References*
1	*Linum usitatissimum* L	Ag/Au	500mg/l	Increase the percentage of somaclonal variation	[33]
2	Lamprocapnos spectabilis	(AuNPs)	50, 75, or 100 ppm	phenotype change	[35]
3		Se	(0, 1, 4, 10, 30, as well as 50 mg/L)	epigenetic alteration of chromatin structure, transcriptional machinery, as well as DNA cytosine methylation	[34]
4	Rice	SiO2	0, 150, 300, as well as 450 ppm)	lower in somaclonal variations for all studied genotypes	[36]
5	chrysanthemum	AgNPs	0, 5, 10, and 20 ppm	variation in pigment, and inflorescence shape	[37]
6	Sweet potato	graphite nanoparticles (GtNPs)	200, 400, and 800 ppm	removing bacterial contaminants, increasing shoot and callus induction, and somaclonal variations	[38]

1.5 CRITICAL EVALUATION & BIOSAFETY

The literature has documented the successful use of NPs in eliciting plant responses; however, this response is generally characterized by a biphasic and concentration-dependent nature. A significant knowledge gap exists with

respect to the long-term physico-chemical stability of NPs in culture media, the uptake efficiency of the plant cells/tissues, and the rate of degradation or persistence of the NPs. Much concern exists with respect to the residual NPs or released ions in the biomass of the plants produced for use as therapeutic or nutraceutical products. Additionally, the above characteristics are rarely considered in studies designed to evaluate NPs for agricultural applications and should be researched much more thoroughly [39,40].

2. METHODOLOGY

This narrative review utilized a systematic strategy to select literature that would provide relevant and comprehensive information about the subjects discussed in this review. We conducted systematic searches for peer-reviewed articles in electronic databases (Scopus, Web of Science, PubMed) from January 2010 through May 2025. The search utilized keywords and Boolean operators: ("nanoparticle" OR "nanomaterial") AND ("plant tissue culture" OR "callus induction" OR "secondary metabolite" OR "somaclonal variation") AND (in vitro*). The search allowed only: (1) primary research articles; (2) Research that specifically used engineered nanoparticles on plant tissue culture systems; and (3) Morphogenesis, production of metabolite, or genetic variation as the outcome of these studies. Non-English articles, review articles, and studies on non-plant systems were removed from consideration. Relevant references of the articles found were also reviewed for this paper.

3. CRITICLE GAPS AND FUTURE RESEARCH DIRECTION

While NPs are a significant opportunity in PTC, there are numerous specific gaps in the literature that require immediate further investigation:

- Uptake and Translocation Mechanisms: How do NPs of different sizes and coatings with varying charges enter plant cells in vitro? Do they enter plant cells through endocytosis, pore formation into plant cells, and/or carrier proteins?
- Fate and Stability: What is the dynamic transformation of NPs when in culture media (agglomeration, dissolution, interaction with media components)? Do NPs degrade, and if so, into what products?
- Comprehensive Biosafety Profiling: There is a need for systematic assessment of NP and ion residues present in regenerated plants and the implications of these materials on human and environmental health. No standardized protocols for assessing NP toxicity for in vitro plant systems exist.
- Precision Elicitations: Develop predictive models based on research measuring NP interaction with specific physicochemical parameters to predict metabolic response and gene expression.
- Long-Term Stability in Gene Expression: Using next-generation sequencing to define the full spectrum of potential genetic and epigenetic changes (from NPs), as well as their inheritance patterns, is needed.
- Formulations with Synergistic Effects: To achieve maximum product yields from NPs, a number of NPs should be able to be applied in combination with other Elicitors (e.g., jasmone, UV), under controlled environments.

4. CONCLUSION

This review summed up the important role that nanoparticles play in several aspects of plant nanobiotechnology, such as callus induction, shoot regeneration, roots, somaclonal variation, and the synthesis of bioactive chemicals for industrial and therapeutic applications. Researchers working in the fields of nanobiotechnology will find this review to be highly interesting and a useful source of information. Additionally, because of their special qualities, nano reservoirs, including graphene, carbon dots, fullerenes, and quantum dots, can be employed in plant tissue growth. However, the field has the potential to develop from being largely descriptive to more of a mechanistic process focused on safety. Filling knowledge gaps on NP uptake, fate, biosafety, and the effects of NPs on long-term gene expression will facilitate evaluating the potential for responsibly translating this technology from a laboratory setting into commercial agriculture. Even though there are still a lot of unanswered concerns, it is evident that plant nanobiotechnology is a cutting-edge, environmentally friendly technology with enormous promises for enhancing crops, delivering genes and agrochemicals, and creating secondary metabolites. Applications involving plant tissue culture also make extensive use of it.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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