Chapter 14
Role of Nanomaterials in Plant Cell and Tissue Culture

Sanaz Feizi

Abstract The plant tissue culture (PTC) technique has been established based on totipotency and regeneration capacity of plant cells by culturing different types of explants on a nutritional culture medium for regenerating the whole organ. It has an economically important place and its use in basic sciences such as genetics, biochemistry, tissue engineering, and biotechnology shows its value. This technique may provide some key applications including plant conservation, higher mass reproduction, genetic manipulation, and biologically active compound production. Nanoparticles (NPs) are small particles with a diameter of 1–100 nm. It is recently believed that many nanoparticles NPs could implicate significant effects on the various aspects of plant tissue culture including somatic embryogenesis, organogenesis, callus induction, sacral modification, genetic transformation, control of microbial pollutants, and the production of secondary metabolites. This chapter has focused on the different effects of several important NPs including metal and metal oxide, polymeric, dendrimers, quantum on the various plant abiotic stresses and then a comprehensive application of them on the amelioration of plant growth, crop production, and cytotoxicity remediation and the mechanism of nanoparticles affecting callus and secondary metabolism would be discussed. Of note, we would highlight different approaches to explore appropriate NPS for the improvement of the potential adaptation of plants under abiotic stresses aiming for their sustainable productivity.

Keywords Callus · Nanoparticles · Organogenesis · Plant tissue culture · Secondary metabolites · Somatic embryogenesis

S. Feizi (✉)
Agrobiotech for Health, Plant Biology and Soil Science Department, Faculty of Biology, University of Vigo, 36310 Vigo, Spain
e-mail: sanaz.feizi@uvigo.es

Department of Plant, Cell and Molecular Biology, Faculty of Natural Sciences, University of Tabriz, 5166616471 Tabriz, Iran

J. M. Al-Khayri et al. (eds.), *Nanomaterial Interactions with Plant Cellular Mechanisms and Macromolecules and Agricultural Implications*,
https://doi.org/10.1007/978-3-031-20878-2_14

14.1 Introduction

Plant tissue culture (PTC) is a vital, eco-friendly, and cost-effective technique implicated in different aspects of plant biology such as cell biology, biotechnology, biochemistry, and genetics (Thorpe 2007). This approach may be utilized for the mass propagation of plant cells, the production of genetically modified and free-disease tissues, and the efficient production of secondary metabolites (Khosroushahi et al. 2006). Moreover, PTC may minimize environmental variations by the use of specific and unique nutrient media in a controlled culture condition, nutrient availability in a homogenous manner, and decreased stress severity (Sakthivelu et al. 2008). Tissue cultures such as cell suspensions, callus, and hairy roots provided several advantages including simple and fast exploration of the effects of microflora and also membrane barriers on the cell and tissues compared with the whole-plant systems (Doran 2009). NPs particle size range is from 1–100 nm which provided a much larger surface area to volume ratio resulting in the enhancement of catalytic reactivity, thermal conductivity, biological activity, and chemical steadiness compared to their bulk forms. Accordingly, NPs could be used in health, cosmetic industries, food supplements, agriculture, electronics, and textile industries (Agarwal et al. 2017; Prasad et al. 2017; Dimkpa and Bindraban 2018). Interestingly, several reports identified the positive effects of nanoparticles (NPs) on the plant cells and tissue cultures in which they might significantly increase the secondary metabolite production, induce callus formation, somatic embryogenesis, organogenesis, and facilitate some genetic modifications (Kim et al. 2013). Moreover, a supplement of NPs can effectively lead to the control of microbial pollutants in plant culture medium (Helaly et al. 2014). Further reports confirmed NPs might facilitate genetic engineering procedures during callus regeneration experiments. NPs such as magnetic-related NPs and carbon nanotubes can mediate the accurate transfer of DNA molecules into the cells by reducing the integrity of plant cell walls (Lv et al. 2020). Ag NPs and Au NPs can induce random changes in the coding sequences of pectin methylesterase enzyme and Mlo-like protein during differentiation of callus of Flaxseed (*Linum usitatissimum.* However, the mechanisms of variations in nuclear genome induced by NPs have still been remain ununderstood (Kokina et al. 2017b).

14.2 Impact of Nanomaterials on Callus Induction

Overall, nanomaterials (NMs) have been categorized into Carbon- and metal-related nanomaterials. Carbon-related NMs have included fullerenes, graphene, and carbon nanotubes (e.g., single-walled carbon nanotubes and multi-walled carbon nanotubes) (Buzea and Pacheco 2017). While metal-based NPs are composed of zero-valent metals (e.g., Au, Ag, and Fe), metal oxides (i.e., nano-CuO, -ZnO, -CeO2, -TiO2, -Fe2O3, and -SiO2), quantum dots (CdSe and CdTe), nano-sized polymers (dendrimers and polystyrene), and metal salts (nano silicates and ceramics)

(Dallavalle et al. 2015). Different reports confirmed that the NPs could significantly improve seed germination and bioactive compound production, enhance plant growth and yield, and intensely increase plant protection capacity (Wang et al. 2016; Ruttkay-Nedecky et al. 2017). Although several metallic NPs are currently utilized in the agriculture industry, the release of these molecules into the environment might impose negative cytotoxic impacts on the living organisms. These toxic effects came from the size, morphology, nature, surface area ratio, composition, and reactivity charcharis of metal-based NPs (Zaka et al. 2016). It is frequently reported that metallic stress (Cu, Cd, Al, Pb, and Ni) could stimulate the phenylalanine ammonia-lyase (PAL) and chalcone synthase enzymes resulting in the induction of plant secondary metabolite production (Singh et al. 2015). It seems the higher tendency of phenolic compounds to the chelate metals are involved in the enhanced biosynthesis of these molecules (Jun et al. 2003). In this context, engineered NPs provided some unique physicochemical properties which facilitated their penetration into plant cells and tissues and subsequent delocalization (Keller et al. 2013). Notably, NPs such as gold, cerium oxide, aluminum oxide, and zero-valent iron might increase plant growth rates, modulate gene expression levels, and induce the synthesis of proteins and other metabolites in the different plant cells and tissues (Jaskulak et al. 2019; Montes et al. 2017; Kim et al. 2014; Kumar et al. 2013; Lee et al. 2010; Yang et al. 2017). The effects of several NPs on physiology, morphology and metabolism-related pathways of plant callus or cell suspension cultures obtained from some recent research are discussed below.

Zinc Oxide (ZnO)

Several studies have been performed to evaluate the impact of biosynthesized zinc oxide NPs on in vitro production of bioactive compounds and the improvement of biomass in different plants. It is reported that low concentrations of ZnO NPs could stimulate callus growth and also enhance regeneration, organogenesis, and decontamination (Mousavi Kouhi and Lahouti 2018; Kavianifar et al. 2018). Upon exposure of plant cells to the ZnO NPs, the production of secondary metabolites has been induced in which they functioned as phytoalexins to protect plant cells and tissues against biotic and abiotic stress (Marslin et al. 2017; Abdel-Lateif et al. 2012). Of note, Zinc Oxide NPs might modulate the antioxidant and macromolecules systems in the callus of *Solanum nigrum*. It is identified that the dry weight of callus was increased upon exposure to the lowest concentration of ZnO NPs. Moreover, the activity of lipoxygenase and antioxidant enzymes were increased at the highest level of ZnO NPs. Although the activity of phenolic and phenylalanine ammonia-lyase compounds was not changed by the treatment of ZnO NPs, the polyphenol oxidase activity was significantly decreased. It should be highlighted that the amino acid, soluble protein and carbohydrates, and also Zn contents were highly enhanced in the callus treated with ZnO NPs (Abdel Wahab et al. 2020). Zn provided vital roles in different biochemical, physiological, and anatomical pathways. ZnONPs have been widely utilized in personal and medical care products, paints, coating materials, UV protectors, and absorber materials. However, these nano molecules might increase health and environmental risks because of their interaction with many biological

and chemical biomaterials (Chithrani et al. 2006). Further research confirmed that the treatment of *Juniperus procera* cells with a suitable amount of biosynthesized ZnO NPs caused a significant enhancement in growth rate, chlorophyll A, and total protein contents (Salih et al. 2021). Interestingly, the treatment of callus of wheat and tobacco with ZnO NPs causes an increment in nutrient and protein contents respectively (Rizwan et al. 2019; Mazaheri-Tirani and Dayani 2020). It is further identified that ZnO NPs could modulate the expression of some genes encoded by certain proteins resulting in turn on/off the expression of some downstream genes (Salama et al. 2019). Also, zinc oxide NPs might increase the CAT activity in the callus of *Punica granatum* and *Prosopis glandulosa* (Farghaly et al. 2020; Hernandez-Viezcas et al. 2011). A strong correlation existed between CAT activity and Zn concentration might be revealed that the CAT enzyme is involved in defense response against ZnO-NPs or BP stress (Hernandez-Viezcas et al. 2011). Moreover, the strong correlations between LOX activity and Zn concentration were also confirmed in which ZnO–NPs could increase O_2^- formation causing oxidative stress (Manke et al. 2013). Upon the ZnO-NPs reaching into the mitochondria, they might induce ROS production by interfering with their reactions resulting in the depolarization of mitochondrial membranes (Xia et al. 2006). Of note, some enzymatic antioxidants were increased under ZnO NPs confirming these enzymes could be enabled plants to neutralize the stress. ZnO NPs provided some positive effects on the protein content of the callus of tomatoes even under salt stress (Alharby et al. 2016). Treatment of *Echinacea purpurea* callus extracts with biosynthesized ZnO NPs enhances secondary metabolite and anticancer activities (Karimi et al. 2018). In different concentrations, zinc as a micronutrient improves the efficiency of callogenesis and regeneration in Panicum virgatum (Shafique et al. 2020). ZnO NPs and ZnO submicron particles have been shown to improve onion *) Allium cepa L. 'Sochaczewska'(* seed germination and seedling growth in vitro. Seeds treated with 800 mgL1 of the NPs had the highest percentage of germination (Fig. 14.1a) (Tymoszuk and Wojnarowicz 2020). Zafar et al. (2016) reported Brassica nigra seed germination and seedling growth are affected with ZnO NPs concentrations ranging from 500 to 1500 mg/L, that also leads to improvement of antioxidative and non-antioxidants activities (Fig. 14.1b).

Silver (Ag)

Silver NPs are considered as one of the most important NPs produced worldwide and provide antimicrobial, cytotoxic, antifungal, physiological, and phytotoxic properties (Keller et al. 2013; Nel et al. 2006). Ag NPs are able to inhibit chronic contamination caused by microorganisms during plant culture experiments (Elechiguerra et al. 2005). These features came mainly from small size and unique phytochemical properties allowing Ag NPs to cross through biological membranes and organs and tissues to improve plant health (Kim et al. 2017). AgNPs significantly enhanced seed germination capacity and seedling growth rate in rice (*Oryza sativa* L., cv. Swarna) (Gupta et al. 2018). This NP has presented different applications in plant tissue technology including simultaneously improvement of callus induction, somatic embryogenesis, organogenesis, genetic transformation, somaclonal variations, and

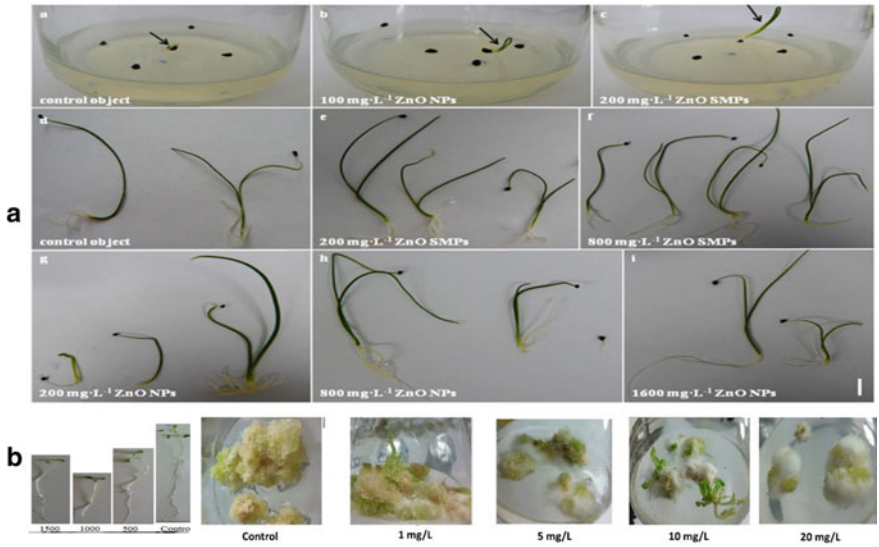

Fig. 14.1 Impact of ZnO NPs on seed germination and stem explants. *Allium cepa L. 'Sochaczewska'* (**a**), *Brassica nigra* (**b**). *Source* Tymoszuk and Wojnarowicz (2020), Zafar et al. (2016)

secondary metabolites production (Lateef et al. 2018; Adebomojo and AbdulRahaman 2020). In addition, AgNPs presented a high potential for improvement of growth, biomass, and secondary metabolites in plant cell cultures (Elechiguerra et al. 2005). It is identified that a suitable concentration of AgNPs can significantly induce the callus formation, the regeneration of shoot and roots, and the nursery phase during the propagation of banana (Musa ssp.) (Huong et al. 2021) of note Ag-SiO_2 stimulates the production of artemisinin in the roots of *Artemisia annua* (Zhang et al. 2013). Moreover, biologically synthesized AgNPs can increase the callus fresh weight and also callus formation in the leaf explants of *Solanum nigrum* (Ewais et al. 2015). Another recent report identified that supplementation of AgNPs and plant growth regulators sustainably enhanced the callus proliferation, biomass, antioxidant, and secondary metabolites production during in vitro culture of *Caralluma tuberculate.* While the sole application of AgNPs produced a higher amount of antioxidants and secondary metabolites (Ali et al. 2019b). On Nicotiana tabacum, hormone-stabilized AgNPs fully promoted the roots (a) control water treatment, (b) IAA, (c) IBA, (d) AgIAA, (e) AgIBA) (Fig. 14.2a). (Thangavelu et al. 2018) Silver NPs in concentrations ranging from 1 to 5 ppm were found to be effective on banana (*Musa spp.*). In vitro shoot cultures on media containing 3 ppm AgNPs also produced a significant number of roots (Fig. 14.2b) (Do et al. 2018).

Gold (Au)

The incorporation of Au NPs into the callus medium of *Arabidopsis thaliana* could improve the seed germination, seedling growth capacity, pod length, and a number of

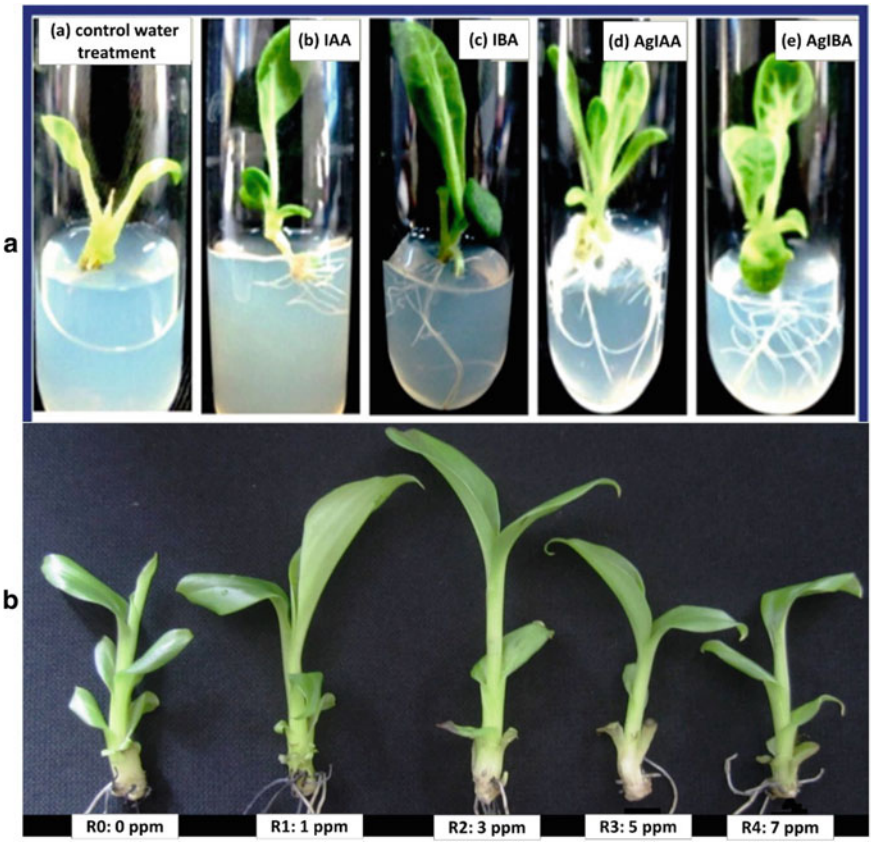

Fig. 14.2 Effect of AgNPs on rooting. *Nicotiana tabacum* (**a**), on banana (Musa spp.) (**b**). *Source* Thangavelu et al. (2018), Do et al. (2018)

seeds. Moreover, the use of Au NPs might enhance the antioxidant enzyme activity in the *A. thaliana* through the decrease of microRNA expression levels miR398 and miR408) (Kumar et al. 2013). Further reports confirmed that the treatment of cell suspension cultures with Au NPs increases the intracellular free amino acid pools (alanine, valine, and γ-aminobutyric acid) and also modulates the extracellular proteins composition (Selivanov et al. 2017).

Copper (Cu)

The treatment of callus culture of *Mentha longifolia* with Cu and Co NPs has a positive impact on the improvement of fatty acid contents in which the linalool and linalyl acetate contents were higher in the treated cells (Talankova-Sereda et al. 2016). Like the other NPs, the use of CuO NPs in the *O. basilicum* callus cultures could elicit the biosynthesis of bioactive compounds with a high antioxidative capacity.

Moreover, the accumulation of flavonoid and phenolic molecules was also significantly improved in the media supplemented with CuO NPs. In addition, the SOD and POD (Peroxidase) activities were highly elicited in the CuO NPs treated cultures compared to the control. Notably, the HPLC data identified that the production of rosmarinic acid, chicoric acid, and eugenol was improved when the callus cultures of *O.basilicum* were treated with CuO NPs (Nazir et al. 2021). (Paramo et al. 2020) suggested that the positive impact of Cu NPs is due to copper showing a greater positive effect in the physio-biochemical processes such as hormone signaling pathways, metabolism, and electron transport reactions. However, the increase in NPs concentrations might show some negative effects on biomass production. Another report showed that CuO NPs could stimulate the in vitro induction of bioactive compounds in the suspension cells of *Stevia rebaudiana* (Javed et al. 2017b). While the use of five levels of CuO NPs caused a significant decrease in fresh and dry weight, water content, amino acids, and potassium contents in the callus cells of *Solanum nigrum* (Abdel-Wahab et al. 2019). Capped CuO NPs were more toxic for the callus cells of *Trigonella foenum-graecum* than uncapped forms causing a higher production of secondary metabolites (ul Ain et al. 2018). It should be noted that CuO NPs could be elicited biomass and bioactive compounds accumulation, and antioxidants biosynthesis in callus cultures of *Ocimum basilicum* (Nazir et al. 2021).

Carbon Nanomaterials (CNMs)

Today, carbon nanomaterials (CNMs) have been attracted much more attention for their application in plant biology. These materials have exhibited positive potential for regulating the plant growth capacity which was a promising future for agriculture. However, the precise mechanism of CNMs in plants is yet well understood especially at the molecular levels (Zhenjie et al. 2020). Until now, the potential different CNMs such as carbon nanotubes and graphene have been evaluated in plant biology research. The appropriate concentration (25–500 $\mu g\ mL^{-1}$) of multi-walled carbon nanotubes can highly improve the callus growth rate in the leaf explants of *Satureja khuzestanica.* Whilst, the higher amounts (100–500 $\mu g\ mL^{-1}$) of these nanotubes might decrease the callus biomass production (Ghorbanpour and Hadian 2015). Similarly, the incorporation of about 100 μg/mL of multi-walled carbon-related nanotubes significantly increased the callus growth cates in the tobacco explants. It is believed these activities are achieved through the upregulation of the genes involved in cell division and extension, cell division, and water transport (Khodakovskaya et al. 2012). Of note, multi-walled carbon nanotubes could intensely improve the nitrogenase activity and also increase gene expression levels involved in the regulation of nodules development (Yuan et al. 2017). However, the treatment of *Arabidopsis* cell cultures with 10–600 mg/L of carbon nanotube treatment was decreased the viability and dry weight of plant cells (Lin et al. 2009). The exposure to low concentrations of single-walled carbon-related nanotubes provided drought stress induced by polyethylene glycol through the activation of some antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD), and Ascorbate peroxidase (APX) and also biosynthesis of secondary metabolites (ie., phenols and proline) in

the seedlings of *Hyoscyamus niger* (Hatami et al. 2017). Graphene-related nanomaterials provided some impressive characteristics such as two-dimensional structure, unique electronic and optical attributes, mechanical flexibility, electrical conductivity, and high and chemical stability resulting in greatly broadened applications in biology, chemistry, and medicine (Shehzad et al. 2016; Shen et al. 2016; Dreyer et al. 2010).

Iron (Fe)

It is reported that the use of FeO NPs in the medium of *Hyoscyamus reticulatus* could increase the production of tropane alkaloid hairy roots through the induction of oxidative stress reactions (Moharrami et al. 2017). Further research identified that SiO2 and Fe NPs could significantly enhance the accumulation of some essential pharmaceutical biologics including rosmarinic acid and xanthomicrol in the hairy roots of *Dracocephalum kotschyi* (Nourozi et al. 2019a, b).

Silicon (Si)

Silicon (Si) as the second most frequent element is enabled of protecting plants from biotic and abiotic stresses, decreasing transpiration losses, and improving their resistance to different diseases (Liang et al. 2007; Ma 2004; Nawrot et al. 2010). It is identified that the treatment of rice cell cultures with silica NPs noticeably reduced Cd toxicity by a decrease in silica NPs size. Moreover, silica NPs could respectively increase and decrease the Si and Cd uptake capacities allowing the alleviation of Cd toxicity in the cells (Cui et al. 2017). It is reported that the fluorescein isothiocyanate-labeled mesoporous silica NPs (MSNs) could successfully interact with hybrid suspension cells of *Liriodendron* through the internalization of MSNs via endocytosis. Owing to admirable biocompatibility, MSNs might be considered as a potential nanocarrier for walled-plant cells (Xia et al. 2013).

Ca

CaO NPs are vital elements that functioned as transducers in several adaptive and developmental reactions in plants. These elements could enhance the tolerance of callus of *Triticale* against salt stress through the improvement of biochemical activity (Yazıcılar et al. 2021).

SnO_2

The cytotoxic effects of SnO2 and Ag/SnO2 NPs on the *tobacco* cell cultures identified the importance of structural modifications on the toxic properties of NPs. Indeed, SnO2 NPs presented low toxicity while Ag-doped NPs have a significant effect in inhibiting the toxicity through modulation of oxidative stress pathways in tobacco cells. Microscopic analyses demonstrated a high level of cell mortality upon treatment with a high level of SnO_2 NPs (e.g., 0.5 mg/ mL) or even a low concentration of Ag/SnO2 NPs (e.g., 0.2 mg /mL). Further experiments showed these components could significantly enhance the accumulation of neutral red stain into the vacuole of NPs-treated tobacco cells inducing the high acidification (Mahjouri et al. 2020).

Polymeric Nanoparticles

Polymeric NPs are colloidal nano molecules ranging from 1 to 1000 nm which are generally prepared from biodegradable polymers (Prabha et al. 2020; Bhattacharjee et al. 2016). Biodegradable polymers mainly utilized for the polymeric NPs fabrications such as poly (lactide) (PLA), poly (amino acids), poly (lactide-co-glycolide), poly (ε-caprolactone) (PCL), (PLGA) copolymers, and several natural polymers especially alginate and chitosan (Asti and Gioglio 2014). Notably, polymeric NPs presented some important advantages such as biocompatibility, biodegradability, simple and easy fabrication process, non-toxicity, non-immunogenicity, and capability to site-specific targeting organs or tissues (Jawahar and Meyyanathan 2012). Recently, polymeric NPs have extensively been implicated in the production of pesticides, herbicides, fertilizer, and plant growth regulators. It is reported that 2,4-D loaded PLGA NPs could significantly increase the growth rate and biomass of Medicago sativa cell suspension cultures compared to its free form (Poyraz et al. 2021). Furthermore, the potential of bulk or nano-chitosan components, as an eco-friendly natural nano-molecule, has been evaluated in morphogenesis, growth, micropropagation, and physiology of Capsicum annuum suspension cells. The treatment of suspension cells with bulk chitosan or synthesized chitosan/tripolyphosphate (TPP) NPs were manipulated morphology and differentiation of some tissues and organs, especially the root architecture. Of note, the appropriate concentration of nano-chitosan might trigger organogenesis through micropropagation (Asgari-Targhi et al. 2018). The chitosan NPs synthesized by *Penaeus semisulcatus* shrimp shells could strongly inhibit some bacterial and fungal pathogens. In addition, chitosan NPs may use to develop pesticides against mosquito vectors in food packaging applications (Thamilarasan et al. 2018).

Dendrimer Nanoparticles

Cationic polyamidoamine (PAMAM) dendrimers as a highly branched NP could be utilized for the improvement of gene delivery capacity into the different cells. In fact, PAMAM may interact with DNA molecules allowing protection from ultrasonic damage. The use of PAMAM could intensely improve the transformation and gene expression efficacy in the *alfalfa* cells (Amani et al. 2018).

Quantum Dots (QDs)

QDs are fluoresce-based NPs expressed with bright and pure colors upon excitation with UV wavelength (Whiteside et al. 2019). The treatment of the suspension culture of *Medicago sativa* with mercaptopropionic acid-coated CdSe/ZnS QDs to the suspension culture significantly reduced cell growth rate. Subsequently, a high accumulation of the CdSe/ZnS QDs in the cytoplasm and nucleus led to dose- and time-dependent production of ROS (Santos et al. 2010). Further data showed these cytotoxic and genotoxic features were induced by the activation of DNA-related repair genes and ROS-eliminating enzymes (Santos et al. 2013). The below table shows the effect of some NPs on plant cell and tissue culture (Table 14.1).

Table 14.1 The effect of some NPs on plant cell and tissue culture

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Ag	Seedling	Rice *(Oryza sativa* L., cv. Swarna*)*		10, 20, 40 ppm	Carotenoid and chlorophyll contents improved	Gupta et al. (2018)
Ag-SiO_2	Root	*artemisinin*	101.8 ± 8.9	900 mg/L	Increased lipid peroxidation and MDA and CAT accumulation	Zhang et al. (2013)
Ag	Callus	*Valeriana officinalis* L	35	25, 50, and 100 μg/mL	Inhibited bacterial infections	Abdi et al. (2008)
Ag	Callus	*Caralluma tuberculata*	40	30, 60 and 90 μg/l	Enhanced callusproliferation and biomass	Ali et al. (2019b)
Ag	Shoot	*Tecomella undulata (Roxb)*		5 to 80 mg/L	Improved the number of fresh shootsper explants	Aghdaei et al. (2012b)
Ag	Callus	*Calendula officinalis*		0.3 mg/L	Increased essential oils production capacity	Abbasi Khalaki et al. (2016)
Ag	Cell suspension	*Echinacea purpurea*		0, 2 and 4 mg/L	enhanced cichoric acid production	Ramezannezhad et al. (2019)
Ag	Cell suspension	*Linum usitatissimum*	18	1, 5, 10, 20, 30, 40, 50 μg/l,	Improved lignans and neo-lignansactivities	Zahir et al. (2019)
Ag	Callus	*banana (Musa ssp)*		0.0, 2.0, 4.0 and 6.0 ppm	Improved callusformation, regeneration, and multiplication	Huong et al. (2021)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Ag	Cell suspension	*Corylus avellana* L		0, 2.5, 5, and 10 ppm	Decreased the viability of the cells and increased radical scavenging activities	Jamshidi et al. (2016)
Ag	Shoot culture	*Vanilla planifolia*	15–35	0, 25, 50, 100 and 200 mg/L	TPC and antioxidant enzymesactivities increased	Spinoso-Castillo et al. (2017)
Ag	Hairy root	*Brassica rapa*			Phenolic compounds enhanced	Chung et al. (2018b)
Ag ion (Ag+)	Hairy root	*Cucumis anguria*		2.0 mg/L	Induced hairy rootand phenolic formation	Chung et al. (2018a)
Ag	Hairy root	*Datura metel*	50–60	20 mg/L	Enhanced atropine contents	Shakeran et al. (2015)
Ag	Cell suspension	*Capsicum frutescens*		3.0 mg/L	Capsaicin production enhanced	Bhat and Bhat (2016)
Ag + Au	callus	*Prunella vulgaris* L		different ratios (1:2, 1:3, 2:1, and 3:1)	Improved TPC and TFC contents	Fazal et al. (2016)
Ag + Au	Cell suspension	*Lavandula angustifolia*	Au = (24) nm and Ag = (27) nm	10–50 mg/dm	Decreased lower molecular weight compounds	Wesołowska et al. (2019)
Ag + plant growthregulators (PGRs)	Callus	*Caralluma tuberculata*	40	30, 60 and 90 μg/l	Improved proliferationand callusbiomass	Ali et al. (2019b)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Au and Ag	Callus	*Linum usitatissimum* L		500 mg /L	Increased somaclonal variationscallus	Kokina et al. (2017b)
Au	*Seedling*	*Arabidopsis thaliana*	24 nm	10 and 80 μg/ml	Enhancedseed germination, vegetative growth, and free radical scavenging capacity	Kumar et al. (2013)
biosynthesized nanosilver (BNS)	Callus	*Ocimum*	12–80 nm	10, 50 and 100 mg/L	Used for surface sterilizationof explantand callus	Adebomojo and AbdulRahaman (2020)
CaO + on exposed to short and long-term salt stress	Callus	*Triticale*		1.5 ppm Ca2 + NPs concentration of 50 g and 100 g NaCl	Suppressed the side effects of NaCl stress	Yazıcılar et al. (2021)
Carbon nanotube	Suspension	*Arabidopsis*		10–600 mg/ L	Cell viability rate and dry weight decreased	Lin et al. (2009)
Co	Cell suspension	*Artemisia annua*	10 nm	0.25, 2.5, and 5 mg/L	Reduced expression levels of SQS and DBR2 genes	Ghasemi et al. (2015)
Cu and Co		*Mentha longifolia*		Copper (0.5 mg/L) and cobalt (0.8 mg/L)	Improved microplant height and growth rate	Talankova-Sereda et al. (2016)
CuO + CaO + zno + Under In Vitro Salt Stress	Callus	*Alfalfa (Medicago Sativa L.)*	CaO = 35 to 160 nm CuO = 20–45 nm ZnO = 17–65 nm	50 mM treatment NaCl with 0.8 ppm NPs	Protectedthe callusagainst NaCl stress	Simsek et al. (2021)
Cu-Au bimetallic	Adventitious root culture	*Stevia rebaudiana*			TPC and TFC increased	Ghazal et al. (2018)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Cu-NPs + copper sulfate		*Ocimum basilicum*	20–40 nm	0.1, 2.5, 5, 7.5, 10, 12.5 and 15 μM	Elevate regenerationcapacity	Ibrahim et al. (2019)
CuO	Cell suspension	*Brassica rapa ssp.*	25–55 nm	50, 250, and 500 mg/L	Reduced total chlorophyll, carotenoid, and sugar contents and improved proline and anthocyanins	Chung et al. (2019)
CuO	Callus	*Solanum nigrum L*	*> 50 nm*	*50, 100 and 150 mg/L*	Decreased fresh weight, amino acid and potassium contents	Abdel-Wahab et al. (2019)
CuO	Shoot and root	*Withania somnifera*		1 ppm	TPC, TFC, and tannins contents improved	Genady, Qaid et al. (2016)
CuO + MnO	Callus	*Ocimum basilicum (Thai basil)*	CuO-NPs (20–50 nm) + MnO-NPs (20–30 nm)	(1, 5, 10, 25, 50, 100 mg/L)	Increased phytochemicals accumulation	Nazir et al. (2021)
$CuSO_4$	Callus	*Verbena bipinnati da Nutt*		5–15 mg/L	Shoot and root lengths, phenolic contents, and fresh weight increased	Genady et al. (2016)
$Fe2O_3$	Hairy root	*Dracocephalum kotschyi Boiss*		75 mg/L	Flavonoid contents increased	Nourozi et al. (2019b)
Fe	Hairy root	*Dracocephalum kotschyi*	100		Flavonoid contents increased	Nourozi et al. (2019a)

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Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Fe_3O_4 ZnO	Hairy root	*Cichorium intybus*		50 mg/L	TPC and TFC improved	Mohebodini et al. (2017)
Fe_3O_4	Hairy root	*Hyoscyamus reticulatus* L		450 and 900 mg/L	Improved scopolamine and hyoscyamine	Moharrami et al. (2017)
$Fe3O_4$	Cell suspension	*Hypericum perforatum* L	0, 50, 100 and 150 ppb		Hyperforin and hypericin enhanced	Sharafi et al. (2013)
Mgo + thidiazuron *(TDZ)*	Seed	*Raphanus sativus* L		20 mg/L	Biomass, TPC, TFC and antioxidant activity increased	Hussain et al. (2019)
Mn_2O_3	Shoot-tip	*Atropa belladonna*		25 mg/L	Increased production of alkaloids and flavonoids	Tian et al. (2018)
MWCNTs	Callus	*Satureja khuzestanica*		0, 25, 50, 100, 250 and 500 μg/ ml	Reduced the callus biomass	Ghorbanpour and Hadian (2015)
MWCNTs	Seedlings	*Salvia verticillata* L		(0–1000 mg /L	Flavonoid contents increased	Rahmani et al. (2020)
nano-garphene oxide(NGO) + under polyethylene glycol-induced dehydration	Callus	*Plantago major* L		100–800 μg/ mL	Reduced growth rate and osmotic potential capacity	Ghorbanpour et al. (2018)
Perlite NPs TiO_2/perlite nanocomposites	Callus	*Hypericum perforatum*	14.51–23.34 and 15.50–24.61 nm	25–200 mg/L	Enhanced the variety, quantity and number of volatile compounds	Ebadollahi et al. (2019)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Poly(amidoamine) dendrimer	Callus	*Agrostis stolonifera* L	4.5 nm		Improved gene transformation efficacy	Pasupathy et al. (2008)
Silver NPs and silver salt ($AgNO_3$)	Callus	Two varieties of wheat *(Triticum aestivum* L.*)*	17 ± 5 nm	0, 20, 40, 60 ppm	Improved the growth rate, callus formation, protein contents, and gene expression levels	Barbasz et al. (2016)
AgNP	Leaves	Chrysanthemum		250 ppm	Teatment for 15–20 min proved optimal for controlling the contamination	Tung et al. (2021)
SiNP	Cell suspension	Rice	19 nm, 48 nm and 202 nm		decreased cadmium toxicity	Cui et al. (2017)
SMF Fe_2O_3	Cell suspension	*Dracocephalum polychaetum Bornm*	30 mT 100 ppm		Flavonoids, lignin, anthocyanins and malondialdehyde production increased	Taghizadeh et al. (2019)
TiO_2	Embryonic callus	*Cicer arietinum*		0/5.1.5,3,4.5.6 mg/L	Total phenolic compounds increased	Al-Oubaidi and Kasid (2015)
TiO_2 and ZnO	Cell suspension	*Linum usitatissimum*	30 10–30	0–150 mg/L 0–120 mg/L	The activity of PAL and CAT enzymes enhanced	Karimzadeh et al. (2019)
TiO_2/perlite	callus	*Hypericum perforatum*	300–350	25–200 mg/L	Increased volatile compounds	Ebadollahi et al. (2019)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
Tio_2, NH_4NO_3, Ag	Cell suspension	*Aloe vera*			Secondary metabolitesproduction improved	Raei et al. (2014)
Zinc and iron nano-oxides	Cell suspension	*Hypericum perforatum*		0, 50, 100 and 150 ppb	Enhanced hyperforin production	Sharafi et al. (2013)
Zinc oxide nano and bulk particles	Callus	*Punica granatum*	100 nm and surface area about 15–25 m^2g-1	0, 10, and 150 μg/mL	Improved CAT, SOD, POD, APX, and PPO activities	Farghaly et al. (2020)
ZnO	Callus	*Panicum virgatum*	90–390	10–50 mg/L	Improvedthe regenerationcapacity and callogenesis	Shafique et al. (2020)
ZnO	Callus	*Solanum nigrum calli*		0, 50 and 100 mg/L used)	The activities of lipoxygenaseand antioxidant enzymesimproved	Abdel Wahab et al. (2020)
ZnO	Callus	*Brassica nigra*	100	1–20 mg/L	Enhanced secondary metabolitecontents in callus and seedlings	Zafar et al. (2016)
ZnO	Callus	*Echinacea purpurea*		75 mg/L	Production of anticancer components and flavonoid contents improved	Karimi et al. (2018)
ZnO	Seedling	*Brassica nigra*		500–1500 mg/L	Prohibitedseed germination	

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
ZnO	Cell suspension	*Linum usitatissimum*	35	1, 5, 10, 20, 30, 40, 50, 75, 100, 200 and 500 μg/l	Increased lignans and neolignans components	Abbasi et al. (2019)
ZnO	Seedling	*Chenopodium quinoa*		0.2, 2, 10, 20 mg/L	Germinationrate, shooting capacity improved	Al Gethami and El Sayed (2020)
ZnO	callus	*Thymus Kotschyanus Tymus daenensis, Thymus vulgaris*		100 and 150 mg/L	Enhanced thymol and carvacrol contents	Mosavat et al. (2019)
ZnO	Shoot culture	*Stevia rebaudiana*	34	0, 0.1, 1.0, 10, 100 or 1000 mg/L	steviol glycosides, avonoid and phenolic contents improved	Javed et al. (2017b)
ZnO	Shoot	*Lilium ledebourii*		20–100 mg/L	Anthocyanin, phenolics and flavonoids increased	Chamani et al. (2015)
ZnO	Hairy root	*Hyoscyamus reticulatus*		100 mg/L	Enhanced total phenolic contents and alkaloids	Asl et al. (2019)
ZnO CuO	Root culture	*Stevia rebaudiana*		20–30 25–30	Steviol glycosides and flavonoids enhanced	Ahmad et al. (2020)

(continued)

Table 14.1 (continued)

Nanoparticle	Type of in vitro culture	Plant species	Size (nm)	Nanomaterial concentration	Role	References
ZnO and CuO	Callus	*Stevia rebaudiana*		1–10 mg/L	Increased total phenolic, flavonoid, antioxidant contents, and DPPH scavenging capacity	Javed et al. (2018)
Zno using Phoenix dactylifera)	Callus	*Juniperus procera*	17 to 36	0.0 mg/L, 80 mg/L and 160 mg/L	Improved callusformation	Salih et al. (2021)
ZnO, SiO_2, and Fe_3O_4	Callus	*Salvadora persica*	25 to 55	of 0.5 or 2 mg/L	Increasing biomass	Fouda et al. (2021)

14.3 Mechanism of Improvement of Secondary Metabolism by Nanoparticles

Elicited plant cell and suspension cultures have attracted more attention worldwide because of their capacity for the production of industrially vital secondary metabolites (Ali et al. 2019a). Plant secondary metabolites are organic components which involved directly in the growth, development, and reproduction of plant cells and tissues. Moreover, these molecules are contributed to different signaling cascades, and also defense pathways against several microorganisms, pathogens, and insects (Hartmann 2007). Most of the secondary metabolites are considered as an enriched resource of pharmaceutical molecules with defensive properties in the human body (Zhao et al. 2005a, b). The biosynthesis of secondary metabolites is dependent on biotic and abiotic factors such as growth rate, physiology, light intensity, temperature, and humidity. Moreover, the secondary metabolite productivity of callus cultures has been especially dependent on culture media composition, pH, agitation, aeration, and light density (Ochoa-Villarreal et al. 2016; Isah et al. 2018). Nowadays, several various biotic and abiotic factors have been evaluated to induce the production and concentration of the secondary metabolites and also increment cell volume in plant suspension cultures (Rao and Ravishankar 2002). Many NPs could be activated through enzymatic pathways which are responsible for secondary metabolites production (Wang et al. 2021). Nanomaterials could be considered a novel effective abiotic for the stimulation of biosynthesis of secondary metabolites (Fakruddin et al. 2012). Different reports have been identified that the nanomaterials could increase the expression of several genes involved in the biosynthesis of secondary metabolites (Ghasemi et al. 2015; Yarizade and Hosseini 2015). Titanium oxide NPs, for example, could distinctly increase the production of gallic acid, cinnamic acid, chlorogenic acid, tannic acid, and o-coumaric acid in the embryonic callus of *Cicer arietinum* (Mohammed 2015). Moreover, the use of silver NPs might increase the concentration of artemisinin in the hairy root cultures of *Artemisia annua* (Zhang et al. 2013). Notably, the growth rate of calli of *Satureja khuzestanica* was significantly improved when treated by gradually increasing concentrations of carbon nanotubes in the plant medium (Ghorbanpour and Hadian 2015). While, in the higher concentration of carbon nanotubes (i.e., 500 mg/L), the highest amounts of H_2O_2, PPO, POD, and secondary metabolic activities were observed. Similarly, the use of about 250 and 1000 mg/L CeO_2 and also indium oxide NPs caused excessive ROS production and PAL, and PPO in the *A. thaliana* suspension cells which revealed the possible function of secondary metabolites against oxidative stresses (Ma et al. 2016). Although NPs could implicate positive impacts on some signaling pathways and modulate the metabolism of secondary compounds, the precise mechanisms of these reactions were not understood. It is believed that the initial responses of different plants to the NPs might be elevated levels of ROS, cytoplasmic calcium and subsequent upregulation of mitogen-activated protein kinase (MAPK) cascades observed during abiotic stresses (Sosan et al. 2016). The increase of Ca^{2+} levels is associated with upregulating some protein signaling pathways in the *O. sativa* roots treated with AgNPs

(Mirzajani et al. 2014). It is hypothesized that AgNPs might impede cell metabolism through binding to the Ca^{2+} receptors, Ca^{2+} channels, and Ca^{2+}/Na^{+} ATPases. Moreover, NPs could minimize Ca^{2+} or signaling molecules in the cytosol upon sensing calcium ions by calcium-binding proteins or other NP-specific proteins (Khan et al. 2017). Further data identified that MAPK phosphorylation and also the activation of downstream transcription factors led to induce of transcriptional reprogramming of secondary metabolism in many plants (Vasconsuelo and Boland 2007; Schluttenhofer and Yuan 2015; Phukan et al. 2016). Although the exact evidence for the contribution of MAPK pathways in plant-NP interactions is yet identified, analogous pathways involved in AgNP-induced signaling reactions were found in the animal and human cell line studies (Eom and Choi 2010; Lim et al. 2012). In this sense, it is believed that plants might utilize MAPK pathways upon exposure to the Ag NPs (Kohan-Baghkheirati and Geisler-Lee 2015). Recent data confirmed that NPs could be regarded as a nutrient resource or an elicitor inducing the overproduction of secondary metabolites (Kim et al. 2017). For instance, the treatment of the tobacco cell suspension cultures with different concentrations of Al_2O_3 NPs could accumulate phenolic molecules (Poborilova et al. 2013). Similarly, the addition of Ag-SiO_2 core–shell NPs into the *Artemisia annua* hairy root cultures could intensely improve artemisinin content (Zhang et al. 2013). Multi-walled carbon nanotubes could significantly induce the production of total phenolics, flavonoids, rosmarinic acid, and caffeic acid in the *Satureja khuzestanica* callus cultures compared to the control experiments (Ghorbanpour and Hadian 2015). The cultures supplemented with zinc nano-oxide showed an increased amount of hypericin and hyperforin (Sharafi et al. 2013). It should be noted that recent genomic data have been found that plants might respond to the internalization of nanomaterials similar to the biotic or abiotic stresses (Khodakovskaya et al. 2012; Kohan-Baghkheirati and Geisler-Lee 2015). Indeed, NPs could be modulated the secondary metabolites production through the induce of several signal transduction pathways including calcium flux, overproduction of ROS, and MAPK phosphorylation reactions (Mahjouri et al. 2018). It seems that NP-induced ROS can function as a signal to trigger the plant's secondary metabolism (Marslin et al. 2017). Plants could produce different types of ROS such as H_2O_2, superoxide, hydroxyl radical, and singlet oxygen during the detoxification mechanism. Different antioxidant enzymes (oxidoreductases and CAT), hormones (e.g., abscisic acid and salicylic acid), and antioxidants with low molecular weight (thiols and ascorbate) are involved in the neutralization of these toxic molecules. Notably, excessive ROS might lead to increase lipid peroxidation capacity, electrolyte leakage, and finally, DNA degradation caused cell death (Dev et al. 2018; Tripathi et al. 2017). It is believed that callus, cell suspension, and hairy root cultures could be considered as an advanced strategy for the production of therapeutically important plant alkaloids (Moreno et al. 1995; Goldhaber-Pasillas et al. 2014). For example, the hairy root cultures of *Catharanthus roseus* caused the significant production of indole alkaloids such as horhammericine, catharanthine, lochnericine, and tabersonine (Li et al. 2011). Moreover, different alkaloids such as ajmalicine, serpentine, antirhine, cathindine, acuamicine, and lochnericine have been successfully obtained from the plant calli, cell suspensions, sprouts, pilose roots, somatic embryos, and vincristine

in sprouts and embryos (van Der Heijden et al. 2004; El-Sayed and Verpoorte 2007; Almagro et al. 2014). In fact, the activation of signaling pathways could modulate the gene expression levels which followed by continuous enzymatic reactions resulting in consecutively change in secondary metabolites production. Previously reported that any change in the activity of phenylalanine ammonia lyase, polyphenol oxidase, and peroxides could modulate the biosynthesis of secondary metabolites (Hatami et al. 2016). The influence of NPs on biosynthesis of secondary metabolites in plant cell and tissue cultures is shown in Fig. 14.3.

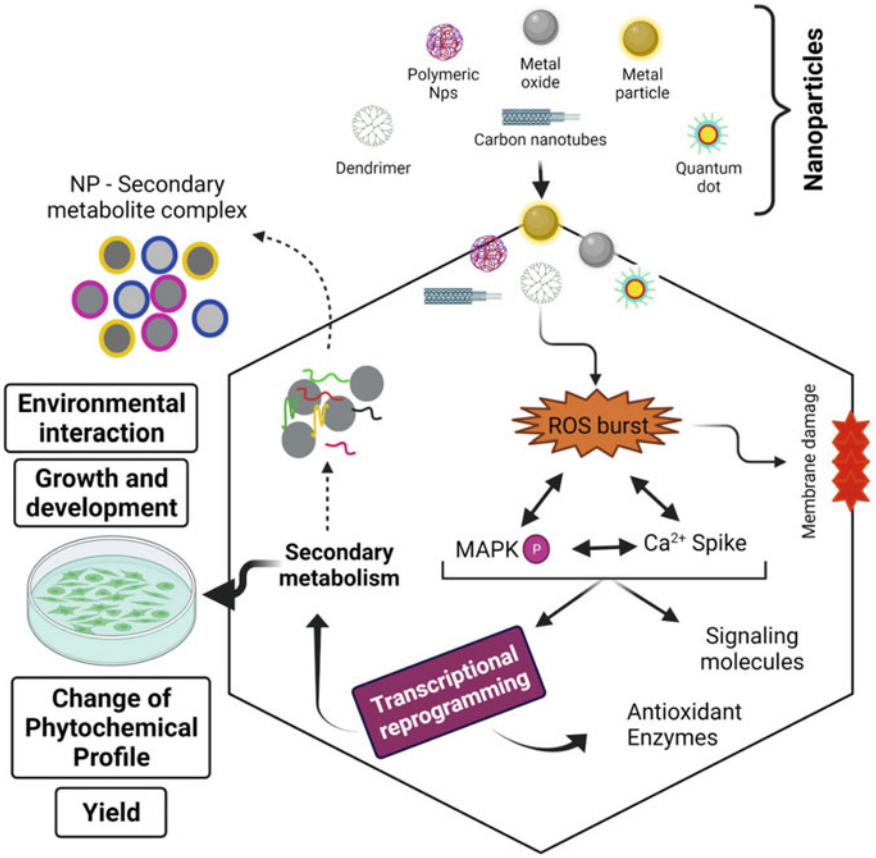

Fig. 14.3 The effect of nanoparticles on the synthesis of secondary metabolites in plant cell and tissue cultures

14.4 Mechanisms of Nanoparticles Affecting Callus

The callus culture could provide the required sterilized and reliable large-scale resources of plant materials for the synthesis of NPs have positive impacts on the callus physiology and secondary metabolites pathways through the production of oxidative stress which eventually activated plant metabolic reactions to inhibit the oxidative outbursts through the production of phytochemicals (Choi and Hu 2008). Biotic and abiotic stresses might suppress cell differentiation during callogenesis through the unwanted production of ROS or the production of toxic metabolites injured directly by the plant cells (Srinivasan 2007). Plant cells could fight against oxidative stress through several enzymes such as SOD, CAT, POD, and APX in which they scavenged the free radicals during cell division (Abbasi et al. 2011). It should be noted that the inclusion of different NPs into the tissue culture media might improve the morphogenetic potential of treated explants (Mandeh et al. 2012). The optimum concentration of AgNPs could improve the callus induction and biomass in the explants of *Phaseolus vulgaris* (Mustafa et al. 2017). While the precise physiological and molecular responses of this impact are yet understood, it is speculated that AgNPs may enhance the nutrient and water uptake capacity from the culture media by mutilating the plant cell wall (Ali et al. 2018). The chemical composition of NPs is mainly responsible for the motivation or inhibition effects of metallic oxide NPs on the callus cells and also the stresses induced by the size, shape, and surface of these NPs. It should be highlighted that the mechanism of transferring NPs across the cell membrane is not well understood, but it is believed that the use of NPs could increase the lipid membrane peroxidation induced by enhancement of ROS production and upregulation of MAPK cascades (Marslin et al. 2017). Moreover, size reduction, surface area enhancement, and capability of apoplastic or symplastic transportation could lead to more electrostatic interactions of many NPs with the living cell membranes resulting in the activation pathways for the biosynthesis of secondary metabolites in the plant cells (Javed et al. 2017a). Upon exposure to NPs, plant cells suffered a series of cascade reactions resulting in oxidative outbursts, ROS generation damage, and subsequent disruption of cell membrane and nuclei. Plants have activated their metabolic pathways such as secondary metabolites induction and MAPK cascades to inhibit intense stress situations and improve the ROS scavenging capacity (Sinha et al. 2011). CAT and APX antioxidant enzymes could significantly scavenge ROS and play a crucial role in the mitigation of oxidative stress (Garg and Manchanda 2009). It should be highlighted that the precise physiological and molecular responses of plant suspension and callus cells to the NPs are still unclear (Bezirğanoğlu 2017; Elmaghrabi and Ochatt 2006).

14.4.1 Impact of Nanoparticles on Quantitative and Qualitative Features of Calli

The treatment of *Salvadora persica* callii with ZnO, SiO2, and Fe3O4NPs increased callus growth rates and improved the production of constituent benzyl isothiocyanate. Further data identified that the increment of benzyl isothiocyanate activity was associated with the decrease of H_2O_2 content and the increase in the activity of superoxide dismutase and peroxidase. Moreover, the genomic DNA stability was reduced when higher doses of NPs utilized (Fouda et al. 2021). CuO, ZnO, and CaO NPs could present an effective approach for the protection of alfalfa callus against NaCl stress (Simsek et al. 2021). The treatment of wheat and *Stevia rebaudiana Bertoni* calli with ZnO NPS could increase proline concentration, flavonoid contents, and antioxidant enzyme (Javed et al. 2018; Barbasz et al. 2016). Exposure of Zn and ZnO NPs on callus cultures of bananas induced a significant decrease in growth rate but it enhanced the total proline associated with CAT, SOD, and POD activities. Despite the antifungal and antibacterial properties, further analyses confirmed NPs have no negative effects on explants regeneration (Helaly et al. 2014; Rad et al. 2020). Ag NPs could present positive effects on plant organogenesis through the inhibition of ethylene production. Upon exposure to Ag NPs, the number of shoots, their lengths, and the percentage of produced shoots were substantially enhanced in the nodal explants of *Tabernaemontana undulata* (Aghdaei et al. 2012c).

14.5 Some Important Applications of Nanomaterials in PTC

14.5.1 Somaclonal Variation

Generally, any changes in chromosome structure and number, DNA sequence, DNA arrangement, and transposable elements activation have been known as somaclonal variation (Kim et al. 2017). Moreover, somaclonal variation is proposed for the description of the plant tissue culture-induced phenotypic and genotypic variation in regenerated plants (Ngezahayo et al. 2007). Indeed, this parameter could evaluate the genetic and epigenetic variation that existed between clonal regeneration and the relative plant (Kaeppler et al. 2000; Wang and Wang 2012). It is identified that the use of gold and silver NPs could in vitro evaluate somaclonal variation in the coding sequence of methylesterase and also Mlo-like protein during tissue developmental stages of donor plant, calli, and regeneration in the *Linum usitatissimum* (Kokina et al. 2017b). Moreover, the treatment of *Vanilla planifolia* plantlets with different concentrations of AgNPs induced changes in repeat units and also polymorphism in its nuclear genome. Interestingly, the polymorphism percentage was enhanced by the increase in the concentration of AgNPs (Bello-Bello et al. 2018). Of note, the addition

of AgNPs to the culture medium induced variation in morphology, anatomy, protein content, and DNA profile of *Solanum nigrum* calli (Ewais et al. 2015). Somaclonal variations might create plants associated with several key features such as higher secondary metabolite production and more resistance to stresses (Kim et al. 2017).

14.5.2 Organogenesis

Different NPs (Au and Ag) could be effective on the inhibition or induction of regeneration capacity and growth of adventitious organs such as roots and shoots through the inhibition of ethylene production (Kim et al. 2017). It is confirmed that tobacco root cells could directly uptake AgNPs resulted in significant adventitious roots formation (Cvjetko et al. 2018). Moreover, the treatment of *S. viarum* and *Gentiana lutea* cells with silver nitrate NPs might induce root formation (Purine et al. 2015; Petrova et al. 2011). Further reports showed that suitable concentrations of AgNPs and AuNPs have positive effects on the random organogenesis in chrysanthemums, gerbera, and cape primrose (Tymoszuk and Miler 2019). It should be noted that shoot induction percentage and also their lengths were significantly improved upon the treatment of stem and nodal explants of *Tecomella undulata* treated with AgNPs (Aghdaei et al. 2012a).

14.5.3 Somatic Embryogenesis

Somatic embryogenesis, developed from somatic cells, is an effective method for micropropagation, regeneration of new plants, and genetic improvement of plant cells (Aghdaei et al. 2012a). Figure 14.4 Cu-NPs could significantly trigger the regeneration capacity of Ocimum basilicum through somatic embryogenesis. Indeed, Cu-NPs presented a higher potential for the production of somatic embryos compared to the plantlets/explant treated with $CuSO_4 \cdot 5H_2O$ (Ibrahim et al. 2019). Notably, ZnO NPs might positively increase the callus and somatic embryo induction (Devasia et al. 2020). In addition, the treatment of rhizome of Panax vietnamensis with Ag NPs could intensely induce somatic embryogenesis and plantlets (Du et al. 2021) (Fig. 14.5a, b).

The use of Phyto molecule-coated *Ulva lactuca* silver NPs (ULAgNPs) could also induce somatic embryogenesis and plant regeneration capacity in the rhizome explants of *Gloriosa superba.* Similarly, Ag NPs could efficiently enhance the percentage of somatic embryogenesis (almost 40%) in the explants of *Begonia tuberousvia* through cell layer culture (Mahendran et al. 2018). Notably, Cu-NPs and also Fe_3O_4-NPs could significantly improve somatic embryogenesis in the explants of *Ocimum basilicum* (84%) and *L. usitatissimum* (100%) when compared to the

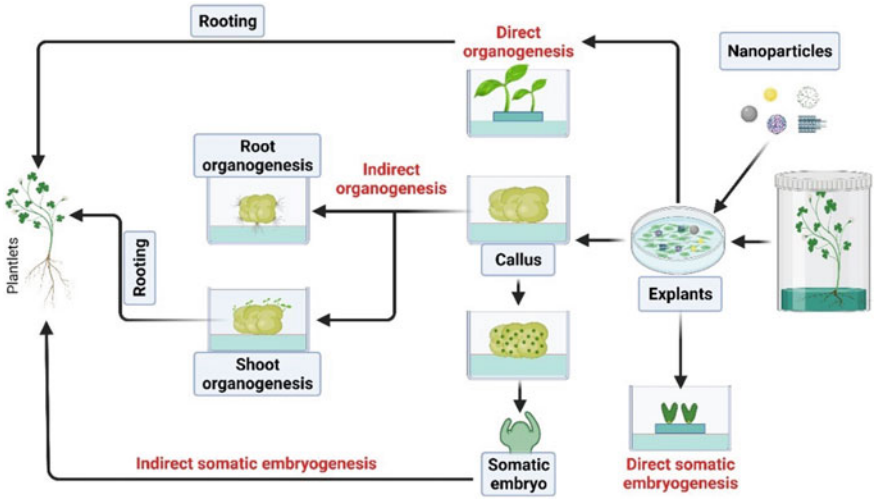

Fig. 14.4 effect of nanoparticles on organogenesis and somatic embryogenesis in plant tissue culture

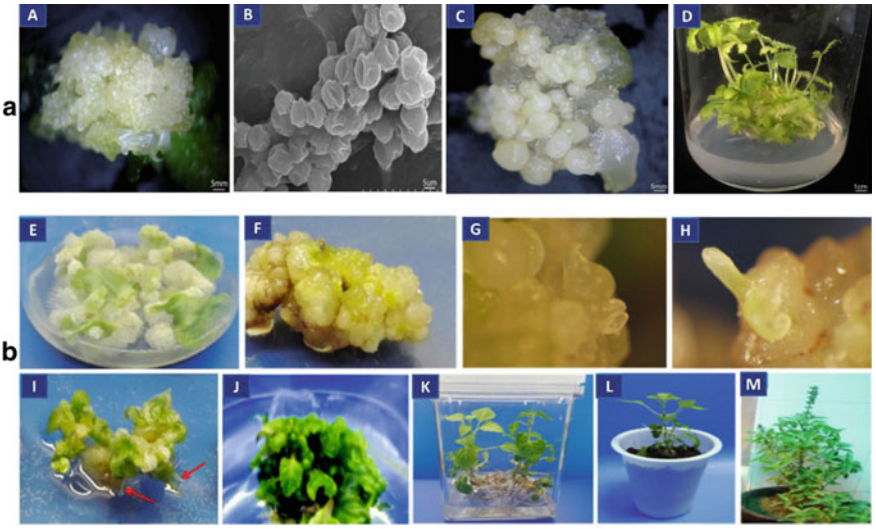

Fig. 14.5 The embryogenic calli induction and somatic embryo. *Panax vietnamensis* (**a**)*, Ocimum basilicum* (**b**)*. Source* (Du et al. 2021; Ibrahim et al. 2019)

control experiments (Ibrahim et al. 2019; Kokina et al. 2017a). However, the precise mechanism of NPs in somatic embryogenesis has not been understood yet, but these molecules might implicate their impacts by modulating the expression of some genes involved in embryogenesis (Kim et al. 2017).

14.5.4 Disinfection

Many NPs could be potentially utilized in superficial disinfection processes in the callus and cell suspension cultures (Sarmast and Salehi 2016). For instance, Ag NPs are effective in significantly decreasing bacterial contamination in the callus of *Vanilla planifolia* (Spinoso-Castillo et al. 2017). Moreover, Au NPs have frequently been utilised as an antimicrobial factor to surface sterilization of callus and explants in tissue culture experiments. The antibacterial, antiviral, antifungal, and antiseptic features of Au NPs have been relied on their potential to attack the wide range of organic processes in microorganisms inducing the disruption of the structure of plasma and cell membranes. These processes could lead to the depletion of intracellular ATP and cell death (Rudramurthy et al. 2016). Interestingly, plant-derived Au NPs could provide a better antimicrobial activity compared to the other NPs synthesized by physical and/or chemical methods. In detail, silver NPs are rapidly and environmental-friendly synthesized through the reduction of aqueous Ag^+ ions using *Dioscorea bulbifera* tuber extracts. The quality of the green AgNPs was evaluated by different approaches such as ultraviolet–visible absorption spectroscopy, high-resolution, and x-ray diffraction. Further data identified that this nanoparticle presented a potent antibacterial property against both gram-positive and gram-negative bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Ghosh et al. 2012).

14.5.5 Genetic Fidelity and Regeneration

Silver nano-complexes have positive impacts on the shoot regeneration capacity and genetic fidelity of in vitro-propagated *Alternanthera sessilis* cells. As a mutagenic factor, NPs could be efficacious for the induction of genotoxic effects in many plants because of their ease of interaction with plant cells (Kulus et al. 2022). Until now, the mutagenicity of ZnONPs and AgNPs was respectively confirmed in the *Allium cepa* and *Chrysanthemum* species (Kumari et al. 2011; Tymoszuk and Kulus 2020). The addition of AuNPs into the medium of *Lamprocapnos spectabilis* explants induced mutation in its genome which was detected by several molecular markers such as RAPD, SCoT, and DAMD markers (Kulus et al. 2022). These mutations mediated by NPs might result in phenotype and physiological variations in plants leading to the creation of new variants with improved characteristics. Moreover, the use of Phyto molecule-loaded silver nano-complex with AdS combination highly increases multiple shoot regeneration capacity in the *A. sessilis* cells (Venkatachalam et al. 2017). It is believed that many NPs especially Ag-related NPs have presented positive impacts on the improvement of regeneration capacity of different plant cell and callus cultures. In fact, NPs could downregulate several genes such as

1-aminocyclopropane-1-carboxylic acid (ACC) and 2-chloroethyl phosphonic acid (CEPA) to induce the pant regeneration pathways (Helaly et al. 2014). Moreover, the supplement of several plant cells such as tobacco, triticale, rape, and wheat with the increasing concentration of $CuSO_4$ NPs could improve the regeneration capacity of shoots (Purnhauser and Gyulai 1993). In addition, regeneration capacity through somatic embryogenesis in different recalcitrant cereal plants (e.g., barley, bread wheat, durum wheat, and rice) were enhanced upon treatment with a suitable concentration of CuNPs (Ibrahim 2012; Ibrahim et al. 2010; Eudes et al. 2003; Fahmy et al. 2012). It is also reported that CuO-NPs could significantly improve callogenesis and regeneration in the *Oryza sativa.* The suitable concentrations for improvement of regeneration capacity and callogenesis were identified as 20 mg/L and 10 mg/L of CuO-NPs (Anwaar et al. 2016).

14.6 Conclusions and Prospects

Today, nanotechnology has highly implicated in many industries especially agriculture, medicine, pharmacology, cosmetics, and environmental conservation. Different NPs have contributed to different aspects of plant biology including orogenesis, embryogenesis, tissue formation, differentiation, and development of plant cells and calli (Fig. 14.6). Notably, NPs especially are involved in the induction of secondary metabolites production and several pharmaceutical components through the up- or down-regulation of some plant genes. Moreover, plant cells and tissues could be considered as a more powerful platform for the production of different green NPs. However, further utmost research is needed to highlight the possible adverse effects of NPs on plant cell and tissue cultures. Plant cell and culture technology could be used as a green bio factory for the production of different valuable NPs. Notably, these green synthetized NPs could be regarded as a more powerful platform for drug delivery approaches provided fewer side effects.

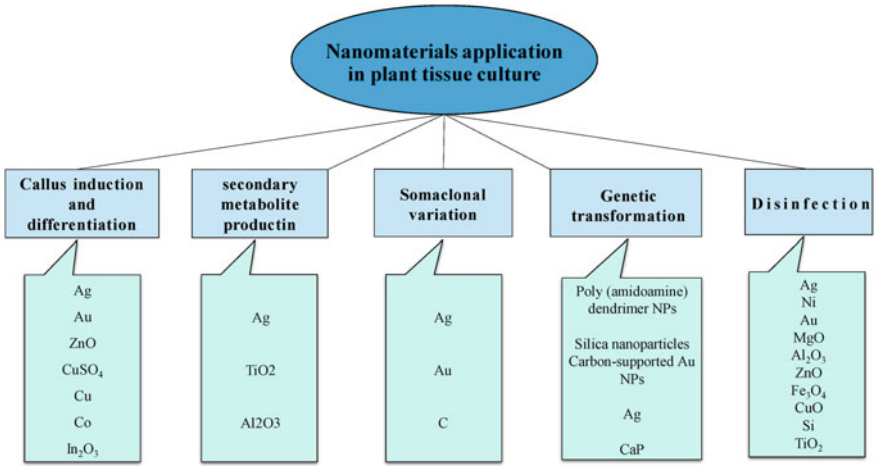

Fig. 14.6 Application of nanoparticles (NPs) in different aspects of plant cell and tissue culture approaches

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