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Metal Nanoparticles in Plant Cell, Tissue and Organ Culture

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Preface

Nanotechnology is considered as an emerging industrial revolution promoting development in all fields, especially biomedicine, energy, environment, agriculture, and information technology. Although this quick development raises questions about potential consequences that would be caused to the environment and living organs, nanomaterials and their effects on plant growth have recently attracted researchers around the world. The positive and negative effects of nanoparticles on plant growth depend on many factors, such as their composition, structure and concentration, and plant species. Utilizing metal nanoparticles in regulating *in vitro* settings is one of the ways to take advantage of the potentials to their fullest. Using metal nanoparticles has proven to be a vast attraction in plant tissue culture. According to numerous studies, *in vitro* rooting, callogenesis, organogenesis, somatic embryogenesis, and other processes can all be positively influenced by the application of non-toxic doses. Metal nanoparticles have indeed been proved being able to enhance the *in vitro* growth in numerous plant species. Nanoparticles also can act as a disinfectant and a direct source of additional micronutrients in the culture medium. In addition, some metal nanoparticles are used as elicitation for producing bioactive compounds in many plants. However, no single published document has yet been known to provide an overview of recent studies conducted in plant cell, tissue, and organ culture nanotechnology. Especially, reference material about this new direction and current applications in research and production is unavailable.

This publication's primary goal is to present in-depth information on current practical studies in the application of nanotechnology in plant cell technology using various experimental methodologies and to highlight some practical examples of their use. The results showed that the supplement of silver (AgNPs), selenium (SeNPs), and silicon dioxide (SiO_2NPs) nanoparticles or replacing metal ion salts in the culture media with iron (FeNPs), cobalt (CoNPs), and copper (CuNPs) nanoparticles showed positive effects to the growth and development of *Phyllanthus amarus*, *Rosa hybrida* 'Baby love', *Dianthus caryophyllus*, *Panax vietnamensis*, *Panax vietnamensis* var. *langbianensis*, *Fragaria* × *ananassa*, *Chrysanthemum morifolium*, *Saintpaulia ionantha*, *Gerbera jamesonii* Revolution Yellow, *Dianthus caryophyllus*, *Passiflora edulis*, *Actinidia chinensis*, and *Limonium sinuatum* through indicators such as number of shoots, shoot height, plantlet height, rooting rate, number of roots, root length, number of new leaves, SPAD, rhizome formation rate,

rhizome size, and fresh and dry weights. The application of AgNPs, FeNPs, CoNPs, and CuNPs has helped overcome abnormal phenomena such as vitrification, leaf yellowing, leaf abscission, and explant browning known in *Fragaria* × *ananassa*, *Phyllanthus amarus*, *Rosa hybrida* 'Baby love', *Passiflora edulis*, *Chrysanthemum morifolium*, *Gerbera jamesonii* Revolution Yellow, and *Dianthus caryophyllus* by reducing the accumulation of ethylene gas in the culture vessel and increasing the activity of antioxidant enzymes. Especially in Chaps. 4 and 12, AgNPs and CuNPs were used as very effective surface disinfectants that can replace traditional disinfectants such as calcium hypochlorite [$Ca(ClO)_2$] and mercury chloride ($HgCl_2$). These alternative disinfectants are environmentally friendly and do not cause adverse effects on human health but are very effective in eliminating fungi and bacteria during the sterilization stage of cultures of *Panax vietnamensis*, *Panax vietnamensis* var. *langbianensis*, *Limonium sinuatum*, *Begonia* × *tuberhybrida*, *Actinidia chinensis,* and *Chrysanthemum morifolium*. In addition, the positive effects of AgNPs on *in vitro* flowering and fruiting of *Passiflora edulis* were also recorded for the first time in Chap. 7. For the first time, several under-studied nanoparticles were applied and brought about as good an effect as SeNPs—a metalloid element—in overcoming the phenomenon of callus formation at the root base and acted as an auxin to help increase the rooting ability in *Passiflora edulis* and *Gerbera jamesonii* Revolution Yellow in Chap. 13. Similarly, the ability of SiO_2NPs in overcoming the plantlet vitrification phenomenon has also been recorded and analyzed in Chap. 14. Simultaneously, the ability of FeNPs and SiO_2NPs to enhance the vigor of plantlets was also clearly demonstrated in Chaps. 11 and 14. Besides being applied in solid media, AgNPs were supplementing the microponic system to improve plantlet quality (Chap. 8). Some metal nanoparticle synthesis methods were also mentioned in Chaps. 2 and 3. The interaction of metal nanoparticles with plants causes changes in the physiology and biochemistry of plants, such as endogenous hormone levels, ethylene gas accumulation, antioxidant enzymes and non-enzymes, biosynthesis of secondary compounds, and the ability to absorb nutrients from the culture medium. Finally, some mechanisms of action of nanoparticles on the explants have also been clarified, thereby giving a better insight into the role of nanoparticles in plant cell technology.

We would like to express our gratitude to all of the authors for their excellent work advancing the scientific understanding of many areas of nanotechnology in plant cell culture. The information in this book should be broadly distributed among readers, promoting crosstalk. The review of this scientific literature will be helpful to scientists, plant breeders, biologists, gardeners, commercial enterprises, and students interested in this new direction in micropropagation.

Dalat City, Vietnam — Duong Tan Nhut
Hochiminh City, Vietnam — Hoang Thanh Tung

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Editors and Contributors

About the Editors

Duong Tan Nhut graduated from the Kagawa University (Japan) with a major in plant biotechnology. He is the Vice Director and President of Scientific Advisory Council of Tay Nguyen Institute for Scientific Research (VAST). He has more than 460 peer-reviewed international and national publications, and he is editor-in-chief of the book *Plant Tissue Culture: New Techniques and Application in Horticultural Species of Tropical Region* by Springer Nature (Singapore, 2022) and contributor of nearly 30 book chapters for Springer. He has delivered numerous oral and poster presentations in various international meetings. He was President of Biology—Agriculture—Life Sciences Council (Vietnam National Foundation for Science and Technology Development) from 2015 to 2017. He is Vice President of Vietnam Plant Physiology Association in 2003 to date, Member of the Council of Dalat University (Vietnam) in 2013 to date, and editorial board member of some international and Vietnam journals. He was awarded the best young scientist award at the international conference on tropical and subtropical fruit trees, Cairns, Australia, in 2000; the first Vietnamese record for asexual propagation of endemic orchid (*Paphiopedilum delenatii*) in 2002; many scientific works (basic research) achieved impressive research in the USA in 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, etc.

Hoang Thanh Tung graduated from the Hue University of Sciences (Vietnam) with a major in plant physiology. He is a researcher at the Institute of Applied Sciences, Ho Chi Minh City University of Technology (HUTECH, Vietnam). He is trained in plant anatomy at Calgary University (Canada) and in the field of bioindustrial science, Tsukuba University (Japan). He is an editor of the book *Plant Tissue Culture: New Techniques and Application in Horticultural Species of Tropical Region* by Springer Nature (Singapore, 2022) and contributor of two book chapters for Springer and has delivered numerous oral and poster presentations in various international meetings.

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Undeniable Positive Impacts of Metal Nanoparticles in Plant Tissue Culture

1

Duong Tan Nhut

1.1 Introduction

Recent decades have seen a significant increase in interest in the use of metal nanoparticles (MeNPs) in plants for a variety of purposes, including disease diagnostics and management, increasing the production of significant secondary metabolites, and enhancing growth and stress resistance (Khan et al. 2022; Krishnani et al. 2022; Landa 2021; Rastogi et al. 2017). Nanoparticles (NPs) with diameters from 1 to 100 nm display distinct properties and can penetrate deep into plant cells (Khan et al. 2019). Many studies on the effects of NPs on plants have been published, and their mechanism of action is still the subject of discussion. The researchers suggested both positive and negative responses of NPs to in vitro plant growth and development. Factors such as particle size and shape, plant species, dosage, method of application, and exposure time are important factors in determining the effects of NPs on plants (Landa 2021).

Plant tissue culture (PTC) is a fundamental component of plant biotechnology, and progress in various areas of biotechnology depends heavily on the improvement of this technique. The PTC technique provides an ideal condition for studying the effects of MeNPs on plant cells. Some studies on the effects of metal and metal oxide nanoparticles on plants have shown toxic effects on plants, while some studies also point to their beneficial role in the form of enhancement of plant growth parameters in vitro (Gao et al. 2023; Tripathi et al. 2017). Many studies have demonstrated that the application of nontoxic concentrations can positively influence many processes such as promoting seed germination, callogenesis, organogenesis, somatic embryogenesis, in vitro rooting, etc. MeNPs have been shown as growth promoters for many plant species in vitro (Mahajan et al. 2022). NPs can act as a disinfectant and a direct source of additional micronutrients in the culture medium

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(Ngan et al. 2020b; Tung et al. 2021c). In addition, some MeNPs are used as stimulants for producing biologically active compounds in many plant species (Inam et al. 2023).

NPs can be transported and accumulated in plants, leading to cytotoxicity. NP-induced phytotoxicity increased reactive oxygen species (ROS) during interactions between NPs and plants, leading to oxidative, DNA, or organelle damage (Gao et al. 2023). In addition, the rapid development of NPs leads to concern about their possible negative effects on plants and ecosystems (Saravanan et al. 2021). Therefore, the possible toxicity risks associated with the application of MeNPs in plants need to be assessed and standardized for hazard level. Under that circumstance, approaching the effects of NPs in controlled in vitro conditions based on PTC techniques is feasible and offers many advantages over direct studies in the environment. This chapter provides an overview of the effects of MeNPs [such as silver nanoparticles (AgNPs), cobalt nanoparticles (CoNPs), iron nanoparticles (FeNPs), and copper nanoparticles (CuNPs), etc.] on PTC in a variety of plants. It covers the current knowledge and prospects that focus on the role of MeNPs in PTC based on our research and the collection of relevant literature available worldwide.

1.2 The Basic Mechanism of the Interaction Between MeNPs and Plants In Vitro

NPs with unique characteristics have advantages in penetrating plant cell wall. The transport of NPs within plants is closely related to the size, intensity, and zeta potential (Syu et al. 2014). NPs interact with plants causing many morphological, physiological, and biochemical changes. Based on many reports, NPs were generally toxic to plants at high concentrations (Rastogi et al. 2017). The researchers from their findings suggested both positive and negative effects on plant growth and development, and the effects of NPs on plants depended largely on the composition, size, surface coating, physical and chemical properties, and dosage used. Based on their transport, properties, and reactivity, NPs can interfere with different metabolic activities to induce effects on plants (Fig. 1.1). In many cases, the exact mechanism of these effects has not been clearly discussed. Hence, more research is needed to determine the exact interaction pathway between NPs for plants.

Some common effects based on ROS production and interference with oxidative mechanisms have been reported in several plant species when exposed to MeNPs (Landa 2021). Protein modification, lipid peroxidation, and DNA damage when plants interact with NPs have been reported under the influence of ROS (Geng et al. 2022). Because of the multifunctional role of ROS, cells have evolved a potent antioxidant mechanism to precisely control ROS levels, including the production of enzymatic and nonenzymatic molecules. Several reports have shown that the production of antioxidant molecules in plants is increased under the influence of NPs, which confirms the regulation of the antioxidant system as a response to the interaction of NPs with plants (Gao et al. 2023).

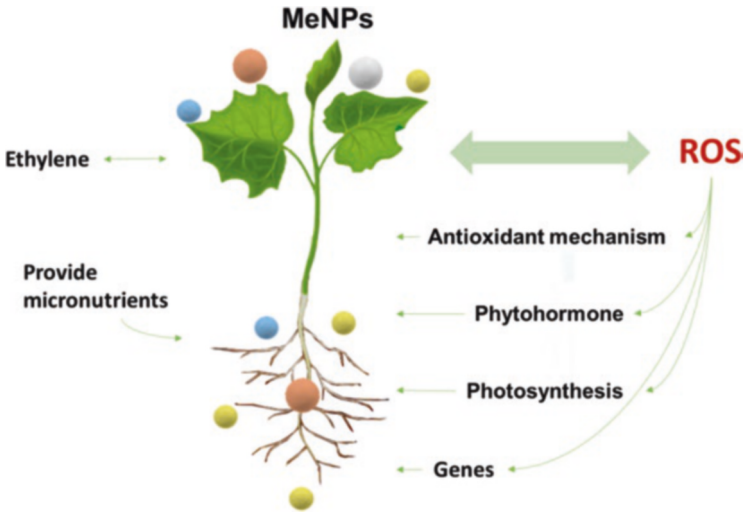

Fig. 1.1 Common mechanisms of MeNPs interaction with plants

ROS is also intricately linked to hormonal signaling and affects each other activity. Changes in the levels of auxin, cytokinin, abscisic acid, or melatonin were observed in plants exposed to certain MeNPs (Cuong et al. 2023b; Phong et al. 2022). It can be seen that NPs affect the hormonal balance in plants, thereby affecting the metabolism of plants. Excess ROS production also impacts electron transport chain processes in mitochondria and chloroplasts. The application of some NPs may be involved in the metabolism and uptake of nutrients, stimulation of chlorophyll biosynthesis and photosynthetic activity, enhancement of stomatal opening, and CO_2 exchange (Tripathi et al. 2017). Low concentrations of FeNPs promoted plant growth at the cellular level by altering the leaf organization, increasing the chloroplast number and grana stacking, and regulating the development of vascular bundles (Yuan et al. 2018).

In addition, some MeNPs such as AgNPs and CoNPs can affect ethylene biosynthesis in plants. At appropriate concentrations, the addition of AgNPs or CoNPs significantly reduced the amount of ethylene accumulated in the culture flask during tissue culture in several plant species, thus affecting plant growth and development in vitro (Cuong et al. 2021; Ngan et al. 2020a). Several other effects are related to metal ions released from NPs, which can be used by plants as micronutrients (Cu, Co, Zn, and Fe). These micronutrients are indispensable in Murashige and Skoog (MS) medium (Murashige and Skoog 1962) and are often added as corresponding salts.

In general, the complex interactions of MeNPs and plants are highly dependent on different experimental conditions, on different responses of different plant species, as well as on the characterization of the NPs. Therefore, the mechanisms responsible for the positive effects of NPs are still an issue that requires more focused research to clarify.

1.3 The Impact of MeNPs on PTC

In vitro culture of plants supplemented with NPs is emerging as an important technology. Some metals are essential micronutrients, or incorporated in many proteins and enzymes, and thus play an important role in plant growth and development. The effectiveness of NPs depends on their concentration and varies between plants. NPs are present in PTC with many roles such as antibacterial agent, source of micronutrients, promoter of callus, organogenesis, somatic embryogenesis, shoot growth, root formation, and elicitation for secondary metabolites (Table 1.1, Fig. 1.2). Common effects of the addition of NPs to the culture medium affect plants in vitro through changes in antioxidant enzyme activity, ROS production, gene expression, and inhibition of ethylene activity (Kim et al. 2017). However, the mechanisms of the promoting or inhibitory effects of NPs on each parameter need to be further investigated.

1.3.1 MeNPs as Explant Surface Disinfectant

Microbial contamination of cultures is an important problem faced in PTC procedures. Some common disinfectants such as hydrogen peroxide, sodium hypochlorite, mercury chloride, and antibiotics are used to decontaminate cultures and limit bacterial growth in tissue cultures. However, these agents can have serious cytotoxic effects on plant cells. Therefore, MeNPs with sterilization properties have great potential as surface disinfectants based on their distinct properties. Many studies demonstrate that MeNPs such as AgNPs and CuNPs are potential disinfectants and can replace conventional disinfectants (Bao et al. 2022; Tung et al. 2018). Among them, AgNPs are common and effective antimicrobial agents against some microorganisms that frequently contaminate plant culture media in vitro in tissue culture studies. The use of NPs brings advantages not only as decontamination agents but also as enhancers of morphological potential, increasing viability and reducing explant browning, which has been demonstrated by many studies (Nartop 2018). In addition, the effectiveness of MeNPs was also reported for the decontamination of seed-based explants. Besides sterilization ability, the application of NPs (usually AgNPs) also significantly improved seed germination parameters and early seedling development. The increase in germination and growth at lower AgNPs concentrations was thought to be related to the enhanced nutrient efficiency and water uptake in the seed treatments. On the other hand, the AgNPs or CuNPs synthesis from biological sources including plants and food waste is a simple production method, without harmful chemicals, reducing costs and reducing adverse impacts on the environment (Ghosh et al. 2020). Hence, decoding the economic viability of using NPs as a source of disinfectants in vitro in PTC is a potential direction.

The NP's effects on removing microbial contaminants in PTC depends on their size, type, and distribution. Some researchers have indicated the survival and subsequent growth negatively affected by NP during the application period. Therefore, it

Table 1.1 Some effects of MeNPs on tissue culture processes in some plant species

Plant species	Concentration of NPs	Effect	Source
Begonia × *tuberhybrida* Voss	0.0465 μg/L CoNPs	Stimulated plantlet growth	Vinh et al. (2023)
Calotropis procera	50–200 mg/L Fe_3O_4NPs	Improved growth and yield of hairy roots by elicitors	Adabavazeh et al. (2023)
Chrysanthemum morifolium	2 mg/L AgNPs	Improved shoot regeneration	Cuong et al. (2023a)
Coriandrum sativum	50–100 mg/L ZnOP NPs (proline-coated ZnO)	Increased shoot length and root length	Hanif et al. (2023a)
Coriandrum sativum Linn	100 mg/L ZnOBt NPs (ZnONPs that were coated with glycine betaine)	Enhanced morphological parameters of plants grown	Hanif et al. (2023a)
Cydonia oblonga mill.	2.5 mg/L ZnONPs	Improved shoot growth	Farhadi et al. (2023)
Gaillardia pulchella Foug cv. 'Torch yellow'	4.0 mg/L AgNPs	Improved shoot regeneration, root induction	Manokari et al. (2023)
Limonium sinuatum (L.) mill. 'White'	200 mg/L AgNPs 1.0 mg/L AgNPs 0.4 mg/L AgNPs	High disinfection Enhanced shoot regeneration Shorten rooting time of plantlets	Cuong et al. (2023b)
Passiflora edulis Sims f. *edulis*	2.0 mg/L AgNPs	Enhanced somatic embryogenesis	Phong et al. (2023b)
Passiflora edulis Sims f. *edulis*	AgNPs	Improved shoot regeneration and somatic embryogenesis	Phong et al. (2023a)
Stevia rebaudiana	12.5 mg/L AgNPs	Increased endogenous levels of diterpenes	Andújar et al. (2023)
Actinidia chinensis planch	200 ppm AgNPs	As a surface disinfectant	Hanh et al. (2022)
Begonia × *tuberhybrida* Voss	0.1–0.2 g/L CuNPs	High disinfection effect Enhance somatic embryogenesis	Bao et al. (2022)
Gerbera jamesonii revolution yellow	2.0 mg/L AgNPs 0.0465 mg/L CoNPs	Enhanced shoot multiplication and rooting, reduced vitrification, yellowing of the leaf	Tung et al. (2022)
Panax vietnamensis	0.7–5.6 mg/L FeNPs	Increased chlorophyll content, biomass, rhizome diameter, and length	Nhut et al. (2022)
Panax vietnamensis Ha et Grushv.	0.2 g/L AgNPs	Enhanced disinfection and somatic embryogenesis	Diem et al. (2022)

(continued)

Table 1.1 (continued)

Plant species	Concentration of NPs	Effect	Source
Panax vietnamensis var. *langbianensis*	0.15% AgNPs	Enhanced disinfection	Anh et al. (2022)
Passiflora edulis Sims f. *edulis*	3.0– 5.0 mg/L AgNPs 7.0 mg/L AgNPs	Improved shoot regeneration and flowering and fruiting	Phong et al. (2022)
Phalaenopsis amabilis	5 μM AgNPs	Improved shoot regeneration, fresh and dry weight	Farrokhzad et al. (2022)
Begonia tuberous	200–300 ppm AgNPs	Increased disinfection and somatic embryogenesis	Tung et al. (2021c)
Chrysanthemum morifolium Ramat cv. Jimba	250 ppm AgNPs	Increased disinfection and plant growth stimulant agents	Tung et al. (2021a)
Fragaria × *ananassa*	200 mg/L AgNPs 0.1–0.5 mg/L AgNPs	Increased disinfection and growth of shoot and shortened the duration of root formation	Tung et al. (2021b)
Panax vietnamensis	1.6 mg/L AgNPs	Enhanced somatic embryogenesis	Cuong et al. (2021)
Populus × *canescens* Aiton. Sm.	0.3 g/L AgNPs 1.5–3 μg/L AgNPs	Increased disinfection and root formation and accelerated the growth of the vegetative part of the shoots	Vasyukova et al. (2021)
Solanum lycopersicum L. 'Poranek' *Raphanus sativus* L. var. *sativus* 'Ramona' *Brassica oleracea* var. *sabellica* 'Nero di Toscana'	50–100 mg/L AgNPs	Germinated seedling	Tymoszuk (2021)
Triticum aestivum L.	0.015 mg/L CuNPs và 4.0 mg/L AgNPs	Stimulated embryogenic calli	Malik et al. (2021)
Chrysanthemum morifolium Ramat. cv. 'Jimba'	75–100 mM FeNPs	Improved plantlet growth	Tung et al. (2020)
Dianthus caryophyllus 'express golem'	0.075 mM FeNPs	Enhanced rooting, antioxidant activities and mineral absorption	Ngan et al. (2020b)
Phoenix dactylifera L., cv. Sewi and Medjool	500 μg/L AgNPs 125, 250 μg/L AgNPs	Increased disinfection, somatic embryogenesis	El-Kosary et al. (2020)
Rosa hybrida L. 'baby love'	2 mg/L AgNPs 4.65 μg/L CoNPs	Increased shoot multiplication and root induction	Ngan et al. (2020a)

(continued)

Table 1.1 (continued)

Plant species	Concentration of NPs	Effect	Source
Betula pubescens	1.5–3 µg/L AgNPs	Increased shoot multiplication and rooting, reduced phytopathogenic contamination of the explants and regenerants	Zakharova et al. (2019)
Chrysanthemum × *grandiflorum* (Ramat.) Kitam. 'Bydgoszczanka' *Gerbera* × *jamesonii* H. Bol 'Suri' *Streptocarpus* × *hybridus* Voss.	10–30 ppm AgNPs	Regenerated less adventitious roots	Tymoszuk and Miler (2019)
Linum usitatissimum	30 µg/L AgNPs	Enhanced lignans, phenolic content, flavonoid content, and biomass	Zahir et al. (2019)
Physalis peruviana L.	0.385 mg/L AgNPs	Promoted seedling biomass	Timoteo et al. (2019)
Stevia rebaudiana	12.5–50 mg/L AgNPs	Enhanced shoot production and length	Castro-González et al. (2019)
Lupinus termis L.	100 ppm AgNPs (synthesis of from the aqueous extract of *Coriandrum sativum*)	Germinated seed	Al-Huqail et al. (2018)
Rosmarinus officinalis L.	AgNPs synthesis from extracts of *Rubia tinctorum* cell	Enhanced surface disinfectant	Nartop (2018)
Saintpaulia ionantha H. Wendl.	0.05% AgNPs	Enhanced surface disinfectant	Nhut et al. (2018)
Panax vietnamensis	2.0 mg/L AgNPs 2.0 mg/L ZnONPs 1.5 mg/L CuNPs	Improved lateral root formation and growth	Linh et al. (2017)

is necessary to study the effects of NPs on explants derived from different plant species to determine the best dosage with no or minimal cytotoxicity.

1.3.2 MeNPs as Growth Stimulants in In Vitro Plant

Many reports have demonstrated the potential role of MeNPs in improving and enhancing tissue culture stages in many plant species. MeNPs are added to the culture medium and act as a stimulant for many processes, such as callogenesis, organogenesis, somatic embryogenesis, rooting, plantlet growth, and flowering and fruiting of in vitro plants (Table 1.1). However, MeNPs added to medium was not

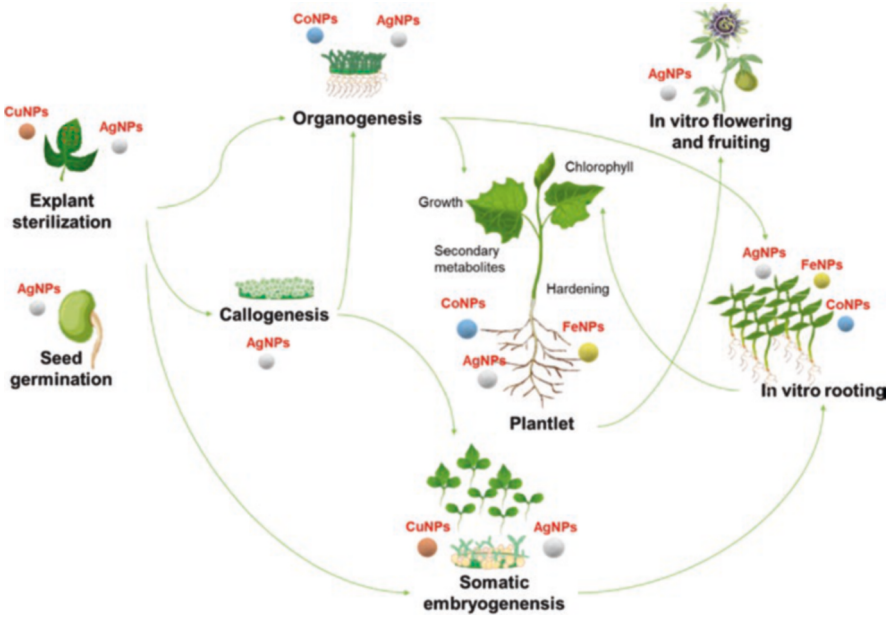

Fig. 1.2 Some common MeNPs have positive effects on several stages in PTC

completely favorable for in vitro plants. The growth in some plants was significantly decreased in high MeNPs concentrations (Farrokhzad et al. 2022).

Induction, proliferation, and transformation of callus in several plant species noted enhancement on medium supplemented with MeNPs. The AgNPs adding to culture medium enhanced callus induction/regeneration of *Oryza sativa* (Manickavasagam et al. 2019). The addition of AgNPs was also reported to significantly promote callus proliferation and embryogenic callus in purple passion fruit (Phong et al. 2023b). MS medium supplemented with biosynthetic AgNPs enhanced the callus formation and the fresh weight of *Solanum nigrum* callus; however, cell wall damage and callus deformation were also observed (Ewais et al. 2015). Significantly higher callus induction/regeneration in wheat (*Triticum aestivum* L.) were obtained from the regeneration medium supplemented with CuNPs compared with $CuSO_4$. Furthermore, combining CuNPs with AgNPs in the induction medium significantly enhanced embryogenic callus induction (Malik et al. 2021).

MeNPs have also been shown to enhance shoot induction, shoot multiplication, and shoot growth in vitro. Carnation micropropagation showed that AgNPs in MS medium increased the number of shoots per explant, plant weight, leaf size, and shoot height (Ngan et al. 2020a). The shoot regeneration efficiency of purple passion fruit was significantly improved by culturing cell thin layer cultures in medium supplemented with AgNPs (Phong et al. 2023a). *Gaillardia pulchella* Foug cv. 'Torch Yellow' shoots were developed based on an medium treatment method that combines AgNPs for a healthier, stronger, and greener appearance (Manokari et al. 2023). Some reports indicated that antioxidant enzymes triggered when explants

treated with AgNPs had an effect on shoot proliferation and thereby enhanced the number of shoots per explant (Saha and Gupta 2018; Sarmast et al. 2015).

Several MeNPs have also been reported to play an active role in somatic embryogenesis in some plants. For example, medium supplemented with AgNPs enhanced somatic embryogenesis in many plants. Culture medium supplemented with AgNPs significantly enhanced callus induction and proliferation in *Panax vietnamensis* (Cuong et al. 2021). The better somatic embryo maturation was obtained in the medium by adding to AgNPs in *Passiflora edulis* (Phong et al. 2023b). The influence of AgNPs in promoting embryo maturation opened a potential research direction of somatic embryogenesis. In addition, the receptor blocking involved in ethylene by AgNPs enabled polyamine synthesis, an essential factor in the maturation of SEs (Bais et al. 2000; Rakesh et al. 2021). CuNPs also enhanced somatic embryogenesis. The explants treated with CuNPs observed the increase of number of somatic embryos and the percentage of somatic embryos (cotyledon-shape) in *Begonia* × *tuberhybrida* Voss (Bao et al. 2022). In addition, CuNPs have been shown to significantly enhance the proportion of explants that produce somatic embryos and the regenerative capacity of *Ocimum basilicum* L. plants through somatic embryogenesis (Ibrahim et al. 2019).

Positive effects of some MeNPs have also been reported on in vitro rooting in several plant species. AgNPs significantly increased root length, and these roots had small diameters in *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam (Tymoszuk and Miler 2019). Similar effects were observed on strawberry (*Fragaria* × *ananassa*) micropropagation and rooting, where adding AgNPs stimulated seedling growth and shortened chrysanthemum root formation time during micropropagation (Tung et al. 2021b). Supplementation of CoNPs at the rooting stage significantly improved the dry weight ratio, number of roots, and root length incarnations (Ngan et al. 2020a). Similarly, FeNPs in the MS medium positively affected the rooting stage and mineral uptake of carnation (Ngan et al. 2020b).

MeNPs added to culture medium also improved plantlet quality of many plants. For instance, AgNPs have been found to be an effective adjuvant to improve chlorophyll index and overcome culturing leaf drop in many plant species (Ngan et al. 2020a). FeNPs significantly enhanced chlorophyll content, rhizome development, and plantlet growth in *Panax vietnamensis* (Nhut et al. 2022). FeNPs added to MS medium increased antioxidant activity in *Chrysanthemum morifolium* Ramat. cv. 'Jimba' and *Dianthus caryophyllus* 'Express golem' in both solid in vitro and microponic cultures, thereby increasing plantlet viability (Ngan et al. 2020b; Tung et al. 2020). Moreover, CoNPs was reported to limit leaf yellowing and leaf drop in roses by inhibiting the activity of defoliation enzymes and ethylene gas biosynthesis, leading to improved survival rates in roses (Ngan et al. 2020a). Many MeNPs have been reported to enhance stress resistance (salt stress, drought stress, cold stress, etc.) in many plant species under in vitro conditions (Al-Khayri et al. 2023; Mozafari et al. 2018).

Several studies have shown that AgNPs have an impact on the reproductive stage in plants. In *Arabidopsis thaliana*, AgNPs slowed flowering, reduced pollen viability, and reduced fruit quality. Furthermore, the downregulation of gene expression

levels responsible for flowering and flower development was also observed (Ke et al. 2020). In contrast, some positive effects of AgNPs have been reported in several plant species in vitro. Ngan et al. (2019) recorded flowering in roses in the culture medium supplemented with AgNPs (Ngan et al. 2019). Phong et al. (2022) also reported a high flowering rate from apical shoots of purple passion fruit in a medium supplemented with AgNPs under in vitro culture conditions. In addition, this study also showed that the addition of AgNPs increased the rate of fruiting and fruit size in this plant (Phong et al. 2022).

1.3.3 Effect of MeNPs on the Production of Plant Metabolites In Vitro

In vitro plant culture has emerged as an important technology for the production of pharmacologically important secondary metabolites and other commercially valuable products. Various strategies were developed to improve the secondary metabolite production in plant cultured in vitro, in which elicitation emerged as a simple and rapid response strategy to enhance the secondary metabolite production. Biotic and abiotic elicitors were used to elicit secondary metabolites. In recent years, nano-stimulators with different types of NPs have been used to produce valuable secondary metabolites. Currently, MeNPs have been used to promote secondary metabolites of different plants cultured in vitro (Singh et al. 2023). AgNPs are promoter secondary metabolites of different plants cultured in vitro (Anjum et al. 2019; Mahajan et al. 2022). Besides, other metal NPs and metal oxides of Au, Zn, Cu, Ti, and Fe are also used to enhance the production of a number of secondary compounds (Inam et al. 2023; Rivero-Montejo et al. 2021).

Many studies have documented the positive regulation of MeNPs on both the content and composition of secondary metabolites in plants. When NPs are applied to plants, the accumulation of secondary metabolites is enhanced in PTC as a natural defense mechanism. Enhancing secondary metabolite content is often reported to positively correlate with enzymatic and nonenzymatic activity involved in the activation of both antioxidant defense mechanisms and secondary metabolism. These protective reactions often lead to an increase or de novo biosynthesis of secondary metabolites that had commercial applications, such as phenolics, flavonoids, alkaloids, terpenoids, anthocyanins, and essential oils (Lala 2021). Fouad and Hafez (2021) were reported the better performance of CoNPs than Co^{2+} in enhancing β-glucuronidase gene expression from *A. tumefaciens* strain in *Catharanthus roseus* suspension culture (Fouad and Hafez 2021). That suggests a potential of MeNP in enhancing therapeutically compound production using transient expression of relevant genes.

The feasibility of applying MeNPs for large-scale production needs to be critically evaluated. The application of NPs as promoters of secondary metabolites depends on the chemical composition, size, shape, concentration, and plant species. Therefore, each production system needs to optimize the relevant parameters for maximum production of secondary metabolites in each particular case. In addition,

the properties of secondary metabolites obtained from plants treated with MeNPs can be investigated in detail to determine their quality and efficacy. The toxicity risks related to MeNP application for secondary metabolite generation must also be rigorously evaluated to achieve maximum secondary metabolite yields with minimal toxic effects on plants and humans or environment. In conclusion, if this technology is properly standardized and regulated, elicitation of plant secondary metabolites by MeNPs holds great promise for plant secondary metabolite production (Lala 2021).

1.3.4 MeNPs as Decontamination Agents and Nutrients Added to Culture Media

In addition to being used as a surface disinfectant, AgNPs are also added directly to the culture medium to limit microbial growth. During the culturing of chrysanthemum plants, the non-autoclaved MS medium treated with AgNPs was uncontaminated after 4 weeks. In addition, chrysanthemums grown in MS medium (without sterilized) treated with AgNPs showed high antioxidant activity and were better adapted to greenhouse conditions (Tung et al. 2021a). Therefore, the research to reduce production costs from the application of AgNPs is a potential direction.

On the other hand, MeNP particles can be added to the culture medium. A number of researches indicated that the addition of micronutrients in the culture medium in the form of NPs has some positive effects on plant growth and development compared with metal salt. Replacing $CoCl_2$ with CoNPs (with a ratio of ¾) in MS medium increased the SPAD index, decreased the ethylene gas content in the culture flask, and decreased the enzyme activities (pectinase and cellulase). In addition, the growth and development of seedlings at the nursery stage on medium supplemented with CoNP was significantly improved (Ngan et al. 2020a). Some positive effects of replacing iron salts with FeNPs on some plant species have been reported. Chrysanthemum plantlets were cultured on MS medium replacing Fe-EDTA with FeNPs significantly improved growth, chlorophyll content, antioxidant enzymes, and acclimatization of in vitro, and hydroponic and microponic systems (Tung et al. 2020). Similarly, 0.075 mM FeNPs replaced by 0.1 mM Fe-EDTA in ½MS medium which had a positive effect on antioxidant activity and mineral uptake of carnation (Ngan et al. 2020b). FeNPs in culture medium have also been shown to have a negative or positive effect on water and mineral absorption, depending on the concentration and culture system (Joseph et al. 2015; Sheykhbaglou et al. 2018). In addition, NPs can also be engineered to facilitate plant growth. For example, AgNPs with auxin rooting hormones as a stabilizing agent enhanced root growth and the rooting capabilities against root growth inhibiting phytopathogens in *Nicotiana tabacum* (Thangavelu et al. 2018). ZnO NPs that were coated with glycine betaine or proline significantly improved the morphological indicators of plants grown under drought stress in *Coriandrum sativum* (Hanif et al. 2023a, b). Therefore, the study and application of the dual effects of MeNPs on plants is a potential research direction.

1.4 Recent Results on Metal Nanoparticles in Plant Cell Technology

Finding a new scientific direction is complex and exciting. New directions in a particular field often give us new directions that benefit science. In plant cell technology, creating a culture medium is challenging to find a suitable medium for plant growth and development artificially for studying plant physiology, biochemistry, genetics, molecular biology, etc. The new direction in this research is to replace metal ions in the culture medium with metal nanoparticles to increase plant cell, tissue, and organ growth. The "industrial revolution" of nanotechnology has been credited with accelerating progress across all industries, including biomedicine, energy, the environment, agriculture, and information technology. Researchers from all over the world have recently become interested in how nanoparticles affect plant growth. There have been investigations into how metal nanoparticles affect plants. Numerous variables, including composition, structure, concentration of nanoparticles, and plant species, have positive and negative effects on plant growth. The research team led by Prof. Dr. Duong Tan Nhut is the first in the world to have studied this issue for more than 10 years and published more than 50 articles in domestic and intentional journals (Q1). This problem has helped to find an exciting new direction in plant cell technology. Metal (AgNPs, FeNPs, CoNPs, CuNPs, MoO_3NPs, SiO_2NPs, ZnONPs, etc.), metalloid (SeNPs), and rare earth (CeO_2NPs) nanoparticles have been helpful in many plant micropropagation applications, acting as a disinfectant for explants, medium sterilization, and acting as plant growth regulators to improve the plant regeneration process, rooting stage, and abnormal phenomena in micropropagation, thereby increasing survival rate of plantlets under nursery conditions. Additionally, using nanoparticles, particularly silver nanoparticles, aided in studying in vitro flowering. Plant physiology and biochemistry are altered due to interactions between metal nanoparticles and plants. Examples include changes in ethylene gas accumulation, endogenous hormone levels, antioxidant enzymes and nonenzymes, secondary compound biosynthesis, plants' capacity to absorb nutrients from the culture medium, etc. A better understanding of the role of nanoparticles in plant cell technology has been provided by clarifying some of the mechanisms of nanoparticles action on the explants. These innovative studies improved the micropropagation of some valuable plant species, including *Panax vietnamensis* Ha et Grushv., *Panax vietnamensis* var. *langbianensis*, *Passiflora edulis*, *Gerbera* L., *Fragaria* × *ananassa*, *Limonium sinuatum* L., *Phyllanthus amarus*, *Begonia* × *tuberhybrida* Voss, *Actinidia chinensis*, *Dianthus caryophyllus* L., *Chrysanthemum morifolium*, and *Saintpaulia ionantha* H. Wendl., creating new medium cultures in micropropagation and advanced plant cell technology studies. We will outline the most recent developments in the use of nanoparticles in plant cell, tissue, and organ culture in this report.

Molecular Biology and Plant Breeding Department (Taynguyen Institute for Scientific Research, Vietnam) and some results of metal nanoparticle applications in explant surface disinfection, culture medium sterilization, and morphogenesis on some plants (Figs. 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 1.10, 1.11, 1.12, 1.13, 1.14, 1.15,

Fig. 1.3 Professor Duong Tan Nhut (right) and Professor Kim Tran Thanh Van (France—the first scientist in the world who introduced the "thin cell layer" concept in the famous ***Nature*** Journal in 1973; third from right) at the Quy Nhon International Biology Conference 2023

Fig. 1.4 Professor Duong Tan Nhut (middle) and his research group (Taynguyen Institute for Scientific Research, Vietnam)

1.16, 1.17, 1.18, 1.19, 1.20, 1.21, 1.22, 1.23, 1.24, 1.25, 1.26, 1.27, 1.28, 1.29, 1.30, 1.31, 1.32, 1.33, 1.34, 1.35, 1.36, 1.37, 1.38, 1.39, 1.40, 1.41, 1.42, 1.43, 1.44, 1.45, 1.46, 1.47, 1.48, 1.49, 1.50, 1.51, 1.52 and 1.53).

Fig. 1.5 Professor Duong Tan Nhut (middle), staffs (green blouse), and students (white blouse) at Molecular Biology and Plant Breeding Department (Taynguyen Institute for Scientific Research, Vietnam)

Fig. 1.6 Professor Duong Tan Nhut (right) and Professor Kee Yoeup Paek (Korea—Father of "big-scale bioreactor" culture system; second from right) at Molecular Biology and Plant Breeding Department (Taynguyen Institute for Scientific Research, Vietnam)

Fig. 1.7 Commercial production laboratory for plant micropropagation at Dalat City (Lam Dong Province, Vietnam)

Fig. 1.8 The application of a nylon bag culture system combined with a millipore membrane filter (pioneered by Professor Duong Tan Nhut) in the production of high-quality plantlets at Dalat Flower Forest Biotechnology Corporation

Fig. 1.9 The application of nylon bag culture system in plant micropropagation at Dalat City (Lam Dong Province, Vietnam)

Fig. 1.10 The application of nylon bag culture system in plant micropropagation at Dalat Flower Forest Biotechnology Corporation

Fig. 1.11 The application of nylon bag culture system in artichoke micropropagation with well growth and development at Taynguyen Institute for Scientific Research

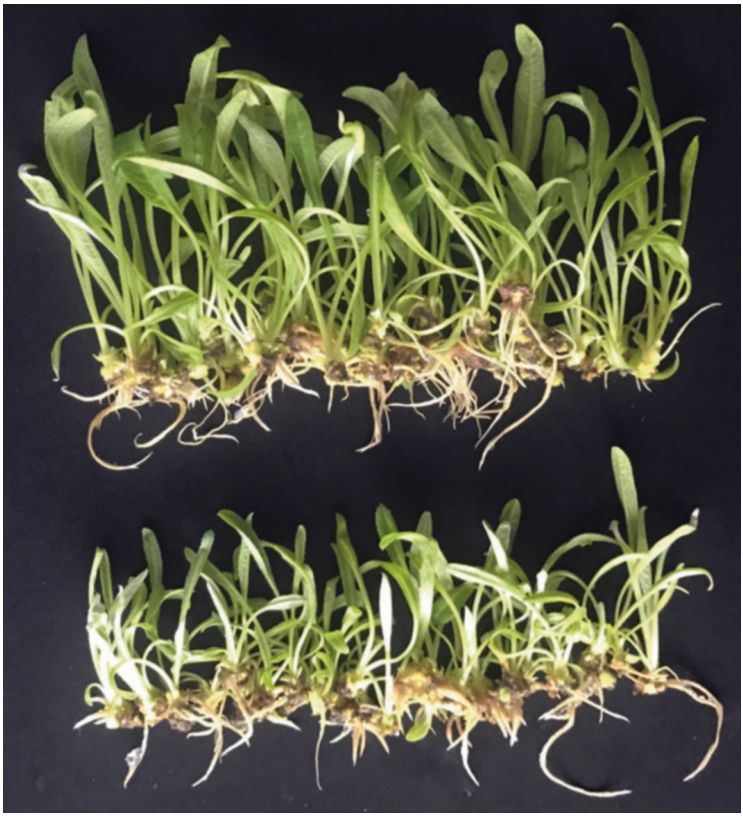

Fig. 1.12 Artichoke plantlets derived from nylon bag culture system

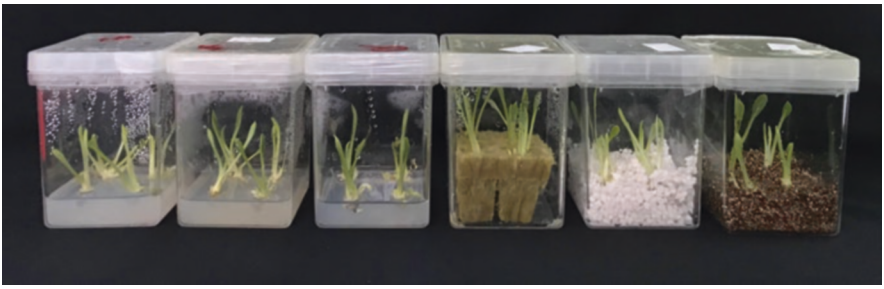

Fig. 1.13 The application of different substrates in artichoke micropropagation at Taynguyen Institute for Scientific Research

Fig. 1.14 Artichoke plantlets acclimatized well in the ex vitro condition

Fig. 1.15 The application of nylon bag culture system in *Panax vietnamensis* Ha et Grushv. micropropagation at Taynguyen Institute for Scientific Research