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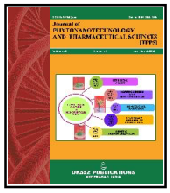

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Nanotechnology and current advances in plant tissue culture

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Abstract

The evaluation of biological techniques like nanotechnology for the production of nanoparticles (NPs) such as silver (Ag) gold (Au), zinc (Zn), and titanium (Ti) from plant extracts has drawn the attention of many researchers. Nanotechnology, as a cutting-edge modern technique, has also entered in the field of plant tissue culture due to their added advantages and being an alternative to the limitations of previously used traditional compounds as disinfectants, and growth manipulators during *in vitro* culture of shoot, root or whole plants. Nanomaterials are now used frequently in plant tissue culture; as sterilizing agents for decontamination of nasty bacteria and fungi, regeneration of callus and shoots, to increase the production of secondary metabolites, to improve *in vitro* seed germination, and somatic embryogenesis. The clear mechanism through which NPs interact at cellular or tissue level during initiation and growth of *in vitro* grown culture and modulate the secondary metabolism of the plant for the production of desired products is not exactly implicated. The current article highlights the multidisciplinary role of NPs application and its current status in the area of plant tissue culture and emphasized to explore the newer theoretical concepts related to mechanism through which the nanotechnology can fully be utilized for the desirable results in the area of plant biotechnology.

1. Introduction

Currently nanotechnology is one of the important research fields in modern science based on the specific properties (size, shape and distribution) of nanoparticles (NPs). The synthesis of nanoparticles has attracted considerable interest in recent decades due to their widespread use in catalysis, sensors, electronics, photonics and medicine (Duan *et al*., 2015). Applications using nanomaterials and NPs are developing rapidly (Jain *et al*., 2009). The field of nanotechnology has been found to be one of the most active areas of research (Kuntal, 2021; Sergeev *et al*., 2008).

Scientists have understood the ability of biological organisms to regenerate metal precursors since the 19th century, but the mechanism remains unknown. Nanotechnology can be used to develop a wide range of products, such as cadmium sulfide quantum dots (Q-dots), hybrid titanium oxide-based electrochemical biosensors, and heparinized oxorubicin-containing nanoparticles, with applications in a wide range of scientific fields, including; optoelectronics, biosensors, nanobiotechnology, and biomedicine (Mehata, 2015; Shetti *et al*., 2019; Neculescu *et al*., 2021). Researchers have turned their attention to biological methods due to the success of synthesizing nanoparticles using natural reducing agents, occlusive agents and stabilizers, and avoiding hazardous chemicals (consumption as well as the use of chemicals) and high energy costs (Korbekandi *et al*., 2009; Luangpipat *et al*., 2011; Arumugam *et al*., 2015).

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Modern nanotechnology uses advances in chemistry, physics, materials science, and biotechnology to create new materials with unique properties because structures are determined on the nanometer scale. Plant tissue culture is also greatly affected by noteworthy applications of nanomaterials. Some applications of NPs in plant tissue culture are made to improve the seed germination, plant growth, provide genetic modifications to plants, improve production of bioactive compounds, and protect plants from the pathogens (Wang *et al*., 2016; Ruttkay-Nedecky *et al*., 2017).

Plant genetic resources are of paramount importance to plant scientists, especially plant breeders and biotechnologists as nature has hidden variability in rare germplasms. One of the best known uses of nanotechnology is in plant tissue culture, and in the field of germplasms preservation. Plant tissue culture can be defined as the cultivation of all types of plant cells, tissues and organs under aseptic conditions. Currently, the plant tissue culture applications cover much more than the clonal propagation (a form of tissue culture), increasing the quantity of quality planting material and to facilitate the large-scale propagation and regeneration of thousands of plants within a short span of time. The technique of plant tissue culture has been extended to include somatic embryogenesis, somatic cell hybridization, virus free plants, mass propagation and the use of bioreactors for the production of desired bioactive substances. Tissue culture, usually, is the manipulation of cells, other tissues so that they can live for long periods of time in laboratory conditions or become intact and growing organisms. Different companies and institutions around the world have invested in or specialized in the regeneration activity, to provide the farmers and other stakeholders a high-quality, healthy planting materials (Husain, 2007; Husain and Anis, 2009; Husain *et al*., 2008, 2010; Martínez *et al*. 2016).

2. Materials and Methods

The information in this article has been compiled from the sources, including recent academic publications, a bibliographic database, and information collected from a variety of databases like PubMed, Science Direct, SCIELO, DOAJ, Science Alert, Semantic Scholar, and Google Scholar.

2.1 Types of nanotechnologies

Wet, dry and computational are the three main types of nanotechnologies and are interdependent for optimal functionality. The wet nanotechnology is predominantly found in water-based systems (Caprarescu *et al*., 2021) and is related with the exploration of living organisms and their components such as; tissues enzymes and membranes (Sofi *et al*., 2019; Sun *et al*., 2019). Inorganic compounds like silicon and carbon are associated with dry nanotechnology. The third one, computational nanotechnology is associated with simulations of nanometer-sized components (Sinha *et al*., 2009).

2.2 Biosynthesis of metal nanoparticles (NPs) with plant extracts

Currently, researchers are attracted to biological synthesis, which uses natural reducing agents, closure and stabilizing reagents, does not use dangerous and expensive chemicals, with low energy costs. The production of NPs by using the plant species is the most trustworthy environmentally sustainable method (Mukherjee *et al*., 2001; Makarov *et al*., 2014). NPs are widely utilized in and agriculture (Ahirwar *et al*., 2019) and medicine (Niculescu and Grumezescu, 2021).

2.2.1 Silver (Ag) nanoparticles

Silver NPs are widely used nanoparticles in new biomedical and industrial applications. These nanoparticles are of great interest to researchers due to their unique properties. The synthesis of silver NPs by biological organisms containing phytochemicals has become an important area for scientists. A variety of unique secondary metabolites derived from plant extracts such as alkaloids, flavonoids, phenolic acids, sugars, and terpenoids play a role in the biological reduction of ionic silver metal into nanoparticles (Rodríguez-León *et al*., 2013; Parlinska-Wojtan *et al*., 2016). Some examples of plant assisted silver NPs are summarized in Table 1.

2.2.2 Gold (Au) nanoparticles

Gold nanoparticles (NPs) are the most attractive metal NPs due to their remarkable applications in disease diagnosis, catalysis, gene expression, nonlinear optics and nano-electronics. These NPs are made by using either phytochemicals or other extract constituents that remain stable for a short period (Singh *et al*., 2010; Aromal and Phili, 2012). Some examples of plant assisted silver NPs are summarized in Table 2.

Table 1: Plant-based synthesis of silver nanoparticles (NPs)

S. No.	Botanical name	Part used	Shape and size (nm)	References
1.	*Acalypha indica*	Leaves extract	Spherical 20-30	Krishnaraj *et al*., 2010
2.	*Capparis zeylanica*	Leaves	Spherical 23	Nilavukkarasi *et al*., 2020
3.	*Mentha piperita*	Leaves extract	Spherical 35	Khatoon *et al*., 2018
4.	*Melia azedarach*	Leaves	Spherical 78	Sukirtha *et al*., 2012
5.	*Ocimum sanctum*	Leaves extract	Spherical 10-20	Philip and Unni, 2011
6.	*Ocimum tenuiflorum*	Leaves	Spherical 25-40	Patil *et al*., 2012
7.	*Tribulus terrestris*	Fruit	Spherical 16-28	Gopinath *et al*., 2012

Table 2: Plant-based synthesis of gold nanoparticles (NPs)

S. No.	Botanical name	Part used	Shape and size (nm)	References
1.	*Aegle marmelos*	Leaves	Spherical 4-10	Jha and Prasad, 2011
2.	*Cuminum cyminum*	Seeds	Spherical 1-10	Sneha *et al*., 2011
3.	*Phyllanthus amarus*	Leaves	Cubic 65-99	Annamalai *et al*., 2011
4.	*Terminalia chebula*	Plant extract	Anisotropic 6-60	Kumar *et al*., 2012
5.	*Stevia rebaudiana*	Leaves	Octahedral 8-20	Mishra *et al*., 2010

2.2.3 Zinc (Zn) nanoparticles

Zinc NPs are widely used in various fields due to their beneficial optical, electrical, dermatological and antibacterial properties (Kamaldeep and Dubey 2012). It got considerable attention due to their high catalytic activity, large surface area, low cost, white appearance, UV-filtering, antifungal, and photochemical properties (Kajbafvala *et al*., 2012; Kumar *et al*., 2013). Zn NPs may be used as effective control tools against mosquito larval populations and have potential applications in the pharmaceutical and biomedical field (Naif Abdullah and Mariadhas, 2018). An inorganic metal oxide of zinc (ZnO) NPs synthesis has been reported in many plant extracts as these contain phytochemicals like terpenoids, polyphenols and saponins that act as reducing and stabilizing agents in the reaction system. These phytochemicals are synthesized in leaf, stem root, fruit and seed and lower the metal's valence to zero, then calcinate it to add oxide (Lingaraju *et al*., 2016; Chaudhuri and Malodia *et al.,* 2017; Gomathi and Suhana, 2021). Additionally, Zn ions interact with the plant polyphenols to form a complex, zinc hydroxide Zn $(OH)_2$, through hydrolysis synthesis of ZnO NPs take place (Bala *et al*., 2015). Some examples of plant assisted silver NPs are summarized in Table 3.

Table 3: Plant-based synthesis of zinc NPs

S. No.	Botanical name	Part used	Shape and size (nm)	References
1.	*Artemisia pallens*	Leaves along with stem	Hexagonal 50-100	Gomathi and Suhana, 2021
2.	*Aloe vera*	Leaves	Hexagonal 22.18	Varghese and George, 2015
3.	*Catharanthus roseus*	Leaves	Spherical 23-57	Savithramma and Bhumi, 2014
4.	*Ruta graveolens*	Stem	Spherical 28	Lingaraju *et al*., 2016
5.	*Zingiber officinale*	Root	Spherical 30-50	Raj and Jayalakshmy, 2015

2.2.4 Titanium (Ti) nanoparticles

Titanium dioxide NPs have attracted much attention due to their high specific surface area, respective electrical band structure, and quantum efficiency, stability and chemical uniqueness. The large-scale synthesis of TiO_2 NPs using biological methods has attracted the attention of researchers due to their low cost, environmental friendliness and reproducibility. Currently, there are many reports on the biosynthesis of TiO_2 NPs using plant parts, algae, microorganisms (bacteria and fungi) and enzymes (Lai *et al*., 2015). Some examples of plant assisted silver NPs are summarized in Table 4.

Table 4: Plant-based synthesis of titanium NPs

S. No.	Botanical name	Part used	Shape and size(nm)	References
1.	*Azadirachta indica*	Leaves	Spherical, 124	Sankar *et al*., 2015
2.	*Mentha arvensis*	Leaves	Spherical, 20-70	Ahmad and Jaiswal, 2020
3.	*Ocimum basilicum*	Leaves	Hexagonal, 50	Salam and Sivaraj, 2014
4.	*Psidium guajava*	Leaves	Spherical, 32.58	Santhoshkumar *et al*., 2014
5.	*Salvia officinalis*	Leaves	Spherical 15-20	Altikatoglu and Attar, 2019

3. Nanotechnology in plant tissue culture

The idea of totipotency by Haberlandt (1902) envisioned the concept of plant tissue culture wherein the plant cells, tissues, and organs in culture can grow into the plants. Plant tissue culture aims to grow plant cells or plant parts (explants) in a defined nutrient medium under controlled, sterile environment. The technique is important for basic sciences and applied fields of plant biology, like morphogenesis, embryogenesis, cytology, nutrition, and germplasms preservation. Plant tissue culture is also valuable for genetic manipulation (biotransformation), large-scale clonal breeding, pathogen-free plants and production of beneficial secondary metabolites (Husain, 2007). The efficient plant regeneration protocols are essential for transgenic development and mass propagation. The success of *in vitro* cultivation of plants depends on factors, such as genotype, physiological state of the donor plant, type of explant, method of surface disinfection, nutrient medium, plant growth regulator, size of culture vessel, and spectral quality, luminous intensity, photoperiod and temperature (Husain, 2007; Husain *et al*., 2009).

Nanotechnology applications with the use of nanomaterials and NPs are developing fast. It is one of the important areas for investigation in sciences of modern materials. NPs have properties that are specific such as size, shape, and distribution. In plant tissue culture, there are several research reports on nanotechnology based on NPs have been widely used (Figure 1) to improve seed germination, enhance plant growth and yield, enable plant genetic modification, improve bioactive compound production and achieve plant protection (Wang *et al*., 2016; Ruttkay-Nedecky *et al*., 2017).

The increased seed germination and seedling growth in *Arabidopsis thaliana* L. was reported by the incorporation of gold NPs into Murashige and Skoog's (1962) medium. The total seed yield was increased by three times over the control with exposure to 24 nm size gold NPs at 10 µg/ml, and the same size of these NPs at both 10 and 80 µg/ ml had significantly improved the vegetative growth, seed germination rate and free radical-scavenging activity (Kumar *et al*. 2013). Stem explant of *Brassica nigra* L. was cultured on MS medium supplemented with zinc oxide NPs in concentrations from 1- 20 mg/l, which resulted in the production of white thin roots with thick root hairs while, at 10 mg/l zinc oxide NPs, shoot emergence, developed calli/roots were also observed (Zafar *et al*., 2016).

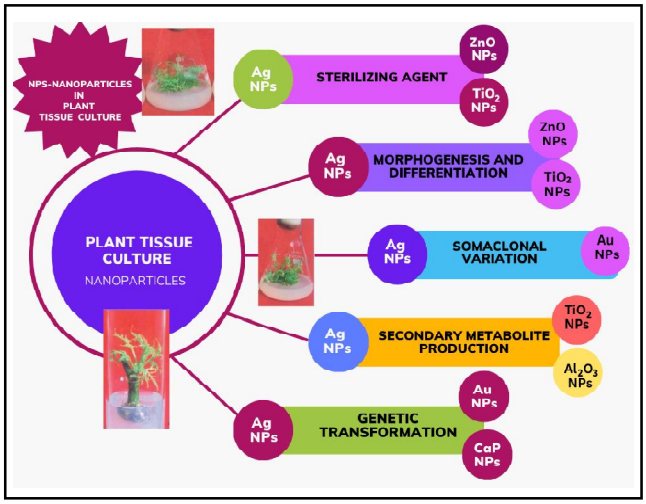

Figure 1: Use of nanoparticles-NPs in plant tissue culture.

Positive effects of silver NPs have been observed on callus induction, shoot regeneration, and explant growth of *Tecomella undulata* (Roxb.). The effect of silver NPs (5-80 mg/l) alone or in combination

with 6-benzyl amino-purine (6 BAP) and indole acetic acid (IAA) was evaluated and the results showed that the addition of silver NPs (10 mg/l) with 2.5 mg/l BAP and 0.1 mg /l IAA in MS medium increased the percentage of explants producing shoots, number of fresh shoots per explant, and also the survival of the plant by the action of ethylene blockage (Aghdaei *et al*., 2012).

The effect of zinc oxide NPs of 34 nm in size at different concentrations (0, 0.1, 1.0, 10, 100, or 1000 mg/l) on production of rebaudioside A and stevioside (steviol glycoside) and antioxidant activities in the tissue culture grown shoots of *Stevia rebaudiana* Bertoni reported by Javed *et al*. (2017). Treatment of licorice seedlings with copper oxide (CuO) and zinc oxide (ZnO) increased the content of anthocyanins, flavonoids, glycyrrhizin, phenolic compounds and tannins (Oloumi *et al*., 2015).

3.1 Involvement of nanomaterials for surface sterilization of explants

The surface disinfection of the explants is the primary concern in plant tissue culture and the problem should be solved initially to proceed with *in vitro* regeneration because, with a lot of pathogens (contamination), the initiation of culture show slow growth and sometimes initial materials destroyed by the nasty pathogens. NPs reduced the microbial contamination in several plants and have shown a great potential for explants surface disinfection. Hwan *et al.* (2017) reported that the use of NPs in the culture medium eliminates bacterial contamination and improves the regeneration potential of the explants, but can also induce somaclonal variation.

Sterile condition is one of the basic requirements for the successful plant tissue culture that depends on the exclusion of exogenous and endogenous contaminating microorganisms (Abdi *et al.,* 2008). Due to presence of sucrose in the medium, the growth of microorganisms (bacteria and fungi) is always faster than the plant parts. Thus, the contamination of bacteria (*Bacillus licheniformis*, *B. Subtilis*, *Corynebacterium* sp., *and Pseudomonas syringae*), and nasty fungi (*Aspergillus fumigate*s, *A. niger*, *Alternaria tenius*, and *Fusarium culmorum*) are the main obstacles during the initiation and maintenance of the culture (Msogoya *et al.,* 2012; Omamor *et al*., 2007; Sivanesan and Park 2015). The subculture process exhibited nearly 5-15% contamination due to inadequate sterilization of explants, growth media, operators, and operational equipments (Omamor *et al*., 2007). Plant organs and tissues such as nodes with axillary buds, shoot tips, cotyledons, hypocotyls, leaf disks, roots, or embryos are sterilized to stop the microbial contamination. The common sterilizing agents are: 0.1% mercury chloride ($HgCl_2$), 70% ethanol (EtOH)), hydrogen peroxide (H_2O_2), calcium hypochlorite (CaOCl), sodium hypochlorite (NaOCl) (5.25% w/v), and silver nitrate ($AgNO_3$). Some specific antibiotics and fungicidal compounds are generally used in the culture medium to stop or remove the unwarranted contaminants from the explants. Use of antibiotics for the prevention and removal of bacterial may cause toxicity to plant tissues of some species and sometimes may become inefficient to remove the contaminants from the plant cell. On one side, the resistance of bacteria and fungi to the sterilizing agents is common. While, on the other side these frequently used sterilant exhibited inhibitory effects on protein synthesis (streptomycin and chloramphenicol) nucleic acid synthesis (rifampicin) and cell wall-membrane synthesis (penicillin) (Abdi *et al*., 2008; Safavi, 2012; Kumar and Loh 2012).

3.2 Nanomaterial-based sterilization in plant tissue culture

Metal and metal oxide bases nanoparticles (NPs) have been established to be helpful for the removal of different strains of bacteria and fungi and are found to be more useful for the sanitization of the contaminated surfaces (Prasad *et al*., 2008; Mahato *et al*., 2009). NPs in particular have shown broad-spectrum antibacterial properties against both gram-positive and gram-negative bacteria (Guzmán *et al*., 2012). Silver (Ag) based NPs are reported to be first used to eliminate the bacterial infestation in plant tissue culture.

A wide range of nanoparticles (NPs) such as silver (Ag), oxides of aluminium, copper, iron, magnesium and zinc (Al_2O_3, CuO, Fe_3O_4, MgO, ZnO), titanium dioxide (TiO_2), gold (Au) nickel (Ni), silicon (Si), silicon dioxide has been well documented for antimicrobial activities against a range of bacteria and fungi (Beyth *et al*., 2015). In plant tissue culture, Ag, TiO_2 and ZnO are frequently used and well demonstrated for the removal of microbial contaminants from the *in vitro* grown cultures (Allahverdiyev *et al*., 2011; Applerot *et al*., 2012; Ebadollahi *et al*., 2019). The application of nanoparticles-NPs such as; Ag, ZnO and TiO_2 as disinfectant is summarized in Table 5.

Table 5: Application of nanoparticles-NPs (Ag, ZnO and TiO_2) in reducing the microbial contaminants in *in vitro* grown culture

Plant name	Nanoparticles (NPs)	Size and concentration	Phytotoxicity	Function in *in vitro* culture	References
Arabidopsis thaliana	Ag (Silver)	– – 100– 2000 mg/l	Yes	Reduced microbial contamination	Mahna *et al*., 2013
Bacopa monnieri	Ag (Silver)	29–33 1.6 × 10–3 to 16 × 10–10	Yes	Rate of contamination reduced	Kalsaitkar *et al*., 2014
Brassica nigra	ZnO (Zinc oxide)	100, 500-1500 mg/l, 1-20mg/l	Yes	Reduced microbial contamination not reported, inhibited seed germination	Zafar *et al*., 2016
Cynodon dactylon	Ag (Silver)	– –, 100 and 200 mg/l	Yes	Restricted microbial growth	Taghizadeh and Solgi, 2014
Hordeum vulgare L.	TiO_2 (Titanium dioxide)	– –, 24.5 10–60 mg/ ml	No	Reduced bacterial contamination	Mandeh *et al*., 2012
Lycopersicon esculentum	Ag (Silver)	—,25–100 mg/l	Yes	Reduced microbial contamination, helped in seed germination	Mahna *et al*., 2013

Musa sapientum, L	Zn and ZnO	100 mg/l dose	—	Eliminated microbial contaminants in banana *in vitro* culture	Helaly *et al*., 2014
Nicotiana tabacum Tobacco	Ag (Silver) TiO_2 (Titanium dioxide)	96, 10 – – 1–15 mg/l - -	Yes	Reduced microbial contamination Exhibited antibacterial activity	Bansod *et al*., 2015 Safavi *et al*., 2011
Rosa hybrida	Ag (Silver)	--,100–400 mg/l	No	Reduced the rate of bacterial contamination	Shokri *et al*., 2014
Solanum tuberosum	Ag (Silver) TiO_2 (Titanium dioxide)	--,10–500 mg/l 10, 1- 2% (w/w)	Yes No	Reduced microbial contamination	Mahna *et al*., 2013 Safavi, 2014
Vanilla planifolia	Ag (Silver)	35, 25–200 mg/l	Yes	Reduced bacterial contamination	Spinoso-Castillo *et al*., 2017

3.2.1 Silver nanoparticles (Ag-NPs) in plant tissue culture

The use of silver nanoparticles (Ag-NPs) has now become common as their production is more economical due to the advancement of nanotechnology. Ag-NPs have exhibited a broad-spectrum antibacterial activity and reduced the fungal and bacterial pathogens in *in vitro* culture (Ismail *et al*., 2017). Based on the fact that contamination is a serious problem in a temporary immersion systems, silver NPs were used in MS liquid medium at different concentrations (0, 25, 50, 100, and 200 mg/l) for *Vanilla planifolia* Jacks. ex Andrews (vanilla) shoot regeneration. The bacterial contamination was reduced at 50, 100, and 200 mg/l of silver NPs, and growth stimulation was observed at 25 and 50 mg/l, while significant inhibition was detected at higher doses, *i.e*.,100 and 200 mg/l (Spinoso-Castillo *et al*., 2017).

The application of Ag-NPs in *in vitro* culture has been well documented for the elimination of microbial contamination during the plant tissue culture (Sarmast and Salehi, 2016). These NPs can inhibit the growth of pathogenic microorganisms such as; *B. cereus C. albicans Enterococcus, E. coli*, *M. luteus*, , *P. aeruginosa S. aureus* (Li *et al*., 2015; Aziz *et al*., 2016). In *Brassica juncea*, Ag-NPs enhanced the seedling growth (Sharma *et al*., 2012). Nanosilver has antimicrobial effects at low concentrations. While nanosilica-silver at a very high concentration injured the cucumber plants (Rico *et al*., 2011; Safavi, 2012).

Bacterial contamination was notably reduced when *Rosa hybrida* explants were treated with 200 mg/l silver nanoparticle (Ag-NPs) solution for 20 min. On the other hand, the augmentation of 100 mg/ 1 Ag-NPs to the medium reduced the rate of bacterial contamination and also phenolic exudation (Shokri *et al*., 2014). Kalsaitkar *et al.* (2014) has reported the significant reduction in internal bacterial contamination in callus cultures of *Bacopa monnieri* by the addition of 160 mg/l of Ag-NPs to the Murashige and Skoog medium.

3.2.2 Zinc oxide nanoparticles (ZnO-NPs) in plant tissue culture

The effect of Zn or ZnO NPs in MS medium at different concentrations (50, 100, 200 mg/l) was observed for the elimination of few bacterial and fungal contaminants and their influence on regeneration of banana *in vitro* cultures. Nine strains of bacterial contaminants and four fungal contaminants were identified in NPs-free MS media of banana *in vitro* cultures, caused the death of the banana explants. The highest percentage of somatic embryogenesis was observed in MS media supplemented with 100 mg/l Zn-NPs followed by ZnO-NPs. Excellent shoot, root, and plantlets regeneration were observed in Murashige and Skoog (1962) MS medium augmented with 100 mg/l nano-Zn and nano-ZnO. Zinc oxide (nano-ZnO) promoted shooting, somatic embryogenesis, and regeneration of banana (*Musa*) plantlets. Zinc oxide (nano-ZnO) also induced proline synthesis and activity of superoxide dismutase, catalase, and peroxidase, thereby improving tolerance to biotic stress (Helaly *et al*., 2014). The regenerated plantlets were acclimatized successfully (98%). Somaclones treated with NPs accumulated more chlorophyll, proline, and antioxidant enzyme and developed higher dry weight accumulation as compared to control. The microbial contaminants in banana *in vitro* culture effectively eliminated by incorporation of Zn and ZnO NPs on MS growth media at different concentrations. Of all concentrations, 100 mg/l was found to be preferable because it showed the better effects on the regeneration of plantlets with good root systems (Helaly *et al.*, 2014).

Zinc oxide (nano-ZnO) also induced proline synthesis and activity of superoxide dismutase, catalase, and peroxidase, thereby improving tolerance to biotic stress (Helaly *et al*., 2014). In *Brassica nigra*, ZnO NPs inhibited seed germination, seedling growth, and callus biomass, while inducing roots from stem explants, and increased secondary metabolite content in callus and shoot and root tissues of seedlings (Zafar *et al*., 2016). Abd-elsalam (2013) reported that ZnO inhibited the fungal growth and led to the death of fungal mats.

3.2.3 Titanium dioxide nanoparticles (TiO_2-NPs) in plant tissue culture

In plant tissue culture, titanium dioxide (TiO_2) exhibited inhibitory action to microorganisms. In *Nicotiana tabacum*, nano-TiO_2 reported to show good potential for removing the bacterial contaminants (Safavi *et al*. 2011). TiO_2 –NPs also reported to reduces microbial contamination in *Solanum tuberosum* and *Hordeum vulgare* (Mandeh *et al*., 2012; Safavi, 2012, 2014).

4. Conclusion

Application of nanotechnology in plant tissue culture is still in its early developmental stages and current research outcome in this area of plant biotechnology is filling the major gaps in our understanding of the subject and its application. Only a few nanoparticles-NPs such as silver (Ag), zinc (Zn), zinc oxide (ZnO) and titanium dioxide (TiO_2) are found to be most useful in controlling microbial contamination in *in vitro* grown cultures, although a variety of NPs are known to be active on microorganisms. Besides surface disinfectant the nanoparticles are also useful in differentiation of calli, production of somaclonal variant, production of secondary metabolites and applications in the production of genetically modified organisms. We know that the plant physiology is well advanced in describing the mechanism during different stages of tissue culture,

whereas the effect of nanoparticles in *in vitro* culture is still not understandable.

Furthermore, multidisciplinary efforts are needed to continue exploring the areas where nanotechnology has a lot to offer in the area of plant biotechnology, especially plant tissue culture.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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