

## APPLICATION OF NANOTECHNOLOGY TO PLANT BIOTECHNOLOGY

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**Abstract:** Nanotechnology is one of the most fascinating and promising science field having ability to transform research in different disciplines of science such as agriculture, medicines, diagnostics and even plant tissue culture. Plant tissue culture is one of the fundamental techniques of plant biotechnology. It not only involved in the micropropagation of endangered plant species but also provide aseptic explants for transformation. But plant tissue culture technique have plethora of methodological obstacles which prevent its full exploitation, such as contamination of explants. This paper mainly presents a review on uses of nanomaterials in plant tissue culture such as decontamination of plant tissue culture and role of NPs in intracellular delivery of biomolecules such as enzymes, proteins and DNA in plant cells.

**Keywords:** Nanoparticles, Decontamination, Intracellular delivery, Plant transformation

## INTRODUCTION

**Abbreviations:** NPs-Nanoparticles

**Nanoparticles for sterilization of explants for *in vitro* culture**

Sterilization of explants and the maintenance of aseptic conditions is an essential part of plant *in vitro* culture. Different microorganisms such as bacteria and fungi grow faster in comparison to plant cells in an *in vitro* culture medium and eventually inhibit the growth of plant cells. Conventionally, plant organs and tissues are sterilized with different antibiotics and antifungal solutions to minimize the contamination; but the microbial resistance to common bactericides and fungicides and their phytotoxic effect, like inhibitory effect on explants' growth rate and survival, somatic embryo induction, multiplication or genetic mutations (Leifert *et al.*, 1992., Teixeira Silva *et al.*, 2003) are limiting factors in sterilization of plant and media materials. Therefore, they are not recommended for use in plant tissue culture techniques (Dabai *et al.*, 2007). So use of nanoparticles proved to be safe and cost effective antimicrobial substitutions. So far different nanoparticles have been used for decontamination of plant tissue culture media in different laboratories. Silver-based compounds such as silver nitrate have long been recognized as highly toxic to microorganisms, and show strong antibacterial, antifungal and antiviral activity due to its capability to release tiny particles of silver ions slowly and silver ions can destroy the cell structure of microorganisms (Sondi and Salopek-Sondi, 2004; Lubick 2008). Adding nano silver particles to culture media at concentration 4mg/l found to be effective in elimination of *in vitro* contaminations of Olive by Rostami and Shahsavari (2009). Nanosilver particles have been successfully used for decontamination of

*Araucaria excelsa* explants in tissue culture by Sarmast *et al* (2011). Al-Ani *et al.*, 2011 reported that the use silver nano particles helps in decreasing bacterial infections in ornamental Blood leaf plant i.e. *Iresine herbstii*. Safavi K. (2014) reported that Titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) act as potential agent to remove bacterial contaminants in potato plant tissue culture.

Mahna *et al.*, 2013 also suggested NS in sterilizing plant seeds and fragile tissues, such as leaf and cotyledon, two important model plants, Arabidopsis and tomato, as well as potato. It has been reported that the nano Zn and nano ZnO particles can effectively eliminate the bacterial and fungal contaminants in banana *in vitro* culture at a dose of 100 mg/L dose because it showed the best effects on the regeneration of plantlets with well-developed root systems (Helaly *et al.*, 2014). In case of *Moringa oleifera in vitro* cultures, Silver & Copper nanoparticles have been found beneficial to get clean cultures, even with high concentrations (Al-Ani *et al.*, 2015). In case of grapevines *in vitro* nanosilver treatments, low rates of burned explants and moderate to high effect on bacterial contamination control and moderate effect on fungi contamination reported (Gouron *et al.*, 2014). Application of 100 and 150 ppm NS both as an immersion and as directly to the culture medium considerably reduces internal as well as external contaminations on *in vitro* establishment of G × N15 (hybrid of almond × peach) rootstock (Arab M. *et al.*, 2014). Abdi *et al.*, 2008 and Abdi., 2012 reported that the application of 100 ppm Nano Silver for 60 min as the efficient decontamination procedure for *Valeriana officinalis* explants. The antimicrobial effect of nanosilver particles has been shown around six hundred microorganisms (Abdi *et al.*, 2008). However, in case of *H. brasiliensis* explants,

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Moradpour *et al.* (2016) reported low concentration of 10 ppm NS at an immersion time of 20 min highly effective in reducing microbial contamination,

increasing the survival and also act as an effective antioxidant and polyphenol adsorbent compared to silver nitrate and activated charcoal.

**Table 1.** Summary of different NPs that have been used in various Plant *in vitro* decontamination

Sr. No.	Type of NPs Used	Type of Plant <i>in vitro</i> Culture	Explant Used	References
1.	Silver	<i>Valeriana officinalis</i> L.		Abdi <i>et al.</i> , 2008
2.	Silver	<i>Olive</i>	Single nodes and shoot apices	Rostami and Shahsavari., 2009
3.	Silver	<i>Iresine herbstii</i>	Stem cutting	Al-Ani <i>et al.</i> , 2011
4.	Silver	<i>Araucaria excelsa</i>		Sarmast <i>et al.</i> , 2011
5.	Silver	<i>Valeriana officinalis</i> L.		Abdi, 2012
6.	Silver	<i>Arabidopsis</i> , <i>Tomato</i> and <i>Potato</i>	Seeds, leaf and cotyledon	Mahna <i>et al.</i> , 2013
7.	Titanium dioxide (TiO <sub>2</sub> )	<i>Potato</i>	Bud	Safavi K., 2014
8.	Silver	<i>Grapevines</i>	Leaf	Gouron <i>et al.</i> , 2014
9.	Silver	G × N15 (hybrid of <i>almond</i> × <i>peach</i> )	Root Stock	Arab M. <i>et al.</i> , 2014
10.	Zn and ZnO	<i>Banana</i>	Shoot tip	Helaly <i>et al.</i> , 2014
11.	Silver and Copper	<i>Moringa oleifera</i>	Stem cutting	Al-Ani <i>et al.</i> , 2015
12.	Silver	<i>H. brasiliensis</i>	Shoot tips and Axillary buds	Moradpour <i>et al.</i> , 2016

### Nanoparticles in plant intracellular delivery of biomolecules

The transgenic plants production is considered as an important tool in plant genetic research. There are different methods of Gene transfer in plants, namely, *Agrobacterium* mediated, chemicals based methods, and physical techniques such as electroporation, microprojectile, etc. Now-a-days, new methods, namely, the nanoparticles-based delivery systems, with better efficacy and stability is presenting an attractive alternative for plant transformation, since nanoparticles can be specifically tailored to deliver a different biomolecules such as DNA, proteins and enzymes to the cell, tissue, or organism of interest when needed (Du *et al.*, 2012). However, Nanoparticles as gene carriers have been mostly used in the mammalian cultured cells (Rojas Chapana *et al.*, 2005., Mattos *et al.*, 2011., Raffa *et al.*, 2012), whereas its application in plant cells is still very limited (Nunes *et al.*, 2011., Serag *et al.*, 2012). Nanoparticles gene carriers possess significant advantages compared with traditional carriers. Firstly, nanoparticles can be used for transformation of both monocotyledons and dicotyledonous plants and any types of organs. Secondly, nanoparticles can efficiently overcome transgenic silencing via controlling the DNA copies that combined to nanoparticles. Thirdly, nanoparticles can be functionalized easily that will further enhance transformation efficiency. Finally, multigene transformation can be achieved easily via nanoparticles without undergoing traditional building method of complex carrier. The key properties of nanoparticles endow this gene carrier with the potential application in plant transformation, and it is promising to solve the existing problem of plant gene engineering such as safety, multigene transformation and so on.

Yu-qin *et al.*, 2012, observed that ZnS nanoparticles modified with positively charged poly-L-lysine

(PLL) successfully delivered GUS-encoding plasmid DNA into tobacco cells by means of ultrasound-assisted method. Torney *et al.*, 2007 reported that honeycomb mesoporous silica nanoparticle (MSN) system with 3-nm pores that can transport DNA and chemicals into isolated plant cells and intact leaves. The MSN loaded with the GFP (Green Fluorescent Protein) gene and its chemical inducer and capped the ends with gold nanoparticles to keep the molecules from leaching out. Uncapping the gold nanoparticles triggered gene expression in the plants under controlled-release conditions. Martin-Ortigosa *et al.*, 2014 observed direct Cre recombinase protein delivery to plant cells using mesoporous silica nanoparticles (MSNs) as carriers into maize (*Zea mays*) cells, the delivery of DNA modifying proteins instead of DNA cassettes into plant cells allows for a genome modifications and editing applications. Hussain *et al.*, 2013 reported the uptake of mesoporous silica nanoparticles functionalised with amine cross-linked fluorescein isothiocyanate (MSN-APTES-FITC) by wheat, lupin and *Arabidopsis* which could be used as a novel delivery system for small molecules in plants. Carbon Nano Tubes(CNTs) with immobilized cellulase can serve as an efficient DNA delivery system for plant cells (Foud *et al.*, 2008). Burlaka *et al.*, 2015 demonstrated the applicability of Single Walled CNTs-based nanocarriers for the transformation of *Nicotiana tabacum*'s protoplasts and walled plant cells and the Multi Walled CNTs-based nanocarriers only for the transformation of protoplasts, because of a limiting role of the cellulose wall against their penetration into the cells. An increase in the genetic transformation frequency for *E. coli* (Rojas Chapana *et al.*, 2005., Raffa *et al.*, 2012) and *Neisseria meningitides* (Mattos *et al.*, 2011) in the presence of MWCNTs has been reported.

**Table 2.** Summary of different NPs that have been used in intracellular delivery of biomolecules in plant cells

Sr. No.	Type of NPs Used	Method used for NPs Delivery	Plant	Reference
1.	ZnS	Ultrasound-assisted	Tobacco	Yu-qin <i>et al.</i> , 2012
2.	Mesoporous silica nanoparticle (MSN)	Biolistic	Tobacco	Torney F. <i>et al.</i> , 2007
3.	Mesoporous silica nanoparticle (MSN)	Biolistic	Maize	Martin-Ortigosa, S. <i>et al.</i> , 2014
4.	Mesoporous silica nanoparticle (MSN)	–	Wheat, Lupin and <i>Arabidopsis</i>	Hussain, H. <i>et al.</i> , 2013
5.	Carbon Nano Tubes (CNTs) with immobilized cellulase	Cell uptake	<i>Arabidopsis thaliana</i> , <i>Glycyrrhiza glabra</i>	Foud <i>et al.</i> , 2008
6.	SWCNTs and MWCNTs	Cell uptake	<i>Nicotiana tabacum</i> L.	Burlaka <i>et al.</i> , 2015

## CONCLUSION

Current applications of nanobiotechnology to plant biotechnology research can serve as a new and potential tool for plant *in vitro* decontamination and plant genome transfection and editing. However, there are many unresolved challenges and issues regarding the biological effects of nanoparticles. Attentions must be given to suitable experimental designs to provide a defensible use of nanoparticles to avoid phytotoxicity due to NPs (Murachov, 2006).

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