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Role of Nanoparticles in Plant Tissue Culture and Secondary Metabolite Production

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Plant tissue culture is an indispensable and valuable technique for investigating morphogenesis, embryogenesis, clonal propagation, generation of pathogen-free plants, gene transfer and expression and the production of secondary metabolites. Plant tissue culture is mainly focused on mass multiplication of plants on appropriate nutrient medium. The technique of *in vitro* culture depends on the totipotency concept, which involves the ability of plant cells to divide and differentiate into a whole plant. The *in vitro* plant culture is regulated by various components, such as physiological status and genotype of the mother plant, explant type, sterilization method, the nutrient medium, growth hormones, and other physical conditions such as temperature, light intensity and day length, quality of light spectrum and size of culture vessels. Optimization of nutrient medium constituents using different combinations of plant growth regulators improves the growth and morphogenesis of plant parts and results in enhanced proliferation of cells, organogenesis, embryogenesis, shoot quality, and production of bioactive metabolites in the cell and tissue cultures.

In vitro, plant cell cultures have emerged as a promising and sustainable method for producing valuable and economically important metabolites in a controlled environment. To enhance metabolite yield, there is a need for strategies involving the addition of elicitors to plant cell culture media. Recent studies explored the potential of nanoparticles (NPs) as elicitors for secondary metabolism productions and alternatives to biotic elicitors like phytohormones and microbial extracts.

Elicitors are the molecules or chemicals that are induced in response to plant stresses such as fungi, bacteria, enzymes, and other biological molecules, and physical attributes like temperature, heavy metals, and salinity. Several studies have demonstrated a significant reduction in microbial contamination after surface sterilization of explants with different NPs. The addition of different NPs to the tissue culture media can result in somaclonal variation and can enhance morphogenetic potential and disinfection of the explants.

Role of nanoparticles in plant tissue culture

1. **Nanoparticles for microbial decontamination of explants:** Microbial contamination at different stages of plant tissue culture is a major problem. The sources of microbial contamination involve the explants themselves and the laboratory conditions. To obtain microbe-free explants, various disinfecting agents such as mercuric chloride, sodium hypochlorite (NaOCl), calcium hypochlorite (CaOCl), silver nitrate (AgNO3), hydrogen peroxide, ethanol, antibiotics, bromine water and fungicides like Bavistin are commonly used. The explant quality is often affected by the concentration and exposure time of the sterilants. A wide range of NPs, including silver, gold, silicon, copper oxide, zinc oxide,

aluminium oxide, titanium dioxide etc.have been reported to have strong antimicrobial activity against contaminating microorganisms in plant tissue culture.

2. **Nanoparticles for differentiation and organogenesis:** Different NPs have been utilized for their positive effects on callus induction, shoot proliferation, shoot multiplication, rooting, somatic embryogenesis, and organogenesis. Various metals and metal oxide NPs can be applied to augment the morphogenetic potential of the different plant species. However, the mechanism underlying the stimulatory or inhibitory effects and influence of different doses and combinations of NPs are often unclear and further focused investigations are needed.
3. **Nanoparticles for genetic transformation in Plant cells:** Plant tissue culture is widely used for the transfer of elite genes and genetic materials for crop improvement using genetic engineering and genome editing approaches. To increase the efficacy of biotransformation and stable transgene integration and expression, transformation methodologies and vectors have been developed and modified. Nanotechnology-based approaches offer safer alternatives for improved delivery of genetic materials and gene constructs to plant tissues. Nanoparticles have been modified in terms of their size, charge, and hydrophobicity to increase the efficiency of delivery into plant cells (Santana et al., 2022).
4. **Nanoparticles for *in vitro* somaclonal variations:** Somaclonal variations are the changes developed *in vitro* in plant cells, tissues, and organs. These are mainly linked with variation in chromosome number, chromosome structure, DNA sequence, DNA methylation, transposable elements activation, and crossing over. Several advantageous characteristics include plant size, leaf variegation, flower color, production of secondary metabolites, fruit ripening, and resistance against biotic and abiotic stresses.
5. **Nanoparticles for elicitation of bioactive compounds:** Plants produce various bioactive compounds or secondary metabolites that can play an essential role in health and even survival in response to different stresses. The extensive use of these compounds in pharmacological sectors, cosmetics, fragrances, dyes, flavours, and other applications has led research on increasing their production through plant tissue culture. Plant tissue culture can provide economical, viable, and sustainable production of different secondary metabolites such as alkaloids, terpenoids, quinones, steroids. By optimizing culture conditions and the culture media composition, and by supplementing the precursors and elicitors, the production level of these compounds can be significantly enhanced. In a culture medium, the added NPs may act as an elicitor and as a nutrient source.
6. **Nanoparticles for cryopreservation of elite Plant materials:** The long-term storage of plant genetic resources including cell lines, tissues, organs, calli, etc., at the temperature of liquid nitrogen (LN) (- 196 ◦C) is known as cryopreservation. Numerous plant species have been preserved using this approach. The different steps include hardening, dehydration, rapid cooling, and rewarming required for storage collectively expose the plant material to osmotic shock, which may induce oxidative stress and cell membrane damage, and also can affect the plants stability. The addition of NPs to the cryoprotectant process can enhance thermal conductivity and relative viscosity, induce vitrification and suppress devitrification, and enhance solution stability during rewarming.

Possible mechanisms of modulating plant secondary metabolism through nanoparticles

The elicitation mechanisms associated with nanoparticles are likely complex and could involve different mediators of signalling pathways and their cross-talk. These events are based on various factors such as morphology, amount, size, and exposure time of NPs, in combination with explant type, plant species, and growth conditions. Therefore, it is challenging to suggest generalised mechanisms for all plant species and NPs used. The possible mechanism of antimicrobial activity of NPs towards various bacteria and fungi largely involves damage to the cell wall and membrane, intracellular penetration, and excessive oxidative stress (Cao et al., 2021)

Once the nanoparticles reach the cell, they may enter the cell via plasmodesmata or plasma membrane receptors. As soon as the NP is inside the cell, it initiates a series of events that remains common for all the plant tissues. It causes ROS bust which further damages the plasma membrane and activates phospholipase-C, an enzyme found at the plasma membrane and cytosol. The PLC hydrolyzes phosphatidyl inositol 4,5 bisphosphate (PIP_2) and produces inositol triphosphate (IP_3) and diacylglycerol (DAG), both of them serve as a secondary messenger for the signalling cascade. The DGA then undergoes phosphorylation by DAG kinase and produces phosphatic acid (PA) which serves as a signaling molecule within the cell. IP_3 gets released into the cytosol and stimulates IP_3-mediated Ca^{2+} channels which leads to an increase in cytosolic Ca^{2+} concentration. Further, the elevated Ca^{2+} concentration is sensed by calcium-binding protein (CABP) or calcium-dependent protein kinase (CDPK). This protein subsequently activates MAPK which activates transcription factors that lead to an increase in secondary metabolites production. Meanwhile, several other chemical signals such as ABA, SA, polyamines, Jasmonates, and NO also play roles in stress response often via similar cross-talk by Ca^{2+} regulate the transcription of target genes by modifying the phosphorylation state of specific transcription factors.

Future outlook

Nanoparticles have demonstrated numerous beneficial uses in plant tissue culture applications, such as positively impacted disinfection, callus induction, organogenesis, biotransformation, somaclonal variations, generation of bioactive compounds and cryopreservation. Additionally, intensive research to further develop sustainable applications of nanomaterials in plant biotechnology for crop improvement as well as the production of bioactive compounds for medicinal and industrial uses should be embraced. Moreover, few studies have shown somaclonal variation in the presence of NPs; therefore, negative impacts on morphogenesis too may also occur under extended exposure. The inclusion of NPs can lead to increased secondary metabolites production in cell, root, and shoot cultures, due to ROS generation, regulation of specific genes, and antioxidant enzyme activation. To ensure safety and sustainability, the fate, transport, and transformation of NPs in plant tissue culture needs to be investigated to enable the development of approaches that avoid the adverse impacts of NPs on plants.

Conclusion

This review summarized the significant role of nanoparticles including crop improvement, maintaining elite cultivars, and sustainable production of bioactive compounds in different areas of plant nanobiotechnology. It is clear that factors such as the type and dose of nanoparticles, plant species, and environmental conditions significantly influence the effects of NPs on plants and plant tissue culture applications. In addition, the nano reservoirs such as graphene, carbon dots and quantum dots, due to their unique properties, can be used in plant tissue culture studies to significantly improve the growth characteristics of plants. Nanoparticle-mediated elicitation offers a versatile and environmentally friendly alternative to traditional methods of secondary metabolites production. It enables precise control over elicitor dosage and application, thereby minimizing resource consumption and reducing the potential for environmental contamination. Additionally, the compatibility of this approach with various plant species and cell cultures makes it a viable option for scaling up production in a cost-effective manner.

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