***Review Article***

**Engineering the Future: Nanoparticle-Based Approaches for Efficient Gene Transfer**

**ABSTRACT**

Plant genetic engineering plays a pivotal role in modern agriculture by enabling the incorporation of beneficial traits such as higher yield, enhanced nutritional quality, and resistance to biotic and abiotic stresses. A crucial step in genetic transformation is gene delivery, which is often species-specific and dictates the success of plant regeneration. Traditional gene delivery methods—biological (e.g., Agrobacterium-mediated), physical (e.g., biolistics, electroporation), and chemical (e.g., PEG and liposome-mediated)—have been widely employed but are limited by challenges such as low efficiency, tissue damage, random gene integration, and labor-intensive protocols. Recent advancements in nanotechnology offer a promising alternative through the use of nanoparticles as gene carriers. These nano-sized materials exhibit unique advantages, including high cargo-binding capacity, ability to cross the plant cell wall, minimal tissue damage, and enhanced transformation efficiency. Their customizable size, shape, and surface properties make them suitable for diverse plant systems and genetic materials. Despite these advantages, efficient delivery of nanoparticle-gene complexes into plant tissues remains an ongoing challenge. This review explores the latest developments in nanoparticle-mediated gene delivery, highlighting methods such as syringe infiltration, vacuum infiltration, biolistics, magnetofection, ultrasound-assisted delivery, passive diffusion, cellular uptake, and foliar spray techniques. By consolidating current knowledge and methodologies, this review aims to support researchers in optimizing plant gene transformation using nanoparticles, paving the way for more efficient and precise genetic engineering in crops.

***Keywords:*** *Nanoparticle-mediated gene delivery, Transformation, Genetic engineering, Nano-biomaterials, Crop improvement*

1. **INTRODUCTION**

Ensuring global food security amidst rapid population growth and the intensifying impacts of climate change has become one of the most urgent challenges of our time. To address this, plant genetic engineering offers a powerful solution by enabling the development of crop varieties with enhanced resistance to biotic and abiotic stressors, improved nutritional profiles, and greater adaptability to changing environmental conditions (Roberts & Mattoo, 2018). At the core of plant genetic engineering lies the ability to deliver foreign genetic material into plant cells either through stable integration into the genome or via transient expression allowing precise control over specific traits and functions.

Traditionally, several transformation techniques have been employed for introducing exogenous DNA into plant cells. These include Agrobacterium-mediated transformation, biolistic or gene gun methods, electroporation, and protoplast transfection (Tripathi & Shukla, 2024). While effective, each method comes with specific limitations such as host specificity, physical damage to tissues, low transformation efficiency, high cost, or labor-intensive protocols. The rigid plant cell wall, in particular, presents a significant barrier to efficient intracellular delivery of genetic material, making transformation in many plant species difficult or impractical (Su et al., 2023). Furthermore, the regeneration of whole plants from transformed tissues remains a time-consuming and species-dependent process.

To overcome these limitations, the use of nanoparticles as carriers for gene delivery has emerged as a promising alternative. Nanoparticles offer unique physicochemical properties that allow them to traverse the plant cell wall and membrane barriers with minimal or no damage. Their versatility in composition, size, shape, and surface functionalization allows for the targeted and efficient delivery of diverse biomolecules—including DNA, RNA, and proteins—into a wide range of plant species and tissues (Lombardo et al., 2020). Importantly, nanoparticle-mediated delivery enables non-invasive approaches such as foliar spray, syringe infiltration, and co-cultivation, offering a platform for scalable, species-independent, and tissue-specific transformation. This review explores the characteristics of various nanoparticles used in plant genetic engineering and highlights recent advances in nanoparticle-mediated delivery systems.

1. **NANOPARTICLES AS EMERGING TOOLS IN PLANT GENETIC ENGINEERING**

Recent advancements in plant transgenic technology have significantly enhanced various agronomic traits such as crop size, grain quality, and the biosynthesis of valuable secondary metabolites. These developments have also deepened our understanding of fundamental plant biology. However, the structural complexity of plant cells—particularly the presence of a rigid cell walls—continues to pose a major challenge for the efficient delivery of foreign genetic material to intracellular targets like the nucleus. Conventional gene delivery methods, such as Agrobacterium-mediated transformation and biolistic (gene-gun) methods, remain the most widely used tools in plant biotechnology (Mubeen et al., 2016). Yet, these techniques have limitations: Agrobacterium is known for its host specificity, restricting its application across diverse plant species, while gene-gun transformation can physically damage plant tissues and often results in multiple or random insertions of the transgene.

In contrast, nanoparticle-mediated transformation—though extensively explored in animal systems—is still in its early stages of implementation in plants. Nanotechnology offers a novel and promising avenue to overcome traditional limitations by enabling the precise and minimally invasive delivery of biomolecules across plant cell barriers. Nanoparticles, typically ranging from 1–100 nm in size, are uniquely positioned to traverse the plant cell wall and membrane due to their small size and customizable surface properties (Hu et al., 2020). These particles have already demonstrated diverse applications across industries such as agriculture, medicine, cosmetics, and electronics, highlighting their versatility and potential. In plant systems, a broad spectrum of nanoparticles has been developed, including nucleic acid-based nanoparticles, carbon-based nanoparticles such as single-walled and multiwalled carbon nanotubes, and metal-based nanoparticles composed of materials like aluminum, zinc, gold, and titanium dioxide (Harish et al., 2022). Additionally, hybrid and composite structures—such as chitosan-complexed carbon nanotubes—are being engineered to enhance delivery efficiency and biocompatibility.

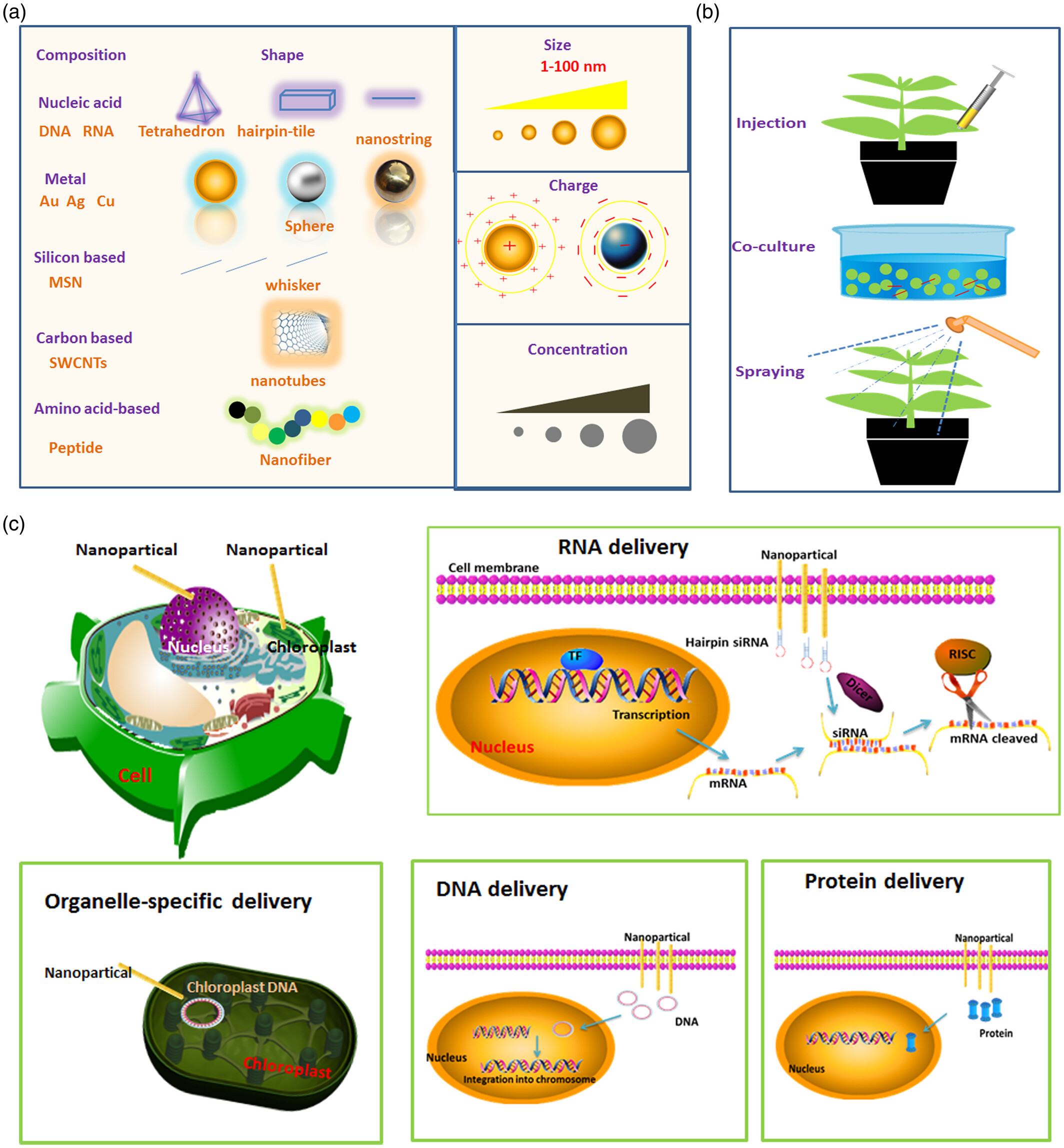
Nanoparticles vary widely not only in their composition but also in shape, surface charge, and mode of application. Their ability to carry DNA, RNA, and proteins into plant cells through methods like injection, co-culture, or foliar spraying introduces an entirely new paradigm for genetic transformation (Levengood et al., 2024). The expanding toolkit of nanomaterials and their successful adaptation for plant biotechnology signals a shift toward more flexible, species-independent, and scalable gene delivery platforms, with promising implications for the future of sustainable agriculture.

The multifunctionality of nanoparticles enables them to serve not only as carriers for genetic material but also as tools for targeted delivery, controlled release, and even real-time tracking within plant tissues. This precision is made possible by engineering nanoparticles with tunable properties such as shape, size, surface charge, and functionalization with targeting ligands. For instance, carbon-based nanoparticles like single-walled carbon nanotubes have shown promise in penetrating plant cells without triggering stress responses or requiring external force, making them ideal for both transient and stable expression of transgenes. Additionally, some nanoparticles have been engineered to target specific organelles, such as chloroplasts, allowing for compartment-specific gene expression—an advancement that holds great potential for improving traits related to photosynthesis and metabolism (Kumawat et al., 2025). As research continues to uncover the interactions between nanoparticles and plant cellular machinery, it becomes increasingly clear that nanotechnology could revolutionize the delivery of biomolecules in plant science. This approach not only broadens the scope of plant genetic engineering beyond current limitations but also opens up opportunities for more sustainable and efficient crop improvement strategies.

1. **NANOPARTICLE CHARACTERISTICS AND DELIVERY APPLICATIONS IN PLANTS**

~~Figure 1 illustrates the multifaceted roles and structural diversity of nanoparticles used in plant biotechnology.~~ One of the key features that determines the efficiency and specificity of nanoparticle-mediated delivery systems is their physicochemical characteristics—namely composition, shape, size, concentration, and surface charge. These properties not only influence cellular uptake and biocompatibility but also dictate the interaction of nanoparticles with plant cell walls and membranes. Panel (a) of Figure 1 categorizes nanoparticles based on their composition into nucleic acid-based (e.g., DNA and RNA nanostructures), metal-based (e.g., Au, Ag, Cu), silicon-based (e.g., silicon carbide whiskers), carbon-based (e.g., SWCNTs, MWCNTs), and amino acid-based materials (e.g., peptides) (Lv et al., 2020). Each category brings unique attributes: for instance, carbon nanotubes facilitate high loading capacity and structural strength, while metal nanoparticles are well-known for their stability and ease of functionalization. The structural variety—ranging from tetrahedrons and hairpin tiles to fibers and whiskers—further expands the scope of interaction with plant tissues, affecting both cargo capacity and cellular localization.

Panel (b) of Figure 1 presents the various methods of nanoparticle-mediated delivery, including direct injection, co-cultivation, and foliar spraying. These approaches enable flexible deployment strategies depending on the tissue type, plant species, and intended application. Injection and syringe infiltration are particularly effective for localized delivery to leaf tissues, while co-culture systems allow stable integration in tissue cultures or callus. Spray applications represent a non-invasive, scalable method suitable for in-field use, as evidenced by their increasing use in delivering siRNA or dsRNA for gene silencing and disease resistance. Panel (c) highlights the multifunctionality of nanoparticles, emphasizing their role in the delivery of a broad spectrum of biomolecules, including DNA, RNA, and proteins (Phogat et al., 2018). Notably, certain nanoparticles are engineered for subcellular targeting—such as chloroplast-directed gene delivery—enabling expression in organelles that are typically difficult to transform via conventional methods. These advanced capabilities offer promising tools for developing transgenic crops, gene editing systems (e.g., CRISPR/Cas9), and transient expression platforms, thereby positioning nanoparticles at the forefront of next-generation plant genetic engineering (Ahmar et al., 2021).

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**Fig. 1. Overview of Nanoparticle Types, Delivery Methods, and Applications in Plant Genetic Engineering (Lv et al., 2020)**

1. **NANOPARTICLE-MEDIATED MOLECULAR DELIVERY IN PLANTS**

Recent advancements in nanotechnology have significantly expanded its applications in plant science, especially in the targeted delivery of genetic material for crop improvement. A wide range of nanoparticles (NPs), differing in their chemical composition, size, surface functionalization, and delivery mechanisms, have been explored for efficient molecular cargo transfer into various plant species. Table 1 summarizes key studies that demonstrate the successful delivery of diverse biomolecules—such as plasmid DNA, small interfering RNA (siRNA), double-stranded RNA (dsRNA), and single-stranded DNA (ssDNA)—into different plant tissues using nanoparticles as carriers.

Carbon-based nanoparticles, particularly carbon nanotubes and quantum dots, are among the most commonly utilized due to their high aspect ratio, biocompatibility, and efficient penetration into plant cells. For instance, Demirer et al. (2020) and Liu et al. (2023) reported effective delivery of siRNA and plasmid DNA into *Nicotiana benthamiana* leaves via syringe infiltration using carbon nanotubes and amine-functionalized carbon dots, respectively. Similarly, Wang et al. (2020a) and Schwartz et al. (2020) demonstrated the utility of polyethyleneimine (PEI)-functionalized carbon dots for delivering genetic cargo across multiple monocot and dicot species using spray or vacuum infiltration methods. Metal-organic frameworks (MOFs), such as ZIF-8 nanoparticles, have also shown promise ~~in~~ as gene delivery systems. Yu et al. (2024) reported successful transgene delivery into Arabidopsis thaliana and Nicotiana benthamiana using ZIF-8 NPs via passive diffusion and infiltration. Additionally, functionalized silica nanoparticles, including mesoporous forms and silica nano powders, have enabled efficient delivery of dsRNA and DNA, as demonstrated in species like *Solanum lycopersicum* and *Nicotiana benthamiana* (Hajiahmadi et al., 2019; Xu et al., 2023).

Other innovative systems, such as virus-like nanocarriers and rosette nanotubes, have been utilized for delivering nucleic acids directly into protoplasts or microspores (Islam et al., 2024; Cho et al., 2020). These systems benefit from mimicking viral entry mechanisms while avoiding the drawbacks associated with viral vectors. Furthermore, the use of chitosan-based and casein-derived nanoparticles represents an emerging frontier in biodegradable, and plant-compatible carriers (Ben-Haim et al., 2024; Xu et al., 2023). Overall, Table 1 illustrates the diversity of nanoparticle types and delivery techniques currently available for plant transformation. It highlights how specific nanoparticle formulations can be tailored to the plant species, target tissue, and cargo type to enhance gene transfer efficiency. These advances underscore the potential of nanotechnology as a versatile tool in sustainable agriculture, offering a non-integrative, species-independent platform for functional genomics, crop protection, and trait improvement.

In addition to traditional leaf infiltration techniques, recent studies have demonstrated the utility of alternative delivery methods such as vacuum infiltration, spray application, and passive uptake for efficient transgene delivery. For instance, Wang et al. (2020a) and Law et al. (2022) utilized vacuum infiltration to introduce PEI-functionalized carbon dots and polymer-coated single-walled carbon nanotubes (SWNTs), respectively, into rice callus and *Arabidopsis* seedlings. These methods offer the advantage of distributing nanoparticles uniformly across plant tissues, enhancing uptake efficiency without physical damage. Spray-based delivery, as shown in the works of Schwartz et al. (2020) and Xu et al. (2023), has emerged as a promising non-invasive approach for field-scale application, especially for foliar delivery of siRNA and dsRNA for transient gene silencing or pathogen defense. Passive diffusion and cellular uptake, often used with protoplasts, roots, or reproductive tissues, also hold promise for delicate tissue types, enabling minimally invasive delivery of nucleic acids as demonstrated by Demirer et al. (2019) and Golestanipour et al. (2018).

Another notable trend in nanoparticle research is the diversification of plant species and tissue types targeted for molecular delivery. While *Nicotiana benthamiana* remains a model system, the extension of delivery strategies to major crops like rice (*Oryza sativa*), wheat (*Triticum aestivum*), tomato (*Solanum lycopersicum*), and mung bean (*Phaseolus radiatus*) reflects growing translational potential. For instance, the delivery of plasmid DNA and RNA into monocot tissues—historically more difficult to transform—has been made possible using carbon dots, graphene quantum dots, and SWNTs. Similarly, studies targeting reproductive structures such as flower buds, pollen, spikes, and microspores (e.g., Molesini et al., 2022; Cho et al., 2020; Yong et al., 2021) open new avenues for nanoparticle-assisted germline transformation and breeding. Collectively, the diverse applications presented in Table 1 reveal how the integration of nanotechnology with plant biotechnology is reshaping strategies for genetic engineering, with the potential to overcome species barriers, improve transformation efficiency, and facilitate precision agriculture.

**Table 1. Evidence of Successful Delivery of Various Molecular Cargoes into Plant Tissues Using Nanoparticles as Carriers.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Nanoparticles** | **Plant Species** | **Targeted Tissue** | **Cargo** | **Method of Insertion** | **Reference** |
| Carbon nanotubes | *Nicotiana benthamiana* | Leaves | siRNA | Syringe infiltration | Demirer et al. (2020) |
| Amine-functionalized mesoporous silica NPs | dsRNA | Sangwan et al. (2023) |
| Zeolitic imidazolate framework-8 (ZIF-8) NPs | Plasmid DNA | Yu et al. (2024) |
| *Arabidopsis thaliana* | Roots | Passive diffusion/Cellular uptake |
| PEI-functionalized carbon dots | *Oryza sativa* | Wang et al. (2020a) |
| *Triticum aestivum* | Leaves | Spray method |
| *Oryza sativa* | Callus | Vacuum infiltration |
| *Phaseolus radiatus* | Leaves | Spray method |
| *Nicotiana benthamiana* | siRNA | Schwartz et al. (2020) |
| *Solanum lycopersicum* |
| *Cucumis sativus* | dsRNA | Delgado-Martín et al. (2022) |
| β-cyclodextrin functionalized quantum dots | *Arabidopsis thaliana* | Plasmid DNA | Santana et al. (2020) |
| Amine-functionalized carbon dots | *Nicotiana benthamiana* | Leaves | Plasmid DNA | Syringe infiltration | Liu et al. (2023) |
| Mesoporous silica nanoparticles | *Solanum lycopersicum* | Spray method | Hajiahmadi et al. (2019) |
| Virus-like nanocarriers | *Arabidopsis thaliana* | Protoplasts | ssDNA | Passive diffusion/Cellular uptake | Islam et al. (2024) |
| PEI-functionalized gold nanoparticles | Leaves | siRNA | Syringe infiltration | Qi et al. (2024) |
| Rosette nanotubes | *Triticum aestivum* | Microspores | Plasmid DNA | Passive diffusion/Cellular uptake | Cho et al. (2020) |
| Graphene quantum dots | Spikes | dsRNA | Gyawali et al. (2024) |
| Carbon quantum dots | *Arabidopsis thaliana* | Leaves | Plasmid DNA | Syringe infiltration | Lin et al. (2023) |
| Carbon nanotubes | *Eruca sativa* | Protoplasts | Passive diffusion/Cellular uptake | Demirer et al. (2019) |
| Casein nanoparticles | *Nicotiana benthamiana* | Leaves | Syringe infiltration | Ben-Haim et al. (2024) |
| Amine-functionalized silica nano powder | *Nicotiana benthamiana* | Leaves, Roots | dsRNA | Syringe infiltration / Passive uptake | Xu et al. (2023) |
| Carbon quantum dots |
| Chitosan quaternary ammonium salt |

**Source: (Shivashakarappa et al., 2025)**

**5. CHALLENGES OF USING NANOPARTICLES IN PLANT GENE TRANSFORMATION**

**5.1. Nanoparticle Properties Limiting Efficiency**

**Physicochemical Characteristics**

Despite the potential of nanoparticles in plant science, their practical application in gene transformation faces numerous technical hurdles. The performance of nanoparticles is influenced by various factors including their size, charge, solubility, and chemical composition (Sukhanova et al., 2018). These parameters determine their dispersion, interaction with biological buffers, and ability to penetrate plant tissues. Stability in colloidal form is especially critical for efficient gene delivery but, can be disrupted by environmental conditions such as ionic strength, pH, and temperature (Zheng & McClements, 2020). Additionally, some plant culture buffers are not compatible with nanoparticle formulations, which can lead to aggregation or inactivation.

**Cargo Loading Capacity**

Another crucial factor is the capacity of nanoparticles to load and transport nucleic acids. Although nanoparticles can accommodate a wide range of DNA/RNA sizes, the delivery efficiency depends on the optimal ratio between the carrier and its cargo (Vaughan et al., 2020). Parameters such as the DNA-to-nanoparticle ratio, exposure time, and buffer conditions must be carefully adjusted depending on the target tissue—whether it is pollen, callus, or embryonic tissue. The current understanding of maximum loading thresholds is still evolving.

**5.2. Uptake Mechanism and Intracellular Fate**

**Barriers to Cellular Entry**

The rigid and selective structure of the plant cell wall is one of the biggest obstacles for nanoparticle-mediated delivery. Since the cell wall contains nanopores with limited dimensions, only certain types of nanoparticles can pass through. Advanced delivery mechanisms have been explored, such as using peptide tags or surface coatings that enable nanoparticles to bypass or interact favorably with cell membranes (Fang et al., 2018). However, the specific mechanisms of entry, movement, and intracellular localization of nanoparticles remain unclear and require further study.

**Nanoparticle Degradation**

Understanding how nanoparticles degrade within plant cells is crucial for evaluating their safety. Some are degraded ~~broken down~~ by oxidative enzymes or reactive molecules such as superoxide and nitric oxide (Sharma et al., 2019). Others may rely on peroxidase-driven pathways or lipid peroxidation products. The presence of such degradation pathways is important to prevent the accumulation of nanoparticles in plant tissues, which could otherwise pose risks to the food chain or the environment.

**5.3. Potential Phytotoxicity of Nanoparticles**

**Toxic Effects on Plants**

While nanoparticles offer a new avenue for plant transformation, they may also have unintended negative effects. Research suggests that certain formulations can hinder seed germination, root elongation, and shoot development. These outcomes are often linked to the generation of reactive oxygen species (ROS), which disturb hormone levels and stress responses in plants (Mittler et al., 2022).

**Gene Expression and Developmental Impact**

In some cases, nanoparticles have been shown to interfere with the expression of genes involved in defense, hormonal regulation, and root architecture. Their presence may trigger cellular stress responses, autophagy, or even programmed cell death, especially in sensitive tissues (Murrow & Debnath, 2013). Therefore, it is essential to design safer nanoparticle formulations that minimize toxicity while preserving transformation efficiency.

**5.4. Regeneration Constraints in Plant Transformation**

**Regeneration Limitation**

One of the most significant barriers in nanoparticle-based gene delivery is the inability to regenerate complete transgenic plants from transformed cells. Unlike Agrobacterium-based methods, nanoparticle delivery is often not integrated into a reliable regeneration system ~~often lacks integration into a reliable regeneration system~~ (Han et al., 2025). This presents a serious bottleneck, as efficient tissue regeneration is crucial for producing stable and inheritable genetic changes.

**Role of Regeneration-Related Genes**

Recent developments suggest that certain developmental genes, such as those controlling meristem formation, can aid regeneration. Inducing the expression of these genes may enhance the ability of transformed cells to develop into full plants, thus overcoming one of the key limitations of nanoparticle-mediated transformation systems (Rani et al., 2025).

1. **FUTURE PERSPECTIVES IN PLANT GENE ENGINEERING**

**De Novo Plant Regeneration**

The traditional approach of regenerating plants through callus formation is often slow and inefficient. New approaches focusing on direct regeneration through targeted gene expression offer a more streamlined alternative. Genes such as WUSCHEL and BABY BOOM, when expressed under tissue-specific promoters, can bypass the callus phase and trigger embryogenesis directly (Xu et al. 2024). This reduces mutation rates and improves the health of regenerated plants.

**Precision Genome Editing**

Genome editing tools like CRISPR/Cas9 are revolutionizing plant science by allowing targeted, efficient, and heritable modifications. Unlike earlier methods such as ZFNs or TALENs, CRISPR offers a more accessible and versatile platform. Techniques have been refined to increase targeting accuracy and reduce off-target effects (Bhardwaj, & Nain, 2021). These tools can be used to enhance yield, stress tolerance, and disease resistance in crops.

**Synergizing with Nanoparticles**

The fusion of genome editing technologies with nanoparticle delivery offers exciting possibilities. Nanoparticles can serve as vehicles for CRISPR components, bypassing conventional barriers and increasing delivery efficiency (Kaupbayeva et al., 2024). When paired with regeneration-promoting genes, this strategy holds promise for rapid and reliable plant transformation, especially in species that are recalcitrant to traditional methods.

Nanoparticle-mediated gene transformation in plants is a promising yet complex field. While the advantages include high cargo capacity and species-independent delivery, challenges such as toxicity, unclear uptake mechanisms, and limited regeneration capabilities must be addressed. By combining advances in genome editing, nanotechnology, and plant regeneration biology, it is possible to envision a future where crop improvement becomes faster, safer, and more precise.

1. **CONCLUSION**

Nanoparticle-mediated gene transformation represents a frontier in plant biotechnology, offering innovative strategies for precise and efficient gene delivery. This technology has demonstrated significant advantages over conventional methods, including species-independent delivery, minimal DNA integration, and the potential for multiplexed gene editing. However, despite these promising attributes, several challenges remain that hinder its widespread application. Key obstacles include the physicochemical limitations of nanoparticles, low uptake efficiency due to plant cell wall barriers, potential phytotoxic effects, and the lack of efficient regeneration systems for transformed cells. Additionally, the intracellular fate, degradation mechanisms, and long-term environmental safety of nanoparticles remain inadequately understood. Advances in genome editing technologies such as CRISPR/Cas9, when integrated with nanoparticle delivery platforms, offer exciting opportunities to overcome these limitations. The incorporation of regeneration-associated genes and targeted promoters further enhances the feasibility of direct organogenesis, reducing dependency on laborious tissue culture techniques. Future efforts should focus on optimizing nanoparticle formulations for biocompatibility, enhancing transformation protocols for recalcitrant species, and ensuring the safe and sustainable use of nanomaterials in agricultural settings. The convergence of nanotechnology, genome editing, and synthetic biology holds great promise for next-generation crop improvement. Continued interdisciplinary research is essential to address current barriers and unlock the full potential of nanoparticle-mediated gene transformation in plants, paving the way for resilient, high-yielding, and climate-smart agricultural systems.

**COMPETING INTERESTS DISCLAIMER**:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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