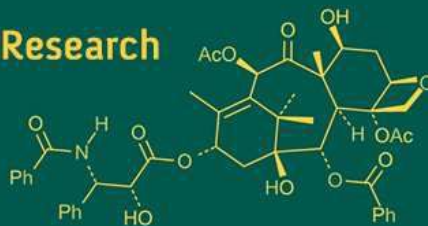


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Applications of nanoparticles in plant genetic transformation and crop improvement

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Nanotechnology has emerged as a powerful and innovative tool in plant genetic engineering by enabling precise manipulation and delivery of biomolecules at the nanoscale. Nanoparticles exhibit unique physicochemical properties, such as a high surface area-to-volume ratio and size-dependent optical, chemical, and biological characteristics, which make them highly suitable for gene delivery applications. The integration of nanotechnology with biological systems, termed nanobiotechnology, has gained significant attention for enhancing the efficiency and versatility of plant transformation strategies. This review highlights the role of nanoparticle-based gene delivery systems, including metal, metal oxide, clay, lipid-based, polymeric, and dendrimer nanoparticles, in plant biotechnology. These nanomaterials can effectively bind, protect, and transport nucleic acids, facilitate cellular uptake, and overcome key physiological barriers such as the rigid plant cell wall. Recent advances demonstrate successful nanoparticle-mediated gene transfer in plants using gold, silica, calcium phosphate, carbon-based, magnetic, and silver nanoparticles. Compared to conventional transformation methods, nanoparticle-assisted approaches offer higher transformation efficiency, reduced tissue damage, genotype-independent delivery, and cost-effective alternatives. Collectively, nanoparticle-enabled genetic engineering represents a promising strategy for accelerating crop improvement programs. By facilitating the precise introduction of stress-resilience, disease-resistance, and yield-related genes, nanotechnology holds substantial potential for developing climate-resilient crops capable of withstanding biotic and abiotic stresses, thereby contributing to sustainable agriculture and global food security.

Keywords: Crop improvement, climate-resilient crops, nanobiotechnology, nanoparticle-mediated gene delivery, nanomaterial-driven transformation, plant genetic transformation

Introduction

Nanotechnology has emerged as a transformative and interdisciplinary field with far-reaching implications for genetic engineering, biotechnology, and agricultural sciences. The conceptual foundation of nanotechnology was first articulated by physicist Richard P. Feynman in his landmark lecture *"There's Plenty of Room at the Bottom"*, in which he envisioned the precise manipulation of matter at atomic and molecular scales (Feynman, 1959) ^[13]. The term *nanotechnology* was later coined by Norio Taniguchi in 1974 ^[26] to describe precision engineering and material processing at the nanometre scale (Taniguchi, 1974) ^[26].

According to the National Nanotechnology Initiative (NNI), nanotechnology involves the understanding and manipulation of matter with at least one dimension in the size range of 1-100 nanometres. Similarly, the European Commission defines nanotechnology as the science and technology of materials and systems that exhibit novel properties and functions due to their nanoscale dimensions (European Commission, 2011) ^[12]. At this scale, materials display size-dependent physical, chemical, and biological properties that are markedly different from their bulk counterparts, enabling innovative applications across multiple disciplines.

Nanotechnology has rapidly expanded into diverse fields, including medicine, molecular and synthetic biology, biochemistry, agriculture, environmental science, and information and communication technologies. The unique behavior of nanomaterials arises primarily from their high surface area-to-volume ratio and the dominance of surface atoms, which

significantly alter surface energy, chemical reactivity, mechanical strength, optical behavior, and electrical conductivity (Guston, 2007; Bhushan, 2017) ^[15, 4]. Unlike bulk materials that exhibit relatively constant properties, nanoscale materials demonstrate enhanced reactivity and tunable functional characteristics, making them particularly attractive for biological applications.

In addition to surface effects, quantum confinement phenomena at the nanoscale impart distinct optical, magnetic, and electronic properties that are absent in larger materials (Bhushan, 2017) ^[4]. These quantum effects enable nanoparticles to interact efficiently with biomolecules such as DNA, RNA, proteins, and lipids, thereby facilitating their application in gene delivery, imaging, sensing, and molecular diagnostics.

Based on composition and structural characteristics, nanomaterials are broadly classified into metal nanoparticles, metal oxide nanoparticles, lipid- and liposome-based nanoparticles, polymer-based nanoparticles, dendrimers, and silica- and carbon-based nanomaterials. Due to their physicochemical tunability, biocompatibility, and functional versatility, these nanomaterials have gained increasing attention in plant biotechnology, particularly for genetic transformation, targeted gene delivery, and crop improvement.

In the context of agriculture, nanotechnology offers promising tools for overcoming limitations associated with conventional breeding and transformation methods. Nanoparticle-mediated delivery systems enable efficient transport of genetic material across plant cellular barriers, including the cell wall, and provide opportunities for precise genetic modification. Consequently, nanotechnology holds significant potential for accelerating the development of climate-resilient crops with enhanced resistance to biotic and abiotic stresses, improved nutrient use efficiency, and sustainable yield stability.

Nanobiotechnology and Its Applications in Agriculture

Nanobiotechnology represents the convergence of nanotechnology and biological sciences, enabling the application and address complex challenges in agriculture, and genetic engineering by enhancing precision, efficiency, and sustainability offers innovative strategies to improve crop productivity, stress tolerance, and resource-use efficiency under changing climatic conditions. A wide range of nanoparticles is employed in nanobiotechnological applications, including metal and metal oxide nanoparticles, clay-based nanomaterials, solid lipid nanoparticles, natural polymer-based nanoparticles, and synthetic dendrimers, which can positively influence plant growth and development by enhancing seed germination, improving nutrient uptake, regulating phytohormone activity, and promoting overall plant health (Barrena *et al.*, 2009) ^[3]. These effects are largely attributed to the ability of nanoparticles to interact efficiently with plant tissues and cellular components.

Recent advances in plant nanobiotechnology highlight the use of nanoparticles as nanocarriers for the targeted delivery of agrochemicals, including fertilizers, herbicides, pesticides, plant growth regulators, and bioactive molecules. Nanocarrier-based delivery systems enable controlled and site-specific release of active compounds, thereby enhancing efficacy while minimizing dosage requirements, off-target effects, and environmental contamination. Such precision

agriculture approaches contribute to sustainable crop management and reduced chemical inputs.

Among various nanomaterials, gold nanoparticles (AuNPs) are among the most extensively studied for biomolecule delivery due to their excellent biocompatibility, low cytotoxicity, and tuneable surface chemistry. AuNPs have been successfully employed for the delivery of proteins and nucleic acids in both plant and animal systems. They can bind and protect DNA from enzymatic degradation and facilitate controlled release of genetic cargo through redox-responsive mechanisms, such as glutathione-mediated dissociation (Han *et al.*, 2000) ^[16]. Nanoparticle-mediated gene delivery has emerged as an alternative to *Agrobacterium* and biolistic methods. Carbon nanotubes and mesoporous silica nanoparticles have enabled DNA, RNA, and CRISPR-Cas components delivery into plant cells without genome integration or tissue damage (Demirer *et al.*, 2019; Cunningham *et al.*, 2018) ^[8, 5]. Recent studies suggest that nanotechnology-enabled approaches can accelerate the development of climate-resilient crops by improving tolerance to abiotic stresses such as drought, heat, salinity, and heavy metal toxicity through enhanced antioxidant defense and stress-responsive gene regulation (Dimkpa and Bindraban, 2018) ^[10]. By integrating nanobiotechnology with plant genetic engineering and modern breeding approaches, it is possible to accelerate the development of climate-resilient, high-yielding, and sustainable crop varieties, thereby addressing global food security challenges.

- Carbon-based nanomaterials, including carbon nanotubes and graphene oxide, have been shown to penetrate plant cell walls and chloroplasts, facilitating transient gene expression and gene silencing in several crop species, demonstrating genotype-independent transformation potential (Giraldo *et al.*, 2014; Demirer *et al.*, 2021) ^[14, 9].
- Gold nanoparticles (AuNPs) have been successfully used for DNA and protein delivery in plants, protecting nucleic acids from degradation and enabling controlled release, thereby improving transformation efficiency (Pissuwan *et al.*, 2011) ^[22].
- Clay nanosheets and layered double hydroxides (LDHs) have been developed as carriers for double-stranded RNA (dsRNA), enabling spray-induced gene silencing (SIGS) for viral and fungal disease control, offering a non-transgenic plant protection strategy (Mitter *et al.*, 2017) ^[19].
- Zinc oxide (ZnO) and silicon nanoparticles have been shown to enhance seed germination, nutrient uptake, photosynthetic efficiency, and antioxidant enzyme activity, leading to improved plant growth and stress tolerance under drought and salinity conditions (Rastogi *et al.*, 2017) ^[24].
- Nano fertilizers based on iron, zinc, nitrogen, and phosphorus nanoparticles have demonstrated higher nutrient-use efficiency, reduced leaching losses, and sustained nutrient release compared to conventional fertilizers, contributing to sustainable agriculture (DeRosa *et al.*, 2010; Kah *et al.*, 2019) ^[7, 17].
- Silver and metal oxide nanoparticles exhibit strong antimicrobial activity against plant pathogens, reducing disease incidence and post-harvest losses, although their dosage-dependent phytotoxicity requires careful evaluation (Rai *et al.*, 2012) ^[23].

- Polymeric and chitosan-based nanoparticles have been widely explored for delivery of pesticides, plant growth regulators, and nucleic acids, owing to their biodegradability, low toxicity, and enhanced plant compatibility.
- Magnetic nanoparticles have been applied for targeted delivery of biomolecules and for tracking nanoparticle movement within plant tissues, opening new avenues for precision agriculture and plant imaging (Tian *et al.*, 2013) [28].

Nanomaterial-Driven Genetic Transformation in Plants

This approach combines advances in nanomaterial engineering with plant biotechnology to enable efficient, precise, and minimally invasive delivery of nucleic acids and genome-editing components into plant cells. The workflow describes the systematic steps involved in nanomaterial-mediated transformation, including nanoparticle synthesis and characterization, surface functionalization and cargo loading, preparation of plant tissues, nanomaterial-assisted delivery, intracellular release and expression of genetic material, and subsequent validation of transformation efficiency and biosafety. Collectively, this framework highlights the potential of nanomaterial-driven strategies as a robust alternative to conventional plant transformation methods for sustainable crop improvement.

- **Synthesis and Characterization of Nanoparticles:** Nanoparticles (e.g., carbon nanotubes, gold, silica, magnetic, polymeric, or clay-based) are synthesized using chemical or biological methods. Physicochemical properties such as size, shape, surface charge, dispersity, and stability are characterized using techniques including transmission/scanning electron microscopy (TEM/SEM), dynamic light scattering (DLS), and zeta potential analysis (Bhushan, 2017; Cunningham *et al.*, 2018) [4, 5].
- **Surface Functionalization and Cargo Loading:** Nanoparticles are functionalized with suitable chemical groups or polymers to enhance biocompatibility and binding efficiency. Genetic cargo (plasmid DNA, siRNA, dsRNA, mRNA, or CRISPR-Cas ribonucleoprotein complexes) is conjugated or adsorbed onto nanoparticles via electrostatic interactions, covalent bonding, or encapsulation. Loading efficiency and cargo stability are assessed prior to delivery (Han *et al.*, 2005; Pissuwan *et al.*, 2011) [16, 22].
- **Plant Material Preparation:** Target plant material such as leaves, embryos, callus, meristems, or protoplasts is prepared under sterile conditions. In some approaches, intact plants or seedlings are used to enable tissue culture-independent delivery (Giraldo *et al.*, 2014; Cunningham *et al.*, 2018) [14, 5].
- **Nanoparticle-Mediated Delivery:** Nanoparticle-cargo complexes are introduced into plant tissues using methods such as foliar infiltration, syringe infiltration, vacuum infiltration, seed imbibition, particle suspension incubation, or magnetofection (for magnetic nanoparticles). Nanoparticles facilitate penetration through the plant cell wall and membrane, enabling intracellular delivery (Torney *et al.*, 2007; Mitter *et al.*, 2017) [29, 19].
- **Release and Expression of Genetic Material:** Once internalized, the genetic cargo is released in a controlled

manner within the cytoplasm or nucleus, depending on nanoparticle design. Transient or stable gene expression, gene silencing, or genome editing is achieved without or with minimal genomic integration (Demirer *et al.*, 2019; Demirer *et al.*, 2021) [8, 9].

- **Validation of Transformation and Gene Expression:** Successful delivery and expression are confirmed using molecular assays such as PCR, qRT-PCR, reporter gene analysis (e.g., GFP, GUS), Western blotting, or sequencing for genome edits. Phenotypic and physiological responses are evaluated where applicable (Torney *et al.*, 2007; Martin-Ortigosa *et al.*, 2012) [29, 20].
- **Assessment of Efficiency and Biosafety:** Transformation efficiency, tissue viability, and potential nanoparticle toxicity are evaluated. Comparative analysis with conventional transformation methods is performed to assess efficiency, reproducibility, and environmental safety (Mitter *et al.*, 2017; Kah *et al.*, 2019) [19, 17].

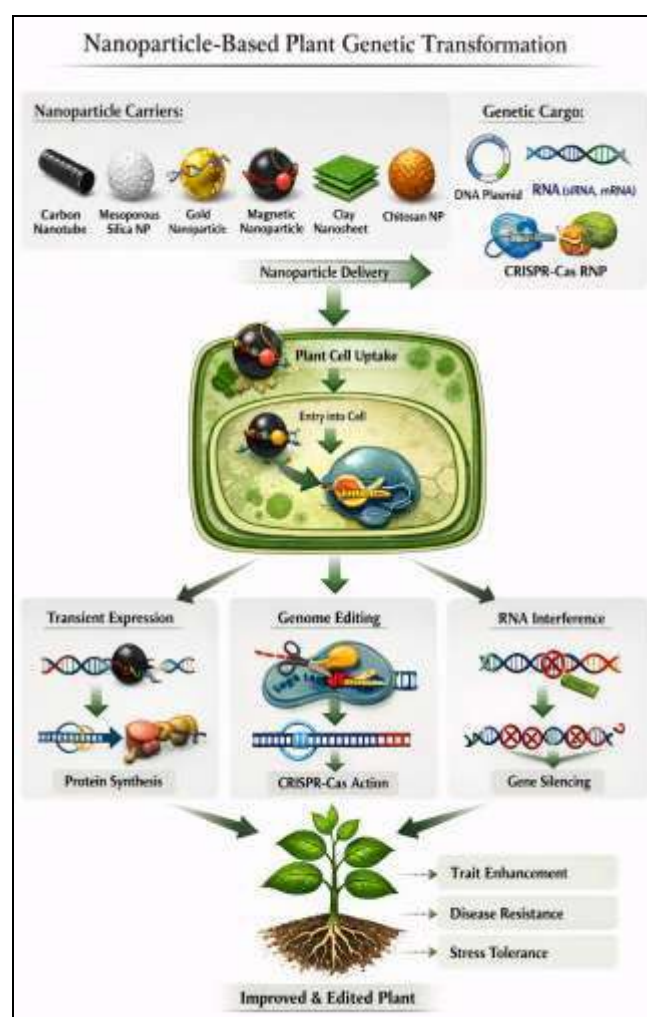


Fig 1: Overview of nanoparticle-mediated plant genetic transformation.

Schematic representation showing different nanoparticle carriers (e.g., carbon nanotubes, silica, gold, magnetic, clay, and chitosan nanoparticles) used to deliver genetic cargo such as plasmid DNA, RNA molecules, and CRISPR-Cas ribonucleoproteins into plant cells. Nanoparticle-assisted delivery enables efficient cellular uptake, resulting in transient gene expression, genome editing, or RNA

interference, ultimately leading to improved plant traits including disease resistance and stress tolerance.

Nanoparticles offer significant potential for improving genetic transformation efficiency by overcoming the biological and physiological barriers associated with conventional gene delivery methods. Positively charged nanoparticles can electrostatically bind the negatively charged phosphate backbone of nucleic acids, forming stable nanocomplexes that protect DNA or RNA from enzymatic degradation and facilitate intracellular transport and nuclear entry (Dizaj *et al.*, 2014) ^[11]. Compared with traditional approaches, nanoparticle-mediated gene delivery enables penetration of the plant cell wall with minimal tissue damage, thereby addressing major limitations of *Agrobacterium*-and biolistic-based transformation systems (Mitter *et al.*, 2017; Zhao *et al.*, 2017; Demirer *et al.*, 2018) ^[19, 31, 9].

Although nanoparticle-assisted gene delivery in plants is still emerging, multiple nanomaterial platforms including calcium phosphate, mesoporous silica, carbon-based nanoparticles, magnetic nanoparticles, and metal nanoparticles—have demonstrated promising results. Mesoporous silica nanoparticles have been successfully used to deliver plasmid DNA into plant cells, resulting in reporter gene expression in isolated cells and intact leaves (Torney *et al.*, 2007) ^[29]. Calcium phosphate nanoparticles have shown higher transformation efficiency than *Agrobacterium tumefaciens* and enabled successful delivery of GFP-encoding plasmids into tobacco cells (Ardekani *et al.*, 2014) ^[2].

Metal-based nanoparticles have also been explored as gene delivery vehicles. Carbon-supported gold nanoparticles improved DNA delivery efficiency, reduced cellular damage, and enhanced regeneration and transformation frequency in multiple plant species compared to conventional gold particles used in gene gun systems (Vijayakumar *et al.*, 2010) ^[30]. Magnetic gold nanoparticles and gold nanoparticle-based biolistics have further enabled effective plasmid delivery and stable transformation in crops such as canola, carrot, and rice (Chang *et al.*, 2013) ^[6]. More recently, silver nanoparticles have emerged as cost-effective microcarriers, significantly increasing transformation efficiency while drastically reducing material costs (Rajkumari *et al.*, 2021) ^[25].

Conclusion

Nanotechnology, and specifically nanobiotechnology, has rapidly evolved into a powerful and versatile platform for next-generation plant genetic engineering and sustainable agriculture. By exploiting the unique physicochemical properties of nanomaterials such as tunable size, surface charge, and functionalization these systems can interface effectively with plant tissues and overcome key biological barriers, particularly the rigid cell wall, to enable precise spatiotemporal delivery of nucleic acids, proteins, and genome-editing components. In this context, nanomaterial-enabled transformation offers distinct advantages over conventional *Agrobacterium*-and biolistic-based methods, including reduced tissue damage and somaclonal variation, broadened genotype and species applicability, DNA-free genome editing via transient delivery of ribonucleoprotein complexes, and, in many cases, enhanced transformation efficiency and reproducibility.

Importantly, the utility of nanotechnology in agriculture extends well beyond genetic transformation. Engineered nanoparticles have been shown to improve nutrient-use efficiency, modulate plant growth and development, and enhance tolerance to abiotic and biotic stresses, thereby contributing to the development of climate-resilient, resource-efficient, and high-yielding crop varieties. By serving as smart carriers for agrochemicals, signaling molecules, and RNA-based biopesticides, nanomaterials can support more precise and reduced-input management strategies, aligning crop production with the principles of sustainable and environmentally responsible agriculture. At the same time, unresolved questions regarding long-term biosafety, environmental fate and accumulation, impacts on non-target organisms and soil microbiomes, and harmonized regulatory frameworks warrant careful, multidisciplinary investigation. Looking ahead, the convergence of nanomaterial engineering with advanced plant biotechnology, multi-omics, systems biology, and modern breeding programs is poised to reshape the paradigm of crop improvement. Rationally designed nanocarriers integrated with genome-editing technologies, high-throughput phenotyping, and data-driven trait prediction could enable highly targeted, rapid, and stackable modifications tailored to diverse agroecological contexts.

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