

Review

Synthetic Nanomaterials for Nucleic Acid Delivery in Plants: Design Principles, Delivery Mechanisms, and Nano–Plant Interactions

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Abstract

Advances in plant genetic engineering hold significant promise for addressing urgent global food security needs. Nevertheless, conventional biomolecule delivery techniques, such as *Agrobacterium*-mediated transformation and biolistics, are hindered by inherent limitations, including narrow host compatibility, inconsistent efficiency, and unintended tissue damage. Nanotechnology has arisen as a transformative approach that exploits the tunable physicochemical properties of nanomaterials to bypass biological barriers, facilitating efficient, non-invasive, and species-independent nucleic acid delivery in plants. While prior reviews have acknowledged nanomaterials as promising delivery vehicles, key mechanistic and functional dimensions remain insufficiently explored. This review summarizes recent breakthroughs in nanomaterial-based delivery systems, emphasizing innovative uptake mechanisms, intracellular trafficking, and systemic transport processes. Additionally, this review explores the dual functionality of nanomaterials, which serve not only as efficient nucleic acid carriers but also as active biostimulants that enhance nutrient assimilation, stress tolerance, and innate immunity in plants. These multifaceted roles underscore the importance of incorporating such dual capabilities into the rational design of plant-specific nanocarriers. By integrating these insights, this work offers a comprehensive framework to support the development of efficient and sustainable nano-delivery platforms for next-generation plant genetic engineering.

Keywords: Plant genetic engineering, nanoparticles, nucleic acid delivery, delivery mechanism, nano-plant interaction.

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Introduction

Given the growing global demand for food and environmental challenges associated with conventional pesticides, plant biotechnology has gained significant importance. Genome editing in plants offers a promising approach to enhance crop yield and strengthen resistance to pathogens and environmental stresses [1,2]. However, the foundation of such genetic engineering lies in the safe and efficient delivery of extracellular genetic material into host plants to precisely modify genomes or regulate genetic machinery. Although established methods, including *Agrobacterium*-mediated transformation, viral vector systems, and biolistics (gene gun), have been widely used for intracellular delivery in plants [3–5], these approaches face limitations such as host range, random integration of transgenes, limited cargo size, and potential tissue damage [6–

8]. Therefore, there is an urgent need to develop innovative strategies to overcome these constraints and enable efficient intracellular transport in plants.

With the rapid advancement of nanotechnology, nanomaterials have emerged as promising platforms for the delivery of extracellular substances such as genes, proteins, pharmaceuticals, and other bioactive compounds into plant cells owing to their distinctive properties, including high surface area-to-volume ratios, tunable size, morphology, and excellent biocompatibility [8–10]. Nanomaterial-based delivery systems facilitate the non-destructive transport of large nucleic acid cargoes across diverse plant species. By offering high loading efficiency and broad host compatibility, these platforms represent a transformative advancement in plant genetic engineering [11,12]. Despite these progress, nanomaterials currently used still exhibit limi-

tations in terms of delivery efficiency and duration, and more investigations involving the long-term safety are yet required. In addition, the interaction between nanomaterials and plants remains poorly understood, particularly given the structural and compositional complexity of plant tissues, as well as the cellular environment, which significantly influences delivery outcomes. For instance, the plant cell wall, composed of cellulose and pectin, serves as a natural barrier that typically permits only particles ranging from 5 to 20 nanometers in diameter to pass through [13,14]. The cuticle and waxy layers on the plant epidermis further impede the delivery of exogenous substances [15–17]. Preliminary studies suggested that nanoparticles (NPs) with ultrasmall sizes, high aspect ratios, and optimized surface charges exhibit enhanced ability to overcome these barriers [18–20]. Moreover, approaches like enzyme-assisted transient degradation of the cuticle have shown promise in improving nanoparticle penetration [21]. A comprehensive understanding of delivery processes at both systemic and cellular levels is crucial for the rational design of nanocarriers. This encompasses the comprehension of transport through xylem and phloem, cell-to-cell movement, cellular uptake, intracellular trafficking, cytosolic release, and subcellular targeting. Acquisition of this knowledge will enhance the delivery efficiency, minimize unintended effects on plant function, and facilitate the development of advanced tools for plant biotechnology and genetic engineering.

This review systematically examines the limitations of conventional plant genetic engineering and offers a comprehensive overview of recent advances in nucleic acid delivery into plants using synthetic nanomaterials. Furthermore, given the limited understanding of NPs' internalization processes in plants, we specifically summarize the latest insights into NPs' uptake, transport, and translocation within plant systems. Additionally, the review systematically analyzes nano-plant interactions and evaluates the biological effects of NPs on plant growth and development. By integrating current progress in nanomaterial applications with mechanistic insights, this work establishes a theoretical foundation for developing efficient and versatile nucleic acid delivery systems, thereby contributing to the advancement of plant genetic engineering and precision agriculture.

Conventional Plant Genetic Engineering Methods

The efficient and precise delivery of nucleic acid molecules into plant cells is crucial for advancing gene editing, regulating gene expression, and screening for desirable crop traits. Nonetheless, the unique architecture of the plant cell wall, coupled with complex physiological barriers and the intrinsic instability of nucleic acids, poses considerable technical challenges to the delivery process. To overcome these obstacles, researchers have developed several biomolecule delivery techniques over the past few

decades, as summarized in Table 1. Biological delivery systems, including *Agrobacterium*-mediated and viral vector-based approaches, leverage natural intrinsic transport machinery to achieve high delivery efficiency [6,22]. However, their utility is limited by host specificity and constraints on cargo size [22,23]. Conversely, physical delivery techniques encompassing electroporation, biolistic particle bombardment, and microinjection circumvent host range restrictions by creating transient openings in cellular membranes for direct cytosolic introduction of nucleic acids [24–26]. Nevertheless, these approaches are often associated with tissue damage and high equipment costs, thereby hindering their widespread practical implementation.

Agrobacterium-Mediated Transformation

Agrobacterium-mediated genetic transformation has been a fundamental and indispensable technique for the precise and efficient introduction of exogenous genes into plant genomes since the 1980s [23,42]. The foundation of this technology was laid when a class of large plasmids in *Agrobacterium tumefaciens* known as Ti (tumor-inducing) plasmids were identified and isolated [43]. Subsequently, in 1977, it was demonstrated that the transfer DNA (T-DNA) of the Ti plasmid possesses the natural ability to deliver foreign DNA into plant cells and integrate it into the host genome [44]. Subsequent studies in 1980 revealed that virulence (*vir*) genes were activated during infection [45]. In this process, the bacterium specifically recognized phenolic compounds released by the wounded plant tissue and subsequently triggered the expression of *vir* genes. The resulting *vir* proteins were crucial for the excision, transfer, and integration of T-DNA into the plant genome [46–49]. To enable biotechnological applications, the tumor-inducing genes were removed from the Ti plasmid, abolishing pathogenicity while retaining its DNA delivery function. [50,51]. This modification has established *Agrobacterium tumefaciens* as a highly effective and widely adopted tool for plant genetic transformation.

Owing to its efficient natural gene delivery capability, *Agrobacterium*-mediated transformation has been widely employed to introduce genes of interest into various plant species such as tobacco and wheat [27,28,52]. Nevertheless, this technique remains constrained by several limitations, including a narrow host range that particularly impedes the transformation of major cereal crops like wheat and corn; random T-DNA integration, which may cause positional effects or unintended gene disruption; and generally low transformation efficiency that exhibits significant variability across plant species and tissue types [6,53]. In response to these challenges, current research endeavors are focused on engineering *Agrobacterium* strains with expanded host infectivity [54,55], as well as integrating CRISPR/Cas systems with *Agrobacterium* virulence genes to improve the precision and efficiency of transgene integration [27,56–58].

Table 1. Traditional genetic transformation methods.

Delivery methods	Cargoes	Principle	Limitation	Ref.
<i>Agrobacterium</i> -mediated	T-DNA	The T4 system employs a set of Vir proteins to excise and mediate the transcellular transport of T-DNA segments, facilitating their integration into the host plant genome.	Random integration into the host genome; limited host range.	[6,23, 27,28]
CRISPR-Cas	Cas9 protein, sgRNA, RNP (Ribonucleoproteins).	The gRNA directs the Cas nuclease to a specific DNA site via base pairing, where it creates a double-strand break (DSB) to trigger targeted genome editing.	Off-target effects; the large size of Cas9; low stability; the plant cell wall barrier.	[7,29]
Virus-mediated	DNA, RNA (siRNA/miRNA)	Viral vectors enable concurrent delivery of packaged nucleic acids to plant tissues through their intrinsic infection machinery.	Limited cargo size	[22,30–33]
Electroporation	Various nucleic acids (DNA, siRNA/miRNA)	Exogenous nucleic acids enter the cells via transient pores in the plant cell membrane induced by high-voltage electric pulses.	Tissue damage; limited cargo-carrying capacity; low delivery efficiency.	[24,34–36]
Biolistic gene gun	Various nucleic acids, RNP	Metal particles coated with nucleic acids are accelerated by high-pressure gas to traverse cellular membranes, facilitating the delivery of genetic material.	Metal particles may induce cytotoxicity; gene rearrangement or multi-copy insertion; tissue damage.	[25,37–39]
Microinjection	Various nucleic acids, Plasmid	Directly delivers nucleic acids (DNA/RNA) into cells using a fine glass micropipette.	Equipment cost; low throughput; cell damage risk.	[26,40, 41]

CRISPR-Cas System

The advent of CRISPR-Cas technology has marked a paradigm shift in plant genome editing. By delivering Cas nucleases (e.g., SpCas9, Cas12a) alongside guide RNAs (gRNAs) or as pre-assembled ribonucleoprotein (RNP) complexes, researchers can achieve precise genetic modifications, including knockouts, insertions, and base editing [29,59,60]. Conventional delivery methods encompass *Agrobacterium*-mediated transformation, biolistics, and protoplast transfection. For example, targeting the *phytoene desaturase* (*PDS*) gene in *Nicotiana benthamiana* (*N. benthamiana*) via *Agrobacterium* carrying the CRISPR-Cas system has successfully yielded albino phenotypes, serving as a robust visual assay for editing efficiency [61]. Despite its utility, *Agrobacterium*-mediated delivery is hampered by random DNA integration and host genotype recalcitrance, especially in monocots [13]. Alternatively, biolistic bombardment has been utilized to co-deliver Cas9 and sgRNA (single-guide RNA) plasmids into rice calli [14]. However, this technique is often limited by low transformation efficiencies and the risk of collateral genomic or cellular damage. These limitations underscore the critical need for the development of novel, high-efficiency, and minimally invasive delivery platforms for CRISPR-Cas systems in plants.

Viral Vectors

Viral vectors, which utilize plant viruses as vehicles for nucleic acid delivery, introduce foreign sequences into plant cells via viral infection and thereby enable either transient gene expression or RNA interference (RNAi). Building on this principle, virus-induced gene silencing (VIGS) was developed to exploit the plant's endogenous post-transcriptional silencing machinery, inducing RNAi against specific host genes and conferring resistance to systemic viral infection [62,63]. Several engineered plant viral vector systems, including tobacco mosaic virus (TMV) [30], tomato golden mosaic virus (TGMV) [31], and tobacco rattle virus (TRV) [32,33] have been adapted for foreign protein expression or RNAi induction [22,64,65]. These platforms are widely applied in the transient production of recombinant proteins such as vaccines, monoclonal antibodies, and industrial enzymes. Despite these advantages, viral vector applications are constrained by a narrow host range, limited transgene capacity, and the potential to trigger host immune responses, which may compromise vector stability and transgene expression [63].

Electroporation

Electroporation is a widely adopted physical delivery technique that employs high-intensity electrical pulses to create transient pores in the cell membrane, thereby facili-

tating the efficient cytoplasmic transfer of DNA and RNA into plant cells [34,66,67]. The technique is valued for its rapid transfection, adaptability to diverse cell types, and ability to process large cell populations simultaneously. Although electroporation has been successfully used to transfer exogenous genes into various plant materials, including protoplasts, suspension cultures, tissue sections, and intact explants [68,69]. Its utility is still constrained by significant drawbacks. The high-voltage electrical pulses might cause considerable physical stress to cells, often resulting in membrane damage, reduced viability, and limited transfection efficiency, especially in sensitive systems such as protoplasts [8,70]. Optimizing parameters like material type, pulse conditions, and buffer composition remains critical to improving its practicality.

Biolistic Particle Delivery

The biolistic method, also known as particle bombardment, is a physical approach for delivering biomolecules into plant cells. It utilizes high-velocity microprojectiles to traverse cell walls and membranes. Early studies demonstrated that microprojectiles propelled at high speeds could penetrate the cell wall and membrane of *Allium cepa* epidermal tissues without causing significant damage, thereby successfully delivering DNA into the cells [71]. This finding established particle bombardment as a promising platform for intracellular DNA delivery in plants. Subsequent work extended its application to monocot systems, where plasmid DNA encoding the chimeric chloramphenicol acetyltransferase (CAT) gene was adsorbed onto tungsten particles and then accelerated via a gene gun to penetrate suspension-cultured maize cells, effectively overcoming cellular barriers [38]. Since then, the technique has been adapted for transforming a wide range of recipient materials, such as protoplasts, callus tissues, and even pollen [12,72,73]. It has also been adapted for the delivery of non-DNA molecules, including small RNAs and mRNAs [39,74,75].

Compared to *Agrobacterium tumefaciens*-mediated plant transformation, this technique allows for the direct use of purified circular or linear plasmid DNA, as well as PCR amplicons for particle bombardment. Additionally, multiple DNA fragments containing different gene of interest cassettes can be mixed and co-bombarded into plant cells or tissues. Although the biolistic method enables physical delivery of nucleic acids into plant cells without the need for biological vectors, its comparatively low efficiency of gene integration often requires extensive screening to isolate transgenic lines exhibiting stable foreign gene expression [76]. Furthermore, the high equipment cost associated with gene gun systems further restricts its broad application [77,78].

Microinjection

Microinjection is a physical delivery technique that enables the direct introduction of DNA or proteins into the cytoplasm of plant cells or embryos through fine micropipettes capable of penetrating the cell wall and plasma membrane. This method allows precise targeting of exogenous genetic material to specific intracellular compartments and thereby facilitates stable genomic integration, transformation, and gene expression [26]. The microinjection system was originally established for delivering DNA into tobacco mesophyll protoplasts. By immobilizing protoplasts in a thin layer of agarose- or alginate-solidified medium and co-injecting FITC-dextran as a tracer, they achieved targeted cytoplasmic delivery of DNA. This approach led to efficient transient expression and stable transformation of the foreign gene [40]. In another study, CRISPR-Cas reagent delivery via electroporation was compared with single-cell microinjection in *Euglena gracilis*. While electroporation of Cas9 ribonucleoproteins (RNPs) yielded low editing efficiency, microinjection enabled precise intracellular transfer and significantly improved editing outcomes [41]. This finding demonstrated its potential for genetic manipulation in photosynthetic microalgae and other recalcitrant plant systems. Despite its broad applicability, the microinjection technique requires specialized equipment and technical expertise, suffers from low throughput, and carries a risk of causing cellular damage, which collectively limit its widespread use [40,79].

While conventional plant genetic engineering has been pivotal for crop improvement, its efficacy is often hindered by limitations such as random genomic integration, low transformation efficiency, and heavy reliance on specialized equipment. To overcome these bottlenecks, nanomaterial-mediated delivery systems have emerged as a versatile alternative. Leveraging tunable physicochemical properties and superior biocompatibility, these nanocarriers can effectively traverse the cell wall and plasma membrane barriers to deliver a wide range of functional biomolecules like DNA, RNA, and protein. Unlike traditional methods, nanotechnology-based strategies often bypass tissue culture requirements, thereby minimizing the risks of gene silencing and unintended DNA integration. Furthermore, their non-invasive nature and genotype-independent delivery broaden the scope for cross-species applications.

Nanomaterial-Mediated Delivery Systems

Nanomaterial-based delivery systems offer distinct advantages, including host species independence and non-invasive delivery compared to conventional genetic techniques. These features enable its broad applicability across diverse plant species from monocots to dicots without causing physical damage. Notably, these systems can efficiently deliver a wide range of nucleic acids, such as DNA, mRNA, siRNA, miRNA, and plasmids [10,21], without size limitations. Moreover, owing to their tunable physicochemical

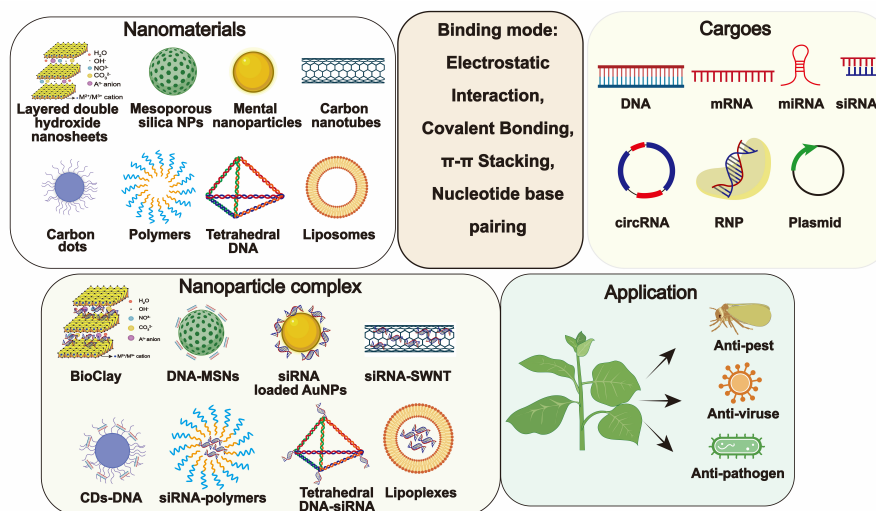


Fig. 1. Schematic representation of nanomaterial-mediated nucleic acid delivery in plants. Nanomaterials can associate with various nucleic acids, such as nucleic acids and proteins, through multiple mechanisms, such as electrostatic interactions, covalent bonding, π – π stacking, and base pairing, thereby enhancing nucleic acid stability and promoting efficient delivery into plants. These interactions facilitate the formation of stable NPs, including a complex formed by layered double hydroxide nanosheets and siRNA (BioClay), DNA-loaded mesoporous silica NPs (DNA-MSNs), siRNA-loaded AuNPs, siRNA-loaded SWNT (siRNA-SWNT), DNA-loaded carbon dots (CDs-DNA), siRNA-loaded polymers (siRNA-polymers), tetrahedral DNA nanostructure-based siRNA carriers (tetrahedral DNA-siRNA), and lipid-based lipoplexes. These nanocomplexes can confer anti-pest, antiviral, and antifungal activities within plant tissues, demonstrating great potential for crop protection in agricultural biotechnology. This figure was created by Adobe Illustrator.

properties, NPs can be precisely engineered to overcome key plant barriers such as the hydrophobic cuticle, rigid cell wall, and variable extracellular and intracellular pH gradients. A variety of nanomaterials, including layered double hydroxides (LDH), mesoporous silica NPs, carbon-based nanomaterials, metal NPs, and polymers, have been developed for plant applications. Nucleic acids can be loaded onto these nanocarriers via electrostatic interactions, covalent bonding, π – π stacking, and base pairing. By optimizing NPs size, surface charge, targeting ligands, and plant physiological responsiveness, these engineered nanocarriers can effectively traverse biological barriers, enabling diverse applications in crop protection, including pest resistance, antiviral strategies, and pathogen defense (Fig. 1). An overview of current nanomaterials developed for nucleic acid delivery in plants is summarized in Table 2 and **Supplementary Table 1**.

LDHs

LDHs consist of cationic metal sheets (e.g., Mg^{2+} , Cu^{2+} , Zn^{2+}) and intercalated anions (e.g., CO_3^{2-} , NO_3^- , Cl^-). Their positive surface charge facilitates the effective binding and intracellular delivery of anionic biomolecules via electrostatic interactions [81,109]. Early studies have demonstrated that layered lactate-modified LDH nanosheets (LDH-lactate-NS), characterized by thicknesses ranging from approximately 0.5 to 2 nm and diameters between 30 and 60 nm, possessed a high adsorption capacity for negatively charged biomolecules [80]. Moreover,

due to their intrinsic anion exchange capacity, LDHs were capable of efficiently releasing intercalated biomolecules into the surrounding environment by partial dissolution or degradation of the LDH structure under acidic pH conditions [84,110,111].

The LDH-nanosheet conjugates were capable of efficiently crossing the cell wall and transporting cargoes into *A. thaliana* seedlings and *N. benthamiana* tobacco bright yellow-2 cells (BY-2) suspension cells [80]. Beyond somatic cells, LDH NPs with diameters up to 50 nm were also proved to facilitate the targeted delivery of dsRNA into tomato pollen. The expression level of the target genes was reduced by 89% 3-day post treatment, indicating successful internalization of the LDH-dsRNA complex into the pollen and subsequent release and gene silencing of dsRNA in the cytosol [82]. Researchers developed a BioClay LDH nanosheet with up to 120 nm in diameter and 5 to 10 nm in thickness for the delivery of dsRNA to protect *N. benthamiana* from virus [83]. The strong positive surface charge and biodegradability of BioClay enabled the efficient adsorption and sustained release of siRNA, facilitating the uptake by plant cells in leaves following topical administration. At least 20 days of antiviral activity was achieved by a single spray of the BioClay due to its long-term sustained release (30 days). Other nucleic acids, e.g., artificial miRNA, could also be delivered by the LDH for antiviral protection [84]. Beyond antiviral applications, LDHs also showed promise

Table 2. Nanomaterial-mediated delivery of nucleic acids in plants.

Delivery method	Cargoes	Administration route	Nano-cargo interaction mode	Loading capacity	Delivery efficiency	Demonstrated host species	Ref.
LDH	ssDNA, dsRNA, DNA, pDNA, miRNA.	Foliar spray	Electrostatic adsorption	<ul style="list-style-type: none"> Completed loading at dsRNA–LDH mass ratio of 1:4. An LDH: siRNA mass ratio of 7:1. 	<ul style="list-style-type: none"> 20 mg/L dsRNA, 89% gene silencing at 3 dps, and a 20-day protection against the virus. DNA was ingested post incubation for 60 minutes. 1.25 μg pDNA, 41.7% symptom expression rate 35 days after inoculation. 	BY-2 (bright yellow-2 cells), homogenous suspension cell line derived from the pith tissue of <i>Nicotiana tabacum</i> , Tomato leaf and pollen, <i>N. benthamiana</i> .	[80–85]
SWCNT	siRNA, DNA, pDNA.	Infiltration, foliar spray, immersing.	π - π stacking, electrostatic adsorption	<ul style="list-style-type: none"> A loading ratio of 100 nM siRNA to 2 mg/L SWCNTs. In a 3:1 PEI-SWCNT: DNA mass ratio 	<ul style="list-style-type: none"> 95% gene silencing at 1 dpi with a siRNA dose of 100 nM. Expression of the transfected plasmid was detected three days post incubation. A 7532-fold upregulation of GFP mRNA expression by pDNA at 3 dpi 	Arugula, Wheat, Cotton, Rice, <i>N. benthamiana</i> .	[86–88]
CDs	siRNA, pDNA	Foliar spray, infiltration, root soaking.	Electrostatic adsorption	<ul style="list-style-type: none"> CDs: siRNA mass ratio of 40:50. 0.5 mg DNA and 1 mL (0.2 mg/mL) CDP. 	<ul style="list-style-type: none"> 12 ng/μL siRNA, >80% reduction of gene expression at 5 dps. 10 ng/μL pDNA, GFP fluorescence was observed 3 dpi. 	Tomato, Rice, Wheat, Mung bean, <i>N. benthamiana</i>	[89–91]
Graphene-based	siRNA, dsRNA	Infiltration, foliar spray.	Electrostatic adsorption	<ul style="list-style-type: none"> siRNA was completely loaded at the ratios of GONs (graphene oxide nanoparticle):siRNA = 0.03:1. 	<ul style="list-style-type: none"> 2 μg siRNA, 97.2 % efficiency at 1 dpi. dsRNA could be continuously released for at least 6 days. 	<i>N. benthamiana</i>	[92–96]
AuNPs	siRNA	Infiltration	Covalent bonding to mercaptan sulfide on AuNPs, Electrostatic adsorption.	<ul style="list-style-type: none"> 25 ng of siRNA per 1 μg of AuNPs. 	<ul style="list-style-type: none"> 2 μM siRNA, 80% knockdown at 1 or 2 dpi in <i>A. thaliana</i>. 100 nM siRNA, 76.5% knockdown at 1 dpi in <i>N. benthamiana</i> 	<i>A. thaliana</i> , <i>N. benthamiana</i> .	[97,98]

Table 2. Continued.

Delivery method	Cargoes	Administration route	Nano-cargo interaction mode	Loading capacity	Delivery efficiency	Demonstrated host species	Ref.
MSNs	DNA	Incubation, bi-olistic.	Electrostatic adsorption	<ul style="list-style-type: none"> The optimal DNA coating for an MSN is 1:10 (w/w). 	<ul style="list-style-type: none"> 1 μg DNA, 7% of the 10^6 cells, transient GFP expression could be observed 36 h after protoplasts were incubated with DNA-coated Type-II MSN in tobacco mesophyll protoplasts. 	Tobacco protoplast, Tobacco or teosinte leaf.	[12,99]
Peptide	dsRNA, siRNA, DNA.	Foliar spray	Electrostatic adsorption	<ul style="list-style-type: none"> At N/P ratio = 2.0 	<ul style="list-style-type: none"> 2 μg siRNA, in <i>A. thaliana</i>, YFP mRNA and protein levels were reduced by 54.1% and 45.5%, respectively, while in tomato, GFP fluorescence and mRNA transcript levels decreased by 83% and 59%, respectively, at 3 dps. 	<i>A. thaliana</i> , Tomato, Soybean, Tobacco chloroplast.	[100–102]
DNA nanos-structure	siRNA	Infiltration	Complementary base pair hybridization	<ul style="list-style-type: none"> One Tetrahedron with a loading capacity of one siRNA molecule per carrier. 	<ul style="list-style-type: none"> 100 nM nanostructure, protein level reduced by about 50% at 3 dpi. 	<i>N. benthamiana</i>	[103, 104]
Polymer	pDNA, miRNA.	Infiltration	Electrostatic adsorption	<ul style="list-style-type: none"> Polymer to DNA ratio of N/P = 3.0. ds-MIRNA: Star polycation=1:1(w/w). 	<ul style="list-style-type: none"> 1.5 μg pre-miRNA enabled relative miRNA amount significantly increased 68.64-fold in <i>A. thaliana</i>; For maize, 2.5 μg miRNA led to over 300-fold increase in miRNA amount compared to the control. 	<i>N. benthamiana</i> , <i>A. thaliana</i> , Maize protoplast.	[11,105, 106]
Liposomes	TMV-RNA, DNA	Incubation, co-cultivation.	Passive encapsulation	<ul style="list-style-type: none"> 30–40% of the TMV RNA was encapsulated 	<ul style="list-style-type: none"> 500 μg of TMV-RNA, ~20% of the tobacco protoplasts were infected after 48 hours; The binding of Liposomes to the pollen tubes was visualized in scanning electron micrographs. 	Tobacco protoplasts, Watermelon pollen tubes.	[107, 108]

in insecticidal applications, such as against whiteflies [112]. Moreover, surface coating modifications can significantly broaden the application potential of LDH NPs. For instance, a recent study showed that lysozyme-coated LDH NPs effectively delivered diverse nucleic acids, including mRNA, dsRNA, siRNA, and pDNA, into various plant tissues, such as roots, leaves, calli, flowers, and developing pollen. This improved delivery was attributed to the ability of the coating to increase cell wall permeability and upregulate genes associated with endocytosis [113]. These findings not only demonstrate the role of LDH in promoting the systemic movement of extracellular nucleic acids within plants but also emphasize their function in mediating the transfer of bioactive molecules from host plants to herbivorous pests through the vascular network.

Carbon-Based Nanomaterial

Carbon nanomaterials, defined as carbon-based materials with at least one dimension smaller than 100 nanometers, encompass various structures, including carbon nanotubes (CNTs), graphene, fullerenes, and quantum dots. These materials exhibit high specific surface area, excellent biocompatibility, and remarkable drug-loading capacity, making them highly attractive for biomedical applications, including drug delivery, cancer therapy, and bioimaging [114,115]. Recently, the unique properties of carbon nanomaterials have also garnered significant interest in plant science, particularly in plant molecular delivery [116]. CNTs and CDs show promise as nanocarriers for plant genetic material delivery, enabling advances in functional genomics and crop biotechnology. Their nanoscale size and modifiable surfaces allow efficient cellular uptake and targeting.

Carbon Nanotubes (CNTs)

Carbon nanotubes are characterized by their remarkably high aspect ratio (length-to-diameter ratio). With diameters ranging from ~0.4 nm (single-walled carbon nanotubes, SWCNTs) to 100 nm (multi-walled carbon nanotubes, MWCNTs) and lengths reaching millimeters to centimeters [117], their one-dimensional tubular and hollow nanostructure enables unique anisotropic properties. The high aspect ratio and large surface area of CNTs enable efficient encapsulation and delivery of genetic material or therapeutic payloads. Their tubular structure, with conjugated π -electron networks, facilitates stable complexation with nucleic acids via π - π stacking interactions [118], enhancing payload stability during delivery. CNTs have been demonstrated to protect DNA from nuclease degradation both *in vitro* and *in vivo* [119]. The high aspect ratio of CNTs also promotes cellular uptake, enhancing intracellular delivery efficiency [120–122]. These unique attributes make CNTs promising carriers for plant biomolecule delivery [115].

SWCNTs have been demonstrated to be capable of overcoming the plant cell wall and membrane barriers, facilitating the efficient internalization of biomolecules into

plant cells [86]. A GFP silencing efficiency of 95% could be achieved when SWCNTs were utilized to deliver siRNA in tobacco [87]. Although CNTs initially were shown to enable effective nucleic acid delivery, their poor aqueous dispersibility and potential cytotoxicity can cause formulation heterogeneity, limiting practical applications in plant systems [123–125]. To address this limitation, targeted surface functionalization of CNTs has been implemented to enhance phytocompatibility and interfacial adhesion, thus expanding their applicability in plant biotechnology [126,127]. It was demonstrated that SWCNTs can facilitate the delivery of plasmid DNA into *N. benthamiana*, *Eruca sativa*, wheat and cotton leaves, as well as arugula protoplasts [128]. Importantly, polyethyleneimine (PEI)-functionalized SWCNTs could enhance DNA binding and nuclease protection capacities through electrostatic interactions, while improving aqueous dispersibility and stability—key attributes that significantly advance their utility in plant nucleic acid delivery [88,128]. Recent studies demonstrated that SWCNTs functionalized with oligohistidine peptides (His₆-SWCNTs) enabled controlled delivery and release of nucleic acids in plants. The release mechanism relied on the deprotonation of imidazole groups within the oligohistidine moiety in the neutral cytosolic environment (pH ~7), facilitating efficient dissociation of the genetic cargo. Using this system, a short tandem-targeting mimetic noncoding RNA (STTM396) was delivered via His₆-SWCNTs to inhibit miR-396 in callus cells, thereby promoting tissue regeneration [129].

Beyond mature plant delivery, SWCNTs also enabled organelle-specific targeting in plants. The SWCNTs were found to be able to transport different cargoes to distinct plant organelles, suggesting their potential for targeted and selective intracellular delivery [130–132]. A recent study developed chitosan-functionalized SWCNTs that release plasmid DNA selectively in response to the alkaline pH of the chloroplast stroma (~pH 8), leading to efficient chloroplast transgene expression [130]. The targeting efficiency of SWCNTs to chloroplasts in *A. thaliana* was significantly improved from 39% to 57% following surface modification with a specific chloroplast-targeting peptide [131]. By integrating SWCNTs with a cross-linked polymer functionalized with the presequence of the mitochondrial cytochrome *c* oxidase subunit IV (Cytcox) and nine lysine-histidine repeats (KH9) peptides, the DNA delivery efficiency into *A. thaliana* mitochondria were enhanced nearly 30-fold compared to conventional methods [132].

CDs

CDs are a class of carbon NPs with a size less than 10 nm, which possess excellent fluorescence properties, remarkable small-size effects, and good biocompatibility [133–136]. These characteristics enable CDs to serve as effective gene carriers, offering new possibilities for precise genetic transformation and efficient gene expression in

plants. Li *et al.* [89] have investigated the toxicity, uptake, and translocation of CDs in plants. Their findings showed that CDs had a negligible impact on the growth of mung beans, indicating low phytotoxicity. Moreover, CDs were transported from roots to stems and leaves through the vascular system of the apoplastic pathway, eventually accumulating in the leaf veins. This research laid the foundation for understanding the behavior of CDs in plants, providing crucial information for their subsequent application as gene carriers.

For genetic transportation, CDs synthesized from branched polyethylenimine (PEI) possess an average diameter of approximately 3.9 nm. Due to the high charge density and robust nucleic acid binding affinity of the PEI precursor, CDs were demonstrated to effectively deliver siRNA into *N. benthamiana* and tomato via foliar spraying. This approach achieved about 80% knockdown of the genes encoding the H and I subunits of magnesium chelatase (*CHLH* and *CHLI*), resulting in visible photo-bleaching [90]. Despite this success, the general application of anionic CDs is often limited by electrostatic repulsion with the plant cell wall.

Cationic surface modification (e.g., with PEI) significantly enhanced the ability of CDs to deliver nucleic acids into plant cells. This strategy improved cellular uptake, enabling robust transgene expression in various crops, including rice, wheat, and mung bean leaves, as well as rice roots. Notably, amine-functionalized CDs (A-CDs) exhibited pH-responsive behavior, surface charge-switching properties, and the capacity to enhance nuclear import of genetic material [91,137]. Furthermore, functionalized CDs loaded with dsRNA have been used for spray-induced gene silencing, effectively controlling pathogens and reducing fungicide use by 90% [138]. These results establish surface-modified CDs as a highly valuable tool for plant biotechnology and crop protection.

Graphene-Based Nanomaterials

Graphene, a two-dimensional (2D) material composed of single-layer sp^2 -bonded carbon atoms arranged in a honeycomb lattice [139], has garnered significant attention in biomedical applications due to its outstanding electrical and thermal conductivity, high specific surface area, and excellent biocompatibility. These exceptional properties make it particularly valuable for medical imaging and gene delivery in mammalian systems [92,93,140,141]. Recently, graphene-based materials have also emerged as promising nanocarriers for plant systems. For instance, sheet-like GONs can complex with siRNA via electrostatic interaction to form nanostructures capable of traversing plant cell walls and achieving up to 97.2% transient gene silencing within 24 hours [94]. Building on this platform, PEI-coated GONs have been shown to adhere to cell walls and deliver antiviral dsRNA, while PEI-functionalized graphene quantum dots (GQDs) enabled foliar delivery of dsRNA for targeted sup-

pression of *Fusarium graminearum* [95,96]. These studies collectively highlight the versatility of engineered graphene materials in plant biotechnology and pathogen control.

Gold NPs (AuNPs)

AuNPs, typically ranging from 1 to 100 nm in diameter, possess unique physicochemical properties due to their nanoscale dimensions. The high specific surface area of AuNPs provides abundant active sites for biomolecule adsorption and conjugation, while the tunable dispersion and low cytotoxicity make them highly suitable for nucleic acid delivery in biological systems [142,143]. AuNPs have been extensively employed for nucleic acid delivery in mammalian cells and animal models [144]. Recent studies explored the potential of AuNPs as efficient carriers for intracellular delivery in plants. Ultra-small AuNPs (1 to 2 nm in diameter) fell below the size exclusion limit of plant cell walls, enabling their passive translocation via apoplastic or symplastic pathways. Engineered positively charged PEI-functionalized AuNPs were shown to deliver siRNA into *A. thaliana*, achieving 80% silencing efficiency of the defense-related *NPR1* gene [97]. Expanding on this work, Zhang *et al.* [98] systematically investigated the influence of PEI molecular weight on siRNA delivery efficiency using 1–2 nm AuNPs. The cationic PEI coating not only facilitated electrostatic binding of nucleic acids but also conferred nuclease resistance, thereby enhancing siRNA stability. Remarkably, these NPs were rapidly internalized by tobacco cells within 0.5–1 h, leading to ~70% silencing of the GFP gene 1 day post infiltration and 32–35% suppression of endogenous target protein expression 3 days post infiltration (Dpi). Collectively, these findings underscore the versatility of AuNPs as a robust nucleic acid nanocarrier platform for plant biotechnology [145].

Mesoporous Silica NPs (MSNs)

Mesoporous silica NPs (MSNs) represent a class of inorganic nanomaterials distinguished by their high specific surface area, exceptional biocompatibility, and facile surface functionalization [146]. These unique characteristics render MSNs particularly suitable for drug delivery applications [147,148]. In plant systems, the nanoscale dimensions of MSNs confer superior penetration capabilities, enabling efficient traversal of biological barriers such as plant cell walls and membranes. A critical consideration in MSN applications is the inherent hydrophobicity of unmodified silica surfaces, which may induce plant cell toxicity or plant cell [149]. To address these limitations, surface modification strategies have been developed to enhance dispersibility while reducing cytotoxicity [150]. A pioneering study demonstrated the synthesis of honeycomb-structured MSNs with a pore diameter of 3 nm, which were functionalized either by covalent attachment of triethylene glycol (TEG) or by capping with gold NPs via amide coupling. The TEG modification enhanced endocytosis in protoplasts

without cytotoxicity. In the case of gold nanoparticle-capped MSNs, the release was controlled through the cleavage of disulfide linkages by dithiothreitol (DTT)-mediated reduction. These engineered NPs successfully delivered DNA into tobacco protoplasts, seedlings, and maize endosperm via particle bombardment [12]. In a related study utilizing biolistic methods, gold-conjugated mesoporous silica nanoparticles (Au-MSNs) were shown to co-deliver proteins and plasmid DNA into both tobacco and teosinte leaf cells [99]. Given the limitations of physical delivery methods, such as tissue damage and high costs, subsequent research demonstrated that functionalized MSNs can deliver DNA into *A. thaliana* roots via simple soaking. Following exposure for 24 h, expression of the reporter gene in root epidermal and cortical cells was detected, demonstrating the capability of MSNs to deliver gene cargo without external force assistance [151]. Notably, the delivery capacity of MSNs has been harnessed for plant protection strategies, including antiviral defense and pest resistance [152]. MSNs have been employed as nanocarriers for the delivery of the *cryIAb* gene into tomato, leading to a 1.36-fold enhancement in resistance to *Tuta absoluta* [153]. Beyond DNA delivery, the application of MSNs has been extended to siRNA delivery for targeted gene silencing. For instance, research has demonstrated that MSNs could effectively deliver siRNA into *N. benthamiana* through foliar spraying. The MSN-based carrier not only shielded siRNA from nuclease degradation but also achieved effective knockdown of multiple target genes (with silencing efficiencies ranging from 90% to 98%) at 4 dps [154]. Polydopamine surface modification enabled MSNs to achieve controlled nucleic acid release. These carriers efficiently load nucleic acid complexes and, when sprayed, remain stable on the weakly alkaline leaves of *N. benthamiana*, protecting the cargo from nuclease degradation. Under mildly acidic conditions, protonation of the polydopamine layer increases electrostatic repulsion, causing its partial detachment and subsequent release of the nucleic acid complexes. At pH 6, the siRNA release rate reached 66.7%, allowing for targeted delivery at specific sites [155].

Peptide

Peptide compounds, composed of amino acids linked by peptide bonds, are versatile biological macromolecules with essential roles in living systems [156]. Notably, certain peptides, such as cell-penetrating peptides (CPPs), possess the unique ability to traverse cellular membranes [157], enabling the intracellular delivery of molecular cargoes in plants. Their potential as nucleic acid delivery vectors has recently drawn significant interest in plant biotechnology. Depending on their structural and functional properties, peptide-based carriers can be categorized into cationic peptides, cell-penetrating peptides, targeting peptides, and stimuli-responsive peptides, which can function either as independent delivery vectors or combine with other nano-

materials to achieve targeted delivery [131,155,158].

Cationic oligopeptides have been developed for the delivery of dsRNA into *N. benthamiana* suspension cells [101]. These peptides effectively protect dsRNA from nuclease degradation and bind it through electrostatic interactions. Their cationic nature enhanced interaction with cell membranes, thereby improving cellular uptake. Once internalized, the dsRNA was released into the cytosol to induce gene silencing [100]. Subsequent studies demonstrated that arginine-rich CPPs facilitated the delivery of plasmid DNA into *Vigna radiata* root cells, leading to successful expression of exogenous genes [159]. To further improve delivery efficiency, polycation was fused to CPPs for transfection in intact plant cells. Fusion peptides exhibit higher transfection efficiency compared to unfused CPPs, indicating that cationic polymers can optimize the delivery properties of CPPs [160]. Furthermore, specific targeting signal peptides enabled the precise delivery of exogenous biomolecules to the plant nucleus [102], mitochondria [161], and chloroplasts [102], facilitating their integration into respective organelles. This approach allows for the precise modulation of plant traits without relying on transgenic techniques, thereby offering a novel strategy for plant genetic improvement.

Polymer-based Nanomaterials

Polymer nanomaterials typically exhibit particle sizes ranging from 1 to 1000 nm, a scale comparable to that of many biomolecules and cellular structures. This dimensional similarity facilitates efficient interactions with biological systems [162]. Their surfaces possess abundant chemically active sites, enabling versatile functionalization with various groups (e.g., carboxyl, amino, and thiol) through surface modification strategies. Such modifications not only promote interactions with biomolecules but also enhance biocompatibility, targeting ability, and specificity [163–165]. Owing to these advantageous properties, polymer nanomaterials hold significant promise for biomedical applications, particularly in drug delivery and gene transfer. In plant delivery systems, polymer nanomaterials serve as versatile carriers for the controlled release of bioactive compounds, enabling precise regulation of plant growth and metabolism. This unique capability renders them particularly valuable for agricultural applications, especially in the targeted delivery of pesticides, fertilizers, and plant protection agents.

Polymer-based carriers are broadly classified into two categories: natural and synthetic polymer nanomaterials. Among natural polymers, chitosan (CS), cyclodextrins, and glucans have been extensively studied [166,167]. CS functions both as a potent antimicrobial agent and an inducer of systemic acquired resistance (SAR) in plants. CS has been applied in pesticide delivery systems, showing significant efficacy in pest control [168,169]. Additionally, CS can serve as an excellent surface modification mate-

rial for NPs, enhancing both targeting precision and biocompatibility [130,170]. Synthetic polymer nanomaterials, particularly polyamidoamine (PAMAM) dendrimers, which are highly branched, symmetric, tree-like polymers, and PEI, have been widely utilized for genetic delivery [171–173]. In turfgrass cells, researchers achieved remarkable transformation efficiency by delivering plasmid DNA using PAMAM carriers. This success relied on precisely optimizing the PAMAM-to-DNA molar ratio and the solution pH [11]. Beyond DNA, polymer nanomaterials like PDMAEMA polymer and cationic poly-aspartic acid-derived polymer were effective for delivering proteins and double-stranded RNA (dsRNA) [174,175]. Other polymers like star polymer (SPc) could not only deliver microRNAs into plants but also enhance plant defense by upregulating pathogen-related genes, thus enhancing plant defense responses [106,176]. The utilization of polymer nanomaterials in plant delivery systems continues to expand significantly, with ongoing advancements particularly enhancing their biocompatibility, delivery efficiency, and target specificity.

Liposomes

Liposomes are self-assembled spherical vesicles characterized by a phospholipid bilayer surrounding an internal aqueous core. This amphiphilic architecture enables the simultaneous encapsulation of hydrophilic payloads, such as nucleic acids, within the aqueous interior and the integration of lipophilic substances within the hydrophobic bilayer. Given that their lipid composition closely mimics the fundamental structure of plant and animal cell membranes, liposomes offer exceptional biocompatibility and minimal cytotoxicity [177,178]. Furthermore, the physicochemical properties of the liposomal surface can be precisely engineered by modulating the ratio of charged lipids—such as cationic DOTAP or anionic DOPG. Such surface-charge tuning is critical for regulating electrostatic interactions with the negatively charged plant cell wall and plasma membrane, thereby influencing cellular internalization and the subsequent efficacy of intracellular delivery [179,180].

In plant biotechnology, the utility of liposome-mediated delivery was early established through the successful encapsulation and functional translocation of viral genetic material. Initial studies demonstrated that liposomes could effectively encapsulate *Tobacco mosaic virus* (TMV) RNA, facilitating its delivery into tobacco protoplasts and resulting in successful viral replication [181]. Subsequent research expanded this application to DNA delivery, showing that plasmids encapsulated within liposomes could be efficiently transferred into protoplasts to drive transient protein expression [107]. To achieve more sophisticated control over cargo release, “stimuli-responsive” liposomes have been developed. By incorporating pH-sensitive lipids such as dioleoylphosphatidylethanolamine (DOPE) and oleic acid (OA), these

vesicles undergo structural destabilization under acidic conditions (pH 6.0), triggering the controlled release of nucleic acids within the intracellular environment [182].

Beyond protoplast-based systems, liposomal carriers have been adapted for the genetic transformation of reproductive tissues. For instance, DNA-loaded liposomes can fuse with germinating watermelon pollen tubes, potentially allowing exogenous genetic material to be integrated into the progeny genome via natural pollination and fertilization [108]. Similarly, this approach has enabled the delivery of *Tomato yellow leaf curl virus* (TYLCV) DNA into tomato protoplasts and callus cultures [183]. Despite these versatile applications, the widespread adoption of liposomes in plant science is hindered by several intrinsic challenges. Their structural integrity is often compromised by poor thermal stability, and the rigid plant cell wall remains a formidable physical barrier to efficient penetration. Furthermore, the current lack of robust *in planta* delivery strategies restricts the practical application of liposomes as carriers for nucleic acids in field-grown crops.

Other Nanomaterials

Nucleic Acid Nanostructure

DNA tetrahedral NPs (TDNs) are three-dimensional structures formed by the self-assembly of four oligonucleotide strands with specifically designed sequences. These structures exhibit excellent biocompatibility, low immunogenicity, and design adaptability, making them ideal candidates for nucleic acid delivery. Furthermore, TDNs can combine with nucleic acids through nucleotide base pairing hybridization, which enables them to protect encapsulated nucleic acids from degradation. DNA nanostructures can penetrate plant cells without external assistance, effectively delivering siRNA to mature plant tissues and achieving targeted gene silencing [103,104]. Similarly, self-assembled single-stranded RNA (ssRNA) constructs based on multi-junction scaffolds offered a promising platform for simultaneous loading and controlled release of multiple siRNAs. These RNA nanostructures show strong stability under physiological and environmental conditions and can be produced cost-effectively, representing a promising platform for scalable nucleic acid delivery in agricultural pest management [184].

Starch NPs (SNPs)

Starch NPs (SNPs), typically ranging from 10 to 500 nm in diameter, are formed through physical, chemical, or biological modification of starch molecules. As naturally abundant, low-cost materials with excellent biocompatibility and modifiability, SNPs have shown great promise for drug delivery, gene delivery, and agricultural applications. Fluorescent SNPs (50–100 nm) with a positive surface charge were developed for cellular tracking. These NPs could effectively bind to DNA and protect it from ultrasound-induced damage and DNase I degradation. Un-

der ultrasound irradiation, the SNP-DNA complexes successfully traversed plant cell barriers and delivered pEGAD plasmid DNA, resulting in GFP expression with a transformation efficiency exceeding 5% [185,186]. These results demonstrate the strong potential of SNPs as plant transformation vectors.

While many nano-mediated delivery systems remain at the proof-of-concept stage in laboratory settings, recent advancements have demonstrated their robust potential in large-scale agricultural applications. For instance, a self-assembled multicomponent nano-bioprotectant was developed to combat potato late blight. By co-delivering dsRNA and plant elicitors via a star polycation platform, this system protected the fragile genetic cargo from environmental degradation. Field trials demonstrated that this approach significantly enhanced oomycete uptake and extended the RNAi protective window compared to conventional methods [187]. Furthermore, functionalized CDs have been employed as a robust platform for spray-induced gene silencing (SIGS). It was demonstrated that CDs-mediated delivery of dsRNA targeting essential genes in *Phytophthora* pathogens significantly enhanced RNA stability and internalization compared to naked dsRNA. This nano-fungicide platform effectively controlled oomycete infections in chili seedlings [138]. Collectively, these successful field-scale demonstrations underscore the transformative potential of nanotechnology in sustainable, large-scale agricultural management.

Delivery Process and Mechanisms

The unique physiological barriers in plants, such as the hydrophobic cuticle, the cell wall, the cell membrane, and the distinct apoplastic and cytosolic pH gradients, significantly influence the delivery efficiency of nanomaterials. Understanding how nanocarriers overcome these plant-specific barriers is essential for developing effective nucleic acid delivery systems. Although previous studies have confirmed the ability of NPs to transport biomolecules into plant cells [188], the mechanisms underlying their cellular internalization remain poorly characterized. Moreover, the transport efficiency of NPs through plant cellular barriers is dictated by key physicochemical properties, including size, morphology, and surface charge [189,190]. NPs can enter plants through foliar application or infiltration via processes such as cuticle penetration, stomatal uptake, extracellular diffusion, and traversal of the cell wall and plasma membrane [14]. Multiple uptake pathways, including endocytosis, direct membrane penetration, pore formation, and transporter protein-mediated internalization, may be involved [191]. Moreover, NPs can move systemically within plants, from roots to leaves or vice versa [192], likely via apoplastic and symplastic pathways. This section summarizes current knowledge on NPs–plant interactions, with a focus on NP absorption, cellular internalization, and systemic translocation (Fig. 2).

Factors Influencing Plant Uptake of NPs

The physicochemical properties of NPs, particularly size, shape, and surface charge, critically govern their uptake and translocation in plants [193–195]. Particle size is a primary determinant of NPs' internalization efficiency [196]. Small NPs typically exhibit superior uptake due to their large specific surface area and enhanced diffusion capacity, enabling penetration through stomata and the cuticle. For instance, it was observed that lettuce roots accumulated higher concentrations of smaller silver NPs (Ag-NPs), with subsequent translocation to shoots, likely due to increased surface area facilitating interactions with root mucilage [197]. Similar size-dependent uptake trends have been documented for foliar absorption [198]. Notably, the size threshold for NPs internalization varies across species; cotton, for example, internalizes NPs below 20 nm in size, whereas maize exhibits a stricter cutoff (below 11 nm) [18].

In addition to size, the surface properties of NPs also significantly influence their delivery efficiency into plant cells [199,200]. Positively charged NPs often show higher cellular uptake owing to electrostatic attraction to negatively charged cell walls and membranes. It was reported that NPs with zeta potentials greater than 15 mV achieved improved foliar delivery, while those smaller than 10 nm with potentials exceeding 40 mV could penetrate protoplasts and localize in chloroplasts [18]. Another study demonstrated that the surface charge characteristics of CDs govern their interaction with cell wall components such as pectin and hemicellulose, thereby determining their ability to penetrate the dual barriers of the cell wall and plasma membrane, and ultimately modulating the specific pathways of cellular internalization [201]. Notably, although positively charged CDs exhibit higher affinity toward protoplasts and may induce alterations in membrane morphology, other studies have shown that negatively charged CDs applied to roots can achieve more efficient root-to-shoot translocation by concurrently utilizing both symplastic and apoplastic transport routes [202]. This suggests that the effects of surface charge may vary depending on plant organ, species, and the local microenvironment of the cell wall. NPs further modulate uptake dynamics. Zhang *et al.* [145] demonstrated that rod-shaped AuNPs bypass cell wall barriers more readily than spherical AuNPs, which face greater restrictions. Beyond intrinsic NPs traits, external factors such as surfactants can enhance uptake by reducing surface tension, solubilizing cuticular waxes, and improving hydration and adhesion [203]. Plant species, growth stage, and environmental conditions also significantly influence NP uptake and translocation efficiency [204].

Transportation of NPs at the Tissue Level

The uptake of NPs by plants primarily occurs through two pathways: root and leaf absorption. Root uptake is primarily mediated by the root tip and root hairs, where the cuticular layer is incompletely developed. The secretion of

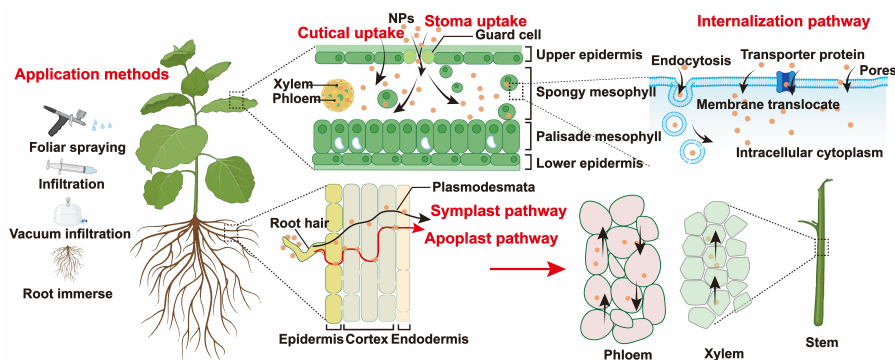


Fig. 2. Proposed mechanisms of nanoparticle uptake and intracellular trafficking in plants. NPs enter plant tissues primarily through stomatal infiltration and cuticular or epidermal absorption, with uptake efficiency governed by physicochemical properties such as particle size and surface charge. Following entry, NPs are translocated intercellularly via apoplastic and symplastic pathways and are further systemically distributed through xylem and phloem. Intracellular delivery is facilitated through diverse mechanisms, including endocytosis, direct membrane penetration, pore formation, and channel protein-mediated transport. These pathways collectively determine the efficiency and specificity of nanoparticle-mediated delivery within plant systems. This figure was created by Adobe Illustrator.

mucilage and organic acids from the root surface imparts a negative charge, which facilitates the binding of positively charged NPs and promotes their accumulation near the root [205,206]. Due to the underdeveloped cuticular layer in the root tip (including the root cap, meristematic zone, and elongation zone) and the relatively porous cell walls of root hairs, NPs with diameters smaller than the pore size of the cell wall can passively diffuse through these pores. Once within the root, NPs are transported to the vascular tissue via the apoplastic or symplastic pathways and subsequently translocated through phloem and xylem, driven by the plant's transpiration and vascular systems [195,207].

As vital organs mediating gas exchange and material transfer between plants and their external environment, leaves also serve as an important entry point for NPs absorption. Stomatal uptake and cuticular penetration are the two primary pathways for foliar NPs absorption, a process intrinsically dependent on leaf structural and physiological characteristics [208,209]. Stomata, the microscopic pores flanked by paired guard cells on the leaf epidermis, function as the major conduits for gas exchange and transpiration. These structures simultaneously represent a critical gateway for nanoparticle internalization. The deposition of NPs on the phyllosphere facilitates their entry into stomatal cavities, driven by pressure differentials and concentration gradients established during stomatal movements [210,211]. This process is modulated by multiple factors, including NPs size, surface charge, stomatal dimensions, and aperture status. Zhu *et al.* [212] systematically investigated the internalization pathways of zinc oxide NPs (ZnO NPs) in wheat leaves, revealing stomatal uptake as the dominant entry mechanism. Their findings demonstrated that internalized ZnO NPs initially accumulate in substantial amounts in the substomatal chambers before subsequent apoplastic translocation. Complementary research on hydrophilic ZnO@MSN NPs in tomato leaves further corroborated the

predominance of stomatal uptake. Notably, the study revealed a 60% higher stomatal density on abaxial leaf surfaces compared to adaxial surfaces, creating spatially heterogeneous nanoparticle absorption patterns [213].

The plant leaf cuticle, a protective layer composed of cutin and waxes, serves as a critical barrier against excessive water and solute loss [214]. The cuticle contains relatively small polar pores (~2 nm in diameter), suggesting that NPs with dimensions below this threshold may penetrate via the cuticular pathway. Notably, NPs with higher hydrophilicity or surface charges demonstrate enhanced penetration efficiency through these layers [18,193]. Additionally, mechanical damage to the leaf surface can create localized entry points, further facilitating nanoparticle uptake through compromised epidermal regions [215]. Previous research has demonstrated that solute transport across the cuticle occurs through two distinct pathways: the lipophilic pathway, which facilitates diffusion of nonpolar solutes through the cuticular matrix [216], and the polar aqueous pathway through which hydrophilic substances and ions are absorbed via pores ranging from 0.5 to 20 nm in radius, which varies significantly between plant species [217,218]. Consequently, NPs smaller than approximately 5 nm can be directly absorbed when deposited on the leaf epidermis [219]. However, studies have also observed the accumulation and subsequent internalization of NPs exceeding 5 nm in diameter, yet the precise mechanisms governing this process remain to be elucidated.

Mechanisms of Nanoparticle Internalization in Plant Cells

The internalization mechanisms of NPs determine the fundamental pathways regulating their cellular interactions. Nevertheless, the efficiency and specificity of these processes are modulated by multiple factors, encompassing the physicochemical attributes of the NPs and the biological characteristics of the cellular microenvironment. These el-

ements collectively influence NPs uptake by regulating endocytic pathway activation, transport efficiency, and intracellular metabolic fate.

Endocytosis: A Central Mechanism for Nanoparticle Internalization

Endocytosis serves as a major route for NPs internalization in plant cells, involving both clathrin-dependent and clathrin-independent mechanisms [220–223]. Evidence from tobacco BY-2 suspension cells indicated that NPs can enter via clathrin-independent endocytosis [224,225], while fluorescent nanobeads exploited both clathrin-dependent and -independent pathways [226], suggesting that NPs surface properties—particularly charge and size may dictate the preferential endocytic route. Fluid-phase endocytosis can also contribute to NPs uptake. For example, lysozyme-coated LDH NPs efficiently deliver RNA and plasmid DNA into plant cells through endocytosis [21]. Similarly, artificial nanospheres and fluorescent quantum dots enter *Firmiana* cultured cells via fluid-phase endocytosis, followed by trafficking to diverse intracellular organelles [227,228]. In maize root tip endodermal cells, actin-dependent fluid-phase endocytosis has also been implicated in NPs internalization [229]. Importantly, the surface charge of NPs has been shown to modulate endocytic pathways. Charged AuNPs, for instance, can enter plant cells via both endocytic and non-endocytic mechanisms [230], while AuNPs with different surface charges exhibit distinct uptake behaviors in tobacco pollen tubes [231], highlighting the potential involvement of charge-specific recognition processes in regulating endocytic mechanisms.

Transporter-mediated Internalization and Direct Membrane Penetration

Membrane-associated proteins and intercellular structures also play critical roles in regulating NPs transport within plant cells. Aquaporins, typically existing as tetramers in biological membranes, facilitate transmembrane transport by providing hydrophilic channels, while hydrophobic amino acid residues within the channel enable interactions with small molecules such as glycerol, thereby assisting their passage. Metal ions can also cross membranes via aquaporins by forming hydrated ion complexes that move along the aqueous channels. In addition, members of the ATP-binding cassette (ABC) transporter family have been implicated in mediating and regulating NPs translocation across cellular membranes, further highlighting the importance of membrane proteins in intracellular NPs trafficking [232–235].

Beyond the energy-dependent pathway, NPs can infiltrate plant cells through direct membrane penetration or translocation. A notable example was the Lipid Exchange Envelope Penetration (LEEP) mechanism proposed by Strano and colleagues, where NPs enter chloroplasts via lipid exchange-mediated membrane fusion [236]. Simi-

larly, researchers proposed that MSNs translocated into *A. thaliana* roots through an energy-independent route following the reorganization of cellulose microfibrils during cell wall relaxation [152]. MWCNTs could traverse both the cell wall and plasma membrane [86,237], while SWCNTs exhibited the ability to directly penetrate protoplasts [238]. This direct translocation process was thought to involve the formation of transient membrane pores induced by NPs [239]. Supporting this, cationic NPs had been shown to create structural defects in lipid bilayers, promoting their transmembrane passage [240]. Rod-shaped AuNPs have been shown to directly deliver siRNA into plant cells without the need for cellular internalization [19]. This suggests that the NPs may facilitate siRNA entry by perturbing the cell membrane.

Intercellular Transport and Systemic Delivery

Plasmodesmata, the specialized intercellular channels that form symplastic connections between plant cells, serve as functional conduits for NPs intercellular trafficking [241–245]. This transport pathway provides a plausible mechanism for tissue-level systemic distribution of NPs in plants. Through plasmodesmata-mediated translocation, NPs can achieve cell-to-cell movement, ultimately facilitating whole-plant systemic dissemination. In addition to symplastic trafficking, NPs can also utilize the apoplastic pathway, which involves movement through extracellular spaces, cell walls, and intercellular gaps without crossing the plasma membrane. This route enables NPs to bypass direct cytoplasmic entry, thereby providing an alternative means to reach the vascular tissues for subsequent long-distance transport.

Researchers proved that foliar application of liposomal NPs enables efficient entry into plant leaves through stomatal pores. Once internalized, these NPs exploited the vascular system to achieve bidirectional systemic transport, which involved acropetal translocation to adjacent leaves and basipetal translocation to the root system [246]. The vascular system, consisting of the xylem and phloem, is a crucial organ for systemic transport in plants. NPs can be translocated acropetally from roots to aerial parts via the xylem, as well as basipetally from leaves to roots and stems through the phloem [247,248]. Studies have shown that CuO NPs (20 to 40 nm) entered maize roots through the apoplastic pathway by exploiting the underdeveloped Casparian strip at the root tip. CuO NPs moved upward via the xylem once they reached the stele and vascular system. Additionally, they underwent reverse transport through the phloem from stems back to roots, demonstrating full systemic mobility within the plant [249].

Surface charge of NPs emerges as a critical determinant of their transport behavior and tissue distribution. For example, negatively charged CDs were capable of traversing root tissues via both apoplastic and symplastic routes, with notable accumulation in plasmodesmata, thereby en-

abling passage across structural barriers such as the Casparian strip [250,251]. In contrast, positively charged CDs were prone to electrostatic interactions with negatively charged constituents of root mucilage and cell walls, leading to their retention in the apoplast and limiting their penetration into deeper tissues and subsequent translocation to aerial parts [202].

Building upon the capacity of NPs for systemic transport within plants, NPs could be utilized for long term distance gene silencing by combining with siRNA or dsRNA. For instance, Guanosine cations (Gu^+) have been employed to self-assemble with siRNA into stable NPs, which effectively exploited both xylem and phloem pathways to mediate root-to-shoot translocation, thereby enabling long-distance gene silencing from roots to the apical meristem. More recently, carbon dot–dsRNA nanocomplexes have exhibited highly efficient phloem-mediated systemic transport, accumulating in distal leaves at concentrations exceeding those of naked dsRNA by more than 1000-fold, thus overcoming the inherent limitations of localized delivery [252,253].

The delivery processes and uptake mechanisms discussed above are more than simple transport events. They directly determine the subsequent biological responses of plants to NPs. The specific way NPs enter the cell, such as endocytosis or through pores, and their resulting distribution within the plant, decide how effectively the cargo is used and the strength of the cellular response. For example, releasing biomolecules into specific cell parts can trigger precise genetic changes or metabolic shifts. At the same time, the characteristics of the NPs themselves may affect plant growth, stress tolerance, or even cause toxicity. Therefore, understanding the link between how NPs are delivered and how the plant responds is essential for improving nano-biotechnology.

Biological Effects of NPs on Plants

In addition to serving as carriers, NPs exert multifaceted effects on plants through both biochemical and biophysical pathways, affecting growth, metabolism, and stress adaptation. Their unique physicochemical properties enable direct interactions with cellular structures and indirect modulation of molecular signaling, thereby shaping plant physiological and developmental outcomes. A comprehensive understanding of these mechanisms (Fig. 3) is essential to better understand the nano-plant interaction for rational design of delivery systems.

Plant growth relies on efficient acquisition of essential nutrients, yet conventional fertilizers suffer from low nutrient use efficiency, leaching, and environmental impact. While engineered NPs offer a promising alternative that enhances nutrient bioavailability and utilization, promoting robust plant growth [254,255]. For example, ZnO and Fe NPs increased Zn and Fe accumulation in wheat grains, boosting chlorophyll synthesis, photosynthetic efficiency,

and yield [256]. The synthesized P-LDHs functioned as phosphate fertilizers by intercalating phosphate into their layered structure and exhibited sustained phosphate release under soil-solution-like conditions [257]. Beyond direct nutrition, NPs can enhance overall mineral assimilation, such as foliar application of ZnO NPs in rice increased Zn, Mn, Cu, Fe, and Mg levels by 15.8–416.9%, indicating systemic modulation of mineral homeostasis [258]. Advances in nanocarrier design further improved long-distance transport. For instance, ZnO NPs encapsulated in MSNs improved Zn absorption and phloem translocation in tomato, while zinc phosphate-coated ZnO NPs promoted stem accumulation and fruit-directed transport in pepper by optimizing apoplastic–symplastic flux coordination [213,248]. Other zinc-containing NPs used for plant delivery, such as zeolitic imidazolate framework-8 (ZIF-8) [259,260], may likewise exert similar biological effects due to the release of Zn^{2+} . Therefore, the nutritional role of the released metal ions should be considered when designing such metal-based nanocarriers.

In addition to their role as nutrient carriers, NPs can physically interact with membranes, cytoskeletons, and organelles, influencing cell division, elongation, and differentiation, thereby further shaping plant morphology and physiology. For example, Nanoscale zero-valent iron (nZVI) promoted root elongation in *A. thaliana* by inducing cell wall loosening [261]. Notably, carboxyl-functionalized single-walled carbon nanotubes (SWCNTs-COOH) have been shown to enhance root elongation in onion and cucumber, likely by facilitating water uptake and modulating cellular processes; in contrast, nonfunctionalized SWCNTs often suppress root growth due to pronounced aggregation and physical obstruction of root tissues [262]. Similarly, hydroxyapatite (HAP) nanorods and titanium dioxide (TiO_2) NPs have been reported to promote early seedling growth [263,264]. The mechanisms underlying nanoparticle-mediated germination enhancement involved improved seed coat permeability, optimized water absorption capacity, and regulated reactive oxygen species (ROS) levels. These combined effects activated critical biochemical pathways for breaking seed dormancy and initiating germination processes [265–267].

Plant primary and secondary metabolism play crucial roles in plant growth and development, as well as in activating defense mechanisms in response to biotic and abiotic conditions [268,269]. As for simulating plant growth, NPs like MWCNT promoted plant growth by regulating enzymatic activities in carbon and nitrogen metabolism, enhancing carbohydrate synthesis and nitrogen assimilation efficiency, while iron oxide NPs (Fe_2O_3) NPs promoted growth by increasing the contents of gibberellins and cytokinins in peanuts [270,271]. CDs and silicon could regulate plant growth and defense by modulating phytohormone pathways: they promoted growth via cytokinins (CKs) and indole-3-acetic acid (IAA), while enhancing

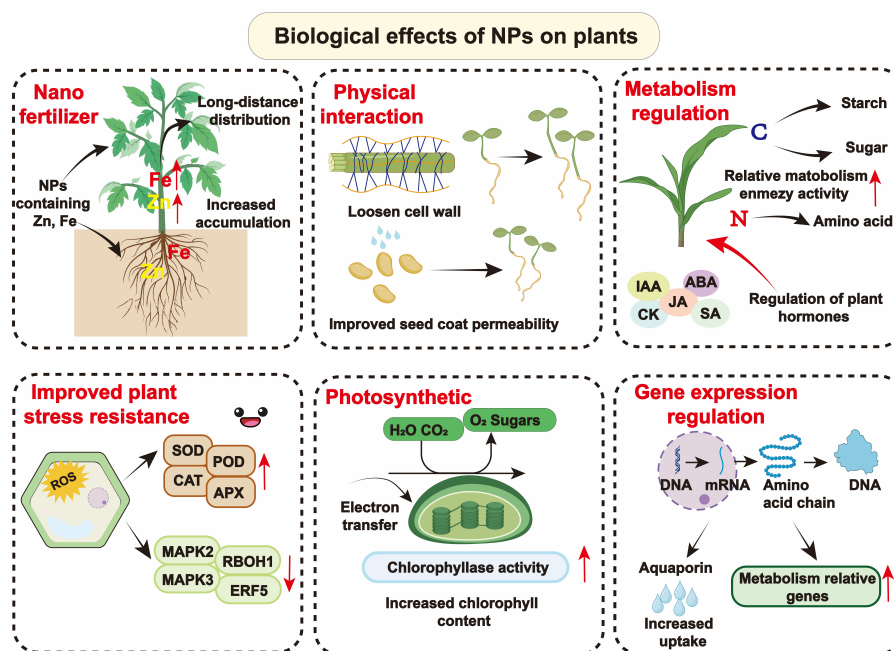


Fig. 3. A schematic representation of interactions between NPs and plants is presented. Engineered NPs influence a range of physiological and developmental processes within plants, such as serving as nano-fertilizers to alleviate nutrient deficiencies, promoting root elongation via nanoparticle-induced remodeling of the cell wall, enhancing seed germination, facilitating targeted delivery to chloroplasts to improve photosynthetic efficiency, and modulating the expression of genes associated with stress responses and development. These complex interactions underscore the potential of nanotechnology as a multifaceted approach for augmenting plant performance and resilience to environmental stressors. This figure was created by Adobe Illustrator.

stress responses through abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) signaling, thus balancing growth and resilience [272–275]. Furthermore, TiO_2 NPs mitigated growth inhibition induced by polystyrene nanoplastics in maize through activation of melatonin signaling, which coordinated carbon partitioning and nitrogen metabolism [276].

NPs also play a pivotal role in enhancing plant resilience to biotic and abiotic stresses, offering critical protection under adverse environmental conditions. NPs Exposure can trigger immune-like responses, including the accumulation of secondary metabolites and activation of systemic acquired resistance (SAR). For example, the star polycation (SPc) could assemble with an immune inducer lentinan (LNT) through hydrogen bonding into the LNT/SPc complex, which could increase the content of salicylic acid (SA) in plants, thereby activating SA-mediated SAR [277]. MWCNT could strengthen the antioxidant defense system of plants by stimulating the activity of superoxide dismutase (SOD) and catalase (CAT), leading to improved reactive oxygen species (ROS) scavenging capacity [278,279]. In addition, NPs can improve plants' resistance to unfavorable environments by inducing the innate immune response and downregulating key signaling genes involved in stress responses, including MAPK2, MAPK3, RBOH1, and ERF5 [272,280,281]. Notably, beyond eliciting host defense, certain NPs, particularly ZnO,

exhibit intrinsic antiviral activity by directly inactivating plant viruses, highlighting their potential as novel nanoscale antiviral agents in sustainable crop protection [280].

As a primary determinant of crop productivity, photosynthesis could also be profoundly influenced by the chlorophyllase activity and chlorophyll biosynthesis [282–284]. Notably, CDs can localize within chloroplasts and form functional conjugates that optimize electron transport in the photosynthetic chain, leading to improved light energy conversion efficiency and increased crop productivity [285,286]. AuNPs have also been shown to increase the rates of electron transport, photophosphorylation, and oxygen evolution, thereby improving the photosynthetic efficiency of isolated chloroplasts in *Vigna radiata* [287]. Furthermore, certain NPs can boost photosynthetic rates significantly. For example, foliar application of Fe and iron oxide NPs (Fe_3O_4) NPs in maize resulted in increased photosynthetic rates by 19.9% and 27.5%, respectively [288]. Photosynthetic efficiency can also be enhanced by light-sensitive materials such as TiO_2 , which stimulated photosynthesis by accelerating the photochemical phase essential for the formation of ATP and NADPH and increasing the content of light-harvesting complex II (LHCII) [289,290].

At the genetic level, A growing body of evidence indicates that certain NPs can enter plant cells and even localize in the nucleus, enabling direct or indirect regulation of genes governing growth, stress responses, and development

[291]. For instance, MWCNTs promoted cellular proliferation in tobacco by upregulating genes involved in cell division and elongation [292]. It could also upregulate the expression of aquaporins, thus improving cellular hydration and plant biomass accumulation [293]. Mg and N co-doped CDs were able to upregulate the gene expressions of enzymes related to chlorophyll synthesis, thus increasing chlorophyll contents [284]. Additionally, specific NPs have been shown to modulate the transcriptional activation of key genes involved in the biosynthesis of essential nutrients, thus controlling plant development [294]. In addition to regulating plant growth, NPs also exhibited potential in modulating plant immune responses through up- or down-regulating relative gene expression [272,295].

Despite the multifaceted benefits of NPs in enhancing nutrient acquisition, stress resilience, and crop yield, their potential phytotoxic effects and disruption of soil ecosystems may also represent critical bottlenecks for large-scale application. These adverse outcomes exhibit significant dependence on dosage and environmental context. Metal oxide NPs (e.g., ZnO, CuO, and MgO) are particularly prone to causing toxicity under high or prolonged exposure [296–298]. At the plant level, toxicity is primarily mediated by oxidative stress. Upon excessive uptake, NPs accumulate in mitochondria and chloroplasts, where they disrupt electron transport chains and trigger a burst of reactive oxygen species (ROS). When ROS production overwhelms the capacity of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), it leads to lipid peroxidation, protein denaturation, and DNA damage, ultimately activating programmed cell death pathways [299–301]. This oxidative damage directly impairs photosynthetic machinery. Specifically, NPs can reduce chlorophyll content, disrupt chloroplast ultrastructure, and inhibit light energy transfer efficiency. Furthermore, altered cell membrane fluidity and permeability significantly weaken photosynthetic performance [300,302]. Beyond physical damage, NPs perturb phytohormone homeostasis—such as suppressing indole-3-acetic acid (IAA) biosynthesis while elevating abscisic acid (ABA) levels—thereby inhibiting seed germination, root elongation, and overall vegetative growth [303].

In addition to direct plant interactions, NPs enter the soil environment through various pathways, including direct agricultural application, foliar leaching, and crop residue return, thereby exerting profound impacts on the soil microbiome [304]. Once introduced, high-dosage NPs interact with soil components and exert significant toxicity on soil microbial communities. Studies indicate that NPs can inhibit the activity of critical soil enzymes, reflecting a general impairment of microbial metabolic functions. Their unique nanoscale dimensions allow for easy penetration of microbial cell membranes, leading to structural damage and the induction of massive reactive oxygen species (ROS) accumulation, which subsequently disrupts

cellular metabolism [305]. This toxic effect is directly manifested through the widespread inhibition of key soil enzymes. Specifically, NPs significantly reduce the activity of urease, which is critical for nitrogen cycling; dehydrogenase, an indicator of overall metabolic vigor; and phosphatase, which regulates phosphorus transformation [306]. The decline in enzymatic activity not only reflects a general impairment of microbial metabolic functions but also weakens core ecological processes, such as biological nitrogen fixation [307]. Furthermore, some metal-based nanomaterials have been shown to suppress soil respiration. These findings highlight the extensive negative impact of NPs on the energy metabolism and functional activity of soil microbial communities [308].

Conclusions

Nanomaterial-mediated genetic transformation has emerged as a powerful alternative to conventional plant genetic engineering, offering unique advantages such as passive traversal of cell walls, efficient delivery of diverse nucleic acids, subcellular targeting, and even sustained release of genetic material. Various nanomaterial platforms, such as LDH, carbon-based nanomaterials, MSNs, and peptide-based carriers, have demonstrated success in facilitating the transport of DNA, RNA, and other biomolecules into a wide range of plant species. These advances enable applications in gene editing, pathogen resistance, and metabolic engineering, highlighting the role of nanotechnology in promoting precise genetic modifications and crop protection. In addition to serving as delivery vehicles, certain nanomaterials can act as nutrient supplements or immune activators, thereby promoting plant growth and enhancing tolerance to biotic and abiotic stresses. These properties position nanotechnology as a transformative tool for precision crop improvement, plant-based biopharmaceutical production, and fundamental research.

Nevertheless, the practical implementation of nanoparticle-mediated delivery systems faces several challenges. The range of nanomaterials suitable for nucleic acid delivery remains limited, and their uptake mechanisms—such as entry through stomata, diffusion across the cuticle, absorption via root meristems, and subsequent apoplastic or symplastic transport—are still not fully elucidated. Delivery efficiency is highly dependent on nanoparticle characteristics, including size, shape, surface charge, and tissue-specific interactions, which often result in inconsistent performance. The mechanisms by which these physicochemical properties govern the delivery process and overall efficiency remain poorly understood. Moreover, the biological interactions between nanomaterials and plants are not yet fully understood. The physiological impact of NPs on plants is highly concentration-dependent. At optimal doses, NPs can function as efficient biostimulants or nutrient carriers that enhance photosynthetic performance and boost plant immunity. However, their

large-scale agricultural application is hindered by potential phytotoxicity and ecological risks. High concentrations or chronic exposure to metal oxide NPs (e.g., ZnO, CuO, and MgO) lead to significant accumulation in plant tissues, triggering the overproduction of reactive oxygen species (ROS). This induced oxidative stress damages the internal structures of chloroplasts and mitochondria, ultimately resulting in programmed cell death. Furthermore, NPs can adversely affect the soil ecosystem by inhibiting essential enzymatic activities and disrupting the symbiotic relationships between plants and beneficial soil fungi. Collectively, these impacts pose a serious threat to long-term soil fertility and the stability of nutrient cycling. To fully harness the potential of nanotechnology in plant biotechnology, future research should prioritize several key directions. First, it is essential to expand the library of biocompatible and multifunctional nanomaterials tailored for a broad spectrum of plant species. This involves precise optimization of nanoparticle properties—such as size, surface charge, functional groups, and responsiveness to plant-specific microenvironments (e.g., pH fluctuations and enzymatic activity). Computational modeling and high-throughput screening offer promising strategies to expedite the design of NPs with improved biocompatibility and delivery efficacy. Concurrently, a deeper mechanistic understanding of nanoparticle uptake, transport, and subcellular localization is urgently needed. Research should focus on clarifying endocytic pathways, systemic mobility through symplastic and apoplastic networks, and interactions with cellular structures. In addition, a comprehensive and systematic assessment of the long-term impacts of NPs on plant physiology, ecological systems, and environmental safety remains imperative. Critical studies on nanoparticle persistence, biodegradability, and potential trophic transfer along the food chain are essential to ensure sustainable and safe integration into agricultural practice. Furthermore, the inherent biological activities of NPs, such as nutrient delivery, immune stimulation, and growth regulation, should be intentionally integrated into carrier design. Leveraging these intrinsic properties can enable dual-functional systems that enhance plant health and development while successfully delivering genetic cargo. The safety of NPs, specifically regarding their potential phytotoxicity and impact on the soil ecosystem, must also be taken into account, as these factors will influence their final large-scale application. Addressing these multifaceted challenges will establish a robust foundation for the rational design of next-generation nanocarriers and support their transition from laboratory innovation to real-world agricultural applications.

List of Abbreviations

NPs, nanoparticles; Dps, days post spraying; Dpi, days post infiltration; LDH, layered double hydroxides; SWCNT, single-walled carbon nanotubes; CDs, car-

bon dots; AuNPs, Gold NPs; MSNs, Mesoporous silica nanoparticles; GONs, graphene oxide nanoparticle; BY-2, bright yellow-2 cells; *N. benthamiana*, *Nicotiana benthamiana*; *A. thaliana*, *Arabidopsis thaliana*; PEI, polyethyleneimine; PAMAM, polyamidoamine; sgRNA, single-guide RNA; gRNA, guide RNA; RNP, ribonucleoproteins; SpCas9, *Streptococcus pyogenes* CRISPR-associated protein 9; Cas12a, CRISPR-associated protein 12a.

Availability of Data and Materials

The data of this study are available from the corresponding author.

Author Contributions

YZ, XYJ, and FW contributed to the conception of the work; YZ and FW contributed to the planning and data analysis; YZ drafted and revised the manuscript; FW critically reviewed the content, provided domain-specific expertise, and revised the manuscript; JYH, ZQZ, XYJ, JXW, and KLS contributed to the data collection and data analysis of the work; CJZ, YS, and YYX critically reviewed the content and participated in revising the manuscript. All authors have read and approved the final version and agree to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.22203/eCM.v059a12>.

References

- [1] Hamdan MF, Karlson CKS, Teoh EY, Lau SE, Tan BC. Genome Editing for Sustainable Crop Improvement and Mitigation of Biotic and Abiotic Stresses. *Plants*. 2022; 11: 2625. <https://doi.org/10.3390/plants11192625>.
- [2] Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, *et al.* Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology*. 2014; 32: 947–951. <https://doi.org/10.1038/nbt.2969>.

- [3] Ramkumar TR, Lenka SK, Arya SS, Bansal KC. A short history and perspectives on plant genetic transformation. *Methods in molecular biology*. 2020; 2124: 39–68. https://doi.org/10.1007/978-1-0716-0356-7_3.
- [4] Li L, Qu R, de Kochko A, Fauquet C, Beachy RN. An improved rice transformation system using the biolistic method. *Plant Cell Reports*. 1993; 12: 250–255. <https://doi.org/10.1007/BF00237129>.
- [5] Zaidi SS, Mansoor S. Viral vectors for plant genome engineering. *Frontiers in Plant Science*. 2017; 8: 539. <https://doi.org/10.3389/fpls.2017.00539>.
- [6] Gelvin SB. Integration of *Agrobacterium* T-DNA into the plant genome. *Annual Review of Genetics*. 2017; 51: 195–217. <https://doi.org/10.1146/annurev-genet-120215-035320>.
- [7] Cunningham FJ, Goh NS, Demirev GS, Matos JL, Landry MP. Nanoparticle-mediated delivery towards advancing plant genetic engineering. *Trends in Biotechnology*. 2018; 36: 882–897. <https://doi.org/10.1016/j.tibtech.2018.03.009>.
- [8] Yan Y, Zhu X, Yu Y, Li C, Zhang Z, Wang F. Nanotechnology strategies for plant genetic engineering. *Advanced Materials*. 2022; 34: e2106945. <https://doi.org/10.1002/adma.202106945>.
- [9] Yong J, Wu M, Carroll BJ, Xu ZP, Zhang R. Enhancing plant biotechnology by nanoparticle delivery of nucleic acids. *Trends in Genetics: TIG*. 2024; 40: 352–363. <https://doi.org/10.1016/j.tig.2024.01.005>.
- [10] Zhang Y, Huang C, Xiong R. Advanced materials for intracellular delivery of plant cells: Strategies, mechanisms and applications. *Materials Science and Engineering: R: Reports*. 2024; 160: 100821. <https://doi.org/10.1016/j.mser.2024.100821>.
- [11] Pasupathy K, Lin S, Hu Q, Luo H, Ke PC. Direct plant gene delivery with a poly(amidoamine) dendrimer. *Biotechnology Journal*. 2008; 3: 1078–1082. <https://doi.org/10.1002/biot.200800021>.
- [12] Torney F, Trewyn BG, Lin VS, Wang K. Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nature Nanotechnology*. 2007; 2: 295–300. <https://doi.org/10.1038/nnano.2007.108>.
- [13] Allará C, Ciccone G, Ciocca M, Vasquez S, Ibba P, Maver M, *et al.* Electronic Nanomaterials for Plants: A Review on Current Advances and Future Prospects. *Advanced Electronic Materials*. 2025; 11: 2500080. <https://doi.org/10.1002/aelm.202500080>.
- [14] Afzal S, Singh NK, Lal AF, Sohrab S, Singh N, Gupta PS, *et al.* Nanostructure and plant uptake: Assessing the ecological footprint and root-to-leaf dynamics. *Plant Nano Biology*. 2024; 10: 100122. <https://doi.org/10.1016/j.plana.2024.100122>.
- [15] Zhang B, Gao Y, Zhang L, Zhou Y. The plant cell wall: Biosynthesis, construction, and functions. *Journal of Integrative Plant Biology*. 2021; 63: 251–272. <https://doi.org/10.1111/jipb.13055>.
- [16] Höfte H, Voxeur A. Plant cell walls. *Current Biology: CB*. 2017; 27: R865–R870. <https://doi.org/10.1016/j.cub.2017.05.025>.
- [17] González-Valenzuela L, Renard J, Depège-Fargeix N, Ingram G. The plant cuticle. *Current Biology: CB*. 2023; 33: R210–R214. <https://doi.org/10.1016/j.cub.2023.01.003>.
- [18] Hu P, An J, Faulkner MM, Wu H, Li Z, Tian X, *et al.* Nanoparticle charge and size control foliar delivery efficiency to plant cells and organelles. *ACS Nano*. 2020; 14: 7970–7986. <https://doi.org/10.1021/acsnano.9b09178>.
- [19] Pinna A, Husted S. Translocation of foliar-applied nanoparticles: A critical review from a plant science perspective. *Physiologia Plantarum*. 2025; 177: e70476. <https://doi.org/10.1111/ppl.70476>.
- [20] Zhu L, Xu W, Yao X, Chen L, Li G, Gu J, *et al.* Cell wall pectin content refers to favored delivery of negatively charged carbon dots in leaf cells. *ACS Nano*. 2023; 17: 23442–23454. <https://doi.org/10.1021/acsnano.3c05182>.
- [21] Yong J, Xu W, Wu M, Zhang R, Mann CWG, Liu G, *et al.* Lysozyme-coated nanoparticles for active uptake and delivery of synthetic RNA and plasmid-encoded genes in plants. *Nature Plants*. 2025; 11: 131–144. <https://doi.org/10.1038/s41477-024-01882-x>.
- [22] Lu R, Martin-Hernandez AM, Peart JR, Malcuit I, Baulcombe DC. Virus-induced gene silencing in plants. *Methods: A Companion to Methods in Enzymology*. 2003; 30: 296–303. [https://doi.org/10.1016/S1046-2023\(03\)00037-9](https://doi.org/10.1016/S1046-2023(03)00037-9).
- [23] Ziemięnowicz A. *Agrobacterium*-mediated plant transformation: Factors, applications and recent advances. *Biocatalysis and Agricultural Biotechnology*. 2014; 3: 95–102. <https://doi.org/10.1016/j.bcab.2013.10.004>.
- [24] Ozyigit II. Gene transfer to plants by electroporation: Methods and applications. *Molecular Biology Reports*. 2020; 47: 3195–3210. <https://doi.org/10.1007/s11033-020-05343-4>.
- [25] Ozyigit II, Yucebilgili Kurtoglu K. Particle bombardment technology and its applications in plants. *Molecular Biology Reports*. 2020; 47: 9831–9847. <https://doi.org/10.1007/s11033-020-06001-5>.
- [26] Matsumoto TK, Gonsalves D. Biolistic and other non-*Agrobacterium* technologies of plant transformation. *Plant Biotechnology and Agriculture*. 2012; 117–129. <https://doi.org/10.1016/B978-0-12-381466-1.00008-0>.
- [27] Zhang Z, Hua L, Gupta A, Tricoli D, Edwards KJ, Yang B, *et al.* Development of an *Agrobacterium*-delivered CRISPR-Cas9 system for wheat genome editing. *Plant Biotechnology Journal*. 2019; 17: 1623–1635. <https://doi.org/10.1111/pbi.13088>.
- [28] Sunilkumar G, Vijayachandra K, Veluthambi K. Preincubation of cut tobacco leaf explants promotes *Agrobacterium*-mediated transformation by increasing vir gene induction. *Plant Science*. 1999; 141: 51–58. [https://doi.org/10.1016/S0168-9452\(98\)00228-3](https://doi.org/10.1016/S0168-9452(98)00228-3).
- [29] Demirev GS, Silva TN, Jackson CT, Thomas JB, W Ehrhardt D, Rhee SY, *et al.* Nanotechnology to advance CRISPR-Cas genetic engineering of plants. *Nature Nanotechnology*. 2021; 16: 243–250. <https://doi.org/10.1038/s41565-021-00854-y>.
- [30] Lacomme C, Hrubikova K, Hein I. Enhancement of virus-induced gene silencing through viral-based production of inverted-repeats. *The Plant Journal: For Cell and Molecular Biology*. 2003; 34: 543–553. <https://doi.org/10.1046/j.1365-313X.2003.01733.x>.
- [31] Kjemtrup S, Sampson KS, Peele CG, Nguyen LV, Conkling MA, Thompson WF, *et al.* Gene silencing from plant DNA carried by a Geminivirus. *The Plant Journal: For Cell and Molecular Biology*. 1998; 14: 91–100. <https://doi.org/10.1046/j.1365-313X.1998.00101.x>.
- [32] Ratcliff F, Martin-Hernandez AM, Baulcombe DC. Technical advance: Tobacco rattle virus as a vector for analysis of gene function by silencing. *The Plant Journal: For Cell and Molecular Biology*. 2001; 25: 237–245. <https://doi.org/10.1046/j.0960-7412.2000.00942.x>.
- [33] Liu Y, Schiff M, Marathe R, Dinesh-Kumar SP. Tobacco Rar1, EDS1 and NPR1/NIM1 like genes are required for N-mediated resistance to tobacco mosaic virus. *The Plant Journal: For Cell and Molecular Biology*. 2002; 30: 415–429. <https://doi.org/10.1046/j.1365-313X.2002.01297.x>.
- [34] Bates GW, Piastuch W, Riggs CD, Rabussay D. Electroporation for DNA delivery to plant protoplasts. *Plant Cell, Tissue and Organ Culture*. 1988; 12: 213–218. <https://doi.org/10.1007/BF00040089>.
- [35] Furuhashi Y, Sakai A, Murakami T, Morikawa M, Nakamura C, Yoshizumi T, *et al.* A method using electroporation for the protein delivery of Cre recombinase into cultured Arabidopsis cells with an intact cell wall. *Scientific Reports*. 2019; 9: 2163. <https://doi.org/10.1038/s41598-018-38119-9>.
- [36] Rakoczy-Trojanowska M. Alternative methods of plant transformation - A short review. *Cellular & Molecular Biology Letters*. 2002; 7: 849–858.
- [37] Altpeter F, Baisakh N, Beachy R, Bock R, Capell T, Christou P, *et al.* Particle bombardment and the genetic enhancement of crops: Myths and realities. *Molecular Breeding*. 2005; 15: 305–327. <https://doi.org/10.1007/s11032-004-8001-y>.
- [38] Klein TM, Fromm M, Weissinger A, Tomes D, Schaaf S, Sletten M, *et al.* Transfer of foreign genes into intact maize cells with high-velocity microprojectiles. *Proceedings of the National Academy of*

- Sciences of the United States of America. 1988; 85: 4305–4309. <https://doi.org/10.1073/pnas.85.12.4305>.
- [39] Martin-Ortigosa S, Wang K. Proteolistics: A biolistic method for intracellular delivery of proteins. *Transgenic Research*. 2014; 23: 743–756. <https://doi.org/10.1007/s11248-014-9807-y>.
- [40] Crossway A, Oakes JV, Irvine JM, Ward B, Knauf VC, Shewmaker CK. Integration of foreign DNA following microinjection of tobacco mesophyll protoplasts. *Molecular and General Genetics MGG*. 1986; 202: 179–185. <https://doi.org/10.1007/BF00331634>.
- [41] Chen Z, Zhu J, Chen Z, Du M, Yao R, Fu W, *et al.* High-throughput sequencing revealed low-efficacy genome editing using Cas9 RNPs electroporation and single-celled microinjection provided an alternative to deliver CRISPR reagents into *Euglena gracilis*. *Plant Biotechnology Journal*. 2022; 20: 2048–2050. <https://doi.org/10.1111/pbi.13915>.
- [42] Bevan M. Binary *Agrobacterium* vectors for plant transformation. *Nucleic Acids Research*. 1984; 12: 8711–8721. <https://doi.org/10.1093/nar/12.22.8711>.
- [43] Zaenen I, van Larebeke N, van Montagu M, Schell J. Supercoiled circular DNA in crown-gall inducing *Agrobacterium* strains. *Journal of Molecular Biology*. 1974; 86: 109–127. [https://doi.org/10.1016/S0022-2836\(74\)80011-2](https://doi.org/10.1016/S0022-2836(74)80011-2).
- [44] Chilton MD, Drummond MH, Merio DJ, Sciaky D, Montoya AL, Gordon MP, *et al.* Stable incorporation of plasmid DNA into higher plant cells: The molecular basis of crown gall tumorigenesis. *Cell*. 1977; 11: 263–271. [https://doi.org/10.1016/0092-8674\(77\)90043-5](https://doi.org/10.1016/0092-8674(77)90043-5).
- [45] Stachel SE, Messens E, Van Montagu M, Zambryski P. Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. *Nature*. 1985; 318: 624–629. <https://doi.org/10.1038/318624a0>.
- [46] Krensek P, Samajova O, Luptovciak I, Doskocilova A, Komis G, Samaj J. Transient plant transformation mediated by *Agrobacterium tumefaciens*: Principles, methods and applications. *Biotechnology Advances*. 2015; 33: 1024–1042. <https://doi.org/10.1016/j.biotechadv.2015.03.012>.
- [47] Lacroix B, Citovsky V. Pathways of DNA transfer to plants from *Agrobacterium tumefaciens* and related bacterial species. *Annual Review of Phytopathology*. 2019; 57: 231–251. <https://doi.org/10.1146/annurev-phyto-082718-100101>.
- [48] Fullner KJ, Lara JC, Nester EW. Pilus assembly by *Agrobacterium* T-DNA transfer genes. *Science*. 1996; 273: 1107–1109. <https://doi.org/10.1126/science.273.5278.1107>.
- [49] Tzfira T, Citovsky V. The *Agrobacterium*-plant cell interaction. Taking biology lessons from a bug. *Plant Physiology*. 2003; 133: 943–947. <https://doi.org/10.1104/pp.103.032821>.
- [50] Li S, Xu B, Niu X, Lu X, Cheng J, Zhou M, *et al.* JAZ8 interacts with VirE3 attenuating *Agrobacterium* mediated root tumorigenesis. *Frontiers in Plant Science*. 2021; 12: 685533. <https://doi.org/10.3389/fpls.2021.685533>.
- [51] Krastanova SV, Balaji V, Holden MR, Sekiya M, Xue B, Momol EA, *et al.* Resistance to crown gall disease in transgenic grapevine rootstocks containing truncated virE2 of *Agrobacterium*. *Transgenic Research*. 2010; 19: 949–958. <https://doi.org/10.1007/s11248-010-9373-x>.
- [52] Bahramnejad B, Naji M, Bose R, Jha S. A critical review on use of *Agrobacterium* rhizogenes and their associated binary vectors for plant transformation. *Biotechnology Advances*. 2019; 37: 107405. <https://doi.org/10.1016/j.biotechadv.2019.06.004>.
- [53] Sharma MK, Solanke AU, Jani D, Singh Y, Sharma AK. A simple and efficient *Agrobacterium*-mediated procedure for transformation of tomato. *Journal of Biosciences*. 2009; 34: 423–433. <https://doi.org/10.1007/s12038-009-0049-8>.
- [54] Goralogia GS, Willig C, Strauss SH. Engineering *Agrobacterium* for improved plant transformation. *The Plant Journal: For Cell and Molecular Biology*. 2025; 121: e70015. <https://doi.org/10.1111/tpj.70015>.
- [55] Singh RK, Prasad M. Advances in *Agrobacterium tumefaciens*-mediated genetic transformation of gram-inaceous crops. *Protoplasma*. 2016; 253: 691–707. <https://doi.org/10.1007/s00709-015-0905-3>.
- [56] Gururavanan P, Vinoth S, Jayabalan N. An improved *Agrobacterium*-mediated transformation method for cotton (*Gossypium hirsutum* L. ‘KC3’) assisted by microinjection and sonication. *In Vitro Cellular & Developmental Biology - Plant*. 2020; 56: 111–121. <https://doi.org/10.1007/s11627-019-10030-6>.
- [57] Ribeiro TP, Lourenço-Tessutti IT, de Melo BP, Morgante CV, Filho AS, Lins CBJ, *et al.* Improved cotton transformation protocol mediated by *Agrobacterium* and biolistic combined-methods. *Planta*. 2021; 254: 20. <https://doi.org/10.1007/s00425-021-03666-5>.
- [58] Wang L, Zhao F, Liu H, Chen H, Zhang F, Li S, *et al.* A modified *Agrobacterium*-mediated transformation for two oomycete pathogens. *PLoS Pathogens*. 2023; 19: e1011346. <https://doi.org/10.1371/journal.ppat.1011346>.
- [59] Mushtaq M, Molla KA. CRISPR Technologies for Plant Biotechnology Innovation. In Ricroch, A., Chopra, S., Kuntz, M. (eds.) *Plant Biotechnology: Experience and Future Prospects*. (pp. 51–67). Springer: Cham. 2021. https://doi.org/10.1007/978-3-030-68345-0_5.
- [60] Zhu H, Li C, Gao C. Applications of CRISPR–Cas in agriculture and plant biotechnology. *Nature Reviews. Molecular Cell Biology*. 2020; 21: 661–677. <https://doi.org/10.1038/s41580-020-00288-9>.
- [61] Nekrasov V, Staskawicz B, Weigel D, Jones JD, Kamoun S. Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nature Biotechnology*. 2013; 31: 691–693. <https://doi.org/10.1038/nbt.2655>.
- [62] Zulfiqar S, Farooq MA, Zhao T, Wang P, Tabusam J, Wang Y, *et al.* Virus-induced gene silencing (VIGS): A powerful tool for crop improvement and its advancement towards Epigenetics. *International Journal of Molecular Sciences*. 2023; 24: 5608. <https://doi.org/10.3390/ijms24065608>.
- [63] Rössner C, Lotz D, Becker A. VIGS goes viral: How VIGS transforms our understanding of plant science. *Annual Review of Plant Biology*. 2022; 73: 703–728. <https://doi.org/10.1146/annurev-arplant-102820-020542>.
- [64] Islam MR, Youngblood M, Kim HI, González-Gamboa I, Monroy-Borrego AG, Caparco AA, *et al.* DNA delivery by virus-like nanocarriers in plant cells. *Nano Letters*. 2024; 24: 7833–7842. <https://doi.org/10.1021/acs.nanolett.3c04735>.
- [65] Boevink P, Santa Cruz S, Hawes C, Harris N, Oparka KJ. Virus-mediated delivery of the green fluorescent protein to the endoplasmic reticulum of plant cells. *The Plant Journal*. 1996; 10: 935–941. <https://doi.org/10.1046/j.1365-3113X.1996.10050935.x>.
- [66] Van Wert SL, Saunders JA. Electroporation and electroporation of plants. *Plant Physiology*. 1992; 99: 365–367. <https://doi.org/10.1104/pp.99.2.365>.
- [67] Miao Y, Jiang L. Transient expression of fluorescent fusion proteins in protoplasts of suspension cultured cells. *Nature Protocols*. 2007; 2: 2348–2353. <https://doi.org/10.1038/nprot.2007.360>.
- [68] Levengood H, Zhou Y, Zhang C. Advancements in plant transformation: From traditional methods to cutting-edge techniques and emerging model species. *Plant Cell Reports*. 2024; 43: 273. <https://doi.org/10.1007/s00299-024-03359-9>.
- [69] Walpuski G, Koziol Ł, Gasulla F, Zaborski P, Zienkiewicz M. Stable transformation of the unicellular lichenic green alga *Coccomyxa solorinae-saccatae* via electroporation. *Symbiosis*. 2024; 93: 115–123. <https://doi.org/10.1007/s13199-024-00985-5>.
- [70] Niazian M, Molaahmad Nalouisi A, Azadi P, Ma’mani L, Chandler SF. Perspectives on new opportunities for nano-enabled strategies for gene delivery to plants using nanoporous materials. *Planta*. 2021; 254: 83. <https://doi.org/10.1007/s00425-021-03734-w>.
- [71] Klein TM, Wolf ED, Wu R, Sanford JC. High-velocity microprojectiles for delivering nucleic acids into living cells. *Nature*. 1987; 327:

- 70–73. <https://doi.org/10.1038/327070a0>.
- [72] Carsono N, Yoshida T. Transient expression of green fluorescent protein in rice calluses : Optimization of parameters for helios gene gun device. *Plant Production Science*. 2008; 11: 88–95. <https://doi.org/10.1626/pps.11.88>.
- [73] Hay I, Lachance D, Aderkas PV, Charest PJ. Transient chimeric gene expression in pollen of five conifer species following microparticle bombardment. *Canadian Journal of Forest Research*. 1994; 24: 2417–2423. <https://doi.org/10.1139/x94-312>.
- [74] Svitashv S, Schwartz C, Lenderts B, Young JK, Mark Cigan A. Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes. *Nature Communications*. 2016; 7: 13274. <https://doi.org/10.1038/ncomms13274>.
- [75] Liang Z, Chen K, Li T, Zhang Y, Wang Y, Zhao Q, *et al.* Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. *Nature Communications*. 2017; 8: 14261. <https://doi.org/10.1038/ncomms14261>.
- [76] Gaba V, Gal-On A. Inoculation of Plants Using Bombardment. *Current Protocols in Microbiology*. 2006; 16B.3. <https://doi.org/10.1002/9780471729259.mc16b03s00>.
- [77] Vuorinen AL, Nieminen A, Gaba V, Sikorskaite S, Valkonen JP. Bi-olistic DNA delivery to leaf tissue of plants with the non-vacuum gene gun (Handygün). *Methods in molecular biology*. 2013; 940: 45–51. https://doi.org/10.1007/978-1-62703-110-3_4.
- [78] Su W, Xu M, Radani Y, Yang L. Technological Development and Application of Plant Genetic Transformation. *International Journal of Molecular Sciences*. 2023; 24: 10646. <https://doi.org/10.3390/ijms241310646>.
- [79] Neuhaus G, Spangenberg G. Plant transformation by microinjection techniques. *Physiologia Plantarum*. 1990; 79: 213–217. <https://doi.org/10.1111/j.1399-3054.1990.tb05890.x>.
- [80] Bao W, Wang J, Wang Q, O'Hare D, Wan Y. Layered double hydroxide nanotransporter for molecule delivery to intact plant cells. *Scientific Reports*. 2016; 6: 26738. <https://doi.org/10.1038/srep26738>.
- [81] Wang Q, O'Hare D. Recent advances in the synthesis and application of layered double hydroxide (LDH) nanosheets. *Chemical Reviews*. 2012; 112: 4124–4155. <https://doi.org/10.1021/cr200434v>.
- [82] Yong J, Zhang R, Bi S, Li P, Sun L, Mitter N, *et al.* Sheet-like clay nanoparticles deliver RNA into developing pollen to efficiently silence a target gene. *Plant Physiology*. 2021; 187: 886–899. <https://doi.org/10.1093/plphys/kiab303>.
- [83] Mitter N, Worrall EA, Robinson KE, Li P, Jain RG, Taochy C, *et al.* Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nature Plants*. 2017; 3: 16207. <https://doi.org/10.1038/nplants.2016.207>.
- [84] Liu Q, Li Y, Xu K, Li D, Hu H, Zhou F, *et al.* Clay nanosheet-mediated delivery of recombinant plasmids expressing artificial miRNAs via leaf spray to prevent infection by plant DNA viruses. *Horticulture Research*. 2020; 7: 179. <https://doi.org/10.1038/s41438-020-00400-2>.
- [85] Yong J, Wu M, Zhang R, Bi S, Mann CWG, Mitter N, *et al.* Clay nanoparticles efficiently deliver small interfering RNA to intact plant leaf cells. *Plant Physiology*. 2022; 190: 2187–2202. <https://doi.org/10.1093/plphys/kiac430>.
- [86] Liu Q, Chen B, Wang Q, Shi X, Xiao Z, Lin J, *et al.* Carbon nanotubes as molecular transporters for walled plant cells. *Nano Letters*. 2009; 9: 1007–1010. <https://doi.org/10.1021/nl803083u>.
- [87] Demire GS, Zhang H, Goh NS, Pinals RL, Chang R, Landry MP. Carbon nanocarriers deliver siRNA to intact plant cells for efficient gene knockdown. *Science Advances*. 2020; 6: eaaz0495. <https://doi.org/10.1126/sciadv.aaz0495>.
- [88] Demire GS, Zhang H, Goh NS, González-Grandío E, Landry MP. Carbon nanotube-mediated DNA delivery without transgene integration in intact plants. *Nature Protocols*. 2019; 14: 2954–2971. <https://doi.org/10.1038/s41596-019-0208-9>.
- [89] Li W, Zheng Y, Zhang H, Liu Z, Su W, Chen S, *et al.* Phytotoxicity, uptake, and translocation of fluorescent carbon dots in mung bean plants. *ACS Applied Materials & Interfaces*. 2016; 8: 19939–19945. <https://doi.org/10.1021/acsami.6b07268>.
- [90] Schwartz SH, Hendrix B, Hoffer P, Sanders RA, Zheng W. Carbon dots for efficient small interfering RNA delivery and gene silencing in plants. *Plant Physiology*. 2020; 184: 647–657. <https://doi.org/10.1104/pp.20.00733>.
- [91] Wang B, Huang J, Zhang M, Wang Y, Wang H, Ma Y, *et al.* Carbon dots enable efficient delivery of functional DNA in plants. *ACS Applied Bio Materials*. 2020; 3: 8857–8864. <https://doi.org/10.1021/acssabm.0c01170>.
- [92] Imani R, Emami SH, Faghihi S. Synthesis and characterization of an octaarginine functionalized graphene oxide nano-carrier for gene delivery applications. *Physical Chemistry Chemical Physics: PCCP*. 2015; 17: 6328–6339. <https://doi.org/10.1039/c4cp04301d>.
- [93] Imani R, Mohabatpour F, Mostafavi F. Graphene-based Nano-Carrier modifications for gene delivery applications. *Carbon*. 2018; 140: 569–591. <https://doi.org/10.1016/j.carbon.2018.09.019>.
- [94] Li S, Li J, Du M, Deng G, Song Z, Han H. Efficient gene silencing in intact plant cells using siRNA delivered by functional graphene oxide nanoparticles. *Angewandte Chemie*. 2022; 61: e202210014. <https://doi.org/10.1002/anie.202210014>.
- [95] Gyawali B, Rahimi R, Alizadeh H, Mohammadi M. Graphene quantum dots (GQD)-mediated dsRNA delivery for the control of fusarium head blight disease in wheat. *ACS Applied Bio Materials*. 2024; 7: 1526–1535. <https://doi.org/10.1021/acsbm.3c00972>.
- [96] Zhang H, Fan G, Leng D, Liu S, Cai L. Graphene oxide nanosheets for delivery of RNAi and plant immune stimulation for sustained protection against plant viruses. *Journal of Agricultural and Food Chemistry*. 2025; 73: 11110–11120. <https://doi.org/10.1021/acs.jafc.4c12485>.
- [97] Lei WX, An ZS, Zhang BH, Wu Q, Gong WJ, Li JM, *et al.* Construction of gold-siRNA NPR1 nanoparticles for effective and quick silencing of NPR1 in *Arabidopsis thaliana*. *RSC Advances*. 2020; 10: 19300–19308. <https://doi.org/10.1039/d0ra02156c>.
- [98] Zhang H, Cao Y, Xu D, Goh NS, Demire GS, Cestellos-Blanco S, *et al.* Gold-nanocluster-mediated delivery of siRNA to intact plant cells for efficient gene knockdown. *Nano Letters*. 2021; 21: 5859–5866. <https://doi.org/10.1021/acs.nanolett.1c01792>.
- [99] Martin-Ortigosa S, Valenstein JS, Lin VSY, Trewyn BG, Wang K. Gold functionalized mesoporous silica nanoparticle mediated protein and DNA codelivery to plant cells via the biolistic method. *Advanced Functional Materials*. 2012; 22: 3576–3582. <https://doi.org/10.1002/adfm.201200359>.
- [100] Chang M, Chou JC, Lee HJ. Cellular internalization of fluorescent proteins via arginine-rich intracellular delivery peptide in plant cells. *Plant & Cell Physiology*. 2005; 46: 482–488. <https://doi.org/10.1093/pcp/pci046>.
- [101] Unnamalai N, Kang BG, Lee WS. Cationic oligopeptide-mediated delivery of dsRNA for post-transcriptional gene silencing in plant cells. *FEBS Letters*. 2004; 566: 307–310. <https://doi.org/10.1016/j.febslet.2004.04.018>.
- [102] Thagun C, Horii Y, Mori M, Fujita S, Ohtani M, Tsuchiya K, *et al.* Non-transgenic gene modulation via spray delivery of nucleic acid/peptide complexes into plant nuclei and chloroplasts. *ACS Nano*. 2022; 16: 3506–3521. <https://doi.org/10.1021/acsnano.1c07723>.
- [103] Zhang H, Zhang H, Demire GS, González-Grandío E, Fan C, Landry MP. Engineering DNA nanostructures for siRNA delivery in plants. *Nature Protocols*. 2020; 15: 3064–3087. <https://doi.org/10.1038/s41596-020-0370-0>.
- [104] Zhang H, Demire GS, Zhang H, Ye T, Goh NS, Aditham AJ, *et al.* DNA nanostructures coordinate gene silencing in mature plants. *Proceedings of the National Academy of Sciences of the United States of America*. 2019; 116: 7543–7548. <https://doi.org/10.1073/pnas.1818290116>.

- [105] Zhang Y, Shin J, Sun H, Chang HF, Martinez MR, Perkins LA, *et al.* High aspect ratio polymer nanocarriers for gene delivery and expression in plants. *Nano Letters*. 2025; 25: 681–690. <https://doi.org/10.1021/acs.nanolett.4c04704>.
- [106] Yang J, Yan S, Xie S, Yin M, Shen J, Li Z, *et al.* Construction and application of star polycation nanocarrier-based microRNA delivery system in Arabidopsis and maize. *Journal of Nanobiotechnology*. 2022; 20: 219. <https://doi.org/10.1186/s12951-022-01443-4>.
- [107] Fraley RT, Dellaporta SL, Papahadjopoulos D. Liposome-mediated delivery of tobacco mosaic virus RNA into tobacco protoplasts: A sensitive assay for monitoring liposome-protoplast interactions. *Proceedings of the National Academy of Sciences of the United States of America*. 1982; 79: 1859–1863. <https://doi.org/10.1073/pnas.79.6.1859>.
- [108] Gad AE, Zeewi B, Altman A. Fusion of germinating watermelon pollen tubes with liposomes. *Plant Science*. 1988; 55: 69–75. [https://doi.org/10.1016/0168-9452\(88\)90043-X](https://doi.org/10.1016/0168-9452(88)90043-X).
- [109] Xu ZP, Zeng QH, Lu GQ, Yu AB. Inorganic nanoparticles as carriers for efficient cellular delivery. *Chemical Engineering Science*. 2006; 61: 1027–1040. <https://doi.org/10.1016/j.ces.2005.06.019>.
- [110] Choy JH, Choi SJ, Oh JM, Park T. Clay minerals and layered double hydroxides for novel biological applications. *Applied Clay Science*. 2007; 36: 122–132. <https://doi.org/10.1016/j.clay.2006.07.007>.
- [111] Li S, Li J, Wang CJ, Wang Q, Cader MZ, Lu J, *et al.* Cellular uptake and gene delivery using layered double hydroxide nanoparticles. *Journal of Materials Chemistry. B*. 2013; 1: 61–68. <https://doi.org/10.1039/C2TB00081D>.
- [112] Jain RG, Fletcher SJ, Manzie N, Robinson KE, Li P, Lu E, *et al.* Foliar application of clay-delivered RNA interference for whitefly control. *Nature Plants*. 2022; 8: 535–548. <https://doi.org/10.1038/s41477-022-01152-8>.
- [113] Kogel KH. Lysozyme-coated LDHs boost trait control. *Nature Plants*. 2025; 11: 9–10. <https://doi.org/10.1038/s41477-024-01874-x>.
- [114] Maiti D, Tong X, Mou X, Yang K. Carbon-based nanomaterials for biomedical applications: A recent study. *Frontiers in Pharmacology*. 2019; 9: 1401. <https://doi.org/10.3389/fphar.2018.01401>.
- [115] Zare H, Ahmadi S, Ghasemi A, Ghanbari M, Rabiee N, Bagherzadeh M, *et al.* Carbon nanotubes: Smart drug/gene delivery carriers. *International Journal of Nanomedicine*. 2021; 16: 1681–1706. <https://doi.org/10.2147/ijn.S299448>.
- [116] Santana I, Wu H, Hu P, Giraldo JP. Targeted delivery of nanomaterials with chemical cargoes in plants enabled by a biorecognition motif. *Nature Communications*. 2020; 11: 2045. <https://doi.org/10.1038/s41467-020-15731-w>.
- [117] Iijima S. Carbon nanotubes: past, present, and future. *Physica B Condensed Matter*. 2002; 323: 1–5. [https://doi.org/10.1016/s0921-4526\(02\)00869-4](https://doi.org/10.1016/s0921-4526(02)00869-4).
- [118] Zheng M, Jagota A, Semke ED, Diner BA, McLean RS, Lustig SR, *et al.* DNA-assisted dispersion and separation of carbon nanotubes. *Nature Materials*. 2003; 2: 338–342. <https://doi.org/10.1038/nmat877>.
- [119] Wu Y, Phillips JA, Liu H, Yang R, Tan W. Carbon nanotubes protect DNA strands during cellular delivery. *ACS Nano*. 2008; 2: 2023–2028. <https://doi.org/10.1021/nn800325a>.
- [120] Alshehri R, Ilyas AM, Hasan A, Arnaout A, Ahmed F, Memic A. Carbon nanotubes in biomedical applications: Factors, mechanisms, and remedies of toxicity. *Journal of Medicinal Chemistry*. 2016; 59: 8149–8167. <https://doi.org/10.1021/acs.jmedchem.5b01770>.
- [121] Sajid MI, Jamshaid U, Jamshaid T, Zafar N, Fessi H, Elaissari A. Carbon nanotubes from synthesis to in vivo biomedical applications. *International Journal of Pharmaceutics*. 2016; 501: 278–299. <https://doi.org/10.1016/j.ijpharm.2016.01.064>.
- [122] Singh B, Lohan S, Sandhu PS, Jain A, Mehta SK. Functionalized carbon nanotubes and their promising applications in therapeutics and diagnostics. In Grumezescu, A.M. (ed.) *Nanobiomaterials in Medical Imaging*. (pp. 455–478). William Andrew: Norwich. 2016. <https://doi.org/10.1016/B978-0-323-41736-5.00015-7>.
- [123] Lu Q, Moore JM, Huang G, Mount AS, Rao AM, Larcom LL, *et al.* RNA polymer translocation with single-walled carbon nanotubes. *Nano Letters*. 2004; 4: 2473–2477. <https://doi.org/10.1021/nl048326j>.
- [124] Firme CP 3rd, Bandaru PR. Toxicity issues in the application of carbon nanotubes to biological systems. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2010; 6: 245–256. <https://doi.org/10.1016/j.nano.2009.07.003>.
- [125] Sayes CM, Liang F, Hudson JL, Mendez J, Guo W, Beach JM, *et al.* Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. *Toxicology Letters*. 2006; 161: 135–142. <https://doi.org/10.1016/j.toxlet.2005.08.011>.
- [126] Chen RJ, Zhang Y, Wang D, Dai H. Noncovalent sidewall functionalization of single-walled carbon nanotubes for protein immobilization. *Journal of the American Chemical Society*. 2001; 123: 3838–3839. <https://doi.org/10.1021/ja010172b>.
- [127] Hersam MC. Progress towards monodisperse single-walled carbon nanotubes. *Nature Nanotechnology*. 2008; 3: 387–394. <https://doi.org/10.1038/nnano.2008.135>.
- [128] Demirel GS, Zhang H, Matos JL, Goh NS, Cunningham FJ, Sung Y, *et al.* High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. *Nature Nanotechnology*. 2019; 14: 456–464. <https://doi.org/10.1038/s41565-019-0382-5>.
- [129] Jeong YY, Kwak H, Jeong W, Lee H, Jeon SJ, Lee HJ, *et al.* Oligohistidine-functionalized single-walled carbon nanotube-guided RNA delivery to improve shoot regeneration efficiency in plant calli. *Advanced Functional Materials*. 2025; 36: e10105. <https://doi.org/10.1002/adfm.202510105>.
- [130] Kwak SY, Lew TTS, Sweeney CJ, Koman VB, Wong MH, Bohmert-Tatarev K, *et al.* Chloroplast-selective gene delivery and expression in planta using chitosan-complexed single-walled carbon nanotube carriers. *Nature Nanotechnology*. 2019; 14: 447–455. <https://doi.org/10.1038/s41565-019-0375-4>.
- [131] Santana I, Jeon SJ, Kim HI, Islam MR, Castillo C, Garcia GFH, *et al.* Targeted carbon nanostructures for chemical and gene delivery to plant chloroplasts. *ACS Nano*. 2022; 16: 12156–12173. <https://doi.org/10.1021/acsnano.2c02714>.
- [132] Law SSY, Liou G, Nagai Y, Giménez-Dejor J, Tateishi A, Tsuchiya K, *et al.* Polymer-coated carbon nanotube hybrids with functional peptides for gene delivery into plant mitochondria. *Nature Communications*. 2022; 13: 2417. <https://doi.org/10.1038/s41467-022-30185-y>.
- [133] Hutton GAM, Martindale BCM, Reisner E. Carbon dots as photosensitisers for solar-driven catalysis. *Chemical Society Reviews*. 2017; 46: 6111–6123. <https://doi.org/10.1039/c7cs00235a>.
- [134] Zheng XT, Ananthanarayanan A, Luo KQ, Chen P. Glowing graphene quantum dots and carbon dots: Properties, syntheses, and biological applications. *Small*. 2015; 11: 1620–1636. <https://doi.org/10.1002/sml.201402648>.
- [135] Wang X, Cao L, Lu F, Mezziani MJ, Li H, Qi G, *et al.* Photoinduced electron transfers with carbon dots. *Chemical Communications: Chem Comm/the Royal Society of Chemistry*. 2009; 25: 3774–3776. <https://doi.org/10.1039/b906252a>.
- [136] Tripathi S, Sarkar S. Influence of water soluble carbon dots on the growth of wheat plant. *Applied Nanoscience*. 2015; 5: 609–616. <https://doi.org/10.1007/s13204-014-0355-9>.
- [137] Liu Y, Yang F, Jing X, Liu X, Wang G, Jian-Ping A, *et al.* A biomimetic nanoparticle for pDNA delivery and expression in plant cells in a pH-dependent manner. *ACS Agricultural Science & Technology*. 2023; 3: 631–641. <https://doi.org/10.1021/acsaagritech.3c00068>.
- [138] Wang Z, Li Y, Zhang B, Gao X, Shi M, Zhang S, *et al.* Functionalized carbon dot-delivered RNA nano fungicides as superior tools

- to control phytophthora pathogens through plant RdRP1 mediated spray-induced gene silencing. *Advanced Functional Materials*. 2023; 33: 2213143. <https://doi.org/10.1002/adfm.202213143>.
- [139] Geim AK, Novoselov KS. The rise of graphene. *Nature Materials*. 2007; 6: 183–191. <https://doi.org/10.1038/nmat1849>.
- [140] Feng L, Liu Z. Graphene in biomedicine: Opportunities and challenges. *Nanomedicine*. 2011; 6: 31–324. <https://doi.org/10.2217/nm.10.158>.
- [141] Bhattacharya N, Cahill DM, Yang W, Kochar M. Graphene as a nano-delivery vehicle in agriculture – current knowledge and future prospects. *Critical Reviews in Biotechnology*. 2023; 43: 851–869. <https://doi.org/10.1080/07388551.2022.2090315>.
- [142] Daniel MC, Astruc D. Gold nanoparticles: Assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chemical Reviews*. 2004; 104: 293–346. <https://doi.org/10.1021/cr030698+>.
- [143] Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. *Small*. 2005; 1: 325–327. <https://doi.org/10.1002/sml.200400093>.
- [144] Rana S, Bajaj A, Mout R, Rotello VM. Monolayer coated gold nanoparticles for delivery applications. *Advanced Drug Delivery Reviews*. 2012; 64: 200–216. <https://doi.org/10.1016/j.addr.2011.08.006>.
- [145] Zhang H, Goh NS, Wang JW, Pinals RL, González-Grandío E, Demir GS, *et al.* Nanoparticle cellular internalization is not required for RNA delivery to mature plant leaves. *Nature Nanotechnology*. 2022; 17: 197–205. <https://doi.org/10.1038/s41565-021-01018-8>.
- [146] Trewyn BG, Nieweg JA, Zhao Y, Lin VSY. Biocompatible mesoporous silica nanoparticles with different morphologies for animal cell membrane penetration. *Chemical Engineering Journal*. 2008; 137: 23–29. <https://doi.org/10.1016/j.cej.2007.09.045>.
- [147] Fang JJ, Trewyn BG. Application of mesoporous silica nanoparticles in intracellular delivery of molecules and proteins. *Methods in Enzymology*. 2012; 508: 41–59. <https://doi.org/10.1016/b978-0-12-391860-4.00003-3>.
- [148] Tang F, Li L, Chen D. Mesoporous silica nanoparticles: Synthesis, biocompatibility and drug delivery. *Advanced Materials*. 2012; 24: 1504–1534. <https://doi.org/10.1002/adma.201104763>.
- [149] Breznan D, Das DD, MacKinnon-Roy C, Bernatchez S, Sayari A, Hill M, *et al.* Physicochemical properties can be key determinants of mesoporous silica nanoparticle potency in vitro. *ACS Nano*. 2018; 12: 12062–12079. <https://doi.org/10.1021/acsnano.8b04910>.
- [150] Perez RA, Singh RK, Kim TH, Kim HW. Silica-based multifunctional nanodelivery systems toward regenerative medicine. *Materials Horizons*. 2017; 4: 772–799. <https://doi.org/10.1039/c7mh00017k>.
- [151] Chang FP, Kuang LY, Huang CA, Jane WN, Hung Y, Hsing YC, *et al.* A simple plant gene delivery system using mesoporous silica nanoparticles as carriers. *Journal of Materials Chemistry B*. 2013; 1: 5279–5287. <https://doi.org/10.1039/c3tb20529k>.
- [152] Sangwan A, Gupta D, Singh OW, Roy A, Mukherjee SK, Mandal B, *et al.* Size variations of mesoporous silica nanoparticle control uptake efficiency and delivery of AC2-derived dsRNA for protection against tomato leaf curl New Delhi virus. *Plant Cell Reports*. 2023; 42: 1571–1587. <https://doi.org/10.1007/s00299-023-03048-z>.
- [153] Hajiahmadi Z, Shirzadian-Khorramabad R, Kazemzad M, Sohani MM. Enhancement of tomato resistance to Tuta absoluta using a new efficient mesoporous silica nanoparticle-mediated plant transient gene expression approach. *Scientia Horticulturae*. 2019; 243: 367–375. <https://doi.org/10.1016/j.scienta.2018.08.040>.
- [154] Cai Y, Liu Z, Wang H, Meng H, Cao Y. Mesoporous silica nanoparticles mediate siRNA delivery for long-term multi-gene silencing in intact plants. *Advanced Science*. 2024; 11: e2301358. <https://doi.org/10.1002/advs.202301358>.
- [155] Ding C, Jiao Y, Li Y, Shen L, Zhang W, Ma H, *et al.* A novel pH-responsive multi-component nanodelivery system with siRNA for plant disease management. *Chemical Engineering Journal*. 2024; 497: 155001. <https://doi.org/10.1016/j.cej.2024.155001>.
- [156] O'Shea DG, Hodgkinson T, Dixon JE, Curtin CM, O'Brien FG. Development of an 'off-the-shelf' gene therapeutic nanoparticle formulation for incorporation into biomaterials for regenerative medicine applications. *European Cells and Materials*. 2024; 47: 152–169. <https://doi.org/10.22203/eCM.v047a11>.
- [157] Fonseca SB, Pereira MP, Kelley SO. Recent advances in the use of cell-penetrating peptides for medical and biological applications. *Advanced Drug Delivery Reviews*. 2009; 61: 953–964. <https://doi.org/10.1016/j.addr.2009.06.001>.
- [158] Ben-Haim AE, Feldbaum RA, Belausov E, Zelinger E, Maria R, Nativ-Roth E, *et al.* DNA delivery to intact plant cells by casein nanoparticles with confirmed gene expression. *Advanced Functional Materials*. 2024; 34: 2314756. <https://doi.org/10.1002/adfm.202314756>.
- [159] Chen CP, Chou JC, Liu BR, Chang M, Lee HJ. Transfection and expression of plasmid DNA in plant cells by an arginine-rich intracellular delivery peptide without protoplast preparation. *FEBS Letters*. 2007; 581: 1891–1897. <https://doi.org/10.1016/j.febslet.2007.03.076>.
- [160] Lakshmanan M, Kodama Y, Yoshizumi T, Sudesh K, Numata K. Rapid and efficient gene delivery into plant cells using designed peptide carriers. *Biomacromolecules*. 2013; 14: 10–16. <https://doi.org/10.1021/bm301275g>.
- [161] Yoshizumi T, Oikawa K, Chuah JA, Kodama Y, Numata K. Selective gene delivery for integrating exogenous DNA into plastid and mitochondrial genomes using peptide–DNA complexes. *Biomacromolecules*. 2018; 19: 1582–1591. <https://doi.org/10.1021/acs.biomac.8b00323>.
- [162] Salit MS. Biopolymer. In Salit, M. S. (ed.) *Tropical Natural Fibre Composites* (pp. 39–52). Springer, Singapore. 2014. https://doi.org/10.1007/978-981-287-155-8_3.
- [163] Beach MA, Nayanathara U, Gao Y, Zhang C, Xiong Y, Wang Y, *et al.* Polymeric Nanoparticles for Drug Delivery. *Chemical Reviews*. 2024; 124: 5505–5616. <https://doi.org/10.1021/acs.chemrev.3c00705>.
- [164] Wang J, Ayano E, Maitani Y, Kanazawa H. Tunable Surface Properties of Temperature-Responsive Polymer-Modified Liposomes Induce Faster Cellular Uptake. *ACS Omega*. 2017; 2: 316–325. <https://doi.org/10.1021/acsomega.6b00342>.
- [165] Tang Z, He C, Tian H, Ding J, Hsiao BS, Chu B, *et al.* Polymeric nanostructured materials for biomedical applications. *Progress in Polymer Science*. 2016; 60: 86–128. <https://doi.org/10.1016/j.progpolymsci.2016.05.005>.
- [166] Allan CR, Hadwiger LA. The fungicidal effect of chitosan on fungi of varying cell wall composition. *Experimental Mycology*. 1979; 3: 285–287. [https://doi.org/10.1016/S0147-5975\(79\)80054-7](https://doi.org/10.1016/S0147-5975(79)80054-7).
- [167] Chouhan D, Mandal P. Applications of chitosan and chitosan based metallic nanoparticles in agrosociences-A review. *International Journal of Biological Macromolecules*. 2021; 166: 1554–1569. <https://doi.org/10.1016/j.ijbiomac.2020.11.035>.
- [168] Liu Y, Zhang J, Li S, Chai L, Chang BH, Malak M, *et al.* Chitosan nanoparticle-mediated delivery of dsRNA for enhancing RNAi efficiency in *Locusta migratoria*. *Pest Management Science*. 2025; 81: 5260–5269. <https://doi.org/10.1002/ps.8880>.
- [169] Kolge H, Kadam K, Ghormade V. Chitosan nanocarriers mediated dsRNA delivery in gene silencing for *Helicoverpa armigera* biocontrol. *Pesticide Biochemistry and Physiology*. 2023; 189: 105292. <https://doi.org/10.1016/j.pestbp.2022.105292>.
- [170] Xu X, Jiao Y, Shen L, Li Y, Mei Y, Yang W, *et al.* Nanoparticle-dsRNA treatment of pollen and root systems of diseased plants effectively reduces the rate of tobacco mosaic virus in contemporary seeds. *ACS Applied Materials & Interfaces*. 2023; 15: 29052–29063. <https://doi.org/10.1021/acsami.3c02798>.

- [171] Yousefi M, Narmani A, Jafari SM. Dendrimers as efficient nanocarriers for the protection and delivery of bioactive phytochemicals. *Advances in Colloid and Interface Science*. 2020; 278: 102125. <https://doi.org/10.1016/j.cis.2020.102125>.
- [172] Zolghadrnasab M, Mousavi A, Farmany A, Arpanaei A. Ultrasound-mediated gene delivery into suspended plant cells using polyethyleneimine-coated mesoporous silica nanoparticles. *Ultrasonics Sonochemistry*. 2021; 73: 105507. <https://doi.org/10.1016/j.ultsonch.2021.105507>.
- [173] Qi J, Li Y, Yao X, Li G, Xu W, Chen L, *et al.* Rational design of ROS scavenging and fluorescent gold nanoparticles to deliver siRNA to improve plant resistance to *Pseudomonas syringae*. *Journal of Nanobiotechnology*. 2024; 22: 446. <https://doi.org/10.1186/s12951-024-02733-9>.
- [174] Zhang Y, Cao Y, Jiang W, Ma Q, Shin J, Sun H, *et al.* Polymeric nanocarriers autonomously cross the plant cell wall and enable protein delivery for stress sensing. *Advanced Materials*. 2024; 36: e2409356. <https://doi.org/10.1002/adma.202409356>.
- [175] Pal G, Ingole KD, Yavvari PS, Verma P, Kumari A, Chauhan C, *et al.* Exogenous application of nanocarrier-mediated double-stranded RNA manipulates physiological traits and defence response against bacterial diseases. *Molecular Plant Pathology*. 2024; 25: e13417. <https://doi.org/10.1111/mpp.13417>.
- [176] Wang X, Zheng K, Cheng W, Li J, Liang X, Shen J, *et al.* Field application of star polymer-delivered chitosan to amplify plant defense against potato late blight. *Chemical Engineering Journal*. 2021; 417: 129327. <https://doi.org/10.1016/j.cej.2021.129327>.
- [177] Wong KY, Wong MS, Liu J. Aptamer-functionalized liposomes for drug delivery. *Biomedical Journal*. 2024; 47: 100685. <https://doi.org/10.1016/j.bj.2023.100685>.
- [178] Thapa Magar K, Boafu GF, Li X, Chen Z, He W. Liposome-based delivery of biological drugs. *Chinese Chemical Letters*. 2022; 33: 587–596. <https://doi.org/10.1016/j.cclet.2021.08.020>.
- [179] Pattini BS, Chupin VV, Torchilin VP. New Developments in Liposomal Drug Delivery. *Chemical Reviews*. 2015; 115: 10938–10966. <https://doi.org/10.1021/acs.chemrev.5b00046>.
- [180] Liu J, Jiang X, Ashley C, Brinker CJ. Electrostatically Mediated Liposome Fusion and Lipid Exchange with a Nanoparticle-Supported Bilayer for Control of Surface Charge, Drug Containment, and Delivery. *Journal of the American Chemical Society*. 2009; 131: 7567–7569. <https://doi.org/10.1021/ja902039y>.
- [181] Rosenberg N, Gad AE, Altman A, Navot N, Czosnek H. Liposome-mediated introduction of the chloramphenicol acetyl transferase (CAT) gene and its expression in tobacco protoplasts. *Plant Molecular Biology*. 1988; 10: 185–191. <https://doi.org/10.1007/BF00027395>.
- [182] Wang CY, Hughes KW, Huang L. Improved Cytoplasmic Delivery to Plant Protoplasts via pH-Sensitive Liposomes I. *Plant Physiology*. 1986; 82: 179–184. <https://doi.org/10.1104/pp.82.1.179>.
- [183] Rosenberg N, Dekel-Reichenbach M, Navot N, Gad AE, Altman A, Czosnek H. Liposome-mediated introduction of DNA into plant protoplasts and calli. *Acta Horticulturae*. 1990; 280: 509–516. <https://doi.org/10.17660/ActaHortic.1990.280.84>.
- [184] Xia Y, Zhang T, Ni X, Zhu Y, Chen Q, Bamu A, *et al.* Engineered RNA nanostructures for scalable and efficient RNAi-based pesticides. *Trends in Biotechnology*. 2025; 43: 3172–3195. <https://doi.org/10.1016/j.tibtech.2025.07.027>.
- [185] Liu J, Wang F, Wang L, Xiao S, Tong C, Tang D, *et al.* Preparation of fluorescence starch-nanoparticle and its application as plant transgenic vehicle. *Journal of Central South University of Technology*. 2008; 15: 768–773. <https://doi.org/10.1007/s11771-008-0142-4>.
- [186] Tejwan N, Kundu M, Ghosh N, Chatterjee S, Sharma A, Abhishek Singh T, *et al.* Synthesis of green carbon dots as bioimaging agent and drug delivery system for enhanced antioxidant and antibacterial efficacy. *Inorganic Chemistry Communications*. 2022; 139: 109317. <https://doi.org/10.1016/j.inoche.2022.109317>.
- [187] Wang Y, Li M, Ying J, Shen J, Dou D, Yin M, *et al.* High-efficiency green management of potato late blight by a self-assembled multi-component nano-bioprotectant. *Nature Communications*. 2023; 14: 5622. <https://doi.org/10.1038/s41467-023-41447-8>.
- [188] Sun D, Hussain HI, Yi Z, Siegele R, Cresswell T, Kong L, *et al.* Uptake and cellular distribution, in four plant species, of fluorescently labeled mesoporous silica nanoparticles. *Plant Cell Reports*. 2014; 33: 1389–1402. <https://doi.org/10.1007/s00299-014-1624-5>.
- [189] Wu H, Li Z. Nano-enabled agriculture: How do nanoparticles cross barriers in plants? *Plant Communications*. 2022; 3: 100346. <https://doi.org/10.1016/j.xplc.2022.100346>.
- [190] Hua Z, Ma S, Ouyang Z, Liu P, Qiang H, Guo X. The review of nanoplastics in plants: Detection, analysis, uptake, migration and risk. *TrAC Trends in Analytical Chemistry*. 2023; 158: 116889. <https://doi.org/10.1016/j.trac.2022.116889>.
- [191] Pérez-de-Luque A. Interaction of nanomaterials with plants: What do we need for real applications in agriculture? *Frontiers in Environmental Science*. 2017; 5: 12. <https://doi.org/10.3389/fenvs.2017.00012>.
- [192] Kohay H, Wielinski J, Reiser J, Perkins LA, Ristroph K, Giraldo JP, *et al.* Nanocarrier foliar uptake pathways affect delivery of active agents and plant physiological response. *Environmental Science. Nano*. 2024; 12: 660–674. <https://doi.org/10.1039/D4EN00547C>.
- [193] Zhang Y, Fu L, Li S, Yan J, Sun M, Giraldo JP, *et al.* Star polymer size, charge content, and hydrophobicity affect their leaf uptake and translocation in plants. *Environmental Science & Technology*. 2021; 55: 10758–10768. <https://doi.org/10.1021/acs.est.1c01065>.
- [194] Su Y, Ashworth V, Kim C, Adeleye AS, Rolshausen P, Roper C, *et al.* Delivery, uptake, fate, and transport of engineered nanoparticles in plants: A critical review and data analysis. *Environmental Science: Nano*. 2019; 6: 2311–2331. <https://doi.org/10.1039/c9en00461k>.
- [195] Wang X, Xie H, Wang P, Yin H. Nanoparticles in plants: Uptake, transport and physiological activity in leaf and root. *Materials*. 2023; 16: 3097. <https://doi.org/10.3390/ma16083097>.
- [196] Zhu J, Wang J, Chen R, Feng Q, Zhan X. Cellular process of polystyrene nanoparticles entry into wheat roots. *Environmental Science & Technology*. 2022; 56: 6436–6444. <https://doi.org/10.1021/acs.est.1c08503>.
- [197] Torrent L, Iglesias M, Margui E, Hidalgo M, Verdaguer D, Llorens L, *et al.* Uptake, translocation and ligand of silver in *Lactuca sativa* exposed to silver nanoparticles of different size, coatings and concentration. *Journal of Hazardous Materials*. 2020; 384: 121201. <https://doi.org/10.1016/j.jhazmat.2019.121201>.
- [198] Luo X, Wang Z, Wang C, Yue L, Tao M, Elmer WH, *et al.* Nanomaterial size and surface modification mediate disease resistance activation in cucumber (*Cucumis sativus*). *ACS Nano*. 2023; 17: 4871–4885. <https://doi.org/10.1021/acs.nano.2c11790>.
- [199] Zhu ZJ, Wang H, Yan B, Zheng H, Jiang Y, Miranda OR, *et al.* Effect of surface charge on the uptake and distribution of gold nanoparticles in four plant species. *Environmental Science & Technology*. 2012; 46: 12391–12398. <https://doi.org/10.1021/es301977w>.
- [200] Sun H, Wang M, Wang J, Wang W. Surface charge affects foliar uptake, transport and physiological effects of functionalized graphene quantum dots in plants. *Science of the Total Environment*. 2022; 812: 151506. <https://doi.org/10.1016/j.scitotenv.2021.151506>.
- [201] Wei L, Wang B, Cao S, Tan H, Long T, Bian C, *et al.* Single-cell insights into carbon dots interactions with plant cell walls for distinct internalization pathways via surface charge modulation. *Carbon*. 2025; 245: 120747. <https://doi.org/10.1016/j.carbon.2025.120747>.
- [202] Chen L, Zhu L, Cheng H, Xu W, Li G, Zhang Y, *et al.* Negatively charged carbon dots employed symplastic and apoplastic pathways to enable better plant delivery than positively charged carbon dots. *ACS Nano*. 2024; 18: 23154–23167. <https://doi.org/10.1021/acsnano.4c05362>.

- [203] Stock D, Holloway PJ. Possible mechanisms for surfactant-induced foliar uptake of agrochemicals. *Pesticide Science*. 1993; 38: 165–177. <https://doi.org/10.1002/ps.2780380211>.
- [204] Zhang Y, Martinez MR, Sun H, Sun M, Yin R, Yan J, *et al.* Charge, aspect ratio, and plant species affect uptake efficiency and translocation of polymeric agrochemical nanocarriers. *Environmental Science & Technology*. 2023; 57: 8269–8279. <https://doi.org/10.1021/acs.est.3c01154>.
- [205] Schwab F, Zhai G, Kern M, Turner A, Schnoor JL, Wiesner MR. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants – Critical review. *Nanotoxicology*. 2016; 10: 257–278. <https://doi.org/10.3109/17435390.2015.1048326>.
- [206] Ali S, Mehmood A, Khan N. Uptake, translocation, and consequences of nanomaterials on plant growth and stress adaptation. *Journal of Nanomaterials*. 2021; 2021: 6677616. <https://doi.org/10.1155/2021/6677616>.
- [207] Gao M, Chang J, Wang Z, Zhang H, Wang T. Advances in transport and toxicity of nanoparticles in plants. *Journal of Nanobiotechnology*. 2023; 21: 75. <https://doi.org/10.1186/s12951-023-01830-5>.
- [208] Avellan A, Yun J, Morais BP, Clement ET, Rodrigues SM, Lowry GV. Critical review: Role of inorganic nanoparticle properties on their foliar uptake and in planta translocation. *Environmental Science & Technology*. 2021; 55: 13417–13431. <https://doi.org/10.1021/acs.est.1c00178>.
- [209] Buick RD, Buchan GD, Field RJ. The role of surface tension of spreading droplets in absorption of a herbicide formulation via leaf stomata. *Pesticide Science*. 1993; 38: 227–235. <https://doi.org/10.1002/ps.2780380218>.
- [210] Kwak SY, Giraldo JP, Wong MH, Koman VB, Lew TTS, Ell J, *et al.* A nanobionic light-emitting plant. *Nano Letters*. 2017; 17: 7951–7961. <https://doi.org/10.1021/acs.nanolett.7b04369>.
- [211] Schreck E, Foucault Y, Sarret G, Sobanska S, Cécillon L, Castrec-Rouelle M, *et al.* Metal and metalloid foliar uptake by various plant species exposed to atmospheric industrial fallout: Mechanisms involved for lead. *Science of the Total Environment*. 2012; 427–428: 253–262. <https://doi.org/10.1016/j.scitotenv.2012.03.051>.
- [212] Zhu J, Li J, Shen Y, Liu S, Zeng N, Zhan X, *et al.* Mechanism of zinc oxide nanoparticle entry into wheat seedling leaves. *Environmental Science: Nano*. 2020; 7: 3901–3913. <https://doi.org/10.1039/d0en00658k>.
- [213] Gao X, Kundu A, Persson DP, Szameitat A, Minutello F, Husted S, *et al.* Application of ZnO nanoparticles encapsulated in mesoporous silica on the abaxial side of a *Solanum lycopersicum* leaf enhances Zn uptake and translocation via the phloem. *Environmental Science & Technology*. 2023; 57: 21704–21714. <https://doi.org/10.1021/acs.est.3c06424>.
- [214] Yeats TH, Rose JK. The Formation and Function of Plant Cuticles. *Plant Physiology*. 2013; 163: 5–20. <https://doi.org/10.1104/pp.113.222737>.
- [215] Domínguez E, Heredia-Guerrero JA, Heredia A. The biophysical design of plant cuticles: an overview. *The New Phytologist*. 2011; 189: 938–949. <https://doi.org/10.1111/j.1469-8137.2010.03553.x>.
- [216] Schreiber L. Polar paths of diffusion across plant cuticles: New evidence for an old hypothesis. *Annals of Botany*. 2005; 95: 1069–1073. <https://doi.org/10.1093/aob/mci122>.
- [217] Tredennick EC, Farquhar GD. Dynamics of moisture diffusion and adsorption in plant cuticles including the role of cellulose. *Nature Communications*. 2021; 12: 5042. <https://doi.org/10.1038/s41467-021-25225-y>.
- [218] Eichert T, Goldbach HE. Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces – further evidence for a stomatal pathway. *Physiologia Plantarum*. 2008; 132: 491–502. <https://doi.org/10.1111/j.1399-3054.2007.01023.x>.
- [219] Eichert T, Kurtz A, Steiner U, Goldbach HE. Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. *Physiologia Plantarum*. 2008; 134: 151–160. <https://doi.org/10.1111/j.1399-3054.2008.01135.x>.
- [220] Chen X, Irani NG, Friml J. Clathrin-mediated endocytosis: the gateway into plant cells. *Current Opinion in Plant Biology*. 2011; 14: 674–682. <https://doi.org/10.1016/j.pbi.2011.08.006>.
- [221] Malinsky J, Opekarová M, Grossmann G, Tanner W. Membrane microdomains, rafts, and detergent-resistant membranes in plants and fungi. *Annual Review of Plant Biology*. 2013; 64: 501–529. <https://doi.org/10.1146/annurev-arplant-050312-120103>.
- [222] Di Rubbo S, Irani NG, Kim SY, Xu ZY, Gadeyne A, Dejonghe W, *et al.* The clathrin adaptor complex AP-2 mediates endocytosis of brassinosteroid INSENSITIVE1 in arabidopsis. *The Plant Cell*. 2013; 25: 2986–2997. <https://doi.org/10.1105/tpc.113.114058>.
- [223] Bar M, Avni A. EHD2 inhibits ligand-induced endocytosis and signaling of the leucine-rich repeat receptor-like protein LeEix2. *The Plant Journal: For Cell and Molecular Biology*. 2009; 59: 600–611. <https://doi.org/10.1111/j.1365-3113.2009.03897.x>.
- [224] Chen L, Wang H, Li X, Nie C, Liang T, Xie F, *et al.* Highly hydrophilic carbon nanoparticles: uptake mechanism by mammalian and plant cells. *RSC Advances*. 2018; 8: 35246–35256. <https://doi.org/10.1039/c8ra06665e>.
- [225] Bandmann V, Homann U. Clathrin-independent endocytosis contributes to uptake of glucose into BY-2 protoplasts. *The Plant Journal*. 2012; 70: 578–584. <https://doi.org/10.1111/j.1365-3113.2011.04892.x>.
- [226] Bandmann V, Müller JD, Köhler T, Homann U. Uptake of fluorescent nano beads into BY2-cells involves clathrin-dependent and clathrin-independent endocytosis. *FEBS Letters*. 2012; 586: 3626–3632. <https://doi.org/10.1016/j.febslet.2012.08.008>.
- [227] Etxeberria E, Gonzalez P, Baroja-Fernández E, Romero JP. Fluid phase endocytic uptake of artificial nano-spheres and fluorescent quantum dots by sycamore cultured cells. *Plant Signaling & Behavior*. 2006; 1: 196–200. <https://doi.org/10.4161/psb.1.4.3142>.
- [228] Etxeberria E, Pozueta-Romero J, Fernández EB. Fluid-phase endocytosis in plant cells. In Šamaj, J. (ed.) *Endocytosis in Plants* (pp. 107–122). Springer Berlin Heidelberg: Berlin. 2012. https://doi.org/10.1007/978-3-642-32463-5_5.
- [229] Baluska F, Samaj J, Hlavacka A, Kendrick-Jones J, Volkmann D. Actin-dependent fluid-phase endocytosis in inner cortex cells of maize root apices. *Journal of Experimental Botany*. 2004; 55: 463–473. <https://doi.org/10.1093/jxb/erh042>.
- [230] Onelli E, Prescianotto-Baschong C, Caccianiga M, Moscatelli A. Clathrin-dependent and independent endocytic pathways in tobacco protoplasts revealed by labelling with charged nanogold. *Journal of Experimental Botany*. 2008; 59: 3051–3068. <https://doi.org/10.1093/jxb/ern154>.
- [231] Moscatelli A, Ciampolini F, Rodighiero S, Onelli E, Cresti M, Santo N, *et al.* Distinct endocytic pathways identified in tobacco pollen tubes using charged nanogold. *Journal of Cell Science*. 2007; 120: 3804–3819. <https://doi.org/10.1242/jcs.012138>.
- [232] Wu B, Beitz E. Aquaporins with selectivity for unconventional permeants. *Cellular and Molecular Life Sciences: CMLS*. 2007; 64: 2413–2421. <https://doi.org/10.1007/s00018-007-7163-2>.
- [233] Maurel C. Plant aquaporins: Novel functions and regulation properties. *FEBS Letters*. 2007; 581: 2227–2236. <https://doi.org/10.1016/j.febslet.2007.03.021>.
- [234] Fitzpatrick KL, Reid RJ. The involvement of aquaglyceroporins in transport of boron in barley roots. *Plant, Cell & Environment*. 2009; 32: 1357–1365. <https://doi.org/10.1111/j.1365-3040.2009.02003.x>.
- [235] Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, *et al.* Understanding biophysicochemical interactions at the nano-bio interface. *Nature Materials*. 2009; 8: 543–557. <https://doi.org/10.1038/nmat2442>.
- [236] Wong MH, Misra RP, Giraldo JP, Kwak SY, Son Y, Landry MP, *et al.* Lipid exchange envelope penetration (LEEP) of nanoparticles for

- plant engineering: A universal localization mechanism. *Nano Letters*. 2016; 16: 1161–1172. <https://doi.org/10.1021/acs.nanolett.5b04467>.
- [237] Wild E, Jones KC. Novel method for the direct visualization of in vivo nanomaterials and chemical interactions in plants. *Environmental Science & Technology*. 2009; 43: 5290–5294. <https://doi.org/10.1021/es900065h>.
- [238] Serag MF, Kaji N, Gaillard C, Okamoto Y, Terasaka K, Jabasini M, *et al.* Trafficking and subcellular localization of multiwalled carbon nanotubes in plant cells. *ACS Nano*. 2011; 5: 493–499. <https://doi.org/10.1021/nn102344t>.
- [239] Chen F, Manandhar P, Ahmed MS, Chang S, Panday N, Zhang H, *et al.* Extracellular surface potential mapping by scanning ion conductance microscopy revealed transient transmembrane pore formation induced by conjugated polymer nanoparticles. *Macromolecular Bioscience*. 2019; 19: 1800271. <https://doi.org/10.1002/mabi.201800271>.
- [240] Leroueil PR, Berry SA, Duthie K, Han G, Rotello VM, McNerny DQ, *et al.* Wide varieties of cationic nanoparticles induce defects in supported lipid bilayers. *Nano Letters*. 2008; 8: 420–424. <https://doi.org/10.1021/nl0722929>.
- [241] Roberts AG, Oparika KJ. Plasmodesmata and the control of symplastic transport. *Plant, Cell & Environment*. 2003; 26: 103–124. <https://doi.org/10.1046/j.1365-3040.2003.00950.x>.
- [242] Geisler-Lee J, Wang Q, Yao Y, Zhang W, Geisler M, Li K, *et al.* Phytotoxicity, accumulation and transport of silver nanoparticles by *Arabidopsis thaliana*. *Nanotoxicology*. 2013; 7: 323–337. <https://doi.org/10.3109/17435390.2012.658094>.
- [243] Zhai G, Walters KS, Peate DW, Alvarez PJ, Schnoor JL. Transport of gold nanoparticles through plasmodesmata and precipitation of gold ions in woody poplar. *Environmental Science & Technology Letters*. 2014; 1: 146–151. <https://doi.org/10.1021/ez400202b>.
- [244] Lucas WJ, Lee JY. Plasmodesmata as a supracellular control network in plants. *Nature Reviews. Molecular Cell Biology*. 2004; 5: 712–726. <https://doi.org/10.1038/nrm1470>.
- [245] Kim JY. Regulation of short-distance transport of RNA and protein. *Current Opinion in Plant Biology*. 2005; 8: 45–52. <https://doi.org/10.1016/j.pbi.2004.11.005>.
- [246] Kanny A, Zinger A, Kajal A, Shainsky-Roitman J, Schroeder A. Therapeutic nanoparticles penetrate leaves and deliver nutrients to agricultural crops. *Scientific Reports*. 2018; 8: 7589. <https://doi.org/10.1038/s41598-018-25197-y>.
- [247] Ma Y, He X, Zhang P, Zhang Z, Ding Y, Zhang J, *et al.* Xylem and phloem based transport of CeO₂ nanoparticles in hydroponic cucumber plants. *Environmental Science & Technology*. 2017; 51: 5215–5221. <https://doi.org/10.1021/acs.est.6b05998>.
- [248] Rodrigues S, Avellan A, Bland GD, Miranda MCR, Larue C, Wagner M, *et al.* Effect of a zinc phosphate shell on the uptake and translocation of foliarly applied Zn nanoparticles in pepper plants (*Capsicum annuum*). *Environmental Science & Technology*. 2024; 58: 3213–3223. <https://doi.org/10.1021/acs.est.3c08723>.
- [249] Wang Z, Xie X, Zhao J, Liu X, Feng W, White JC, *et al.* Xylem- and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.). *Environmental Science & Technology*. 2012; 46: 4434–4441. <https://doi.org/10.1021/es204212z>.
- [250] Spielman-Sun E, Lombi E, Donner E, Howard D, Unrine JM, Lowry GV. Impact of surface charge on cerium oxide nanoparticle uptake and translocation by wheat (*Triticum aestivum*). *Environmental Science & Technology*. 2017; 51: 7361–7368. <https://doi.org/10.1021/acs.est.7b00813>.
- [251] Koelmel J, Leland T, Wang H, Amarasiwardena D, Xing B. Investigation of gold nanoparticles uptake and their tissue level distribution in rice plants by laser ablation-inductively coupled-mass spectrometry. *Environmental Pollution*. 2013; 174: 222–228. <https://doi.org/10.1016/j.envpol.2012.11.026>.
- [252] Lin S, Zhang Q, Bai S, Yang L, Qin G, Wang L, *et al.* Beyond species and spatial boundaries: Enabling long-distance gene silencing in plants via guanidinium-siRNA nanoparticles. *Plant Biotechnology Journal*. 2025; 23: 1165–1177. <https://doi.org/10.1111/pbi.14575>.
- [253] Delgado-Martín J, Delgado-Oliden A, Velasco L. Carbon dots boost dsRNA delivery in plants and increase local and systemic siRNA production. *International Journal of Molecular Sciences*. 2022; 23: 5338. <https://doi.org/10.3390/ijms23105338>.
- [254] Ali SS, Darwesh OM, Kornaros M, Al-Tohamy R, Manni A, El-Shanshoury AE-RR, *et al.* Nano-biofertilizers: Synthesis, advantages, and applications. In Rakshit, A., Meena, V.S. (eds.) *Biofertilizers: Advances in Bio-Inoculants* (pp. 359–370). Elsevier: Netherlands. 2021. <https://doi.org/10.1016/B978-0-12-821667-5.00007-5>.
- [255] Kaur H, Hussain SJ, Mir RA, Chandra Verma V, Naik B, Kumar P, *et al.* Nanofertilizers – Emerging smart fertilizers for modern and sustainable agriculture. *Biocatalysis and Agricultural Biotechnology*. 2023; 54: 102921. <https://doi.org/10.1016/j.beab.2023.102921>.
- [256] Rizwan M, Ali S, Ali B, Adrees M, Arshad M, Hussain A, *et al.* Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and cadmium concentration in wheat. *Chemosphere*. 2019; 214: 269–277. <https://doi.org/10.1016/j.chemosphere.2018.09.120>.
- [257] Everaert M, Warrinnier R, Baken S, Gustafsson JP, De Vos D, Smolders E. Phosphate-exchanged Mg–Al layered double hydroxides: A new slow release phosphate fertilizer. *ACS Sustainable Chemistry & Engineering*. 2016; 4: 4280–4287. <https://doi.org/10.1021/acssuschemeng.6b00778>.
- [258] Guo S, Hu X, Wang Z, Yu F, Hou X, Xing B. Zinc oxide nanoparticles cooperate with the phyllosphere to promote grain yield and nutritional quality of rice under heatwave stress. *Proceedings of the National Academy of Sciences of the United States of America*. 2024; 121: e2414822121. <https://doi.org/10.1073/pnas.2414822121>.
- [259] Yu P, Zheng X, Alimi LO, Al-Babli S, Khashab NM. Metal-organic framework-mediated delivery of nucleic acid across intact plant cells. *ACS Applied Materials & Interfaces*. 2024; 16: 18245–18251. <https://doi.org/10.1021/acsami.3c19571>.
- [260] Gao Z, Rensing C, Wang J, Shen C, Elzaki MEA, Li X, *et al.* Increased and synergistic RNAi delivery using MOF polydopamine nanoparticles for biopesticide applications. *Nature Communications*. 2025; 16: 6384. <https://doi.org/10.1038/s41467-025-61604-5>.
- [261] Kim JH, Lee Y, Kim EJ, Gu S, Sohn EJ, Seo YS, *et al.* Exposure of iron nanoparticles to *Arabidopsis thaliana* enhances root elongation by triggering cell wall loosening. *Environmental Science & Technology*. 2014; 48: 3477–3485. <https://doi.org/10.1021/es4043462>.
- [262] Cañas JE, Long M, Nations S, Vadan R, Dai L, Luo M, *et al.* Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species. *Environmental Toxicology and Chemistry/SETAC*. 2008; 27: 1922–1931. <https://doi.org/10.1897/08-117.1>.
- [263] Zheng L, Hong F, Lu S, Liu C. Effect of nano-TiO₂ on strength of naturally aged seeds and growth of spinach. *Biological Trace Element Research*. 2005; 104: 83–92. <https://doi.org/10.1385/BTER:104:1:083>.
- [264] Santás-Miguel V, Arias-Estévez M, Rodríguez-Seijo A, Arenas-Lago D. Use of metal nanoparticles in agriculture. A review on the effects on plant germination. *Environmental Pollution*. 2023; 334: 122222. <https://doi.org/10.1016/j.envpol.2023.122222>.
- [265] Shah T, Latif S, Saeed F, Ali I, Ullah S, Abdullah Alsahli A, *et al.* Seed priming with titanium dioxide nanoparticles enhances seed vigor, leaf water status, and antioxidant enzyme activities in maize (*Zea mays* L.) under salinity stress. *Journal of King Saud University – Science*. 2021; 33: 101207. <https://doi.org/10.1016/j.jksus.2020.10.004>.
- [266] Rhaman MS, Tania SS, Imran S, Rauf F, Kibria MG, Ye W, *et al.* Seed priming with nanoparticles: An emerging technique for improving plant growth, development, and abiotic stress tolerance.

- Journal of Soil Science and Plant Nutrition. 2022; 22: 4047–4062. <https://doi.org/10.1007/s42729-022-01007-3>.
- [267] Cappetta E, Del Regno C, Conte M, Castro-Hinojosa C, Del Sol-Fernández S, Vergata C, *et al.* An Integrated Multilevel Approach Unveils Complex Seed–Nanoparticle Interactions and Their Implications for Seed Priming. *ACS Nano*. 2023; 17: 22539–22552. <https://doi.org/10.1021/acsnano.3c06172>.
- [268] Abasi F, Raja NI, Mashwani ZU, Amjad MS, Ehsan M, Mustafa N, *et al.* Biogenic silver nanoparticles as a stress alleviator in plants: A mechanistic overview. *Molecules: A Journal of Synthetic Chemistry and Natural Product Chemistry*. 2022; 27: 3378. <https://doi.org/10.3390/molecules27113378>.
- [269] Zhao J, Davis LC, Verpoorte R. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances*. 2005; 23: 283–333. <https://doi.org/10.1016/j.biotechadv.2005.01.003>.
- [270] Rui M, Ma C, Hao Y, Guo J, Rui Y, Tang X, *et al.* Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). *Frontiers in Plant Science*. 2016; 7: 815. <https://doi.org/10.3389/fpls.2016.00815>.
- [271] Hu Y, Zhang P, Zhang X, Liu Y, Feng S, Guo D, *et al.* Multi-wall carbon nanotubes promote the growth of maize (*Zea mays*) by regulating carbon and nitrogen metabolism in leaves. *Journal of Agricultural and Food Chemistry*. 2021; 69: 4981–4991. <https://doi.org/10.1021/acs.jafc.1c00733>.
- [272] Mukarram M, Petrik P, Mushtaq Z, Khan MMA, Gulfishan M, Lux A. Silicon nanoparticles in higher plants: Uptake, action, stress tolerance, and crosstalk with phytohormones, antioxidants, and other signalling molecules. *Environmental Pollution*. 2022; 310: 119855. <https://doi.org/10.1016/j.envpol.2022.119855>.
- [273] Kumar V, Naik IS, Das B, Singh A, Nayak P, Mohapatra C, *et al.* Nanoparticles in plant system: A comprehensive review on their role in diverse stress management and phytohormone signalling. *Plant Stress*. 2025; 18: 101000. <https://doi.org/10.1016/j.stress.2025.101000>.
- [274] Mascarenhas R, Mathur T, Maheshwari J, Nagella P. Interaction of nanomaterials with plant macromolecules: Nucleic acid, proteins and hormones. In Al-Khayri J.M, Alnaddaf, L.M. (eds.) *Nanomaterial Interactions with Plant Cellular Mechanisms and Macromolecules and Agricultural Implications* (pp. 231–271). Springer: Cham. 2023. https://doi.org/10.1007/978-3-031-20878-2_9.
- [275] Wang Z, Tang J, Zhu L, Feng Y, Yue L, Wang C, *et al.* Nanomaterial-induced modulation of hormonal pathways enhances plant cell growth. *Environmental Science: Nano*. 2022; 9: 1578–1590. <https://doi.org/10.1039/D2EN00251E>.
- [276] Yang X, Feng K, Wang G, Zhang S, Zhao J, Yuan X, *et al.* Titanium dioxide nanoparticles alleviates polystyrene nanoplastics induced growth inhibition by modulating carbon and nitrogen metabolism via melatonin signaling in maize. *Journal of Nanobiotechnology*. 2024; 22: 262. <https://doi.org/10.1186/s12951-024-02537-x>.
- [277] Jiang Q, Xie Y, Zhou B, Wang Z, Ning D, Li H, *et al.* Nanomaterial inactivates environmental virus and enhances plant immunity for controlling tobacco mosaic virus disease. *Nature Communications*. 2024; 15: 8509. <https://doi.org/10.1038/s41467-024-52851-z>.
- [278] Yang Z, Deng C, Wu Y, Dai Z, Tang Q, Cheng C, *et al.* Insights into the mechanism of multi-walled carbon nanotubes phytotoxicity in *Arabidopsis* through transcriptome and m6A methylome analysis. *The Science of the Total Environment*. 2021; 787: 147510. <https://doi.org/10.1016/j.scitotenv.2021.147510>.
- [279] Razavizadeh R, Adabavazeh F, Gholipour Z. Application of multi-walled carbon nanotubes in *Calotropis procera* in vitro cultures for improved element uptake and enhanced production of monoterpenes and antioxidants. *Plant Cell, Tissue and Organ Culture (PCTOC)*. 2025; 161: 14. <https://doi.org/10.1007/s11240-025-03014-5>.
- [280] Cai L, Liu C, Fan G, Liu C, Sun X. Preventing viral disease by ZnONPs through directly deactivating TMV and activating plant immunity in *Nicotiana benthamiana*. *Environmental Science: Nano*. 2019; 6: 3653–3669. <https://doi.org/10.1039/C9EN00850K>.
- [281] Almutairi ZM. Effect of nano-silicon application on the expression of salt tolerance genes in germinating tomato (*Solanum lycopersicum* L.) seedlings under salt stress. *Plant Omics Journal*. 2016; 9: 106–114. <https://doi.org/10.3316/informit.888088806058398>.
- [282] Lei Z, Mingyu S, Chao L, Liang C, Hao H, Xiao W, *et al.* Effects of nanoanatase TiO₂ on photosynthesis of spinach chloroplasts under different light illumination. *Biological Trace Element Research*. 2007; 119: 68–76. <https://doi.org/10.1007/s12011-007-0047-3>.
- [283] Raliya R, Tarafdar JC, Biswas P. Enhancing the mobilization of native phosphorus in the mung bean rhizosphere using Zn nanoparticles synthesized by soil fungi. *Journal of Agricultural and Food Chemistry*. 2016; 64: 3111–3118. <https://doi.org/10.1021/acs.jafc.5b05224>.
- [284] Li Y, Xu X, Lei B, Zhuang J, Zhang X, Hu C, *et al.* Magnesium-nitrogen co-doped carbon dots enhance plant growth through multifunctional regulation in photosynthesis. *Chemical Engineering Journal*. 2021; 422: 130114. <https://doi.org/10.1016/j.cej.2021.130114>.
- [285] Chandra S, Pradhan S, Mitra S, Patra P, Bhattacharya A, Pramanik P, *et al.* High throughput electron transfer from carbon dots to chloroplast: a rationale of enhanced photosynthesis. *Nanoscale*. 2014; 6: 3647–3655. <https://doi.org/10.1039/C3NR006079A>.
- [286] Jing X, Liu Y, Liu X, Zhang Y, Wang G, Yang F, *et al.* Enhanced photosynthetic efficiency by nitrogen-doped carbon dots via plastoquinone-involved electron transfer in apple. *Horticulture Research*. 2024; 11: uhae016. <https://doi.org/10.1093/hr/uhae016>.
- [287] Das S, Debnath N, Pradhan S, Goswami A. Enhancement of photon absorption in the light-harvesting complex of isolated chloroplast in the presence of plasmonic gold nanosol—a nanobionic approach towards photosynthesis and plant primary growth augmentation. *Gold Bulletin*. 2017; 50: 247–257. <https://doi.org/10.1007/s13404-017-0214-z>.
- [288] Li P, Wang A, Du W, Mao L, Wei Z, Wang S, *et al.* Insight into the interaction between Fe-based nanomaterials and maize (*Zea mays*) plants at metabolic level. *The Science of the Total Environment*. 2020; 738: 139795. <https://doi.org/10.1016/j.scitotenv.2020.139795>.
- [289] Zheng L, Su M, Wu X, Liu C, Qu C, Chen L, *et al.* Effects of nano-anatase on spectral characteristics and distribution of LHClI on the thylakoid membranes of spinach. *Biological Trace Element Research*. 2007; 120: 273–283. <https://doi.org/10.1007/s12011-007-8025-3>.
- [290] Skiba E, Pietrzak M, Michlewska S, Gruszka J, Malejko J, Godlewska-Żyłkiewicz B, *et al.* Photosynthesis governed by nanoparticulate titanium dioxide. The *Pisum sativum* L. case study. *Environmental Pollution*. 2024; 340: 122735. <https://doi.org/10.1016/j.envpol.2023.122735>.
- [291] Li H, Huang J, Lu F, Liu Y, Song Y, Sun Y, *et al.* Impacts of carbon dots on rice plants: Boosting the growth and improving the disease resistance. *ACS Applied Bio Materials*. 2018; 1: 663–672. <https://doi.org/10.1021/acsabm.8b00345>.
- [292] Khodakovskaya MV, de Silva K, Biris AS, Dervishi E, Villagar-cia H. Carbon nanotubes induce growth enhancement of tobacco cells. *ACS Nano*. 2012; 6: 2128–2135. <https://doi.org/10.1021/nn204643g>.
- [293] Martínez-Ballesta MC, Chelbi N, Lopez-Zaplana A, Carvajal M. Discerning the mechanism of the multiwalled carbon nanotubes effect on root cell water and nutrient transport. *Plant Physiology and Biochemistry: PPB/Société Française de Physiologie Végétale*. 2020; 146: 23–30. <https://doi.org/10.1016/j.plaphy.2019.11.008>.
- [294] Ramezani M, Asghari S, Gerami M, Ramezani F, Karimi Abdolmaleki M. Effect of silver nanoparticle treatment on the expression of key genes involved in glycosides biosynthetic pathway in *Stevia rebaudiana* B. *Plant. Sugar Tech*. 2020; 22: 518–527. <https://doi.org/10.1007/s12355-019-00786-x>.

- [295] Ikhajagbe B, Igiebor FA, Ogwu MC. Growth and yield performances of rice (*Oryza sativa* var. *nerica*) after exposure to biosynthesized nanoparticles. *Bulletin of the National Research Centre*. 2021; 45: 62. <https://doi.org/10.1186/s42269-021-00508-y>.
- [296] Ghosh M, Jana A, Sinha S, Jothiramajayam M, Nag A, Chakraborty A, *et al.* Effects of ZnO nanoparticles in plants: Cytotoxicity, genotoxicity, deregulation of antioxidant defenses, and cell-cycle arrest. *Mutation Research. Genetic Toxicology and Environmental Mutagenesis*. 2016; 807: 25–32. <https://doi.org/10.1016/j.mrgentox.2016.07.006>.
- [297] Javed R, Khan B, Sharafat U, Bilal M, Galagedara L, Abbey L, *et al.* Dynamic interplay of metal and metal oxide nanoparticles with plants: Influencing factors, action mechanisms, and assessment of stimulatory and inhibitory effects. *Ecotoxicology and Environmental Safety*. 2024; 271: 115992. <https://doi.org/10.1016/j.ecoenv.2024.115992>.
- [298] Gowtham HG, Shilpa N, Singh SB, Aiyaz M, Abhilash MR, Nataraj K, *et al.* Toxicological effects of nanoparticles in plants: Mechanisms involved at morphological, physiological, biochemical and molecular levels. *Plant Physiology and Biochemistry: PPB/Société Française de Physiologie Végétale*. 2024; 210: 108604. <https://doi.org/10.1016/j.plaphy.2024.108604>.
- [299] Gong C, Wang L, Li X, Wang H, Jiang Y, Wang W. Responses of seed germination and shoot metabolic profiles of maize (*Zea mays* L.) to Y2O3 nanoparticle stress. *RSC Advances*. 2019; 9: 27720–27731. <https://doi.org/10.1039/C9RA04672K>.
- [300] Wang X, Yang X, Chen S, Li Q, Wang W, Hou C, *et al.* Zinc Oxide Nanoparticles Affect Biomass Accumulation and Photosynthesis in *Arabidopsis*. *Frontiers in Plant Science*. 2016; 6: 1243. <https://doi.org/10.3389/fpls.2015.01243>.
- [301] Qian H, Peng X, Han X, Ren J, Sun L, Fu Z. Comparison of the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model *Arabidopsis thaliana*. *Journal of Environmental Sciences (China)*. 2013; 25: 1947–1955. [https://doi.org/10.1016/S1001-0742\(12\)60301-5](https://doi.org/10.1016/S1001-0742(12)60301-5).
- [302] Abdel Latef AAH, Zaid A, Abu Alhmad MF, Abdelfattah KE. The Impact of Priming with Al2O3 Nanoparticles on Growth, Pigments, Osmolytes, and Antioxidant Enzymes of Egyptian Roselle (*Hibiscus sabdariffa* L.) Cultivar. *Agronomy*. 2020; 10: 681. <https://doi.org/10.3390/agronomy10050681>.
- [303] Sun J, Wang L, Li S, Yin L, Huang J, Chen C. Toxicity of silver nanoparticles to *Arabidopsis*: Inhibition of root gravitropism by interfering with auxin pathway. *Environmental Toxicology and Chemistry/SETAC*. 2017; 36: 2773–2780. <https://doi.org/10.1002/etc.3833>.
- [304] Rajput V, Minkina T, Sushkova S, Behal A, Maksimov A, Blicharska E, *et al.* ZnO and CuO nanoparticles: a threat to soil organisms, plants, and human health. *Environmental Geochemistry and Health*. 2020; 42: 147–158. <https://doi.org/10.1007/s10653-019-00317-3>.
- [305] Aruoja V, Pokhrel S, Sihtmäe M, Mortimer M, Mädlar L, Kahru A. Toxicity of 12 metal-based nanoparticles to algae, bacteria and protozoa. *Environmental Science: Nano*. 2015; 2: 630–644. <https://doi.org/10.1039/C5EN00057B>.
- [306] Xu C, Peng C, Sun L, Zhang S, Huang H, Chen Y, *et al.* Distinctive effects of TiO2 and CuO nanoparticles on soil microbes and their community structures in flooded paddy soil. *Soil Biology and Biochemistry*. 2015; 86: 24–33. <https://doi.org/10.1016/j.soilbio.2015.03.011>.
- [307] Sotomayor-Ramírez D, Espinoza Y, Acosta-Martínez V. Land use effects on microbial biomass C, β -glucosidase and β -glucosaminidase activities, and availability, storage, and age of organic C in soil. *Biology and Fertility of Soils*. 2009; 45: 487–497. <https://doi.org/10.1007/s00374-009-0359-x>.
- [308] Lin J, Ma K, Chen H, Chen Z, Xing B. Influence of different types of nanomaterials on soil enzyme activity: A global meta-analysis. *Nano Today*. 2022; 42: 101345. <https://doi.org/10.1016/j.nantod.2021.101345>.

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