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Abstract

Nanobiotechnology significantly enhances plant genetic engineering procedures by using nanocarriers such as metal, carbon-based, and polymeric nanoparticles to transfect and transport nucleic acids and proteins to deliver genes with maximum efficiency and translation. It improves the ability of the plants to be transformed using Agrobacterium, poses gradual serious limitations concerning the transfer of genes, and enhances the tolerance of plants to stress. One example is gene editing Crispr/Cas which uses nanoparticles to promote appropriate procedures.In addition, by using nanosensors and nanodevices in practical work it is rather possible to control in the real-time province of gene expression as well as everything occurring around the modified organism, which in turn helps to increase the efficiency of producing genetic modifications. This development can also improve the quality of the harvested crops, and the production yield, play a role in fight against food shortage that is prevalent in the global world like today.

Keywords: Nanoparticles, Plant Genetic Engineering, Genetic Modification, Biotechnology, Agricultural Innovation, Gene Delivery.

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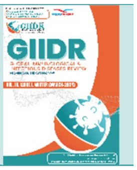

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Title

Nanoparticles Mediated Plant Genetic Engineering: Emerging Field with Promising Applications

Abstract

Nanobiotechnology significantly enhances plant genetic engineering procedures by using nanocarriers such as metal, carbon-based, and polymeric nanoparticles to transfect and transport nucleic acids and proteins to deliver genes with maximum efficiency and translation. It improves the ability of the plants to be transformed using Agrobacterium, poses gradual serious limitations concerning the transfer of genes, and enhances the tolerance of plants to stress. One example is gene editing Crispr/Cas which uses nanoparticles to promote appropriate procedures.In addition, by using nanosensors and nanodevices in practical work it is rather possible to control in the real-time province of gene expression as well as everything occurring around the modified organism, which in turn helps to increase the efficiency of producing genetic modifications. This development can also improve the quality of the harvested crops, and the production yield, play a role in fight against food shortage that is prevalent in the global world like today.

Keywords: Nanoparticles, Plant Genetic Engineering, Genetic Modification, Biotechnology, Agricultural Innovation, Gene Delivery

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Contents

Introduction

According to the UN, by the end of this year, the population will stand at 9. By 2050, the global population will grow to 7 billion and raise the need for agriculture. Climate change poses threats that aggravate social problems such as increased droughts, floods, and increased temperatures in our society. Skillful temperature, humidity, and rain-sensing systems for the betterment and safety of agricultural production present the solutions of the future (Hassan and Siddiqui, 2024).

The employment of nanoscale aspects in plant genetic engineering is of paramount importance to nanobiotechnology since it assists in the deposition of genetic materials as well as in the genetic modification and observation which is interconnected to agricultural yield and total food security as posited by Behl et al . , (2024). The numerous existing nanoparticles like liposomes, polymeric nanoparticles, carbon-based nanoparticles, etc., can effectively deliver nucleic acids, proteins, enzymes, and other bioactive molecules into plant cells with low degradation (Fashola et al., 2021). This makes it possible to have precise regulation of gene expression together with tools such as CRISPR/Cas when enhancing the desirable characteristics of crops, disease and stress robustness, and plant defense mechanisms of the same quality with optimum set time.

History of Nanobiotechnology:

Nanotechnology is a relatively new study that allows many types of substances to be developed including particulate matter which has one dimension smaller than 100 nm (nm)(Saleh, 2020) (Rind et al., 2023). The implementation of nanoparticles is new in agriculture and it requires additional research. The concept of nanotechnology was initially made public by Nobel Prize-winning American scientist Richard Feynman in 1959. Feynman gave a speech titled "There's Plenty of Room at the Bottom" at the Institute of Technology in California at the American Physical Society's annual conference(Bayda*et al.*, 2019) (Pisano and Durlo, 2023). Approximately The word was originally used by a Japanese physicist named Norio Taniguchi fifteen years after Feynman's presentation "nanotechnology" to refer to semiconductor processes taking place at the nanoscale. The early 21st century saw an increase in interest in the developing areas of nanoscience and nanotechnology. President Bill Clinton spoke in support of funding studies in this emerging discipline on January 21, 2000, at a Caltech speech (Hulla*et al.*, 2015). Ancient Egyptians used synthetic chemical procedures to create PdS2 nanoparticles with a diameter of around 5 nm for making hair dye. Fig; etc The term “nanotechnology” was described in the following way by Professor Norio Taniguchi of Tokyo Science University in a 1974 paper: “Nanotechnology’ mainly consists of the processing of segregation, consolidation, and deformation of materials by one atom or by one molecule.”

Figure 1

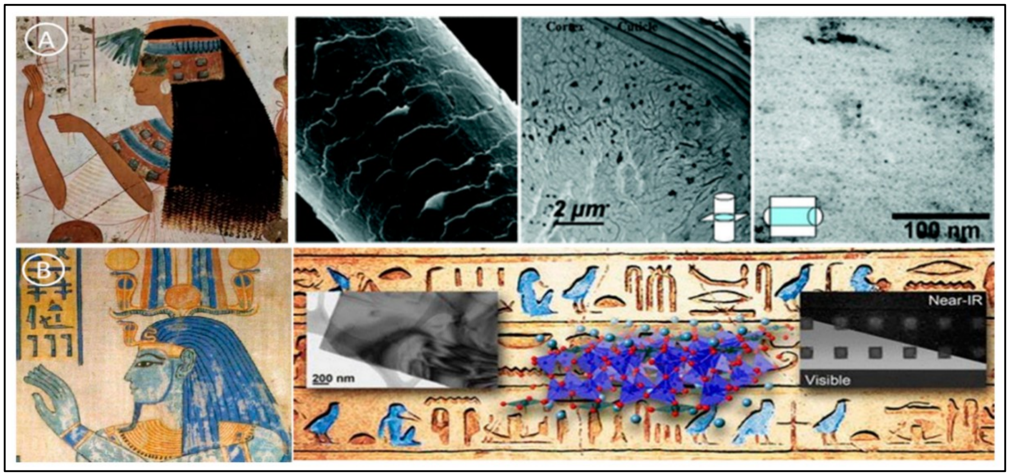


Fig.1. Human-made nanomaterials from earlier societies. (A) PdS2 NPs were made by Egyptians and used as a hair dye (Walter et al., 2006). © American Chemical Society, 2006; (B) Egyptians created Egyptian blue, or nanosheets of SiO2 and CaCuSi4O10, with a thickness of less than 5 nm.(Johnson-McDaniel et al., 2013) © American Chemical Society, 2013.

Characteristics of Nanoparticles

Physical: The surface plasmon resonance and light-interacting properties of nanoparticles made up of gold in nanospheres, having a size range from 20 to 100 nm are the primary areas of investigation in this study. Due to their singular characteristics and historical applications, these properties pique the interest of scientists (Sajid et al., 2020; Shaheen et al., 2023; Bora et al., 2024).

Chemical: Chemical properties dictate how NPs are used in chemical and biological engineering. The substance qualities of nanoparticles additionally change in light of their size and are dependent. Chemical compositions such as toxicity, oxidation, reduction, sensitivity, antifungal, and antibacterial properties are present in the stability of nanoparticles.

Conventional Methods of Transformation

Another area of biotechnology that has come to focus as early as the 1970 but perhaps intensified especially in the 1980s is the genetic transformation for crop improvement. From the study, the different techniques such as Agrobacterium-mediated transformation have improved crops such as tobacco, cotton, and maize as well as rice. Difficulties consist of low efficiency and random integration; therefore, techniques offering basic, affordable, and safe approaches toward constructing a cell with multiple copies of genes are required. Plants are transformed indirectly through the assistance of soil bacteria which include Agrobacterium species through plasmids in the target cells (Alekseeva& Kuznetsov, 2023; Gull& Jander, 2023; Adachi et al., 2021).

The ability to cause a crown gall disease is associated with the presence of Ti (tumor-inducing plasmid). This is the large (> 200kb) that carries the numerous genes involved in the infective process(Kuzmanovićet *al*., 2023). Crown gall tumor in plants is caused by Agrobacterium tumefaciens by transferring a segment of DNA (transferred DNA or T-DNA) from tumor-inducing (Ti) plasmid to the plant chromosomal DNA. This DNA segment is between 15 and 30kb in size(about 10% of plasmid size), depending on the strain type. T-DNA genes are involved in opine synthesis as well and they impart cancerous properties. After that, it was realized that the Ti-plasmid can help to insert a foreign gene into the plants if the new genes are inserted into the T-DNA region. Scientists used the disarmed Ti- plasmids as there is no role of cancerous genes in T-DNA transfer, only two 25bp repeat sequences found at the right & left borders are involved in the DNA transfer. Any DNA present between these two repeats is treated as T-DNA and can be transferred to plants. Infectivity is only controlled through virulence genes(Brown, 2020)(Azizi-Dargahlou and Pouresmaeil, 2023) T-DNA is integrated into plant genome when enters into the nucleus by illegitimate recombination a process likely mediated by host factors(Stepchenkova*et al*., 2023).To improve the agrobacterium-mediated transformations scientists have developed a binary vector, super binary vector, and ternary vector for efficient work in dicots and monocots(Johnson *et al*., 2023). The type of agrobacterium, types of crops, types of explants, and types of vectors determine the efficiency of agrobacterium-mediated transformation.

However, tre sre still so many challenges that need to be addressed, including

1. transformation of economically important plant species, which are highly recalcitrant to *Agrobacterium*-mediated plant transformation,
2. use of *Agrobacterium* for site-directed recombination to avoid random T-DNA integration,
3. introduction of multiple "stacked" transgenes(Ziemienowicz, 2014)

Despite the fact that Agrobacterium is broadly utilized for quality exchange, its constraints require elective strategies. Direct quality exchange strategies, like polyethylene glycol (PEG) treatment, precipitate DNA onto protoplasts (Fizree et al., 2023; Duan et al., 2023). Microparticle siege liked for safe harvests like cotton and maize, permits without vector, multi-quality exchange to any organelle, including chloroplasts and mitochondria (Gao and Nielsen, 2012). Be that as it may, it can cause explant harm, lower plant improvement effectiveness, and quality quieting because of high duplicate numbers. It is likewise expensive because of the utilization of gold particles.

Nanoparticles – Mediated Biomolecule Delivery

Nanomaterials, containing nanoparticles with aspects of 1 to 100 nm, display exceptional actual properties contrasted with large counterparts, making them promising for quality exchange in plants (Modena et al., 2019). Using nanoparticles works with the conveyance of transgenes, upgrading plant science and horticulture, with quality vehicles considered to be significant for crop improvement and illness the board (Gad et al., 2020). Overcoming the size of the cell wall's exclusive limit of 5-20 nm remains a test (Zhang et al., 2019), especially for conveying biomolecules to establish organelles like plastids (Cunningham et al., 2018). Without force nanoparticle conveyance presents an answer, offering the potential for moving DNA, RNA, and proteins to propel plant hereditary designing (Wang et al., 2019). Techniques include streamlining nanoparticle size and surface properties for proficient cell wall infiltration and freight transport, expecting to reform plant biotechnology and yield improvement.

Types of Nanoparticles

Numerous applications exist for various nanoparticles, including silicon-based, metal-based, and peptide-based NPs. This study investigates pollen magnetofection, a method that allows for the direct production of transgenic seeds without the need for regeneration by transporting DNA into pollen through the use of magnetic fields. Even though it looks promising for crops like cotton, it's still not clear how effective it is. (Zhao et al., 2017; Mohamed et al., 2019) Genetic delivery of maize remains restricted to specific genotypes and requires lengthy tissue culture. In order to fertilize maize's female florets inbred lines, magnetic nanoparticles (MNPs) loaded with DNA that encodes either RFP or GUS resistance were introduced into pollen grains. The results show that using our genotype-independent pollen transfection technique, it was possible to successfully transfer exogenous DNA to superior maize inbred lines that exhibited normal expression and resistance to tissue culture-mediated changes. (Wang *et al.*, 2022). We delivered DNA plasmids in plants of multiple species using modified carbon nanotube nanoparticles. This produced high levels of protein expression without the need for transgenic insertion and also RNA delivery into plants without its degradation. NP-mediated gene transformation offers benefits such as short cycle, increased expression efficacy, and biosafety to avoid creating heritable progenies (Lv*et al*.,2020)

Figure 2

Nanoparticles that are employed to carry genetic material, pesticides, and nutrients (fertilizer) into plants (Sembada and Lenggoro, 2024)

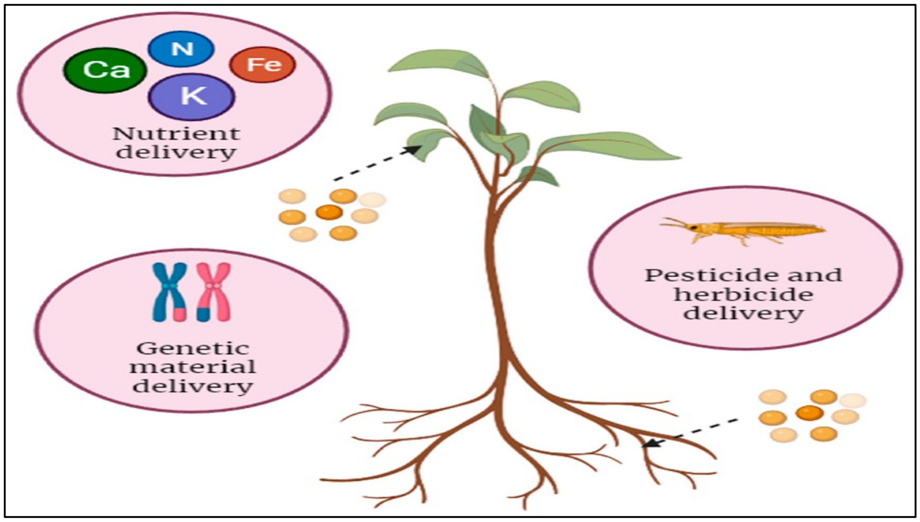
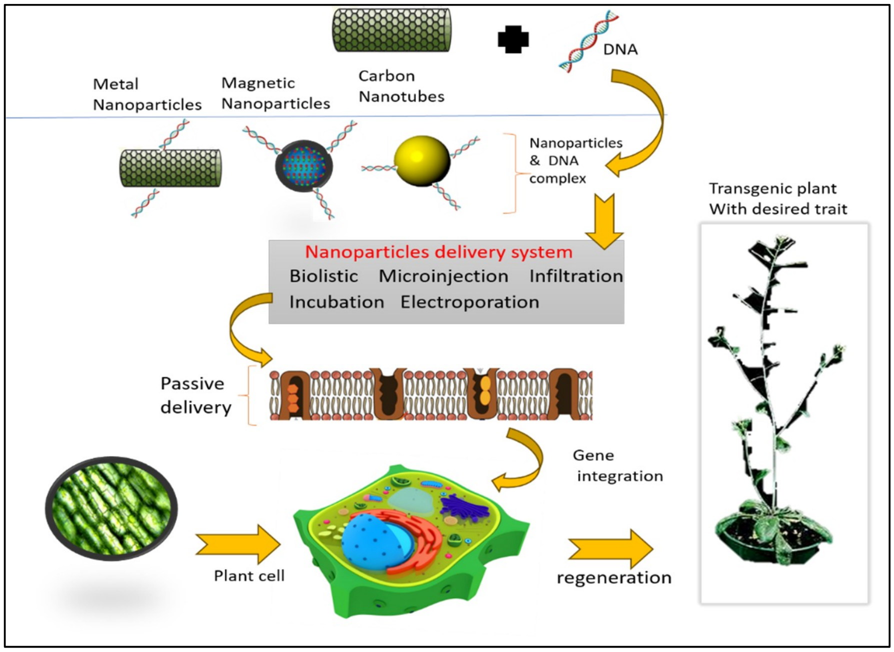


Figure 3

Different nanoparticles used in the genetic transformation of plants



Delivery Challenges

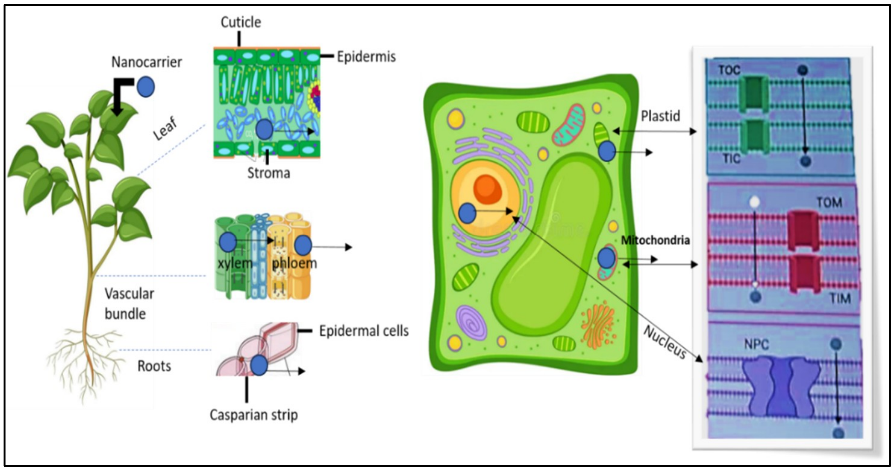
Several techniques including chemical treatment, electrical forces, nanoparticles, and others, have been tried to alter GMO in microbial and mammalian cells. The current methods are, however, limited in several ways and this is why nanoparticles present a more viable solution to the problem by improving cell wall penetration, adaptability to the type of payload, and efficiency of delivery across the plant kingdoms. Nanobiotechnology appears as an innovation of choice in genetic engineering since it offers a remedy to delivery concerns in myriad biological systems.

- Efficiency is more than Agrobacterium-mediated transformation and also the bombardment of particles
- Stable transformation and integration in plant genome
- Avoid random integration of transgene that may disrupt the endogenous plant genome
- The gene of interest will go to the target (Roberts *et al*.,2020)

There could be difficulty during the delivery of nano cargo complex in plant systems but seen success in microbial, Animal, and Mammalian cells because of basic variations in each system's biological barriers.

Figure 4

Delivery barriers



Applications of Nano Biotechnology

Genetic Transformations

The use of NP-based transformation is a promising approach for tackling the limitations of traditional transformation methods, including limited species applicability and susceptibility to cell damage (Khanna*et al*., 2023) Numerous nanomaterials nanoparticles with metal nanoparticles, or mesoporous silica nanoparticles, among others, have been designated to transport nucleic acids in plant cells, despite the fact that hard cell wall is a significant obstacle to the biomolecules transfer in the plant cell (Yadav*et al*., 2023). Nanoparticles may target genetic material to inaccessible plant tissues, cells, and subcellular places, making this promising. According to recent research, plant meristematic areas allow editing in target tissues(Tandon*et al*., 2023). In addition nanoparticles cargos resistance against degradation. It not only delivers the plasmid DNA but can be used as a freight carrier in mature plants to deliver siRNA directly and silence genes (Zhang *et al*., 2020). To serve as a vehicle for the transport of siRNA, we created polyethyleneimine functioning gold particles (PEI-AuNPs) with fluorescence and the scavenging of ROS. Defense-regulated gene silencing using the PEI-AuNPs delivery method reduced bacterial population, balanced ROS concentration, increased antioxidant enzyme activity, and improved chlorophyll fluorescence performance, increasing the resilience of plants against disease. The opportunity plant nanobiotechnology in order to safeguard farming output and the advantages of AuNP-based RNA interference in enhancing plant disease resistance. (Wu*et al*., 2024). We report the encapsulation and delivery of dsRNA in cationic poly-aspartic acid-derived polymer (CPP6) into plant cells for physical characteristics and the immune system's reaction to bacterial infections (Palet *al.*, 2024)(Sembada and Lenggoro, 2024). Nanotubes safeguard siRNA from nucleases and convey it into plant cells, accomplishing 98% quality hushing proficiency. This empowers RNA conveyance for plant biotechnology applications, with a critical potential for practical genomic studies and farming turn of events (Cai et al., 2023).In the table below you can see that the protein delivery is medicated by nanoparticles.

Figure 5

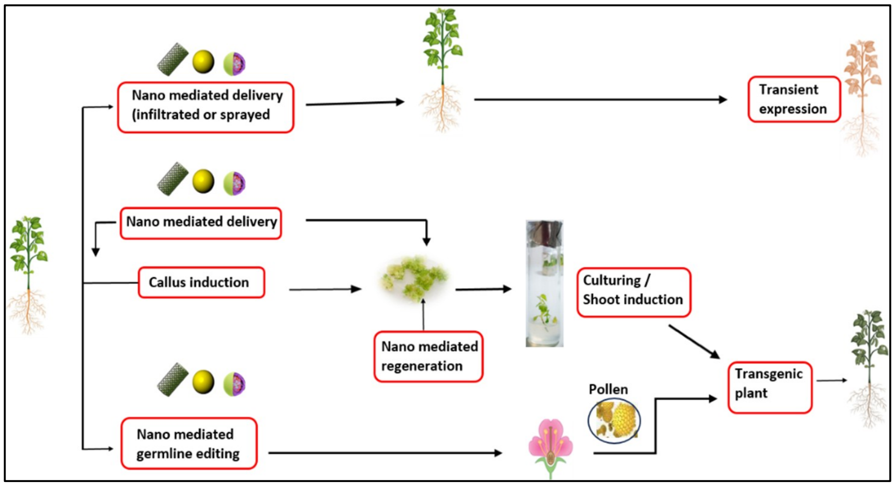


Table 1

nanoparticle-mediated protein delivery

Nanoparticles	Crops	Protein Cargo	References
mesoporous silica nanoparticle (Au-MSN)	Onion and tobacco	Tobacco and onions as mesoporous silica nanoparticles (Au-MSN) Research is conducted on increased BSA and eGFP	(Martin‐Ortigosa *et al.*, 2012)
MSNs that are gold-plated	maize (*Zea mays*)	re recombinase protein	(Martin-Ortigosa *et al.*, 2013)
Gold microparticles	Onion's epidermis and leaves of tobacco	GFP, , BSA, GUS, , trypsin	(Martin-Ortigosa and Wang, 2014)
Tpl CPP complexes	Rape seed and wheat	Gus protein	(Chen *et al.2015*)

Table 2

The NANOBIOTECHNOLOGY Mediated Genetic transformation in various crop species

Nanoparticles	Crops	Cargo	Role	References
Layered double hydroxide (LDH) clay nanosheets	N. benthamiana, Tomato,Vigna mungo (L.),	dsRNA, microRNA, siRNA	A single spray of LDH-encapsulated dsRNA offers a 20-day virus defense; three amiRNAs target distinct TYLCV regions for transcript silencing.	Layered double hydroxide (LDH) clay nanosheets

Nanoparticles	Crops	Cargo	Role	References
magnesium/iron-layered double hydroxides (MgFe-LDH) nanosheets	A few crops like soybean and sunflower	dsRNA	S. sclerotiorum lesion expansion was considerably slowed down by magnesium or iron labeled double hydroxide (MgFe-LDH) nanosheet filled with dsRNA segments that had been transcribed both.	magnesium/iron-layered double hydroxides (MgFe-LDH) nanosheets
polymeric nanocarriers	Rice, Arabidopsis, and Tobacco	dsRNA	Physiological traits and defense against bacterial diseases	polymeric nanocarriers
Fluorescent gold nanoparticles	Arabidopsis	siRNA	To provide plants siRNA to strengthen their resistance against Pseudomonas syringae. It enhanced the performance of chlorophyll fluorescence.	Fluorescent gold nanoparticles
Carbon nanotube (CNT)	N. benthamiana E. sativa T. aestivum Rice leaves and seeds	GFP, Plasmid DNA delivery CRISPR Cas- 9	Plant scientists have modified to produce species-independent modified single-walled carbon nanotubes with surface chemistry designed for plasmid DNA transport.	Carbon nanotube (CNT)
(PSWNTs)	Tobacco	Vaccine delivery	Plant viral disease prevention	(PSWNTs)
(DNA-CNTs),	(*Spirodela polyrhiza*)	DNA delivery	Duckweed potential as a powerhouse in synthetic biology	(DNA-CNTs),
polyethyleneimine (PEI)--coated nanoparticles with carboxylated SNWTs	Litopenaeus vannamei (L.vannamei)	CRISPR-Cas9 delivery	Gene editing	polyethyleneimine (PEI)-coated nanoparticles with carboxylated SNWTs

Different Nanoparticles in Plant Engineering

Carbon Nanomaterials

The exceptional mechanical, electrical, optical, and thermal capabilities of engineered carbon nanostructures make them highly suitable for an extensive variety of uses. The main constituents of the carbon nanomaterial family are carbon nanotubes (CNTs), carbon dots (CDs), graphene oxide, and nanodiamonds (Zhang *et al.*, 2016)(Zakaria *et al.*, 2022). Carbon nanomaterials internalization was started in 2009(Liu *et al.*, 2009). Because of its compact size and great tensile strength could be the better option for bypassing the cell wall. The effective transport of DNA in a range of plants, such as cotton, wheat, arugula, and N. benthamiana by CNTs. Lignin-Loaded Carbon Nanoparticles against Fusarium verticillioides in Maize(El-Ganainy*et al.*, 2023).Carbon nanostructures in chloroplast(Santana*et al.*, 2022). Through electrostatic contact, functionalized carbon dots (CDs) are complicated with the screened dsRNAs (dsRNA-CDs) against*Phytophthora infestans* and*Phytophthora sojae*. To the Enhancement of increased photosynthetic efficiency in plants through plastoquinone-mediated electron transfer using nitrogen-doped carbon dots (Jing*et al.*, 2024). Nicotiana tabacum, Spinacia oleracea, Arabidopsis thaliana mesophyll protoplasts, mature plants of Eruca sativa and Nasturtium officinale, we exhibit chloroplast-targeted transgene transport and temporary expression. This delivery mechanism of the chloroplast transgene via nanoparticles offers several benefits over conventional delivery methods and might potential transformation method for plant bioengineering and biological investigations Mitochondria offers agronomic traits, but the delivery into mitochondrial genome less to low efficiencies, limiting in genetic engineering. CNTApproaches for the advancement of organelle biotechnology

Figure 6

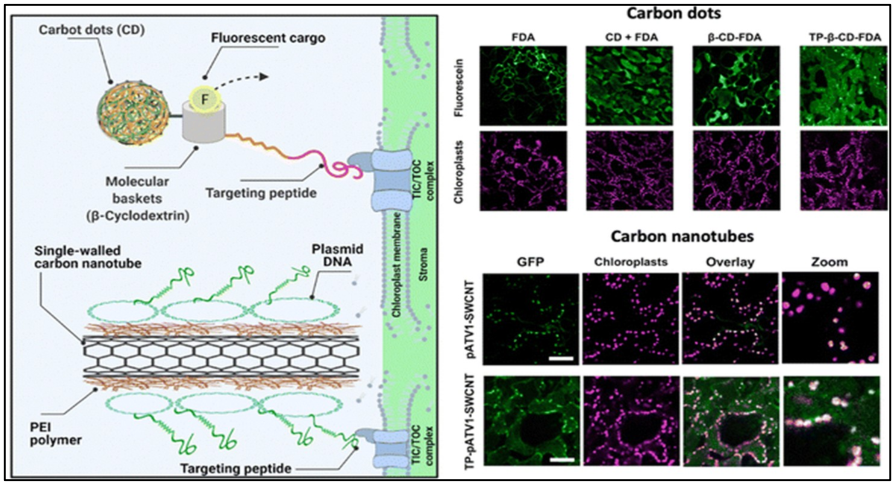
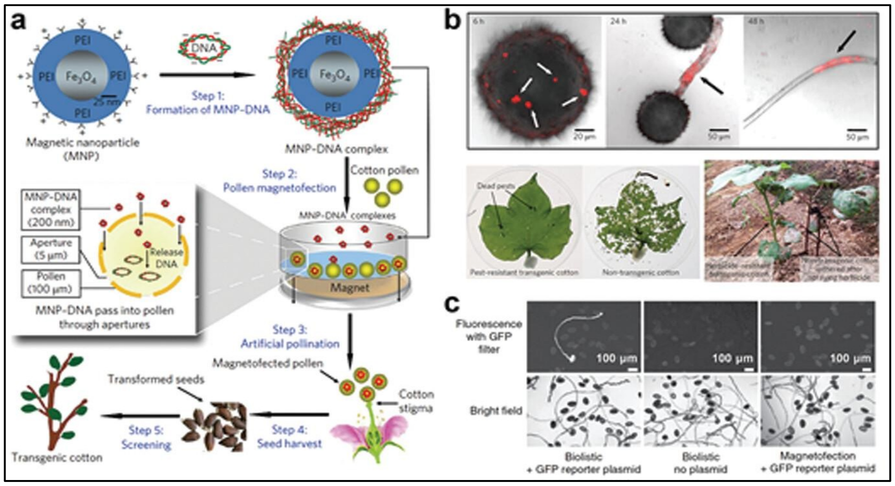


Fig.6. NM-mediated transport of chloroplasts by including a peptide specific to chloroplasts. In order to facilitate cargo delivery into the chloroplasts of A. thaliana leaves, It was possible to observe dye uptake in the chloroplasts by delivering a fluorescent dye via carbon dots in conjunction with a biorecognition motif specific to the TIC/TOC complex and a molecular basket. This was also applied to PEI-attached CNTs that shared a similar biorecognition motif and had been attached to a peptide containing a DNA binding domain. after being complexed with pDNA. After seven days of exposure, the reporter GFP construct was seen to be expressed. There is a 50 μm scale bar. Permission to use this adaptation is provided by the American Chemical Society (Santana et al., 2022).

Metallic Nanoparticles

Large-scale and small-scale metallic delivery methods have been extensively used for transporting genetic material within the systems of animals, with gold nanoparticles. It is the most extensively studied for delivering biomolecules. For many years, tiny gold particles have been used in plants to carry molecules via a process called biolistic delivery(Duan*et al.*, 2021). In order to transfect such siRNA into the intact plants of Aloë Vera, we functionalize polyethyleneimine gold nanoclusters as stated earlier and referred to as PEI-AuNCs. Such nanoclusters can, therefore, be considered to have gene knockdown since a phenomenon as such can be clearly proven with the help of such. Additionally, we also prove that due to its size, it cannot penetrate into the PEI-AuNC; thereby, confirming that siRNA has better protection against RNase degradation as compared to a plant cell. In this study, we used AuNPs in conjunction with AmiRNA technology to target specific genes in plants. Fluorescent gold nanoparticles deliver siRNA against Pseudomonas syringae (Wu *et al.*, 2024)(Khan*et al.*, 2024), and also a role as a nanophytovirology to detect plant viruses(Warghane*et al.*, 2024). AuNPs-siRNA$_{NPR1}$ silenced 80% of the *NPR1* gene in *Arabidopsis*(Lei *et al.*, 2020). The highest efficacy of transformation was documented in *Lilium regale* pollen by using nanomagnetic beads to DNA plasmid (Zhang *et al.*, 2023). By Pollen magnetofection, transgenic seed production without regeneration. Existing maize gene delivery strategies were time-consuming. Thus, we present an updated by using nanoparticles with magnetic properties (MNPs) coated with DNA expressing bialaphos resistance (bar), improved green fluorescent protein (EGFP), □-glucuronidase gene, or red fluorescent protein (RFP) (Wang *et al.*, 2022). See Fig 6 for how pollen magnetic nanoparticles are carried out

Figure 7



Five Steps Includes in Pollen Magnetofection

Fig.7. 1) MNP-DNA complex creation; 2) pollen magnetofection using cotton pollen; 3) artificial pollination using magnetofected pollen grains; 4) harvesting of seeds; and 5) screening of transgenic plants. A,b) Reproduced with permission from Zhao et al. (2017). B) Temporal monitoring of fluorescent MNPs labeled with Lumogen F Red 305 in the pollen grains and tubes within 48 hours. Used by permission (Vejlupkova et al., 2020) Copyright 2017, Springer Nature

Silicon NPS

Numerous reports on silicon-based delivery methods in animal systems. The Am-MSNs/pDNA compound demonstrated strong stability and effectively shielded confined pDNA from cellular nucleases' destruction. No cytotoxic effects on A. thaliana protoplasts. Much more transformation efficiency was made possible by the Am-MSN-50 (LU *et al.*, 2022). The MPI promoter-controlled functionalized MSNs with the appropriate particle size and cryIAb gene delivery into the tomato plants and the putative transgenic seeds were collected. Due to its biodegradability, and biocompatibility, prefer over conventional methods(Juneja*et al.*, 2021). Also act as against *Fusarium graminearum* (Kaziem*et al.*, 2022)

Genome Editing Applications of Nanoparticles

Crispr-cas9 is an advanced technology for genetic engineering because it enables targeted alteration in the genome of an organism. Nanomaterials improve gene editing, which has been considered a difficult technique using conventional techniques. They have targeted endonucleases namely Meganucleases with recognition sequences of 20–30 kb (Tröder and Zevnik, 2021; Li et al., 2024) and Zinc-finger nucleases (ZFNs), which has revolutionized genetic modification (Sufyan et al., 2023). The novel Fanzor technique also develops genome editing even further (Writer, 2023).

In order to effectively employ nanoparticles in plant bioengineering, steady change in genes and expression to allow producing productive transgenic plants. When CNTs are used for delivery, CRISPR plasmids will express themselves momentarily to help prevent the negative effects of repeated copy insertions. BsTargeted tissue genome editing using nanoparticles random integration allows for the transgene-free engineering of crops grown vegetatively and can create permanent edits in the plant genome (Wang *et al.*, 2019). Rice seed and embryos using CNT-delivered CRISPR-Cas for gene editing SWCNTs are thought to be promising delivery systems for the CRISPR-Cas9 genome editing tool into plant cells (Aliet *al.*, 2022). CRISPR-Cas9 delivery nanoparticles Gold nanoparticles, DNA nanostructures, polymer-based nanoparticles, lipid-based nanoparticles, and so forth (Duan et al., 2021). The production of aromatic rice for specific Rice Gene Editing Through Pollen Magnetofection Assisted by Magnetic Nanoparticles (Shen *et al.*, 2023). Exosome/Liposome, A DNA "nanoclew" Cationic Lipid Nanoparticles Hybrid Delivery of CRISPR/Cas Reagents (Alghuthaymiet *al.*, 2021).

Figure 8

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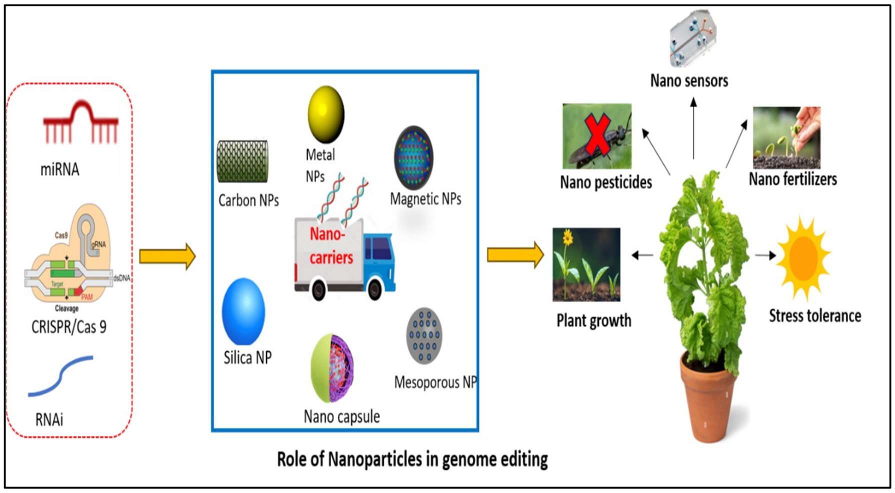

Role of Nanoparticles in genome editing

Table 3

Successful examples of NANOBIOTECHNOLOGY-based delivery of CRISPR/Cas

Editing	Target genes	Nanoparticles	References
Knockout	*BAFFR*	Polyethyleneimine–cyclodextrin	(Li *et al.*, 2018)
Knockout	Polo-like kinase 1 (PLK-1)	Catalic lipid nanoparticles modified with phospholipid and polyethylene glycol (PLNP)-based delivery systems	(Zhang *et al.*, 2017)
Knockout	To knock out PD-L1	Stearyl polyethyleneimine complexed with plasmids as the core of human serum albumin nanoparticles	(Chenget *al.*, 2018)
Homology-directed repair	*CXCR4*	Cas9 ribonucleoprotein can be delivered using a delivery vehicle made of gold nanoparticles attached to DNA and complex with cationic endosomal disrupting polymers.	(Lee *et al.*, 2017)
Knockout	GFP	DNA nano clew	(Wang *et al.*, 2016)
Knockout	*CD38*	Nanoscale zeolitic imidazole frameworks (ZIFs)	(Alsaiariet *al.*, 2017)

Editing	Target genes	Nanoparticles	References
K456 nockout	*GFP*	Gold-Nanoparticle-Mediated Laserporation	(Bošnjaket *al.*, 2018)
Knockout	*H11*	Self-Assembled DNA Nanoclews	(Sun *et al.*, 2015)

Challenges for Nanoparticle Application for Genome Editing in Plant Species

No doubt Nano-biotechnology has potential however, caution must be used when handling nanoparticles size.

- The dosage of these nanoparticles has also been identified as an important criterion for gene transfer due to issues of reactivity and stability.
- A low dosage might hamper the functionalization and poor cargo carriage while a high dosage poses danger to the cells by inducing oxidative stress.
- Information concerning the biosafety of nanoparticles is important, for instance, the effects or toxicity side effects.

Table 4

Other Applications of Nanobiotechnology for Crop Improvement

Nanoparticles	Delivery method	Crop and dose	Role	References
ZnO-NPs	foliar spray	.5, 1 and 5g L−1 fortnight gap on rice crop	Foliar spray of ZnONPs enhanced plant growth, yield traits, zinc content, and soil microbial activity, and exhibited antibacterial effects against rice blight pathogen.	(Bala *et al.*, 2019) (Jaithon *et al.*, 2024)
Cerium oxide nanoparticles	By soil	(25 nm and 50 nm)	Three species of spontaneous plants were observed in a germination experiment and a pot soil investigation to see how they responded to varying concentrations of nCeO2 with varying dimensions. In the early phases of plant development, CeO2 treatments promote root elongation and raise the percentage of germination.	(Lizzi, 2020)
Silver nanoparticles (AgNPs)	By foliar applications	(20, 40, 80 and 100 ppm)	Silver nanoparticles (AgNPs) inhibited 75.93% of B. fabae, effectively increasing growth and yield while protecting faba beans from chocolate spot disease.	(El-Fawyet *al.*, 2024)

Nanoparticles	Delivery method	Crop and dose	Role	References
chitosan-based nanoparticles	By spray mediated	concentrations of nanoparticles (CS, CSAg, and CSCu) 1, 10 and 20ppm	Capsicum spp. leaves enhanced physiological traits, increased chlorophyll (20–75%) and carotenoids, boosted secondary metabolites, and provided 70–85% protection against thrips.	(Mawale and Giridhar, 2024)
TiO2 NPs	Treatment in lab	(15 mg L− 1).	TiO2 nanoparticle on cytological, physiological, and expression of genes alterations.	(Ghouri *et al.*, 2024)
Carbon nanomaterials	By soil	1000 mg/kg and exposure time limited to 50–100 days	MWCNTs enhance soil microbial diversity and promote crop growth, showing promise for increased agricultural output.t.	(Zuo *et al.*, 2024)
FA and ZnO NPs (FZ-50)	Soil	indicated as 20% FZ, 50% FZ, and 80% FZ with mass proportions of 1:5, 1:2, and 4:5.	ZnONPs elevated soil and mung bean zinc levels boosting production, and nitrogen-fixing ability without inducing oxidative stress harm.	(Guo *et al.*, 2024)
Carbon-based NMs	Spray	200 mg L− 1	200 mg L− 1 carbon-based NMs protect against TMV, enhancing photosynthetic efficiency and inducing defense responses.	(Adeel *et al.*, 2021)
Nanoparticles of Zinc Oxide	Foliar sprays	ZnO-NPs at 50 mg/L (ZnO-NPs1) and 100 mg/L (ZnO-NPs2)	ZnO-NPs were tested for their effects on the antioxidant defense mechanism activity and tomato development indices under ToMV stress.	(Sofy*et al.*, 2021)
chitosan–gu		Nano CSGA-M-1.0 at 1.5 ppm (which	Control of Solanum tuberosum L. Early	(Kumar*et al.*, 2022)

Nanoparticles	Delivery method	Crop and dose	Role	References
m acacia (CSGA) polymers to form nanocomposite (NC) CSGA-M		includes 1.0 mg/mL mancozeb)	Blight and Stem Rot by Mancozeb-Loaded Chitosan-Gum Acacia Nanocomposites	

Figure 10

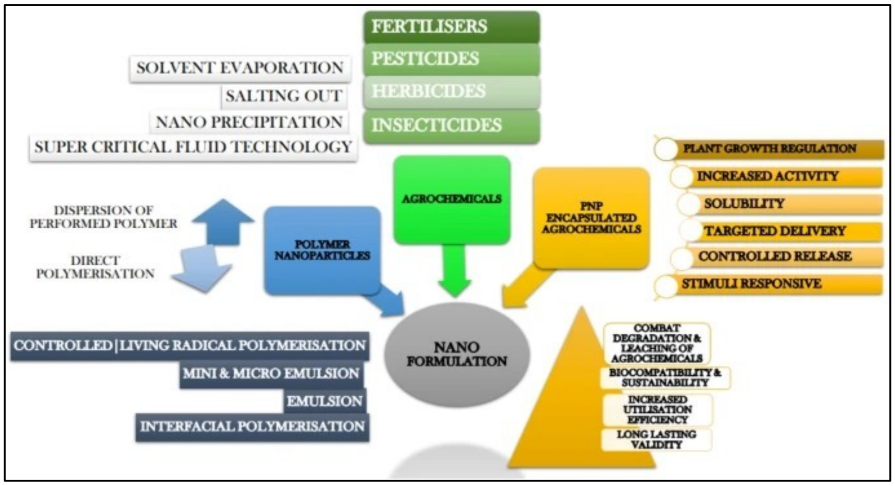

Table 5

Recent examples of Nanoparticles Application in plant species

Nanoparticles	Delivery method/Role	References
Mesoporous silica NPs	By Foliar spray and genome editing	(El-Shetehyet *al.*, 2020)(Deng et *al.*, 2024)
Silicon nanoparticles	▪ Against pests and pathogens, is an option, ▪ Detoxification of heavy metals, antifungal activity	(Naiduet *al.*, 2023)(Ulhassanet *al.*, 2023)(Nabilet *al.*, 2024)
Nanoselenium and nanosilicon	Nutrition and disease protection of crop species	(Sohrawardyet *al.*, 2022)
Loaded Azoxystrobin and Pectin Nanoparticles of Fe3O4	▪ Increase Resistance of Rice to Sheath Blight, ▪ In order to look into how Fe3O4 nanoparticles (Fe-NPs) affect sunflower seed germination	(Menget *al.*, 2024)(Kornarzy□skiet *al.*, 2020)
Multi-walled carbon nanotube	▪ Alleviating the adverse effects of environmental stresses on plants, ▪ Shows positive progression in the bio-fabrication of L-Dopa in Hybanthus enneaspermus suspension cells	(Krá□ová and Jampílek, 2023)(Rahmani and Radjabian, 2024)(2023)(Parthasarathy et *al.*, 2024)
Metal oxide-nanoparticles	To determine the properties of soil	(Peng et *al.*, 2020)(Suazo-Hernández et *al.*, 2023)
Chitosan nanoparticle	▪ - Chitosan nanoparticles foliar application sped up finger millet growth, activating defense enzymes. ▪ - Enhance wheat yield during drought stress. ▪ - Improve grape plant yield under salinity stress.	(Mishra et *al.*, 2023)

Nanoparticles	**Delivery method/Role**	**References**
Liposome NPs	▪ Assist in improving the uptake and distribution of active substances to boost autumn barley's resilience, vitality, and yield (*Hordeum vulgare*),	(Heged□s*et al.*, 2022)

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