

Review Article

# Nanoparticles in Plant Genetic Engineering: Innovative Tools and Future Prospects for Enhanced Crop Traits and Agricultural Sustainability

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## Abstract

The global agricultural sector faces unprecedented challenges in meeting the projected food demand of 9.7 billion people by 2050, exacerbated by the adverse impacts of climate change, such as increased droughts and temperature extremes. Nanobiotechnology, the synergistic integration of nanotechnology and biotechnology, offers transformative solutions in plant genetic engineering to enhance agricultural sustainability and ensure food security. Nanobiotechnology exploits the unique physicochemical properties of nanomaterials, enabling the precise delivery of genetic materials, advanced gene editing, and real-time monitoring of cellular processes. Innovative nanoparticle-mediated methods facilitate the transfer of nucleic acids, proteins, and other biomolecules into plant cells, overcoming the limitations of conventional genetic transformation methods such as *Agrobacterium*-mediated transformation and gene gun technologies. For example, magnetic nanoparticles and carbon nanotubes have shown promise in genotype-independent genetic material delivery and efficient transgene expression. This review highlights groundbreaking applications of nanobiotechnology, including enhanced delivery of CRISPR/Cas9 components for accurate gene editing, nanoscale sensors for intracellular process monitoring, and the use of mesoporous silica nanoparticles for stable gene silencing. Despite these advancements, barriers such as nanoparticle biocompatibility, potential toxicity, and scalability in agricultural systems must be addressed. Regulatory frameworks ensuring the safe adoption of nanomaterials in agricultural practices are equally critical. Nanobiotechnology holds the potential to revolutionize plant genetic engineering by enabling precise trait manipulation, increased crop resilience, and reduced environmental impact. Leveraging these advancements can foster sustainable agricultural practices and mitigate the challenges posed by global food demands and climate change.

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**Received:** 8 January 2025; **Accepted:** 22 February 2025; **Published:** 10 April 2025



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## Keywords

Nanobiotechnology, Plant Genetic Engineering, Nanoparticle-Mediated Transformation, Crispr/Cas9, Sustainable Agriculture, Climate Change Mitigation

## 1. Introduction

The anticipated increase in the global population to 9.7 billion by 2050, as predicted by the United Nations, heightens the agricultural sector's challenge to fulfill rising needs for food, feed, and biofuel production. Simultaneously, the escalating impacts of climate change, characterized by heightened occurrences and severity of droughts, floods, and temperature extremes, pose formidable challenges to crop productivity and global food security. In response, urgent calls for innovative solutions to bolster agricultural output and mitigate environmental stressors echo across scientific and policymaking arenas. One promising avenue of exploration lies in the development of advanced sensor-equipped systems that meticulously monitor external parameters such as temperature, humidity, and precipitation [1]. Nanobiotechnology, the convergence of nanotechnology and biotechnology, has garnered significant attention for its transformative potential across various fields, including agriculture. In plant genetic engineering, nanobiotechnology offers innovative tools and methodologies that revolutionize our ability to manipulate and enhance crop traits with unprecedented precision and efficiency [2].

This review provides a detailed overview of the role of nanobiotechnology in plant genetic engineering, highlighting its applications, challenges, and potential impacts on agricultural sustainability and global food security. Nanobiotechnology encompasses the design, synthesis, characterization, and application of nanomaterials and nanoparticles at the nanoscale level, typically ranging from 1 to 100 nanometers. These nanoscale structures possess unique physical, chemical, and biological properties that differ from their bulk counterparts, making them highly versatile and effective tools for various biological applications. In plant genetic engineering, nanobiotechnology offers several distinct advantages over traditional methods, including enhanced delivery of genetic materials, precise gene editing capabilities, and real-time monitoring of cellular processes (Behl *et al.*, 2024). One of the key applications of nanobiotechnology in plant genetic engineering is the delivery of nucleic acids, proteins, and other bioactive molecules into plant cells. Nanoparticles serve as efficient carriers for these biomolecules, protecting them from degradation and facilitating their uptake into plant cells [3]. Various types of nanoparticles, such as liposomes, polymeric nanoparticles, and carbon-based nanomaterials, have been engineered to deliver genes, siRNAs, and other genetic elements into plant tissues with high efficiency and specificity.

This targeted delivery enables precise manipulation of gene expression, allowing researchers to introduce desired traits into crops or modulate endogenous gene expression for improved agronomic traits [4]. Furthermore, nanobiotechnology enables advanced gene editing techniques, such as CRISPR/Cas systems, to precisely modify plant genomes with unparalleled accuracy. Nanoparticle-mediated delivery of CRISPR components, including Cas proteins and guide RNAs, enhances the efficiency and specificity of gene editing, enabling targeted modifications of specific genes associated with crop improvement, disease resistance, and stress tolerance. Additionally, nanoscale sensors and nanodevices provide real-time monitoring of gene expression, metabolic pathways, and environmental factors within plant cells, offering insights into plant physiology and enabling optimization of genetic engineering strate [5].

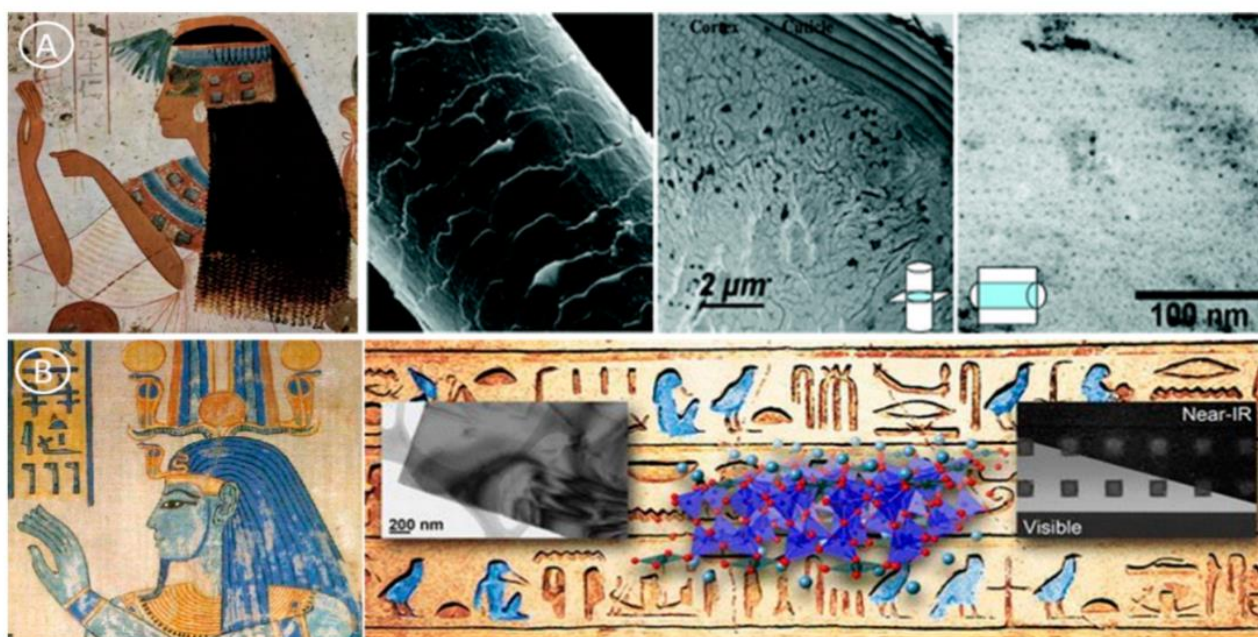
Despite its tremendous potential, the widespread adoption of nanobiotechnology in plant genetic engineering faces several challenges, including safety concerns, regulatory issues, and scalability of production. The environmental impact of nanomaterials and their potential toxicity to plants and ecosystems require careful assessment to ensure sustainable agricultural practices. Moreover, regulatory frameworks governing the use of nanomaterials in agriculture need to be developed to address safety, ethical, and societal concerns associated with their use [6]. In conclusion, nanobiotechnology holds immense promise for revolutionizing plant genetic engineering and contributing to agricultural sustainability and food security. By leveraging the unique properties of nanomaterials and nanoparticles, researchers can develop novel strategies for enhancing crop productivity, improving stress tolerance, and mitigating the impacts of climate change on agriculture. However, addressing the challenges and uncertainties surrounding the use of nanomaterials in plant genetic engineering is essential to realize the full potential of nanobiotechnology in shaping the future of agriculture [7].

## 2. History of Nanobiotechnology

Nanotechnology is an emerging study that enables the creation of a wide variety of substances, such as particulate matter that have minimum one dimension measuring less than 100 nanometers (nm) [8, 9]. It encompasses a wide range of materials with physical and chemical properties. The implementation of nanoparticles is new in agriculture and it require

additional research [5, 10, 11]. 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 [12-15]. 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 [16]. The early 21st century saw an increase in interest in the developing areas of nanoscience and nano-

technology. President Bill Clinton spoke to support of funding studies in this emerging discipline on January 21, 2000, at a Caltech speech [17]. Ancient Egyptians used synthetic chemical procedures to create PdS<sub>2</sub> nanoparticles with a diameter of around 5 nm for making hair dye [18]. Figure 1. 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, separation, consolidation, and deformation of materials by one atom or by one molecule."



**Figure 1.** Human-made nanomaterials from earlier societies. (A) PdS<sub>2</sub> NPs were made by Egyptians and used as a hair dye [18]; (B) Egyptians created Egyptian blue, or nanosheets of SiO<sub>2</sub> and CaCuSi<sub>4</sub>O<sub>10</sub>, with a thickness of less than 5 nm [19].

### 3. Characteristics of Nanoparticles

**Physical:** The optical and magnetic properties are the physical characteristics of nanoparticles. The scientific world is highly intrigued by the optical characteristics of metal nanoparticles (NPs), and their application has a long history dating back to the mid of the 1800s [20-22]. Scientists interested in the optical properties of spherical gold nanoparticles because of their light-interacting features. This study examined surface plasmon resonance using gold nanospheres ranging from 20 to 100 nm in diameter [23, 24]. Its brittleness durability, flexibility, resilience, hardness, fatigue capacity, elasticity, ductility, rigidity, and yield stress are the ten main components of a metal's mechanical properties. The superior mechanical properties of nanomaterials stem from the quantum, surface, and volume effects of their nanoparticles [25, 26].

### 4. Conventional Methods of Transformation

For the development of improved crop varieties genetic transformation research is becoming more and more popular. Beginning in 1970, the first recombinant DNA molecules were produced using biochemical scissors known as restriction enzymes [27-29]. There have been numerous events done to improve crops. Different techniques for genetic transformation such as *Agrobacterium mediated* have been done to improve tobacco, cotton, sugarcane, maize, soybean, rice, barley, Arabidopsis, oil palm, wheat, and tomato [30-34]. Although there are several genes transfer technologies accessible today, there are still drawbacks, such as low effectiveness in transformation and random integration locations. A number of conditions need to be taken into account in order to produce genetic transformation using a reproducible technique [35].



Transformation methods must be easy, low cost, safe, can introduce several genes at same time, low copy number. Indirect or direct transformation are the two main categories of common genetic transformation techniques [36, 37]. Through the use of bacteria that are able to transmit indirect transformation methods insert plasmids and transfer genes to a higher plant species into the target cell. Two soil-native bacteria called *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* are the most commonly employed microorganisms [38, 39].

The ability to cause a crown gall disease associated with the presence of Ti (tumor inducing plasmid). This is the large (> 200 kb) that carries the numerous genes involved in infective process [40]. *Agrobacterium tumefaciens* causes crown gall tumors in plants by transferring a segment of DNA (transferred DNA or T-DNA) from its tumor inducing (Ti) plasmid to the plant chromosomal DNA [41]. This segment of DNA is between 15 and 30 kb in size (about 10% of plasmid size), depending on the strain type. T-DNA genes are involved in opines synthesis as well as they impart the 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 inserted into the T-DNA region. Scientist used the disarmed Ti- plasmids as there is no role of cancerous genes in T-DNA transfer, only two 25 bp repeat sequence found at left and right border are only involved in the DNA transfer. Any DNA present between these two repeats treated as T-DNA and can be transferred to plants. Infectivity is only controlled through virulence genes [42-44]. Upon entering the nucleus, the T-DNA is integrated into plant genome by illegitimate recombination a process likely mediated by host factors [45]. To improve the *agrobacterium* mediated transformations scientist has developed a binary vector, superbinary vector and ternary vector for efficient work in dicot and monocots [46]. Type of *agrobacterium*, types of crops, types of explants and types of vectors determine the efficiency of *agrobacterium* mediated transformation.

However, there still many challenges to be addressed, including (i) transformation of economically important plant species, which are highly recalcitrant to *Agrobacterium*-mediated plant transformation, (ii) use of *Agrobacterium* for site-directed recombination to avoid random T-DNA integration, (iii) stable integration of the transgene and consistent inheritance in further generations without loss or alteration of expression, and (iv) introduction of multiple “stacked” transgenes [47, 48].

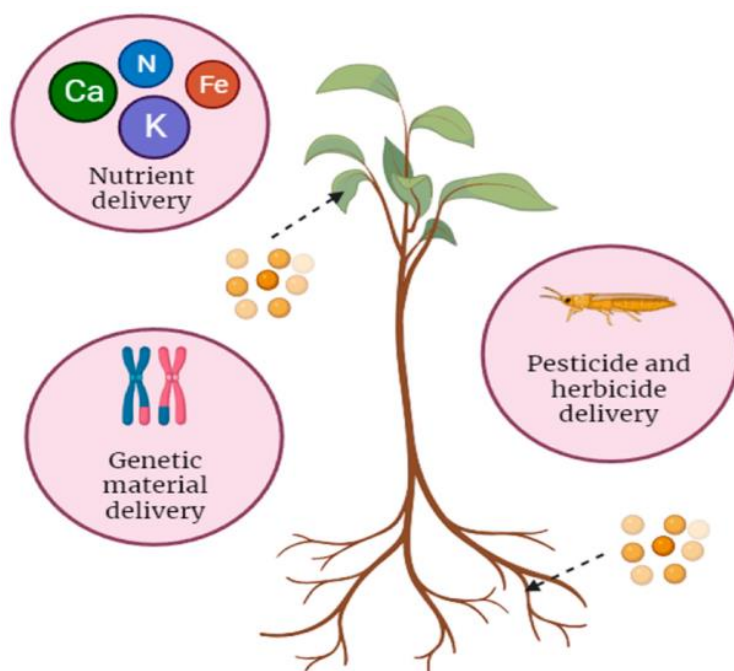
Although *Agrobacterium* is the cost effective and most commonly used method but due to certain limitation there was the need for the search of the alternative methods for gene transfer. Direct gene transfer methods allowed the transfer of supercoiled DNA integration at the chromosome due to re-

combination. Direct methods include chemical method like polyethylene glycol (PEG), polymeric, negatively charged substance which is supposed to cause DNA to precipitate onto protoplast surfaces [49, 50]. Electroporation sometimes is used to increase the transformation frequency. Another direct gene transfer method is gene gun mediated plant transformation [51]. Using metal beads, such as gold and tungsten particles, as carriers, the DNA is directly and mechanically integrated into the host genome by the direct gene transfer method [38].

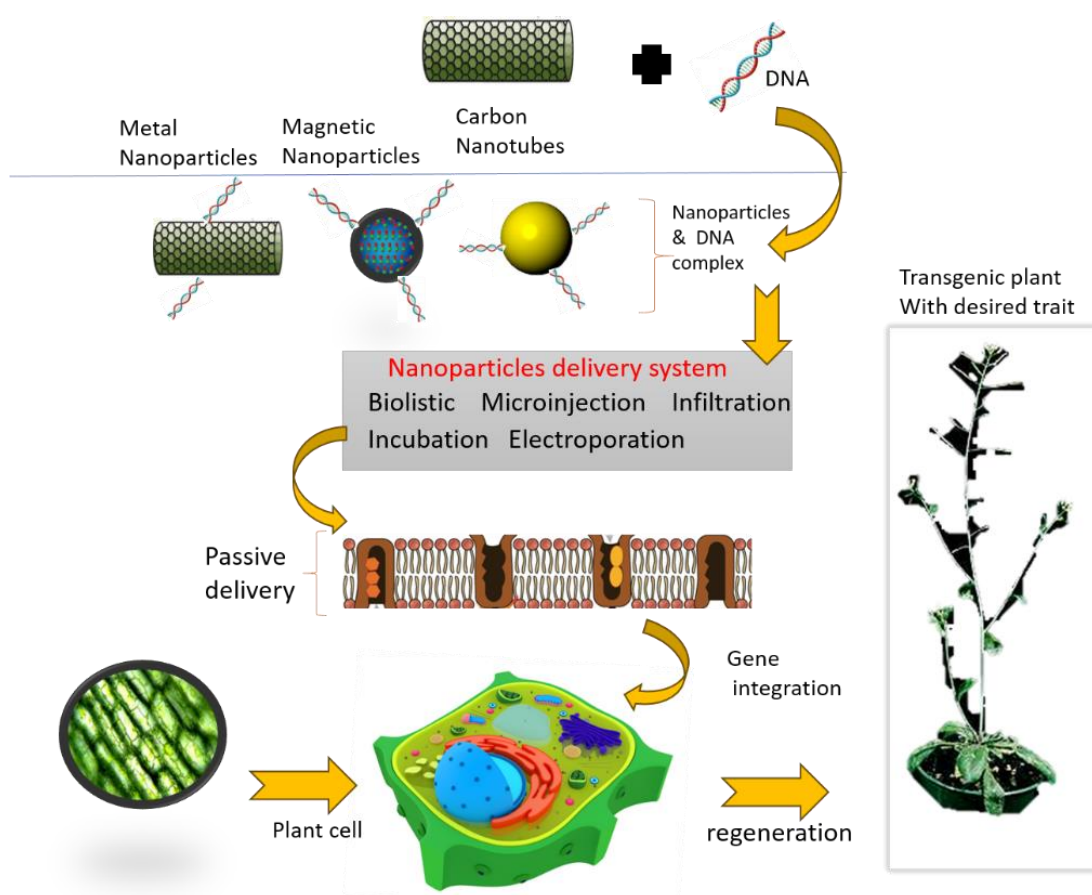
## 5. Nanoparticles – Mediated Biomolecule Delivery

### 5.1. Types of Nanoparticles

There are different varieties of nanoparticles, such as metal-based NPs, silicon-based NPs, clay nanosheets, peptide-based NPs, DNA nanostructures, and polymer-based NPs, Carbon nanotubes, silver nanoparticle, Mesoporous silica nanoparticles etc. Every type of nanoparticles has own characteristics and functions like that stable and transient transformation. Specifically, several crop species, including cotton, provide challenges in terms of regeneration. In this investigation, we provide pollen magnetofection, which allows for the direct production of transgenic seeds without the need for regeneration [52]. This technique transported magnetic nanoparticle-loaded exogenous DNA into pollen using a magnetic field. Transgenic plants were created from transformed seeds by magnetofected pollen [53]. But its efficiency is still controversial based [54]. Maize genetic delivery techniques are restricted to particular genotypes and require tissue culture, which takes time. Here, a novel, genotype-independent transfecting technique for maize is reported. In order to fertilize female florets of maize inbred lines, magnetic nanoparticles (MNPs) loaded with DNA that encodes either RFP, GUS resistance were introduced into pollen grains [52]. 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. [55]. We delivered DNA plasmid in plants of multiple species using modified carbon nano tube nano particle. This produced high levels of protein expression without the need for transgenic insertion and also the RNA delivery into plants without its degradation [56, 57]. NP-mediated gene transformation offers benefits such as, short cycle, increased expression efficacy, and biosafety to avoid creating heritable progenies [58].



**Figure 2.** Nanoparticles that are employed to carry genetic material, pesticides, and nutrients (fertilizer) into plants [59].



**Figure 3.** Different nanoparticles used in genetic transformation of plants.

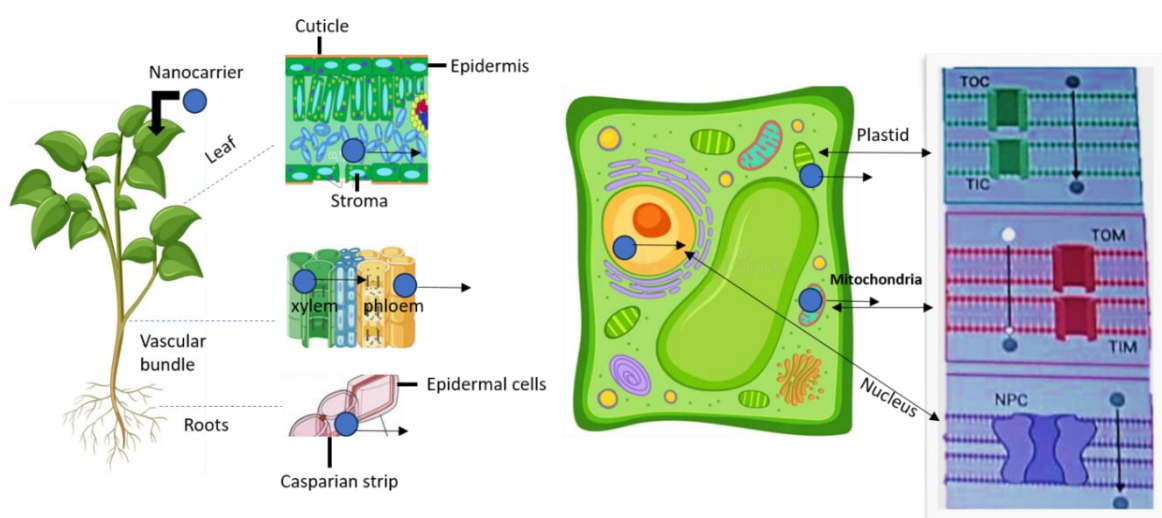
## 5.2. Delivery Challenges

Various ways exist to GMOs, microbes and mammalian cell. Genetic transformation to these targets has been attempted by several methods, including chemical processing, electroporation, sonoporation, polymer transfection, microinjection, gene gun, and also by nanoparticles [60]. But their manipulations in plants are still limited. Their applications in protoplast could be successful but not still in intact plants and also limited transformation efficiency [33, 61-63]. Nanoparticles (NPs) that have been engineered are an intriguing alternative since they provide a tool for plant genetic modification that is species-independent and (possibly) biocompatible. However, the information obtained from delivering biomolecules to animals may serve as a model for delivering biomolecules to plant systems, thereby speeding up progress in using nanoparticles to transport biomolecules to plants. Delivery facilitated by NP (nanoparticles) may effectively address the three primary constraints of present delivery methods. The strategies used in plant systems include managing the size of nanoparticles to effectively pass wall of cell, adjusting their charge and surface features to transport various cargo, and ensuring their effectiveness over a wide range of plant species. The delivery

challenges in any biological systems are similar but the nanobiotechnology could be the best choice of genetic engineer due to the following reasons

- 1) Efficiency is more than *Agrobacterium* mediated transformation and also particle bombardment
- 2) Stable transformation and integration in plant genome
- 3) Avoid from random integration of transgene that may disrupts the endogenous plant genome
- 4) Protection of plasmid DNA against endonucleases degradation
- 5) Gene of interest will go the target [64]

There could be difficulty during delivery of nanocargo complex in plant system but seen successful in microbial, Animals and Mammalian cells because of basic variations in each system's biological barriers. Moreover, it is important to focus on the uptake and incorporation of nanomaterials in plants, as well as the potential consequences. This analysis offers an optimistic outlook on plant nanoscience, presenting alternatives for future food shortages and energy emergencies [65]. Nanotechnology improvements provide a superior option for the secure and exceptionally effective transportation of biological molecules, including i.e miRNA, CRISPR-Cas, and RNAi, without causing harm to plant's cells [66].



**Figure 4.** Delivery barriers.

## 6. Applications of Nanobiotechnology

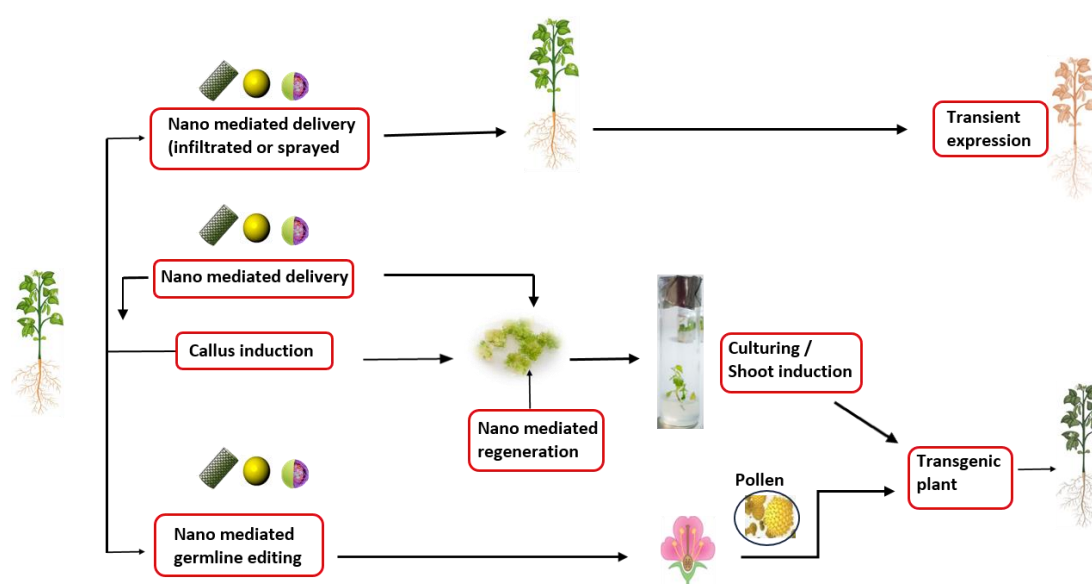
### 6.1. Genetic Transformations

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 [67, 68]. Numerous nanomaterials nanoparticles with metal nanoparticles, or mesoporous silica nanoparticles, among others, have been demonstrated to

transport nucleic acids in plant cells, despite the fact that hard cell wall is a significant obstacle to the biomolecules transfer in plant cell [69]. It's delivery in plants has attracted interest in recent years owing to its potential to overcome some of the limitations of conventional plant genetic modification approaches [70]. 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 [71]. 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 [72]. 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 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. [25]. 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 [73,

59]. We show that nanotubes silenced endogenous genes by delivering siRNA intracellularly and protecting it from the breakdown of nucleases. This work demonstrates that RNA delivery to intact cells is a prerequisite for many plant biotechnology applications, which could be made possible using nanotubes [70]. MSN-mediated siRNA delivery has a high potential for use in plant functional genomic studies and agricultural development as a method for long-term multi-gene silencing (98% silencing efficiency) [74]. However, there are many obstacles in the way of wall of cell penetration, internal delivery, the endosomal escape, and nanoparticles chemistry and design when it comes to the development of nanoparticle-mediated protein delivery [75]. In table below you can see that the nanoparticle mediated protein delivery.



**Figure 5.** In the given figure, have shown the complete process of nanoparticles in genetic engineering.

**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 bovine serum albumin (BSA) and green fluorescent protein (eGFP) (Martin-Ortigosa et al., 2012).	[76]
gold-plated MSNs	maize (Zea mays)	Re-recombinase protein	[77]
Gold microparticles	Onion's epidermis and leaves of tobacco	GFP, BSA, GUS, trypsin	[78]
TpI CPP complexes	Wheat and rapeseed	Gus protein	[79]

Without external force, different plant species' chloroplasts receive plasmid DNA through the use of single-walled carbon nanotubes. In mature *Spinacia oleracea* and *Eruca sativa* plant

also our findings reveal transgenic that targets chloroplasts in separated *Arabidopsis thaliana* mesophyll protoplasts transport and temporary expression [80].



**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	The application of a single spray of dsRNA encapsulated on LDH provided effective defence against viruses for a minimum duration of 20 days. In order to silence the transcripts that the virus produces, we created three amiRNAs that target distinct TYLCV areas.	[81-83]
magnesium/iron-layered double hydroxides (MgFe-LDH) nanosheets	Few crops like soybean and sunflower	dsRNA	S. sclerotium lesion expansion was considerably slowed down by magnesium or iron labbele double hydroxide (MgFe-LDH) nanosheet filled with dsRNA segments that had been transcribed both.	[84]
polymeric nanocarriers	Rice, Arabidopsis and Tobacco	dsRNA	Physiological traits and defense against bacterial diseases	[73]
Fluorescent gold nanoparticles	Arabidopsis	siRNA	To provide plants siRNA to strengthen their resistance against Pseudomonas syringae. Boosting the activity of antioxidant enzymes and enhancing the performance of chlorophyll fluorescence.	[25]
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.	[56, 85, 86]
(PSWNTs)	Tobacco	Vaccine delivery	Plant viral disease prevention	[87]
(DNA-CNTs),	(Spirodela polyrhiza)	DNA delivery	Duckweed potential as a powerhouse in synthetic biology	[88]
polyethylenimine (PEI)-coated nanoparticles with carboxylated SNWTs	Litopenaeus vannamei (L. vannamei)	CRISPR-Cas9 delivery	Gene editing	[89]
Gold nanoparticles covered with silicon dioxide modified by a cationic polymer called poly-ethylenimine (PEI-Au@SiO <sub>2</sub> )	Cannabis sativa	Transcription factors	Following the diffusion of fluorescent-tagged transcription factors proteins found in Cannabis leaf cell nuclei and soybeans genes responsible for biosynthesizing important terpene and cannabinoid metabolites—were observed.	[90]
calcium nutrition nanoagent	Tomato	Calcium	Boost the rate at which leaves photosynthesize to manage tomatoes' atypically rapid development. This work is the first successful application of a nano-delivery method for improved transportation of calcium and antiviral defenses, which will help to improve nutrient-use efficiency and has promising field application possibilities.	[91]

## 6.2. Different Nanoparticles in Plant Engineering-Carbon Nanomaterials

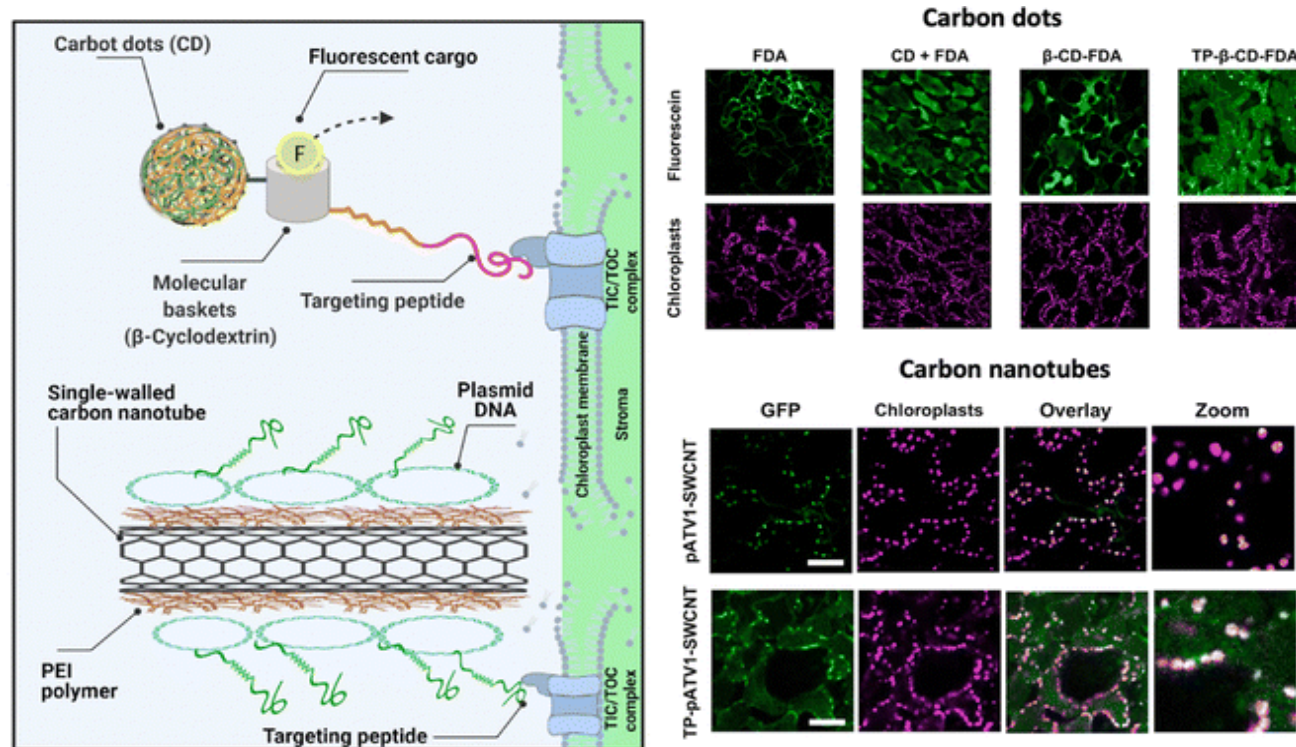
The exceptional mechanical, electrical, optical, and thermal

capabilities of engineered carbon nanostructures making 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, graphene oxide, and nanodiamond [92, 93]. Carbon nanomaterials internalization was started in 2009 [94]. 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 [95]. Lignin-Loaded Carbon Nanoparticles against *Fusarium verticillioides* in Maize [96]. The impact of multiwalled carbon nanotubes on Birch's stress resistance gene expression and growth [97]. Carbon nanostructures in chloroplast [98]. Through electrostatic contact, functionalized carbon dots (CDs) are complicated with the screened dsRNAs (dsRNA-CDs) against *Phytophthora infestans* and *Phytophthora sojae* [99]. To the Enhancement of increased photosynthetic efficiency in plants through plastoquinone-mediated electron transfer using nitrogen-doped carbon dots [100]. In this study, successfully produced single-walled carbon nanotubes complexed with chitosan by the lipid exchange envelope penetration technique. In many plant species,

carbon nanotubes with a single wall effectively transfer the DNA plasmid to the chloroplasts with no additional chemical or biological support. *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 [101]. Mitochondria offers agronomic traits, but delivery in to mitochondrial genome less to low efficiencies, restricting in genetic engineering [102]. But carbon nanotube-polymer hybrid modified deliver DNA in to mitochondrial genome over thirty times more efficient than current techniques [103].



**Figure 6.** NM-mediated transport of chloroplasts by including a peptide specific to chloroplasts. 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 seven days of exposure, the reporter GFP construct was seen to be expressed. There is a 50  $\mu\text{m}$  scale bar. The American Chemical Society provides permission to use this adaptation. [98].

### 6.3. CNT Approaches for the Advancement of Organelle Biotechnology-Metallic Nanoparticles

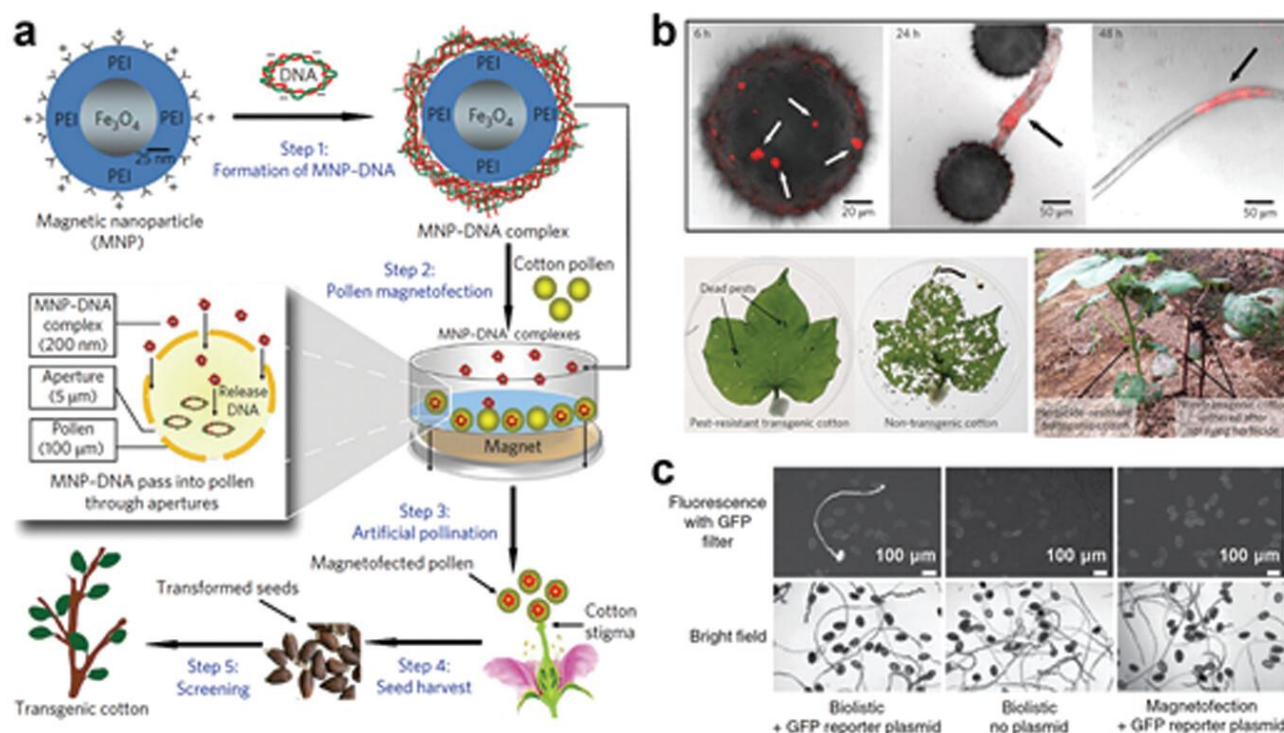
Large-scale and small-scale metallic delivery methods have been extensively used for transporting genetic material in animal systems, with gold nanoparticles being the most ex-

tensively studied for delivering biomolecules. For many years, tiny gold particles have been used in plants to carry molecules via a process called biolistic delivery [104]. Various types of nano particles been used in combination with nucleic acids. Nanoparticles of noble metals provide an alternate option, with gold nanoparticles (AuNP) being the most popular [105]. In this study, we create polyethylenimine-functionalized gold nanoclusters (PEI-AuNCs) for the purpose of delivering

siRNA into intact plants. Our results demonstrate that gene knockdown is achieved by these nanoclusters. Additionally, we show that the PEI-AuNC combination is tiny enough to evade the plant cell and protects siRNA from RNase breakdown. Consequently, AuNCs allow for gene knockdown with no discernible toxicity, achieving efficiencies of up to GFP and ROQ1 showed  $76.5 \pm 5.9\%$  and  $76.1 \pm 9.5\%$ , accordingly [106]. In this study, we used AuNPs in conjunction with AmiRNA technology to target specific genes in plants. Our findings demonstrate that AuNPs to achieve the effect of ATG6 silencing in *Arabidopsis* [107]. Fluorescent gold nanoparticles to deliver siRNA against *Pseudomonas syringae* [108, 109], also role as a nanophytovirology to detect the plant viruses [110]. AuNPs-siRNA<sub>NPRI</sub> silenced 80% of the *NPRI* gene in *Arabidopsis* [111]. The highest efficacy of transformation was documented in *Lilium regale* pollen by using

nanomagnetic beads to DNA plasmid [112]. By Pollen magnetofection, transgenic seed production without regeneration. Exogenous DNA that was combined with magnetic nanoparticles into pollen, in the presence of magnetic field [53]. GUS and GFP delivery by nanoparticles also carried out [113]. Existing maize gene delivery strategies rely on labor- and time-intensive tissue culture approaches and are restricted to particular genotypes. Thus, we present an updated by using nanoparticles with magnetic properties (MNPs) coated with DNA expressing bialaphos resistance (*bar*), improved green fluorescent protein (EGFP),  $\beta$ -glucuronidase gene, or red fluorescent protein (RFP) [55]. Systematic research of NP-pollen interactions across species will enhance understanding of factors affecting pollen transformation. See the figure 6 how pollen magnetic nanoparticles is carried out.

Five steps include in Pollen magnetofection:



**Figure 7.** 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. Ab) Reproduced with permission from Zhao et al. [53]. B) Temporal monitoring of fluorescent MNPs labeled with Lumogen F Red 305 in the pollen grains and tubes within 48 hours. Used by permission [113].

**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 [114]. The MPI promoter-controlled functionalized MSNs with the appropriate particle size and cryIAb gene delivery in to the tomato plants and the putative transgenic seeds were collected. Due to its biodegradability, biocompatibility, prefer over conventional methods [115, 116]. Also act as against

*Fusarium graminearum* [117].

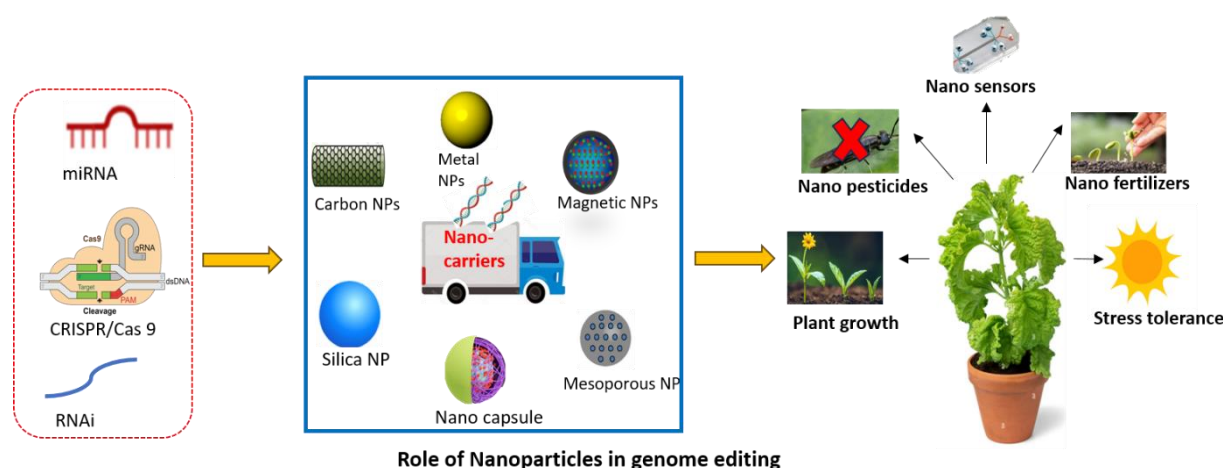
## 6.4. Applications of Nanoparticles in Genome Editing

Genome editing is an emerging genetic engineering technique that enables the precise modification of certain target genes inside an organism's genome [118-120]. Genetic engineer is getting benefits to implement nanomaterials for gene editing purpose to get around the drawbacks of traditional

ways of transformation like less gene stability and low efficiency. Currently, there are four categories of designed nucleases that are used to generate targeted double-strand breaks (DSBs). Like Meganucleases found in yeast in 1985, and recognition sequence of about 20–30 kb on average [121, 122]. Since zinc-finger nucleases (ZFNs) was the first protein reagents to target specific locations, their introduction resulted in a substantial change in the area of genetic modification [123-126], also the new genome editing technique Fanzor [127].

In order to effectively employ nanoparticles in plant bio-engineering, steady change in genes and expression to allow producing productive transgenic plants. When CNTs are used for delivery, CRISPR plasmids will express themselves momentarily help prevent the negative effects of repeated copy insertions. BsTargeted tissue genome editing using nanoparticles random integration, allow for the transgene-free engineering of crops grown vegetatively, can create permanent edits in plant genome [128]. Rice seed and embryo using

CNT-delivered CRISPR-Cas for gene editing [85]. SWCNTs are thought to be promising delivery systems for the CRISPR-Cas9 genome editing tool into plant cells [129]. Mesoporous silica nanoparticles (MSNs), investigated as carriers for CRISPR-Cas9 components [130]. CRISPR-Cas9 delivery nanoparticles Gold nanoparticles, DNA nanostructures, polymer-based nanoparticles, lipid-based nanoparticles, and so forth [104]. The production of aromatic rice for specific Rice Gene Editing Through Pollen Magnetofection Assisted by Magnetic Nanoparticles [131]. Exosome/Liposome, A DNA “nanoclew” Cationic Lipid Nanoparticles Hybrid Delivery of CRISPR/Cas Reagents [132]. Chitosan for Cas9 RNPs with 12.50% Carboxymethyl chitosan has a high effectiveness and is not cytotoxic for Cas9 mRNA and sgRNA and Lipid nanoparticles like Amino-ester-derived lipid, for Cas9 mRNA and sgRNA, FTT lipids, DOPE, cholesterol, and 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 [133-136].



**Figure 8.** Role of Nanoparticles in genome editing.

**Table 3.** Successful examples of NANOBIO TECHNOLOGY based delivery of CRISPR/Cas.

Editing	Target genes	Nanoparticles	References
Knockout	BAFFR	Polyethyleneimine–cyclodextrin	[137]
Knockout	Polo-like kinase 1 (PLK-1)	Catalic lipid nanoparticle modified with phospholipid and polyethylene glycol (PLNP)-based delivery systems	[138]
Knockout	To knock out PD-L1	Stearyl polyethylenimine complexed with plasmids as the core of human serum albumin nanoparticles	[139]
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.	[140]
Knockout	GFP	DNA nanoclew	[141]
Knockout	CD38	Nanoscale zeolitic imidazole frameworks (ZIFs)	[142]
Knockout	GFP	Gold - Nanoparticle - Mediated Laserporation	[143]



Editing	Target genes	Nanoparticles	References
Knockout	H11	Self-Assembled DNA Nanoclews	[144]

### 6.5. Challenges for Nanoparticles Application for Genome Editing in Plant Species

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

The optimisation of the appropriate dosage or concentration of nanoparticles or functionalized materials for coating is crucial due to their strong reactivity and limited stability. In cases of low concentration, there may be insufficient func-

tionalization or inadequate contact between the nanoparticles and the cargo, resulting in limited delivery. Moreover, an increase level of NP concentration might lead to the deposition of these nanomaterials, resulting in cellular oxidative stress. Hence, the choice of appropriate dosage is really vital.

Necessary to have a complete knowledge of the biosafety aspects of the nanoparticles used for transfer of gene in a specific tissue. Research should be conducted on the potential side effects of nano-sized materials.

**Table 4.** Other Applications of Nanobiotechnology for crop improvement.

Nanoparticles	Delivery method	Crop and dose	Role	References
ZnO-NPs	foliar spray	0.5, 1 and 5 g L <sup>-1</sup> fortnight gap on rice crop	ZnONPs were present on the leaf lamina close to stomatal openings, as seen by the leaf surface following foliar spray. The growth and yield characteristics were dramatically increased by applying ZnONPs (@ 5 g L <sup>-1</sup> ) topically. At 5.0 g L <sup>-1</sup> The highest levels of soil microbial populations and enzyme activities, such as dehydrogenase activity and viable cell counts, were determined after ZnONPs treatment. Treatments with ZnONPs boosted plant Zn content and reversed symptoms of Zn insufficiency ZnO NPs treatments at two-fold inhibitory concentration (IC500) values substantially increased rice plant root length, dry biomass, and chlorophyll a levels and also blight pathogen ( <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> ) through antibacterial evaluations.	[145, 146]
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 nCeO <sub>2</sub> with varying dimensions. In the early phases of plant development, CeO <sub>2</sub> treatments promote root elongation and raise the percentage of germination.	[147]
silver nanoparticles (AgNPs)	By foliar applications	(20, 40, 80 and 100 ppm)	The effectiveness for controlling chocolate spot disease silver nanoparticles used. Silver nanoparticles (AgNPs), at a concentration of (ppm), demonstrated a 75.93% inhibition of the pathogenic fungus <i>B. fabae</i> . The AgNP were effectively increasing growth and yield while shielding faba bean plants from the chocolate spot disease.	[148]
chitosan-based nanoparticles	By spray mediated	1, 10, and 20 ppm concentrations of nanoparticles (CS, CSAg, and CSCu)	Plants of the <i>Capsicum</i> spp. genus produced leaves that enhanced physiological characteristics and provided 70–85% protection against thrips. Additionally, it increased the amount of carotenoids, chlorophyll (20–75%), enhance secondary metabolite production and disease resistance.	[149]
Iron nanoparticles	Foliar applications	1 and 2 mM Fe nanoparticles/HA.	Improved the photosynthetic capacity and essential oil yield of pot marigold, increased pigments content and photosystem II efficiency, phenols and flavonoids content and antioxidant ac-	[150]



Nanoparticles	Delivery method	Crop and dose	Role	References
TiO <sub>2</sub> NPs	Treatment in lab	(15 mg L <sup>-1</sup> ).	tivity in the leaves. TiO <sub>2</sub> nanoparticle on cytological, physiological, and expression of genes alterations.	[151]
Carbon nano-materials	By soil	1000 mg/kg and limiting exposure time to 50–100 days	Increasing the soil microbial diversity. Compared to other CNMs, MWCNTs are less harmful to microbial populations, making them a better choice for promoting crop growth. To sum up, CNMs have a lot of promise to increase agricultural output.	[152]
Calcium silicate nanoparticles	Soil	20 ppm, a pH of 8.1, and a temperature of 298 K. 54.54 ppm, a pH of 6.08, and a temperature of 308.5 K.	In the present study, various calcium silicate nanoparticles (NPs) were examined to decrease the impact of salinity on the grown plants by adsorbing Na <sup>+</sup> and borate (BO <sub>3</sub> <sup>-3</sup> ) from water and soil.	[153]
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.	Increased soil and mung beans fruit zinc concentrations, as well as increased mung bean production and plant nitrogen-fixing capacity, did not significantly harm plants through oxidative stress. (Guo	[34]
Carbon-based NMs	Spray	200 mg L <sup>-1</sup>	200 mg L <sup>-1</sup> carbon-based NMs Spray play a protective function against single-stranded RNA TMV, preventing systemic infection of apical tissues and TMV replication by enhancing photosynthetic efficiency and inducing TMV defense responses.	[154]
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.	[155]
chitosan–gum acacia (CSGA) polymers to form nanocomposite (NC) CSGA-M		Nano CSGA-M-1.0 at 1.5 ppm (which includes 1.0 mg/mL mancozeb)	Control of Solanum tuberosum L. Early Blight and Stem Rot by Mancozeb-Loaded Chitosan-Gum Acacia Nanocomposites	[156]

Table 4. Continued.

Nanoparticles	Delivery method/Role	References
Mesoporous silica NPs	By Foliar spray and genome editing	[157, 158]
Silicon nanoparticles	Against pest and pathogens, is option, Detoxification of heavy metals, antifungal activity	[159-163]
Nanoselenium and nanosilicon	Nutrition and disease protection of crop species	[164]
Nanoparticles of Fe <sub>3</sub> O <sub>4</sub> Loaded with Azoxystrobin and Pectin	Enhance Resistance of Rice to Sheath Blight, In order to look into how Fe <sub>3</sub> O <sub>4</sub> nanoparticles (Fe-NPs) affect sunflower seed germination	[165, 166]
Multi-walled carbon nanotube	Alleviating the adverse effects of environmental stresses on plants, To better understand the possible roles of phytohormones in the production of phenolic acids in <i>S. verticillata</i> , Increased the amount of volatile compound in growing basil in green house, improved callogenesis performance and callus biomass, Shows positive progression in the bio-fabrication of L-Dopa in <i>Hybanthus enneaspermus</i> suspension cells	[167-171]
Metal oxide- nanoparticles	To determine the soil properties	[172, 173]
Chitosan nanoparticle	Applying chitosan nanoparticles foliarly accelerated finger millet development and acti-	[174-176]

Nanoparticles	Delivery method/Role	References
	vated the plant’s defense enzymes. Promote yield increment during drought stress in wheat, improve the grape plant yield under salinity stress.	
Liposome NPs	Assist in improving the uptake and distribution of active substances to boost autumn barley’s resilience, vitality, and yield (Hordeum vulgare), Stimulates the ABA Pathway and PR Gene in Wheat.	[177, 178]

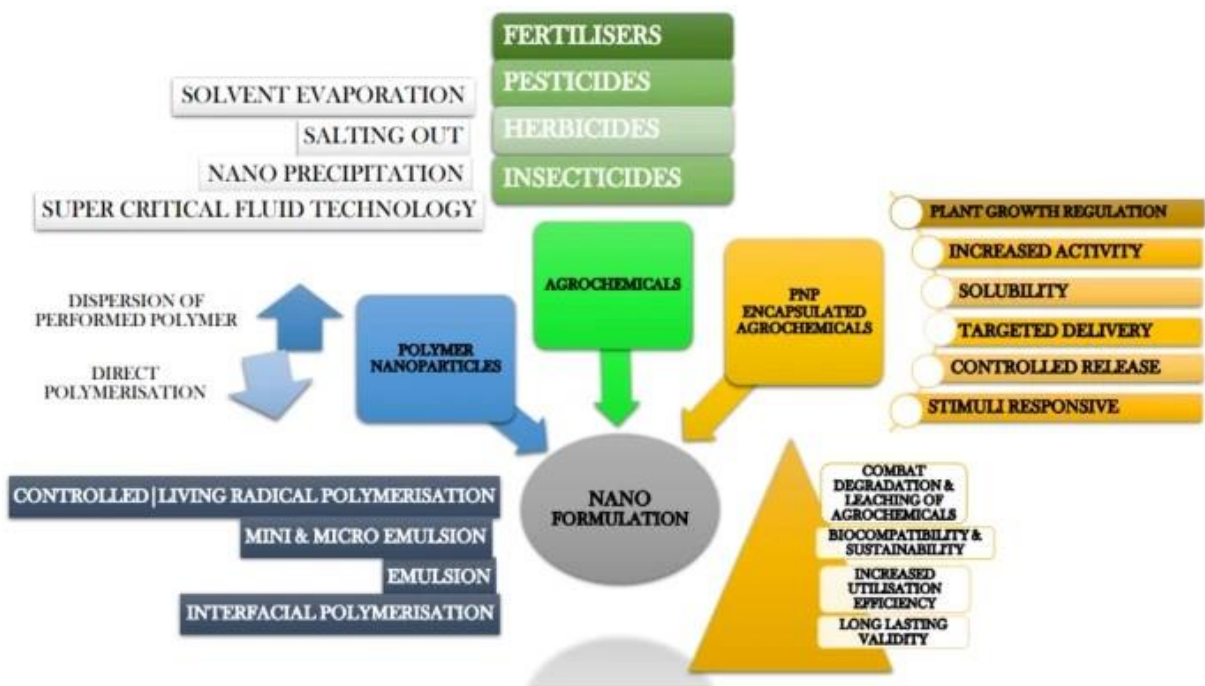


Figure 9. Recent examples of Nanoparticles Application in plant species.

7. Conclusion

Nanobiotechnology has emerged as a transformative field in plant genetic engineering, offering innovative solutions to address the global challenges of agricultural sustainability, food security, and climate change mitigation. The unique properties of nanomaterials, such as their size, stability, and precise functionality, provide unparalleled advantages over traditional methods in genetic transformation, gene editing, and real-time cellular monitoring. Techniques such as nanoparticle-mediated delivery of nucleic acids and CRISPR/Cas systems demonstrate significant potential to enhance crop productivity, stress resilience, and disease resistance. Despite its immense promise, the widespread adoption of nanobiotechnology in agriculture is hindered by challenges such as safety concerns, nanoparticle toxicity, and scalability issues. Furthermore, the development of robust regulatory frameworks and ethical guidelines is essential to ensure the safe and sustainable integration of nanotechnologies in agricultural systems. Collaborative efforts between scientists, policy-makers, and industry stakeholders are vital to address these

challenges and promote global acceptance of these technologies. Future research should focus on developing eco-friendly and biodegradable nanoparticles to mitigate potential environmental risks, optimizing large-scale production methods for agricultural applications, and refining nanoparticle-based delivery systems for improved gene-editing efficiency. Additionally, further exploration of nanobiotechnology’s role in precision agriculture, including nanosensors for real-time crop health monitoring and stress detection, can significantly enhance agricultural sustainability. By addressing these critical research gaps, nanobiotechnology can revolutionize plant genetic engineering, equipping researchers with advanced tools to enhance crop performance, overcome environmental constraints, and meet future food demands in an era of rapidly changing climate conditions.

Abbreviations

NPs	Nanoparticles
Ti	Tumor Inducing
PEG	Polyethylene Glycol
MNPs	Magnetic Nanoparticles

GMOs	Genetically Modified Organism
CNTs	Carbon Nanotubes
CDs	Carbon Dots
RFP	Red Fluorescent Protein
DSBs	Double-strand Breaks
ZFNs	Zinc-finger Nucleases
MSNs	Mesoporous Silica Nanoparticles
ZIFs	Zeolitic Imidazole Frameworks

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## Conflicts of Interest

The authors declare no conflicts of interest.

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