Multifunctional Hybrid Nanomaterials for Sustainable Agri-Food and Ecosystems

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CHAPTER

6 Nanomaterials for gene delivery and editing in plants: Challenges and future perspective

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6.1 Introduction

Nanotechnology plays an important role in agriculture, including pesticide delivery such as avermectin using porous hollow silica (15 nm) (Li et al., 2007), fertilizer delivery such as NPK controlled delivery using chitosan (78 nm) (Corradini et al., 2010), genetic material delivery such as DNA using gold (10–15 nm) (Torney et al., 2007a,b), pesticide sensors such as paraoxon using silica (100–500 nm) (Ramanathan et al., 2009), and pesticide degradation such as imidacloprid using titanium oxide (30 nm) (Guan et al., 2008). The field of nanotechnology has witnessed impressive advances in many aspects such as the synthesis of nano-scale matter and the use of its exotic physicochemical and optoelectronic properties. Nanogenomics-based strategies has enabled plant breeders better accuracy breeding has opened up interesting new opportunities for selecting and transferring genes, which has not only minimized the time required to eradicate unneeded genes but has also allowed the breeder to access important genes from distant plants. Singh et al. (2011) found that nanotechnology provides a good model to explore important properties of metal in the shape of nanoparticles that have future applications in diagnostics, cell labeling, biomarker contrast agents, antimicrobial agents, and nanodrug systems. Some metallic nanomaterials such as silver and gold are recognized as plasmonic particles have a remarkable ability to absorb and scatter light outside of their physical cross-sections. This ringing frequency depends on the particle shape, size,

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electron distribution, particle consistency, and the surrounding dielectric conditions. It supplies the beneficial information for the properties of the particles. Nanomaterials exhibit transitional dimensions between bulk materials, molecules, and atoms. Nanoparticles can be engineered and they are called intentional and unintentional. They can be referred to as nanocrystals when they have a crystal structure and as quantum dots (QDs) if they are semiconducting (Masciangioli and Zhang, 2003). Engineered nanoparticles cover a wide range of substances that include elemental particles and inorganic compounds such as zinc sulfide (ZnS). Metallic nanoparticles are formed from elements such as nickel, iron, zinc, gold, titanium, silver, palladium, platinum, and iridium (Connor et al., 2005). Materials used in QDs include inorganic cadmium selenide (Green and Howman, 2005). Inorganic nanomaterials are considered less toxic and possess versatile properties such as more availability, excellent biocompatibility, functionally rich, higher potential in target delivery, controlled release of target drugs, and driving forces for delivery (Kim et al., 2006). Nowadays, there are numerous nanomaterials including gold nanoparticles, starch nanoparticles, single-walled carbon nanotubes (SWCNTs), silica nanoparticles, QDs, and magnetic nanoparticles that have been extensively applied in the interdisciplinary fields of molecular genetics and genomics. A complete evaluation of the trendy research on specific nonviral transport materials for the shipping of clustered regularly interspaced short palindromic repeat (CRISPR-Cas) devices is offered, which include lipid nanoparticles, polymeric materials, hydrogels, gold nanoparticles, graphene oxide (GO), metal-organic frameworks, cell-penetrating peptides (CPP), black phosphorus DNA, and DNA. A change in physicochemical characters of nanocarriers, protoplasts, and plasma membranes might also extensively enhance the liposomal- and nanoparticle-encapsulated gene into plant cell (Rai et al., 2015). In this chapter, in addition to the status of gene delivery utilizing various nanomaterials for gene transfer in plants, we will also highlight the challenges and prospects of the CRISPR/Cas9 system combined with nanotechnology for futuristic plant biotechnology (Fig. 6.1).

6.2 Nanoparticle synthesis

Three methods are used to synthesize nanomaterials:

Chemical depreciation is the dominant method for nanoparticle synthesis using inorganic and organic reduction factors. The operation requires three main constituents: a reducing agent, a metal origin, and a covering/settling factor. Stubbs et al. (2016) reported that nanoparticle synthesis involves the microemulsion process, UV origin photo lowering irradiation procedures, and electrochemical artificial procedures. Chemical procedures have the merit of a big yield in contrast to physical procedures.

The advantages of physical synthetic procedures in contrast to chemical procedures include physical processes, the symmetry of nanoparticle delivery, and the absence of solvent contamination. NPs in emulsion has generated the

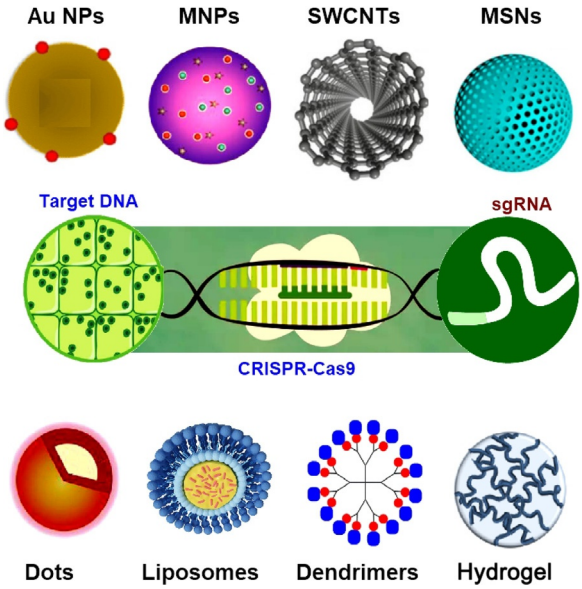

FIG. 6.1

Nanomaterials used for CRISPR-Cas plant genome editing and gene delivery.

use of laser ablation of mineral bulk particles (Eftaihab et al., 2010). However, the defects of the physical process involve time, harmful environmental effects, and required space.

Biological processes of nanoparticle synthesis control the biological molecules and enzymes. Jayaweera et al. (2017) found that microorganisms such as fungi have the ability to produce nanomaterials through a biological process. For example, silver NPs have been produced from *Pseudomonas* sp. Eftaihab et al. (2010) reported that the merits of these processes are the strong constancy of the Ag nanoparticles, which is different from that produced by the chemical process. The advantages of biological processes include less cost and a safer process compared to other processes for nanoparticle synthesis.

6.3 Phytonanotechnology and engineered nanoparticles

The implementation of engineered nanoparticles in agriculture has the potency to change normal plant production techniques, enhance the target-specific delivery of biomolecules (nucleic acids, activators, and proteins), and organize the usage of agrochemicals (pesticides and nitrogenous fertilizers). Nel et al. (2006) found that engineered NPs have specific physicochemical characteristics (less surface area, enhanced reactivity, irregular surface structure). Nair et al. (2010) determined that nanoparticles can have time-controlled, programmed, target-specific,

multifunctional capabilities while also being self-organized. ENPs can deliver agrochemicals in an "on-demand" way, either for defense against pathogenic microorganisms or for nutritional need. In addition, nanoparticles play an important role in genetic material and protein transmission.

6.4 Bioconjugation

Bioconjugation means that the structure is formed of a metal substance and adsorbed or chemically adhered biomacromolecules. In view of the many-sided physicochemical features of inorganic nanomaterials involving broad availability and abundant functionality, in combination with their distinct optical, catalytic, and electronic features, the conjugation of biological molecules and inorganic nanomaterials has created a new way in the expansion of advanced functional materials with obviously enhanced properties and broad applications. Several inorganic nanomaterials containing metals, metal oxides, and Quantum Dots (QDs) have been produced by different synthetic methods and immobilized or hybridized with biomolecules such as DNA, antibodies, and enzymes, each through noncovalent or covalent interactions. The mechanism in the formation of conjugates, biological molecules, and inorganic nanoparticles remains incompletely unclear. It can include electrostatic interactions among positively charged regions of biomolecules and a negatively charged particle, hydrophobic interactions, and covalent binding of the nanomaterial to the sulfhydryl groups (—SH) of the biomolecule. Bioconjugated compounds can open a new route as an active antimicrobial factor by their mode of action. If a pesticide-resistant microorganism is immune to a particular pesticide molecule, then the nanomaterial can show its impact against the microorganism by attacking the site and mode of action.

6.5 Gene delivery

The main and inherent challenge confronting plant gene delivery is biomolecule transfer into plant cells through the inflexible and multilayered cellular wall. Contemporary techniques suffer from a host of various boundaries, low transformation efficiencies, toxicity, and inevitable DNA integration into the plant host genome. Nanomaterials are the most appropriate candidates to eradicate the current limitations of delivering biomolecules into plants. Nanomaterials have been investigated for gene transport into plant cells. Gene delivery plays an important role in the evolution of pathogens and pests in crops through modification of gene behavior (Gelvin, 2003). Gene vectors are mostly divided into two divisions: biological and synthetic vehicles. In the biological part, viral vectors give active delivery (Takahashi et al., 2009; Walther and Stein, 2000). Conventional synthetic vehicles involving cationic lipids (Cho et al., 2010), dendrimers (Dufes et al., 2005), and polymers (Wu et al., 2012) have been greatly used for intracellular gene delivery. An

efficient nanocarrier is required to supply strong protection for DNA to avoid lysis with nuclease enzymes, effective penetration inside cell tissues, and deliverance of the genetic material in its active form inside the cell nucleus (Thomas and Klibanov, 2003). Mesoporous silica nanoparticles are able to deliver genetic material inside plant cells (Torney et al., 2007a,b). Conventional gene delivery methods have some disadvantages such as less integrity of the transferred DNA, cell destruction, restricted range of the plant species, and toxicity, so engineered nanoparticles provide a new vehicle for the transfer of nucleic acid, activators, and proteins inside plant cells as well as additional safety.

Torney et al. (2007a,b) reported that the use of silica nanoparticles to transfer genetic materials inside tobacco cells offers a distinct advantage. Martin-Ortigosa et al. (2014) found that enzymes or proteins can be transferred by mesoporous silica nanoparticles (MSNs) in plants, and they can also be beneficial for phytochemical analyses and gene alterations. In addition to MSNs (Hussain et al., 2013; Martin-Ortigosa et al., 2014), Au nanoparticles (Martin-Ortigosa et al., 2012a; Wu et al., 2011), QDs (Etxeberria et al., 2006), carbon-coated magnetic nanoparticles (Corredor et al., 2009; González-Melendi et al., 2008), and starch nanoparticles (Liu et al., 2008a,b) can be used for gene delivery in plants. The engineered nanoparticles have some advantages such as simple in use, efficacious, capable of co-delivering several biomolecules to the specific site such as genes, nucleic acid (Torney et al., 2007a,b), and proteins (Martin-Ortigosa et al., 2012b). Nanoparticles can be taken up by plants and transferred to other plant cells by utilizing different insoluble engineered nanoparticles, such as silica NPs, QDs, and titanium dioxide NPs. Engineered nanoparticles offer modern methods for transferring bioactive materials such as gene delivery, cellular differentiation, and visualizing (Fig. 6.2). Using nanoparticle-bear visualizing factors such as fluorescence (Liu et al., 2009a,b; Serag et al., 2011a,b), researchers may notice the motion of the delivered gene. Inorganic nanoparticles are produced as synthetic vehicles that offer various advantages relative to conventional lipid-based vehicles, involving tunable size and surface characteristics, multifunctional abilities, and the ability to translate the physical characteristics of the metal core to the delivery vector (Arsianti et al., 2010; Chen et al., 2005; Harashima et al., 2012; Kam et al., 2005; Lee et al., 2009; Prato et al., 2004). Inorganic nanoparticles are described as gene delivery vectors such as:

6.5.1 Quantum dots

QDs of Cd, Se, or ZnS nanoparticles with conjugated amino acids are recognized as traveling through the vascular system of plants (Schwab et al., 2016). Quantum dots (QDs) are brilliant fluorescent materials for bioimaging, tracking, gene insertion and drug delivery applications (Jamieson et al. 2007). The issue of using these particles is their toxicity, which is restrained by their implementations in agriculture systems. These substances displayed extremely effective QD cellular labeling as compared

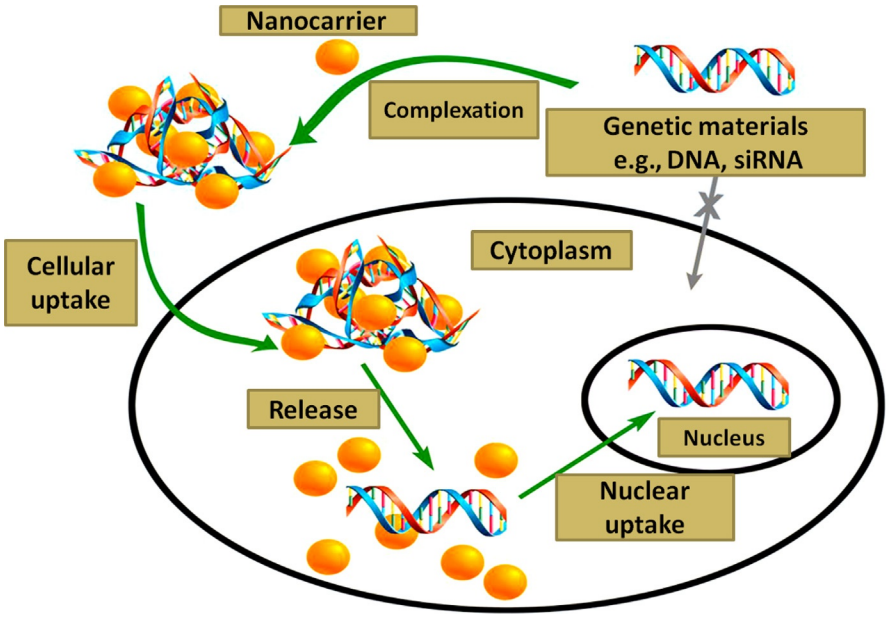

FIG. 6.2

Nanocarrier-mediated delivery of bioactive molecules into plant cells.

Modified from Loh, X.J., Lee, T.C., Dou, Q., Deen, G.R., 2016. Utilizing inorganic nanocarriers for gene delivery. Biomaterials Sci. 4 (1), 70–86 with permission from Elsevier.

to ordinary techniques. QDs have been applied to the integration of genetic cloth transduction, ratiometric oxygen sensing, and cell-precise labeling.

6.5.2 Silica nanoparticles

MSNs have without difficulty functionalizable surfaces and rich textural properties, inclusive of tunable pore size (2–20 nm), huge surface area ($>1000\,m^2\,g^{-1}$), and pore volume. MSN applications have been established in many genetic engineering fields. The pioneering work of using MSNs to transfer some active biomaterials into plant cells was achieved in vivo by Dr. Kan Wang (Martin-Ortigosa et al., 2012b). MSNs were used to codeliver DNA and chemical substances (Torney et al., 2007a,b) further to DNA and proteins (Martin-Ortigosa et al., 2012b) to plant cells by biolistics. Martin-Ortigosa et al. used MSNs as carriers to deliver Cre recombinase into maize (*Zea mays*) cells (Martin-Ortigosa et al., 2014). For example, the MSN study from The novel structures of organically functionalized MSNs with 3-nm can offer innovative possibilities in target-specific delivery of proteins, nucleotides and chemicals in plant biotechnology (Torney et al., 2007a,b). Silica in its nanoforms, such as nanosilica and mesoporous silica nanoparticles, is particularly crucial in the delivery genes and plasmid cells. Magnetic nanoparticles particularly release their load in

the plant cells (Zhu et al., 2008). Functionalized MSNs were employed to expand an MSN-mediated plant transient gene expression system. Within the MSN device, nanoparticles served as transgenic carriers to deliver foreign parts from DNA into intact *Arabidopsis thaliana* roots without the aid of mechanical force. Gene expression is detected inside the epidermal layer and within more internal cortical and endodermal root tissues via each fluorescence and antibody labeling (Chang et al., 2013). These particles are effective materials in agricultural implementations, such as transferring genetic materials and drugs to the proposed site in the plant. This is due to their potential to penetrate plant cells, synthesis at cost-effective rates, and material consistency. Mesoporous silica nanoparticles have low toxicity and additionally are more efficient in the protection of the genetic material from lysis inside plant tissues (Li et al., 2018). The main and promising technique of genome modifying mediated thru MSNs has been recently proposed. MSNs was applied as carriers to transfer Cre recombinase in maize embryos, carrying loxP sites combined into chromosomal DNA. After the biolistic introduction of engineered MSNs in plant tissues, the loxP was properly recombined into maize cells (Valenstein et al., 2013).

6.5.3 CNMs for gene delivery and genome editing

Unlike mammalian cells, genetic transformation in a plant cell is complex; plant cell walls act as a sturdy barrier that inhibits the entry of every other external agent. The cellular wall is made from pass-connected polysaccharides, which are different among plant and animal cells. The plant cellular wall structure limits the use of any sort of nanomaterial for genetic cargo delivery. In an investigation, the uptake of fluorescent QDs directly into the plant cells became completed after 24 h of starving; however, unfortunately, the gene was no longer delivered (Etxeberria et al., 2006). Liu et al. (2009a,b) mentioned the SWNT penetration into walled plant cells, *Nicotiana tabacum* bright yellow (by way of −2) cells, a popular plant cell version that validated efficaciously delivered DNA and small dye molecules into intact plant cells. Serag et al., 2011a carried out the capability of multiwalled CNTs (MWCNTs) to traverse through a plant cell membrane via escape endocytosis. Further, MWCNTs are capable of localizing within the area of the nucleus, plastids, and vacuoles.

With the use of two types of carbon nanotubes, SWCNTs at a concentration of 20 μg/mL and MWCNTs at a concentration of 15 μg/mL, the *N. tabacum* L. protoplasts were genetically transferred with the plasmid construct pGreen 0029. The SWCNT-based nanocarriers verified their applicability for the transformation of protoplasts and walled plant cells (Burlaka et al., 2015). The engineered peptide with its unique cationic and hydrophobic domains and the arginine functionalized with SWCNTs (Arg-SWCNTs) because of its nanocylindrical form can pass through plant cell barriers. Engineered chimeric peptides and Arg-SWCNTs were examined as easy, rapid, and secure gene providers for tobacco intact root cell transfection. Arg-SWCNTs could effectively transfer GFP-expressing plasmids to root cells (Golestanipour et al., 2018). Efficacious diffusion-based plasmid DNA and small interfering RNA (siRNA) are delivered into various mature species of plants with

a set of pristine and chemically functionalized high aspect ratio nanomaterials (Demirer et al., 2018a,b). Demirer et al. (2018a,b) evolved and optimized a nanomaterial-based delivery tool that could transfer functional biomolecules into each model and crop plant species with high efficiency. For the first time, we demonstrated effective transient gene expression and silencing in mature plants thru CNT-based delivery of purposeful biomolecules. Demirer et al. (2018a,b) proved that pristine and chemically functional nanotubes efficaciously deliver DNA and protein expression without transgene integration in mature plants of *Nicotiana benthamiana*, *Eruca sativa*, *Triticum aestivum*, and *Gossypium hirsutum* leaves and arugula protoplasts. CNTs not only facilitated the delivery of biomolecules into intact plant cells, but they additionally protected the polynucleotides from nuclease degradation. Kwak et al. (2019) tried a novel method to deliver transgenes into the chloroplast, particularly via nanotubes. In his study, he designed chitosan-complexed SWCNTs that selectively delivered plasmid DNA to the chloroplasts of mature *E. sativa*, *Nasturtiu mofficinale*, *N. tabacum*, and *Spinacia oleracea* plants and in isolated *A. thaliana* mesophyll protoplasts. The delivery was a success and exhibited transient expression. Kwak and coworkers indicated gene delivery in plants via visualization of the transitive expression of a marker gene, following infusion of the nanoparticle carriers to the leaf lamina. Kwak produced four groups of chitosan-complexed SWCNTs by using noncovalent coating and covalent alteration of the nanotube sidewall to reinforce plasmid DNA (pDNA) loading and deliver overall performance to the chloroplasts. Nontoxic chitosan-wrapped SWCNTs may navigate plant cell walls, the plasma membrane, and double lipid bilayers of chloroplasts, thanks to their excessive surface charge. Chitosan can be complicated by negatively charged pDNA through electrostatic exchanges to preserve DNA from nuclease degradation. This nanoparticle-mediated chloroplast transgene delivery method may additionally have vast applications because it is simple, cheap, and applicable throughout different species (Lyu, 2019). CNT-mediated DNA and RNA delivery to plants is simpler and quicker than agrobacterium-mediated plant transformation, amenable to multiplexing, and can be applied on a large scale, allowing its broad-scale adoption (Demirer et al., 2018a,b).

Some inorganic nanoparticles are natural capability CRISPR component carriers due to the fact that they've already been used for similar purposes. Examples of these contain AuNPs, CNTs, bare mesoporous silica nanoparticles (MSNPs), and dense silica nanoparticles (SiNPs). The use of AuNPs for CRISPR/Cas9 delivery was described earlier while CNTs (Bates and Kostarelos, 2013), MSNPs (Luo et al., 2014), and SiNPs (Luo and Saltzman, 2000) have been used for many gene delivery applications; the use of these carriers for Cas9 delivery has yet to be mentioned. CRISPR-Cas systems are efficiently implemented throughout an extensive range of prokaryotic and eukaryotic species for effective genome editing. In the future, the Innovative Genomics Institute (IGI) research group needs to apply new techniques to deliver CRISPR-Cas9 genome editing methods. The carbon nanotube would be lined with DNA that codes for the Cas9 protein and the guide RNA, which work together to edit a particular gene (Demirer et al., 2018a,b). The genome editing

tools are presented to edit the plant's DNA, leaving no footprint. Which means that unlike with some current plant genome-editing strategies, no strange DNA would be inserted into the plant's genome. Nanomaterials consisting of carbon nanotubes show an awesome capacity to function as delivery tools of various genetic materials inside plant cells and plastids because of their potential to navigate the plant cell wall, cellular membrane, and organelle membranes. The enhancement and sizable adoption of CNTs in plant mobile biology call for further research into internalization mechanisms, the limits of what CNTs can convey and supply efficaciously, a detailed analysis of cytotoxicity, and the CNT destiny in cells after transporting their cargo (Demirer et al., 2018a,b). Our destination activities will explore these elements of CNT-mediated shipping to broaden an inexpensive, facile, and robust delivery method that could transfer genetic fabric into all phenotypes of any plant species with excessive efficiency. This, in sequence, will increase the proficiency of plant genome engineering systems with several applications within agriculture, therapeutics, the environment, and the pharmaceutical and power industries.

6.5.4 Gold nanoparticles

Gold nanoparticles (AuNPs) are utilized because of their ability to become carriers of multifunctional gene vendors as well as their ease of synthesis, biocompatibility, and well-described surface chemistry. The nano-sized composite carrier has numerous advantages over different techniques due to its small size as well as matrices embedded with gold NPs (AuNPs) and their own higher transformation performance. Some research groups have demonstrated the use of DNA-functionalized gold nanoparticles for both the delivery of nucleic acid therapeutics and the modulation of gene expression in plant cells. AuNPs can bind with DNA or peptides via electrostatic magnetism and deliver their inner plant cells (Cunningham et al., 2018; Hao et al., 2013; Mortazavi and Zohrabi, 2018; Rosi et al., 2006; Ye and Loh, 2013; Ye et al., 2015). Rosi et al. (2006) determined that the shipping of DNA by the use of AuNPs revealed splendid gene delivery efficiency. Those nanoparticles established a maximal knockdown of gene expression, more immunity to nuclease, a complex bonding affinity for target DNA, and irregular toxicity in comparison to different gene vehicles. There are few reviews on the applications of gold nanoparticles for gene transfer in rice cells (Rai et al., 2012). The mycogenic intracellular gold nanoparticles (5–25nm) synthesized by Aspergillus ochraceus combined with carbon nanoparticles have been used effectively to deliver plasmid DNA to Nicotina tabacum via a gene gun (Vijayakumar et al., 2010). The very same work also explained efficient DNA delivery into the monocot *Oryza sativa* and a hard dicotyledon tree species, *Leucaena leucocephala*, with minimal plant cell damage. The nano-sized composite carrier has several merits over commercial micrometer-sized gold particles used in gene gun delivery. First, due to their small size, matrices embedded with gold nanoparticles have higher transformation efficiency. Additionally, they need less gold and plasmid to obtain the same transformation level efficiency. Moreover, this approach has comparatively low toxicity to plant cells

(Vijayakumar et al., 2010). Nevertheless, gold-nanoparticle-embedded carbon matrices still need the support of a gene gun to successfully transfer DNA genetic material into plant cells. The biolistic cotransformation system using gold nanoparticles can be applied as a successful technique for gene delivery into rice varieties. Magnetic gold nanoparticles (mGNPs) with uniform length and morphology synthesized by way of our sonication treatment approach have been covalently with fluorescein isothiocyanate (FITC) molecules. Driven by an exterior magnetic field, FITC-labeled nanoparticles have been delivered into plant cells with and without cell wall partitions (Hao et al., 2013). Moreover, it requires less gold and plasmid to gain equal transformation performance. The technique notably lowers the toxicity of plant cells (Cunningham et al., 2018). Separate plasmids have been used within the transformation and have been correctly included inside the genome of an aromatic Iranian rice cultivar. The transgenic traces didn't show any atypical physiologic characteristics and confirmed a growth pattern much like that of the nontransgenic parental line (Mortazavi and Zohrabi, 2018). Gold nanoparticles with their high surface-to-volume ratio, easy DNA attainable geometry inside monolayers, and tunable hydrophilic characteristics offer a promising platform for gene transport. A gold-nanoparticle-based nonviral vector acts as the gene delivery device inclusive of double-stranded DNA covalently linked to NIR-absorbing, plasmon-resonant gold nanoshells.

6.5.5 Magnetic nanoparticles

Later, distinctive sorts of nanoparticles have been progressively utilized in plant transformation applications that can act as a transgenic vehicle for DNA, RNA, and oligonucleotides in various plant cells (Dyab et al., 2018; Rai et al., 2012). But the investigation of nanovendors for gene transport in plants remains a promising field, with an awful lot of potential for the future of plant biotechnology and genome editing. Magnetic nanoparticles right now are the nanomaterial sorts broadly used as special biomolecular providers for enzymes, proteins, and nucleic acids, assisted through an exterior magnetic field (Cordero et al., 2017). Employing these sorts of nanoparticles was examined in industrial biological implementations consisting of biosensors and diagnostics. These particles were suggested for different approaches, such as chemical and protein immobilization (Cao et al., 2003). Magnetic nanomaterials are applied as vectors for genetic fabric transduction; on this approach, the gene is mixed with nanoparticles and delivered to a targeted location in the interior of plant cells. Liu and his group moreover extensively utilized particular fluorescent-labeled enhanced nanoparticles as a gene shipping device to deliver specific genes in plant cells (Agotegaray et al., 2016; Liu et al., 2008a,b). Such gene-nanoparticle conjugation was designed in such a way that it binds and transports shuttle genes throughout the natural obstacles of plant cells similar to the cell wall, cell membrane, and nuclear membrane of the plant cells through inducing on-the-spot small pore channels with the assist of ultrasound waves. Via this method, researchers investigated how diverse specific genes can be effortlessly combined on the fluorescence-labeled nanoparticle surface at identical times and transported into

plant cells. Alternatively, different elements depending on the kind of plant organ and its anatomy may have an effect on nanoparticle dissemination. Part of this kind of research was targeted at designing precise magnetic providers for unique treatment needs, and several secure and effective magnetic nanocarriers were formed (Hola et al., 2015; Mishra et al., 2015). A successful strong genetic transformation in plants has been carried out in cotton flowers by magnetic nanoparticles (MNPs). A β-glucuronidase (GUS) reporter gene–MNP complex was penetrated into cotton pollen grains by way of magnetic force, without compromising pollen viability. Through pollination with magnetofected pollen, cotton transgenic plants were effectively produced and exogenous DNA was positively combined into the genome, successfully expressed, and stably inherited in the offspring acquired with the assistance of selfing cross (Zhao et al., 2017). On the grounds of in prior report reviews that indicated the use of magnetic nanoparticle-based total gene transfer, diverse mechanistic ideas may be suggested to make the efficiency of this unique nonviral gene-blanketed biotransformation method better. The magnetic nanoparticle offers a vast field for gene delivery in plant studies, in particular in plant disorder treatment (Mohamed and Abd-Elsalam, 2019.)

6.6 Challenges

The effective and extensive applications of novel hybrid nanomaterials in molecular genetic nanotechnology depend substantially on robust nanoparticle synthesis and engineering methodologies. The development steps of nanomaterials in agroecosystem applications include design, synthesis, surface variation, and bioconjugation. Each of these steps is essential in figuring out the general performance of nanoparticles. There are still many troubles and challenges that need to be addressed and conquered. As an example, no matter the current development at the bioconjugation of nanomaterials, researchers still want better techniques to attain reasonable reproducibility, robust surface coating, and bendy functionalization and bioconjugation techniques due to the complex surface chemistry of nanomaterials. Furthermore, the possible toxicity of some nanomaterials to the human body still needs clarification. To overcome those issues, surface modifications and progressed hybrid nanomaterials are taken into consideration. After immobilization of different functional and biocompatible compounds, nanomaterials would produce fewer agglomerations and less injury toward cells while displaying greater effectiveness for research in vivo. In the meantime, the regular manner for assembling nanomaterials and oligonucleotides should help to facilitate the recognition of target molecules. DNA-templated or as-prepared hybrid nanomaterials may additionally be a solution.

6.7 Future perspectives

Nanobiotechnology offers appealing tools for delivering and modifying some bioactive materials because nanoparticles can be accurately tailored to deliver a specific biomolecule to the plant cell, tissue, or organism of interest (Du et al.,

2012). Several recent reviews has proposed, however, that nanotechnologies may additionally conquer the barrier of the cell wall and decrease the drawbacks associated with present transgene delivery systems (Joldersma and Liu, 2018). Demirer et al. (2018a,b) and Mitter and coworkers has each produced proof that positively charged nanoparticles are able to be linked with the negatively charged backbones of nucleic acids and directed into the plant cell (Mitter et al., 2017). During the journey into and inside the cell, nanoparticles protect DNA from enzymatic assault; however, on delivery to the cellular nucleus, the nanoparticles liberate the external nucleic acids. The mechanisms explaining the release and delivery of the transgene into the normal genetic processing machinery of the plant aren't yet understood; however, the implications are thought-provoking. A successful stable genetic transformation has been accomplished in cotton vegetation by magnetic nanoparticles (MNPs). A β-glucuronidase (GUS) reporter gene–MNP complex has been infiltrated into cotton pollen grains with the aid of magnetic force, without compromising pollen viability (Fig. 6.3). Throughout pollination via magnetofected pollen, cotton

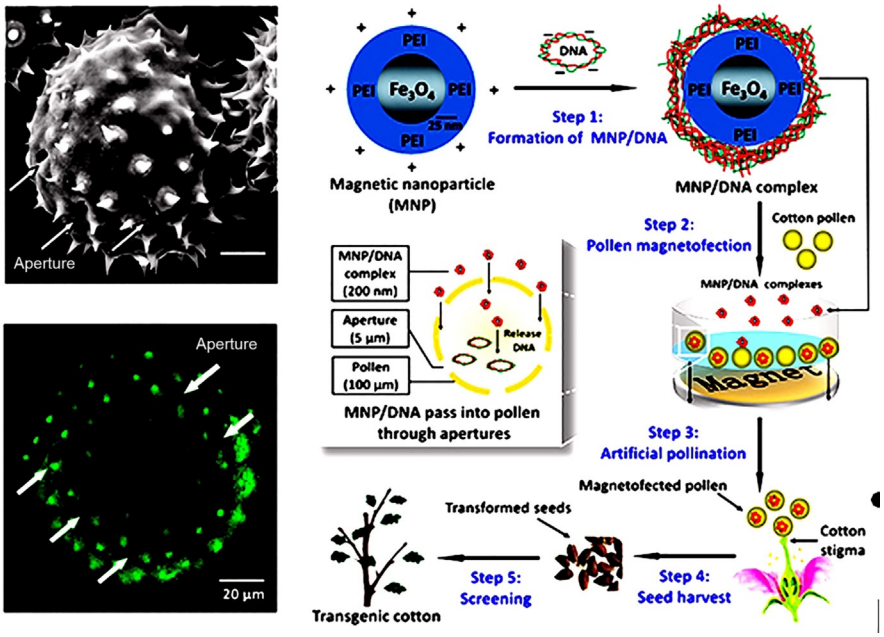

FIG. 6.3

A diagram illustrating the processes and principles of the pollen magnetofection protocol developed to insert a DNA nanoparticle complex into plants via pollen grains.

Reproduced from Zhao, X., Meng, Z., Wang, Y., Chen, W., Sun, C., Cui, B., et al., 2017. Pollen magnetofection for genetic modification with magnetic nanoparticles as gene carriers. Nat. Plants 3, 956–964 with permission from Springer.

transgenic plants were efficaciously generated and exogenous DNA became effectively integrated into the genome, efficiently expressed, and stably inherited within the offspring obtained with the aid of selfing cross (Zhao et al., 2017). The primarily pollen-based transformation technology in cotton is a "pollen magnetofection" to at once produce transgenic seeds devoid of tissue culture. In this system, magnetic nanoparticles loaded with pure plasmid DNA carrying purposeful genes have been added into pollen by pollen apertures in the occurrence of a magnetic discipline. Later, these magnetofected pollens have been used for pollination to provide modified seeds. Pollen magnetofection is a genotype-unbiased transformation system; moreover, exogenous DNA turns out to be well integrated into the genome and stably expressed within the successive generations (Zhang et al., 2019). Regardless of the significant growth in plant molecular genetics, the delivery of exogenous DNA and/or enzymes for genome modifying remains a huge challenge. New strategies based totally on nanoparticle-mediated clustered frequently interspersed palindromic repeats–CRISPR related protein (CRISPR-Cas9) technology, as those were tested in other organic systems (Glass et al., 2018; Lee et al., 2017; Sanzari et al., 2019; Wan et al., 2019). Past and recent research on nanoparticles primarily based on CRISPR/Cas9 gadget delivery for genome modifying was discussed in detail (Deng et al., 2019). Identify nanoparticle composites that are exceptionally efficient for plant cell internalization, and make use of those nanoparticles to deliver DNA, RNA, and Cas9-gRNA RNP to plants and calli in a species-independent manner (Glass et al., 2018; Mohamed and Abd-Elsalam, 2019). Recently, an innovative nanoplex-mediated plant transformation method may open up novel potentials in gene transfer in plants, which will enable developing a great number of transgenic plants. A new technique for gene transformation using amino acids improved AuNPs, where a plant cell-penetrating amino acid (CPA) nanoplex (AuNPs-CPAs-pDNA-CPAs) was employed as a gene vehicle for gene transfer. The nanoplex may be applied to effective gene and protein transporters. It is an appreciated method for the transfer of genes of interest or significant industry proteins into tobacco plants or other plant species for higher yield (Bansod et al., 2019). CRISPR systems, hybrid nanomaterials, and pollen magnetofection are rapidly becoming more effective, flexible, and accurate to encounter numerous requirements for targeted gene amendments. These advances are paving the way to design dream plants for the future (Bansod et al., 2019; Deng et al., 2019; Mao et al., 2019; Zhang et al., 2019).

6.8 Conclusion

A nanoparticle-mediated transgene delivery technique will simplify plant biotechnology, as this type of system offers a simple and low-cost procedure to accomplish multiple requirements in excessive throughput reports, particularly when these nanoparticles pass in plant life spontaneously. The main existing applications of

nanomaterials for gene delivery and editing in some crops may be open different and safer possibilities for smart insertion of biomolecules for new approaches in plant genetic engineering. Thus, there is a still need for the improvement of nano- or microparticles, and delivery methods for biolistic gene transfer in various plants to enhance seed growth or boost plant promotion and crop protection. More research is also required to enhance the regeneration and plant transformation responses of a wide variety of plant tissues and cultivars. Pollen magnetofection is easy, quick, culture-free, genotype-independent and with the capacity of multigene delivery. Current methods can transform virtually all crops, tremendously facilitating the breeding procedures of new types of transgenic plants. Hybrid nanomaterials and pollen magnetofection exposed a new age in agri-food sectors because they have essential roles such as the synthesis of new antimicrobials and recombinant protein in plant cells, engineering crops for better production and cleaner biofuels, fertilizers, increasing soil health, and developing crops that are protected from drought, pests, herbicides, and disease. Focusing on how these nanomaterials have the ability to passively permeate the cell walls can help us develop much better tools in the foreseeable future so that we are able to implement other fascinating methods such as CRISPR for the production of modified crops. However, we may still find several important challenges ahead. We may answer a few of these challenges by incorporating options for the efficient distribution of varied genes along with other editing tools to many plants, the design and fabrication of novel hybrid nanomaterials, pollen magnetofection, and CRISPR strategies.

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