Chapter 1
Application of Nanotechnology in Genetic Improvement in Crops

M. Sheikh Mohamed and D. Sakthi Kumar

Abstract Plant genetic engineering is at the epicenter of environmental sustainability endeavors, synthesis of natural products of commercial and pharmaceutical value, while also addressing the ever-expanding food and energy requirements of an ever-growing global population impacting the global climate in more ways than one. Though the importance of this field has been emphasized, still progress remains at a slow pace, partly owing to the drawbacks of conventional plant biotransformation approaches such as limited host range, below par transformation efficiencies, cellular toxicity, in addition to foreign DNA integration into the host genome. These drawbacks are additionally supported by the presence of plant cell wall which has been a limiting checkpoint for essential exogenous biomolecules entry. Nanotechnology, with its well-established impact on the materials, electronics, energy, and medical sectors, has found a new role in plant genetic engineering. Nanomaterials with their unique physicochemical properties have attracted immense interest from plant scientists and are the solution to the conventional limitations faced in plant biotransformation. A wide array of nanoparticles have been already studied for carrying and delivering sensitive cargo (DNA, RNA, proteins) to specific cells without any damage to the cargo or the host. This chapter deals with such nanomaterials, their success, challenges and future scope in agriculture.

1.1 Introduction

Increasing global food security triggered by man-made and natural causes has been exerting tremendous pressure on the agricultural sector. The past century was witness to numerous technological innovations, such as the development of hybrid varieties, systemic development, and use of highly efficient synthetic chemicals that helped the growth in crop production (Ray et al. 2013; Fischer et al. 2014).

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However, a major boost was seen with the advent of biotechnology, which propelled the production of crops that were resistant to various biotic and abiotic factors including viruses, pests, drought, salinity, etc. (Altman and Hasegawa 2011). Though all this assisted greatly in addressing the global food management, numerous factors, natural and man-made, have been stalling the progress or alternatively creating sudden and excessive demands, varying by geographic locations, which have been posing new challenges for this sector (FAO 2018). Therefore, rises the need for a more advanced technological system for alleviating the problems faced by the agricultural community which can work in conjunction with the old system (conventional) and independently on its own.

Due to the various revolutionary applications in fields as diverse as medicine, materials science, electronics, energy, etc., nanotechnology has emerged as an indispensable asset in the modern world (De et al. 2014). It is only befitting that it is accredited as one of the six "Key Enabling Technologies" by the European Commission (European Commission Communication 2009). Investigations on the applications of nanotechnology in agriculture pertaining to sustainability, resistant/tolerant varieties, increased productivity, post-processing, packaging, and distribution, etc. have been going on for many years, but has only over the past decade began to take prominence, which is evident from the increased number of scientific publications and patents in this field (Rodrigues et al. 2017; Lowry et al. 2019; Mohamed and Kumar 2016a, b). The essence of nanotechnology lies in minimizing the essentials and delivering them to exactly the desired location with high precision, thus greatly reducing costs, decreasing undesirable wastage of resources, limiting offsite interactions, and having explicit and increased effects (Prasad et al. 2017). In agriculture, this pertains to significantly reducing the application of chemicals, instead employing a smarter delivery mode of active ingredients facilitating better and efficient essential resource (water, nutrients, etc.) management (Zhao et al. 2018). A more exciting and enticing application prospect of nanotechnology is in the field of plant breeding and genetic transformation (Elemike et al. 2019; Cunningham et al. 2018).

Genetic improvements of crops generally involve the cellular level diversion of genes to a desired location for target gene expression, which could be for a variety of purposes like imparting resistance to pest(s), tolerance to climatic anomalies or to induce additional productivity traits. These genetic alterations are intended for improving the overall quality of crops (Abdallah et al. 2015). A general routine of two distinct steps are followed during genetic engineering of plants: (1) transient transformation—the introduction of desired DNA into plant cells, and (2) stable transformation—subsequent integration of foreign DNA with plant genome. Though each step poses its own challenges, the proper integration of the transgene into the host genome is the most crucial part to produce transgenic plants with desired traits (Bates et al. 1990). This process of transformation is generally conducted under the protective environment of sterile laboratory conditions and engineered plants need to regenerate from transformed tissues which again introduces newer challenges.

Introduction or transfer of genes has been conventionally achieved by (1) natural method—using natural agents as *Agrobacterium* species, viruses, etc., (2) direct

method—protoplast fusion, transfection, etc., (3) physical method—electroporation, particle bombardment, microinjection, etc., and (4) chemical method—gene transfer using plasma membrane destabilizing/precipitating agents as PEG, PVA, $NaNO_3$, DEAE, dextran, DMSO, etc. (Demirer and Landry 2017). Although successful, these conventional methods were plagued with limited transformation rates due to various reasons, the most prominent being utilization of excessive energy and chemicals inflicting damage to DNA and cells. Circumventing these issues would result in an efficient process and a more productive outcome.

Nanotechnology-based processes promise effective solutions for such limitations. Various nanocarriers such as liposomes, polymeric and inorganic nanoparticles (NPs), carbon nanotubes (CNTs), and quantum dots (QDs) have been successfully tested for their abilities to carry genes of interest to the desired host system with commendable success rates (Cunningham et al. 2018). This chapter will dwell into the implications (past, present, and future) of nanotechnological interventions in the field of plant genetic engineering while comparing the conventional modules as well.

1.2 Conventional Plant Biotransformation Techniques

Methods for plant biotransformation have been adapting to the various advancements in time to achieve better and stable gene expression outcomes. Though technological advancements have been implemented, conventional gene delivery approaches are still being used (Cardi et al. 2017). These conventional methodologies fall under three predominant classes: physical (biolistic particle bombardment/gene gun, electroporation—using electric field pulses to create pores in cell membranes and microinjection), chemical (PEG-mediated), or biological (Agrobacterium-mediated) with their own advantages and limitations specific to the mode of application (Table 1.1). Yet, the limitations outweigh the advantages and hence the need for promising alternatives. Nanotechnological interventions that are being developed to circumvent these limitations of conventional approaches and simultaneously enhancing overall process efficiency are discussed in this chapter.

1.3 Why Nanotechnology?

With conventional plant gene delivery methods facing serious issues, prospective alternatives that are cost-effective, easy, robust, non-toxic, and that can transfer genes into all phenotypes irrespective of the species become a necessity. Nanotechnology promises augmentation of agricultural productivity by highly specific and reliable genetic transformation methods at the cellular level (Sekhon 2014).

The nano-approach advertises numerous advantages over the conventional practices, with regards to its ease of operation and high efficiency (Mishra et al.

Table 1.1 Conventional gene delivery methods, advantages, and drawbacks

Conventional gene delivery methods		Advantages	Drawbacks
Physical methods	Biolistics (gene gun)	• Facile process • Large size/quantity of DNA transfer possible	• Compromised DNA integrity • Poor efficiency and short life span of expression • Damage to host
	Electroporation	• Rapid • Cost-effective	• Limited to certain plant species • Compromised DNA integrity • Damage to host
	Microinjection	• Direct injection into plant protoplasts or cells • Suitable for use in large cells • Effective and easy gene transfer to the nucleus • Direct introduction of gene by penetrating the cell membrane	• Not suitable for small cells or tissues. • Infrequent gene transfer method in plant biotransformation • Low efficiency
Chemical methods	PEG-mediated	• High-efficiency protoplast transfection	• Highly restricted protoplast regeneration into whole and fertile plants
Biological methods	Agrobacterium-mediated	• Cost-effective • High efficiency • Stable transformation	• Limited to certain plant species
	Virus-mediated	• Useful for transient and whole-plant expression • Independent of host range limitation	• Non-selectivity • Slow process • Low DNA size carrying capacity

2017). For example, a thousand times less amount of DNA is required for the detection of expression than the conventional methods. Transient DNA-free genome editing (including gene silencing) is enabled in a controlled fashion. Co-delivery of multiple biomolecules (DNA activator, DNA proteins, different genes), simultaneously to the target, is facilitated. The surface of the gene nanocarriers can be easily functionalized using certain bio-recognition entities for specific, targeted delivery. The non-toxic nature of nanocarriers and their local translocation to individual cells, organs, or tissues is an additional advantage.

Particles with size ranging from 1 to 1000 nm are regarded as nanoparticles or nanocarriers (Nair et al. 2010, 2012). The distinctive small size and unique surface

properties project them as versatile carriers for genes and other biomolecules. NP-based biotransformation proposes numerous benefits: (i) faster gene transfer than Agrobacterium-based process, (ii) DNA encapsulated in protective layers therefore resistant to damage/inactivation, (iii) gene mutation due to energy-induced DNA damage (typically in physical methods) is greatly reduced, (iv) greater control over transformation process, and (v) amalgamation of nano- and physical methods is possible leading to effective and improved gene transfer. So far, a wide range of nanomaterials such as liposomes, CNTs, QDs, and silica NPs have been used for plant gene engineering (Nair et al. 2010).

In contrast to the animal system where the application of nanotechnology for gene delivery has been widely demonstrated, in plants, owing to the presence of a rigid cell wall which restricts the transport of molecules into plant cells, research has been slightly sparse (Rai et al. 2012). NPs have interesting value as intracellular biomolecule delivery materials, such as (i) their biological membranes trespassing ability, (ii) ability to carry, shield and release various payloads, and (iii) attain specific targeting by facile surface modification (Sokolova and Epple 2008). The NP-based gene delivery in plants is limited not only by the cell wall but also by the general in vitro plant studies. The regeneration capacity in in vitro cultures varies greatly across species, genotype, and even in individual plant depending on the developmental age of source tissue. As of now, regardless of the delivery method, all stable transformations need progeny regeneration from embryogenic calli. Therefore, delivery and regeneration optimizations are essential for improving the efficiency of stable transformation across a wide range of plant species.

1.4 Nanocarriers for Plant Biotransformation

NPs for the delivery of biomolecules (nucleotides, activators, proteins) to plants in vitro and in vivo (Mohamed and Kumar 2016a) are being explored at a much-enhanced pace than ever before. NP-mediated genome editing to deliver green fluorescent protein (GFP) gene-containing plasmid and functional recombinase into plant tissues has been a great success. One prominent example is that of mesoporous silica NPs (MSNs) for delivery of DNA and its activator into tobacco plant via isolated cells and intact leaves (Torney et al. 2007). Furthermore, MSNs, single-/multi-walled CNTs (SWCNTs/MWCNTs), gold NPs (AuNPs), magnetic virus-like NPs (MVNPs), carbon-coated magnetic NPs, QDs, and starch NPs have been employed in this regard (Mohamed and Kumar 2016b; Nair et al. 2012).

1.4.1 Silica Nanoparticles

Looking at a few detailed examples, MSNs-DNA complex was delivered into intact roots of *Arabidopsis thaliana* without any involvement of mechanical force

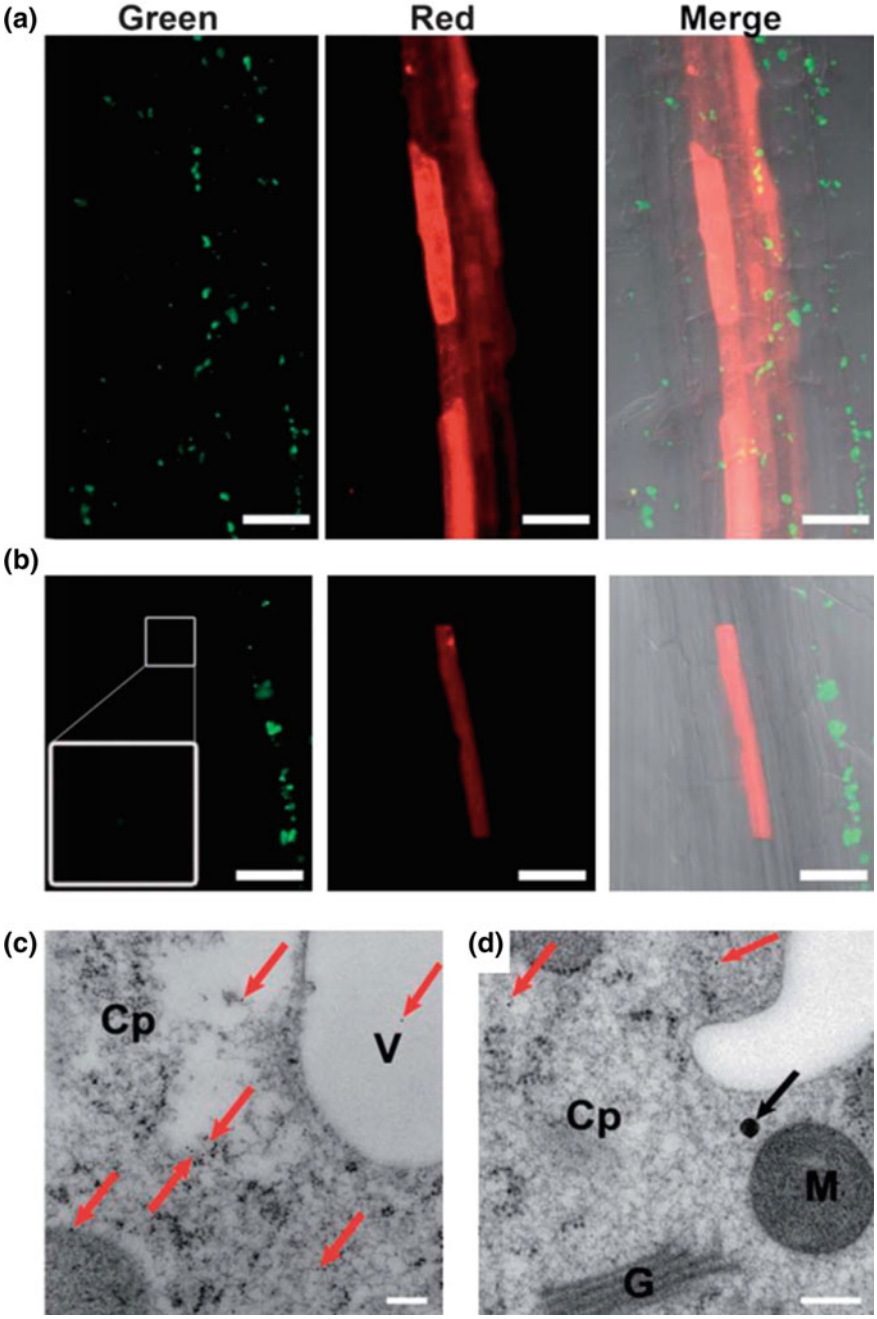
(Chang et al. 2013). Fluorescence labeling along with antibody detection was used to confirm the gene expression from the epidermal and inner cortical and endodermal (not possible with conventional physical methods) root tissues. Simple co-culture was enough for MSNs to infiltrate the cell wall resulting in transient gene expression in intact Arabidopsis roots, thereby negating the need for physical injury causing conventional biolistic approaches (Fig. 1.1).

Biolistics mediated co-delivery of AuNP functionalized MSNs (Au-MSN), with large average pore diameters (10 nm) and fluorescently labeled protein, bovine serum albumin (BSA) along with plasmid DNA, an enhanced green fluorescent protein (eGFP) into *Allium sativum* (onion) plant tissues was reported (Ortigosa et al. 2012). A proof-of-concept experiment demonstrated Au-MSN delivery and release of proteins and plasmid DNA in the same cell after trespassing the cell wall via biolistic approach. This was the first example of co-delivery of active non-denatured proteins and plasmid DNA (eGFP fluorescence) to plant cells providing new insights into NP-mediated cargo delivery to plants.

Genome editing can be carried out without direct delivery of DNA as such, rather with proteins or enzymes that can initiate/facilitate biochemical analysis or genome modifications. This can negate DNA (transgene)-genome integration and result in precisely modified "non-transgenic" plants. In this regard, gold-plated MSNs-protein (Cre recombinase) complex were used to deliver the protein via biolistics into *Zea mays* (maize) cells harboring loxP sites flanking a selection gene and a reporter gene (Ortigosa et al. 2014). With the release of Cre protein from MSNs, recombination of loxP sites followed by elimination of both genes occurred. Fertile plants were regenerated from selected recombination events with nearly 20% of embryos developing calli with recombined loxP sites. This technology is a DNA-free module for genome editing where MSNs can be modified to accommodate specific enzymes and target desired tissue.

Torney and co-workers utilized honey-comb-like MSNs to transport DNA and chemicals into *Nicotiana tabacum* via biolistics (Torney et al. 2007). MSNs were loaded with the gene and its inducer, and to further prevent unwanted escape from MSN pores, the ends were capped with AuNPs through disulfide bonds. The significance of disulfide bonds is in cellular environments, where they are reduced, resulting in the release of DNA, conditions which can be precisely controlled. Optimal DNA/MSN ratio was found to be 1:10 (w/w), wherein, a stable DNA-MSN complex was formed, without any free DNA in solution post 2 h of incubation. Transient GFP expression was observed post 36 h of DNA-MSN incubation

Fig. 1.1 MSN-mediated gene delivery. **a**, **b** Confocal microscopic images of DNA-MSN complex (1: 100 ratio) treated (48 h at 24 °C in 1/2 MS) Arabidopsis root cells. Gene expression (mCherry protein; red) was observed in endodermal (**a**) and cortical (**b**) cells. TMAPS/F-MSNs were present in cells expressing mCherry (**b**, green channel). Scale bars: 50 mm. **c**, **d** TEM of immunogold-labeled mCherry protein in root cells after incubation with DNA-MSN complexes. Red arrows show the gold-labeled mCherry proteins. Presence of TMAPS/F-MSNs (black arrow) and mCherry protein (red arrows) in the same cell (**d**). Scale bars are 200 nm. Cp, cytoplasm; M, mitochondrion; V, vacuole; G, Golgi apparatus. Reproduced with permission from RSC ▶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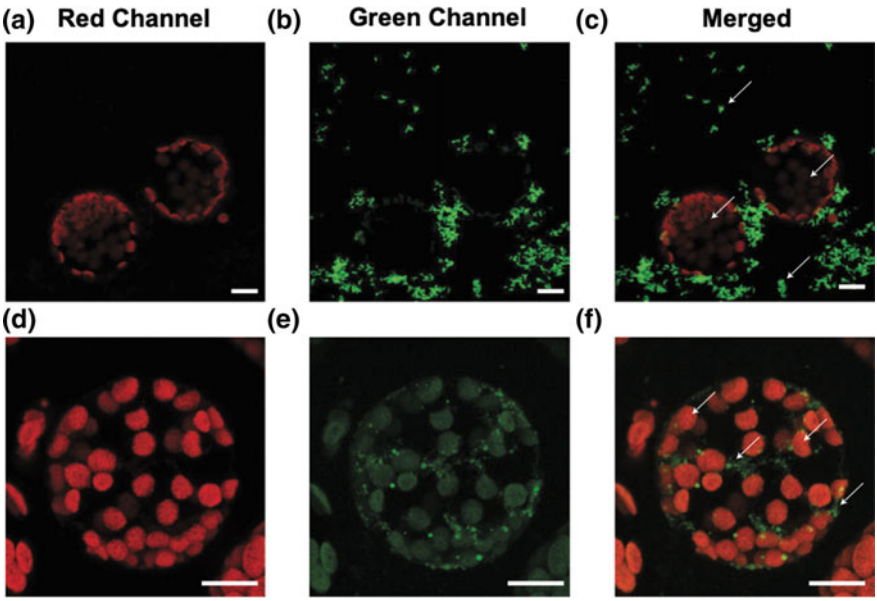

Fig. 1.2 Confocal imaging of MSN uptake by tobacco mesophyll protoplasts. Protoplasts incubated with **a–c**, type-I MSNs (single focal plane images) and **d–f**, type-II MSNs (three-dimensional reconstruction images). No uptake of type-I MSNs was observed, but type-II MSNs were internalized. Both MSNs are functionalized with fluorescein and visible in green (thick arrows). Auto-fluorescing chloroplasts in the protoplasts are in red (thin arrows). Reproduced with permission from NPG

(Fig. 1.2). This stable nanoconstruct can be utilized for genetic transformation of plants or to induce pathogen-activated defense response.

1.4.2 Calcium-Based Nanoparticles

In another work, 20–50 nm calcium phosphate (CaP) NPs carrying a reporter gene (pCAMBIA1301) was employed (Naqvi et al. 2012). Gel electrophoresis was used to determine pCAMBIA1301 encapsulation and pH-dependent dissolution of CaPNPs. The encapsulation of DNA in CaPNPs was highly beneficial in protecting it from intracellular nucleases. A transformation efficiency of 80.7% was achieved with the nanocarrier when compared to *Agrobacterium tumefaciens* (54.4%) and naked DNA (only 8%), clearly highlighting the superiority of the NP-mediated genetic transformation over the conventional module. The authors suggest the release of plasmid DNA from CaPNPs in the cell, perhaps facilitating nuclear entry as opposed to the case of *A. tumefaciens* infection where transgenic GUS (b-glucuronidase) integrates into genomic DNA by non-homologous recombination. Also, this nanoconstruct did not cause any injury to the plant cell while delivery (Fig. 1.3).

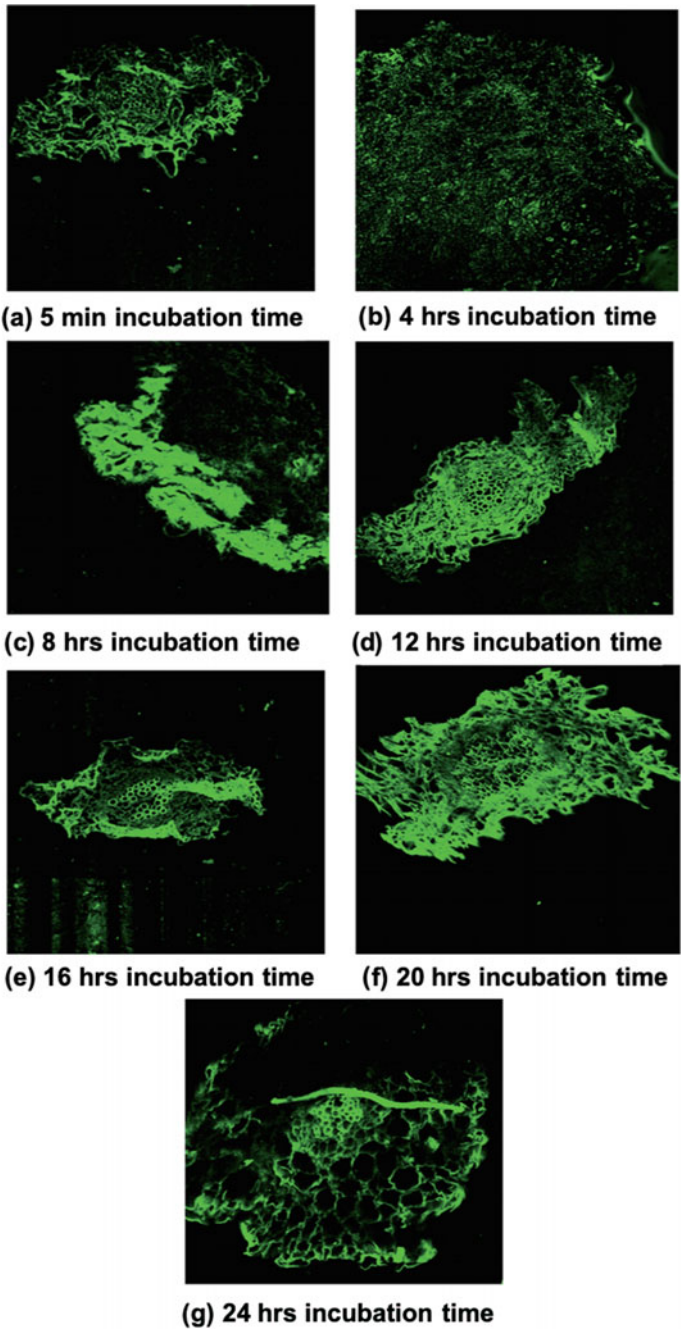

Fig. 1.3 Confocal scanning laser microscopy of FITC–dextran loaded CaP nanoparticles incubated with hypocotyl section of *Brassica juncea* L. cv. Pusa Jaikisan at different time intervals, i.e., **a** 5 min, **b** 4 h, **c** 8 h, **d** 12 h, **e** 16 h, **f** 20 h, and **g** 24 h. Reproduced with permission from RSC

In a similar kind of work, some researchers developed CaPNPs (25–55 nm) and successfully delivered pBI121 harboring GFP driven by 35S promoter-encoding plasmid DNA in tobacco plants (Roy et al. 2003). Due to pDNA being anionic, easy encapsulation into CaPNPs was achieved. The pBI121 with GFP-loaded CaPNPs was an alternative non-viral vector for easy and efficient gene delivery while providing protection from cellular nuclease enzymes.

It is known that gene encapsulation into biocompatible carriers augments the biotransformation process and provides protection to the gene, cell, and enzymes. Genetic manipulation of *Cichorium intybus* L. with CaPNPs loaded with pBinAR containing trans-*hmgr* gene was performed (Rafsanjani et al. 2016). A transformation efficiency of 9.6% was recorded, with the transgenic plants showing higher levels of chlorophyll, soluble protein and esculin, attributed to the higher expression of *hmgr* transgene and increased activity of HMGR enzyme. These nanoparticles could be useful in producing genetically engineered plants of desired and enhanced traits.

1.4.3 Magnetic Nanoparticles

Magnetic gold nanoparticles (mGNPs) covalently bound to fluorescein isothiocyanate (FITC) molecules were delivered into *Brassica juncea* (canola) cells with/without cell walls under an external magnetic field (Hao et al. 2013). The translocation of NPs was confirmed from sectional transmission electron microscopy (TEM) images and further by confocal images where green fluorescence in the protoplasts and walled cells was observed, with a delivery efficiency of 95%. Further, canola cells with/without cell walls were targeted with plasmids-mGNPs complex. Post 48 h culture, blue color (X-Gluc) developed in the protoplasts, indicating the expression of the GUS gene. Meanwhile, intact cells (with cell wall) exhibited a green color, possibly a mix of blue and yellow from the suspension cells themselves. The positive gene expression and non-cytotoxic nature of mGNPs even with the toxic dye FITC, project this nanocarrier as an efficient gene and biomolecule delivery vector.

It is a fact that majority of the genetic modification modules depend on tissue regeneration from culture, often associated with long and arduous processes. In addition, certain crop species pose difficulty in regeneration, for example, cotton. Therefore, a novel transformation technique, pollen magnetofection, was developed for the direct production of transgenic seeds, bypassing regeneration (Zhao et al. 2017). Herein, exogenous DNA-loaded magnetic nanoparticles (MNPs) were directly delivered into pollen under a magnetic field. These magnetofected pollen were utilized for pollination, resulting in transgenic plants from transformed seeds. The successful integration of exogenous DNA into the genome was expressed and inherited in the offspring. The advantages of such a system are that it does not require laborious culture procedures and is genotype independent. Additionally, it is a facile and rapid process with multi-gene transformation capabilities and suitable

for almost all crops, with an imminent impact in developing new varieties of transgenic crops.

1.4.4 Dendrimers

Cationic polyamidoamine (PAMAM) dendrimers are nanoparticles with a tunable number of branches and unique molecular features, making them one of the most promising nanocarriers for gene delivery applications. A diethylenetriamine core hyperbranched PAMAM (hPAMAM)-G2 dendrimer was used to conjugate with DNA (ssDNA-FITC-hPAMAM) and tested with delivery to alfalfa cells. Additionally, the efficacy of ultrasound (US)-mediated delivery of hPAMAM-G2 and gene expression (gusA) was probed (Amani et al. 2018). The electrophoresis retardation analysis (DNA-dendriplex dissociation) portrayed partial DNA protection from sonication damage with N/P ratios (where N is the number of nitrogen atoms in dendrimer and P is the number of phosphorus atoms in DNA) < 2 and with better DNA protection corresponding to increasing N/P ratios. Sonication increased the ssDNA delivery by 36%, significantly higher than without sonication group. Also, the N/P ratio was essential in determining the efficiency of transfection and gusA gene expression with the highest efficiency (1.4%) recorded at an N/P ratio of 10. The US (120s) and hPAMAM–DNA combination proved to be optimal with the highest gusA gene transfection and expression of 3.86%.

A similar work involving supramolecular complexes of G4 poly(amidoamine) dendrimers with GFP-encoding plasmid DNA via electrostatic interaction, to *Agrostis stolonifera* L. (creeping bentgrass) was reported. The fluorescently (TRITC) labeled dendrimers were recorded to enter the cells of turfgrass and expressing the GFP gene in the plant cells as observed by confocal fluorescence microscopy. It was concluded that the efficiency of transfection might be further enhanced with optimization of conditions as cell culture media pH and the molar ratio (N/P) of dendrimer/DNA (Pasupathy et al. 2008).

1.4.5 Liposomes

Liposomes are fascinating particles which bear a close resemblance to the plasma membrane of cells, mostly synthesized by components found inside the living system, thereby rendering them high biocompatibility. Their flexibility and ability to carry large payloads garner them unprecedented scientific appreciation. Deshayes et al. developed negatively charged liposomes encapsulating an *Escherichia coli* plasmid, pLGV23neo, carrying a kanamycin resistance gene (Deshayes et al. 1985). PEG was used to facilitate the fusion of liposomes with tobacco mesophyll protoplasts. Kanamycin-resistant clones were isolated from transfected cultures at an average frequency of 4×10^{-5} with the leaf protoplasts being resistant to

kanamycin at 100 µg/mL. Aminoglycoside 3′-phosphotransferase II, a pLGV23neo encoded enzyme, was detected in the leaf extracts. At least one of the transformants had approximate 3–5 copies of the kanamycin resistance encoding genes inserted in their genome, making the experiment a commendable success.

In another work, Caboche and co-workers prepared unilamellar liposomal vesicles encapsulating pUC8CaMVCAT plasmid vector with the CAT gene (chloramphenicol acetyl transferase) and successfully introduced the gene in tobacco protoplasts (Caboche and Lurquin 1987). Ballas et al. synthesized tobacco mosaic virus RNA (TMV RNA)encapsulated liposomes (formulation of phosphatidylcholine/cholesterol) (Ballas et al. 1988). The surface of the so-developed liposomes was functionalized by quaternary ammonium detergent, di-isobutyl cresoxyethoxyethyl dimethyl benzylammonium (DEBDA [OH–]), thereby introducing surface hydroxyl groups which facilitated efficient TMV RNA transfer into tobacco and petunia protoplasts, within 48 h.

Wiesman et al. synthesized liposomes utilizing vernonia oil (a natural epoxidized triglyceride)-derived cationic amphiphilic compounds for DNA encapsulation (Wiesman et al. 2007). Sawahel utilized lipofectin for effective transfusion of *N. tabacum* and *N. plumbaginifolia* mesophyll protoplasts (Sawahel 2002). During their experiments, they confirmed that the CaMV-35S promoter controlled ß-glucuronidase gene had lower transformation efficacy than the PEG-mediated method.

1.4.6 Gold Nanoparticles

Due to their unique properties at the nanoscale, gold nanoparticles have found extensive applications in sensors and other biomedical applications. Though their use in plant biotransformation has been limited, it is on a steady rise. AuNPs-embedded carbon matrices were synthesized by heat treatment of the intracellular biogenic AuNPs produced by the fungus *Aspergillus ochraceus*. Vijayakumar and co-workers utilized these AuNPs (5–25 nm) for the successful delivery of plasmid DNA into *N. tabacum* utilizing gene gun (Vijayakumar et al. 2010). A similar DNA delivery demonstration was conducted in the same work with *Oryza sativa* (a monocot) and *Leucaena leucocephala* (a hard dicot tree species) with negligible cellular damage. Compared with conventional gene gun approaches using micrometer Au particles, it was established that the small size of AuNPs assists in easy and efficient embedding in the matrices which in turn enhances the transformation efficiency. On the other hand, less raw material (gold and plasmid) is needed to achieve the same level of transformation efficiency, aided by lower toxicity levels as well.

DNA-coated AuNPs were used for stable transformation of commercially important cultivars of *Glycine max* L. (soybean) callus. Soybean embryos (immature) were targeted with highly accelerated DNA-NP complex. Subsequent protoplast cultures were initiated under controlled conditions for the introduced

neomycin phosphotransferase II gene. Kanamycin-resistant calli were obtained from the transfected cultures. Foreign gene expression was confirmed with enzyme assays and Southern blot hybridization (Christou et al. 1988).

1.4.7 Carbon Nanotubes

Liu et al. (2009) were supposedly the first to exploit CNTs for delivery of genes to plants. The independent (externally unaided) cellular uptake of both SWCNT/FITC and SWCNT/ssDNA-FITC conjugates was demonstrated in *N. tabacum* cells, validating the CNTs ability to penetrate intact plant cell walls and cell membranes (Liu et al. 2009). Later, Giraldo et al. (2014) injected the ssDNA-SWCNTs into the underside of *A. thaliana* leaves, using a syringe. They found that certain CNTs (defined size and charge) penetrated cell membranes without any external assistance (gene gun). The near-infrared (NIR) fluorescence emission of SWCNTs was used to understand their transport and localization in plant cells. Leaf tissue cross sections revealed the localization of ssDNA-SWCNTs in the leaf lamina and veins in intra- and extracellular parenchyma tissues and in chloroplasts (Fig. 1.4). These observations were depictive of successful trespassing of the plant cell wall, cell membrane, as well as the chloroplast membrane. Curiously, the leaf life span and chlorophyll content seemed to be unaffected by the infiltrated ssDNA-SWCNT solution, an assurance of non-cytotoxic nature of the complex (Giraldo et al. 2014).

Demirer and co-workers recently utilized covalently functionalized or pristine SWCNTs and MWCNTs for an efficient DNA delivery and robust transient protein expression in mature *Eruca sativa* (arugula) leaves. In the same study, cell wall-free arugula protoplasts were used for CNT-based transient protein expression, with a transformation efficiency of 85%. Again, in the same study a remarkable 95% gene (GFP) silencing efficiency was recorded for CNT scaffold-siRNA complex in *N. benthamiana* plant cell cytosol. The group was the first to demonstrate the efficient transient gene expression and silencing in mature plants by the use of CNT-biomolecules complexes. The results could spur high-throughput plant genetic transformation applications. The same group developed an efficient diffusion-based delivery of biomolecules with pristine/chemically functionalized SWCNTs and MWCNTs into tissues/organs of intact plants of several species. In *N. benthamiana* (Nb), *E. sativa* (arugula), *Triticum aestivum* (wheat), and *Gossypium hirsutum* (cotton), efficient delivery of DNA followed by robust protein expression (devoid of transgene integration) was achieved. Additionally, a gene silencing efficiency of 95% was observed with small interfering RNA (siRNA) delivery to Nb leaves. Here again, the nanomaterials apart from ferrying biomolecules into plant cells were able to safeguard polynucleotides from nuclease degradation. The results encourage species-independent, passive delivery of biomolecules, devoid of transgene integration, for various biotechnological applications (Demirer et al. 2019).

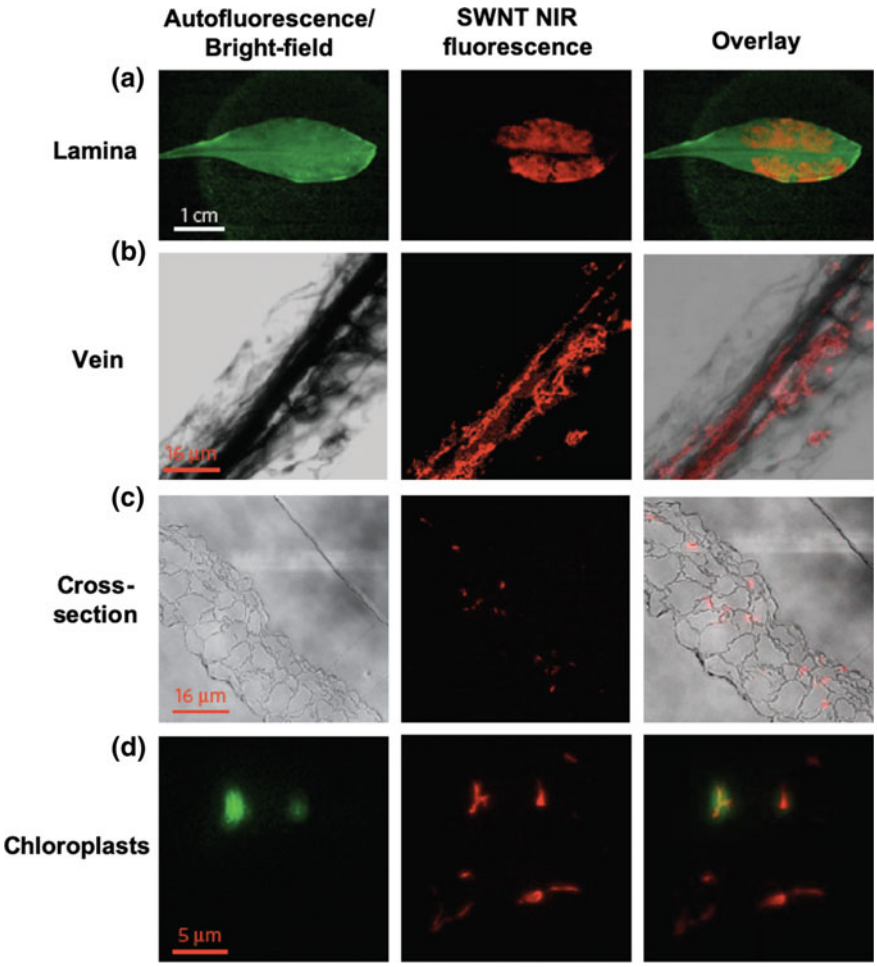

Fig. 1.4 Nanoparticle transport inside isolated chloroplasts and leaves. **a** CRi Maestro images of ss(AT)15–SWNTs within the leaf lamina of *A. thaliana*. **b–d**, Co-localization of ss(AT)15–SWNTs near leaf veins (**b**), in parenchyma cells (**c**), and chloroplasts in vivo (**d**). Reproduced with permission from NPG

1.4.8 Other Nanoparticles

ZnS nanoparticles (3–5 nm) modified with positively charged poly-L-lysine were utilized for transformation of tobacco cells by delivering GUS-encoding plasmid DNA (pBI121), assisted by ultrasound (Fu et al. 2012). Molecular analysis as polymerase chain reaction (PCR), Southern blot, and GUS histochemical staining was performed on regenerated plants. Higher transformation efficiencies (43.42, 48.57, and 52.67%) were recorded with ZnS NPs-DNA complex as compared to

conventional US-based transformation route (30–40%). This is primarily attributed to the shielding effect provided to the DNA by ZnS NPs. The stable expression results by ZnS NPs demonstrate their role as gene protector and carriers (Fu et al. 2012).

Quantum dots have been widely used as fluorescent bio labels in in vitro cell and in vivo animal imaging studies and off late for theranostics (therapy and diagnostic) as well (Mohamed et al. 2016). In plant sciences, QDs are mainly utilized for tracking the fate of NPs-DNA complexes during/post cell transfection and subsequently in plant transformation. Water-soluble CdSe QDs were synthesized for labeling chitosan-DNA NPs via electrostatic interaction. This CdSe-chitosan-DNA complex was utilized for the genetic transformation of *Jatropha curcas* cells. Full-length PCR amplification of the reporter gene (GFP) revealed that the DNA was not degraded in *J. curcas* cells transfected with the nanocomplex rather the target DNA was integrated into the plant genome as evidenced with fluorescence detection of the complex in the transformed calli (Wang et al. 2011).

Virus resistance in plants can be imparted by the topical application of pathogen-specific double-stranded RNA (dsRNA), an attractive alternative to transgenic RNA interference (RNAi). However, spraying naked dsRNA onto plants exposes its instability, thereby limiting practical application. Mitter et al. loaded dsRNA in layered double hydroxide (LDH) clay nanosheets which were non-toxic as well as biodegradable. The advantages of such a carrier include stable and robust dsRNA loading that does not wash off, presents sustained release from the nanocarrier and can be detected even after 30 days of application from sprayed leaves. LDH degradation, dsRNA uptake by cells, and homologous RNA silencing were recorded (Fig. 1.5). On comparison of sprayed and newly emerged unsprayed leaves, a single spray of dsRNA-LDH complex proved to provide effective virus protection for at least 20 days. The results witnessed in this work translate to the near future development of RNAi-nanosprays for a diverse range of applications (Mitter et al. 2017).

Starch, owing to its biocompatible and biodegradable nature, has been studied in the nanoform for gene delivery applications. The starch NPs-gene complex was efficient enough to cross the cell wall, via US-assisted induction of transient pores in the cell wall, cell/nuclear membrane. Working in the same area, Liu and co-workers synthesized poly-L-lysine coated starch NPs (50–100 nm) in water oil microemulsion and surface modified with fluorescent $[Ru(bpy)_3]^{2+}\cdot 6H_2O$ conjugated to pEGAD plasmid DNA. They demonstrate the DNA protective effects of starch NPs in the DNA-NP complex from US damage and DNase I cleavage in plant suspension cells of *Dioscorea zingiberensis*. It was concluded that starch NPs could successfully be used in plant transformation as efficient nanocarriers of nucleic acids, assisted by US (Liu 2008).

Apart from improving yield, imparting disease resistance via gene transformation is also an essential aspect of sustainable agriculture. Chitosan NPs were encapsulated with thionin genes (AT1G12660 and AT1G12663) from *Arabidopsis thaliana*, in pEGAD vector and delivered to potato plants (Abdel-Razik et al. 2017). Thionin is an antifungal protein with action against pathogenic fungi of potato as

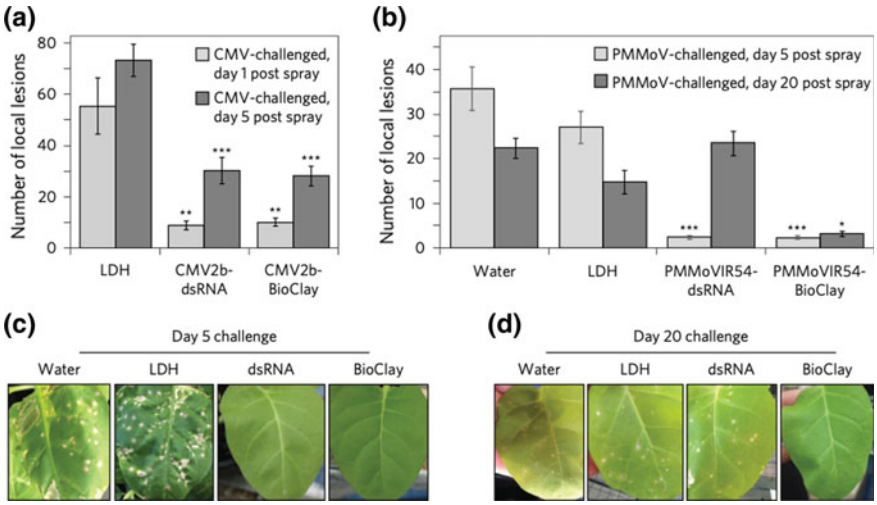

Fig. 1.5 BioClay (dsRNA-LDH) spray provides protection against viruses in local lesion assays. **a** Local lesions caused by CMV inoculation on cowpea. Plants at the two-leaf stage were sprayed with LDH, CMV2b-dsRNA, and CMV2b-BioClay on day 0 (n = 8–16 leaves per treatment group). Plants were mechanically inoculated with CMV at 1- or 5-day post-treatment. Lesions were counted 10 days post viral challenge (pvc). **b** Local lesions caused by PMMoV inoculation on N. tabacum cv. Xanthi. Plants were sprayed with either water, LDH, PMMoVIR54-dsRNA, or PMMoVIR54-BioClay on day 0 (n = 10–25 leaves per treatment group). Plants were mechanically inoculated with PMMoV at either 5- or 20-day post-treatment and necrotic lesions were counted 10 days pvc. **c**, **d** Images are showing the extent of necrotic lesions on N. tabacum cv. Xanthi leaves challenged with PMMoV 5-day post-spray treatment (**c**), and 20-day post-spray treatment (**d**). $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ are significant using the Kruskal–Wallis test with post-hoc Nemenyi test for multiple comparisons between samples compared with LDH. Data represent mean ± s.e.m. Reproduced with permission from NPG

Alternaria alternata and *Rhizoctonia solani*. Two prominent potato cultivars (lady and spunta) were chosen for transformation studies. RT-PCR was used for confirmation of thionin transgene expression in transgenic plants. Two assays were performed on transgenic potatoes to test resistance to the pathogenic fungi, (i) spore suspension effect on potato organs and (ii) effect of thionin on spore germination inhibition. New transgenic cultivars were highly resistant to the fungal infection symptoms as compared to non-transgenic normal plants. These chitosan NPs-DNA complex can be an effective tool to augment disease/pest resistance in various crop species.

While further extensive research is required to fine-tune the properties and functionalization of nanoparticles, the current scenario seems promising for aggressive application of NP delivery platforms for biomolecules to plants, addressing the limitations of traditional methodologies. Biomolecular delivery to plants is marred with limitations as low gene-editing efficiency, tissue/organ damage, species limitations, cargo limitations, etc. As compared with traditional

modules, NP-based biomolecule delivery promises higher-throughput plant genome editing via DNA, single-guide RNA (sgRNA), and RNP delivery, thus opening new avenues for exploration in this exciting field.

Plants have remained elusive to the advances of technology in genetic transformation techniques, especially due to the rigid cell wall hindering the delivery of essential gene modification capable cargo to mature plants. This curiously reveals a vast scope for potential in designing much more efficient nanocarriers with distinct cargo (nucleic acids, proteins) ferrying capabilities and optimal physicochemical features for efficient and independent bypass of the inherent barriers in intricate plant tissues.

1.5 Patented Technologies

Though limited, there has been a rise in patent applications in the field of nanocarrier-based biomolecule delivery to plants, especially with regard to enhancing specific traits of desired plants via transformation. Recent patents 20110065092A1 (gene-encapsulated non-viral particle) and US6534484 (gene-encapsulated liposome for biotransformation) highlight the importance and necessity of nanotechnology in biotransformation. Few more patents assigned to nanotechnology-based biotransformation are enlisted in Table 1.2.

1.6 Concluding Remarks and Future Perspectives

Engineering of plants at the gene level has impacted the global society in an unimaginable manner and has accelerated scientific ingenuity to develop crop variants with enhanced features as resistance to pests and diseases, tolerance to stress (abiotic/biotic), and enrichment of nutritional values. Advanced site-specific genome editing technologies have fine-tuned the precision with which genetic augmentation of plants is accomplished. Conventional approaches are greatly limited by the presence of the rigid cell wall, which makes penetration of biomolecules near to impossible without significant damage or loss of action. Agrobacterium has been the most revered plant genetic transformation tool but has limited host species range and is consistently under regulatory oversight in many countries. Biolistic and PEG transfection, though boast some perks over their biological counterpart, face severe difficulties in healthy plant regeneration from transformed tissues with low-editing efficiency. NPs are now established in in vitro mammalian cultures with a large population finding its way to human trials and further clinical applications. On the other hand, they are still in infancy when it comes to application in plants with much scope for investigations in future plant biotechnology and genome engineering. Initial reports have shown the potential of properly synthesized and functionalized analogues that can deliver biomolecules to

Table 1.2 Few nanoparticle-based patents on plant biotransformation

Patent title	Patent number	Invention
Plant delivery inventions		
T-DNA/protein nanocomplexes for plant transformation	US20120070900A1	DNA/protein nanocomplex for transfecting eukaryotic cells, wherein the nanocomplex comprises an expressible nucleotide molecule complexed with a VirD2 protein molecule, and a RecA protein molecule complexed with the nucleotide molecule/VirD2 protein molecule complex. The nucleotide molecule/VirD2 protein molecule/RecA protein molecule complexed may be further complexed to a cell-penetrating peptide. The DNA/protein nanocomplex can be used for delivering the expressible nucleotide molecule to target plant cells, in particular, monocot plant cells exemplified by wheat cells, corn cells, triticale cells, barley cells, and rye cells
Delivering compositions of interest to plant cells	US2011203013 (A1)	Nanoparticle–microparticle mixture can be used to provide a composition of interest to a plant or plant cell
Compositions and methods for delivery of a polynucleotide into a plant	WO2016196738A1	Compositions and methods for delivering a polynucleotide from the exterior surface of a plant or plant part into the interior of a plant cell. More specifically, the present disclosure relates to compositions comprising at least one polynucleotide and at least one agent that is able to disrupt at least one barrier of the plant or plant part
DNA-loaded supported gold nanoparticles, process for the preparation and use thereof	EP2462227A1, WO2011016053 (A1)	Relates to carbon embedded nanogold particles with sharp edges useful for gene delivery
Compositions and methods for safe delivery of biologically active plant transformation agents using non-fibrous silicon carbide powder	WO2007050715A2	A biologically active nucleic acid may be directly delivered into plant cells in an improved and operator-safe fashion using encapsulated or complexed nucleic acid formed into nanoparticles, using

(continued)

Table 1.2 (continued)

Patent title	Patent number	Invention
		carborundum powder, a nucleic acid-binding protein with nuclear localization signals (NLSs), or both
Other general inventions extendable to plant gene delivery		
Transfection reagents	US7915450B2	Cationic lipids and their compositions for macromolecule delivery to cells
Use of non-viable particles comprising an internal control (IC) nucleic acid	US20110065092A1	Non-viable particles (particularly liposomes, particles made of viral protein coat or synthetic polymers, non-viable genetically modified organisms), comprising an IC nucleic acid sequence in nucleic acid-based analysis
Lipid-encapsulated polyanionic nucleic acid	US8021686B2	Preparation of a lipid-nucleic acid composition
Method of inserting DNA into living cells	US4394448A	DNA/fragments of DNA insertion into live cells, by means of encapsulation in a lipid vesicle with subsequent contact with cells, facilitating insertion
Cationic lipids for intracellular delivery of biologically active molecules	EP0523189B1	Cationic lipids for efficiently delivering biologically active agents (drugs, proteins, polynucleotides, peptides, etc.) by assisting in transport across membranes or by promoting adhesion to biological surfaces
Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer	US5976567A	Nucleic acid introduction into cells via lipid-nucleic acid particles
Methods for encapsulating plasmids in lipid bilayers	US6815432B2	In vitro and in vivo transfection of cells using plasmid-lipid particles

plants in vitro and in vivo with better efficiency than conventional methods, though still some of them require external aid (i.e., gene gun). Not only the nanocarriers, but the auxiliary systems such as the ultrasound and the magnetic field generation systems should also be developed to better support the nanocarriers in enhancing their inherent potentials to the extent possible. The steps to the future are happening now, with numerous researchers fully engrossed in finding the perfect nanosystem for delivering the most sensitive of biomolecules across the most rigid cell walls to achieve the best transient gene expression efficiency so far. Slow, but sure,

nanotechnology with its vast trove of nanocarriers and delivery mechanisms is the hope for fighting the various agricultural issues plaguing this planet now.

References

Abdallah NA, Prakash CS, McHughen AG (2015) Genome editing for crop improvement: challenges and opportunities. GM Crops Food 6(4):183–205

Abdel-Razik AB, Hammad IA, Tawfik E (2017) Transformation of Thionin genes using chitosan nanoparticle into potato plant to be resistant to fungal infection. IOSR J Biotech Biochem 3:01–13

Altman A, Hasegawa P (2011) Plant biotechnology and agriculture: prospects for the 21st century, 1st edn. Academic Press, Cambridge

Amani A, Zare N, Asadi A, Asghari R (2018) Ultrasound-enhanced gene delivery to alfalfa cells by hPAMAM dendrimer nanoparticles. Turk J Biol 42(1):63–75

Ballas N, Zakai N, Loyter A (1988) Liposomes bearing a quaternary ammonium detergent as an efficient vehicle for functional transfer of TMV-RNA into plant protoplasts. Biochimica et Biophysica Acta (BBA) Biomembranes 939:8–18

Bates GW, Rabussay D, Piastuch W (1990) Transient and stable expression of foreign DNA introduced into plant protoplasts by electroporation. Methods Mol Biol 6:309–322

Caboche M, Lurquin PF (1987) Liposomes as carriers for the transfer and expression of nucleic acids into higher plant protoplasts. Methods Enzymol 148:39–45

Cardi T, D'Agostino N, Tripodi P (2017) Genetic transformation and genomic resources for Next-generation precise genome engineering in vegetable crops. Front Plant Sci 8:241

Chang FP, Kuang LY, Huang CA, Jane WN, Hung Y, Hsing YC, Mou CY (2013) A simple plant gene delivery system using mesoporous silica nanoparticles as carriers. J Mater Chem B 1:5279–5287

Christou P, McCabe DE, Swain WF (1988) Stable transformation of soybean callus by DNA-coated gold particles. Plant Physiol 87(3):671–674

Communication from the Commission to the European Parliament (2009) Preparing for our future: developing a common strategy for key enabling technologies in the EU, European Commission

Cunningham FJ, Goh NS, Demirer GS, Matos JL, Landry MP (2018) Nanoparticle-mediated delivery towards advancing plant genetic engineering. Trends Biotech 36(9):882–897

De A, Bose R, Kumar A, Mozumdar S (2014) A brief overview of nanotechnology. In: Targeted delivery of pesticides using biodegradable polymeric nanoparticles. Springer, New Delhi

Demirer GS, Landry MP (2017) Delivering genes to plants SBE supplement. Plant Synth Biol CEP, 40–45

Demirer GS, Zhang H, Matos JL, Goh NS, Cunningham FJ, Sung Y, Chang R, Aditham AJ, Chio L, Cho MJ, Staskawicz B (2019) High aspect ratio nanomaterials enable delivery of functional genetic material without DNA integration in mature plants. Nat Nanotechnol 14 (5):456–464

Deshayes A, Herrera-Estrella L, Caboche M (1985) Liposome-mediated transformation of tobacco mesophyll protoplasts by an Escherichia coli plasmid. EMBO J 4:2731–2737

Elemike EE, Uzoh IM, Onwudiwe DC, Babalola OO (2019) The role of nanotechnology in the fortification of plant nutrients and improvement of crop production. Appl Sci 9(3):499

EP0523189B1: Cationic lipids for intracellular delivery of biologically active molecules

Fischer T, Byerlee D, Edmeades G (2014) Crop yields and global food security: will yield increase continue to feed the world?. ACIAR, Australia

FAO (2018) The future of food and agriculture. Trends and challenges. http://www.fao.org/3/a-i6881e.pdf

Fu YQ, Li LH, Wang PW, Qu J, Fu YP, Wang H, Sun JR (2012) Delivering DNA into plant cell by gene carriers of ZnS nanoparticles. Chem Res Chin Univ 28(4):672–676

Giraldo JP, Landry MP, Faltermeier SM, McNicholas TP, Iverson NM, Boghossian AA, Reuel NF, Hilmer AJ, Sen F, Brew JA, Strano MS (2014) Plant nanobionics approach to augment photosynthesis and biochemical sensing. Nat Mater 13:400–408

Hao Y, Yang X, Shi Y, Song S, Xing J, Marowitch J, Chen J, Chenac J (2013) Magnetic gold nanoparticles as a vehicle for fluorescein isothiocyanate and DNA delivery into plant cells. Botany 91(7):457–466

Liu Q, Chen B, Wang Q, Shi X, Xiao Z, Lin J, Fang X (2009) Carbon nanotubes as molecular transporters for walled plant cells. Nano Lett 93:1007–1010

Liu J (2008) Preparation of fluorescence starch-nanoparticle and its application as plant transgenic vehicle. J Cent South Univ Technol (Engl. Ed) 15:768–773

Lowry GV, Avellan A, Gilbertson LM (2019) Opportunities and challenges for nanotechnology in the agri-tech revolution. Nat Nanotechnol 14:517–522

Mishra S, Keswani C, Abhilash PC, Fraceto LF, Singh HB (2017) Integrated approach of agri-nanotechnology: challenges and future trends. Front Plant Sci 8:471

Mitter N, Worrall EA, Robinson KE, Li P, Jain RG, Taochy C, Fletcher SJ, Carroll BJ, Lu GQ, Xu ZP (2017) Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. Nature Plants 3:16207

Mohamed SM, Kumar DS (2016a) Methods of using nanoparticles, plant nanotechnology principles and practices. Springer International Publishing, Switzerland

Mohamed SM, Kumar DS (2016b) Effect of nanoparticles on plants with regard to physiological attributes, plant nanotechnology principles and practices. Springer International Publishing, Switzerland

Mohamed SM, Poulose AC, Veeranarayanan S, Romero Aburto R, Mitcham T, Suzuki Y, Sakamoto Y, Ajayan PM, Bouchard RR, Yoshida Y, Maekawa T, Sakthi Kumar D (2016) Plasmonic fluorescent CdSe/Cu_2S hybrid nanocrystals for multichannel imaging and cancer directed photo-thermal therapy. Nanoscale 8(15):7876–7888

Nair R, Mohamed MS, Gao W, Maekawa T, Yoshida Y, Ajayan PM, Kumar DS (2012) Effect of carbon nanomaterials on the germination and growth of rice plants. J Nanosci Nanotechnol 3:2212–2220

Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS (2010) Nanoparticulate material delivery to plants. Plant Sci 179:154–163

Naqvi S, Maitra AN, Abdin MZ, Akmal Md, Arorad I, Samim Md (2012) Calcium phosphate nanoparticle mediated genetic transformation in plants. J Mater Chem 22:3500–3507

Ortigosa SM, Peterson DJ, Valenstein JS, Lin VSY, Trewyn BJ, Lyznik LA, Wang K (2014) Mesoporous silica nanoparticle-mediated intracellular cre protein delivery for maize genome editing via IoxP site excision. Plant Physiol 164(2):537–547

Ortigosa SM, Valenstein JS, Lin VSY, Trewyn BJ, Wang K (2012) Gold functionalized mesoporous silica nanoparticle mediated protein and DNA codelivery to plant cells via the biolistic method. Adv Funct Mater 22:3576–3582

Pasupathy K, Lin S, Hu Q, Luo H, Ke PC (2008) Direct plant gene delivery with a poly (amidoamine) dendrimer. Biotechnol J 3(8):1078–1082

Prasad R, Bhattacharyya A, Nguyen QD (2017) Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. Front Microbiol 8:1014

Rafsanjani MSO, Kiran U, Ali A, Abdin MZ (2016) Transformation efficiency of calcium phosphate nanoparticles for genetic manipulation of Cichorium intybus L. IJBT 15(2):145–152

Rai M, Deshmukh S, Gade A (2012) Strategic nanoparticle-mediated gene transfer in plants and animals—a novel approach. Curr Nanosci 8:170–179

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS One 8(6):e66428

Rodrigues SM, Demokritou P, Dokoozlian N, Hendren CO, Karn B, Mauter MS, Sadik OA, Safarpour M, Unrine JM, Viers J, Welle P, White JC, Wiesnerde MR, Lowry GV (2017) Nanotechnology for sustainable food production: promising opportunities and scientific challenges. Environ Sci Nano 4:767–781

Roy I, Mitra S, Maitra A, Mozumdar S (2003) Calcium phosphate nanoparticles as novel non-viral vectors for targeted gene delivery. Int J Pharm 250(1):25–33

Sawahel WA (2002) The production of transgenic potato plants expressing human alpha-interferon using lipofectin-mediated transformation. Cell Mol Biol Lett 7:19–29

Sekhon BS (2014) Nanotechnology in agri-food production: an overview. Nanotechnol Sci Appl 7:31–53

Sokolova V, Epple M (2008) Inorganic nanoparticles as carriers of nucleic acids into cells. Angew Chem Int Ed Engl 47(8):1382–1395

Torney F, Trewyn BG, Lin VSY, Wang K (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nat Nanotechnol 2:295–300

US20110065092A1: Use of nonviable particles comprising an internal control (ic) nucleic acid

US20110203013A1: Delivering compositions of interest to plant cells

US20120070900A1: T-DNA/protein nano-complexes for plant transformation

US4394448A: Method of inserting DNA into living cells

US5976567A: Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

US6534484B1: Methods for encapsulating plasmids in lipid bilayers

US7915450B2: Transfection reagents

US8021686B2: Lipid-encapsulated polyanionic nucleic acid

Vijayakumar PS, Abhilash OU, Khan BM, Prasad BLV (2010) Nanogold-loaded sharp-edged carbon bullets as plant-gene carriers. Adv Funct Mater 20(15):2416–2423

Wang Q, Chen J, Zhang H, Lu M, Qiu D, Wen Y, Kong Q (2011) Synthesis of water-soluble quantum dots for monitoring carrier-DNA nanoparticles in plant cells. J Nanosci Nanotechnol 11(3):2208–2214

Wiesman Z, Dom NB, Sharvit E, Grinberg S, Linder C, Heldman E, Zaccai M (2007) Novel cationic vesicle platform derived from vernonia oil for efficient delivery of DNA through plant cuticle membranes. J Biotechnol 130:85–94

WO2007050715A2: Compositions and methods for safe delivery of biologically active plant transformation agents using non-fibrous silicon carbide powder

WO2011016053: DNA loaded supported gold nanoparticles, process for the preparation and use thereof

WO2016196738A1: Compositions and methods for delivery of a polynucleotide into a plant

Zhao X, Cui H, Wang Y, Sun C, Cui B, Zeng Z (2018) Development strategies and prospects of nano-based smart pesticide formulation. J Agric Food Chem 66(26):6504–6512

Zhao X, Meng Z, Wang Y, Chen W, Sun C, Cui B, Cui J, Yu M, Zeng Z, Guo S, Luo D, Cheng JQ, Zhang R, Cui H (2017) Pollen magnetofection for genetic modification with magnetic nanoparticles as gene carriers. Nat Plants 3:956–964