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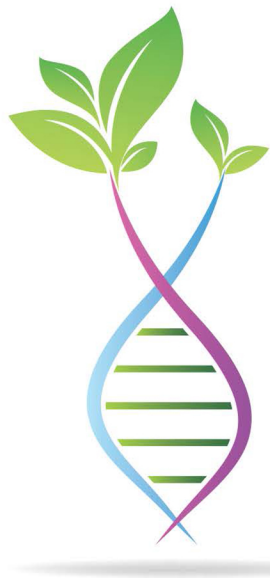

Yaroslav B. Blume
Editor

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Chapter 2

APPLICATION OF NANOMATERIALS FOR GENETIC ENGINEERING OF PLANT CELLS

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ABSTRACT

Modern strategies of plant engineering enabled a creation of plants with improved nutritional characteristics, producers of secondary metabolites, as well as plants resistant to diseases, pest, and other stressing factors. The efficiency of genetic transformation of plants remains a challenge due to limitations of intracellular transportation of genes and other biomolecules through the cell wall of plant cells,

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damaging of cells/tissues, gene disruption, and high cost of application of the transformation methods. Protocols of rapid regeneration are needed for many techniques of plant transformation that are species- and tissue-dependent, as well as rather laborious. The mineral nanoparticles (mesoporous silica NPs, metal oxide, calcium phosphate), carbon nanotubes, and cationic polymers have been proposed for plant transformation. In this review, the results of literature search and our experience on practical development of efficient gene transfer techniques based on using nanomaterials and applicable for plants are presented. When used in plants, several of such techniques still require a particle bombardment, application of the electromagnetic field, preparation of protoplasts for transformation, and regeneration of the obtained transformants. While small size of the applied nanocarriers of the nucleic acids, their positive charge, physical and chemical properties of their surface, as well as an ability to traverse a wall of plant cell without an external force make them an attractive remedy for genetic engineering in plants.

Keywords: plant cells, gene delivery, nanomaterials, metallic nanoparticles, carbon nanotubes, cationic polymers

INTRODUCTION

Efficient techniques of genetic engineering of plants are needed for agricultural research, biotechnology, and phytomedicine. Modern strategies of plant engineering enabled a creation of plants with improved nutritional characteristics, producers of secondary metabolites, as well as plants resistant to diseases, pest, and other stressing factors. The Agrobacterium-mediated delivery, biolistic, electroporation, ultrasound, and polyethylene glycol (PEG)-mediated techniques were reported as the main methods for plant transformation (Cunningham et al. 2018, Rafsanjani et al. 2012, Ziemienowicz et al. 2012, Tripathi et al. 2017, Wang et al. 2016).

Table 1. Methods for gene delivery in plants

Method	Short characteristics	Limitations	Comments	Reference
Biological-based method				
Agrobacterium-mediated delivery	*Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* are commonly used for gene transfer. Genetic transformation occurs via T-DNA export with the selection marker from bacterium to plant, targeting, and insertion into the plant nuclear genome. The T-strand (a single-stranded copy of the T-DNA sequence) is then transported to the nuclear and integrated at random positions into the plant nuclear genome via non-homologous recombination	• random integration into plant genome; • transgene silencing events; • damaged potential; • high risk of bacterial infection; • exhibits high host-specificity; • is mostly applied to dicotyledon, efficiency for monocotyledon dropped	• indirect method; • requires the regeneration protocols; • requires tissue culture (except Arabidopsis) to generate progeny; • applied modifications (application of acetosyringone, site-specific recombination strategies, transformation with multiple T-DNAs)	Cunningham et al. 2018, Ziemienowicz et al. 2012, Rafsanjani et al. 2012, Chang et al. 2013
Instrument (physical)-based method				
Biolistic method (gene gun or particle bombardment)	DNA-coated particles are used for plant cells and tissues bombardment by pressurized helium gas. Applied to wall-containing plant cells	• damage to both target tissue and genes, • low penetration depth, • random integration; • low transformation efficiency (mostly 2-20%); • high cost	• requires laborious regeneration protocols which are highly species-dependent; • can be used for plastids, or mitochondria transformation	Southgate et al. 1995, Rivera et al. 2012, Maliga 2017, Cunningham et al. 2018, Rafsanjani et al. 2012

Table 1. (Continued)

Method	Short characteristics	Limitations	Comments	Reference
Electroporation	The applied electrical impulse enhance the pore size and improve cellular uptake of genes	• possibility of application only for protoplasts; • damage to target tissue; • nonspecific transport of material through pores	• relatively high efficiency, however, the frequency of stable transformation is much more lower; • requires regeneration protocols; • application is limited only to a few species; • applied modification (post-pulse addition of ascorbic acid)	Rivera et al. 2012, Cunningham et al. 2018, Rafsanjani et al. 2012
Ultrasound-mediated	Ultrasound is applying to pierce instantaneous channels on the cell wall, cell membrane and nucleus membrane, which are helpful for the introduction of exterior genes into cells	• cell damage	• the ultrasound intensity and time exposure should be taken into account with different plants; • requires the regeneration protocols	Rivera et al. 2012, Cunningham et al. 2018, Rafsanjani et al. 2012
Chemical-based method				
Polyethylene glycol	Fusion of protoplasts under the influence of carrier charge interaction. PEG enhances the protoplasts permeation	• damage to the protoplast; • used only for protoplasts transformation; • inefficient regeneration protocols	• requires regeneration protocols	Maliga 2017, Sun et al. 2018, Yu et al. 2017, Rafsanjani et al. 2012

An efficiency of genetic transformation of plants remains a challenge due to limitations of intracellular transport of genes and other biomolecules through the plant cell wall, damaging of cells/tissues, gene disruption, and high cost of the applied methods (Cunningham et al. 2018, Tripathi et al. 2017, Wang et al. 2016). Transformation protocols have been developed for a number of plant species (Cunningham et al. 2018). Most of the conventional plant transformation methods target the nuclear genome and do not target the chloroplast or mitochondrial genomes. The dominating number of plant transformation protocols require the regeneration of transformed cells, protoplast, calli or suspension cells to tissue (Chang et al. 2013). Regeneration protocols are species and source tissue-dependent, time-consuming, and complicated laborious processes (Zhao et al. 2017). Thus, optimization of gene delivery and tissue regeneration protocols is obligatory in order to improve efficiency of genetic transformation of plants. Cell membranes and cell wall are main barrier for efficient genetic engineering as cytosolic or nuclear localization of genes is necessary for its effective function. Size exclusion limits (SELs) for cell wall were indicated as less than 50 nm (Cunningham et al. 2018, Hao et al. 2013). These barriers and limitations (Table 1) can be circumvented through application of chemical or mechanical (bombardment, ultrasound application) aids (Schwab et al. 2016).

NANOMATERIALS USED FOR GENE ENGINEERING IN PLANTS

A big progress has been achieved in the development of novel nanomaterials (NM) for intracellular transportation of different substances (plasmid DNA, si/miRNA, proteins, drugs, and other bio-molecules) into the mammalian cells. NMs have been used for protection of cargos, controlled targeted release, and multi-targeted delivery of these substances (Zhao et al. 2017). Size, charge, shape, bio-functionalization, and tensile strength of the NMs can influence their intracellular transportation. A

direct penetration (energy-independent) and endocytosis (energy-dependent) are two principal pathways for cellular uptake of the NMs. Passive type of delivery of biomolecules to plant cells is a promising approach for species-independent and minimally invasive genetic transformation of plants (Cunningham et al. 2018). Based on the morphology and chemical properties, the NMs are typically classified as metallic, magnetic, polymeric, lipid, and carbon-based (Figure 1).

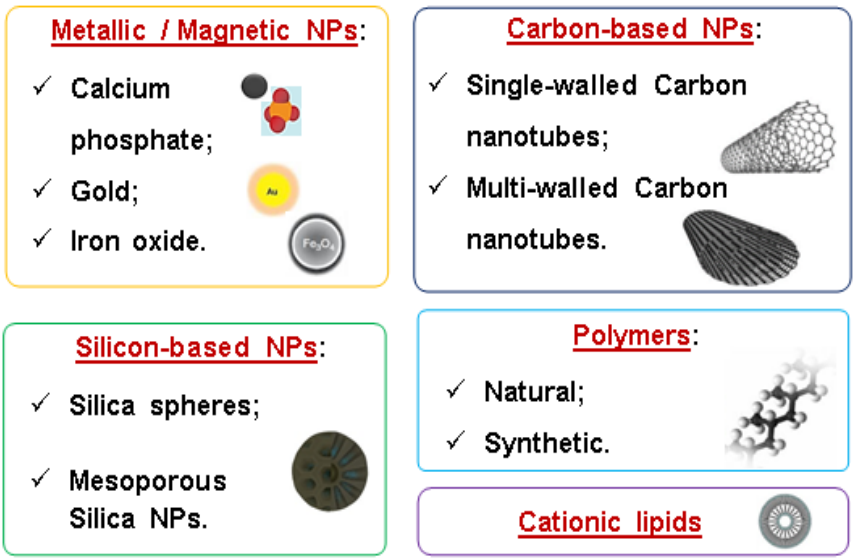

Figure 1. Commonly used nanomaterials for genetic material delivery to cells. NPs – nanoparticles.

A literature search demonstrated a broad application of the NMs for gene transfer to plant cells. Some of NMs-based strategies of delivery into plants still require a particle bombardment, application of the electromagnetic field, or preparing protoplasts for transformation (Cunningham et al. 2018, Rivera et al. 2012, Maliga 2017). However, small size of such carriers, their charge and other properties make them attractive in genetic engineering of plant cells. The metallic nanoparticles (mesoporous silica NPs, metal oxide, calcium phosphate), carbon

nanotubes, and cationic polymers have been proposed for plant transformation (Cunningham et al. 2018, Rafsanjani et al. 2012).

MESOPOROUS SILICA NANOPARTICLES

Mesoporous silica nanoparticles (MSN) are attractive vectors for delivery of various bio-molecules into the mammalian and plant cells. Additional functionalization of the MSN leads to a controlled intracellular transport, DNA/RNA affinity, magnetization and changes of surface areas and pore size (Martin-Ortigosa et al. 2012). DNA forms electrostatic complexes with the MSN, and such complexes can be used for conducting transient and stable plant transformation.

Torney et al. (2007) functionalized the MSN with the triethylene glycol (TEG) and used them for transformation of *Nicotiana tabacum* protoplasts. They demonstrated a delivery of plasmid DNA (pDNA) containing gene coding for the green fluorescent protein (GFP) through bombarding gold NP-capped MSNs to *N. tabacum* cotyledons. The authors also used the MSNs-DNA complexes to bombard immature embryos of the maize (*Zea mays*) (Torney et al. 2007).

Martin-Ortigosa et al. (2012) described using the biolistic method with gold-plated MSNs (Au-MSNs) for co-delivery of pDNA, fluorescently-labeled bovine serum albumin (BSA) or eGFP to *Allium cepa* epidermis cells. They also delivered plasmid DNA containing *AmCyan1* gene, Cre-encoding plasmid DNA and Cre protein delivery to maize (*Z. mays*) immature embryos (Martin-Ortigosa et al. 2014).

Chang et al. (2013) applied N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (TMAPS)-modified MSNs for delivery of pDNA coding for red fuorescence protein (mCherry) into intact *Arabidopsis thaliana* roots without an additional mechanical force (a bombardment or ultrasound). pDNA was adsorbed on the TMAPS/F-MSNs via electrostatic interactions and applied for genetic transformation. Gene expression was detected in the epidermal, cortical, and endodermal cells of *Arabidopsis* roots. There was no toxicity of the MSNs reported for

the tobacco protoplasts. The plastids of the vascular tissue were also treated with the MSNs for targeted plant transformation (Chang et al. 2013).

MAGNETIC NANOPARTICLES

The wall of plant cell limits intracellular uptake of the NPs, and the magnetic field can be used to improve cellular uptake of the DNA associated with the NPs (Hao et al. 2013).

Gold-coated magnetic iron oxide NPs (magnetic gold NPs, mGNPs) with covalently bound plasmid DNA (coding for β-glucuronidase (GUS) gene) were capable of genetic transformation of the protoplasts of canola (*Brassica napus*), however, they were not effective in transformation of the walled cells (Hao et al. 2013). The results of transmission electron microscopy (TEM) study showed that mGNPs did not pass through a canola cell wall. These mGNPs were reported as biocompatible and non-cytotoxic carriers of plasmid DNA. They were also applied for FITC delivery, and FITC-pDNA co-delivery to protoplasts of the canola and carrot (*Daucus carota*) (Hao et al. 2013).

Polyethyleneimine-coated Fe_3O_4 MNPs were effective for delivery of the GUS plasmid DNA into cotton (*Gossypium hirsutum*) pollen. The magnetic field was applied for transportation of pDNA-MNP complex into pollen before pollination. Transgenic seeds were selected and the transgenic cotton plants were obtained in the presence of the kanamycin. The MNPs were relatively non-toxic for cotton pollen, and at least 80% of viable pollen was observed. The MNPs also protected DNA from its degradation by the DNase I. Molecular-genetic study of the transgenic cotton confirmed DNA integration into the plant genome and its inheritance in the T1-T3 offspring. Pollen magnetofection was successfully applied for two cotton varieties with seven different pDNA vectors. Circular pDNA was found to be significantly more effective in pollen transformation than the linearized plasmid. Such technique was also used for creation of the transgenic pepper (*Capsicum annuum*) and pumpkin

(*Cucurbita moschata*). However, further optimization of pollen magnetofection is needed for its application toward other flowering plants (Zhao et al. 2017).

CALCIUM PHOSPHATE NANOPARTICLES

The NPs of calcium phosphate (CaP) have been effectively used for gene delivery to the mammalian cells (Khan et al. 2016, Mostaghaci et al. 2016, Kovtun et al. 2009). Such NPs can be transferred into these cells via a fluid-phase endocytosis. In addition, calcium ions provided a cytosolic stability of the DNA and relieved the nuclear membrane penetration (Lee et al. 1998).

Naqvi et al. (2012) reported about using CaP NPs as a carrier for pDNA containing GUS gene into the hypocotyl explants of *Brassica juncea* L. cv. Pusa Jaikisan. The NPs of pDNA-CaP complexes were prepared in aqueous core of the Aerosol-OT/hexane/water reverse micellar system. The efficiency of transient transformation reached 80.7% compared to 54.4% when *A. tumefaciens* transformation was applied (Naqvi et al. 2012). It was shown that a transgenic GUS integrates into the plant genomic DNA by means of a non-homologous recombination, as it was reported in the case of *Agrobacterium*-mediated infection. CaP NPs were non-toxic for the hypocotyls cells demonstrating normal morphology and cytoskeletal structure. The pDNA encapsulated by the CaP was protected from the DNAse I degradation (Naqvi et al. 2012).

Sone et al. (2002) described an ability of calcium alginate beads referred to as “bio-beads” to deliver GFP-coding plasmid to protoplasts isolated from the BY-2 suspension culture of the tobacco cells. A transient expression of the GFP was detected.

The biocompatible fluorescent quantum dots (QDs) were used to label chitosan-DNA MPs via the electrostatic interaction. Complexes with the GFP-coding plasmid were used for transformation of *Jatropha curcas* L. callus cells. The results of the PCR analysis confirmed that target DNA was integrated into the plant genome (Wang et al. 2011).

CARBON NANOTUBES

Carbon nanotubes (CNTs) were applied as carriers for gene delivery into plant cells (Demirer et al. 2019, Burlaka et al. 20015, Burlaka et al. 2011). Demirer et al. (2019) have detected a delivery of pDNA containing GFP gene into the mature tobacco (*N. benthamiana*), arugula (*Eruca sativa*), wheat (*Triticum aestivum*), and cotton (*G. hirsutum*) leaves. The applied pristine or multi-walled CNTs were covalently functionalized with the polyethylenimine (PEI). These CNTs demonstrated low toxicity and they were capable of delivering pDNA into plant cells without an additional mechanical aid. The CNTs were also effective in transformation of protoplasts isolated from the arugula leaves. GFP expression was found to be higher when DNA-CNTs were prepared via the electrostatic interaction compared to that induced by complexes prepared by means of the dialysis. Furthermore, the CNTs protected DNA against nuclease degradation (Demirer et al. 2019).

Burlaka et al. (2015) have used both single-walled CNTs (SWCNTs) and multi-walled CNTs (MWCNTs) for transformation of protoplasts, callus and leaf discs of the tobacco *Nicotiana tabacum* L. These CNTs were additionally functionalized with the double-stranded plasmid DNA, bovine serum albumin (BSA, a globular protein of Mr = 64 kDa), and with a commercial aqueous vitreous humor extract (VHE). A transient expression of the yellow fluorescent protein (YFP) reporter gene was detected in the tobacco protoplasts. The *N. tabacum* callus and leaf explants were transformed by the SWCNTs-pDNA and MWCNTs–pDNA complexes, and the obtained transformants were selected on the kanamycin-containing medium. The SWCNTs were capable of transforming both protoplasts and walled plant cells, while the MWCNTs could transform only the protoplasts because of a limiting effect of the cellulose wall against their penetration into the plant cells.

Kwak et al. (2019) designed a chitosan complex for pDNA delivery to chloroplasts of different plants without an additional chemical or the biolistic aid. Chitosan is a biodegradable and biocompatible copolymer of N-acetyl glucosamine and glucosamine linked by 1,4-glycosidic bonds.

Chitosan can be obtained by the deacetylation of chitin, the component of the exoskeletons of the crustaceans, fungi, and bees. Chitosan is mostly soluble in aqueous acidic medium (Shi et al. 2017). It demonstrated low toxicity and high cationic charge. However, low transfection efficiency is the main challenge at its application for gene delivery (Moreira et al. 2009, Morris et al. 2010). Kwak et al. (2019) demonstrated transgene delivery and transient expression in mature *N. tabacum*, *Eruca sativa*, *Nasturtium officinale*, *Spinacia oleracea* chloroplasts, as well as in the isolated *Arabidopsis* mesophyll protoplasts.

CATIONIC POLYMERS

The polymeric carriers of different structure (Figure 2) were proposed as non-viral DNA and RNA vector systems in various biotechnological and biomedical investigations (Tomlinson et al. 1996, Lv et al. 2006). The cationic polymers possess good water solubility and protocols of their synthesis and modification are relatively easy and not costly (Zakeri et al. 2018). There are linear, branched, and dendrimer-like polymers. Most of the cationic polymers have primary, secondary or tertiary amine functional groups that can be protonated. The positively charged molecules can be located in the backbone or side chains in structure of the cationic carrier.

Application of the NMs in plant engineering is limited by low activity of their endocytosis (Chang et al. 2013, Fan et al. 2015). Cellular internalization of the cationic NPs was reported to be faster than that of the anionic NPs (Cunningham et al. 2018). The cationic nanocarriers electrostatically interact with negatively charged DNA/RNA. Thus, a positive charge of the cationic NMs is one of their significant preferences. Besides, the cationic NMs act as endosome disruption agents that improves an endosomal escape of the engulfed complexes containing nucleic acids (Cunningham et al. 2018).

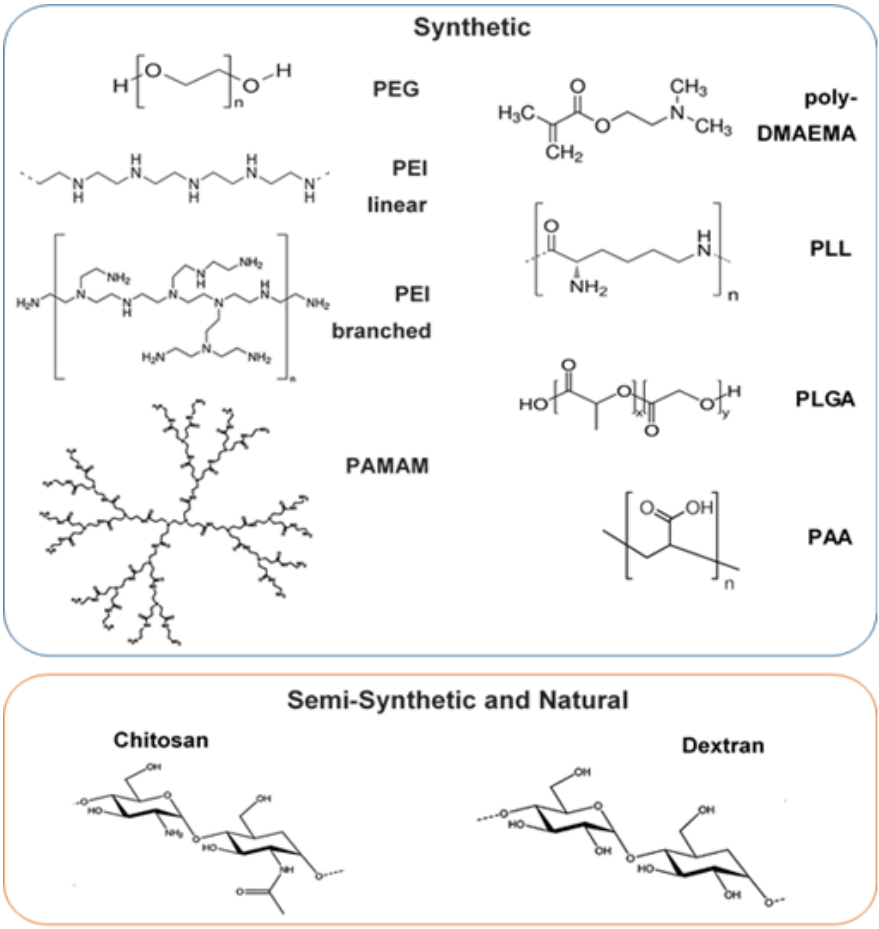

Figure 2. Formulas of commonly used polymers for gene delivery. PEG – polyethylene glycol; PEI – polyethylene imine; PAMAM – polyamidoamine; DMAEMA – dimethylaminoethyl methacrylate; PLL – poly-L-lysine; PLGA – poly(lactic-co-glycolic acid; PAA – poly-acrylic acid.

The polymeric carrier-based gene delivery is a multistage process. The cationic polymers protect the genetic material from the aggressive acidic environment of cell-uptake vesicles and promote a release of genetic material to the cytoplasm (Zakeri et al. 2018). The target cells can endocytose positively charged particles by electrostatic interactions *in vitro* to achieve effective gene delivery (Figure 3). Alternatively, the polymeric carriers can use the non-endocytic pathways (fusion and penetration). The

molecular weight, size, shape and zeta potential of nanomaterials, concentration of nucleic acid/carrier complexes at the cell surface and even and the phase of cell cycle are important to improve cellular uptake of DNA polyplexes and gene transfer (Xiang et al. 2012, Kamaly et al. 2012, Kim et al. 2013, Zakeri et al. 2018). After endosome release, the polyplexes must enter the nucleus for further gene expression. Pichon et al. (2010) reported that anionic microtubules promoted the cytoplasmic movement of DNA. Next, nucleic acids can enter nucleus through nuclear pores passively (molecules smaller 10 nm) or via active transport using specific nuclear localization signals in the non-dividing cells. In dividing cells, nuclear permeability is enhanced that makes nucleus entry easier for the polyplexes. Glover at al. (2010) reported that approximately 0.1% of DNA microinjected to the cytosol can pass to the nucleus, and that amount increases to approximately 1% when the carriers of the nucleic acid were applied. The polyplex dissociation in the nucleus is a final requirement for efficient gene expression (Zakeri et al. 2018).

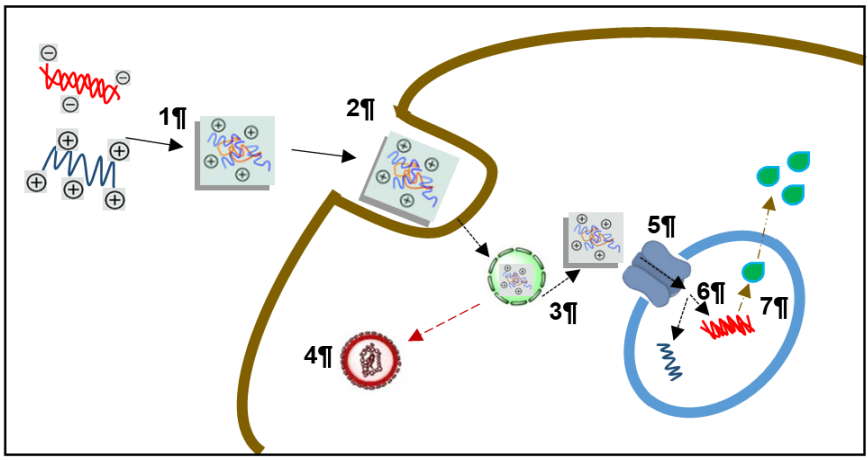

Figure 3. General scheme of the cationic polymer-based gene delivery: 1 – cationic polymer/nucleic acid complex formation; 2 – cellular uptake of complex; 3 – escape of polyplex from endosome; 4 – lysosome degradation of polyplex; 5 – nucleus entry of polyplex; 6 – dissociation of complex; 7 – gene expression and product export.

Many strategies (synthesis of biodegradable carriers, graft polymerization, adding other polymers with a desired functionality to the base polymer (e.g., PEGylation), bonding polymer with several functional groups) were introduced in order to improve a gene delivery efficiency using cationic polymers and reduce their cytotoxicity. Chemical modification of the inorganic nanoparticles with functional groups or even with polymers can be applied to promote gene transfer (Shi et al. 2017, Mousavi et al. 2016, Pandey et al. 2017, Zakeri et al. 2018, Fan et al. 2017, Lai et al. 2018).

POLY-L-LYSINE COATED NANOPARTICLES

Starch NPs coated with the poly-L-lysine were applied for genetic transformation of *Dioscorea zingiberensis* C.H. Wright calli mediated by the ultrasound (Liu et al. 2008). 5% efficiency of transient expression of the GFP was demonstrated. These NPs were capable of protecting DNA from the DNase I cleavage and ultrasound damage. An efficiency of transformation based on using a linear pDNA was higher than that at using circular pDNA. Thus, shorter linear segments might easier combine with the genomic DNA of plant cells and provide better gene expression (Liu et al. 2008). PLL is a positively charged synthetic polypeptide proposed as a potential gene delivery carrier. PLL is partly biodegradable carrier because of the lysine presence and amide linkages. PLL is also used for functionalization of other gene delivery vectors (Shi et al. 2017).

POLYETHYLENEIMINE

Li et al. (2011) successfully applied PEI for transient transformation of *A. thaliana* protoplasts with pDNA coding for the GFP. The authors analyzed different N/P ratios (1:10) for plant transformation. The maximal transformation efficiency (approximately 65% of GFP-positive protoplasts)

was observed under application of PEI/DNA complex with N/P = 5. An efficiency of the PEI-based transformation of *A. thaliana* protoplasts was higher than that at using the PEG. At higher concentrations, the PEI was toxic for *A. thaliana* protoplasts.

PEI is widely used for gene delivery to the mammalian cells. PEI contains amines isolated by the ethylene groups. This is a hydrophobic molecule due to its rich ethylene-backbone. PEI contains primary, secondary, and tertiary amines at an approximate 1:2:1 ratio (Olden et al. 2018). Branched or linear PEI with different molecular weight were used for gene delivery. Generally, linear PEI is a less toxic polymer with higher transfection efficiency, while the branched PEI possesses higher chemical reactivity (Zakeri et al. 2018). PEI with a molecular weight of 25 kDa was reported to be an optimal polymer for gene delivery (Meleshko et al. 2017, Zakeri et al. 2018, Shi et al. 2017). PEI has high charge density and chain flexibility. It electrostatically interacts with pDNA to form polyplexes with a size of 10–100 nm which can be absorbed via endocytosis. A widespread application of PEI for gene delivery is limited due to its cytotoxicity (Lai et al. 2014, Goyal et al. 2014, Lazarus and Singh 2016, Meleshko et al. 2017, Zakeri et al. 2018, Forkato et al. 2017). Generally, the application of PEI with higher molecular weight resulted in both higher transfection efficiency and higher cytotoxicity (Yu et al. 2016, Meleshko et al. 2017, Zakeri et al. 2018).

POLY-DIMETHYLAMINOETHYL METHACRYLATE POLYMERS

We applied N,N-dimethylaminoethyl methacrylate (DMAEMA)-based polymers of different molecular structure for delivery of plasmid DNA into the moss (*Ceratodon purpureus*) and tobacco (*N. tabacum*) protoplasts (Finiuk et al. 2014, Finiuk et al. 2017). The poly-DMAEMA-block- poly-1-vinyl-2-pyrrolidone (NVP)-co-butyl acrylate (BA)-co-2-aminoethyl methacrylate hydrochloride (AEM), poly-DMAEMA-block-poly(NVP-co-vinyl acetate (VA)-co- 2-hydroxyethyl methacrylate (HEMA))- block-

oligo(NVP-co-BA-co-AEM), PEG-block- poly(NVP)- block-poly-DMAEMA and poly(NVP)-block -PEG-block - poly(NVP)- block-poly-DMAEMA were prepared and used for gene delivery to plant cells. The poly-DMAEMA nanocarriers interact electrostatically with pDNA and protect it against nuclease degradation. These nanocarriers are effective in a delivery of plasmid DNA into the moss and tabacco protoplasts resulting in both transient and stable transformation of *C. purpureus*, as well as a transient expression of the reporter yellow fluorescent protein (YFP) gene product in protoplasts of *N. tabacum*. Application of the poly-DMAEMA nanocarriers for transformation of *C. purpureus* protoplasts was more effective than the application of the polyethylene glycol 6,000 (PEG). We have shown that the diblock-copolymers consisting of the water-soluble flexible block including chains of vinyl-pyrrolidone and monomer with initial primary amine group and a chain of the poly-DMAEMA were more effective agents than PEG-containing block polymers for gene transfer into the moss and tobacco protoplasts. The application of PEG-containing polymers as a transformation agent for protoplasts was associated with an increased damage of the tobacco protoplasts and *A. cepa* cells (Finiuk et al., 2017). Such effects might be due to PEG-induced reversible destabilization of plasma membrane of targeted cells (Burris et al. 2016, Chakrabarty et al. 2008, Masani et al. 2014).

The poly-DMAEMA-based cationic polymers are perspective non-viral vectors for gene delivery. Poly-DMAEMA contains only tertiary amines in its structure. Positively charged polyplexes promote an electrostatic binding to the negative cell membrane and cellular uptake via endocytosis (Olden et al. 2018, Fan et al. 2017). These polymers are pH sensitive and hydrophilic compounds (You and Auguste 2008). Liu and collaborators reported that the length/amount of poly-DMAEMA chains in the polymer structure affected gene delivery properties and cytotoxicity of carriers. A long poly-DMAEMA chain provides better DNA binding and condensation capability. The lower DMAEMA block number resulted in a higher efficiency of gene delivery to the mammalian cells *in vitro* and in a lower cytotoxicity of the applied copolymer (Liu et al. 2015).

POLYAMIDOAMINE DENDRIMER

Polyamidoamine (PAMAM) dendrimer is another potential gene delivery carrier. It is highly branched, water soluble, and biocompatible polymer. PAMAM forms stable complexes with nucleic acids (dendriplexes) that preserves the DNA/siRNA from degradation (Abedi-Gaballu et al. 2017). PAMAM-based carriers are characterized by good endosomal escape (Chen et al. 2017, Ullah et al. 2017).

Jiang et al. (2014) demonstrated a successful delivery of G2/dsRNA to the *Arabidopsis* root cells by means of the cationic fluorescent polyamidoamine (PAMAM) dendrimers. However, such transformation led to a significant reduction in the expression of important developmental genes that resulted in the phenotype changes.

PAMAM dendrimeric nanocarriers were applied for delivery of the GFP-encoding plasmid DNA to the turfgrass (*Agrostis stolonifera* L.) calli without an additional external aid (Pasupathy et al. 2008). A formation of the PAMAM dendrimer-pDNA complexes occurs via the electrostatic interactions. The level of transient GFP expression in the calli cells reached 48.5%. An increased dendrimer concentration significantly reduced the uptake of PAMAM-pDNA by the turfgrass cells. The transformation efficiency might be improved by the pH optimization in culture medium and a selection of molar ratio of the pDNA/dendrimer for complexation (Pasupathy et al. 2008).

OTHER CARRIERS USED FOR PLANT TRANSFORMATION

Silva et al. (2010) have successfully applied fluorescent conjugated polymeric amine-containing poly-1,4-phenylene-based NPs (CPNs) for delivery of the siRNAs to protoplasts of tobacco BY-2 suspension cells. The CPNs in 5-25 μM dose were essentially non-toxic for the protoplasts with a little impact on their viability over a 72 h treatment period. The results of the microscopy and flow cytometry study showed that the CPNs penetrated into protoplasts in 2 h after the delivery. The investigators also

demonstrated that the CPNs were effective for delivery of siRNAs for NtCesA-1 gene whose product is involved in cellulose biosynthesis pathway.

Summarizing, the NP-mediated delivery of genetic materials to plant cells might be useful for overcoming several limitations of other known techniques of gene delivery to these cells. Potential reasons for that are as following:

1. size and surface properties (geometry/chemistry) of the developed NMs may enhance an effectiveness of gene transfer through a cell wall;
2. controlled surface charge and other properties of these NMs allow to carry various cargo (DNA, RNA, proteins) to nucleus and organelles of the targeted cells;
3. developed NMs can reduce cell/tissue damage and protect genetic materials against degradation by the nucleases.

Thus, the NMs demonstrate great potential as gene delivery systems especially in plants that are not as easily transformed as the microorganisms and the mammalian cells. Such conclusion is important for further development of agricultural biotechnology and production.

PERSPECTIVES

The application of specific nuclease systems (ZFNs, TALENs, and CRISPR-Cas) may be helpful for overcoming challenges of efficient and stable gene editing in plants, since other conventional methods of gene engineering in plants are either destructive or species- and tissue-specific and are not applicable for targeting germ line cells. Furthermore, a global regulatory oversight for genetically modified organisms (GMO) is motivating scientists to develop gene transfer methods in which the delivered gene is expressed transiently and is not integrated in genome (Demirer et al. 2019).

Nuclease systems of genome editing refer to techniques in which genes are changed or removed by the engineered nucleases. These enzymes perform the targeted double-stranded breaks (DSBs) at specific locations of the host genome. After that, cell undergoes a homology-directed repair (HDR) or non-homologous end joining (NHEJ) in order to repair the cut. The NHEJ is a random, error-prone repair process leading to a gene knockout. The HDR is a non-random repair process allowing a precise editing by introducing customized homologous recombination sequences for targeted mutations, gene knock-out and gene knock-in. The CRISPR (clustered regularly interspaced short palindromic repeat)-Cas (CRISPR associated) system demonstrated a success in genome editing in several plants such as *A. thaliana*, tobacco (*N. benthamiana, N. tabacum*), rice (*Oryza sativa*), wheat (*T. aestivum*), corn (*Z. mays*), and tomato (*Solanum lycopersicum*) (Bortesi and Fischer 2015, Arora and Narula 2017, Feng et al. 2014). Application of the NMs at using nuclease systems of genome editing will open a new era in plant biology and biotechnology.

CONCLUSION

Genetic engineering of plants has a great potential especially for development of crops with an enhanced nutritional value, improved growth characteristics, resistance to disease and environmental stresses. However, an efficiency of the conventional methods of genetic engineering in plants stays limited. This is primarily caused by an existence of a wall in plant cells that blocks a delivery of genetic materials to these cells, and it is expected that such barrier could be circumvented by the nanocarrier of the genetic materials. Application of *Agrobacterium* is a preferred method for genetic transformation in plants, however, it demonstrates effectiveness only in a limited range of species and is characterized by a random integration of genes in plant genome. Besides, utilization of *Agrobacterium* for gene delivery possesses high damaging potential, as well as a high risk of the bacterial infection. Gene delivery using the biolistic particles and PEG-mediated transformation demonstrated difficulties in the regeneration

of transformed cells/tissues and generally shows low efficiency. The results of our literature search and own experience suggest a significant contribution of the NMs in development of efficient gene transfer techniques applicable for plants. Several NMs-based gene delivery strategies proposed for a use in plants still require a particle bombardment, application of the electromagnetic field, preparation of protoplasts for transformation and regeneration of the obtained transformants. While small size of the nanocarriers, their positive charge, physical and chemical properties of their surface, as well as an ability to traverse a wall of plant cell without an external force make them an attractive remedy for genetic engineering in plants.

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