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Role of nanoparticles in management of plant pathogens and scope in plant transgenics for imparting disease resistance

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Abstract: Current efforts are focused on the search for efficient methods of pathogen management that will not result in damage to the environment or cause an imbalance in the existing biota. One of the strategies for this is the use of nanoparticles in agriculture for disease management. This review presents a summative view on the various applications of nanoparticles in conferring disease resistance to crops and the possibility of using nanoparticles as carriers of genetic material for the generation of disease resistant crops. Nanoparticles are directly being used for the control of pathogens. Nanoparticles have been used as antiviral, antifungal and antibacterial agents. The nano-encapsulation of pesticides in controlled release matrices is one of the most promising research areas for the future. Nano-encapsulation has been shown to increase the efficiency of pesticides, reduce their volatilisation and decrease the toxicity and environmental contamination in crops. Nano-encapsulated agrochemicals or biomolecules can be engineered to be released in a controlled manner and in a target-specific location. Nanoparticles also have great scope in the field of transgenics vis-à-vis pathogen resistance. The field of agriculture can be revolutionised by the use of nanoparticles for imparting disease resistance in crops. The field is so versatile that the possibilities are endless.

Keywords: gene-editing; nano-encapsulation; plant protection; gene silencing; disease management; nano-pesticide

The management of pathogens is necessary for the protection of agricultural products against pre- and post-harvest diseases. The prevention of these diseases, which are mainly caused by bacteria, viruses and fungi, is a problem that remains unresolved. The exploitation of host-plant resistance is the most commonly used strategy for the management of diseases in plants. Conventional breeding requires a source of disease resistant genes with desirable disease resistance and entails growing and examining a large population of crops over multiple generations (Vincelli 2016; Dong & Ronald 2019). Transgenics is the deliberate modification of the trails of an organism by manipulation of the genetic material. Transgenics can be a method of choice to overcome the limitations posed by conventional breeding (Sun et al. 2019). Transgenic plants exhibit long-lasting resistance to one or multiple pathogens through the introduction of gene(s) from distantly or non-related species and reduces the chances of pathogen developing resistance (Wally & Punja 2010). However, due to consumer anxieties and

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legislation from various countries about the safety of transgenic crops, the benefits of transgenic technology have not been fully exploited (van Esse et al. 2019). Another issue that needs to be addressed is the environmental impact of the excessive use of chemical pesticides. One of the alternatives that is finding increasing application in this regard is nanotechnology. Nanotechnology involves tailoring materials at an atomic level to attain unique properties, which can be suitably manipulated for the desired applications (Gleiter 2000). Most natural processes also take place on a nanometre scale regime. Therefore, the coming together of nanotechnology and biology can address several problems and can revolutionise the field of agriculture. The new chemical and/or physical properties of nano-scale particles provide useful functions that are being rapidly exploited in medicine, biotechnology, electronics, material science and the energy sectors, among others. The agricultural sector is also concerned with these promising developments, where continuous innovation is strongly needed because of the increasing global food security and climate change challenges. Nanotechnology provides an exciting avenue in searching for solutions to several agricultural and environmental challenges, such as sustainability, improved varieties and increased productivity.

In the recent past, agricultural nanotechnology has shown to be growing especially for disease management and crop protection (Sastry et al. 2010; Gogos et al. 2012). Nanoparticles interact at the molecular level in living cells and nanoparticles are employed in agriculture with the ambition that these particles will impart some beneficial effects to the crop. Nanotechnology derived devices are being explored in the field of plant breeding and genetic transformation (Torney et al. 2007). Numerous advantages in the use and application of nanoparticles have been observed. Various nanoparticles have also been used as nano-fertilisers. The application of nanoparticles to crops increases their growth and yield. Biologically synthesised silver nanoparticles have been employed to improve the seed germination as well as seedling growth of *Boswellia ovalifoliolata,* which is an endemic, globally threatened medicinal tree species (Savithramma et al. 2012). The effects of SiO_2 nanoparticles and TiO_2 nanoparticles on soybean seeds have been investigated. It was observed that a mixture of nanoparticles led to an increase in the nitrate reductase in the soybean which, in turn, increased its germination and growth (Lu et al. 2001). Similar results were seen with ZnO nanoparticles on the growth of *Vigna radiata* and *Cicer arietinum* seedlings (Mahajan et al. 2011). The colloidal solution of ZnO nanoparticles has been used as fertiliser (Mfon et al. 2017). Nano-fertilisers have the added advantage of being a plant nutrient which is more than a fertiliser because it not only supplies nutrients for the plant, but also revives the soil to an organic state without the harmful factors of a chemical fertiliser. In addition to this, nano-fertilisers are used in very small amounts when compared to conventional fertilisers, which leads to the lower contamination of the soil with chemicals (Selivanov & Zorin 2001; Raikova et al. 2006). In addition to this, using nano-fertilisers reduces the chemical residue in the grown vegetables and reduces the harmful effects if they are consumed raw or at an immature stage (Mfon et al. 2017).

Nanoparticles for disease management

Inorganic nanoparticles are non-toxic, hydrophilic biocompatible and highly stable. Examples of inorganic nanoparticles are calcium phosphate nanoparticles, gold nanoparticles, iron oxide nanoparticles, zinc oxide nanoparticles, silver nanoparticles, etc. (Paul & Sharma 2010; Kurtjak et al. 2017). Inorganic nanoparticles have ions and these ions are antimicrobial agents. Zinc and copper lead to reactive oxygen species (ROS) generation to kill the engulfed pathogen. Gold and silver are very toxic to bacteria at low concentrations. The application of nanoparticles leads to a disruption in the cell membranes of pathogens. A nanoparticle application also leads to the generation of reactive oxygen species (ROS) and free radicals and metal ions. In addition to this, nanoparticles of very small size also intercalate into the DNA (Figure 1). Metals are known to bind to thiol or the amine moiety of cellular proteins and lead to the deactivation and precipitation of proteins. The high attraction of metal ions by proteins leads to an increase in the cellular concentrations and eventually cell death (Mittapally et al. 2018). Polymeric nanoparticles (PNPs) are used for the production of nano-capsules or nanospheres and are composed of various natural or synthetic polymers. Many of these polymers are biodegradable and non-toxic (Stanisic et al. 2018). Examples of polymers used to prepare PNPs include cellulose, gelatine, chitosan, alginate, etc.

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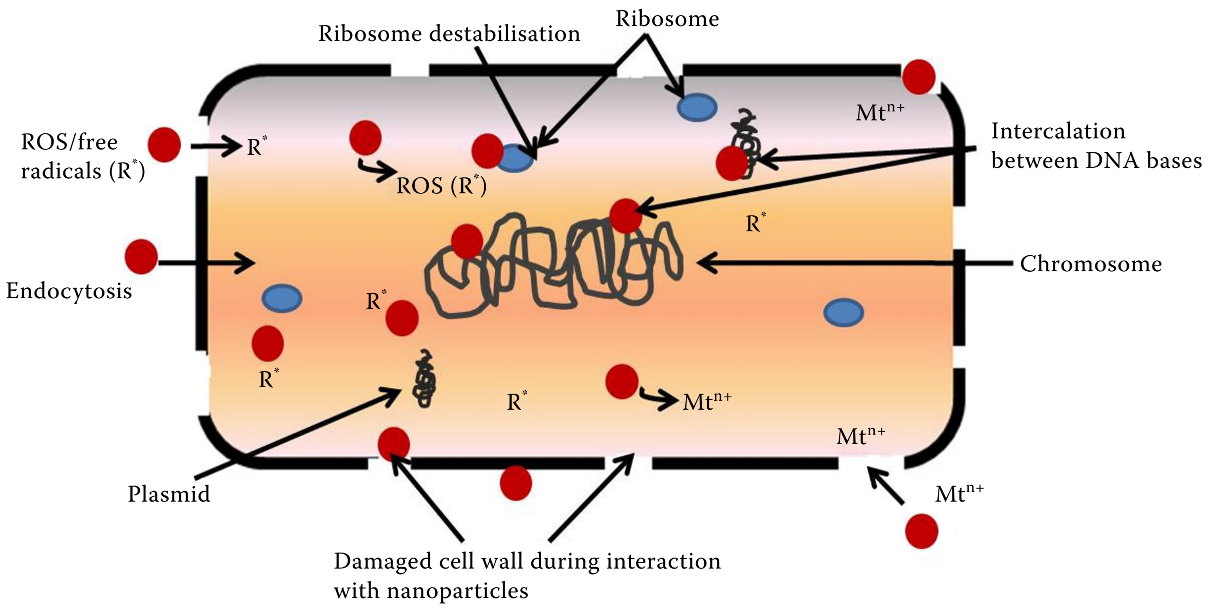

Figure 1. Multiple effects of nanoparticles on bacterial cells

PNPs range in size from 10 nm to 1 µm. They are colloidal particles and solid in nature. Nanospheres have a matrix system where the drug is uniformly dispersed. Nano-capsules have the drug embedded in a cavity which, in turn, is surrounded by a polymeric membrane. These polymers that make up the PNPs are hydrophilic, are made up of carbohydrates or proteins and are hydrophobic (Sharma 2019). PNPs provide an excellent vehicle for the delivery of inorganic nanoparticles. PNPs can be easily modified according to the target site and can also be controlled by their size, morphology and surface charge (Huang et al. 2016). Also, inorganic nanoparticles inside a PNP show low aggregation and reduce concerns regarding the cytotoxicity (Surudžić et al. 2016; Zare et al. 2017). Nanoparticles can be directly applied to the seeds, foliage or roots in order to protect the plants from a pathogen invasion. These nanoparticle actions can be comparable to chemical pesticides. Nanomaterials can also be used for the controlled release of various substances by using them as carriers for chemicals like pheromones, systemic acquired resistance inducing chemicals, active ingredients of pesticides, etc. Nanoparticles have been shown to have a suppressive effect on pathogens such as bacteria, fungi and viruses. Nanoparticles have been shown to have multiple effects in pathogen cells. These nanoparticle properties lead to the cell death in pathogens. Plant pathogens, in turn, are limiting factors in the production of food materials. Magnetic nanoparticles can be utilised for the site-specific delivery of systemic plant protection substances for the treatment of diseases, or for the targeted delivery of nucleic acids in particular regions of the plants. The movement of internalised nanoparticles can be tracked externally by the use of external magnets.

Antifungal. Silver nanoparticles exhibited antifungal effects against *Candida albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata* and *Aspergillus brasiliensis* (Bryaskova et al. 2011). Silver nanoparticles also inhibited the activity of *Alternaria alternate*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea* and *Curvularia lunata* (Krisnaraj et al. 2012). ZnO nanoparticles were fungicidal to *B. cinerea* and *Penicillium expansum*. The nanoparticles were seen to prevent the development of fungal hyphae and also prevented the development of the conidia and conidiophores. Platelet shaped ZnO particles have been seen to have more antifungal activity when compared to rods and nanoparticles against *Fusarium solani* (Pariona et al. 2020). Chitosan has been studied for the formation of microparticles and nanoparticles owing to its biodegradable and biocompatible properties, its non-toxicity to animals and humans and its antimicrobial activity (López-León et al. 2005; Akamatsu et al. 2010; Zhou et al. 2011). In addition, an increase in the biological activity of chitosan in a solution, when present in the form of microparticles or nanoparticles, has also been reported (Du et al. 2008; Huang et al. 2009).

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These chitosan nanoparticles have also demonstrated a fungistatic effect both *in vitro* and *in vivo* against a wide variety of fungi, such as *Aspergillus niger* (Martínez-Camacho et al. 2010), *A. parasiticus* (Cota-Arriola et al. 2011), *Alternaria alternate, Botrytis cinerea, Colletotrichum gloeosporioides, Rhizopus stolonifer* (El Ghaouth et al. 1994; Bautista et al. 2006). It has also been shown to have a fungicidal effect on *Fusarium oxysporum*, *R. stolonifer*, *Penicillium digitatum* and *C. gloeosporioides*. Cu/Cu_xO nanoparticles were studied for their antifungal activity against *Fusarium oxysporum*. An inhibition of radial growth was observed with these nanoparticles along with changes in the hypha morphology, membrane damage and production of ROS (Hermida-Montero et al. 2019). Metalloid and metal oxide nanoparticles have been seen to be effective against Fusarium wilt of watermelons. Nanoparticles of B, CuO, MnO, SiO, TiO, and ZnO were used in a foliar spray for this study performed by Elmer et al. (2018).

Antiviral. Silver nanoparticles have been seen to be effective against viral diseases. These nanoparticles were evaluated as an antiviral agent to induce systemic acquired resistance against *Tomato mosaic virus* and *Potato virus Y*. The sap of the infected tomato plants examined under a transmission electron microscope showed evidence of silver nanoparticles binding to coat the protein of the virus particle. Also, the induction of systemic acquired resistance against *Tomato mosaic virus* and *Potato virus Y* was confirmed chemically. A spray application of a 50-ppm aqueous solution of silver nanoparticles on cluster bean leaves inoculated with *Sunn-hemp rosette virus* showed complete suppression of the disease. Plant resistance against the viral disease caused by *Barley yellow mosaic virus* particles has been induced by introducing a therapeutically effective amount of poly-dispersed gold nanoparticles to the plant. The gold nanoparticles were introduced via a mechanical abrasive. The average effective diameter of the nanoparticles was between about 0.5 nm and 200 nm. Alkubaisi et al. (2015) observed that the gold nanoparticles melt and dissolve the virus particles conferring resistance to the plant. Chitosan nanoparticles have antiviral activity. Chitosan induces resistance in plant tissues by protecting them against infections caused by the *Alfalfa mosaic virus*, *Snuff mosaic virus*, *Peanut mosaic virus*, *Potato mosaic virus* and *Cucumber mosaic virus* (Pospieszny et al. 1991; Kochkina et al. 1994; Chirkov 2002). ZnO nanoparticles and silica nanoparticles have been used against *Tobacco mosaic virus* (TMV) *in vitro* and *in vivo*. Pre-treatment for two hours with these nanoparticles *in vitro* results in substantial aggregation and breakage of the virus particles. Similarly, under *in vivo* conditions, the mixtures of these nanoparticles were inoculated onto Tobacco plants. Two days after inoculation, a significant decrease in the virus colonisation was observed when compared to the control (Cai et al. 2019). The antiviral activity of biologically synthesised ZnO nanoparticles against TMV has also been studied. The double foliar application of the prepared nanoparticles, 24 h before and after the TMV inoculation showed a 90.21% reduction in the viral accumulation level and disease severity (Abdelkhalek & Al-Askar 2020).

Antibacterial. Due to their ultra-small size, high surface area and high reactivity, nanoparticles affect the activity of microorganisms. Silver nanoparticles, synthesised by *Solanum trilobatum* and *Ocimum tenuiflorum*, have been shown to inhibit the colonisation of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumonia*. Zinc nanoparticles show an antimicrobial effect on *P. aeruginosa* (Jayaseelan et al. 2012). CuO nanoparticles have also been shown to have antimicrobial activity against various bacterial strains (Azam et al. 2012). Chitosan nanoparticles have been shown to have antimicrobial properties against a wide variety of bacteria, viruses and fungi. Antimicrobial effects have been observed on Gram-negative bacteria such as *E. coli*, *P. aeruginosa*, *Salmonella typhimurium*, *P. fluorescens* and *Vibrio parahaemolyticus*, etc. (Muzzarelli 1983; No et al. 2002; Rabea et al. 2003; Sun et al. 2007; Palma-Guerrero et al. 2010).

Nano-encapsulation

Nanoparticles have been used to develop effective agricultural formulations and commonly act as carriers to encapsulate, absorb or attach active molecules. The most commonly used nanoparticles, viz silica nanoparticles, chitosan nanoparticles, solid lipid nanoparticles and layered double hydroxides, are used as carriers of fungicides, insecticides, herbicides and RNA-inducing molecules (Worrall et al. 2018). These nanoparticles have been effectively used as carriers for plant disease management. Nanoparticles offer great advantages for the effective delivery of these agrochemi-

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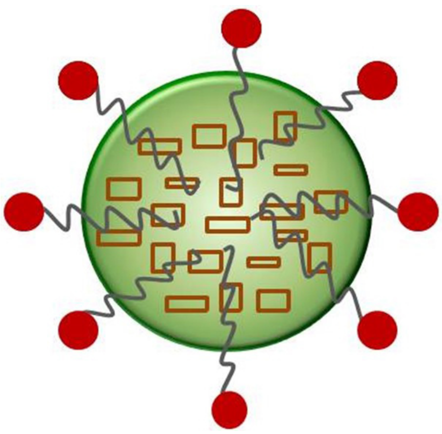
cals due to their easy attachment, large surface area and fast mass transfer (Ghormade et al. 2011). Through capsulation, absorption, surface ionic or weak bond attachments, micronic or sub-micronic particles are incorporated into the agrochemicals (Shang et al. 2019). The nano-encapsulation of pesticides in controlled release matrices is one of the most promising research areas for the future. Nano-encapsulation has been shown to increase the efficiency of pesticides, reduce their volatilisation and decrease the toxicity and environmental contamination in crops (González-Melendi et al. 2008; Sopeña et al. 2009). This has also been shown to lead to a reduction in the quantity of pesticides required for disease control and also minimises the adverse effects of the pesticides on humans and the environment. Nano-emulsions serve as a better pesticide delivery system because of their small size, low viscosity, high surface area, kinetic stability and optical transparency (Xu et al. 2010) (Figure 2). Agri-nanotechnology, therefore, holds the promise of the controlled release of agrochemicals and site-targeted delivery of various macromolecules. The administered chemicals and macromolecules can help in improving the plant disease resistance, efficient nutrient utilisation, and enhanced plant growth.

Nanoparticles for gene delivery and transgenics

To reduce the losses to crops caused by plant pathogens, plant biologists have used many methods to engineer resistant plants. RNA silencing-based resistance has been a powerful tool that has been used to engineer resistant crops during the last two decades. Based on this mechanism, diverse approaches have been developed thus far. RNA silencing has been utilised to produce plants that are resistant to plant viruses, such as RNA and DNA viruses, viroids, insects, and to fungal pathogens. RNA interference (RNAi) is a process through which the synthesis of targeted proteins is downregulated by interfering in the expression of the genetically encoded message. The interference of the genetically encoded message is an RNA mediated and post-transcriptional gene regulation process (Fire et al. 1998). Usually, viral vectors are used for the delivery of siRNAs or miRNAs into plants. Viral vectors transiently and directly express the siRNA in the plant and do not rely on plant transformation. The most commonly used viral vector for delivery of siRNAs is the bipartite *Tobacco rattle virus* (Ratcliff et al. 2001; Dinesh et al. 2003). In addition to the *Tobacco rattle virus*, the *Cabbage leaf curl virus* vector has also been developed for expressing synthetic and endogenous miRNAs in plants

(A)



(B)

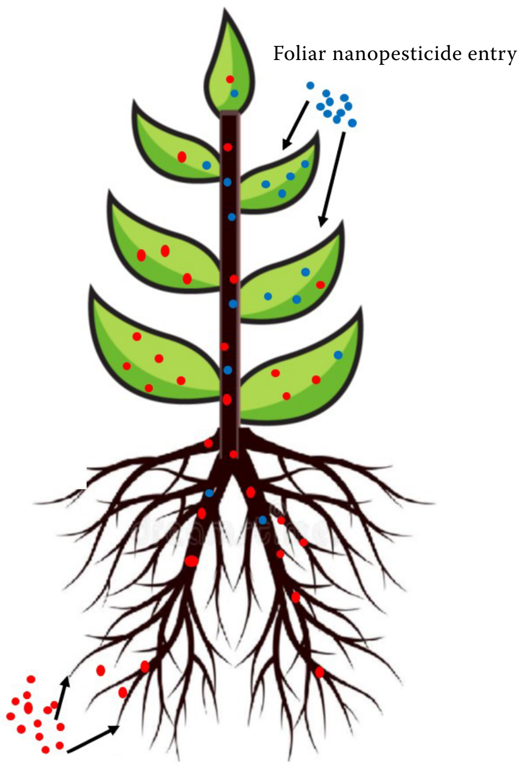


Figure 2. (A) Schematic representation of a nano-emulsion. (B) Nano-emulsions as a pesticide delivery system. Nano-pesticides can be applied via the foliar route as well as the root route

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(Tang et al. 2010). There are various disadvantages in using viral vectors for post-transcriptional gene silencing (PTGS), one of them being that viruses are limited in their host range. Viral vectors do not infect all the tissues uniformly, although they might spread systemically. In addition to these drawbacks, the phenotype attributed to PTGS is mixed with the onset of virus symptoms like the mosaic pattern of the disease and mild leaf curling. These disadvantages of using viral vectors make it imperative to look for alternative, more efficient methods that can be used for PTGS in plants in order to confer greater pathogen resistance to crops.

Nanoparticles offer a solution in overcoming numerous challenges in siRNA delivery. Cationic lipid or polymer nanoparticles form a condensed complex with nucleic acids and, hence, have been used to transport anionic nucleic acids into the cells (Behlke 2006). This leads to the stabilisation and protection of the siRNA from enzymatic degradation by the nucleases. Cationic materials can also help nanoparticles escape sequestration in endosomes/lysosomes. Nitrogen groups in cationic polymer polyethyleneimines become protonated in the acidic pH environment of the endosome/lysosomes. This, in turn, helps in the endosomal escape by leading to the Cl^- influx in response to the protonation of polymer polyethyleneimines. This results in an increase in the osmotic pressure and swelling. The swelling finally leads to the organelles bursting and the delivery of the siRNA nanoparticles. This phenomenon is referred to as the "proton sponge effect". Nanoparticle interactions with DNA can lead to antisense gene regulation by bringing about hybridisation with the mRNA of interest and thereby preventing protein production. The DNA nanoparticles bind to a target mRNA strand and prevent translation into proteins via the steric inhibition of the ribosome by the nanoparticle (Figure 3).

The application of nanoparticle technology in plant pathology can provide new ways to help in crop protection. It would be very desirable

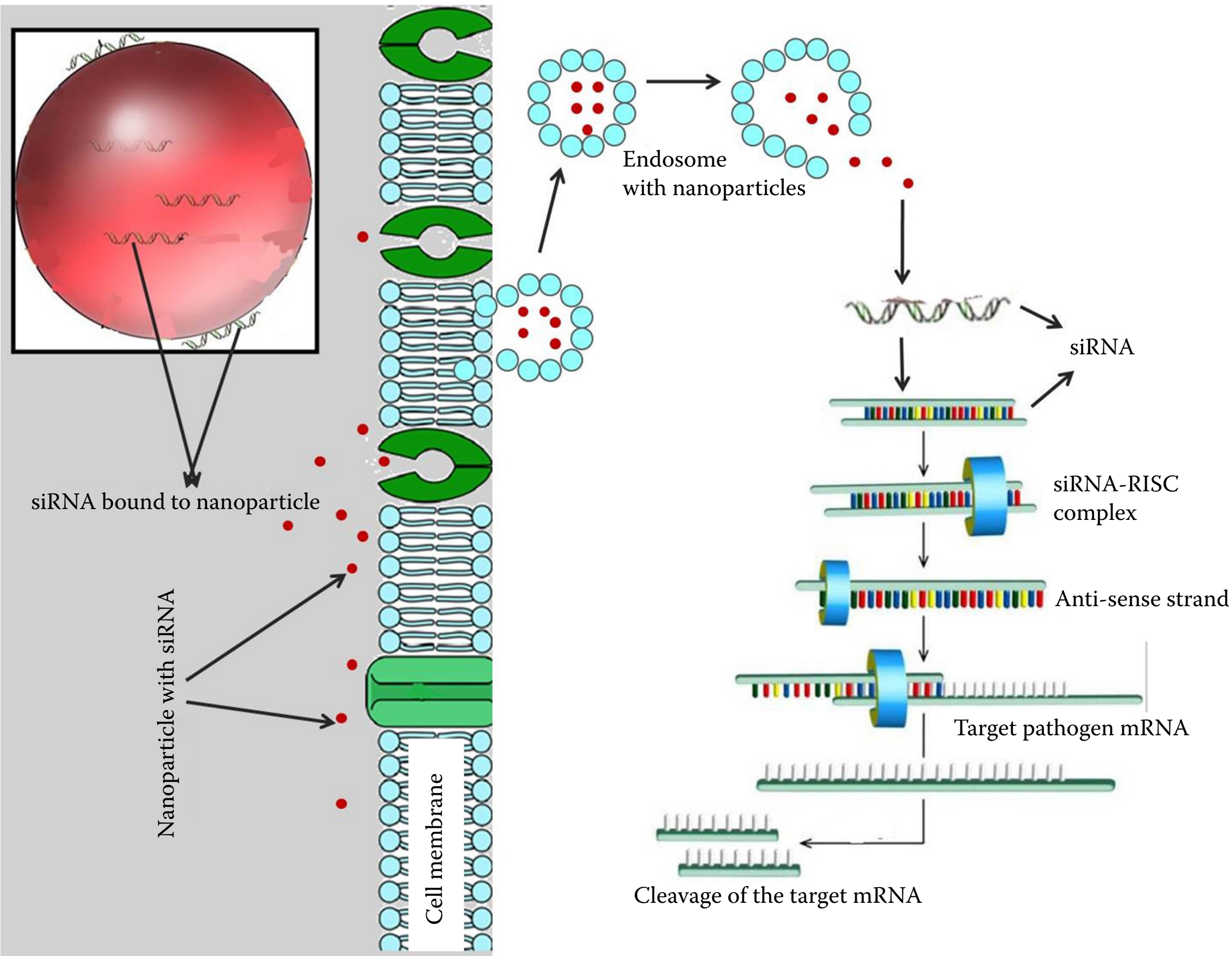

Figure 3. Nanoparticles as delivery systems for siRNA; application in gene silencing and disease resistance

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to utilise nanoparticles for either identifying possible remedies to various viral diseases or identifying resistant genes within the biological cell system of the plant to improve resistance against viral infections. A method of inhibiting plant viruses using nanoparticles would solve a great deal of the aforementioned problems. Gene delivery constitutes one of the most critical steps in gene manipulation (Bhat et al. 2017). The size and function of viral vectors has been mimicked for the development of nonviral gene delivery systems based on biocompatible nanostructured materials (Niemeyer 2001; Davis & Shin 2008), such as, inorganic nanoparticles (De et al. 2008), carbon nanotubes (Liu et al. 2007), etc. to provide an alternative approach to the problems in viral gene delivery. Inorganic nanoparticles including silica (Torney et al. 2007), iron oxide (Medarova et al. 2007) and CdSe (Derfus et al. 2007) have recently been exploited as alternate nonviral vectors. Gold nanoparticles provide particularly attractive scaffolds for the creation of transfection agents (Sandhu et al. 2002; Thomas & Klibanov 2003; Rosi et al. 2006). Gold colloids are bio-inert, non-toxic, and readily synthesised and functionalised (Daniel & Astruc 2004). Recently, gold nanoparticles with a core diameter of 2 nm and an overall diameter of 6 nm have been designed. These gold nanoparticles resemble histone octamers, where the nucleosome core proteins are 6 nm in diameter. These gold nanoparticles also resemble histones in shape, size and surface functionality (Ghosh et al. 2008a).

Recently, layered double hydroxide (LDH) clay nanosheets have been developed that have been used for the delivery of dsRNA to plants. Complexes of dsRNA-LDH are formed on the nanosheets. These are referred to as a bioclay. This bioclay is topically sprayed onto the plants. The dsRNA from the bioclay has been detected on the leaves even after 30 days of initial topical spray, where it provides a sustained release of dsRNA on the leaves and RNAi-based systemic protection to the sprayed plants (Mitter et al. 2017). Besides dsRNAs, siRNAs have also been delivered either to cell cultures through nanosecond pulsed laser-induced stress or to protoplasts as conjugated with polymer nanoparticles (Tang et al. 2006; Silva et al. 2010). Gold nanoparticles (Ghosh et al. 2008b; Kim et al. 2013) have been seen to serve as attractive materials for DNA and RNA delivery (Xu et al. 2006; Bhattacharya & Mukherjee 2008; Roca & Haes 2008). Gold nanoparticles can be fabricated in a scalable fashion with small sized dispersity (Sun & Xia 2002; Shenhar & Rotello 2006). It is possible to achieve functional diversity in gold nanoparticles readily by the creation of multifunctional monolayers. This allows for multiple functional moieties, such as nucleic acids, to be placed onto the particle surface (Ryan et al. 2007; Bowman et al. 2008). Attachment of nucleic acids to gold nanoparticles is an effective mean of transporting gene-silencing oligonucleotides, where the modification will not hamper the biological activity. Antisense RNA on gold nanoparticles has a higher affinity for the complementary nucleic acids than the linear counterparts. This forms a key determinant of gene-silencing efficiency (Jin et al. 2003) (Figure 4).

Transgenic plants have been successfully generated in a species-independent manner, carrying out the gene transfer by bombardment of DNA-absorbed gold particles. This process has been successfully applied in the soybean callus. The genetically modified plants showed that the exogenous DNA could be inherited in a Mendelian manner over two generations (Christou et al. 1988). The delivery of DNA and chemicals via honeycomb mesoporous silica nanoparticles internalised in plant cells has also been reported. It was observed that mesoporous silica nanoparticles can deliver the gene and the chemical that triggers the gene expression simultaneously. The mesoporous silica nanoparticles were capped with gold nanoparticles so that their momentum is increased after acceleration. The DNA transferred by this method was seen to be expressed in tobacco and maize tissues. The minimum amount of DNA required in this method, in order to detect marker expression, was observed to be 1 000-fold less than that required for conventional delivery methods. Therefore, this efficient DNA delivery method can have a multitude of applications in protoplast-based gene expression studies (Torney et al. 2007).

Nanobiotechnology can offer the use of nanoparticles in improving plant resistance by trans-genetically multiplying genes and improving the plant resistance (McKnight et al. 2003; Torney et al. 2007; Rai et al. 2012). Insertion and integration of plasmid DNA in the plant genome has already been successfully confirmed through gene expression (Filipenko et al. 2007). Nanoparticles have also been used as a vehicle to deliver siRNA in plant

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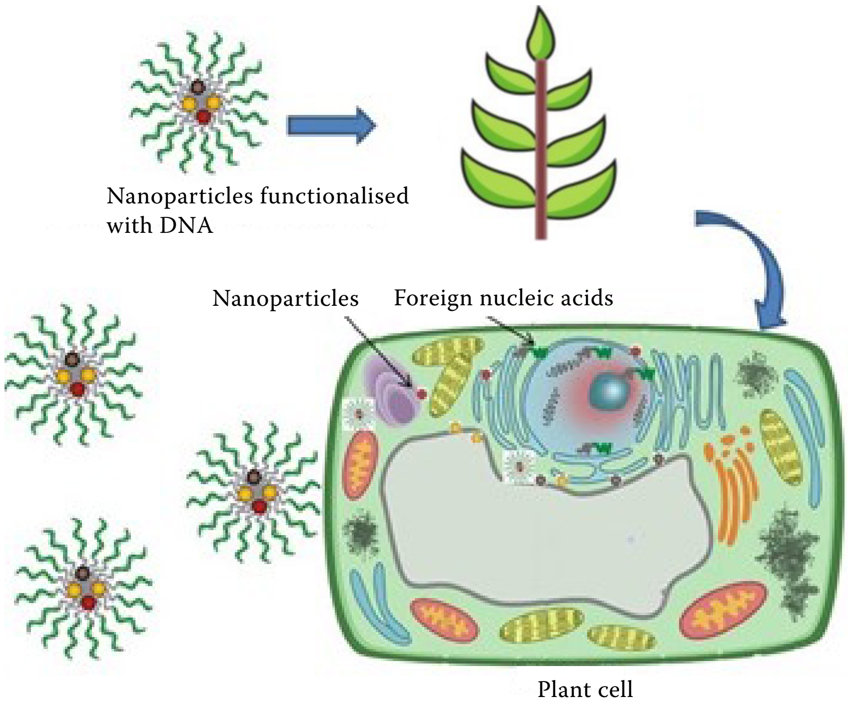

Figure 4. Nanoparticles engineered with biomolecules as a delivery system in plant cells

cells. This has helped in studying the cellular pathway (Silva et al. 2010). Amine-conjugated polymeric nanoparticles have been used as vehicles to deliver siRNAs targeting specific genes in the cellulose biosynthesis pathway. It was observed that *NtCesA-1*, which is a factor involved in cell wall synthesis in the whole plant, also has a role in the cell wall regeneration of isolated protoplasts. In addition to this, multiwall carbon nanotubes have been shown to induce changes in the gene expression in tomato leaves that were previously unknown. It has been shown that up-regulation of the stress-related genes in tomato leaves and roots, particularly those induced by pathogens and the water-channel *LeAqp2* gene takes place when treated with carbon nanotubes (Khodakovskaya et al. 2011).

Genetic modification via nanoparticles mediated plant transformation has the potential to improve plants by increasing the disease resistance capabilities (McKnight et al. 2003). Nanotechnology can provide new methods for crop disease management by targeting specific phytopathology problems in agriculture such as in plant-pathogen interactions. The introduction of resistance genes in plant cells using nanotechnology methods can lead to the development of resistant varieties which, in turn, will minimise expenses on the agrochemicals required for disease control (Bouwmeester et al. 2009).

CONCLUSION

Nanotechnologies, until now, have mostly found applications focused on either medical research or animal science. With the increasing challenges in agriculture, new technologies are always needed to increase the crop yield in order to meet the increasing demand for food all over the world. Improvement in plant protection is imperative so that the ever-increasing demands are met. The versatility of nanotechnology can be applied to plant science research and disease management as well. The application of nanotechnology and nanoparticles to agricultural science can help to further the investigation of plant genomics and gene functions. Nanoparticles can be used for gene delivery in plants, for the development of disease resistant plants and improvement of crop species. Nanoparticles engineered with nucleotides will have many advantages over viral nucleotide delivery via virus induced gene silencing. Nanoparticle mediated gene delivery can be used in the future for the improvement of crops and make them resistant to pathogens as well as pests. The smart delivery of nucleotides, such as siRNAs, to plants can be the future application of engineered nanoparticles. These nanoparticles with their nucleotides can be used for engineering disease resistance.

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