



The Nanoparticles in plants: Review Paper

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ABSTRACT

The successful application of various nanoplateforms in medicine under in vitro conditions has generated some interest in agri-nanotechnology. This technology holds the promise of controlled release of agrochemicals and site targeted delivery of various macromolecules needed for improved plant disease resistance, efficient nutrient utilization and enhanced plant growth. Processes such as nanoencapsulation show the benefit of more efficient use and safer handling of pesticides with less exposure to the environment that guarantees ecoprotection. The uptake efficiency and effects of various nanoparticles on the growth and metabolic functions vary differently among plants. Nanoparticle mediated plant transformation has the potential for genetic modification of plants for further improvement. Specifically, application of nanoparticle technology in plant pathology targets specific agricultural problems in plant-pathogen interactions and provide new ways for crop protection. Herein we reviewed the delivery of nanoparticulate materials to plants and their ultimate effects which could provide some insights for the safe use of this novel technology for the improvement of crops.

1. Introduction

Changes in agricultural technology have been a major factor shaping modern agriculture. Among the latest line of technological innovations, nanotechnology occupies a prominent position in transforming agriculture and food production. The development of nanodevices and nanomaterials could open up novel applications in plant biotechnology and

agriculture [1]. Currently, the main thrust of research in nanotechnology focuses on applications in the field of electronics [2], energy [3], medicine and life sciences [4,5]. Experiences gained from these fields facilitate the development of genetically modified crops, plant protecting chemicals and precision farming techniques. Nanotechnology permits broad advances in agricultural research, such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful byproducts through enzymatic nanobioprocessing, disease prevention and treatment

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in plants using various nanocides [6]. Nanoscale devices with novel properties make the agricultural systems “smart”. Such devices are capable of responding to different situations by themselves, thus taking appropriate remedial action. These smart systems deliver chemicals in a controlled and targeted manner as similar to the proposed use of nanodrug delivery in humans [7,8]. “Smart Delivery Systems” in agriculture should possess combinations of time controlled, specifically targeted, highly controlled, remotely regulated/preprogrammed/self-regulated and multifunctional characteristics to avoid biological barriers for successful targeting. Agriculture and food system security, novel delivery systems for disease treatment, new tools for molecular and cellular biology and new materials for plant pathogen detection are some of the nanotechnological links to agriculture and food science. Besides these, plants and/or their extracts provide a biological synthesis route of several metallic nanoparticles which is more ecofriendly and allows a controlled synthesis with well defined size and shape [9,10]. This review presents some of the recent developments in plant science and agriculture that covers the application of nanoparticles for more effective and safe use of chemicals for plants, plants as source of nanoparticle synthesis, the effects of different type of nanoparticles on the growth and metabolic functions of different plants and nanoparticle mediated plant genetic transformation.

2. Nanoformulations in agriculture

Nanotechnology offers an important role in improving the existing crop management techniques. Agrochemicals are conventionally applied to crops by spraying and/or broadcasting. Usually only a

very low concentration of chemicals, which is much below the minimum effective concentration required, has reached the target site of crops due to problems such as leaching of chemicals, degradation by photolysis, hydrolysis and by microbial degradation. Hence repeated application is necessary to have an effective control which might cause some unfavorable effects such as soil and water pollution. Nano-encapsulated agrochemicals should be designed in such a way that they possess all necessary properties such as effective concentration (with high solubility, stability and effectiveness), time controlled release in response to certain stimuli, enhanced targeted activity and less ecotoxicity with safe and easy mode of delivery thus avoiding repeated application [11–14]. The control of parasitic weeds with nanocapsulated herbicides thereby reducing the phytotoxicity of herbicides on crops is a best example [15]. Properly functionalized nanocapsules provide better penetration through cuticle and allow slow and controlled release of active ingredients on reaching the target weed. Nanoencapsulation of chemicals with biodegradable materials also makes the concentrated active ingredients safe and easy to handle by the growers. Surface modified hydrophobic nanosilica has been successfully used to control a range of agricultural insect pests [16,17]. Properly functionalised lipophilic nanosilica gets absorbed into the cuticular lipids of insects by physiosorption and damages the protective wax layer [made of various fatty acids and lipids that acts as an effective barrier for the evaporation of water] and induces death by desiccation [18,19]. The use of such nanobiopesticide is more acceptable since they are safe for plants and cause less environmental pollution in comparison to conventional chemical pesticides. In 1997 a research program was initiated to access

the ability of nanospheres to improve the bioavailability of a new insecticide RPA 107382 to plants [13]. The aim of the study was to obtain stable nanoparticles with the smallest size and largest amount of encapsulated active ingredients by nanoprecipitation. Stable polymeric nanospheres having size of about 135nm and encapsulation rate of 3.5% were obtained. Biological studies were performed on cotton plants infested with aphids to estimate the direct contact efficacy of nanosphere formulations on insects. The systemic effect of nanoformulation was studied from their ability to penetrate through the plant and reach the sap. The nanosphere formulations performed better than the reference to control the infestation at all the doses used due to their enhanced systemicity. The use of porous hollow silica nanoparticles (PHSN), with a shell thickness of nearly 15nm and a pore diameter of 4–5 nm, for providing shielding protection to pesticides from degradation by UV light was reported [20]. PHSN carriers improved the photostability of the pesticide, avermectin, loaded into the inner core and avermectin showed a typical sustained-release pattern from the carrier. Hence, such carriers have a promising future in the sustained-release pattern applications of various photosensitive components. The effects of slow/controlled-release fertilizers (for regulated, responsive and timely delivery) cemented and coated by nanomaterials; clay-polyester, humus-polyester and plasticstarch on crops were studied with wheat [21,22]. It was found that these nanocomposites were safe for wheat seed germination (over 99% germination), emergence and growth of seedlings. Leaching experimental results showed that nitrogen release rate of fertilizer coated by plastic-starch composites was the lowest and the release rate of coated slow-

release fertilizers were lower than that of the cemented slow-release fertilizers. The successful use of silver nanoparticles [Ag NPs] in diverse medical streams as antifungal and antibacterial agents [23,24] has led to their applications in controlling phytopathogens. Ag NPs with broad spectrum of antimicrobial activity reduce various plant diseases caused by spore producing fungal pathogens [25]. The effectiveness of Ag NPs can be improved by applying them well before the penetration and colonization of fungal spores within the plant tissues. The small size of the active ingredient [diameter of 1–5 nm] effectively controls fungal diseases like powdery mildew [26]. However it was also observed that a very high concentration of nanosilica–silver produced some chemical injuries on the tested plants (cucumber leaves and pansy flowers). The use of Ag NPs as an alternative to pesticides for the control of sclerotium forming phytopathogenic fungi was also investigated [27]. Exposure of fungal hyphae to Ag NPs caused severe damage by the separation of layers of hyphal wall and collapse of hyphae. Detrimental effects of Ag NPs on unidentified fungal species of the genus *Raffaelea* causing mortality of oak trees was also investigated and studies showed harmful effects of Ag NPs on conidial germination [28]. The efficacy of Ag NPs in extending the vase life of gerbera flowers was also studied and the results show inhibited microbial growth and reduced vascular blockage which increased the water uptake and maintained the turgidity of gerbera flowers [29,30]. Improved nanoparticle delivery systems need to be developed for specifically targeting the infected tissues alone which still needs more focused studies. Studies also showed the use of biocide-containing polymeric nanoparticles for introducing organic wood preservatives and fungicides into

wood products thereby reducing wood decay [31–35]. Hence nanoparticulate formulations have great potential as novel agrochemicals with high specificity and improved functions.

3. Mode of entry, formation, transport and effects of different nanoparticles in plants

Plants provide a potential pathway for the transport of nanoparticles to the environment and serve as an important route for their bioaccumulation into food chain. Various studies are now ascertaining the effects of different nanoparticles on plant growth and metabolic functions.

3.1. Entry of nanoparticles into plants

Plant cell wall acts as a barrier for easy entry of any external agent including nanoparticles into plant cells. The sieving properties are determined by pore diameter of cell wall ranging from 5 to 20nm [36]. Hence, only nanoparticles or nanoparticle aggregates with diameter less than the pore diameter of the cell wall could easily pass through and reach the plasmamembrane [37,38]. There is also a chance for enlargement of pores or induction of new cell wall pores upon interaction with engineered nanoparticles which in turn enhance nanoparticle uptake. Further internalization occurs during endocytosis with the help of a cavity like structure that form around the nanoparticles by plasma membrane. They may also cross the membrane using embedded transport carrier proteins or through ion channels. In the cytoplasm, the nanoparticles may bind with different cytoplasmic organelles and interfere with the metabolic processes at that site [39]. When nanoparticles are applied on leaf surfaces, they enter through the stomatal openings or through the

bases of trichomes and then translocated to various tissues [40–42]. However, accumulation of nanoparticles on photosynthetic surface cause foliar heating which results in alterations to gas exchange due to stomatal obstruction that produce changes in various physiological and cellular functions of plants [43]. Studies on the mechanism of uptake and formation of nanoparticles within plants have also led to more investigations on the use of plants as source for nanoparticle synthesis.

3.2. Plants as nanofactories

The use of biological systems for the synthesis of nanoparticles is gaining increased attention since it is more esuriently compared to various physical and chemical methods [10,44,45]. Among various biological systems, plants provide an easy and safe green route for the synthesis of various metal nanoparticles and for this the metal of interest should be present in the growth medium of plants and be efficiently transported through the plant root cells. The formation and growth of gold nanoparticles [Au NPs] inside live alfalfa plants and sesbania seedlings grown in gold enriched media was reported [46,47]. Microscopic and X-ray absorption studies confirmed the nucleation and growth of nanoparticles inside plants. The uptake of silver ions and their reduction and distribution as Ag NPs within cellular structure of somemetallophytic plants has also been investigated [48–50]. Copper biomineralisation with some wetland plants that transform copper into metallic nanoparticles at soil-root interface with the help of some endomycorrhizal fungi were also reported that could reduce copper toxicity in contaminated soils [51]. The formation of nanoparticles of an alloy of gold–silver–copper using plant was also reported [52]. This opens up a great

scope of using plants for the production of mixed metal nanoparticles of specific compositions. The mechanism of formation of nanoparticles; whether they are formed outside in the media and then translocated to plants or whether they are formed by the reduction of metal salts within the plants itself still needs more clarification [47,48,53]. The uptake and translocation of nanoparticles across root cells [in which several active and passive transport processes involve] depends on the type of metal ions and plant species. The amount of nanoparticle accumulation in plants also varies with reduction potential of ions and the reducing capacity of plants that depends on the presence of various polyphenols and other heterocyclic compounds present in plants [54,55]. Different studies regarding the extracellular biological synthesis of nanoparticles using leaf extracts [56–63] and dead tissues of various plants [64–67] were also reported. Hence, compared to the chemical synthesis of nanoparticles, this method provides a better and safe means of nanoparticle production with defined size and shape of our interest.

3.3. Impact of different nanoparticles on plants

3.3.1. Effects of carbon-based nanomaterials

Increased applications of engineered carbon-based nanomaterials create concerns about their toxicity to humans and animals [68,69]. Such carbon nanomaterials (such as single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotube (MWCNTs), carbon buckyballs, etc.) also found increased applications in the field of agriculture and food. This raised questions regarding the safety of using such nanomaterials with crops. Various studies showed contradictory results on the phytotoxicity of carbon nanomaterials in plants. The effects of functionalized SWCNTs (fCNTs; functionalized with

poly-3-aminobenzenesulfonic for high dispersibility) and non-functionalized SWCNTs (CNTs) on root elongation of 6 different crop species (cabbage (*Brassica oleracea*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), onion (*Allium cepa*) and tomato (*Solanum lycopersicum*)) were studied to understand their toxicity to crops [70]. CNTs enhanced root elongation in onion and cucumber and nanotubes sheets were formed by both fCNTs and CNTs on cucumber root surface due to their interaction with root surface; however none entered into the roots. Cabbage and carrot were not affected by either form of nanotubes. Root elongation in lettuce was inhibited with fCNTs and tomato was found to be most sensitive for CNTs with significant root length reduction. However a very recent work reported on the effects of MWCNTs on the seed germination and growth of tomato plants showed a positive response upon interaction with nanotubes [71]. Their results showed increased water uptake by seeds in the presence of MWCNTs which enhanced the germination process. Some other studies also support the positive effects of suspensions of MWCNTs on seed germination and root growth of six different crop species (radish (*Raphanus sativus*), rape (*Brassica napus*), rye grass (*Lolium perenne*), lettuce (*Lactuca sativa*), corn (*Zea mays*) and cucumber (*Cucumis sativus*)) [72]. The works conducted in our laboratory also supported the positive effects of carbon nanotubes on germination and growth of plants. We studied the effects of both SWCNTs [in purchased form and purified form] and MWCNTs on the germination of rice seeds and observed an enhanced germination for seeds germinated in the presence of nanotubes (Fig. 1). In zucchini plants, no negative effects were observed on seed germination and root

elongation in the tested range of MWCNTs whereas a decrease in the biomass of plants was observed during further growth in the presence of SWCNTs [73]. Hence the response of plants to nanomaterials varies with the type of plant species, their growth stages and the nature of nanomaterials since the studies showed contradictory effects of even the same nanomaterial in different plants at different developmental stages. Many other studies investigated the potential toxicity of MWCNTs in plant cells. The interactions of MWCNTs with rice suspension cells had led to aggregation of cells so that a self-defense response was observed among the cells itself to escape from the detrimental effects of MWCNTs [74]. Some cells having immediate interaction with nanotubes act as a barrier thus saving the rest of cells.



Fig. 1. Effects of different carbon nanotubes on the germination of rice seeds. Both SWCNTs and MWCNTs are added to plant growth media @ 30_g/ml of the media. Enhanced germination was observed for seeds germinated in presence of carbon nanotubes compared to the control [unpublished data].

The attachment of MWCNTs to the proteins and polysaccharides of cell wall results in a signaling cascade which increased the production of certain compounds necessary for cell wall thickening. Such hypersensitive responses are common in plants during other biotic and abiotic stresses that adversely affect plant growth. Further studies by the same group reported the detachment of plasma membrane from the cell

wall of those cells interacted with MWCNTs leading to cell death. Accumulation of reactive oxygen species (ROS) was induced by MWCNTs resulting in increased oxidative stress and decreased cell proliferation, which eventually led to complete cell death [75]. Penetration of MWCNTs inside the cells was not reported in above studies. As in rice suspension cells, MWCNTs were also found to be toxic for Arabidopsis T87 suspension cells [76]. The cells cultured in media with loose agglomerates of MWCNTs (as received product without any further treatment) and fine-agglomerates of MWCNTs (obtained after ultrasonication of the loose agglomerates of MWCNTs) had decreased dry weight, less viability, lower chlorophyll content and superoxide dismutase activity which showed the detrimental effects of MWCNTs on Arabidopsis suspension cells. The fine-agglomerates of MWCNTs inhibited cell growth more than the loose MWCNTs. This was explained by the fact that the plant cell cultures have greater tendencies to form clumps with variable number of cells. Loose agglomerates of nanotubes with larger size could not easily enter into such cell clumps whereas the distribution of fine-agglomerates in clumps is easier due to their small size and greater interaction with cell wall proteins and polysaccharides in an irreversible manner. This study clearly showed the size dependent toxicity of nanotubes on plant cells. Attachment of such extracellular materials on cell clumps led to hypersensitive responses and cell death occurs either by apoptosis or necrosis. The uptake, accumulation and transmission of natural organic matter (NOM) [37]-suspended carbon nanomaterials in rice plants was also reported [77]. Fullerene C70 or MWCNTs were suspended in NOM solution to produce C70-NOM and MWCNTs-NOM to reduce the hydrophobicity of engineered

nanomaterials. Rice seeds were germinated in different concentrations of C70-NOM or MWCNTs-NOM and then the seedlings were grown in greenhouse till maturity. First generation seeds were then used to raise the second generation plants without any addition of nanomaterials. Microscopic images of various tissues of both first and second generation rice plants at different developmental stages showed the occurrence of C70 nanomaterials whereas little MWCNTs was observed inside the cells (Fig. 2). These results showed the transmission of C70 to the progeny through the seeds of first generation. C70 nanoparticles might enter the plant roots via osmotic pressure and capillary forces and enter through the cell wall pores and translocated through intercellular plasmadesmata. Since the aggregates were mostly found near the vascular system, there is a possibility of hindrance to the uptake of water and nutrients which is not good for the potential growth of the plants. Delay in flowering and reduced seed set was observed in plants incubated both with C70-NOM and MWCNTs-NOM at a concentration of 400 mg/L.

It is unpredictable how different nanomaterials interact with plants and how they modify the genetic and molecular mechanisms of plants. Such an interaction varies with type and time of exposure to nanomaterials and hence these points need to be considered while assessing nanotoxicity studies. The ability of carbon nanomaterials to penetrate the cell wall and cell membrane of intact plant cells were also recently reported. *Nicotiana tabacum* L. BY-2 cells were used as models for the uptake of oxidized SWCNTs (less than 500 nm length) labeled with fluorescein isothiocyanate (FITC) [78]. It was observed that FITC alone was not easily taken up by the cells whereas SWCNT/FITC was translocated

effectively into intact cells by fluid phase endocytosis [79]. This result clearly pointed out the ability of SWCNTs to penetrate the cell wall of plant cells. Further studies showed the ability of nanotubes to carry single-stranded DNA into intact plant cells by incubating the BY-2 cells in a medium with SWCNT/ssDNA-FITC [78]. A pronounced difference was observed in the distribution of SWCNT/FITC and SWCNT/ssDNA-FITC in the cell. The former was localized in the vacuolar region and latter in the cytoplasmic strands thus showing their ability to deliver different components to different cellular compartments. However the mode of entry of nanotubes through the cell wall remains mysterious. The orientation of nanotubes with respect to the plant cell wall might be important for their penetration which still needs more studies. Moreover, intense studies on potential toxicity of the nanotubes on plant cells are relevant for their safe use as nanotransporters.

3.3.2. Effects of magnetic nanoparticles

The main advantage of using magnetic nanoparticles is that they allow a very specific localization of the particles to release their load, which is of great interest in the study of nanoparticulate delivery for plants. A few works have been reported regarding the uptake, translocation and specific localization of magnetic nanoparticles (less than 50 nm) in pumpkin plants [80–82]. Magnetization signals of various strengths were observed from different portions (ranging from roots to leaves) of the treated plants (Fig. 3) which clearly indicates the successful translocation of nanoparticles in the entire plant system irrespective of the area of application. No toxicity on plant growth had been detected thus suggesting the safe use of such nanoparticles for nanoparticulate delivery in plants.

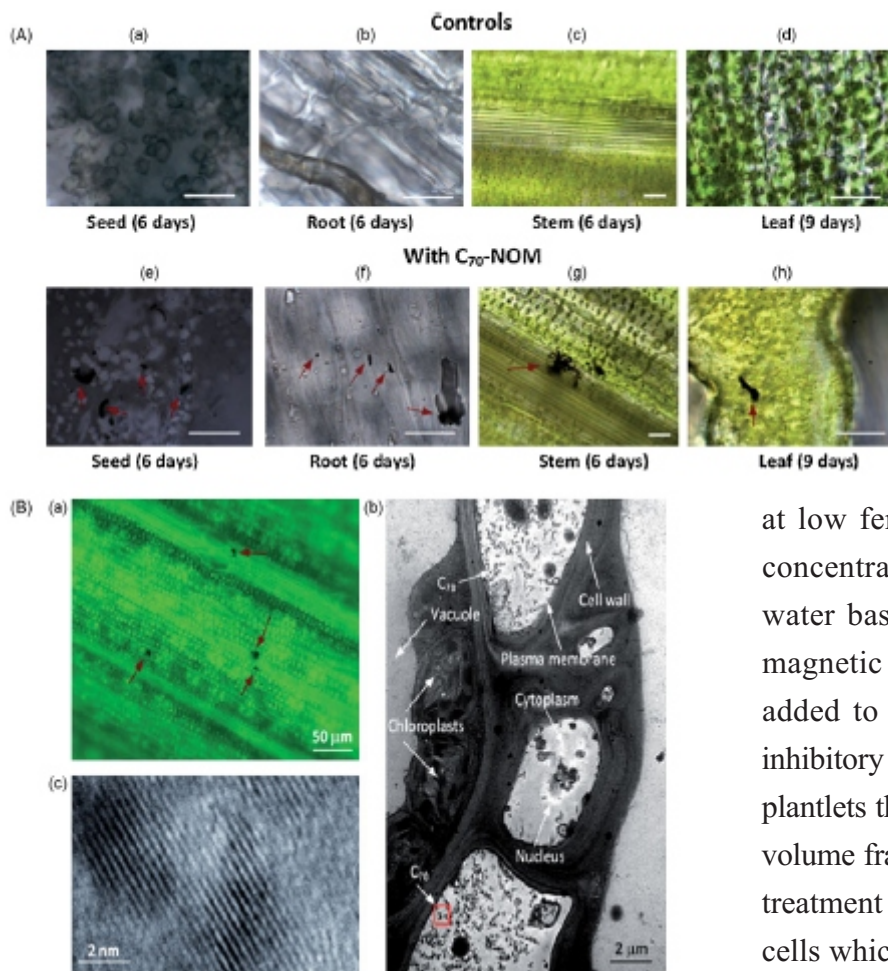


Fig. 2. Bright field images of rice plants showing C70 uptake. (A) Bright field images of root and leaf portions of 1-week-old rice seedlings. Control plants without any C70 (a-d) and treated plants showing C70 uptake (e and f). Arrows indicate the aggregation of nanoparticles in corresponding C70 treated plant tissues (scale bar: 20 μ m). (B)(a) Bright field image of the leaf portion of a second generation rice plant. C70 aggregates were mostly found near the leaf vascular system. (b) TEM image of the leaf cellsshowing C70 particles (C70: 20mg/L). (c) TEM image of C70 particles with higher magnification (adopted from Ref. [77]).

Magnetic nanoparticles could be used as capping agents for porous carriers of various chemicals so that their specific localization and uncapping process has been carried out using external magnets, hence releasing the payload at target site. Such methods are possible for specific treatments in fruit trees or high-input crops under green house conditions since it is easy to provide an external magnetic exposure that triggers the payload release [81,82]. In recent years, great attention has been paid to genetic effects of ferrofluids; that lead to chromosomal aberrations in young plants [83–86]. The influence of magnetic nanoparticles coated with tetramethylammonium hydroxide (TMA-OH) (as a stabilizing agent) on the growth of maize plants in early growth stages was studied [87]. 'Chlorophyll a' level was increased

at low ferrofluid concentrations while at higher concentrations it was inhibited. In another work, water based magnetic fluid obtained by coating magnetic nanoparticles with perchloric acid was added to germinated maize seeds [88]. A slight inhibitory effect was observed on the growth of the plantlets that led to brown spots on leaves at higher volume fractions of magnetic fluid. The excess iron treatment generated some oxidative stress in leaf cells which in turn affected photosynthesis and led to decreased metabolic process rates. They also studied the oxidative stress induced by the ferrofluid concentration on living plant tissues [89]. Maize seeds were germinated in the presence of magnetic fluid followed by exposure to electromagnetic field called, LM-EMF samples. For LM-EMF samples, a decrease in assimilatory pigments was observed with increased volume fraction of magnetic fluid solution. Exposure to electromagnetic field produced some local heating on the applied region due to the electromagnetic field energy absorbed by internalized magnetic nanoparticles in plant tissues that in turn affect the redox reactions involved in photosynthesis process. A pronounced increase in nucleic acid level was observed in LM-EMF samples due to the regeneration reactions of plant metabolism processes against local heating of vegetal tissues produced by

the electromagnetic field energy. There is also a chance for the magnetic nanoparticles to produce some magnetic influences on the enzymatic entities involved in different photosynthetic and developmental stages. Hence it is necessary to have an idea of suitable concentration ranges of ferrofluids before designing the biotechnological tools for plant cultures thus having better yield of biochemical mutant types with improved photosynthetic pigment levels.

3.3.3. Effects of metal and metal - oxide based nanomaterials

3.3.3.1. Effects of nano-TiO₂.

The effects of nano-TiO₂ on the germination and growth of spinach seeds were studied. These nanoparticles improved light absorbance and promoted the activity of Rubisco activate thus accelerated spinach growth [90–97]. Nano-TiO₂ [anatase] improved plant growth by enhanced nitrogen metabolism [98] that promotes the absorption of nitrate in spinach and, accelerating conversion of

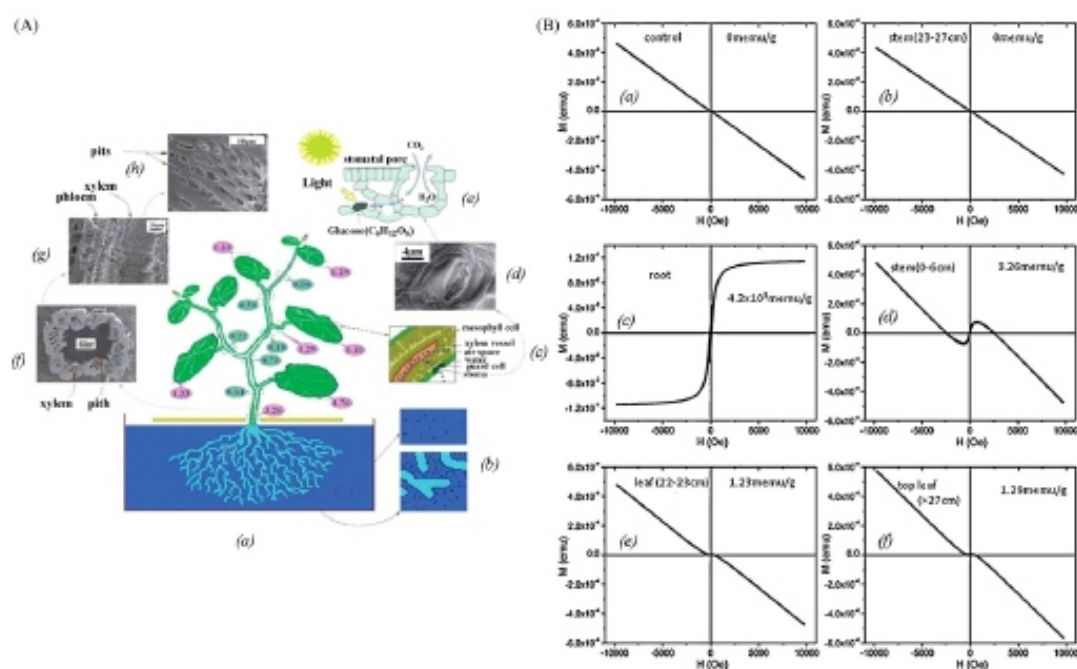


Fig. 3. Uptake, translocation and accumulation of manufactured Fe₃O₄ nanoparticles by pumpkin plants. (A) A schematic pumpkin plant and VSM measured magnetization at various sampling locations, marked as numbers in the unit of 10⁻³ emu/g⁻¹. Strong magnetic signals (>1.0 memug⁻¹) were detected in all leaf specimens regardless of their distances from the roots, while signals were much weaker from the stem tissues samples except those close to the roots. Although large numbers of the suspended particles accumulated on root surfaces, it is clear that Fe₃O₄ particles were taken up by the pumpkin plants and moved into the various tissues. The strongest magnetization (3.26 memug⁻¹) was detected right above the roots which might be due to nanoparticle agglomeration. (B) Selected VSM (Vibrating Sample Magnetometer) measurements of pumpkin plant tissues for Fe₃O₄ nanoparticles. (a) Control plant (root), (b) treatment plant stem at height of 23–27 cm, (c) treatment plant root, (d) treatment plant stem at height of 0–6 cm, (e) treatment plant leaf at height of 22–23 cm, and (f) treatment plant leaf above 27 cm. The background diamagnetic signal is linear with magnetic field. The magnetization at H= 10,000 is determined after the background signal is removed. No magnetization signal was detected from the control plants whereas most of the tissue samples of the treatment plants showed magnetization of various strengths (adopted from Ref. [81]).

inorganic nitrogen into organic nitrogen, thereby increasing the fresh weights and dry weights. Studies also showed the effects of nitrogen photoreduction on the improved growth of treated spinach plant [99,100]. Effects of nano- TiO_2 [anatase] on the content of light harvesting complex II (LHC II) on thylakoid membranes of spinach was studied and it showed an increase in LHC II content [101,102]. These promote energy transfer and oxygen evolution in photosystem II (PS II) of spinach [103]. It has also been found that nano-anatase TiO_2 promoted antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonyl-dialdehyde content and enhance the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and thereby increase the evolution oxygen rate in spinach chloroplasts under UV-B radiation [104]. The ability of nano-anatase TiO_2 to improve the light harvesting complex content of plants is highly comparable with the use of TiO_2 -quantum dot (QD) assembly [105] for the conversion of solar energy. Uptake and distribution of QD by the plant cells can be exploited for efficient and increased solar energy trapping that might improve the photo synthetic efficiency of plants and the photoluminescence property of quantum dots can be used for cell imaging too.

3.3.3.2. Effects of aluminium based nanoparticles

Nano-aluminum has been widely used in various industries and hence there is greater chance for the interaction of such nanomaterials with higher plants that constitute a major portion of the ecosystem. Pure alumina nanoparticles (13 nm) without any modifications reduced root elongation in studied plants (corn (*Zea mays*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), carrot (*Daucus carota*) and

cabbage (*Brassica oleracea*)) [106], thus potentially retarding the growth of plants. However, it was surprisingly observed that when nanoparticles were loaded with phenanthrene (a major constituent of polycyclic aromatic hydrocarbons), their toxicity significantly decreased showing no adverse effects on roots of plants. This showed the relevance of proper surface modifications which reduce the phytotoxicity of nanoparticles. The impact of aluminum oxide and aluminum oxide with carboxylate ligand coating particles (100nm in size) on plants (California red kidney bean (*Phaseolus vulgaris*) and rye grass (*Lolium perenne*)) was studied and no adverse effect on the growth of the plants was observed [72,107]. Aluminum concentration in rye grass was increased 2.5-fold above control tests whereas no uptake of aluminum was observed in kidney beans which inferred the difference in uptake and distribution efficiency of even the same kind of nanoparticles by different plants.

3.3.3.3. Effects of zinc based nanoparticles

The use of metal based nanoparticles like ZnO for increased permeability and creation of new holes in bacterial cell wall [108,109] has paved way for the use of such nanoparticles for studying their cell internalization and further translocation in plant cells. The seed germination and root growth study of zucchini seeds in hydroponic solution containing ZnO nanoparticles showed no negative effects [73] whereas the seed germination of rye grass and corn was inhibited by nanoscale zinc (nano-Zn (35 nm)) and zinc oxide (nano-ZnO (15-25 nm)) respectively [72]. Root growth of radish and rape incubated in nano-Zn suspension was significantly inhibited; however such an inhibition was not detected while soaked in nano-ZnO suspension due to the selective

permeability of seed coat. The same research team continued their work Ortiz sphere dissolution of ZnO nanoparticles to investigate its contribution to phytotoxicity [110]. In another work, ryegrass plants were grown with ZnO nanoparticles and Zn^{2+} ions in nutrient solution and it was observed that ZnO nanoparticles and Zn^{2+} had toxic effects at higher concentrations. Zn^{2+} ions were more toxic than ZnO nanoparticles resulting in yellowing and complete death of the plant at higher concentrations. Electron microscopy images confirmed the uptake of ZnO nanoparticles and showed the damage of epidermal and cortical cells upon nanoparticle intake by cells. They also caused injury to the endodermal and vascular cells which result ryegrass growth inhibition. After entering the cells the nanoparticles transport from one cell to other through plasmadesmata. Sometimes aggregation of nanoparticles may happen resulting in blocking of pores and channels, hence more research is needed to reduce the risk assessment and to clarify the ecotoxicity. Studies should also focus on the generation of novel nanomaterials enlarging the pore size of plants' cell wall upon their interaction with wall proteins and polysaccharides resulting in their successful transfer to plant system.

3.3.3.4. Effects of copper nanoparticles (Cu NPs).

The effects of Cu NPs on the seedling growth of mung bean and wheat were studied using plant agar culture media for easy dispersion of nanoparticles without any precipitation [111]. Mung bean was found to be more sensitive to Cu NPs than wheat and growth inhibition of seedlings was observed. Transmission electron microscopy (TEM) images confirmed the entry of copper nanoparticles across the cell membrane. Bioaccumulation of NPs increased with its concentration in growth media and

their bioavailability to the test plants was estimated by calculating the bioaccumulation factor. This study demonstrated plant agar test as a good protocol to test phytotoxicity of nanoparticles which is hardly water soluble. Also, studies on the effects of Cu NPs on the growth of zucchini plants showed reduced length of emerging roots [73]. However, the germination of lettuce seeds in the presence of Cu NPs showed an increase in shoot to root ratio compared to control plants [112]. Different flora and fauna responds differently to nanomaterials and hence, it is necessary to evaluate the safe effective concentration of each group of nanoparticles before their application that reduce the risks of ecotoxicity to a great extend.

3.3.3.5. Effects of silver nanoparticles (Ag NPs).

As mentioned earlier, Ag NPs have several antimicrobial functions to control various phytopathogens [25–28]. Studies on the seed germination and root growth of zucchini plants in hydroponic solution amended with Ag NPs showed no negative effects whereas a decrease in plant biomass and transpiration was observed on prolonging their growth in presence of Ag NPs [73]. The cytotoxic and genotoxic impacts of Ag NPs were studied using root tips of onion. It was investigated that Ag NPs impaired the stages of cell division and caused cell disintegration [113]. All such studies throw light to the need for a more cytotoxic and genotoxic evaluations by considering the properties of nanoparticles, their uptake, translocation and distribution in different plant tissues.

4. Nanogenetic manipulation of agricultural crops

Nanobiotechnology offers a new set of tools to manipulate the genes using nanoparticles, nanofibres

and nanocapsules [114–118]. Properly functionalized nanomaterials serve as a platform to transport large number of genes as well as chemicals that trigger gene expression in plants.

4.1. Exploring nanotechnology for delivering genetic materials into plants

Nanofibre arrays which can deliver genetic material to cells quickly and efficiently have potential applications in drug delivery, crop engineering and environmental monitoring. Reports came on the integration of carbon nanofibres which are surface modified with plasmid DNA with viable cells for controlled biochemical manipulations in cells [118,119]. The successful delivery and integration of plasmid DNA was confirmed from the gene expression. This process has similarity with microinjection method of gene delivery [120–122] and hence possible with the plant cells in which the treated cells could be regenerated into whole plant that would express the introduced trait. It is possible to make DNA tethered on carbon nanofibers without allowing them to integrate into host genome but still allowing some transcriptions of the tethered genes and hence this technique does not pass modified traits to further generations. This differ it from the existing genetic engineering methods and a onetime modification of the cells is possible. The application of fluorescent labeled starch-nanoparticles as plant transgenic vehicle was reported in which the nanoparticle biomaterial was designed in such a way that it bind and transport genes across the cell wall of plant cells by inducing instantaneous pore channels in cell wall, cell membrane and nuclear membrane with the help of ultrasound [123].

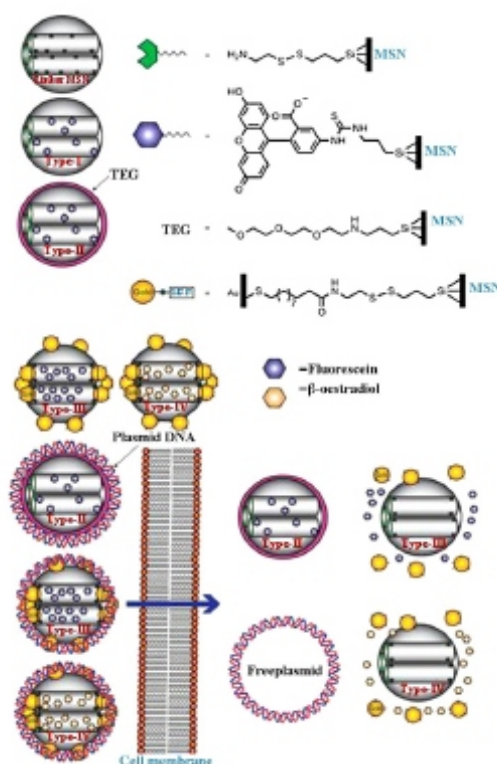


Fig. 4. Internalization of mesoporous silica nanoparticle [MSN] by plant cell. Type I: Fluorescein labeled MSN; Type II: Type I MSN coated with Triethylene Glycol (TEG) polymer; Type III: fluorescein labeled MSN capped by gold nanoparticles; Type IV: β -estradiol loaded MSNs with gold nanoparticle capping. After action of the gene gun, mesoporous silica nanoparticles (MSNs) [small circles], carrying the small effector molecule (β -estradiol) within the gold-capped structure and externally coated with plasmid DNA, penetrate the cell wall and, in some cases, enter the cytoplasm. The MSNs are uncapped by incubating the plant cells with dithiothreitol (DTT). This releases the β -estradiol effector molecules and activates the expression of plasmid DNA in the nucleus. Surface functionalized mesoporous silica nanoparticles (MSNs) are successfully used for the intracellular controlled release of genes and chemicals into plant cells. This will help in the future investigations of plant genomics and gene function as well as improvement of crops (adopted from Ref. [117])

It is possible to integrate different genes on the nanoparticle at the same time and the imaging of fluorescent nanoparticle is possible with fluorescence microscope thus understanding the movement of exterior genes along with the expression of transferred genes. Hence successful generation of pores on cell wall and cell membrane by suitable agents help in nanoparticle mediated DNA transfer that might be more successful in regenerative calli and soft tissues. The ability of surface functionalized mesoporous silica nanoparticles (MSNs) to penetrate plant cell walls also opens up new ways to precisely manipulate gene expression at single cell level by delivering DNA and its activators in a controlled fashion [117]. It was reported that a honeycomb MSN system with 3-nm pores transport DNA and chemicals into isolated plant cells and intact leaves. MSNs were loaded with gene and its chemical inducer and the ends were capped with gold nanoparticles to protect the molecules from leaching out. Uncapping of gold nanoparticles enabled release of chemicals and triggered gene expression in plants under controlled-release conditions (Fig. 4). In preliminary experiments protoplasts were incubated with fluorescently labeled MSNs. It was found that surface modification of MSNs with triethylene glycol was necessary to penetrate the cells. Such modification of MSNs also allowed plasmid DNA to adsorb on MSN surface. After entering the protoplasts, the plasmid DNA was released from the MSNs and the green fluorescent protein (GFP) marker encoded in the DNA was expressed in the cell which was detected by microscopy. In this method the minimum amount of DNA that is required to detect marker expression was 1000-fold less than that required for the conventional delivery method. This efficient delivery method has pronounced

applications in various protoplast-based gene expression studies. Nowadays gene gun or particle bombardment is one of the popular tools to deliver DNA into intact plant cells [124,125]. Particles used for bombardment are typically made of gold since they readily adsorb DNA and are non-toxic to cells. Since MSNs are too light, it is difficult for delivering foreign DNA attached on MSNs by gene gun method. This problem was solved by capping MSNs with gold nanoparticles which increased their momentum after acceleration by the gene gun. Experiments showed that the plasmid DNA transferred by gene gun method using gold-capped MSNs was successfully expressed in intact tobacco and maize tissues. The major advantage is the simultaneous delivery of both DNA and effectors molecules to the specific sites that results in site targeted delivery and expression of chemicals and genes respectively. This is how the nanoparticle mediated plant transformation differs from the conventional genetic engineering methods (like electroporation, microinjection, etc). Future scopes include pore enlargement and multifunctionalization of MSNs which provide even better possibilities in target-specific delivery of proteins, nucleotides and chemicals in plant biotechnology.

5. Conclusion and future perspectives

Application of nanotechnology in agriculture, even at its global level, is at its nascent stage. Nanoscience is leading to the development of a range of inexpensive nanotech applications for enhanced plant growth. Nanoparticles and nanocapsules provide an efficient means to distribute pesticides and fertilizers in a controlled fashion with high site specificity thus reducing collateral damage. In the context of plant-

pathogen interaction, application of nanoparticle technology and efficient transportation of substances, such as systemic chemicals, to specific sites provide novel solutions for the treatment of plants. If it is possible to have a distribution of properly functionalized nanoparticles throughout the plant vascular system and guide them to targeted sites; then these nanoparticles can be successfully used to unload chemicals (fungicides, insecticides, etc.), or other substances (plant hormones, elicitors, nucleic acids) into localized areas of plant tissues. This could help to carry on several studies at physiological, biochemical and genetic levels. Plant mediated synthesis of metal nanoparticles provide a safe synthesis route with better control over morphology of nanoparticles. The interaction of plant cell with the nanoparticles results in modification of plant gene expression and associated biological pathways which ultimately affect plant growth and development. Hence future studies should also highlight the needs to clarify the nanotoxicity to plants, possible uptake and translocation of nanoparticles by plants, and physical and chemical properties of nanoparticles in rhizosphere and on root surfaces.

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