Chapter 5
Effects of Nanoparticles on Plant Growth and Development

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Abstract Nanomaterials provide great opportunities in the field of agriculture because of their unique physicochemical properties. The interaction of nanoparticles with plants results in several physiological, morphological, and genotoxic changes, and their understanding is important for the effective use of nanotechnology in agriculture. Researchers suggested both positive and negative responses of nanoparticles on plant growth and development depending upon the properties of nanomaterials, mode of application as well as plant species. Studies on the uptake, translocation and biotransformation, and risks of application of nanomaterials on agriculturally important crops are recent research focus for understanding the physiological, biochemical, and molecular mechanisms of plants in relation to nanoparticles.

Keywords Nanoparticles · Plants · Uptake · Translocation · Growth · Phytotoxicity

5.1   Introduction

The increased use of engineered nanoparticles (ENPs) in different fields results in their accidental release to terrestrial, aquatic, and atmospheric environments. Plants are important component of all ecosystems, and interaction of ENPs with plants results in uptake and accumulation into plant biomass and decides the fate and transport of nanoparticles (NPs) in the environment. Recently, an increased number of studies have been highlighting the potential effects of ENPs to plants (Ruffini and Roberto 2009; Nair et al. 2010; Xingmao et al. 2010; Karl-Josef and Simone 2011). NPs get adsorbed on different plant surfaces, and their subsequent uptake occurs

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through micrometer- and nanometer-scaled plant openings. NPs can get into plant body using different paths, and the uptake rate depends on the size, shape, concentration, and surface charge of NPs (Tarafdar et al. 2012). The aerial part facilitates the interaction of airborne NPs on shoot surfaces. Also, there is a chance for the portion of engineered NPs released into environment to get dispersed by wind and reaching the leaves of plants (Espinosa and Oliva 2006). Plants interact with atmospheric NPs through the leaves, and hence, leaf stomata and hydathodes play an important role in the foliar uptake of NPs. The stomatal pathway is highly capacitive due to its large size exclusion limit above 10 nm and its high transport velocity; however, the chances of variability in permeability make this pathway highly unpredictable (Eichert et al. 2008). Sometimes, only very less percentage of stomata contributes to the uptake process which can be increased by the repeated wetting and drying of a foliar-applied solution. The NPs that penetrate leaf surface through the stomata or hydathodes traverse cell walls of palisade parenchyma and reach the leaf phloem and translocate to other plant parts. NPs can also get deposited on the cell walls of substomatal cavity or nearby cells. Other pathways for association of nanomaterials with aerial parts include cuticle, bark surfaces, and stigma. The epicuticular structures increase the deposition of NPs in epicuticular cavities. Also, the trichomes on shoot surfaces provide greater chance of NP deposition. The size of particles and its concentration play an important role in the uptake and distribution of NPs in plant system. Small lipophilic NPs can be taken up into apolar fluid areas of the cuticle that contains both apolar and polar uptake pathways. The uptake of larger particles occurs through cuticle-free areas such as stomata, hydathodes, and stigma of flowers.

The soil NPs interact with root system of plants, and the adsorption of NPs on plant roots facilitates their incorporation into cell wall and further uptake into cell. Most of the primary roots have a suberized exodermis and endodermis, and the apoplastic bypass flow of solutes and water from the soil to the central cylinder is prevented by suberized exodermis. However, apoplastic bypass is possible with the newly formed lateral roots that break through the cortex. Hence, the NPs could enter at the region of lateral roots formation into the xylem through the cortex and the central cylinder. The plant cells with negative surface charge permit the transport of negative charge surface compounds into apoplast (Nowack and Bucheli 2007). It was hypothesized that the negatively charged NPs could enter the apoplasm of the root cortex and eventually into xylem, but they are not taken up by the cells. Compounds can also enter into xylem through wounded cells or holes and can be further transported to shoots. This has been reported as a dominant process for the uptake of metal complexes and their subsequent translocation to shoots (Nowack et al. 2006; Tandy et al. 2006). This chapter reviews the studies on the interaction of engineered NPs with different plant species, uptake, translocation, and its phytotoxic effects with special mention to germination effects and seedling parameters.

5.2 Effects of Different Nanoparticles in Plant System

5.2.1 Effects of Metallic Nanoparticles

Metallic NPs have great potential in nanotechnology and have wide range of applications in engineering and biomedical sciences, and hence, it is important to study the fate and long-term effects of these nanomaterials on the environment. There are different ways in which the metal NPs can be taken up by plants. They can either be imported as NPs itself or the metal NPs can be oxidized to metal ions in soil solution and imported as ions followed by their reduction in plant system (Rico et al. 2011).

5.2.1.1 Effects of Gold Nanoparticles

The emergence of improved technologies with gold NPs provides great promise for future applications in various fields. However, the interaction of these NPs with biological systems creates new concerns on toxicological effects due to their unique physiochemical properties. Even though gold is not an essential nutrient for plant growth, there is greater chance for the contamination of soils with gold due to their increased application and hence greater exposure of plants to gold at significantly higher levels.

Understanding the interaction of NPs with plants is important for accessing their toxicity. Zhu et al. (2012) studied the effects of surface charge on the uptake and distribution of gold NPs in four different plant species, rice (*Oryza sativa*), radish (*Raphanus sativus*), pumpkin (*Cucurbita mixta* cv. white), and ryegrass (*Lolium perenne*). Plant seedlings were exposed to NP solution hydroponically for 5 days, and it was observed that the uptake and distribution of NPs were dependent on the surface charge and plant species in which the positively charged NPs were taken up by the plant roots and negatively charged NPs were translocated efficiently to plant shoots. Higher accumulation of NPs was observed in ryegrass and radish roots than in rice and pumpkin roots. The effects of NP properties on the uptake and translocation showed a strong interaction between NPs and cellular biomolecules. The uptake of NPs is also size selective as it was reported that the gold NP aggregates were observed in root cytoplasm of tobacco (*Nicotiana xanthi*) when exposed to 3.5-nm gold nanospheres and not when exposed to 18-nm gold NPs (Sabo-Attwood et al. 2012). Seedling toxicity experiments were also carried out using Arabidopsis (*Arabidopsis thaliana* L.) plants with different concentrations of $KAuCl_4$ (Taylor et al. 2014). It was observed that the germination was not affected by any of the concentrations of gold, whereas root growth was inhibited with increased $KAuCl_4$ concentration. The physiological and genetic responses of Arabidopsis to gold NPs (AuNPs) were also investigated. It was reported that the root lengths of the seedlings grown on nutrient agar media with 100 ppm AuNPs were reduced by 75 %. Roots and shoots showed the presence of oxidized gold, whereas reduced gold as NPs were observed only in root tissues.

The growth profile and yield of Indian mustard (*Brassica juncea*) on treatment with five different concentrations of AuNPs by foliar spray were studied under field conditions (Arora et al. 2012). It was reported that the germination percentage of Indian mustard was improved up to treatment with 25 ppm Au NPs than the control seeds and it could be due to the increased seed capsule permeability, which in turn allowed more water and di-oxygen into the cells. An increase in the number of leaves per plant was also observed with the treatment of AuNPs, and maximum increase was observed on treatment with 10 ppm AuNPs. It was also observed that the average leaf area was not increased with treatment of NPs. An improved growth profile was occurred for treated plants with increased plant height, average stem diameter, and number of branches per plant, and maximum increase was reported for treatment with 10 ppm AuNPs. An increased average yield with increased number of pods per plant was also reported on NP treatment.

A few works have been reported with tobacco (*Nicotiana tabacum* cv. xanthi) and wheat (*Triticum aestivum*) plants describing their interaction with gold nanoparticles. Tobacco and wheat seedlings were treated hydroponically with 10-, 30-, and 50-nm-diameter gold nanomaterials coated with tannate or citrate for 3 or 7 days for wheat and tobacco, respectively, for studying the bioavailability of NPs with different size and surface chemistries to plants (Judy et al. 2012). This study provided information on the role of plant cell wall pores in the uptake of NPs and also investigated the influence of soil components on the intrinsic properties of nanomaterials and their further transport to plants.

5.2.1.2 Effects of Silver Nanoparticles

Silver has wide range of industrial applications as well as for medical and antibacterial purposes and hence more likely to get exposed to humans and environment which highlights the needs to study the effects of silver nanoparticles (AgNPs) in plants. The phytotoxicity of AgNPs (29 nm) on cucumber (*Cucumis sativus*) and lettuce (*Lactuca sativa*) was studied with seed germination tests, and a reduced effect on germination index was reported for cucumber seeds (Barrena et al. 2009). For lettuce seeds, the germination index seemed to be comparable with the controls. In the same study, a positive effect was observed with AuNPs of size 10 nm. The impact of AgNPs on the root elongation of greenhouse-grown radish and lettuce with barley (*Hordeum vulgare*) as the reference plant was investigated under hydroponics and soil conditions (Gruyer et al. 2014). Under hydroponics condition, a positive response on root elongation was observed in barley at low concentration of AgNPs, whereas a significant reduction in root length was observed on treatment with higher concentration of AgNPs. For lettuce, a reduction in root length was observed, and for radish, no significant variation had been reported on treatment. No negative effects were seen on treatment with AgNPs for the root length of all the three plants exposed to soil. The growth parameters of common bean (*Phaseolus vulgaris*) and maize (*Zea mays*) were studied with

different concentrations of AgNPs in which enhanced growth was observed at low concentrations and inhibitory effects with higher concentrations of NPs (Salama 2012). An increased root length, shoot length, and chlorophyll content were reported up to treatment with 60 ppm AgNPs after which declined growth parameters and chlorophyll content for higher concentrations. Similar observations were also reported for mung bean (*Vigna radiata*) and sorghum (*Sorghum bicolor*) plants on treatment with AgNPs (Namasivayam and Chitrakala 2011). Studies on the phytotoxic effects of AgNPs on rice plants reported uptake of NPs through roots, thus resulted in intracellular damage (Mazumdar and Ahmed 2011). The effects of AgNPs of three different sizes ranging from 1 to 20 nm and concentration ranging from 1 to 100 ppm on the germination of ryegrass, barley, and flax (*Linum usitatissimum*) were studied, and differently sized NPs affected differently on plant species. The smallest sized particle had an inhibitory effect on ryegrass even at very low concentration, whereas the intermediately sized particles had less inhibitory effects at low concentration but greater at higher concentration particularly with barley. Flax seeds were not at all affected by any type and/or any concentrations of AgNPs (El-Temsah and Joner 2010). Since different types of plant species behaved differently to same type of NPs of different size and concentration, seed germination tests solely cannot be relied for the analysis of environmental impact of AgNPs. Size-dependent toxicity studies of AgNPs were also carried out with Italian ryegrass (*Lolium multiflorum*), and it was reported that smaller AgNPs significantly reduced the growth with shorter roots and shoots and less biomass as compared to plants treated with larger NPs of similar concentration (Yin et al. 2011). This showed that the toxicity of AgNPs is highly influenced by total NP surface area. This study also reported that on exposing the seedlings to 40 ppm of gum arabic (GA)-coated AgNPs, the seedlings failed to develop root hairs with vacuolated and collapsed cortical cells and broken root cap which might be due to the loss of gravitropism in roots due to reduced auxin transport. Studies on the phytotoxicity of AgNPs with mung bean and sorghum reported adverse effects on seedling growth (Lee et al. 2012). Experiments were carried out both in agar and in soil media for highlighting the importance of media effect in nanotoxicity. Nanoparticle concentration-dependent growth inhibition was observed for both the plants in agar media; however, mung bean plants were not affected much in soil media, whereas a slight reduction in growth rate was observed for sorghum plants, which clearly showed the influence of exposure media on dissolution, bioavailability, and phytotoxicity of NPs. Wang et al. (2013a, b) reported stimulatory effects on root elongation, fresh weight, and evapotranspiration of poplars (*Populus tremula*) and Arabidopsis on using a narrow range of sublethal concentration of AgNPs, whereas above a certain level of concentration all forms of silver were phytotoxic and accumulation of silver in different plant parts varied with the plant species.

Developmental responses of maize and cabbage (*Brassica oleracea*) exposed to citrate-coated AgNPs and zinc oxide (ZnO) NPs were evaluated, and comparative toxicity profiles were developed with their corresponding ionic salts (Pokhrel and Dubey 2013). The toxicity due to NPs on the germination and root elongation of

both maize and cabbage was observed to be lesser than that occurred due to free ions. Anomalies were observed in maize root anatomy on treatment with citrate nano-silver and nano-ZnO, and this confirmed that the NPs might cause different biological interactions and their toxicity outcome is different from those occurred due to their specified ion effect. Uptake of silver by maize seedlings was found to be higher for treatment with $AgNO_3$ than with citrate nano-silver. Changes in the gene expression of plants on treatment with AgNPs and silver ions were also studied with Arabidopsis for analyzing the molecular mechanism of plant response to different contaminants. Both upregulation and downregulation of significant genes had been observed in response to AgNPs and silver ions which are associated with several stress responses in plants in which the upregulated genes are connected with metal and oxidative stresses and downregulated genes to pathogen and hormonal stimuli (Kaveh et al. 2013). These studies highlight the need for more studies to get enough information for better explaining the toxicity differences between metal-based NPs and their free ions. Effects on plant physiological responses and gene expression with NPs of different morphology were also investigated with AgNPs of triangle, spherical, and decahedral shape on Arabidopsis plants (Syu et al. 2014). This study reported the highest degree of root growth promotion and lowest accumulation of Cu/Zn super oxide dismutase with decahedral AgNPs, whereas the spherical AgNPs induced null effect on root growth promotion with highest level of Cu/Zn superoxide dismutase and anthocyanin accumulation. Protein accumulations were induced by all three morphologies of AgNPs and also activated gene expression in Arabidopsis. The phytotoxic and genotoxic effects of AgNPs on germinating wheat seedlings were also investigated, and negative effects were observed with higher concentrations of AgNPs which resulted in reduced seedling growth and morphological variations in root tip cells (Vannini et al. 2014). This study also suggested that the toxicity of AgNPs had come from the release of silver ions from AgNPs, which supported many of the above-mentioned reports. No DNA polymorphism was observed in wheat seedlings with NP treatment in the studied range of concentrations, whereas variations were observed in the expression of several proteins controlling primary metabolism and defense mechanisms. The fate and transport of AgNPs in the aquatic environment was studied by investigating their effects on aquatic plants. An important study was conducted with two different types of AgNPs, PVP-AgNPs (polyvinylpyrrolidone-coated silver nanoparticles), and GA-AgNPs (gum Arabic-coated AgNPs), on a mixed wetland plant community (Yin et al. 2012). Direct exposure and soil exposure experiments were carried out, and magnitude of variation on germination rate was more pronounced under direct exposure with higher concentration of GA-AgNPs treatment. The response of plant growth with NP treatment also varied with plant species. Higher rate of growth inhibition with increased concentrations of AgNPs was also reported for the aquatic plant swollen duckweed (*Lemna gibba*) which demonstrated toxicity due to the accumulation of AgNPs in the aquatic environment (Oukarroum et al. 2013).

5.2.2 Effects of Metal Oxide Nanoparticles in Plants

5.2.2.1 Effects of TiO$_2$, ZnO, SiO$_2$, Al$_2$O$_3$, CeO$_2$, and CuO Nanoparticles

The extensive production of TiO$_2$ worldwide resulted in their greater release to the environment with consequent contamination of plants, soils, and water systems. The end of life cycle of NPs was simulated, and it was reported that the greatest concerns were associated with the use of TiO$_2$ NPs and their fate and effects on ecosystem. Several studies reported the impact of TiO$_2$ NPs on plant development and physiology with contradictory results. The size-dependent distribution of TiO$_2$ NPs in wheat plants was reported by Larue et al. (2012a, b). It was suggested that NPs had not accumulated in plant roots above a threshold diameter of 140 nm and the size was limited to 36 nm for easy translocation to shoots from roots. It was also reported that the accumulation of NPs was not affecting the germination of seeds and or total biomass of plants. The same group also studied the effects of TiO$_2$ NPs on wheat and rapeseed (*Brassica napus*) plantlets grown under hydroponic conditions (Larue et al. 2012a, b). The plants were exposed to NPs both through root and through leaf exposure. Nanoparticle agglomerates were observed in plantlets under both root and leaf exposure methods, and higher Ti amount was found in rapeseed than in wheat. An increased root elongation was observed at early development stages on treatment with TiO$_2$ NPs; however, null effect on germination, evapotranspiration, and total plant biomass was observed. An increase in the catalase amount and decrease in the ascorbate peroxidase were reported on growing cucumber plants in sandy loam soils treated with 750 and 500 mg/kg of TiO$_2$, respectively (Servin et al. 2013). Translocation of TiO$_2$ from roots to fruits without biotransformation was also reported in this study. Enhanced seed germination and vigor of wheat were reported by employing proper concentrations of nano-TiO$_2$, whereas inhibitory effects were reported with bulk TiO$_2$ and neutral effects for higher concentration of nano-TiO$_2$ (Feizi et al. 2012). Larue et al. (2011) also studied the effects of TiO$_2$ NPs on wheat, rapeseed, and Arabidopsis, and results showed the uptake of NPs by plants. They also reported that the germination rate and root elongation were not affected with the studied type of NPs. Hence, the effects of titanium on plants show significant size, concentration, and species dependence. Studies also investigated the effects of TiO$_2$ NPs on agronomic traits such as plant height, ear weight, ear number, seed number, final yield, biomass, gluten, and starch content under water-deficit conditions, and improved agronomic traits were reported for TiO$_2$ NPs at 0.02 % (Jaberzadeh et al. 2013). In maize plants, the changes of photosynthetic pigments upon nano-TiO$_2$ spraying at different stages of plant growth and development were studied, and it was observed that nano-TiO$_2$ has significant effects of total content of chlorophyll, carotenoids, and anthocyanin and maximum effect was recorded on spraying nano-TiO$_2$ at reproductive stage of the plants (Morteza et al. 2013).

Du et al. ([2011](#)) studied the effects of TiO_2 and ZnO NPs on wheat growth and soil enzyme activities. The wheat plants were harmed by both the NPs with reduced plant biomass and affected soil environment by decreasing the activity of some of the soil enzymes such as protease, catalase, and peroxidase. The mechanism of toxicity induced by both the NPs is different. The TiO_2 NPs were retained in soil for longer time and hence greater probability to get attached to root cell wall, thus caused changes in the microenvironment and generated reactive oxygen species (ROS), which might damage cells. For ZnO NP treatment, greater uptake of Zn by plants had occurred due to high solubility of ZnO NPs in soil. The effects of nano-TiO_2 and nano-ZnO on rice seed germination showed no reduction in the percent seed germination from both NPs; however, nano-ZnO produced some detrimental effects on rice roots at early seedling stage with stunted and reduced number of roots (Boonyanitipong et al. [2011](#)). No negative effects were observed with nano-TiO_2 on root growth. On exposing the tomato (*Solanum lycopersicum*) plants to TiO_2 NPs and Fe_3O_4 NPs in the same experiment, an abnormal proliferation of root hairs was observed for seedlings exposed to higher concentration of TiO_2 NPs when compared to seedlings exposed to Fe_3O_4 NPs and control seedlings. No changes in shoot morphology and no seedling toxicity were observed on NP treatment in this experiment (Giordani et al. [2012](#)).

Arabidopsis is a model plant to study the developmental phytotoxicity of metal oxide NPs. Four different metal oxides such as aluminum oxide (nAl_2O_3), silicon dioxide ($nSiO_2$), magnetite (nFe_3O_4), and zinc oxide (nZnO) have been selected for studying their effects on three important toxicity indicators such as seed germination, root elongation, and number of leaves (Lee et al. [2010](#)). nZnO was found to be the most phytotoxic metal oxide NP followed by nFe_3O_4, $nSiO_2$, and nAl_2O_3. A significant reduction in germination was observed for nZnO NPs which could be related to their small size, monodispersity, and greater solubility facilitating their transport to intracellular spaces through seed coat pores. The greater particle size of other metal oxide NPs limited their inhibitory effect on seed germination. This study highlighted the significance of elemental composition and particle diameter on developmental phytotoxicity. Increased root elongation was observed with nAl_2O_3 in all the tested concentrations, whereas inhibitory effects were observed with other metal oxide NPs except for low concentration of $nSiO_2$. Hence, the same type of NP could cause different effects on same plant species with varied concentrations as reported in case of $nSiO_2$. This is one of the first reports for positive effects of nAl_2O_3 on plant growth, whereas all previous reports highlighted their negative or neutral effects on plant growth (Yang and Watts [2005](#); Lin and Xing [2007](#)). The degree to which NPs affect seed germination was also studied with cucumber seeds using Fe_3O_4, TiO_2, and carbon nanoparticles up to 5000 µg/ml, and inhibitory effects were observed with reduced root elongation especially at higher concentrations (Mushtaq [2011](#)). Studies on the effects of nanoscale ZnO on the germination, seedling vigor, plant growth, flowering, chlorophyll content, pod yield, and root growth of the peanut (*Arachis hypogea*) plants showed positive effects on all the studied parameters at a concentration of 1000 ppm ZnO and inhibitory effects at higher concentrations of 2000 ppm which revealed the

judicious usage of these particles on plants (Prasad et al. 2012). The effects of ZnO NPs and Zn^{2+} ions were also studied in alfalfa (*Medicago sativa*), tomato, and cucumber (*Cucumis sativus*) (De La Rosa et al. 2013). It was noticed that at higher concentrations of 1600 ppm of ZnO NPs, there was an increase in cucumber germination by 10 %, whereas tomato and alfalfa germination were reduced by 20 and 40 %, respectively. There was wide variation in the toxicity level of each plant species to different concentrations of NPs and difference in plant species might be the reason for differences in NPs uptake, tolerance, and toxicity. This study highlighted the phytotoxicity and uptake of ZnO NPs and Zn^{2+} ions by plants and also investigated the possible ZnO and Zn^{2+} biotransformation in plant tissues (Figs. 5.1 and 5.2; Table 5.1).

Mukherjee et al. (2014) also studied the physiological effects of ZnO NPs in soil cultivated green peas. Toxicological effects were measured by analyzing various parameters such as plant growth, Zn accumulation, chlorophyll production, activity of stress enzymes. An increased root elongation was observed for all studied concentrations of ZnO NPs, whereas the shoot growth was unaffected. The chlorophyll content in the leaves got reduced on treatment with ZnO NPs compared to the
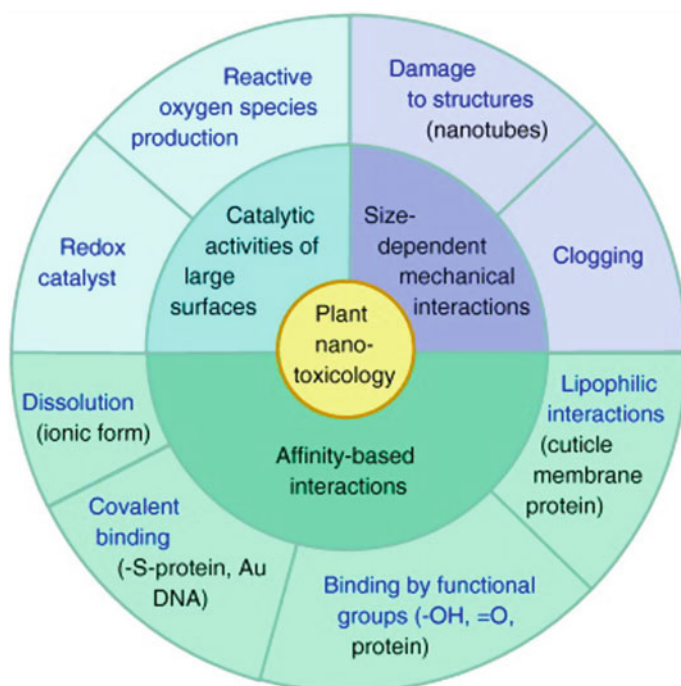
Fig. 5.1 Categorization of nanoparticle-dependent toxicity mechanisms. Different shades of the same color indicate related mechanisms subsumed to (*i*) size-dependent mechanical interactions; (*ii*) catalytic activities of large surfaces; and (*iii*) affinity-based interactions. Abbreviation: Au, gold [Reprinted with permission from Karl-Josef and Simone (2011)]
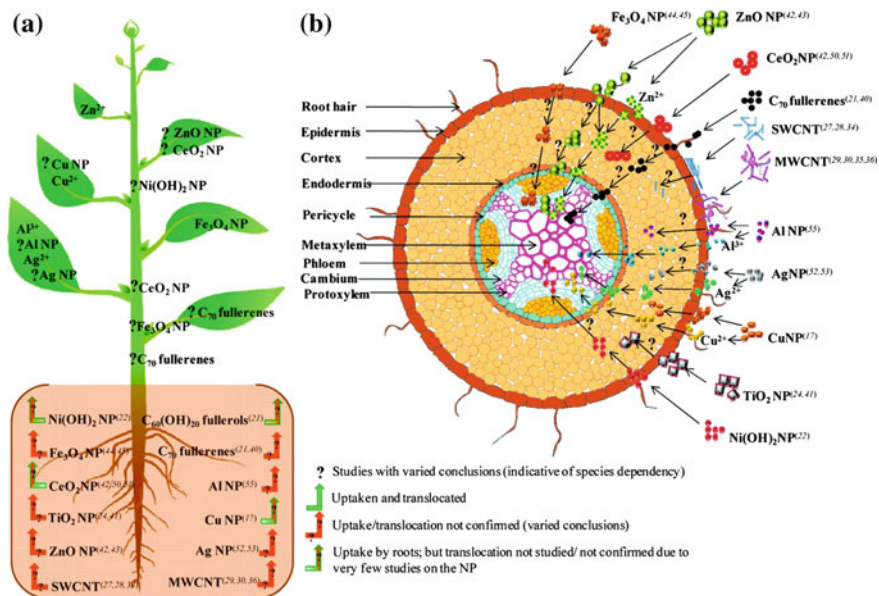
Fig. 5.2 Schematic representation of uptake, translocation, and biotransformation pathway of different nanoparticles in plant system (**a**) shows the selective uptake of nanoparticles by plants and (**b**) shows the transverse cross section of the root absorption zone showing the differential nanoparticle interaction on exposure [Figure reprinted with permission from Rico et al. (2011)]

controls and an increase in the H_2O_2 content indicated the nanotoxicity effects. Lin and Xing (2008) also reported the phytotoxicity of ZnO NPs with reduced biomass, shrunken root tips, and highly vacuolated and collapsed root epidermal and cortical cells. The root exudates could make changes in the zeta potential and aggregate size of ZnO NPs which affects their phytotoxicity mechanism.

Germination assay was also carried out with SiO_2 and Mo NPs in rice plants, and a positive effect on rice seed germination was observed on NP treatment. For SiO_2 NPs, further growth of rice seedlings was not affected with increased root and shoot length, whereas inhibited root growth and elongation was observed with higher concentrations of Mo NPs (Adhikari et al. 2013). Root necrosis was reported due to increased adsorption of Mo NPs on root system which resulted in toxicity. This study showed both positive and negative effects of different NPs at different concentrations on the same plant species. The beneficial effects of nano-SiO_2 on the germination of tomato seeds were reported by Siddiqui and Al-Whaibi (2014). This study supported the beneficial use of nanomaterial in sustainable agriculture. The phytotoxicity of SiO_2 NPs on Arabidopsis plants grown hydroponically was studied, and SiO_2 NPs did not exhibit severe phytotoxicity except for pH-dependent phytotoxicity with reduced development and chlorosis for plants exposed to NPs with high negative zeta potential. Accumulation of NPs was observed in Arabidopsis root cells in a size-dependent manner (Slomberg and Schoenfisch

Table 5.1 Phytotoxicity of important NPs on some major food and agricultural crops

Crops	Type of nanoparticles	Effects on plants	Reference
Mustard greens	AuNPs	Improved germination rate (with 25 ppm AuNPs) and improved total growth profile (at 10 ppm AuNPs)	Arora et al. (2012)
Arabidopsis	AuNPs	Reduced root length	Taylor et al. (2014)
Barley, Ryegrass	Ag NPs	Reduction in germination and shoot length with small-sized particles	El-Temsah and Joner (2010)
Cucumber	AgNPs	Reduced germination	Barrena et al. (2009)
Lettuce, Barley	AgNPs	Reduced root length for lettuce Increased root elongation for barley at low concentration AgNPs and reduced root length at higher concentration AgNPs	Gruyer et al. (2014)
Common bean, Corn, Mung bean, and Sorghum	AgNPs	Improved growth parameters at low concentration and inhibitory effects at high concentration	Salama (2012), Namasivayam and Chitrakala (2011)
Mung bean, Sorghum	AgNPs	Reduced seedling growth	Lee et al. (2012)
Onion, Tomato, and Radish	N-TiO$_2$	Positive effect on germination at 100 and 200 ppm N-TiO$_2$	Stampoulis et al. (2009)
Wheat	TiO$_2$	Not affecting germination and total biomass	Larue et al. (2012a, b)
Wheat	TiO$_2$ and ZnO	Reduced plant biomass	Du et al. (2011)
Corn	Al$_2$O$_3$	Reduced root length	Lin and Xing (2007)
Carrot, Cabbage, Cucumber, and Maize	Al$_2$O$_3$	Reduced root growth	Yang and Watts (2005)
Arabidopsis	Al$_2$O$_3$	Increased root elongation	Lee et al. (2010)
Soybean	ZnO	Reduced growth	Yoon et al. (2014)
Peanut	ZnO	Improved germination and seedling vigor at 1000 ppm and inhibitory effects at 2000 ppm	Prasad et al. (2012)
Tomato, Alfalfa	ZnO	Reduced germination	de la Rosa et al. (2013)
Green peas	ZnO	Increased root elongation	Mukherjee et al. (2014)
Rice	SiO$_2$	Improved seed germination and seedling growth	Adhikari et al. (2013)

Table 5.1 (continued)

Crops	Type of nanoparticles	Effects on plants	Reference
Tomato	CeO_2	Promoted plant growth and fruit maturity at low concentrations	Wang et al. (2012a, b)
Arabidopsis	CeO_2	Reduced chlorophyll at higher concentration	Ma et al. (2013)
Cucumber	CeO_2, ZnO	No negative impact on whole-plant life cycle	Zhao et al. (2013)
Maize	CuO	No inhibition on seed germination	Wang et al. (2012a, b)
Sunflower	Fe_3O_4	Reduced photosynthetic pigments	Ursache-Oprisan et al. (2011)
Soybean	SPIONs	Increased chlorophyll content	Ghafariyan et al. (2013)
Tomato	CNTs	Increased number of flowers and fruits	Khodakovskaya et al. (2013)
Rice	SWCNTs, MWCNTs, C_{60}	Improved seed germination, water uptake, healthier seedlings	Nair et al. (2012)
Barley, Soybean	MWCNTs	Improved seed germination	Lahiani et al. (2013)
Onion, Cucumber	Poly-3-amino benzenesulfonic acid Functionalized SWCNTs	Enhanced root elongation	Canas et al. (2008)
Lettuce	Poly-3-amino benzenesulfonic acid Functionalized SWCNTs	Inhibited root elongation	Canas et al. (2008)
Maize	MWCNTs	Improved growth	Tiwari et al. (2014)
Arabidopsis, Rice protoplasts	SWCNTs	Programmed cell death	Shen et al. (2010)
Maize	C_{60} Fullerenes	Reduced biomass	Torre-Roche et al. (2013)
Bitter melon	Fullerol	Increased biomass, fruit yield, and improved phytomedicines content	Kole et al. (2013)
Zucchini	MWCNTs	Reduced biomass	Stampoulis et al. (2009)
Lettuce	MWCNTs	Reduced root length	Lin and Xing (2007)

2012). Studies also reported increased germination percentage, dry weight, silica accumulation, and nutrient alleviation in seeds exposed to nano-SiO_2 under hydroponic conditions (Suriyaprabha et al. 2012). This highlighted the use of nano-SiO_2 as a highly utilizable source for plants.

The biotransformation of ZnO and CeO_2 nanoparticles on soybean was investigated, and its effects on germination and seedling growth along with the impact on DNA stability were studied (Lopez-Moreno et al. 2010a, b). Soybean germination was not affected by either of the NPs except at 2000 mg/L of CeO_2. However, both the NPs differentially affected the elongation of roots. Increased root elongation was observed for treatment with CeO_2 NPs, whereas treatment with ZnO NPs showed maximum elongation at 500 mg/L and minimum at 4000 mg/L. This study also reported the uptake of both NPs with the highest Zn accumulation occurred at 500 mg/L ZnO NPs and Ce accumulation increased with increase in the external concentration of CeO_2 NPs. The phenotypic response of tomato plants from seed germination to fruit maturity to low concentrations of CeO_2 NPs were documented, and the results indicated that the CeO_2 NPs at the studied concentrations promoted plant growth and fruit production (Wang et al. 2012a, b). However, translocation of Ce from roots to shoots and fruits presented a high level of risk to human health through dietary exposure. Uptake and toxicity studies of nano-ceria on alfalfa, maize, cucumber, and tomato plants reported reduced germination rate of maize, tomato, and cucumber at higher concentrations of nano-ceria (Lopez-Moreno et al. 2010a, b). It was also observed that the root growth was promoted by nano-ceria in cucumber and maize, whereas reduced in alfalfa and tomato and nano-ceria promoted shoot elongation in all studied plant species with all studied concentrations.

It is important to analyze the phytotoxic effects of NPs through foliar application also as most of the nanotoxicity studies are focusing on root exposure to NPs. Hong et al. (2014) studied the translocation and physiological impacts of foliar-applied CeO_2 NPs on cucumber plants. Hydroponically grown cucumber plants were treated with nano-ceria powder through aerial application. Cerium was detected in different tissues of treated plants suggesting the translocation of Ce from leaves to other plant parts. This is an important study which showed that atmospheric NPs could be taken up by plants which poses a threat to environment and health. However, another study reported that there was no evidence of translocation of CeO_2 NPs in maize plants as no nanoparticles were detected in the newly grown leaves of already treated plants. This suggests that the biological barriers of plants are more resistant toward easy entry and translocation of NPs than the mammalian barriers (Birbaum et al. 2010). Ma et al. (2013) investigated the physiological and molecular responses of CeO_2 and indium oxide (In_2O_3) NPs on Arabidopsis. This study is the first report investigating differential regulatory response through changes in the expression of glutathione and sulfated metabolic pathways in response to exposure to rare earth oxide NPs. In this study, it was also reported that the chlorophyll content was reduced at higher concentrations of CeO_2 NPs; however, it was unaffected on exposure to In_2O_3 NPs. Zhao et al. (2012) reported that the treatment of CeO_2 NPs on maize plants increased the accumulation of H_2O_2 in phloem, xylem, bundle sheath cells, and shoot epidermal cells. The integrity of membranes was not compromised on NP treatment as no ion leakage of reported in either roots or shoots. The net photosynthetic rate of the leaves, transpiration, and conductance of stomata were also not affected on CeO_2 NP treatment. Increased production of stress-related parameters in maize plants on NP treatment helped

them to survive against nanotoxicity. Changes in the nutritional property of cilantro (*Coriandrum sativum*) with significant uptake and translocation on treatment with CeO_2 NPs were also reported which showcased their entry and impacts in the food chain (Morales et al. 2013). The transgenerational studies with CeO_2 NPs showed that the second-generation seedlings grown from the seeds obtained from CeO_2-treated tomato plants were smaller and weaker with lesser biomass, lower water transpiration, and higher reactive oxygen species content and also accumulated higher amount of ceria (Wang et al. 2013a, b). This study demonstrated the multigenerational effects of engineered NPs on plants.

The toxicity of CuO NPs to maize was studied by germination tests, and no inhibition was observed on the germination of seeds. Their transport and redistribution were also investigated, and it was found that the NPs were transported to shoots via xylem and back-translocated from shoots to roots through phloem (Wang et al. 2012a, b). The fate of metal oxide NP as a function of size by comparing the behavior of CuO and ZnO NPs with corresponding microparticles in sand matrix with and without wheat plants was studied and greater root toxicity was observed on interaction with smaller particles. It was noticed that several factors from sand and plant modified the aggregation and dissolution of both NPs and microparticles, which decides their route of accumulation and fate in the environment (Dimkpa et al. 2013).

5.2.2.2   Effects of Magnetite Nanoparticles

Increased biological applications of magnetic NPs as multifunctional agents for targeted cell delivery and medicinal imaging have opened avenues for their applications in plant biology. However, studies on their toxicological effects on plants and bioaccumulation in food chain still need to be more addressed for their improved use as smart treatment delivery vehicles in plants. The effects of magnetic NPs coated with perchloric acid on the early ontogenic stages of maize plants were studied, and a slight inhibitory effect on plantlet growth was observed. The toxicity symptoms led to the development of brown spots on leaf surface and excess iron treatment generated oxidative stress on leaf cells which in turn affected photosynthesis with decreased rate of metabolism (Racuciu and Creanga 2009). In sunflower (*Helianthus annuus*) seedlings, it was observed that the chlorophyll content was reduced up to 50 % on the application of low concentrations of magnetic NPs. Magnetic NPs negatively affected the biosynthesis of photosynthetic pigments thus affecting the chlorophyll content (Ursache-Oprisan et al. 2011). The effect of SPIONs (super paramagnetic iron oxide nanoparticles) on soybean has been studied, and it was reported that the SPIONs, which were translocated in soybean, increased the chlorophyll levels with no trace of toxicity (Ghafariyan et al. 2013). The total and nitric nitrogen content of lettuce due to treatment with magnetic nanofluids showed that the treatment influenced both the total and nitric nitrogen content. The total nitrogen content was found to be higher in the treated plants, and the nitric nitrogen in treated plants is lower when compared to the

control plants (Pirvulescu and Sala 2012). Feizi et al. (2013) carried out experiments to study the biological responses of muskmelon (*Cucumis melo*) to magnetic field and AgNPs in comparison with commercial fertilizers under field conditions, and the results indicated that the plants treated with AgNPs in magnetic field had the highest fruit yield with improved early ripening.

Zhu et al. (2008) studied the uptake of magnetic NPs (20 nm in size) by pumpkin seedlings in hydroponic solutions and the signals for magnetic NPs were detected in roots, stems, and leaves of pumpkin plants using vibrating sample magnetometer. However, there was difference in the uptake of NPs in different growth medium, and no uptake was observed in soil medium, whereas reduced uptake of NPs by plants was found when grown in sand. This might be due to the difference in the adherence of magnetic NPs to soil and sand. The physiological effects of magnetite NPs on perennial grass and pumpkin plants grown under hydroponic conditions were also investigated, and it was found that the tested NPs were not translocated to the plants as no magnetization was detected in the shoots of treated plants (Wang et al. 2011). The size of NPs used in this test was larger than the cell wall pores which limited their entry. The uptake of magnetic carbon-coated bioferrofluid through the roots of four crop plants, pea, sunflower, tomato, and wheat was studied, and it was reported that the ferrofluid reached the vascular cylinder, moved through the xylem vessels, and reached the entire aerial portions of the plants in less than 24 h (Cifuentes et al. 2010). The same group also reported the penetration and transportation of magnetic carbon-coated NPs through the aerial parts of cucumber (González-Melendi et al. 2008; Corredor et al. 2009). Krystofova et al. (2013) studied the effects of magnetic NPs and modified magnetic NPs on tobacco BY-2 cell suspension cultures. They studied the effects of NPs on growth, proteosynthesis, and antioxidant activity of cells, and it was observed that the effects of magnetic NPs on growth of cell suspension culture were moderate, whereas noticeable changes were detected in all biochemical parameters. Hao et al. (2013) reported the use of magnetic gold NPs as a carrier for the delivery of fluorescein isothiocyanate (FITC) and plasmids into canola cells with and without cell wall which would benefit the development of transgenic plants. All such reports on the uptake and distribution of magnetic-based NPs in plant system opened up great opportunities to explore them for site targeted delivery of chemicals and other substances with an external control using strong magnets.

5.2.3   Effects of Carbon-based Nanomaterials

Carbon-based nanomaterials, such as single-walled carbon nanotubes (SWCNTs), multiwalled carbon nanotubes (MWCNTs), buckyballs (C_{60}), have several unique mechanical and structural properties and hence having potential applications in biomedical engineering and medicinal chemistry rather than its large-scale applications in electronics. However, concerns on the toxicity of these nanomaterials are the major limiting factor for its large-scale applications in medicine and agriculture.

Several works were reported with conflicting results for the interaction between carbon nanomaterials and biological systems, especially with animals (Cui et al. 2005; Fabbro et al. 2012; Das et al. 2013) but very limited works on plant system.

The effects of carbon nanotubes on plant phenotype and soil microbial community were studied, and it was observed that the tomato plants grown in soil supplemented with carbon nanotubes produced twice the amount of flowers and fruits when compared to control plants. The soil microbial community was also checked, and phylogenetic analyses indicated that the relative abundances of *Bacteroidetes* and *Firmicutes* got increased, and a decrease was observed for *Proteobacteria* and *Verrucomicrobia* with increasing concentration of CNTs (Khodakovskaya et al. 2013). The effects of engineered carbon nanomaterials of various dimensionalities on rice seed germination were studied, and an increase in germination rate with increased water uptake was observed for treated seeds than the control seeds (Nair et al. 2012). The treated seedlings also appeared to be healthier than the control plants in the studied range of concentration of carbon nanomaterials. In barley and soybean, it was observed that MWCNTs accelerated the seed germination and no negative effects were observed on further development of plants grown from exposed seeds (Lahiani et al. 2013), and it was observed that the expression of genes encoding water channel proteins increased in treated seeds than the control seeds. Canas et al. (2008) functionalized SWCNTs with poly-3-amino benzenesulfonic acid and studied the effects of both functionalized and non-functionalized SWCNTs on root growth of six crop plants, cabbage, carrot (*Daucus carota*), cucumber, lettuce, onion (*Allium cepa*), and tomato. Root elongation was enhanced in onion and cucumber and inhibited in tomato with non-functionalized nanotubes and functionalized nanotubes inhibited root elongation in lettuce. Cabbage and carrots were not affected by both types of nanotubes. Nanotubes were found to be adsorbed on the surface of roots with little uptake in this study. Studies on the effects of MWCNTs on red spinach (*Amaranthus dubius*), lettuce, rice, chili (*Capsicum* spp.) cucumber, okra (*Abelmoschus esculentus*), and soybean showed varied effects on root and shoot growth of different plant species and toxicity of nanotubes on seed germination, and growth was observed at higher concentrations and little effect was observed on chili, soybean, and okra (Begum et al. 2012). The beneficial effects of MWCNTs at low concentrations to maize plants were studied, and growth enhancement was correlated with improved water delivery by MWCNTs (Tiwari et al. 2014). Similar effects were reported for mustard and gram plants too (Mondal et al. 2011; Tripathi et al. 2011). Lin et al. (2009) studied the uptake and translocation of natural organic matter (NOM)-modified C_{70} and MWCNTs, and aggregates of NOM-C_{70} were found near the vascular system of stem which suggested their uptake along with water and nutrients though xylem; however, only minimal uptake of MWCNTs which is limited to roots. The uptake of water, nutrients, and overall plant development could hinder at higher concentrations of MWCNTs due to the blockage of plant roots and roots hairs by the surface adsorbed nanotubes. Khodakovskaya et al. (2012) demonstrated that the growth of tobacco cell culture had been enhanced with MWCNTs in a wide range of concentrations. The expression of tobacco aquaporin

gene and the production of corresponding protein increased in cells exposed to MWCNTs when compared to control. Also the expression of marker gene for cell division and cell wall extension was upregulated on treatment with MWCNTs. These results suggested the role of CNTs in regulating cell division and plant growth with applications in enhanced production of plant cell cultures in plant biotechnology and pharmaceutical industry. The adverse cellular responses of SWCNTs to Arabidopsis and rice protoplasts were investigated, and it was found that the oxidative stress generated had led to programmed cell death and the survival of cells was highly dose dependent (Shen et al. 2010). The effects of fullerene exposure on the uptake and accumulation of dichlorodiphenyldichloroethylene (p, p′-DDE, a common agricultural contaminant) by three different plants, zucchini (*Cucurbita pepo*), soybean, and tomato were investigated (Torre-Roche et al. 2012). An increased contaminant level in shoots was observed for zucchini, whereas decreased p, p′-DDE level in soybean shoots and not much change has been observed for tomato. However, the total plant p, p′-DDE level got increased for all plant species on exposure to fullerene which calls for more studies on nanoparticle-contaminant interactions.

The ability of SWCNTs to traverse across the plant cell wall and cell membrane was first reported by Liu et al. (2009). This has opened novel methods to deliver DNA and other molecules to intact plant cells. Liu and his group also studied changes in the cell wall of tobacco cells under the repression of water soluble carboxy-fullerenes. Disruption in cell wall and cell membrane was observed on the adsorption of fullerenes which led to complete inhibition of cell growth (Liu et al. 2013). An increased glycosyl residue was observed in the cell wall of fullerene-treated plants cells with elevated levels of reactive oxygen species. Serag et al. (2011a, b) investigated the ability of FITC-labeled MWCNTs to penetrate the cell membrane of periwinkle (*Catharanthus roseus*) protoplasts, and their internalization mechanism was studied with the help of confocal imaging and TEM techniques. The direct penetration mode helped MWCNTs to bypass endosomes and hence opens new avenues in designing endosomes escaping nanotransporters for plant cells. A size-dependent translocation of MWCNTs to different cellular structures such as nucleus and plastids was also observed which can be utilized for delivering molecular cargoes specifically into target compartments. They also explained a functional approach for the controlled subcellular distribution of FITC-labeled SWCNTs and studied the nature of vacuolar uptake, cytoplasmic accumulation in different subcellular structures, and finally the cellular elimination. Such studies on trafficking of SWCNTs through subcellular membranes are important in site specific delivery of biomolecules for plants that are currently recalcitrant to genetic transformation (Serag et al. 2011a, b). The same group also investigated the ability of cup stacked carbon nanotubes (CSCNTs) with cellulase immobilized on its side walls and tips to penetrate plant cell walls by producing local lesions with the help of cellulase. CNTs can hence be successfully utilized as nanotransporters to plant cells without completely removing the cell wall of plants (Serag et al. 2012a, b). The role of carbon nanotubes in oxidative cross-linking of monolignols during lignin biosynthesis in plant cells was also studied, and this

provided information on the post-uptake behavior of CNTs inside the cell which can be more helpful in plant defense research and possible detoxification mechanisms in cells (Serag et al. 2012a, b). Torre-Roche et al. (2013) studied the effects of MWCNTs or C_{60} fullerenes on the uptake of weathered pesticides by maize, zucchini, tomato, and soybean, and the results showed that the pesticide accumulation varied with the type of plant species, type of nanomaterial, and its concentration. Studies on the effects of fullerol on the biomass, fruit yield, and phytomedicine content of bitter melon (*Momordica charantia*) reported increased biomass with large and bigger fruits with improved content of anticancerous phytomedicines (Kole et al. 2013). Recently, the researchers from MIT reported the engineering of plant chloroplasts with SWCNTs in which the nanotubes were passively transported and interacted with the lipid bilayers of plant chloroplasts. A triple fold increase in photosynthetic activity was reported with enhanced electron transport rates. They also demonstrated the use of plants as biochemical detectors with the help of interaction of plants with modified nanotubes (Giraldo et al. 2014). This novel research area called nanobionics could bring more applications of nanotechnology in plant biology.

5.3 Conclusion

For the sustainable development of nanotechnology, it is important to understand the ecotoxicological effects of engineered nanomaterials on environment. The current chapter reviewed the uptake, translocation, accumulation, and phytotoxic effects of different nanoparticles depending on the plant species and size, type, chemical composition, functionalization, concentration, and stability of nanoparticles. Nanoagriculture could utilize nanotechnology in the best possible ways for the improved growth and development of plants. However, still there is a big gap in the knowledge about effects of different nanomaterials in plants as it is depending upon several interrelated factors such as different properties of nanoparticles and also the type of plant species. Some plants are capable of uptaking nanoparticles and accumulating them in different plant tissues. Their effects in plants vary with plant growth stage, time of exposure, method of uptake, and also various physical and chemical properties of plants. Researchers reported both positive and negative effects of nanomaterials on plant system. Some nanoparticles improved the seed germination and stimulated growth parameters in some plants, however, produced contradictory effects on others. Several studies have reported significant phytotoxicity due to the direct exposure to specific type of nanoparticles, and this emphasizes the need for ecologically responsible disposal of nanoparticle containing wastes. This highlights the necessity for more experimental studies extending over several generations of plants that are required for understanding the long-term effects of nanoparticles on ecosystem and for the safe and effective use of nanomaterials at judicious concentrations.

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