

# Chapter 14

## Impact of Nanomaterials on Plant Physiology and Functions



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### 14.1 Introduction

Nanotechnology has generated a variety of nanoparticles (NPs) with differences in size, shape, surface charge, and surface chemistry (Albanese et al. 2012). Among the various types of nanomaterials synthesized and released in the environment, metallic NPs are found in greater amount. The metal-based NPs have been extensively used in different applications (Husen and Siddiqi 2014a; Siddiqi et al. 2016, 2018; Husen 2017) which are directly or indirectly related to humans (Zhu and Njuguna 2014) and the environment (Remédios et al. 2012). The systematic design and engineering of NPs pose risks through inhalation, dermal penetration, and environmental persistence to humans as well as environment (Maurer-Jones et al. 2013). Small size and large surface area of NPs make them more prone for causing toxicity in the ecosystem (Ostiguy and IRSST (Québec) 2006). Plants, the most abundant species in the ecosystem, possess the highest likelihood to experience adverse or positive effects of NPs (Maiti et al. 2015). The presence of NPs in the air, water, and soil shows inevitable effects on plants. NPs are absorbed directly or indirectly by rootless or rooted surface by any of the physical or chemical processes. NPs are transported to leaves and other parts of plants through stem and may get accumulated in seeds (Maiti et al. 2015). Plants originating from seeds having NP concentration above bioconcentration may develop toxicity. NPs also play vital role in the protection of plants against different abiotic stresses as these can mimic the role of antioxidant enzymes (Rico et al. 2013a, b; Wei and Wang 2013; Siddiqi and Husen 2016, 2017). They may also enhance the photosynthesis rate by suppressing the

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osmotic and oxidative stresses (Haghighi and Pessarakli 2013; Qi et al. 2013). On the other hand, plants also show toxic effects depending upon the plant species and the type and concentration of NPs used (Begum et al. 2011; Slomberg and Schoenfisch 2012). For instance, the presence of NPs in growth media inhibits the seed germination rate, root and shoot length, plant biomass, and even the level of nutrients (Barhoumi et al. 2015; Da Costa and Sharma 2016; Wang et al. 2015). Exposure of plants to NPs has detrimental effects on cell biosynthesis, cell organization, electron transport, and energy pathways of the plant system (Landa et al. 2012; Van Aken 2015). Extensive studies have been carried out to investigate the interaction of NPs with plants, but the role of NPs in plant physiology and functions still remains ambiguous and unclear.

This chapter addresses the role of metallic NPs in plant physiology affecting the various functions of plant systems. The mechanism of NPs uptake, transport, and accumulation in the plant and plant parts and the role of NPs associated factors in relation to major physiological processes such as photosynthesis, water and nutrient uptake, respiration, transpiration, and seed germination have been highlighted. Both the negative and positive effects of metallic NPs on the activation of plant growth and development have been discussed with special focus on the NP-mediated gene delivery to plants as well as the phytotoxicity of NPs.

## 14.2 NPs Interactions with Plants

As already mentioned, the wide applicability and use of metal NPs in our daily life are directly proportional to the release of NPs in the environment (Maurer-Jones et al. 2013). NPs discharged into the environment possess a great tendency to interact with the terrestrial or aquatic plants through the atmosphere, soil, or water (Rico et al. 2011). The mechanism of NPs interactions with plants covers mainly three phases: uptake, translocation, and accumulation. The interactions of NPs with plants depends upon the type of plant species, type of NPs, the chemical composition, surface functionality, properties, shape and size of NPs, and many more factors (Rico et al. 2011). The uptake of nanomaterials by plants is a current topic of research in the scientific world, as most of the data available till date provides a view merely in the formative stage. This is because the protocols followed to measure the quantity of NPs entering in the plant tissues are not yet well-defined (Remédios et al. 2012). The lack of proper information regarding how the NPs enter the plant tissues, their transport, and then accumulation in various food chains leads to a defective understanding of the phenomena. The metal-based nanomaterials include Ag, Au, Al<sub>2</sub>O<sub>3</sub>, Cu, Zn, ZnO, TiO<sub>2</sub>, CeO<sub>2</sub>, SiO<sub>2</sub>, Fe, Fe<sub>2</sub>O<sub>3</sub>, CdS, ZnSe, etc. Several reports are available on the uptake of these different kinds of NP by the plant systems and their effect on the physiology and growth of plants.

### 14.3 Uptake, Translocation, and Bio-distribution of Metallic NPs in Plants

In general, uptake of NPs occurs when a particle penetrates the cell walls of plants. The plant cell wall acts as a semipermeable barrier that regulates the trafficking of materials across the membrane through various pores to the plant cells. Several routes have been proposed in the literature describing the uptake of NPs by the plants. Pore sizes of the plant cell wall vary depending upon the plant species but generally range from 5 to 20 nm (Miralles et al. 2012; Fleischer et al. 1999). To pass through these pores, the size, orientation, and properties of NPs play a critical role. NPs or their aggregates having a size smaller than the diameter of the cell wall pores could easily penetrate the cell wall and reach the plasma membrane (Navarro et al. 2008). NPs may enter into plant cells by binding to carrier proteins and ion channels, through endocytosis, by binding to organic acids present in the culture media, or by forming complexes with the transporters of the plasma membrane (Kurepa et al. 2010; Rico et al. 2013a, b). Once the NPs reach the cell cytoplasm, these may combine with various cellular organelles and obstruct the metabolic reactions at that particular site (Jia et al. 2005). Although in vitro studies have been performed using isolated plant cells to determine the NPs uptake and interactions, these studies did not prove enough to explain the interactions of NPs with the whole plant. Most of the studies have suggested that NPs uptake by plants is associated with the absorption of nutrients from the culture media in which plants are grown (Lin et al. 2009). The uptake of NPs by the plant mainly occurs through seed coat, roots, and leaves. It takes place through the root tips in many species, though NPs can also enter the plant tissues through the stomatal openings present on the leaf surface. The mucilage and exudates excreted by root caps and root hairs also assist in the NP uptake in various plant species (Chichiricò and Poma 2015; Schwab et al. 2016). Details of the uptake through different routes are described below.

Metallic NPs are being rapidly discharged into the environment and used extensively in agriculture system over the past few years. Studies on the uptake, translocation, and accumulation of iron oxide ( $\text{Fe}_2\text{O}_3$ ) NPs in pumpkin plant grown in the aqueous media showed that NPs were taken up by the roots and then transported to leaves through the stem of the plant (Zhu et al. 2008). In wheat plant (*Triticum aestivum*) grown in the hydroponic culture, uptake of the citric acid-coated  $\text{Fe}_2\text{O}_3$  NPs ( $20 \text{ mg L}^{-1}$ ) took place by roots through the apoplastic pathway (Iannone et al. 2016). Leaves and roots of arugula (*Eruca sativa*) and escarole (*Cichorium endivia*) were exposed to Pt NPs to study root-to-leaf and leaf-to-root translocation. Inductively coupled plasma mass spectrometry results showed that for both plants, the proportion of Pt translocated from roots to leaves (99% for arugula and 28% for escarole) was higher than that from leaves to roots (<1% for both plants) (Kranjc et al. 2018). It was depicted that foliar surface free energy affects Pt NPs adhesion, uptake, and translocation from leaves to roots in arugula and escarole. Similarly, the fluorescence and transmission electron microscopy images revealed that the magnetic  $\text{Fe}_2\text{O}_3$  NPs penetrated the corn (*Zea mays*) root epidermis and migrated from the epidermal

layer to endodermal cells through the apoplastic route (Li et al. 2016a, b). It was hypothesized that the macromolecular exudates excreted by root cells might be responsible for the NPs accumulation in the root epidermis (Li et al. 2016a, b). A different study regarding the uptake and accumulation of the anatase titanium dioxide  $\text{TiO}_2$  NPs (12 nm) has shown the uptake of these NPs by wheat plant roots from the hydroponics media. After 7 days of exposure,  $\text{TiO}_2$  NPs were observed in the parenchyma cells of the root and also in the vascular cylinder, indicating the transfer of NPs from media to vegetal tissues of the wheat (Larue et al. 2011). In another study, the uptake and transport of  $\text{TiO}_2$  NPs were studied in two crops (*Phaseolus vulgaris* and *T. aestivum*), a wetland species (*Rumex crispus*), and a floating aquatic plant (*Elodea canadensis*). All the rooted plants showed higher concentrations of Ti in roots. Ti from these NPs was translocated from roots to shoots in *R. crispus*. It was proposed that this species might increase the availability of metal NPs by producing siderophores (which have the tendency to bind metals) or by altering the rhizosphere pH (Jacob et al. 2013).

The uptake and transport of silver nanoparticles (AgNPs) of 20 nm size in *Arabidopsis thaliana* seedlings grown in gel media have been studied by Geisler-Lee et al. (2014), who noticed the presence of AgNPs on root surface and root tips and also in the root hair cells 14 days after planting. By the 17th day, AgNPs reached the vascular tissues, viz., xylem and phloem, and got transported through them to the whole plant. The AgNPs accumulated in cotyledons quite early, entered the stomatal pores at day 14, and then heavily accumulated in stomata and pavement cells by the 17th day of planting. The study thus showed the accumulation and a long-distance transport of AgNPs in *A. thaliana* (Geisler-Lee et al. 2014). Another study revealed the uptake of tannate and citrate-coated gold (Au) NPs of 10, 30, and 50 nm size in tobacco *Nicotiana* and *Triticum aestivum* plants. The uptake behavior of Au NPs was different in the two species. Au NPs were found in the leaf midrib of tobacco plant in detectable concentrations, whereas the wheat leaf showed no evidence of Au NPs accumulation. The authors suggested that there is no requirement of passive movement through the pores of the cell wall for NPs uptake inside the plants (Judy et al. 2012). Similarly, polyvinylpyrrolidone (PVP)-coated Au NPs of diameter 40 nm were applied to tomato seeds at concentrations of  $0.2 \text{ mg L}^{-1}$  and  $5 \text{ mg L}^{-1}$ , and the tomato plants obtained were capable of absorbing the 40 nm-sized Au NPs and transporting them as intact NPs to the plant shoots (Dan et al. 2015).

A recent study (Antisari et al. 2015) described the uptake and translocation of metal oxides  $\text{CeO}_2$ ,  $\text{Fe}_3\text{O}_4$ ,  $\text{SnO}_2$ , and  $\text{TiO}_2$  and metallic Ag, Co, and Ni NPs in tomato plants grown in a soil and peat mixture.  $\text{Fe}_3\text{O}_4$  and  $\text{TiO}_2$  did not show any uptake by plant organs. Ag, Co, and Ni accumulated in leaves, and Fe, Ce, and Sn were accumulated in roots, while Ni was found in stems. Ag and Co were translocated from roots to the aboveground organs, whereas no translocation was observed in the case of Ce, Sn, and Ti. The translocation of metal NPs could be associated with the absorption behavior of water and nutrients in plant roots (Antisari et al. 2015). Similarly, CuO NPs exposed to *Z. mays* were studied for their transport and bio-distribution in the plant. The results demonstrated that CuO NPs were taken up by the plant roots. The evidence of translocation of NPs was provided by the

presence of NPs in the xylem saps which might be responsible for the transport of particles from roots to shoots (Wang et al. 2012). NPs could also be translocated back to roots from shoots by the phloem. In general, CuO NPs existed in the cell wall of epidermal cells, intracellular spaces, and the cytoplasm of cortical cells. Their presence was also observed in the nucleus at 100 mg L<sup>-1</sup> concentration. The study suggested that NPs may pass through epidermis and cortex to enter the intracellular space via the apoplastic pathway; further, the presence of endosome confirmed that CuO NPs entered the plant cells by endocytosis (Wang et al. 2012). Another study of zinc oxide (ZnO) NPs (30 nm) in relation to maize plants has shown that ZnO NPs were present in the epidermal cells, cortex, and root tips of the plant. Few NPs also entered the vascular cylinder of tap roots through the primary root-lateral root junction. No NPs were observed in shoots, thus indicating a lack of NPs translocation to the aerial plant parts (Lv et al. 2015) (Table 14.1).

**Table 14.1** Role of metal and metal oxide nanoparticles in plant growth and development

Type of nanoparticles (NPs)	Plant	Concentration of NPs used	Effect on plants	Reference
AgNPs	<i>A. thaliana</i>	300 mg L <sup>-1</sup>	Inhibition of root elongation and leaf expansion, decreased photosynthetic efficiency	Sosan et al. (2016)
AgNPs	Wheat, cowpea, and <i>Brassica</i>	50 and 75 ppm	Improvement in growth, root nodulation and shoot parameters	Mehta et al. (2016)
AgNPs	<i>Lycopersicon esculentum</i>		Drastic decrease in germination index, root and shoot length	Mehrian et al. (2016)
AgNPs	Maize	100 mg kg <sup>-1</sup>	Increase in biomass	Sillen et al. (2015)
Cu NPs and AgNPs	<i>Cucurbita pepo</i>	–	Decrease in biomass and transpiration	Musante and White (2012)
AgNPs	<i>Raphanus</i>	500 mg L <sup>-1</sup>	Decrease in root and shoot length, decrease in water content	Zuverza-Mena et al. (2016)
AgNPs	<i>Brassica</i>	50 ppm	Positive effect on root and shoot length and seedling index	Sharma et al. (2012)
AgNPs	Rice	10 or 100 mg L <sup>-1</sup>	Decrease in growth and germination of seedlings	Thuesombat et al. (2014)
CeO <sub>2</sub>	<i>Arabidopsis thaliana</i>	250 ppm	Increase in biomass	Ma et al. (2013)
Fe NPs	Barley	300 mg L <sup>-1</sup>	Reduction in germination rate	El-Temsah and Joner (2012)
Alumina NPs	<i>Lemna minor</i>	0.3 g L <sup>-1</sup>	Increase in root length and accumulation of biomass	Juhel et al. (2011)

(continued)

**Table 14.1** (continued)

Type of nanoparticles (NPs)	Plant	Concentration of NPs used	Effect on plants	Reference
ZnO	<i>Arachis hypogea</i>	1000 ppm	Enhancement in stem and root growth, high yield	Prasad et al. (2012)
ZnO	Soybean	500 mg L <sup>-1</sup>	Increase in root growth of the plant	Lopez-Moreno et al. (2010)
Fe, Co, and, Cu NPs	Soybean	<300 mg hectare <sup>-1</sup>	Increase in yield of the crop plant	Ngo et al. (2014)
Zeravalent Fe	Peanut	40 and 80 μmol L <sup>-1</sup>	Stimulation of seedling development and growth	Li et al. (2015a, b)
ZnO, FeO, and ZnCuFe-oxide NPs	<i>Vigna radiata</i>	–	Improvement in shoot growth of seedlings	Dhoke et al. (2013)
Cu NPs	Lettuce	0.013% (w/w)	Improvement in shoot/ root ratio	Shah and Belozeroova (2009)
CuO	<i>Triticum aestivum</i>	500 mg kg <sup>-1</sup>	Increase in plant biomass	Dimkpa et al. (2012)
TiO <sub>2</sub>	Wheat	2 and 10 ppm	Promotion of seed germination	Feizi et al. (2012)
Au NPs	<i>Gloriosa superba</i>	1000 μM	Enhancement in seed yield	Gopinath et al. (2014)
TiO <sub>2</sub>	Fennel	60 ppm	Positive effect on seed germination and shoot dry weight	Feizi et al. (2013)
ZnO	<i>Vigna radiata</i>	20, 40, 60 and 100 mg	Increase in germination rate, fresh and dry weights of roots and shoots	Jayarambabu et al. (2014)
Au NPs	<i>Brassica</i>	10–25 ppm	Increase in seed yield as well as increase in total sugar content	Arora et al. (2012)
Al NPs	Raddish, rape	2000 mg L <sup>-1</sup>	Improvement in growth of plant roots	Lin and Xing (2007)

Quantum dots (QDs) are another important kind of metal-based NPs used in various applications. The impact of these NPs after their release in the environment is necessary to understand. Thereby, few recent studies have explored the uptake, translocation, and bioaccumulation of CdSe/CdZnS QDs in plants. In a study, *A. thaliana* leaf petiole was exposed to three types of CdSe/CdZnS coated with anionic, cationic, and neutral coatings. It was found that the anionic NPs were taken up by the leaf petiole and roots and distributed uniformly in the plant leaves. The cationic and neutral QDs showed destabilization in the plant growth media. The cationic QDs failed to translocate readily to the distal parts of plant leaves possibly due to their aggregation. Confocal microscopic studies demonstrated that

the anionic and neutral charged QDs get accumulated within cells of the main root, root hairs, and leaf veins (Koo et al. 2014). In another study of *A. thaliana* exposed to CdSe/CdZnS QDs, intact QDs were not internalized by the plants. Analysis of Cd and Se in the roots and leaves showed that QDs were mainly present on the external surfaces of roots and were not transported to the plant leaves (Navarro et al. 2012). The strong adsorption of QDs on the root surface was supposed to be due possibly to the mechanism of van der Waals interactions as well as the cross-linking of CO<sub>2</sub> groups on the plant roots and QDs. The uptake of QDs occurs when QDs are conjugated to nitrogenous organic compounds like arginine, glycine, and chitosan (Silver and Ou 2005).

## 14.4 Effect of NPs Uptake on Plant Physiology and Functions

The main processes that constitute plant physiology are water uptake, transpiration, photosynthesis, plant nutrition, respiration, seed dormancy and germination, and plant growth and movements. Since, the NPs are widely used in various products that are released into the environment, it is necessary to study the impact of these NPs on plant physiology. Studies have shown both the positive and negative effects of NPs on the physiological parameters of plants.

### 14.4.1 Seed Germination

Water is important for seed germination, a process by which a plant grows from the seed through the emergence of radical and plumule. NPs present in the plant growth media highly regulate the water imbibitions by the seed coat and thus affect seed germination. In a recent study (Andersen et al. 2016), TiO<sub>2</sub> NPs and CeO<sub>2</sub> NPs were found to affect seed germination as well as the timing of cotyledon development in ten different plant species. Exposure of TiO<sub>2</sub> NPs significantly altered the germination rate in five species, although the germination was enhanced in some species and suppressed in some others. CeO<sub>2</sub> NPs did not show alteration of germination in any of the ten species (Andersen et al. 2016). The mechanism behind alteration in germination rate may be related to the photocatalytic activity of these NPs (Ma et al. 2012). Another study investigated the influence of ZrO<sub>2</sub>, SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and TiO<sub>2</sub> NPs on maize seed germination. Al<sub>2</sub>O<sub>3</sub> and TiO<sub>2</sub> reduced the germination percentage, while SiO<sub>2</sub> has enhanced it under all the growth conditions. The uptake of metal NPs by seeds was the maximum for SiO<sub>2</sub>, followed by TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and then ZrO<sub>2</sub> (Karunakaran et al. 2016). Corn seedlings exposed to 400 mg L<sup>-1</sup> concentration of ZnO NPs (24 ± 3 nm) for 15 days showed a significant reduction of 40 and 53% in seed germination at exposure temperature of 20 and 25 °C, respectively (López-Moreno et al. 2017). The effect of AgNPs on the germination (%) of pea, rice, and

maize plants varied with the size of the NPs. AgNPs of all sizes (77.5, 111.7, 68.6, and 98.9 nm) showed a 100% higher germination in peanut, whereas AgNPs of 77.5 and 111.7 nm showed lesser germination in maize and rice, as compared to controls (Prasad et al. 2016). Seed germination was greatly influenced upon the application of metallic NPs like Ag, Au, and Cu. The seed germination was 98.6% for Ag, 69.6% for Cu, and 56.5% for Au NPs suspensions, compared to the control, after 35 days of inoculation (Hussain et al. 2017). Similarly, effect of AgNPs at concentration (0, 10, 20, 30, and 40  $\mu\text{g mL}^{-1}$ ) was investigated on the rate of seed germination in lentils. At low concentrations (10  $\mu\text{g mL}^{-1}$ ), AgNPs promoted seed germination, while adverse effects were seen at higher concentrations (Hojjat and Hojjat 2016). Influence of metal NPs (Si, Pd, Cu, and Au) has also been studied on seed germination in lettuce plants; the Pd and Au NPs at low concentrations whereas Cu and Si NPs at high concentrations could enhance seed germination after a 15-day exposure and ultimately affected the root and shoot growth of the plants (Shah and Belozeroва 2009). Another study focused on the germination of lettuce seeds exposed to low concentrations of four metal NPs (CuO, Fe<sub>2</sub>O<sub>3</sub>, ZnO, and MnO<sub>2</sub>) showed that CuO NPs inhibited seed germination and the MnO<sub>2</sub> and Fe<sub>2</sub>O<sub>3</sub> NPs enhanced the growth of seedlings, whereas no alteration was caused by ZnO NPs, as compared to their respective controls (Liu et al. 2016). Spinach seeds treated with 50  $\mu\text{g mL}^{-1}$  solutions of graphene oxide NPs prompted early and extensive seed germination and increased the mass of spinach, compared to seeds treated with 0 as well as 200  $\mu\text{g mL}^{-1}$  NP concentration (He et al. 2018).

#### **14.4.2 Water Absorption**

Water uptake by plant roots is an important process to carry out the various metabolic functions in the plant. Plants absorb water from the media (e.g., the soil) in which they are grown. The nanomaterials present in the plant growth media or soil greatly alter the water absorption capacity of plant roots by regulating the gene expression of water channel proteins involved in uptake process. In a recent study, effect of a short-term exposure of the nano zerovalent iron and Fe<sub>2</sub>O<sub>3</sub> NPs on the water uptake capacity and root water content of tomato plants was investigated (Martinez-Fernandez and Komárek 2016). Fe<sub>2</sub>O<sub>3</sub> NPs at a concentration of 100 mg L<sup>-1</sup> have significantly reduced water uptake by plant roots (40% less water content than in the control), whereas at the same concentration, the nanoscale zerovalent iron did not show any effect on the water uptake. Thus, the potential adherence of Fe<sub>2</sub>O<sub>3</sub> NPs to the root surface could be responsible for the observed effects. Hence the difference in relative effect of two types of NPs could be due to their degree of aggregation on the surface of roots, as well as their oxidation products. It was presumed that at this concentration, Fe<sub>2</sub>O<sub>3</sub> NPs got accumulated in the epithelial root cell wall causing blockage of water uptake (Martinez-Fernandez and Komárek 2016). Similarly, TiO<sub>2</sub> NPs also decreased the water transport of maize roots because the aggregates of NPs formed in the cell



wall of roots blocked the water uptake (Asli and Neumann 2009). Also, TiO<sub>2</sub> NPs could interfere with the pores of the root cell wall leading to a reduction in the water uptake and ultimately inhibited the growth of tobacco roots. Similarly, cobalt oxide NPs affected the water uptake by blocking the water channels through adsorption, whereas ZnO NPs permeated the onion root cells and damaged their morphology (Ghodake et al. 2011).

### 14.4.3 Mineral Uptake

Since the NPs are released frequently in the soil, it is a prerequisite to study the impact of NPs on the nutrient uptake by the plants. Micro- and macronutrients absorbed by the plant roots from the soil have a great role in various physiological processes such as photosynthesis, nitrogen fixation, and nitrogen metabolism. The *Raphanus sativus* sprouts were exposed to AgNPs at a concentration of 500 mg L<sup>-1</sup> to study the impact of NPs on the nutrient uptake. The Ca and Mg uptake was found to decline by 33% and 19%, respectively. Even the uptake of micronutrients (B, Zn, Mn, and Cu) was found impaired at this concentration of AgNPs. It was believed that the accumulation of AgNPs at high concentration might block the diffusion pathways or Ca and Mg channels, affecting their active absorption by the plants (Zuverza-Mena et al. 2016). Similarly, the pumpkin plants exposed to the neodymium oxide (Nd<sub>2</sub>O<sub>3</sub>) NPs at 100 mg L<sup>-1</sup> showed significantly reduced levels of S, Ca, K, and Mg. On the other hand, when tannic acid was used in addition to Nd<sub>2</sub>O<sub>3</sub> NPs, plants restored the levels of these nutrients, because the tannic acid aided in altering the surface charge of Nd<sub>2</sub>O<sub>3</sub> NPs, which reduced the likelihood of NPs binding onto the roots of the plants (Chen et al. 2016).

Another study (Peralta-Videa et al. 2014) conducted on the soil-cultivated soybean has shown that CeO<sub>2</sub> (1000 mg kg<sup>-1</sup>) exposure increased the P and Cu contents but decreased the Ca content of pods, as compared to controls. However, plant exposure to ZnO NPs resulted in a greater Zn, Mn, and Cu accumulation. Both these NPs altered the nutritional uptake in soybean to a different extent. It was suggested that CeO<sub>2</sub> interferes with the uptake of those nutrients which play a role in nitrogen metabolism and photosynthesis of plants (Peralta-Videa et al. 2014). In contrast, application of TiO<sub>2</sub> NPs (500 mg kg<sup>-1</sup>) to cucumber plants grown in sandy loam soil caused accumulation of about 35% more K and 34% more P than in the control plants (Servin et al. 2013). The authors reported that TiO<sub>2</sub> NPs have a similar positive effect as do the plant hormones cytokinins and gibberellins that favor K and P uptake by plants. Likewise, exposure to three types (unmodified, hydrophilic, and hydrophobic) of TiO<sub>2</sub> NPs has been found to affect the homeostasis of essential elements in *Ocimum basilicum* plant. At 500 mg kg<sup>-1</sup> concentration, the unmodified NPs increased the Cu (104%) and Fe (90%) contents, and hydrophilic NPs increased Fe content (90%), while hydrophobic ones increased Mn (339%) but reduced Ca (71%), Cu (58%), and P (40%) contents, with reference to the control (Tan et al. 2017). Another study described the effect of Cu/CuO NPs (10 and 20 mg L<sup>-1</sup>) on

nutrient uptake in lettuce plants showing that these NPs significantly increased the uptake of Al, Zn, S, and Fe whereas decreased that of Mg, Mn, and P (Trujillo-Reyes et al. 2014). Lettuce plants exposed to the other kind of core/shell Fe/Fe<sub>2</sub>O<sub>3</sub> NPs (10 mg L<sup>-1</sup>) were found to increase the uptake of Al. The uptake was less affected by the Fe/Fe<sub>2</sub>O<sub>3</sub> NPs than by the Cu/CuO NPs, which might be due to less damage of the cellular membrane by the former NPs and consequently a lesser reduction in selectivity for the nutrient uptake (Trujillo-Reyes et al. 2014). Leaf of *Capsicum annum* plants exposed to 125 mg kg<sup>-1</sup> of CuO NPs was found to show lower concentration of P by 42% than in plants exposed to the bulk CuO. In fruit samples upon 500 mg kg<sup>-1</sup> CuO NPs exposure, the P concentration was significantly lower than the bulk CuO at 62.5 and 125 mg kg<sup>-1</sup> exposure. CuO NPs at 500 mg kg<sup>-1</sup> also reduced the amount of Zn in leaves compared to bulk CuO treatment (Rawat et al. 2018). The effect of citrate-coated Au NPs has also been studied on the uptake of micro- and macronutrients by the barley plants. Au NPs slightly increased the uptake of Ca when at 10 mg L<sup>-1</sup> concentration and significantly increased the uptake of K when at 5 mg L<sup>-1</sup> concentration. The uptake of micronutrients Zn and Fe increased, whereas that of Mn decreased after the plant's exposure to Au NPs (Feichtmeier et al. 2015).

#### ***14.4.4 Root Water Transport and Transpiration***

Maintenance of the water transport capacity of roots is essential to meet the water utilization for evaporation and leaf growth in plants. Reduction in water supply by the roots may cause xylem tension, leaf growth inhibition, stomatal closure, reduced transpiration, and, ultimately, plant wilting (Neumann 2008). Imbalance in the root water transport may occur as a result of environmental stress. Any material present in the external water sources has to pass through the cell wall pores of the epidermal layer of roots to reach the root xylem through the parallel transport of molecules via both the symplastic and apoplastic pathways. Plant cell walls act as semipermeable membranes to allow the passage of water or only the small molecules through them and block the movement of the larger ones (Steudle and Peterson 1988). Therefore, the presence of NPs in the soil water sources may affect the root hydraulic conductivity and ultimately the transpiration. In a study, TiO<sub>2</sub> NPs applied to *Z. mays* caused a concentration-dependent inhibitory effect on the root hydraulics in a progressive manner and ultimately decreased the transpiration rate. Physical interaction between the NPs and the root cell wall, buildup of the interfacial viscosity, and further decrease in the pore diameter were considered to be reasons behind the inhibition of the root hydraulic conductivity (Asli and Neumann 2009). AgNPs and Cu NPs have reduced the transpiration rate of *Cucurbita pepo* by 66–84% and 60–70%, respectively, compared to untreated controls (Musante and White 2012). In other study, treatments of 500 and 100 mg L<sup>-1</sup> of AgNPs decreased the transpiration rate of *Cucurbita pepo* by 41% (Stampoulis et al. 2009).

### 14.4.5 Stomatal Conductance and Gas Exchange

In a study, exposure of *A. thaliana* plants to ZnO NPs (300 mg L<sup>-1</sup>) reduced the stomatal conductance and intracellular CO<sub>2</sub> as compared to controls (Wang et al. 2015). CeO<sub>2</sub> and TiO<sub>2</sub> NPs applied to soil-grown *Clarkia unguiculata* plants inhibited CO<sub>2</sub> assimilation in plants (Conway et al. 2015). Similarly, application of CeO<sub>2</sub> and ZnO NPs (at 0, 400 and 800 mg kg<sup>-1</sup> dosage) on maize (*Zea mays*) plants revealed that CeO<sub>2</sub> NPs did not affect the stomatal conductance and gaseous exchange parameters, while ZnO NPs (at 800 mg kg<sup>-1</sup>) reduced the stomatal conductance by 15%, compared to controls, 20 days after exposure (Zhang et al. 2015). In contrast, *Cucurbita pepo* plants grown under saline (NaCl) stress showed a decreased gas exchange and stomatal conductance due to excessive accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions. However, addition of SiO<sub>2</sub> NPs alleviated the adverse effects of NaCl treatment by enhancing the gas exchange parameters in plants (Siddiqui et al. 2014). A recent study has described the effect of positive-charged and negative-charged CeO<sub>2</sub> NPs on the physiology of soybean. At 100% field capacity, both types of NPs significantly increased the stomatal conductance to a similar extent (Cao et al. 2018).

### 14.4.6 Photosynthetic Parameters

The effect of metal and metal oxide NPs on the chlorophyll content and photosynthesis rate of plants has been widely studied. Of late, the effect of mesoporous SiO<sub>2</sub> NPs on the chlorophyll and photosynthetic activity of treated wheat and lupin plants was investigated, showing an increase in chlorophyll (both *a* and *b* pigments) and the maximum photosynthetic activity when plants were exposed to 500 mg L<sup>-1</sup> of NPs (Sun et al. 2016). It was believed that the application of Si increased the expression of genes (*HemD* and *PsbY*) that might be related to chlorophyll biosynthesis. Enhanced expression of these genes might result in greater activity of photosystem II and the electron transfer rate, which led to enhanced chlorophyll content (Song et al. 2014; Li et al. 2015a, b). Application of 0.1, 0.2 and 0.4% anatase TiO<sub>2</sub> NPs on *Ulmus elongata* seedlings lowered the net photosynthetic activity, as compared to control plants. It also led to reduction and blockage of electron flow from Q<sub>a</sub> to Q<sub>b</sub> (Gao et al. 2013). At 60 mg kg<sup>-1</sup> concentration, TiO<sub>2</sub> NPs of less than 20 nm increased the chlorophyll content in wheat (*Triticum aestivum*) plants by 32.3% in comparison to the control. However, there was a decrease of 11.1% at 100 mg kg<sup>-1</sup> concentration, as the plants were not able to tolerate NPs at concentrations beyond 60 mg kg<sup>-1</sup> (Rafique et al. 2018). In contrast, TiO<sub>2</sub> NPs increased the regulated photosystem II (PS II) energy dissipation and decreased the nonregulated PS II energy dissipation during heat stress in tomato plants and then promoted photosynthesis (Qi et al. 2013).

In a similar way, application of ZnO NPs (300 and 400 mg L<sup>-1</sup>) on *A. thaliana* showed that ZnO NPs significantly lowered the chlorophyll *a* and *b* content by 50%, as compared to controls. The net photosynthetic activity was also reduced by more than 50% at 300 mg L<sup>-1</sup> concentration of these NPs. Even the expression of chlorophyll synthesis genes and photosystem structure genes was reduced fivefold (Wang et al. 2015). CeO<sub>2</sub> NPs at a concentration of 400 or 800 mg kg<sup>-1</sup> showed no effect on the net photosynthetic activity after 10, 15, and 20 days of germination in maize (Zhao et al. 2012a, b). In a similar manner, CeO<sub>2</sub> and ZnO NPs did not affect the chlorophyll content and net photosynthetic rate of cucumber plants (Zhao et al. 2013). On the other hand, CuO NPs of <50 nm size when applied to barley leaves at concentrations of 0.5, 1.0, and 1.5 mM showed no noticeable change in chlorophyll contents on the 10th day of growth. A sudden loss of chlorophyll contents was, however, observed on the 20th day of growth (Shaw et al. 2014). In another study, exposure of the aquatic plant *Lemna gibba* L. to CuO NPs for 48 h inhibited the photosynthetic rate due to inactivation of reaction centers of PS II and a decline in electron transport (Perreault et al. 2014). AgNPs showed a concentration-dependent fluorescence quenching of chlorophyll in soybean plants and also reduced the photosynthetic pigments. The fluorescence quenching of chlorophyll may be attributed to the process of electron transport from excited chlorophyll molecules to AgNPs (Falco et al. 2015). A recent study reveals that at 100% field capacity, the content of chlorophyll *a* was increased by 18 and 20% in soybean exposed to uncoated CeO<sub>2</sub> NPs and PVP-coated CeO<sub>2</sub> NPs, respectively. However, the uncoated CeO<sub>2</sub> NPs reduced chlorophyll *b* content by 12% and 21% at 55% and 100% field capacity, respectively, whereas the reduction caused by PVP-coated CeO<sub>2</sub> NPs at 55 and 100% field capacity was up to 15 and 12%, respectively. The net photosynthetic rate was significantly higher in the CeO<sub>2</sub> NP-treated plants than in their respective controls (Cao et al. 2018).

## 14.5 Factors Affecting Behavior of NPs

It is clear from the above discussion that the use of metal-based NPs has a direct impact on physiology and growth of plants. Let us now examine the important factors that decide the behavior of NPs toward plant physiology.

### 14.5.1 Size, Shape, and Type of NPs

Size and shape of NPs are the most important factors which have noticeable impact on plant performance (Siddiqi and Husen 2016, 2017). NPs of the same metal having different sizes and shapes may have different behaviors toward the plant physiological processes. In a study of rice plants exposed to AgNPs of different sizes (20, 30–60, 70–120, and 150 nm), the uptake was increased when the seeds

were treated with small (20 nm)-sized AgNPs. Treatment with AgNPs of 150 nm diameter resulted in leaf cell deformation (Thuesombat et al. 2014). *Nicotiana tabacum* L. cv Xanthi (tobacco) and *Triticum aestivum* (wheat) have been exposed to tannate (T-MNPs)- or citrate (C-MNPs)-coated Au NPs of 10, 30, and 50 nm sizes. All these MNPs bioaccumulated in tobacco, but no bioaccumulation of MNPs was observed in wheat (Judy et al. 2012). In another study, ZnO and CeO<sub>2</sub> NPs were exposed to soybean plants. Presence of CeO<sub>2</sub> NPs was observed in plant roots, but no ZnO NPs were present in roots, demonstrating the differential effect of different types of metal NPs exposed to plants (Lopez-Moreno et al. 2010). Similarly, the effect of four different metallic NPs (Al<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub>, Fe<sub>3</sub>O<sub>4</sub>, and ZnO) was different on the development of *Arabidopsis thaliana*; ZnO NPs were most phytotoxic, followed by Fe<sub>3</sub>O<sub>4</sub>, SiO<sub>2</sub>, and Al<sub>2</sub>O<sub>3</sub>. ZnO NPs inhibited the germination of seeds, and the effect was size dependent (Lee et al. 2010). A similar study evaluated the effect of anatase and rutile TiO<sub>2</sub> on flax seeds. The anatase TiO<sub>2</sub> were more toxic than the rutile TiO<sub>2</sub> due to differences in their crystalline behavior (Clement et al. 2013). Different types of carbon nanomaterials, namely, fullerenes, single-walled carbon nanotube (CNT), multi-walled CNT, and graphene oxide NPs, showed different effects on the physiological parameters, viz., concentration of phytohormones, activity of antioxidant enzymes, and extension growth of plant axis in rice (*Oryza sativa*) grown in loamy soil (Hao et al. 2018).

### 14.5.2 Surface Coating or Surface Functionality of NPs

Surface characteristics of NPs are important with reference to phytotoxicity of NPs. Five plant species, *Zea mays* (corn), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Brassica oleracea* (cabbage), and *Daucus carota* (carrot), treated with monomolecular layers of alumina NPs, showed that phytotoxicity of alumina NPs was reduced appreciably by the loading of 10% monomolecular layers of phenanthrene. Alumina NPs loaded with phenanthrene were less toxic than the non-loaded NPs (Yang and Watts 2005). Likewise, corn plants treated with bare and alginate-coated cerium oxide (CeO<sub>2</sub>) NPs in soil exhibited that alginate coating on NPs surface increased the uptake of Ce in plants (Zhao et al. 2012a, b). In another study, the surface of ultra-small TiO<sub>2</sub> NPs was modified with alizarin red S and sucrose and the effect was seen in the intact *Arabidopsis thaliana*. The nano-conjugates traversed the cell walls to enter plant cells and accumulated in specific subcellular locations (Kurepa et al. 2010). One more study demonstrated the effect of surface ligands, i.e., cysteamine, cysteine, and thioglycolic acid, on the uptake of Au NPs in tomato and rice. The negatively charged cysteine-coated Au NPs were more easily internalized by roots and then transferred to plant shoots as compared to the thioglycolic acid-coated Au NPs of similar size and having similar charge. It was noted that the uptake and translocation of NPs depend more upon the type of surface ligand rather than the surface charge (Li et al. 2016a, b). Similarly, citric acid coated and uncoated CeO<sub>2</sub> NPs could increase the shoot length and total chlorophyll content in

tomato plants, whereas the same were reduced after incubation of plants with the bulk cerium oxide and cerium acetate. The ligand coating on the surface of NPs reduced the Ce uptake by plants and did not have any effect on the translocation of NPs (Barrios et al. 2016).

### 14.5.3 Surface Charge of NPs

Surface charge (cationic, anionic, or neutral) of NPs is a major deciding factor for their uptake by the plant parts. The positively charged Au NPs were most readily taken up by plant roots, while the negatively charged NPs were most efficiently translocated from roots to shoots (Zhu et al. 2012). CeO<sub>2</sub> NPs functionalized with positive-charged, negative-charged, and neutral dextran coatings were assessed for their uptake by wheat seedlings. A 15–20% reduction from Ce(IV) to Ce(III) was found in both roots and leaves. Due to their high affinity with the negative-charged cell walls, CeO<sub>2</sub>(+) NPs adhered in a strongest way to the plant roots. After 34 h, plants exposed to CeO<sub>2</sub>(–) and CeO<sub>2</sub>(0) NPs showed higher leaf Ce concentrations than the plants exposed to CeO<sub>2</sub>(+) NPs (Spielman-Sun et al. 2017).

### 14.5.4 Concentration/Dose of NPs

Concentration of NPs has a significant effect on the growth and development of plants (Siddiqi and Husen 2016, 2017). Exposure to 24 nm-sized green synthesized Au NPs at concentrations of 10 and 80 µg mL<sup>–1</sup> enhanced the shoot length of *A. thaliana* seedlings by 1.42- and 1.64-folds in comparison to the control (Kumar et al. 2013). Cucumber plants exposed to CeO<sub>2</sub> or ZnO NPs at concentrations of 0, 400, and 800 mg kg<sup>–1</sup> have indicated that remained unaffected with reference to plant growth, gas exchange, and chlorophyll content. However, at 800 mg kg<sup>–1</sup> concentration, CeO<sub>2</sub> NPs reduced the yield, compared to the control (Zhao et al. 2013). The hydroponically grown lettuce treated with core–shell (Fe/Fe<sub>3</sub>O<sub>4</sub>, Cu/CuO NPs) at 10 and 20 mg L<sup>–1</sup> and FeSO<sub>4</sub>·7H<sub>2</sub>O and CuSO<sub>4</sub>·5H<sub>2</sub>O at 10 mg L<sup>–1</sup> indicated no effect of iron ions/NPs on the physiological parameters. Conversely, Cu ions/NPs decreased the water content, root length, and dry biomass of the lettuce plants (Trujillo-Reyes et al. 2014). Treatment with 2000 mg L<sup>–1</sup> and 4000 mg L<sup>–1</sup> CeO<sub>2</sub> NPs has induced genotoxic effects in soybean plants (Lopez-Moreno et al. 2010). *Lemna minor* exposed to media with various concentrations of TiO<sub>2</sub> NPs showed that superoxide dismutase activity was decreased when TiO<sub>2</sub> NPs concentration was higher than 200 mg L<sup>–1</sup> and the plant cell membrane experienced serious damage at 500 mg L<sup>–1</sup> TiO<sub>2</sub> NP concentration (Song et al. 2012). Similarly, TiO<sub>2</sub> NPs have a significant effect on seed germination, plant growth, and development of switchgrass (*Panicum virgatum*) in a dose-dependent manner (Boikov et al. 2018).

The well-known model plant, *A. thaliana*, when exposed to different concentrations of CeO<sub>2</sub> and indium oxide (In<sub>2</sub>O<sub>3</sub>) NPs, indicated that CeO<sub>2</sub> NPs at 250 ppm can significantly increase the plant biomass, but at 500–2000 ppm the plant growth was reduced by 85%. CeO<sub>2</sub> NPs at 1000 and 2000 ppm reduced the chlorophyll production by nearly 60% and 85%, respectively, whereas anthocyanin production was enhanced three- to fivefold. Malondialdehyde (MDA) production was unaffected by the exposure to 250–500 ppm CeO<sub>2</sub> NPs, but it increased by 2.5-fold at 1000 ppm (Ma et al. 2013). Application of CeO<sub>2</sub> NPs at 0–1000 mg kg<sup>-1</sup> and ZnO NPs at 0–500 mg kg<sup>-1</sup> reduced the nutritional value of soybean plants. Compared to the control, CeO<sub>2</sub> NPs at 1000 mg kg<sup>-1</sup> could cause significantly less accumulation of Ca and more of P and Cu in pods, while 100 mg kg<sup>-1</sup> ZnO NPs led to a higher accumulation of Zn, Mn, and Cu (Peralta-Videa et al. 2014). The higher concentration of NPs had a more negative effect on seedling growth (Thuesombat et al. 2014). Growth of roots of buckwheat treated with ZnO NPs and CuO NPs at 2000 and 4000 mg L<sup>-1</sup>, respectively, was reduced considerably in comparison to the control (Lee et al. 2013).

### 14.5.5 Type of Plant Species Exposed

NPs of the same type often have different impact on different plant species (Husen and Siddiqi 2014b, c; Siddiqi and Husen 2016, 2017). A study revealed that radish and ryegrass roots generally accumulated more amounts of Au NPs (14–900 ng mg<sup>-1</sup>) than rice and pumpkin roots (7–59 ng mg<sup>-1</sup>). Accumulation was statistically significant in rice shoots (1.1–2.9 ng mg<sup>-1</sup>), while none of the Au NPs accumulated in the shoots of radish and pumpkins (Zhu et al. 2012). NPs bioaccumulation has also been reported to be plant species-dependent possibly due to interspecific differences in the nature of chemical exudations from roots (Judy et al. 2012). In another study, effect of Cu NPs on the growth of *Polyboroides radiatus* and *Triticum aestivum* was studied and found that *P. radiatus* was more sensitive to Cu NPs than *T. aestivum* (Lee et al. 2008).

### 14.5.6 Nature of Growth Media

The medium used for plant cultivation also has an effect on the phytotoxicity of NPs. The toxicity and bioavailability of AgNPs to *Polyboroides radiatus* and *Sorghum bicolor* were measured in the agar and soil media. Growth of *S. bicolor* was reduced in a concentration-dependent manner in the agar medium. In the case of soil, growth of *P. radiatus* was not significantly affected, but *S. bicolor* showed a slightly reduced growth rate (Lee et al. 2012). In another study, it was shown that the soil organic matter plays a vital role in the mobility and bioavailability of CeO<sub>2</sub> NPs in the soil solution (Zhao et al. 2012a, b). The pumpkin and wheat plants were

exposed to CeO<sub>2</sub> NPs dissolved in media containing gum arabic or fulvic acid. None of the plants showed a reduced growth or any toxic response. CeO<sub>2</sub> NPs were found to be translocated shoots in pumpkin but not in wheat plants. The presence of fulvic acid and gum arabic acid also affected the amount of NPs associated with roots (Schwabe et al. 2013).

## 14.6 Effect of NPs-Mediated Gene Delivery on Plant Growth

Metallic NPs, nanofibers, and nanocapsules offer a new set of tools to manipulate the genes. Appropriately functionalized NPs serve as a platform to transport a large number of genes as well as chemicals that trigger gene expression in plants (McKnight et al. 2003; Radu et al. 2004; Roy et al. 2005; Torney et al. 2007; Xia et al. 2009). Fluorescent-labeled starch NPs were used for transport of genes across the plant cell wall. Integration of different genes on NPs and, at the same time, imaging of the fluorescent NPs were possible with fluorescence microscope so as to monitor the movement of exterior genes along with the expression of genes (Liu et al. 2008). SiO<sub>2</sub> NPs capped with Au NPs have been used for particle bombardment in plant cells. The results have demonstrated that the plasmid DNA transferred by the gene gun method, using the gold-capped SiO<sub>2</sub> NPs, was successfully expressed in the intact tobacco and maize tissues. The main benefit was the simultaneous delivery of both DNA and effector molecules to the specific sites that resulted in the site-targeted delivery and expression of chemicals and genes, respectively (Torney et al. 2007). In another study, plasmids were covalently bound to magnetic NPs and successfully delivered to canola cells. The expression of plasmid delivery after 48 h in suspension culture was confirmed by the appearance of blue-color expression of GUS gene due to staining with 5-bromo-4-chloro-3-indolyl-β-d-glucuronic acid (X-Gluc) in the plant protoplasts (Hao et al. 2013). Likewise, SiO<sub>2</sub> NPs served as a vehicle for the delivery of foreign DNA into the roots of *Arabidopsis thaliana*. The expression of DNA was noticed in the epidermal layer and also in the inner most cortical and endodermal regions of plant roots (Chang et al. 2013). Another study has described the role of gold-coated SiO<sub>2</sub> NPs for co-delivery of protein and DNA into the onion plant cells by the particle bombardment method. The expression of marker gene (enhanced green fluorescent protein) and fluorescently labeled BSA protein was detected in the onion epidermal cells (Martin-Ortigosa et al. 2012). Thus, the metallic NPs serve as candidates suitable for the plasmid/DNA delivery to plant cells.

## 14.7 Phytotoxicity of NPs

NPs are used in a wide variety of consumer products such as cosmetics, wound dressings, textiles, and so on. After the end usage of NPs, these are discharged into the environment inevitably, leading ultimately to the pollution of water



bodies and soil. Plants also get exposed to NPs in the aquatic and terrestrial environments (Choudhury et al. 2016). Airborne NPs possess the tendency to attach to leaves and other aerial parts of plants, whereas roots interact with NPs through the waterborne or soil materials. Once present on the leaf surface, NPs penetrate the plants via the bases of trichomes or through stomata and then get translocated to tissues of different plant systems. The main damage to the ecosystem due to NP deposition is related to the alteration of competition pattern among the species, which results in a drastic effect on plant biodiversity (Choudhury et al. 2016). More sensitive species may be eliminated, while the growth, flowering, and fructification of other species may be favored. This situation has brought the phytotoxicity issues of NPs to the fore.

Higher plants strongly interact with their atmospheric and terrestrial environments. These interactions are expected to be affected by their exposure to NPs (Navarro et al. 2008). Most of the studies undertaken on the effects of NPs on higher plants have been confined to seed germination and root elongation test (Lin and Xing 2007; Yang et al. 2006; Zheng et al. 2005). TiO<sub>2</sub> NPs have shown positive effects on germination of aged spinach seeds as well as on the growth of seedlings (Zheng et al. 2005). Similarly, TiO<sub>2</sub> NPs significantly promoted the growth of spinach and accelerated the nitrogen assimilation (Yang et al. 2006). TiO<sub>2</sub> NPs were not much toxic to willow trees, and the toxic effects did not follow a clear dose-dependent relationship, probably due to the formation of aggregates and their subsequent sedimentation (Seeger et al. 2009). Similarly, effects of TiO<sub>2</sub> NPs on the photochemical reaction of chloroplasts of *Spinacia oleracea* were analyzed (Hong et al. 2005), which showed that the nano-TiO<sub>2</sub> treatment could enhance the Hill reaction and the activity of chloroplasts, which further accelerated the FeCy reduction and oxygen evolution. Moreover, noncyclic photophosphorylation activity was higher than the cyclic photophosphorylation activity during the TiO<sub>2</sub> treatment, which also increased the germination rate and vigor indexes (Zheng et al. 2005). The plant dry weight also increased due to increase in the chlorophyll formation and the ribulose biphosphate carboxylase/oxygenase activity and, consequently, in the photosynthetic rate. Gao et al. (2006) have shown that Rubisco carboxylase activity was 2.67 times higher in the nano-anatase TiO<sub>2</sub>-treated *Spinacia oleracea* than that in the control. The molecular mechanism of the carbon reaction promoted by nano-anatase TiO<sub>2</sub> during photosynthesis is still not clearly understood. Reverse transcription PCR and northern blotting experiments have evidenced that mRNAs encoding the small and large subunit of Rubisco were promoted in the NPs-treated plants (Xuming et al. 2008). Accordingly, the protein expression of Rubisco from the nano-anatase-treated spinach was increased by 40%, as compared to the control.

The phytotoxicity of AgNPs was investigated in crop plants *Phaseolus radiatus* and *Sorghum bicolor* grown in the agar and soil media. AgNPs showed a concentration-dependent growth inhibition of *P. radiatus* and *S. bicolor* seeds in agar media and also caused browning and necrosis of root tips. The phytotoxicity was relatively less in soil media due to the reduced bioavailability of AgNPs in soil than in agar (Lee et al. 2012). In a similar study, exposure of glucoxyilan-mediated

green synthesized AgNPs (40 nm) and Au NPs (6 nm) did not show any significant effect on germination of radish seeds. These two types of NPs behaved differently toward the root growth of radish seedlings, where AgNPs induced a stimulatory effect on root length in a concentration-dependent manner, while Au NP exposure caused no significant change in the root length (Iram et al. 2014). Zn and ZnO NPs have shown effect on the root and shoot growth in radish, rape, and ryegrass. The phytotoxicity of ZnO NPs was not directly related with their limited dissolution in the bulk nutrient solution or rhizosphere (Lin and Xing 2007). In the aquatic plant *Hydrilla verticillata*, growth in early stages was inhibited after exposure to ZnO NPs at a high concentration of 1000 mg mL<sup>-1</sup>, whereas *Phragmites australis* indicated a decline in growth rate after few weeks of exposure. It was concluded that high dosage of ZnO NPs caused significant phytotoxicity in these aquatic plants (Song and Lee 2016). In another study to determine the toxicity of ZnO NPs in the corn and cucumber plants, root length was found to reduce by 17% and 21%, respectively, while seed germination remained unaffected by the ZnO NPs at a concentration of 1000 mg mL<sup>-1</sup> (Zhang et al. 2015).

In a study of the phytotoxicity and bioavailability of Cu NPs to *Phaseolus radiatus* and *Triticum aestivum*, growth rate of both the crop plants was found to drop as a result of exposure to NPs. Bioaccumulation was concentration-dependent, and the contents of NPs in plant tissues increased with increasing NPs concentration in the growth media. *T. aestivum* showed a relatively greater accumulation of Cu NPs in roots due to its peculiar root morphology (Lee et al. 2008). Of late, toxicity of Cu NPs was evaluated in 10–15 days old alfalfa and lettuce seedlings grown in hydroponics. Plant size and the nutrient content of tissues got reduced, whereas enzyme activity was altered in both the plants (Hong et al. 2015). In *A. thaliana* grown in agar media, CeO<sub>2</sub> NPs caused stimulatory effect at a low dose, while high NP concentrations led to adverse effects on antioxidant system, photosystem, and the overall plant growth (Yang et al. 2016). The toxicity effects of Al<sub>2</sub>O<sub>3</sub> NPs (13 nm) were investigated in a time- and dose-dependent manner on the root growth and development of *T. aestivum*. There was a decrease in root elongation with increase in the concentration of NPs applied. Moreover, a decrease in the total protein content, an increase in peroxidase activity and accumulation of lignin, and a damage to root cortex were also recorded (Yanik and Vardar 2015).

The magnetic NPs coated with tetramethylammonium hydroxide had a magnetic influence on the enzyme structures implied at different stages of photosynthesis during the early growth of *Zea mays* plants. Small concentrations of aqueous ferrofluid solution added to the culture medium showed a stimulating effect on growth, while the larger amounts induced an inhibitory effect (Racuciu and Creanga 2007). Toxic effects of semiconductor L-cysteine capped CdS NPs (15–20 nm) were examined on the aquatic plant *Spirodela polyrrhiza*, which experienced significant reduction in plant growth as well as changes in the activity of antioxidant enzymes (peroxidase and superoxide dismutase), demonstrating the phytotoxicity of the synthesized NPs (Khataee et al. 2014). Earlier, phytotoxicity and biotransformation of La<sub>2</sub>O<sub>3</sub> NPs were assessed in cucumber roots. The NPs over a concentration of 200 mg L<sup>-1</sup> affected the root elongation and growth rate. The organic acids

secreted from the root cells could possibly cause dissolution of the NPs applied. Moreover, the biotransformation of  $\text{La}_2\text{O}_3$  NPs from spherical shape to needlelike form was observed in the intercellular spaces and middle lamella of the root tissues (Ma et al. 2011).

## 14.8 NPs Exposure Causes Oxidative Stress in Plants

There are limited reports in the literature regarding the oxidative stress caused or altered by NPs in the plant tissues. Reactive oxygen species (ROS) are free radicals which contain atoms of oxygen and are generally formed inside several plant structures due to various metabolic pathways (Anjum et al. 2015a, b; Aref et al. 2016). Various environmental factors are responsible for increasing the ROS in the cellular milieu of plants, leading to stressful conditions. Increase in ROS inside plant cell membranes may cause several damages and disturb normal activity of plants (Gill and Tuteja 2010; Anjum et al. 2012). Heavy metal ions present in soil or water may affect the plant metabolism and cause oxidative stress (Lopez-Moreno et al. 2010). Excessive increase in ROS levels may elicit detoxification mechanisms involving both enzymatic and nonenzymatic systems that tend to prevent oxidation of biological molecules like proteins or lipids (Gill and Tuteja 2010). A difference in balance of both the ROS production and detoxification leads to oxidative stress. Oxidative stress caused in plants by metal NPs has been mentioned in several studies (Anjum et al. 2015a, b).

In a study, wheat (*T. aestivum*) plants exposed to  $\text{Fe}_2\text{O}_3$  NPs were evaluated for any oxidative damage by monitoring the activity of antioxidant enzymes. It came out that the activity of enzymes such as ascorbate peroxidase, superoxide dismutase (SOD), guaiacol peroxidase, and catalase (CAT) was increased significantly in the roots and aerial parts of NPs-treated plants (Iannone et al. 2016). Similarly, *R. sativus* exposed to  $\text{Fe}_2\text{O}_3$  NPs ( $1 \text{ mg mL}^{-1}$  concentration) showed a 219.5% increase in the ROS inside the cells. Consequently, activities of CAT, SOD, and the glutathione content also increased, showing that  $\text{Fe}_2\text{O}_3$  NPs generated heavy oxidative stress which was countered by the antioxidant enzymes in order to develop the defense system (Saqib et al. 2016). AgNPs of size 20 nm (20 and 50  $\text{mg mL}^{-1}$ ) applied to *Vigna radiata* L. (mung bean) for 21 days produced ROS and increased the formation of hydrogen peroxide and lipid peroxidation, which caused cellular damage (Nair and Chung 2015). In contrast, AgNPs synthesized by synthetic route caused a decrease in SOD activity in *E. crassipes* at all the doses used, whereas activity of CAT was increased. In water hyacinth plant, the activity of catalase (CAT) and peroxidase (POD) was decreased with increase in the concentration of biosynthesized AgNPs applied. The study suggested that synthetic AgNPs could produce more oxidative stress in comparison to the biosynthesized AgNPs, which was combated by modulation of antioxidant enzymes accordingly (Rani et al. 2016).

CuO and ZnO NPs in *Cucumis sativus* significantly increased the activity of SOD, CAT, and POD enzymes. The activity of SOD and POD in root cells was

increased by more than 50% than in the control (Kim et al. 2012). Similarly, oxidative stress in the sand-grown wheat plants treated with CuO and ZnO NPs was evidenced by increased lipid peroxidation and oxidized glutathione in roots, leading to increased ROS production (Dimkpa et al. 2012). A significant increase in the levels of SOD and POD was found in response to ROS generation in cotton plants caused by the low dose of ZnO NPs carrying phycomolecule ligands present in the extract of algae *Halimeda tuna* (Venkatachalam et al. 2017). On the other hand, ZnO NPs at higher concentration induced the oxidative stress in *S. lycopersicum* through the generation of ROS. The activity of SOD was increased at lower concentrations of ZnO NPs, whereas lipid peroxidation measured in terms of malondialdehyde increased with increase in the ZnO NP concentration (Singh et al. 2016). In rice plants, H<sub>2</sub>O<sub>2</sub> content increased by 162% over the control on exposure to 500 mg L<sup>-1</sup> CeO<sub>2</sub>. At 125 mg L<sup>-1</sup>, the activity of SOD, glutathione reductase, and dehydroascorbate reductase was significantly reduced, causing damage to cellular membranes (Rico et al. 2013a, b). Similarly, radish plants grown with 125 mg of CeO<sub>2</sub> NPs showed enhanced CAT and ascorbate peroxidase activity in root tubers but a reduced one in leaves (Corral-Diaz et al. 2014). *Medicago sativa* cells exposed to MPA-CdSe/ZnS QDs at 10, 50, and 100 nM showed increased activity of SOD, CAT, and glutathione reductase enzymes, possibly due to the release of Cd from the degradation of CdSe/ZnS QDs. The ROS generated in the plants after the treatment with QDs was thus overcome by mobilizing the ROS-scavenging mechanisms through enzymatic actions (Santos et al. 2013).

## 14.9 Conclusions

A variety of metallic NPs released in our environment have a great impact on the living beings such as humans, plants, and animals. Uptake, translocation, and accumulation of metallic NPs occur in plant roots, stem, leaves, and other plant parts. Uptake of NPs affects plant physiological processes such as photosynthesis, respiration, mineral and water uptake, and transpiration. This leads to variations in seed germination rate, plant growth, and biomass. Plants' exposure to NPs has both positive and negative effects on plant growth and development, depending on several factors such as size and shape of NPs, plant species exposed, concentration of NPs, and duration of exposure. Delivery of plasmids/DNA to plant cells mediated by NPs carriers and also the phytotoxicity and oxidative stress caused in plants by application of NPs are of utmost importance. It is a prerequisite to decide the various parameters related to NPs (size, shape, concentration of use, exposure duration, etc.) before their applicability in the plant systems so as to avoid toxicity effects and get the beneficial response.

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