Chapter 2
Nanoparticles: Physiology, Chemistry, and Biochemistry

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1 Introduction

In the last two decades, the use of engineered nanoparticles increases in industrial, agricultural, and biomedical sectors (Maurer-Jones et al. 2013). The engineered nanoparticles are of different shapes and sizes that alter their properties from the naturally occurring materials (Auffan et al. 2009; Joshi et al. 2019; Singh et al. 2020). The extensive use of these nanoparticles makes their presence in the

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environment inevitable. The water bodies and soil are the most likely to get contaminated with excess nanomaterials that can enter indirectly from industrial discharge or directly from the nanomaterials used for agriculture. So, it is crucial to understand the impact of nanoparticles on different plant species before their release in the environment.

Over the last two decades, the application of nanotechnology in the agricultural field has been proven as an effective tool to ensure food security in a sustainable manner (Singh et al. 2021). Nanofertilizers are more efficient for crop production as they ensure high nutrient use efficiency (Zulfiqar et al. 2019). Site-directed, controlled delivery of these nanofertilizers minimizes the use of fertilizers and increases crop production in a highly efficient manner. The emergence of biopesticides offers the protection of plants without much damage to the natural ecosystem as it offers a reduced amount of active ingredient and high efficacy (Kookana et al. 2014). On the other hand, the use of nanosensors can help to detect the microclimate change in the crop field and can alert the farmer before any biotic or abiotic stress hits the threshold and affect crop production (Pérez-de-Luque et al. 2012; Pérez-de-Luque and Hermosín 2013). The development of nanosensors has improved human control over soil and plant health and contributed to precision farming and sustainable agriculture (Chen et al. 2016).

The increasing use of NPs is directly related to their accumulation in the environment. Besides the NPs used in agriculture, various other NPs that are used in different industries also enter in air, water, and soil by different means. Industrial wastes tend to end up in water bodies that increase the chances of NPs contamination. Soil is the ultimate sink of NPs, which receives the NPs directly or indirectly (Remédios et al. 2012). Plants are the base of the ecosystem and directly in contact with soil, water, and air. Any negative effects of nanomaterials on plants can ultimately affect animals and human beings (Judy et al. 2012; Hawthorne et al. 2014; De la Torre Roche et al. 2015; Tangaa et al. 2016). Hence, it is imperative to intensively study the role of NPs on the plant, before their intensive use in the natural environment. Also, it is important to examine the effect of NPs on microflora present in soil because in the nature, growth and development of plants are affected by them. Any positive or negative effect on them can ultimately affect the crop plants. In this chapter, we will describe the effect of various NPs on plants physiology and their effect on plant microbial associations.

2 Entry and Translocation of Nanoparticles (NPs) in Plant System

Nanoparticles are more reactive than their molecular form due to their smaller size and higher surface to volume ratio (Taylor et al. 2015). The uptake of NPs by plants is dependent on several factors including the nature of nanoparticles and

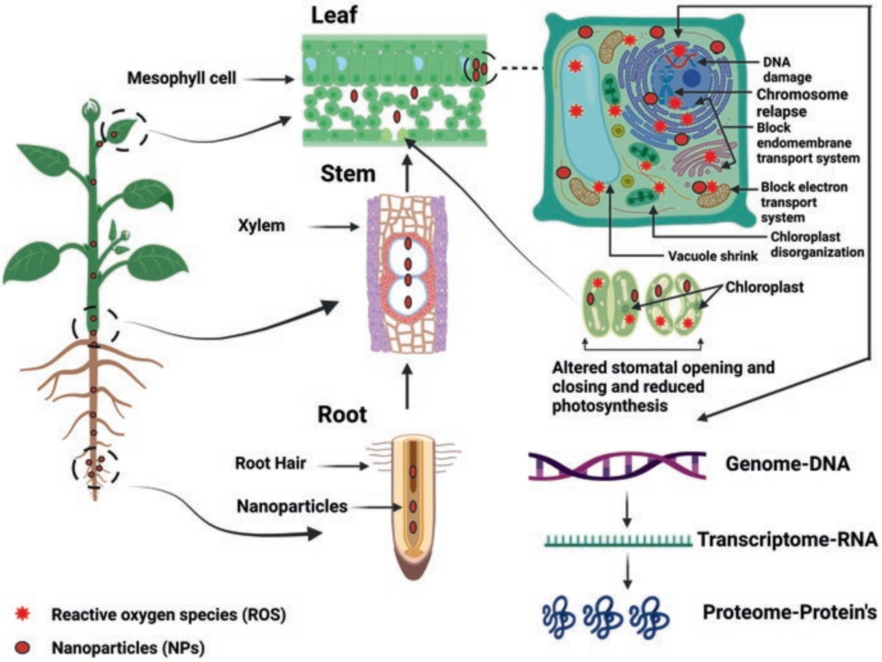

Fig. 2.1 Diagrammatic representation of translocation nanoparticle inside the plant cell from root to leaf, and effect of nanoparticle at a physiological, biochemical, and molecular level

their interaction with environment, along with the physiology of plant species (Pérez-de-Luque 2017).

The NPs are generally taken up by plant roots and translocated through vascular tissues to aerial parts of the plant (Fig. 2.1). The uptake and penetration of NPs in plant cells is directly dependent on their size. The small NPs, ranging between 5 and20 nm, can penetrate through the plant cell wall (Dietz and Herth 2011). Few studies reported about the maximum dimensions allowed by plants for the movements and accumulation of NPs inside the plant cells. Usually, 40–50 nm is the size limit of NPs to enter in the plant cells (González-Melendi et al. 2008; Corredor et al. 2009; Sabo-Attwood et al. 2012; Taylor et al. 2015). The morphology of NPs and the means of delivery are also important for their transport in plants. Raliya et al. (2016) had demonstrated that the morphology of gold nanoparticles (nAu) plays a significant role in its translocation in watermelon. The chemical property of NP is an additional factor influencing its interaction with plant cells. The attachment of NPs to the surface of negatively charged plant cell wall is directly dependent on the surface charge of NPs. Zhu et al. (2021) found that the absorption of ZnO onto the leave surface and cell wall of wheat was enhanced by the smaller size and positive charges. In order to understand the uptake, translocation, and accumulation of NPs in plants, standardized laboratory experiments are needed to correlate the physicochemical properties of NPs with their effect on plant tissues.

The NPs of smaller size (3–5 nm) can pass directly through the root epidermis or they penetrate to the plant root along with osmotic pressure and capillary force. The small pores in the cell wall of roots permit the entrance of small-sized NPs while some NPs could form new pores in the root cell wall and enter through it (Du et al. 2011). After crossing the cell wall, NPs enter in extracellular spaces and make their passage to the vascular system. After reaching the vascular bundle, the NPs enter in xylem and they are transferred to shoot and other aerial parts (Ali et al. 2021). When the NPs enter in the cell, they travel from cell to cell through plasmodesmata (Perez-de-Luque 2017). The NPs that cannot enter in the cell are accumulating on the Casparian strip (Wang et al. 2012; Raliya et al. 2016).

NPs can also enter through the stomata or cuticle present on the aerial part of the plant, especially leaves (Larue et al. 2014). The foliar absorption of NPs is dependent on the leaf morphology. The presence of cuticle, wax, trichrome, and leaf exudates are some important factors, which affect the adherence and entrance of NPs from leaves (Larue et al. 2014). The cuticle allows the entrance of NPs smaller than 5 nm while the NPs of 10 nm range can enter through stomata (Ali et al. 2021). The NPs entered through aerial tissues are transported through phloem along with sugar transportation and can accumulate in root, grains, fruits, and young leaves (Wang et al. 2013; Raliya et al. 2016).

The translocation and accumulation of NPs depend upon the plant species and the characteristics of nanomaterial. Accumulation of the same NP was observed in different parts in different plant species (Cifuentes et al. 2010; Zhu et al. 2012). For example, the accumulation of nAu was found in the shoots of *Oryza sativa* while in *Cucurbita pepo* and *Raphanus raphanistrum* nAu accumulation was not found in shoots (Zhu et al. 2012).

3 Physiological Alterations in Plants in Response to Nanoparticles

After entering in plant system, the NPs interact with plants at the cellular and subcellular level, resulting in various morphological and physiological changes. In order to access the effect of NPs, percentage of germination, root elongation, total biomass, and leaf numbers are generally considered physiological parameters (Lee et al. 2010). NPs can affect the plant physiology positively or negatively in a dose-dependent manner and also dependent on plant species.

NPs cover a wide range of materials, but only few of metal and metal oxides nanoparticles are extensively used in various industries. NPs of silver, titanium and its oxide, zinc oxide, iron oxide, aluminum oxide, etc., are the few NPs that are entering in the environment due to their excessive use in different processes. Hence, it is important to understand their physiological impact on the plant. Various methods of NP application, such as foliar application, seed treatment, application in soil, growth media, or in hydroponics, were used to examine the impact of NPs on plant

Table 2.1 Nanoparticles (NPs) and their effect on different plant species

Type of NPs	Concentration	Crop species	Physiological effect	References
nAg	0.5–5 mg/kg	*Triticum aestivum*	Increased branching in roots Increased ROS generation	Dimkpa et al. (2013)
nAg	0.5–10 mg/kg	*Spirodela polyrhiza*	Increased ROS accumulation and activities of peroxidase, SOD, and glutathione	Jiang et al. (2014)
nAg	0.01–1 mg/kg	*Capsicum annuum*	Reduced plant growth Elevated cytokinin concentration	Vinkovic´et al. (2017)
nAg	40 mg/l	*Lolium mutiforum*	Damaged epidermis and root cap	Yin et al. (2012)
nAg	10 mg/l	*Eruca sativa*	Increased root length	Vannini et al. (2013)
nAg	10–100 ppm	*Bacopa monnieri*	Reduction in total protein content in root and shoots	Krishnaraj et al. (2012)
nAl_2O_3	10–1000 mg/l	*Triticum aestivum*	Increased activities of catalase and SOD at 200 and 500 mg/l concentrations	Riahi-Madvar et al. (2012)
$nCeO_2$	500 mg/kg	*Oryza sativa*	Decrease in starch, glutalin, lauric acid, valeric acid, prolamin Fe and S in rice grains	Rico et al. (2013)
$nCeO_2$	100, 400 mg/kg	*Triticum aestivum*	Reduced chlorophyll level Increase in catalase and SOD activities Changed root and leaf cell microstructure, delayed flowering, and high protein content in grains	Du et al. (2015)
$nCeO_2$	62.5–500 mg/kg	*Solenum lycopersicum*	Induced catalase activity and chlorophyll content in leaves Increased stem length	Barrios et al. (2016)
$nCeO_2$	0–500 mg/kg	*Phaseolus vulgaris*	Increased antioxidant activities in aerial tissues	Majumdar et al. (2016)
nCu	200–1000 mg/l	*-Phaseolus radiates* *-Triticum aestivum*	Reduced growth of roots and seedlings Roots were more affected than shoots *T. aestivum* showed more responsive than *P. radiates*	Lee et al. (2008)
nCuO	2.5–1000 mg/l	*Oryza sativa*	Decreased thylakoid number/grana Decreased photosynthesis rate, transpiration rate, stomatal conductivity Increased activities of SOD and ascorbate peroxidase	Costa and Sharma (2016)
nCuO	10–200 mg/l	*Lemna minor*	Increased activities of catalase, SOD and peroxidase Induced lipid peroxidation Reduced plant growth	Song et al. (2016)

(continued)

Table 2.1 (continued)

Type of NPs	Concentration	Crop species	Physiological effect	References
nCuO	20, 50 mg/l	*Arabidopsis thaliana*	Among Col-0, WS-2, and Bay-0, Col-0 was the most sensitive ecotype nCuO inhibited the growth of all ecotypes Presence of nCuO was detected from root to seeds	Wang et al. (2016a, b, c)
nCuO	3–300 mg/l	*Triticum aestivum*	Shorting of division and elongation zones in root	Adams et al. (2017)
nFe_2O_3	5–20 mg/l	*Triticum aestivum*	Exposed plants to NPs had a positive response for preventing oxidative damage	Iannone et al. (2016)
nFe_2O_3	20–100 mg/l	*Zea mays*	Germination index was found to be dose-dependent. Exposure to 20 and 50 mg/L of NPs increased the germination, but exposure to 100 mg/L of NPs decreased it	Li et al. (2016)
TiO_2	300 mg/l	*Zea mays*	Inhibited leaf growth Root water transport was physically affected	Asli and Neumann (2009)
TiO_2	100 mg/l	*Linum usitatissimum*	Reduced seed germination, root length, and root biomass	Clément et al. (2013)
TiO_2	200 mg/l	*Mentha piperita*	Negatively affect the germination percentage and shoot length Increase in chlorophyll and carotenoid content	Samadi et al. (2014)
TiO_2	0–1000 mg/kg	*Solenum lycopersicum*	Dose-dependent increase in height, root length, and biomass 100 mg/kg of TiO_2 positively affect the lycopene content and fruit size Chlorophyll content was increased on 750 mg/kg of TiO_2	Raliya et al. (2016)
ZnO	10–1000 mg/l	Lolium perenne	Inhibited root elongation in dose-dependent manner Seedling biomass decreased at >20 mg/L concentration	Lin and Xing (2008)
ZnO	200–800 mg/l	*Allium cepa*	Increased cytotoxicity in root cells. Increased DNA degradation Increased ROS accumulation and activity of glutathione peroxidase, but decreased catalase activity	Ghosh et al. (2016)

physiology (Table 2.1). Also, various concentrations and sizes of NPs were used to find the threshold level of NPs for different species (Raliya et al. 2016).

Silver nanoparticles (nAg) are extensively used in the preparation of antimicrobial agents, food packaging materials, fabrics, paint, detergents, etc. (Rai et al. 2009; Wijnhoven et al. 2009). Through the industrial waste, nAg can easily enter in the ecosystem and ultimately can affect the plants. It has been observed that the lower concentration of nAg (30 µg/ml) cannot affect the rice, while the higher concentration can affect the cell structure (Mirzajani et al. 2013). Also, it has been observed that the nAg with lower size (˃30 nm) has higher toxic effect on plant and inhibits the growth of root and shoot in different plant species. On contrary, the nAg particles with higher size were able to enhance the growth of root and shoot (Jasim et al. 2017). These results indicate that the penetration and accumulation of nAg in the plant cell can cause a negative impact on plant growth, while its presence in the surrounding has a positive effect on plants.

The higher concentration of NPs can negatively affect plant growth. The increased concentration of NPs can cause stress in plants and in turn, can elevate the production of reactive oxygen species (ROS) as a defense response. There are several studies showing that nAg and nCu/CuO can elevate the level of ROS and various antioxidants in plants (Dimkpa et al. 2013; Jiang et al. 2014; Nair and Chung 2014; Shaw et al. 2014; Cvjetko et al. 2017; Tripathi et al. 2017). These NPs can also affect the expression of genes related to the synthesis of antioxidant. For example, in *Arabidopsis thaliana,* the expression of various genes related to oxidative stress was changed by applying a high concentration of nCuO (Nair and Chung 2014). The application of NPs can also affect the level of hormones, such as auxin and cytokinin (Yin et al. 2012; Vinkovi'c et al. 2017).

Titanium oxide (TiO_2) is known for its photocatalytic property and hence, foliar application of it has shown a positive impact on plants. Foliar application of TiO_2 in tomato resulted in an enhanced growth of the plant with an increase in fruit yield and chlorophyll content (Raliya et al. 2016). Similar to nAg and nCu, at high concentration the phytotoxic response of TiO_2 was observed in plants (Rafique et al. 2014). Application of TiO_2 in colloidal form was observed to inhibit transpiration rate and root growth by physically affecting the root-water interaction (Asli and Neumann 2009).

These studies suggest that the NPs can have impacts on the physiology, morphology, and biochemistry of plants. The effects of NPs on plants can depend upon their size, concentration, and mode of application. It was also observed that the response of the same NPs can differ in genetically diverse plant species (Lee et al. 2008; Van et al. 2016; Wang et al. 2016b). These studies also indicate that the internalization and high accumulation of NPs in plant cells can cause phytotoxic effects in plants, but at lower concentration these NPs can improve plant growth and yield. Application of NPs at lower concentration can help the plant to mitigate various stresses (Jaberzadeh et al. 2013).

4 Molecular and Biochemical Changes in Plants in Response to Cellular Internalization of Nanoparticles

Once the NPs internalize within the plant cells, they start interfering with various metabolic processes. Various reports show that the excess accumulation of NPs in the cell causes oxidative stress and increased production of ROS (Hossain et al. 2015).

NPs present within the cell can enter in the cell organelles. The electron transport chain present on the membrane of mitochondria and chloroplast can be interrupted by the NPs, which ultimately cause oxidative burst (Faisal et al. 2013; Ghosh et al. 2016; Rastogi et al. 2017). This oxidative burst causes the increased production of ROS that ultimately causes the lipid peroxidation, DNA-strand breaks, and cell death (Van Breusegem and Dat 2006; Cvjetko et al. 2017; Saha and Dutta Gupta 2017). The production of ROS also activates the stress regulatory mechanism in plants. The balance between production and scavenging mechanism of ROS determines the level of stress tolerance in plant species (Sharma et al. 2012). The increased level of ROS induces the antioxidant mechanism of cell and as a result, the level of various antioxidant enzymes (peroxidase, catalase, superoxide dismutase (SOD), etc.) and non-enzymatic molecules (ascorbate, glutathione, carotenoids, tocopherols, etc.) increase, to maintain the cellular oxidative state (Sharma et al. 2012).

There are several reports showing an increase in the activities of antioxidant enzymes associated with the application of NPs in plants (Table 2.1). Effects of nickel oxide nanoparticles (nNiO) were examined on tomato. Increased level of ROS, SOD, glutathione, and lipid peroxidation was observed in nNiO-treated tomato plants (Faisal et al. 2013). Application of nAg affected the photosystem II in *Spirodela polyrhiza* that decreases the fixation of solar energy and promotes the production of ROS in chloroplasts (Jiang et al. 2014). Similarly, nCuO damaged the PSII in rice. The osmotic and oxidative stress was confirmed in nCuO-treated rice plants by the increased level of malondialdehyde, proline, SOD, and ascorbate peroxidase (Costa and Sharma 2016).

Hormonal signaling is liked with the ROS and integrated with the stress signaling pathway. The synthesis of various hormones is differentially regulated by various environmental stresses (O'Brien and Benková 2013). In *Arabidopsis*, the level of auxin and cytokinin was decreased in shoot, while the level of zeatin, salicylic acid, and abscisic acid (ABA) was increased in presence of nZnO (Vankova et al. 2017). In response to nFe_2O_3 exposure, an increase in indole acetic acid and ABA was reported in the roots of non-transgenic and transgenic rice (Gui et al. 2015). On contrary, a decrease in phytohormone level was found in seedlings of rice in response to nanocarbon tubes (Hao et al. 2016). These outcomes indicate that the NPs can influence the hormonal synthesis in plants. It has been hypothesized that plants sense the presence of NPs as stress and in response to that they synthesize more stress-related hormones (Vankova et al. 2017). Plant roots are directly in contact with the NPs present in soil/water; hence they are more adversely affected than shoots (Pokhrel and Dubey 2013; Qian et al. 2013; Shaw et al. 2014; Tripathi et al. 2017; Vinkovi'c et al. 2017).

Molecular changes in plants due to NPs are dependent upon their uptake and translocation. NPs of smaller size can penetrate and internalize in the cells by endocytosis. Interaction of NPs with cell organelles like mitochondria, chloroplast, etc., can cause oxidative stress (Fig. 2.1). Various molecular and biochemical changes occur in the plant due to this oxidative stress. The growth and survival of plants depend upon the level of ROS generated in response to NP stress. ROS production at an optimal level can enhance the synthesis of various antioxidant compounds and provide resistance to various stresses. On the other hand, a higher level of ROS can induce apoptosis, cell damage, and ultimately affect plant growth and yield (Foyer and Shigeoka 2011). Hence, intensive studies on the translocation and accumulation of NPs in different plant species at different concentrations are required before their use in the natural environment.

5 Biochemical Alterations in Primary and Secondary Metabolites

In the previous section of this chapter, we mentioned the increase in ROS production as a result of NP-plant interaction. There are several evidences showing ROS-mediated signaling for secondary metabolite production (Simon et al. 2010; Jacobo-Velazquez et al. 2015). Also, ROS can act as a signaling molecule of brassinosteroids (BRs), jasmonic acid (JA), salicylic acid (SA), ethylene, etc., that can modulate the synthesis of secondary metabolites (Wu and Ge 2004; Xia et al. 2009; Maruta et al. 2012; Baxter et al. 2014; Zhang et al. 2016).

Although there is a link between ROS and secondary metabolite production, there are very few reports showing the production of secondary metabolites can be affected by NPs. However, there are few studies related to the alteration in secondary metabolites in plants in response to NPs (Table 2.2). There are many evidences showing the change in SA, JA, BR level, etc., in plants, which are indirectly liked with secondary metabolite production. The level of SA was increased while JA was decreased in *Arabidopsis* treated with nZnO (Vankova et al. 2017). nAg can reduce the production of ethylene biosynthesis by affecting the production of ACC and ACC oxidase 2 (Syu et al. 2014).

Proteomic studies of some plant species exposed to NPs had shown the change in expression of primary and secondary metabolites. Application of Al_2O_3, ZnO, and Ag nanoparticles caused alteration in the amount of 104 proteins related to secondary metabolites in soybean roots (Hossain et al. 2016). Application of nZnO increased the proline content, soluble phenols, and phenylalanine ammonia-lyase in tomato (Pejam et al. 2021). In *Artemisia annua*, the expression of artemisinin was increased by 3.9-fold after the application of nAg in root culture (Zhang et al. 2013). An increase in diosgenin concentration was obtained in fenugreek after nAg

Table 2.2 Effect of nanoparticles (NPs) on primary and secondary metabolite production

NPs	Plant species	Organ/ tissue	Effects	References
nAg	*Calendula officinalis*	Areal parts	Enhanced saponin, decreased anthocyanin, flavonoid, and carotenoid	Ghanati and Bakhtiarian (2014)
nAg	*Prunella vulgaris*	Calli culture	Increased total phenols, DPPH-radical scavenging activity	Fazal et al. (2019)
nAg	*Corylus avellana*	Hazel cells	Increased taxon, baccatin, and lipid peroxidation. Decreased total soluble phenols and total flavonoids	Jamshidi and Ghanati (2017)
nAg, $nAgNO_3$	*Cucumis anguria*	Hairy roots	Increase in total phenols (caffeic, syringic, hydroxybenzoic acid, chlorogenic, β-resorcylic, ferulic, vanillic acid, protocatechuic, t-cinnamic, o-coumaric, and ρ-coumaric)	Chung et al. (2018)
nAg	*Isatis constricta*	Plantlets	Increase in tryptanthrin and indigo that reduced upon 10 and 15 days post-treatment	Karakas (2020)
nAg	*Caralluma tuberculata*	Calli culture	Increase in total phenols, SOD, CAT, APX, etc.	Ali et al. (2019)
nAu	*Prunella vulgaris*	Calli culture	Increase in total phenols, flavonoids, and DPPH-radical-mediated scavenging	Fazal et al. (2019)
$nCeO_2$	*Solanum lycopersicum*	Fruits	Increase in lycopene, decrease in reducing sugar and starch content	Barrios et al. (2016)
nCu	*Solenum lycopersicum*	Fruits, leaves and seedlings	Increase in lycopene content and activity of catalase	Juarez-Maldonado et al. (2016)
nCu	*Capsicum annuum*	Fruits	Increase in antioxidant content, total phenol content, and flavonoids	Pinedo-Guerrero et al. (2017)
nCu	*Cucumis sativus*	Fruits	Increase in various amino acids, fructose, fructose, proline, and benzoic acid. Decrease in lysine and methionine.	Zhao et al. (2017)
nCuO	*Withania somnifera*	Shoots and roots	Increase in antioxidants, total phenols, and flavonoid content	Singh et al. (2018)
nSiO2, nTiO2	*Tanacetum parthenium*	Leaves	Increase in parthenolide, increased expression of *COST*, *TpGAS*, and *TpCarS* genes related to biosynthesis pathways of β-caryophyllen and parthenolide	Khajavi et al. (2019)
nSiO2, nTiO2	*Argania spinos*	Callus culture	Enhance tocopherol accumulation	Hegazi et al. (2020)

(continued)

Table 2.2 (continued)

NPs	Plant species	Organ/ tissue	Effects	References
nSe	*Apium graveolens*	Stem and leaves	Increase in total antioxidant capacity, total phenols, proteins, vitamin C, jasmonic acid, aspartic acid, chlorophyll, beta-carotenes, glutamic acid, flavonoids, soluble sugar, ariginine, tryptophan, and proline	Li et al. (2020)
nTiO2	*Abelmoschus esculentus*	Roots, stem, and leaves	Increase in activity of SOD, but reduced glutathione reductase (GR) and ascorbate peroxidase (APX) activities in roots. Increased level of malondialdehyde and GR activity, but reduced activity of APX in leaves	Ogunkunle et al. (2020)
nZnO	*Stevia rebaudian*	Shoots	Increase in total reducing power, total antioxidant activity, total flavonoids, total phenols, rebaudioside A, stevioside content, and inhibition of DPPH	Ahmad et al. (2020)
nZnO	*Momordica charantia*	Shoots	Increased antioxidant enzyme activities, carbohydrate, phenols, carotenoids, flavonoids, anthocyanins, and proline content	Sharifi-Rad et al. (2020)
nZnO	*Camelina sativa*	Root-shoot	Increase in total phenols, phosphorus, calcium, zinc, carotenoids, and anthocyanins. Decrease in antioxidant capacity and total flavonoids	Hezaveh et al. (2020)

application (Jasim et al. 2017). Effects of NPs were examined on lower non-vascular members of plant kingdom. It was found that the application of $nTiO_2$ can increase the secretion of phenolic compound in *Arthrospira platensis* (cynobacteria) and *Haematococcus pluvialis* (micoalga) (Comotto et al. 2014).

Although, the studies suggest that NPs are capable of modulating the synthesis of secondary metabolites but the exact mechanism is not clearly understood. Synthesis of secondary metabolites is a defense mechanism of plants against any stress. NPs, after entering in the cell, might interfere with the electron transport system present in mitochondrial and chloroplast membrane, create oxidative stress, and ultimately generate ROS, which can generate secondary metabolite, directly or indirectly.

6 Effect of Nanoparticles in Rhizospheric Environment

Soil is the habitat of diverse microorganisms, which are associated with plants growth and development. Among these soil microorganisms, rhizobia and mycorrhiza are the most important microorganisms that interact with the roots of plants in rhizosphere. Almost all the vascular plant roots establish the symbiotic relationship with arbuscular mycorrhizal fungi (AMF) (Brundrett and Tedersoo 2018). This association helps the plant to access less available minerals in soil, viz. phosphate, improve plant growth, and tolerance to abiotic/biotic stress (Hildebrandt et al. 2007; Smith et al. 2009; Miransari 2010). On the other hand, the symbiotic association of rhizobium with legumes plays a significant role in atmospheric N-fixation. Knowing the importance of rhizobium and mycorrhizal symbiotic association with plants to maintain the health of soil, it is crucial to examine the effect of NPs on these microorganisms.

There is only hand full of studies showing the effect of NPs on symbiotic association of rhizobium and mycorrhiza with plants. Most of the studies show that the presence of NPs has negative impact on rhizobium and mycorrhiza while few show the positive effect of NPs on these microorganisms (Table 2.2). The effect of NPs on mycorrhiza and rhizobium is dependent upon the physicochemical properties of NPs, concentration of NPs, species of fungus and bacteria, and the properties of soil (Tian et al. 2019).

The impact of NPs on mycorrhizal colonization or rhizobial association with plants is dependent on the size and concentration of soil, microbial species, and physicochemical properties of soil. The concentration and size of nanoparticles have a crucial impact on these symbionts. To understand the mechanism by which NPs can affect these microorganisms in the natural environment, it is important to characterize NPs in the possible exposure conditions.

Soil microorganisms are crucial to sustainable agriculture and their association with plants is vital for the growth and development of plants. Hence, it is important to examine the effect of NPs on these microorganisms, individually as well as the effect of NPs on their association with plants. At this point, we know that NPs can affect the symbiotic association of plant-mycorrhiza and legume-mycorrhiza in a positive and negative way. In most of the experiments, unrealistically high concentrations of NPs are used to examine the effect on mycorrhiza (Tian et al. 2019). Also, the experiments with rhizobia were performed in soil-less media (Tian et al. 2019). For a better understanding of the impacts of NPs on soil microorganisms, more studies are required that are closed to natural environmental conditions.

7 Major Concerns

Nowadays, nanoparticles are used in every industry and becoming the part of our lives. The ultimate sink for these NPs is our environment, i.e., air, water, and soil and from there it enters in plants. Hence, it is imperative to observe the impact of NPs on plants. Several studies have reported that the lower concentrations of NPs can positively affect plant growth while its high concentration can cause phytotoxicity. Excessive use of NPs can increase their concentration in the environment. So, it is important to characterize the NPs, their stability in the environment.

Table 2.3 Effects of nanoparticles (NPs) on plants in rhizospheric environment

NPs	Symbiotic partners	Effect	References
nAg	White clover-*Glomus caledonium*	+ve	Feng et al. (2013)
nAg	Faba bean-*Rhizobium leguminosarum*	−ve	Abd-Alla et al. (2016)
nAg	Faba bean-*Glomus aggregatum*	−ve	Abd-Alla et al. (2016)
nAg	Alfaalfa-*Sinorhizobium meliloti*	−ve	Mohaddam et al. (2017)
nAg	Tomato-AMF	−ve	Noori et al. (2017)
nAu	Tomato-AMF	−ve	Judy et al. (2015)
nCeO	Soybean-*Bradyrhizobium japonicum*	No effect	Priester et al. (2012)
nCeO	Red clover-AMF	No effect	Moll et al. (2016)
$nCu(OH)_2$	Bean- *Rhizobium leguminosarum*	−ve	Baijukya and Semu (1998)
nFe_3O_4	Soybean-*Bradyrhizobium japonicum*	+ve	Ghalamboran (2011)
nFe_3O_4	Soybean- *Rhizobium* sp.	No effect	Burke et al. (2015)
nFe_3O_4	Pea- *Rhizobium leguminosarum*	Varied from −ve to +ve, dependent upon the dose and days of inoculation	Sarabia-Castillo and Fernández-Luqueño (2016)
nFeO	Clove-*Glomus caledonium*	+ve to no effect (dose dependent)	Feng et al. (2013)
nMo	Chickpea-*Bradyrhizobium japonicum*	+ve	Taran et al. (2014)
$nTiO_2$	Pea- *Rhizobium leguminosarum*	−ve	Fan et al. (2014)
$nTiO_2$	Wheat-AMF	No effect	Kingenfuss (2014)
$nTiO_2$	Soybean- *Rhizobium* sp.	No effect	Burke et al. (2015)
$nTiO_2$	Soybean-AMF	No effect	Burke et al. (2015)
$nTiO_2$	Reds clove-AMF	No effect	Moll et al. (2016)
$nTiO_2$	Pea- *Rhizobium leguminosarum*	−ve	Sarabia-Castillo and Fernández-Luqueño (2016)
$nTiO_2$	Rice-AMF	−ve	Priyanka et al. (2017)
nZnO	Soybean-*Bradyrhizobium japonicum*	No effect to +ve	Priester et al. (2012)
nZnO	Pea- *Rhizobium leguminosarum*	−ve	Huang et al. (2014)

(continued)

Table 2.3 (continued)

NPs	Symbiotic partners	Effect	References
nZnO	Tomato-AMF	No effect	Watts-Williams et al. (2014)
nZnO	Maize-*Funneliformis mosseae*	−ve	Li et al. (2016)
nZnO	Soybean-*Funneliformis mosseae*	−ve	Jing et al. (2016)
nZnO	Pea- *Rhizobium leguminosarum*	−ve	Sarabia-Castillo and Fernández-Luqueño (2016)
nZnO	Maize- *Glomus caledonium/versiforme*	No effect to −ve (dose-dependent)	Wang et al. (2016a, b and c)

The assessment of the impact of NPs on plants is very complicated as it is species-specific (Pérez-de-Luque 2017). Also, the results obtained from experiments done in control conditions, using hydroponics or soil-less media, can differ while using in the field. Crop fields are inhabited by several microorganisms that affect plant growth. Application of NPs in the field can influence the microflora (Table 2.3) so their impact on the plant can differ in the field from the laboratory experiments.

Before the use of NPs in agriculture, experimentation is needed in more realistic conditions. The physicochemical changes of NPs in soil and their long-term effects on mycorrhiza and rhizobia should be investigated. The mechanism of translocation and internalization of NPs in different plant tissues and induction of secondary metabolite pathways by them are still not clearly explained. Studies suggested that NPs can be used to improve the synthesis of bioactive compounds (Table 2.2). This can be a great strategy to enhance the synthesis of commercially important bioactive compounds. But, the potential impact of the NPs as an elicitor of secondary metabolite production needs more studies to assess the overall impact on ecosystem. Furthermore, to bring the NPs in regular agricultural practices, large-scale experiments are required for the better understanding of the NP-plant interaction on the basis of concentration, size, the exposure period of NPs, and its impact on other components of soil.

8 Conclusion

Technological innovation is essential to fill the gap between food production and the exponentially increasing world population. Nanotechnology is one of the promising fields with broader applications. An increase in the use of NPs in industries is ultimately going to end up in our environment. Once they enter in soil, they will affect our crops and ultimately become a part of our food chain. Hence, it is imperative to thoroughly investigate the impacts of commonly used NPs on plants along with their microenvironment. The studies showed that they can affect plant growth and its physiology in positive, negative, or in neutral ways. Also, the effect of the same NPs can vary from species to species.

Similar to plants, the effect of NPs on the microbiome also varies from species to species and can affect positively or negatively in a dose-dependent manner. Before the use of NPs in large-scale crop field, it is crucial to examine the holistic effect of that NP in particular species along with its effect on microbial population present along with that crop. This chapter shows that the study of NPs in agriculture is expanding. Although, more studies are needed to explain the mode of interaction of NPs with different bio-molecules and their impact on gene expressions.

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