Chapter 1
Nanoparticles and Plant Interaction with Respect to Stress Response

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1.1 Introduction

Nanotechnology is an incipient multidirectional technique with extensive applications in cancer remedies, drug delivery, microelectronics, biosensors, and cosmetic production, and also in agricultural fields (Nel et al. 2006; Singh et al. 2016, 2019; Arif et al. 2018; Shweta et al. 2018, 2017; Vishwakarma et al. 2018; Rastogi et al. 2019a, b). However, unspecified discharge of nanoparticles (metallic) into ecological communities has increased worldwide apprehension about their probable toxicity. The length of nanoparticles generally ranges from 1 to 100 nm in at least two dimensions, so that these are extremely fine particles with more surface area (Nowack and Bucheli 2007). Compared to molecules and bulk materials, nanoparticles are intermediate in size. The distinctive physical as well as chemical properties of nanoparticles result from their detachment in bulk material into reduced and smaller pieces (Jefferson 2000). However, because of their nanoscale size, their surface area thus increases, which makes them extremely catalytic or reactive.

The haphazard release of nanoparticles into natural environments from industrial effluents leads to their bulk production (Brunner et al. 2006; Owen and Handy 2007). Thus, most produced nanoparticles consist of heavy metals, toxifying water and soil with metallic nanoparticles, now a concern in environmental degradation. Plant interaction with excess nanoparticles in water and soil may cause the uptake and accumulation of the nanoparticles in the plant, leading to their ultimate conveyance to the ecosystem. However, nanoparticles may persist and be accumulated

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within the plant, thus causing physical as well as chemical damage to various parts of the plant. Generally, nanoparticles pass into the plant root system through junctions of the lateral roots, thereby reaching the xylem tissue through the pericycle and the cortex (Dietz and Herth 2011). Moreover, although the cell wall restricts the entrance of nanoparticles into the plant body, cell walls have a definite pore size, allowing the transference of nanoparticles smaller than the pore size of the cell wall (Fleischer et al. 1999; Navarro et al. 2008). The degree of penetration is subject to the surface characteristics and dimensions of the nanoparticles. Undeniably, the smaller nanoparticles can pass through the cell wall easily. Larger nanoparticles are unable to pass through the cell wall and thus cannot disturb the metabolic pathways of the cell (Verano-Braga et al. 2014). However, flower stigmas, hydathodes, and stomata possess larger cell-wall openings, so that larger-size nanoparticles can pass through and possibly affect the plant. Chemical and physical interactions of nanoparticles with plants could be a natural or induced phenomenon. Chemical interfaces encompass the production of reactive oxygen or nitrogen species (ROS/RNS) (Nel et al. 2006), disruption in cell membrane transport activity of ions (Auffan et al. 2008), injury from oxidative stress (Foley et al. 2002; Jalil et al. 2017), and peroxidation of lipids present in the cells of the plant (Kamat et al. 2000). The ensuing admittance into the body of the plant through the cell wall subsequently allows nanoparticles to associate and work as metallic ions, reacting with carboxyl and sulfhydryl groups and eventually modifying protein activity. The effect of nanoparticle applications in shown in Fig. 1.1.

However, while studying engineered nanomaterials that arbitrate ecotoxicity, several artifacts that often lead to misconceptions of outcomes should be considered (Petersen et al. 2014). These probable aspects consist of noxious scum in engineered nanomaterials, their apposite storage and dissemination in a particular medium. Furthermore, engineered nanomaterials imply unintended effects on plant

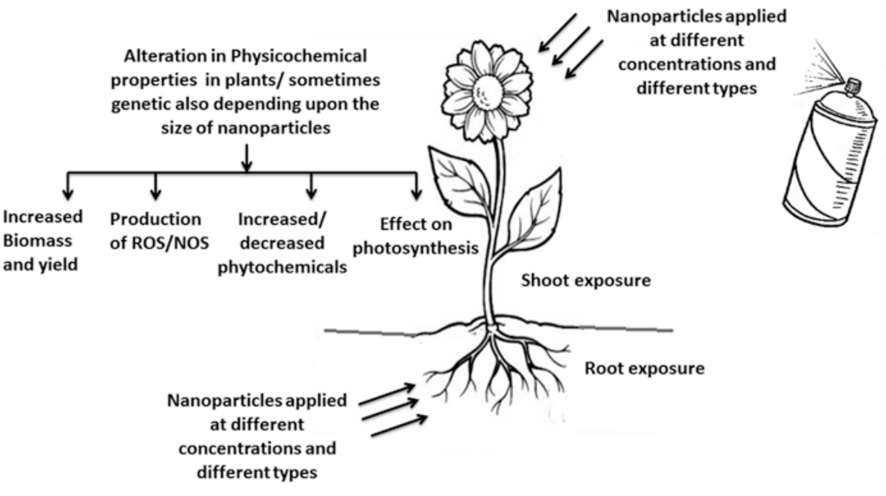

Fig. 1.1 Effect of nanoparticles on plants, leading to stress conditions

growth and development by depletion of nutrients with time and the diffusion of the engineered nanomaterials in organisms. Additionally, the dissolution, settling, and agglomeration properties of engineered nanomaterials cause diverse variations all through the study period that are difficult to evaluate accurately. Characteristics such as greater superficial area with distinct physical as well as chemical properties allow engineered nanomaterials to freely interact with ions physicochemically within the nutrient medium, thereby causing unintended toxic reactions such as wilting and chlorosis (Slomberg and Schoenfisch 2012; Begum and Fugetsu 2012; Jalil et al. 2018). Furthermore, as engineered nanomaterials interact with organic acids in the roots of the plant, the pH of the media falls, thereby modifying the nutrient quantity and properties of the engineered materials (Marschner 1995). The ineffectiveness of such interactions and their effects can be observed as an incongruous elucidation of phytotoxicity, and eventually a fictitious impression of engineered nanomaterials is understood (Ma et al. 2010).

1.2 The Nanoparticle and Its Role in Plant Stress

It is been reported that nanoparticles generally possess desired and undesired outcomes on various plant species because of their configuration, dissimilar size, altered physicochemical properties, and the concentrations of different nanoparticles used (Ma et al. 2010; Tripathi et al. 2017). It was reported that carbon nanotubes (multi-walled) substantially exaggerated the upregulation of stress-related gene expression in tomato seed germination and further affected the progress of the seedlings (Khodakovskaya et al. 2009; Singh et al. 2016; Koul et al. 2018). In a finding by Lee et al. (2010), Al_2O_3 nanoparticles were found to be less toxic than ZnO, SiO_2, or Fe_2O_3 nanoparticles when used to treat *Arabidopsis thaliana*. Earlier investigations on algae also underscored the noxious effects of nanoparticles (Arouja et al. 2009). Several nanoparticles such as CeO_2, ZnO, TiO_2, and Ag nanoparticles were observed to be deposited on the cell-wall surface as well as on the surface of the organelles, which causes stimulation in the form of stress response from oxidative stress within the cell (Buzea et al. 2007). In another study of *Cucurbita pepo*, the outcome of showed that seed germination was not altered by treatment with Cu, Si, Ag, and ZnO nanoparticles. On the other hand, their complementary bulk constituents and Cu nanoparticles cause alteration in root length when estimated against the control and a powder of bulk copper (Stampoulis et al. 2009). Treatment with ZnO nanoparticles affected root length in rice plants, but TiO_2 nanoparticles had no effect on the roots (Boonyanitipong et al. 2011). In another study, Riahi-Madvar et al. (2012) revealed effects on the roots of *Triticum aestivum* by treating the plant withAl_2O_3 nanoparticles at different concentrations, although the nanoparticles did not influence seed germination, shoot length, or fresh weight/dry weight ratio. Treatment of rice seedlings with CuO nanoparticles had an effect on enzymatic activity. Enzymatic antioxidant values were shown to be elicited (Shaw and Hossain 2013). An analogous experiment was conducted by Shaw et al. (2014)

wherein treatment of *Hordeum vulgare* with CuO nanoparticles caused an effect on photosynthetic activity and also on the antioxidants. It was elucidated that the decreased growth of shoot and root length caused poor photosynthetic activity. Furthermore, Atha et al. (2012) showed CuO nanoparticles cause decreased growth and DNA damage in *Raphanus sativus*, *Lolium perenne*, and *Lolium rigidum* plants. Rico et al. (2013) revealed effects on photosynthetic activity, enzymatic activity, and ascorbate and thiol levels when CeO_2 nanoparticles were used to treat rice seedlings. Formation of ROS/RNS and H_2O_2 when *Spirodela punctata* plants were treated with Ag and ZnO nanoparticles showed the significant toxicity of these nanoparticles (Thwala et al. 2013). Among the numerous metallic nanoparticles, ample consideration has been paid to Ag nanoparticles because of their distinctive biological and physicochemical properties when compared to various other large-size nanoparticles (Sharma et al. 2009). The fungicidal and bactericidal properties of Ag nanoparticles have widespread application as an indispensable constituent in various products at domestic, nutritional, and industrial levels (Tran et al. 2013). Silver nanoparticles when compared to silver-based compound nanoparticles possess an enlarged surface area accessible for microbe interface; further, it was reported that such are more noxious to various fungi and bacteria and several viruses as well. Similar to alternate metallic nanoparticles, Ag nanoparticles possess more impact on prompt stress reactions (ROS/RNS) in various microorganisms, algae, animals, and plants (Jiang et al. 2012). Conversely, the stimulus of Ag nanoparticles on plants is basically governed by such factors as plant species, growth and developmental stage, type and concentration of nanoparticles used, and experimental parameters such as period of treatment, humidity and temperature, and method of nanoparticle treatment (Vannini et al. 2013). Ag nanoparticles are one of the most widely examined nanoparticles whose toxic effects have been investigated in numerous plant crops (Stampoulis et al. 2009; Jiang et al. 2012; Kumari et al. 2009). Several studies have revealed that Ag nanoparticles were disadvantageous for plant growth as compared to the growth-enhancing possession of Ag nanoparticles in wetland plants (Yin et al. 2012), *Proteus vulgaris* and *Zea mays* (Salama 2012), *Brassica juncea* (Sharma et al. 2012), and *Eruca sativa* (Vannini et al. 2013). Ag nanoparticles showed a chromotoxic outcome on mitotic cell division in *Allium cepa* (Kumari et al. 2009). Further, Ag nanoparticles intermingle with proteins (are membrane bound) and stimulate various metabolic pathways, which restricts cell propagation (Roh et al. 2012; Gopinath et al. 2010). Some of the numerous effects of nanoparticles on plants are summarized in Table 1.1.

1.3 Mechanistic Interaction of Nanoparticles in Plant Stress

Recent studies showed that all the interactions of nanoparticles may be determined and noted to be affected by plant species, nanoparticle type and size, and the chemical structure, constancy, and functional aspects of the nanoparticles. The interaction of nanoparticles with plants leading to stress can be classified into different phases of nanoparticle uptake, translocation within the plant, accumulation in different

Table 1.1 Response of different plants toward different kinds of nanoparticles

Plants	Types of nanoparticles applied	Response	References
Boswellia ovalifoliolata, Egeria densa, Juncus effusus, Quercus robur	Silver	Improved germination rate; enhanced enzymatic antioxidants; no effect on chlorophyll; no phytotoxicity	Savithramma et al. (2012); Yuan et al. (2018b); Olchowik et al. (2017)
Gloriosa superba, Arabidopsis thaliana	Cerium oxide	Toxic; Increased plant growth	Arumugama et al. (2015); Ma et al. (2013)
Gum karaya, *Phyllanthus amarus, Cassia alata*	Copper oxide	Therapeutic applications; Positive antimicrobial activity	Jayalakshmi and Yogamoorthi (2014); Vellora et al. (2013); Acharyulu et al. (2014)
Quercus robur	Copper	Positive antimicrobial activity; no phytotoxicity	Olchowik et al. (2017)
Euphorbia condylocarpa	Palladium	As a catalyst	Nasrollahzadeha et al. 2015
Oryza sativa, Triticum aestivum, Lycopersicon esculentum	Titanium dioxide	Phytocatalyst Elicitation of chlorophyll	Ramimoghadam et al. (2014); Mahmoodzadeh et al. (2013); Qi et al. (2013)
Glycine max, Vigna radiata	Iron oxide	Improved productivity and quantity	Dhoke et al. (2013); Sheykhbaglou et al. (2010)
Capsicum annuum	Iron	Promoted plant growth; alleviated iron deficiency	Yuan et al. (2018a, b)
Arachis hypogaea	Zinc oxide	Improved productivity	Prasad et al. (2012)
Cucumis sativus	Gold	Improved germination rate	Barrena et al. (2009)
Lycopersiconm esculentum	Carbon nanotubes	Improved germination rate	Morla et al. (2011)
Solanum lycopersicon	Nickel oxide	Induced apoptosis in roots; enhanced antioxidants	Faisal et al. (2013); Soares et al. (2016)
Lemna minor	Alumina	Enlarged plant growth; improved productivity and quantity	Juhel et al. (2011)

cells and tissues, and desired or undesired outcomes. Some of the studies revealing such conditions are discussed next.

1.3.1 Phytotoxicity Mechanism of Nanoparticles

Investigation related to phytotoxicity in higher plants is imperative for elucidating the toxic effect of nanoparticles. Both undesirable and desirable or insignificant effects have been elucidated concerning the potential noxiousness of nanoparticles to various plants (Bystrzejewska-Piotrowska et al. 2009; Sohaebuddin et al. 2010; Muller et al. 2005; Jalil et al. 2018). Various studies point toward nanoparticle toxicity (Ghodake et al. 2010; Stampoulis et al. 2009). A noticeable variation in germination rate and growth was detected in the seeds of rice when exposed to carbon nanomaterials, mainly carbon nanotubes (Wang et al. 2012; Smirnova et al. 2012; Tan and Fugetsu 2007). In the experiment, water content in carbon nanotube-treated seeds was compared to that in control seeds, with better water content observed in the treated seeds. The germinating seeds were supplemented with carbon nanotubes to elucidate the effect on further developmental stages. The findings show significant use of carbon nanotubes to improve the growth of rice seedlings (Smirnova et al. 2012). In another example using Al_2O_3 nanoparticles, root length elongation was hindered in soybean, carrot, cabbage, cucumber, and corn (Kollmeier et al. 2000; Yamamoto et al. 2001; Tian et al. 2007; Ryan et al. 1992), whereas nanoparticles of ZnO were found to be maximally toxic, impeding the growth rate of the roots in various plants (Stella et al. 2010; Ma et al. 2009; Huang et al. 2002). Nanoparticles of ZnO were observed to be noxious in high concentrations when used to treat *Arabidopsis thaliana* plants, where a decreased germination rate was observed, with Al_2O_3, SiO_2, and Fe (II, III) oxide nanoparticles showing a moderate effect (Bin Hussein et al. 2002; Wang et al. 2004; Dwivedi and Randhawa 1974). With consideration for the toxicological aspect, the ratio of particle size to surface area is a vital physical property of a nanoparticle; the lesser the particle size, the greater the surface area. Hence, more of its atoms or molecules are exhibited externally than internally (Fugetsu and Parvin 2011; Begum et al. 2011). Several studies revealed surface properties of nanoparticles to be more toxic with a higher toxicity level than finer particles of the same material (Clarke and Brennan 1989; Kashem and Kawai 2007): this has been experimentally demonstrated by the use of diverse types of nanoparticles, such as cobalt, nickel, titanium dioxide, and carbon black. It was observed that TiO_2 nanoparticles with a minimum size less than 30 nm consequently are 43 fold more inflammatory than nanoparticles larger than 200 nm (Feizi et al. 2012; Castiglione et al. 2011; Qiu et al. 2013). Numerous investigations revealed that nano-sized particles are somewhat more toxic than micro-sized particles (Currie and Perry 2007). It was elucidated that one of the important parameters of noxiousness of nanoparticles is surface area. For instance, crystalline TiO_2 did not exhibited more severe toxicity than shown by TiO_2 nanoparticles (Stephen et al. 2012; Han et al. 2010). Generally, the present phytotoxic outline of nanoparticles is somewhat hypothetical; initially, the effects of nanoparticle properties are not well understood and further investigation on toxic effects is necessary, particularly on valuable food crops (Groppa et al. 2008). Thus, it can be concluded that various investigations have revealed that direct exposure to a particular kind of nanoparticles instigated a noteworthy phytotoxic effect, underscoring the necessity for environmental accountability for discarding wastes containing nanoparticles. Further studies on the influence of nanoparticles on valuable food crops and on the environment are required.

1.3.2 Uptake Mechanism of Nanoparticles

Investigations on the mechanism of uptake of nanoparticles in plants lack reliable and widely acceptable information (Nevius et al. 2012). Previous observations showed that nanoparticles may adhere to the plant root system and cause physico-chemical changes during uptake within the plant (Hartley and Lepp 2008; Taylor and Foy 1985). Currently, various investigations emphasize revealing the interface mechanism of nanoparticles toward plants (Besson-Bard et al. 2009; Zhang et al. 2017; Ma et al. 2018). Nanoparticle uptake and accumulation may differ depending on the difference in size and type of nanoparticle within the plant. Undeniably, validation of the mechanism of nanoparticle uptake is very restricted, and it depends on the concentration applied (Smirnova et al. 2012). Thus, most of the investigations reported do not yield similar outcomes for diverse forms (shapes and sizes) of nanoparticles (John et al. 1972). The majority of information is related to metal-based nanoparticles such as TiO_2, ZnO, Ag, Au, or Fe that correspond to a particular germination stage of the plant. Various possibilities have been suggested for nanoparticle uptake by the cells of the plants. Studies have suggested that nanoparticles move in plant cells by binding with protein biomolecules or ion channels, or through the process of endocytosis by means of new pores formed, finally binding to some organic molecule (Maine et al. 2001; Kurepa et al. 2010). For such investigations, carbon nanotubes have been preferred over other nanoparticles (Smirnova et al. 2012). However, it was reported that nanoparticles when compared to the bulk metals cause more reactivity by the greater surface area to mass ratio (Yuan et al. 2011). Subsequently, the nanoparticles might align with membrane transporters to form complexes as the root absorbs these and transports the particles into the plants. Thus, nanoparticles have been identified that can recognize ion transporters and be readily taken up by the plant (Tani and Barrington 2005). Selectivity among types of plants and the uptake of nanoparticles, which is still not very clear, is an area of further investigation.

1.3.3 Translocation Mechanism of Nanoparticles

Several investigations supported that the translocation of nanoparticles is determined by the quantity delivered and the species of plant (Yang and Ma 2010). Specific nanoparticles move swiftly within the plant, forming interactions with other biomolecules. Thus, the other nutrients are estimated according to the translocation of the nanoparticles applied (Zhu et al. 2008). The mechanism of translocation is instigated by the permeation of nanoparticles, into first the cell walls and then the plasma membrane of the cells. Through conduction by the plant xylem, the uptake mechanism and nanoparticle transferences take place in the shoot system

(Pola et al. 2012; Birbaum et al. 2010). The pore size of the cell wall is a vital criterion for the selection of nanoparticles, determining which nanoparticles can penetrate. As was investigated in *Allium porrum*, nanoparticle penetration was swifter in stomata than in the leaf (Birbaum et al. 2010).

1.3.4 Interaction Mechanism of Nanoparticles Leading to Stress

For the past few decades, the phytotoxicity of nanoparticles has been extensively investigated in several plant species, mainly focusing morpho-physiologically and biochemically. Nevertheless, only a few experiments have been focused on nanoparticle interaction with biomolecules with consideration of proteomics and causes of stress in the plant. Mirzajani et al. (2014) revealed by proteomic technique (gel-based) that the interaction of Ag nanoparticles on *Oryza sativa* causes toxicity. This investigation, based on root proteomics, elucidated that Ag nanoparticle-associated proteins were mainly related to the oxidative stress pathway, transcription, cell-wall synthesis, ion signaling and its regulation, division of cells, and degradation of protein. The effect of nanoparticles on the cell leading to such alterations is shown in Fig. 1.2. It was further observed that elicitation of enzymatic antioxidants such as peroxidases, glutathione-*S*-transferase, L-ascorbate, and superoxide dismutase induces enhanced formation of ROS under Ag nanoparticle treatment stress (Vannini et al. 2013). When Ag nanoparticles and $AgNO_3$ compounds were applied to *Erruca sativa* roots, both forms of silver produced alterations in the proteins associated with cellular homeostasis and redox regulation. These outcomes showed that the noxiousness of Ag nanoparticles mainly derives from its distinctive physicochemical characteristics (Vannini et al. 2013). Under flooding stress, the toxicity mechanism of Ag nanoparticles was studied in early stages of *Glycine max* plants, showing that proteins associated with signaling pathways, and the metabolism of cells and stress response, were altered. Furthermore, glyoxalase, an enzyme related to the detoxification pathway, was also degraded by Ag nanoparticle treatment (Mustafa et al. 2015a, b). In another study by Mustafa et al. (2015a, b), wherein the effects of Ag, ZnO, and Al_2O_3 nanoparticles were compared for treating *Glycine max* plant under flooding stress, protein synthesis was degraded, and glycolysis and lipid metabolism were also affected. Such investigations exhibit the interaction of nanoparticles with plants, but more research is needed to fully elucidate such effects. Thus, the desired as well as undesirable effects of nanoparticles can be observed on plants depending upon the requirements. Future investigations on interaction of nanoparticles with plants would help elucidate better understanding, implying specific nanoparticles can be applied to plants for desired outcomes, which may lead to better agricultural yields.

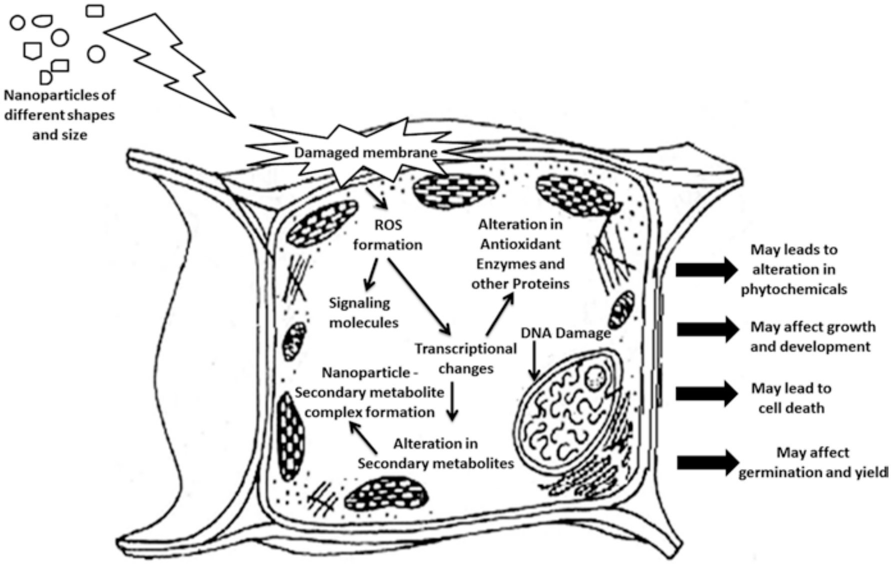

Fig. 1.2 Systematic representation of effect of nanoparticles within the plant cell leading to stress conditions

1.4 Conclusions and Future Prospects

The investigations conducted so far mostly concern plant reactions to a particular nanoparticle stress displaying an abundance of proteins associated with ROS, signaling caused by stress, pathways related to plant hormones, oxidation-reduction within the cell, and detoxification. Investigations on nanoparticles causing phytotoxicity showed that nanoparticle size is an important aspect in the type and degree of response within the plant cell. Further investigations are needed to fully elucidate whether metallic nanoparticles wield their noxious effects because of their distinctive characteristics or the loose metallic ions. Furthermore, exploration intended to recognize and illustrate subcellular organelles for elucidating the detailed alterations within the cell helps to understand the stress mechanism caused by nanoparticles. Additionally, metabolomics and transcriptomics techniques can have great prospects to fully elucidate the stress response toward nanoparticles. All this information would give us a wide explanation of the response mechanism of plants to stress caused by nanoparticles. Such investigations on plant stress tolerance mechanisms toward nanoparticles can lead to better plant yields for the production of specific valuable phytochemicals. These data, based on the interaction of nanoparticles with plants, would help elucidate improved understanding about the plant responses, which would suggest particular nanoparticles for plants for anticipated outcomes. This information can be advantageous for the agricultural perspective in improved yields and increased production of secondary metabolites that are beneficial in the pharmaceutical and nutraceutical industries.

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