

Manzer H. Siddiqui  
Mohamed H. Al-Whaibi  
Firoz Mohammad *Editors*

# Nanotechnology and Plant Sciences

Nanoparticles and Their Impact on  
Plants

 Springer

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Nanoparticles and Their Impact on Plants

*Editors*

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# Preface

The world population is increasing at an alarming rate. While it has already crossed the seven billion line, it is expected to continue rising in the near future. To feed the teeming humanity in the new millennium, a huge responsibility lies on the shoulders of plant scientists to discover newer ways of enhancing crop production. Along with the inputs from classical breeding, molecular breeding, and biotechnology sciences, will nanotechnology also help in this venture? Will the birth of so-called ‘Nanobiotechnology’ science prove a boon? Nanotechnology in a naïve sense may appear as a paradigm of the physical sciences. This is however an understatement of the potential of nanotechnology. As it turns now, the advanced and modern nanotechnology science is equally relevant to life sciences and may play a major role in improving the quality of human life in the future years. Based on nanotechnology principles, novel inventions are being made everyday in the field of medicine. Nanoparticles are receiving much attention because of their unique physicochemical properties. The nanoparticles are thus being employed as “smart” delivery systems in life sciences. No wonder, the Noble laureate in Physiology Paul Ehrlich referred these compounds as “magic bullets”. In agriculture, nanoparticles are proving important as compound fertilizers and nanopesticides. Most excitingly, it is shown in recent years that nanoparticles may act as chemical delivery agents for targeting molecules such as genes/DNA to specific cellular organelles like nuclei in plants.

Considering that gaining a deeper understanding of the role of nanotechnology in relation to plant systems is of paramount importance, we felt that a dedicated book on bringing together varied aspects of plant and nanotechnology is the need of the hour. Our book *Nanotechnology and Plant Sciences: Nanoparticles and Their Impact on Plants* presents a holistic view of the use of nanoparticles in complex and dynamic aspects of plant research. The inclusion of nanoparticles in commercial products and industrial applications has significantly increased. To further extend these commercial gains, it is important to understand the interaction mechanisms between the nanoparticles and biological systems at the molecular level. The latter aspect has been emphasized in this book. As a new emerging field, nanobiotechnology unlocks new frontiers in genetic engineering science. However,

the information available on the use of nanoparticles in genetic transformation of plants is still scarce. We have tried to bring together the views of experts of these subjects under one platform of this book to address the above issues.

This book has 14 chapters written by experts with considerable experience in the area of research. The contents of each chapter are based on the research findings of active workers in nanotechnology. The book covers various important topics related to nanoparticles and plants. It provides an understanding of the mechanisms involved in the response of plants to nanoparticles. We firmly hope that the readers of this book will be exposed to new challenges and at the same time new vistas of future line of action in the area of plants and nanotechnology. We believe that students and researchers of plant molecular biology, plant physiology, agriculture, botany, biochemistry, biotechnology, environmental biology, microbiology, and forestry will be hugely benefitted by the contents of this book. We also hope that NGOs dealing with civic problems caused by rapid environmental degradation will find this book useful. The book will lead to a better understanding of the interdisciplinary field of functional biology and nanoparticles. The aim of writing this book was to bring together all possible approaches to tackle the aim of the improvement of current crops and introducing crop plants into areas not currently being used for cultivation. We have tried our best to realize these goals in bringing out this book and now we want the readers to evaluate how far we have been successful in this aim.

The editors convey their heartfelt gratitude to all the contributors for their excellent, informative, and up-to-date contributions and for their consistent support and cooperation. We are particularly grateful to Christina Eckey, Senior Editor, Plant Sciences, Springer and Anette Lindqvist, Project Coordinator for their continuous support and technical advice.

We also thank Dr. Anil Grover for his critical assistance and for encouraging me from time to time during the preparation of this important book.

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Riyadh, Saudi Arabia  
Aligarh, India

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# Chapter 1

## Chemistry, Biochemistry of Nanoparticles, and Their Role in Antioxidant Defense System in Plants

C.M. Rico, J.R. Peralta-Videa and J.L. Gardea-Torresdey

**Abstract** As time passes, engineered nanoparticles (ENPs) are more frequently found in medical and consumer products, as well as in industrial and agricultural applications. The intensive production, use, and disposal of ENPs-containing wastes increase the likelihood of emission of such products to the environment. During the last two decades, a body of scientific literature has shown that ENPs interact with living components of ecosystems in different ways. The literature indicates that ENPs impact on plant growth, cell structure, and physiological and biochemical functions. In this chapter we discuss the stress induced by ENPs on higher plants. Although some references about carbon-based ENPs are included, most of the references are related to metal-based ENPs. The discussion is mainly focused on the effects of ENPs on photosystems and the mechanisms of generation/scavenging of reactive oxygen species (ROS). Effects on the enzymes catalase (CAT), guaiacol peroxidase (GPOX), ascorbate peroxidase (APOX), superoxide dismutase (SOD), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) are discussed. Information about low molecular weight antioxidant thiols (GSSG or GSH) and ascorbate is also included.

**Keywords** Engineered nanomaterials · Vascular plants · Physiology · Biochemistry · Reactive oxygen species

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## 1.1 Introduction

Metabolic processes in aerobic organisms, like plants, generate reactive oxygen species (ROS) molecules as intermediate products of the reduction of ground state oxygen ( $O_2$ ) to water (Apel and Hirt 2004; Karuppanapandian et al. 2011). Oxygen is reduced for energy production and in the process, the following ROS are sequentially produced: Singlet oxygen ( $^1O_2$ ), superoxide radical ( $O_2^{\bullet-}$ ), hydroperoxy radical ( $HO_2^{\bullet}$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical ( $OH^{\bullet}$ ) (Apel and Hirt 2004; Karuppanapandian et al. 2011).

Plants continually produce ROS in structures such as chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum (ER), and plasma membranes (Karuppanapandian et al. 2011). Likewise, ROS molecules do not build up because they are continually removed nonenzymatically or enzymatically by a complex defensive system. Components of the defensive system have been classified according to their catalytic activity, molecular weight, compartment where they act, and level of defense or mechanism of action (Pradedova et al. 2011).

There are several biotic and abiotic factors that alter the equilibrium between production and removal of ROS. Insect attacks are among the most studied biotic stressors. At the penetration point, there is a local hypersensitive response and subsequent production of phytoalexins and other pathogenesis related proteins in preparation for programmed cell death (PCD). This process generates the production of ROS (De Gara et al. 2003). According to De Gara et al. (2003), excess ROS produces alterations in the “levels and/or redox state of ascorbate and/or glutathione (GSH), as well as in the activity of their redox enzymes.” Phosphorylated proteins have shown to be another response to biotic stress in plants. Huang et al. (2011) quantified changes on phosphoproteins in *Arabidopsis thaliana* leaves treated with compounds mimicking biotic stresses. They found and characterized 75 phosphoproteins very likely associated with biotic stressors. Tyagi et al. (2014) found that rice plants invaded by bacteria and fungi showed upregulation of OsSAP1 and OsSAP11. The functional role of OsSAP1 in plant defense responses has been explored through overexpression in transgenic plants (Tyagi et al. 2014). Additionally, the gene family GF14 of rice plants is up-regulated under pathogen attack; while in other plants, phytohormones like ethylene, salicylic acid, and jasmonic acid increased under biotic stress (Fraire-Velázquez 2011).

Abiotic stress is produced by a series of factors like extreme temperatures, chemical compounds, unbalances in water conditions, and excess of heavy metals. Mizoi et al. (2012) reviewed recent literature about plant stress responses under temperature and water conditions. According to the literature, plants have binding proteins that activate the expression of abiotic stress-response genes. Responses to water and temperature stresses are regulated by a large family of transcription factors named AP2/ERF that shares a well-conserved DNA-binding domain. Calcium-dependent protein kinases,  $Ca^{2+}$ , and ROS are also well-characterized signaling molecules upregulated under abiotic and biotic stresses (Fraire-Velázquez 2011). The genetic pathway includes several gene families upregulated

under abiotic stress. Hashimoto et al. (2004) reported that in rice, the gene RO-292 is upregulated under salt and drought stresses.

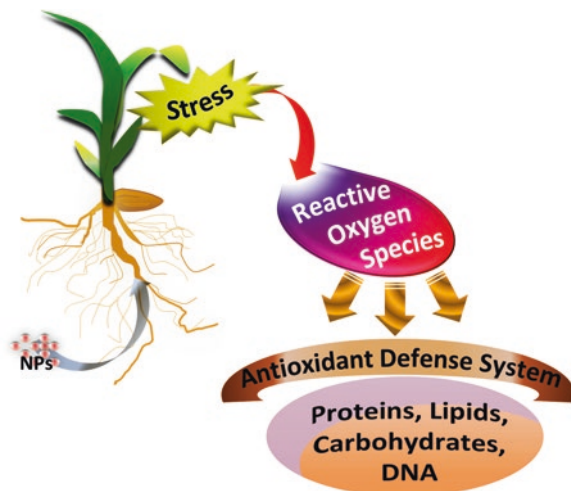
Heavy metals are well known abiotic plants' stressors. In a recent review, Ovecka and Takac (2014) highlighted the strategies used by plants to manage the stress imposed by heavy metals. These authors concluded that the response of plants to heavy metal stress is genotype-specific but "to some extent, modulated by environmental conditions." Several metal transporters have been found to be involved in maintaining heavy metal homeostasis in plant cells. Detailed information about families of transporters like ZIP, HMA, CDF, NRAMP, phytochelatins, and others, was analyzed by Ovecka and Takac (2014). Studies have also been aimed to study the effects of heavy metals on ROS production and proton pumps at vacuolar and plasma membrane levels. Kabała et al. (2008) reported that Cd, Cu, and Ni did not affect the proton pumps; however, these metals modified the structure and properties of plasma membrane fatty acids. Lipid peroxidation is another typical effect of oxidative stress imposed by heavy metals in plants. In barley (*Hordeum vulgare* L.),  $\mu\text{M}$  concentrations of Cu (10), Zn (4), Cr (4), Ni (4), Pb (0.1), and Cd (22) were found to induce lipid peroxidation (Juknys et al. 2012). Moreover, Zn and Cd reduced the dry biomass production at concentrations as low as 0.1 and 3  $\mu\text{M}$ , respectively (Juknys et al. 2012). Other types of stress produced by heavy metals include reduction in photosynthesis (Cu, Zn, Cd), changes in root ultrastructure and architecture (Al, Cd, Cu), and alteration in cellular ionome (Cd, Pb, U) (Viehweger 2014). Silver is another heavy metal that has shown to cause stress in plants, even at low concentration. Kaveh et al. (2013) reported that after 10 days of exposure to 5 mg  $\text{Ag}^+/\text{L}$ , there were 84 genes upregulated and 53 genes downregulated in *A. thaliana*. Some of the upregulated genes were linked to oxidative stress and some of the downregulated genes were linked with response to pathogens and hormonal stimuli.

## 1.2 Nanoparticles and Their Interaction with Plants

Nanoparticles (NPs), natural or manmade, are materials with at least two dimensions between 1 and 100 nm (ASTM 2012). Manmade NPs (engineered nanoparticles, ENPs) can be carbon-based or metal-based (Peralta-Videa et al. 2011). Carbon-based are of two main types, fullerenes and carbon nanotubes; while metal-based are grouped in metals, metal oxides, and quantum dots (Peralta-Videa et al. 2011). Among the most produced and used metal-based ENPs are zinc oxide ( $n\text{ZnO}$ ), titanium dioxide ( $n\text{TiO}_2$ ), gold ( $n\text{Au}$ ), silver ( $n\text{Ag}$ ), cerium oxide ( $n\text{CeO}_2$ ), and copper ( $n\text{Cu}$ ) NPs (Keller et al. 2013). Other NPs like  $n\text{Mn}$ ,  $n\text{Fe}_3\text{O}_4$ ,  $n\text{CuO}$ , and  $n\text{CoFe}_2\text{O}_4$  are also widely used.

Investigations have shown that both carbon-based and metal-based ENPs are able to produce stress, generating excess ROS with the potential to affect proteins, lipids, carbohydrates, and DNA in plants (Fig. 1.1). Carbon nanotubes, one of the carbon-based ENPs have been reported to induce ROS accumulation enhancing lipid peroxidation in cell culture (Liu et al. 2010) and seedlings' root tips (Liu et al. 2013). On the

**Fig. 1.1** Stress induced by engineered nanoparticles and the antioxidant defense system in plants



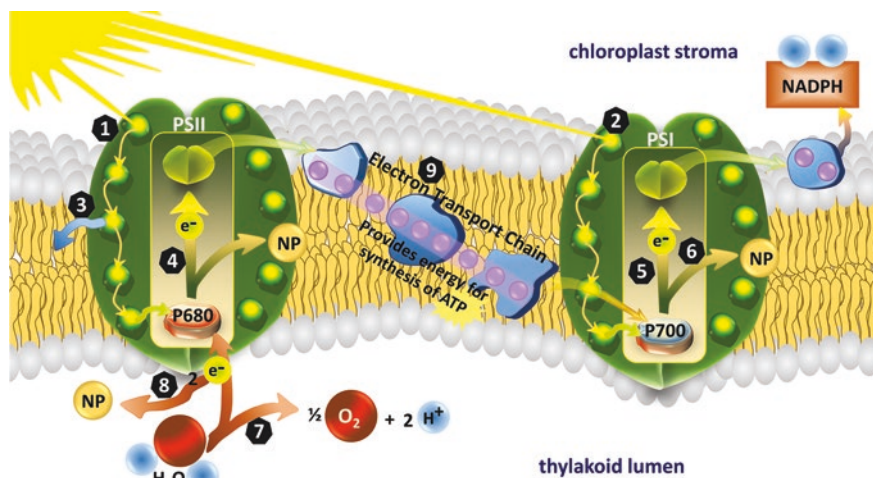
other hand, metal-based ENPs and/or the released ions from the NPs have been found to produce stress inducing ROS accumulation in plants. For instance, several reports indicate that *nAu* and *nAg* affect photosynthesis in different ways (Barrazzouk et al. 2005; Bujak et al. 2011; Olejnik et al. 2013).

Other NPs like *nCeO<sub>2</sub>*, *nFe<sub>3</sub>O<sub>4</sub>*, *nCoFe<sub>2</sub>O<sub>4</sub>*, and *nTiO<sub>2</sub>* have also shown to affect chlorophyll and ROS generation (Mingyu et al. 2007; Ursache-Oprisan et al. 2011; Rico et al. 2013b). Studies have shown that both rutile and anatase crystalline phases of *nTiO<sub>2</sub>* were found to generate ROS in spinach (Fenoglio et al. 2009). The stress imposed by *nZnO* and *nCuO* has been associated to the NPs and released Zn and Cu ions (Shi et al. 2011; Kumari et al. 2011; Lee et al. 2013; Nair and Chung 2014). On the other hand, contradictory results have been reported about ROS generation by *nCeO<sub>2</sub>* (Rico et al. 2013b; Gomez-Garay et al. 2014). In the following sections we discuss the stress imposed by ENPs/released ions on plants and their defensive mechanisms.

### ***1.2.1 Interaction of ENPs with the Photosynthetic Machinery***

Photosynthetic efficiency is a convenient parameter to detect stress induced by biotic and abiotic factors. Disturbance in the photosynthetic activity results in oxidative stress in plants. NPs alter the photosynthetic efficiency, photochemical fluorescence, and quantum yield in plants; thus, knowledge on the interactions of NPs with the photosynthetic machinery provides understanding on NP-induced oxidative stress and antioxidant defense system in plants. The current knowledge on the influence of NPs on plants photosystems is summarized in Fig. 1.2.





Legend	NPs	Chemistry/mechanism involved	Plants	References
1,4	TiO <sub>2</sub> -A	Increased light absorption and quantum yield in PSII	<i>Spinacea oleracea</i> , <i>Lycopersicon esculentum</i>	Mingyu et al. 2007, Yang et al. 2007, Lei et al. 2007ab, Qi et al. 2013
1,2,4,5	TiO <sub>2</sub> -A	Decreased light absorption	<i>Ulmus elongata</i>	Gao et al. 2013
1,2	Au	Increased light absorption due to plasmon resonance effect	<i>Glycine max</i>	Falco et al. 2011
1,2	CdSe/ZnS	Decreased light absorption	<i>Chlamydomonas sp.</i>	Lin et al. 2009
1,2	CeO <sub>2</sub>	Decreased light absorption and photochemical efficiency	<i>Medicago arborea</i>	Gomez-Garay et al. 2014
1,2,4,5	CuO	Decreased light absorption and quantum yield in PSII	<i>Lemna gibba</i>	Perreault et al. 2010
4	TiO <sub>2</sub> -R	No effect on quantum yield in PSII	<i>Vicia faba</i>	Foltete et al. 2011
3	CuO, TiO <sub>2</sub> -A	Enhanced non-photochemical fluorescence	<i>L. gibba</i> ; <i>Ulmus elongata</i>	Perreault et al. 2010, Gao et al. 2013
2,5	CdSe/ZnS	Enhanced light absorption and improved quantum yield in PSI	<i>C. reinhardtii</i>	Jung et al. 2010
4	Ag	Improved quantum yield in PSII	<i>Brassica juncea</i>	Sharma et al. 2012
6	Au	Decreased quantum yield due to chlorophyll to nanoparticles electron/energy transfer	<i>G. max</i>	Falco et al. 2011
7	Mn, TiO <sub>2</sub> -A	Enhanced splitting of water and evolution of oxygen	<i>S. oleracea</i> , <i>Vigna radiata</i>	Pradhan et al. 2013, Lei et al. 2007ab
9	Ag	Enhanced the production of secondary quinone electron acceptors in the electron transport chain (ETC), but inhibited electron transport	<i>Chlamydomonas reinhardtii</i>	Matorin et al. 2013
	Mn, TiO <sub>2</sub> -A	Improved the photophosphorylation activity in ETC	<i>S. oleracea</i> , <i>V. radiata</i>	Gao et al. 2013, Lei et al. 2007ab, Pradhan et al. 2013
	CuO, TiO <sub>2</sub> -A	Decreased ETC activity	<i>U. elongata</i> ; <i>C. reinhardtii</i>	Gao et al. 2013, Saison et al. 2009
	CeO <sub>2</sub>	Increased ETC activity	<i>M. arborea</i>	Gomez-Garay et al. 2014

Fig. 1.2 Influence of nanoparticles on the photosystems

### 1.2.1.1 Metal Nanoparticles

The implications of metal nanoparticles in the chemical energy production of a photosynthetic system have been explored. The experiment designed by Govorov and Carmeli (2007) where photosynthetic reaction center of a photosystem I (PSI) was bound to *n*Au and *n*Ag, revealed two competing effects affecting the photosystem efficiency: improved light absorption by chlorophyll molecule due to plasmon resonance effect of metal nanoparticles, and decreased quantum yield by photosystem due to enhanced chlorophyll to metal nanoparticles energy transfer. Similarly, electron transfer from excited fluorophore to *n*Au or *n*Ag has been

reported (Barrazzouk et al. 2005; Nieder et al. 2010; Beyer et al. 2011; Bujak et al. 2011; Matorin et al. 2013; Olejnik et al. 2013).

Falco et al. (2011) determined the effects of *nAu* on photosystem II (PSII) chlorophyll *a* fluorescence quenching in soybean leaves. The chlorophyll was extracted and after mixing with *nAu* of different sizes (5, 10, 20 nm) and different concentrations (0, 3.6, 7.2, 10.6, 14.0, 17.3  $\mu\text{M}$ ), the absorbance at 538 nm (the characteristic absorption band of *nAu* surfaces) and fluorescence spectra at typical PSII region (625–800 nm) were measured. Data revealed that absorbance and fluorescence quenching increased at increased *nAu* concentration. The absorbance increased primarily due to higher amount of *nAu* that absorbs light, whereas fluorescence quenching was enhanced due to more *nAu* available for electron transfer. On the other hand, the lowest absorbance was recorded at the highest *nAu* size while the highest fluorescence quenching was registered at the lowest *nAu* size. Low *nAu* size enhanced fluorescence suppression due to its higher surface area that could adsorb large amount of chlorophyll molecule which facilitates better the chlorophyll to metal nanoparticles electron transfer. Similarly, *nAu* (8 nm) increased fluorescence quenching in a chlorophyll solution which was attributed to the enhanced electron transfer from excited chlorophyll molecules to *nAu* (Barazzouk et al. 2005).

In the same study, Falco et al. (2011) investigated the chlorophyll fluorescence in soybean leaves in vivo. Soybean seeds were inoculated with *nAu* and allowed to germinate until the cotyledon, unifoliate, and trifoliate leaves appeared. Chlorophyll fluorescence was measured directly on the surface of each of cotyledon, unifoliate, and trifoliate leaves. Results showed a shifting of fluorescence band to the higher wavelength and a *nAu*-induced quenching of chlorophyll fluorescence. The fluorescence was also measured when the *nAu* was deposited directly either on the surface or bottom surface of the leaves, and the results showed a similar *nAu*-enhanced fluorescence quenching in leaves.

In a similar study, Matorin et al. (2013) examined the influence of *nAg* on the photosynthetic activity of green algae *Chlamydomonas reinhardtii*. They found that *nAg* had no direct effects on PSI, but inhibited the electron transfer in PSII, and enhanced the production of secondary quinone electron acceptors ( $\text{Q}_\text{B}$ ). These observations were opposite to those reported by Sharma et al. (2012a) wherein *nAg* improved the quantum efficiency of PSII in *Brassica juncea*.

The modulations in photochemistry of *Vigna radiata* exposed to *nMn* have been extensively investigated by Pradhan et al. (2013). The analysis of photoreduction activities in isolated chloroplasts revealed that *nMn* modulated the activity of PSII by enhancing the splitting of water and evolution of oxygen, and improving the photophosphorylation activity of electron transport chain (ETC). Related studies also showed the effects of quantum dots on the photosynthetic activity in *Chlamydomonas* sp. Lin et al. (2009) exposed *Chlamydomonas* sp. to CdSe/ZnS quantum dots and found that QDs decreased light absorption that reduced the photosynthetic activity in the algae. In contrast, Jung et al. (2010) examined the photosynthetic process in PSI purified from *C. reinhardtii* and integrated with CdSe/ZnS QDs. They found that QDs could absorb light and transfer energy efficiently to PSI.

### 1.2.1.2 Metal Oxide Nanoparticles

Chlorophyll *a*, the major photosynthetic pigment in plants, is more sensitive to photodegradation than other pigments, and could be a more useful indicator of NPs toxicity compared with growth characters. For example, *nCeO<sub>2</sub>* did not induce apparent signs of toxicity but severely decreased the chlorophyll content in rice (*Oryza sativa*) (Rico et al. 2013a). *nFe<sub>3</sub>O<sub>4</sub>* and *nCoFe<sub>2</sub>O<sub>4</sub>* also showed no toxic effects on sunflower seedlings, but the chlorophyll content decreased, relative to the control, by 50 % in *nFe<sub>3</sub>O<sub>4</sub>* and 28 % in *nCoFe<sub>2</sub>O<sub>4</sub>* treatments (Ursache-Oprisan et al. 2011). A related study also showed that *Scenedesmus obliquus* exposed to 50 mg/L *nSiO<sub>2</sub>* exhibited a marked reduction in chlorophyll *a*, despite its normal growth (Wei et al. 2010). In contrast, superparamagnetic iron oxides nanoparticles (SPIONs) improved chlorophyll levels without trace of toxicity in soybean (Ghafariyan et al. 2013).

Chlorophyll *a/b* ratio is considered a global indicator of photosynthetic activity. It is an indicator of plants response to light and N availability (Hikosaka and Terashima 1996). Ursache-Oprisan et al. (2011) found that *nFe<sub>3</sub>O<sub>4</sub>* and *nCoFe<sub>2</sub>O<sub>4</sub>* (20–100 µL/L) did not affect the germination rate nor caused toxic effects in sunflower; however, chlorophyll ratio in both *nFe<sub>3</sub>O<sub>4</sub>* and *nCoFe<sub>2</sub>O<sub>4</sub>* decreased significantly, compared to the control. On the contrary, Rico et al. (2013a) found that *nCeO<sub>2</sub>* increased the chlorophyll ratio, despite decreased chlorophyll content in rice, whereas Ghafariyan et al. (2013) did not find changes in chlorophyll *a/b* ratio in soybean exposed to SPIONs, indicating that the photosynthetic efficiency was not affected. However, these studies did not clarify the mechanisms causing the changes in chlorophyll *a/b* ratio.

The chlorophyll *a* fluorescence in *Lemna gibba* exposed to 0.1–0.4 g/L *nCuO* has been investigated (Perreault et al. 2010). The study showed that *nCuO* markedly decreased the quantum yield which inhibited the photosynthetic processes causing retardation in plant growth. *nCuO* also strongly suppressed the photochemical fluorescence quenching and greatly enhanced the non-photochemical fluorescence quenching, indicating major modifications in PSII photochemistry. Overall, the findings illustrated that *nCuO* decreased conversion of absorbed light energy via PSII electron transport. Similarly, Lalau et al. (2014) reported that *nCuO* caused disruption of mitochondria, dilation of chloroplast membrane, distortion of stroma and grana of the chloroplasts, and alteration of photosynthetic pigments in *Landoltia punctata*. *nCuO* coated with polyacrylic acid also severely damaged the PSII electron transport system in the unicellular algae *C. reinhardtii* (Saison et al. 2009). Here, the toxicity of *nCuO* was attributed to its dissolution and release of copper ions.

Nano-anatase (*nTiO<sub>2</sub>-A*) generally improved the photosynthetic activity in plants due to its large specific surface area, high thermal conductivity, and high photocatalytic ability (Mingyu et al. 2007; Lei et al. 2007a, b; Yang et al. 2007). Studies have shown that *nTiO<sub>2</sub>-A* (5 µM) treatment of spinach (*Spinacia oleracea*) chloroplast resulted in enhanced light absorption in chlorophyll *a*, fluorescence quantum yield in PSII, electron transfer activities, and oxygen evolution rate (Mingyu et al. 2007;

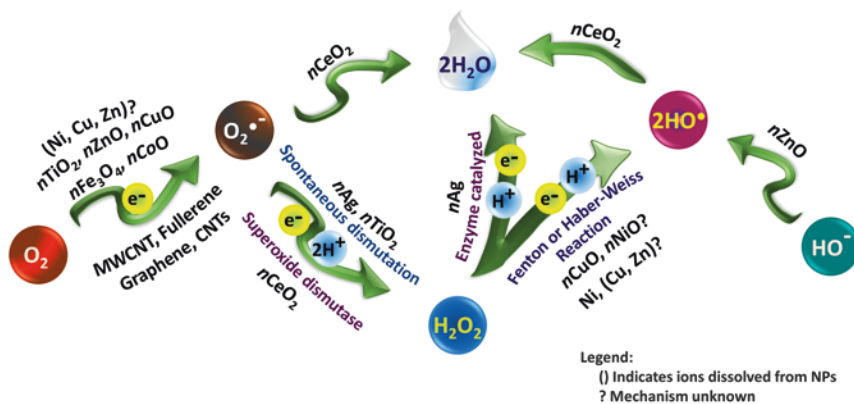
Yang et al. 2007). Spinach and tomato exposed to  $n\text{TiO}_2\text{-A}$  exhibited superior efficiency in the absorption, transfer, and conversion of light in PSII (Lei et al. 2007a, b; Qi et al. 2013). Lei et al. (2007b) hypothesized that the photogenerated electron holes in  $n\text{TiO}_2$ ,  $h^+$ , capture electrons from water which accelerated water photolysis and oxygen evolution in PSII. On the contrary, foliar application of 0.1–0.4 %  $n\text{TiO}_2\text{-A}$  in *Ulmus elongata* exposed to light intensity of 800 and 1,600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  resulted in lower PSII quantum yield, chlorophyll fluorescence, photochemical quenching, and electron transfer rate, but higher non-photochemical quenching and water loss, relative to the control (Gao et al. 2013). The marked reduction in photosynthetic activity was due to increased water loss caused by decreased mesophyll activity. These researchers also believed that  $n\text{TiO}_2\text{-A}$  reduced electron transfer rate by blocking the electron transfer from  $Q_A$  to  $Q_B$ . In a related study, altered  $n\text{TiO}_2$  (nano-rutile coated with aluminum hydroxide and dimethicone films) at 5–50 ppm concentrations did not change the PSII maximum quantum yield in *Vicia faba*, even after 48-h exposure (Foltete et al. 2011).

Fluorescence emission analysis of 8-month old *Medicago arborea* exposed to  $n\text{CeO}_2$  (100–400 mg/L) has been performed (Gomez-Garay et al. 2014). The study revealed that, relative to control, the 100 and 200 mg/L  $n\text{CeO}_2$  treatments reduced the photochemical efficiency. At 200 mg/L,  $n\text{CeO}_2$  increased the fluorescence levels of fully oxidized and completely reduced plastoquinone electron acceptor pool ( $Q_A$ ), indicating that the PSII was damaged and the electron transport system was impaired. These results are in stark contrast to those observed when isolated chloroplasts were incubated with  $n\text{CeO}_2$  wherein the ROS scavenging ability of  $n\text{CeO}_2$  protected the chloroplasts from ROS damages and improved its photosynthetic activity (Boghossian et al. 2013; Giraldo et al. 2014).

### 1.2.2 Reactive Oxygen Species (ROS) and Oxidative Damage

Reactive oxygen species generation is a toxicological mechanisms of heavy metals (Sharma et al. 2012b) and NPs (Begum et al. 2011; Rico et al. 2013a) in plants. Under various biotic and abiotic stresses, the amount of ROS could increase and result in oxidative damage and cell death in plants. The effects of NPs on the oxidative stress in plants have been widely investigated using techniques that measure either just  $\text{H}_2\text{O}_2$  or ROS in general. Oxidative damage, also referred to as cell death, is commonly measured by lipid peroxidation (thiobarbituric acid reactive species, TBARS), electrolyte leakage (conductivity test,  $\text{K}^+$  leakage), and propidium iodide fluorescence assay.

Literature review would show that the effects of NPs on ROS generation and oxidative damage in plants have been widely investigated. ROS generation and oxidative damage are believed to cause toxicity in NP-treated plants; however, there is still a great lack of understanding on how the chemical properties of NPs induce ROS production and membrane damage in plants. The available reports on the mechanism of NPs on ROS generation or scavenging are summarized in Fig. 1.3.



NPs	Chemistry/mechanism involved	References
CeO <sub>2</sub>	Alternates between Ce <sup>4+</sup> and Ce <sup>3+</sup> to scavenge O <sub>2</sub> <sup>•-</sup> and •OH, and mimics the superoxide dismutase activity	Bhogossian et al. 2013, Heckert et al. 2008, Horie et al. 2011, Xia et al. 2008
TiO <sub>2</sub>	Produces free radicals (O <sub>2</sub> <sup>•-</sup> , HO• and CO <sub>2</sub> <sup>•-</sup> ) in light or dark conditions; Ti <sup>4+</sup> /Ti <sup>3+</sup> oxidize/reduce O <sub>2</sub> <sup>-</sup> /O <sub>2</sub> <sup>•-</sup> to O <sub>2</sub> /H <sub>2</sub> O <sub>2</sub>	Fenoglio et al. 2009, Lei et al. 2008
ZnO	Traps electron from •OH and produce HO•	Li et al. 2008, McLaren et al. 2009
NiO	Probably produces HO• via Haber-Weiss reaction similar to Ni ions. However, the reaction is not confirmed.	Faisal et al. 2013
CuO	Produces HO• via Fenton reaction.	Fubini et al. 2007
Ag	Improves redox reactions by acting as electron relay center	Mallick et al. 2010
Fe <sub>3</sub> O <sub>4</sub> , CoO	Block aquaporins and disturb respiration	Wang et al. 2011, Ghodake et al. 2011
Fullerene, CNTs, Graphene	Not clear, probably due to aggregating on root surface and blocking aquaporins	Begum et al. 2011, Tan et al. 2009, Liu et al. 2010, Liu et al. 2013

Fig. 1.3 Proposed mechanisms on how nanoparticles generate/scavenge reactive oxygen species

### 1.2.2.1 Metal Oxide Nanoparticles

The ROS scavenging ability of *n*CeO<sub>2</sub> has been widely investigated. *n*CeO<sub>2</sub> possess vacant oxygen sites on the surface lattice giving them the ability to alternate between the Ce<sup>4+</sup> and Ce<sup>3+</sup> oxidation states and scavenge O<sub>2</sub><sup>•-</sup> and HO• in the process (Boghossian et al. 2013). The ROS generation and oxidative damage in rice seedlings germinated in *n*CeO<sub>2</sub> (62.5, 125, 250, and 500 mg/L) for 10 days were studied (Rico et al. 2013b). Results revealed that, relative to the control, *n*CeO<sub>2</sub> decreased the H<sub>2</sub>O<sub>2</sub> concentration at 62.5 mg/L probably due to the radical scavenging ability of *n*CeO<sub>2</sub> (Heckert et al. 2008; Horie et al. 2011; Xia et al. 2008). The H<sub>2</sub>O<sub>2</sub> content increased steadily from 125 to 500 mg/L treatments that was attributed to increased *n*CeO<sub>2</sub> SOD mimetic activity at increased

$n\text{CeO}_2$  concentration (Rico et al. 2013b). Gomez-Garay et al. (2014) also found that low  $n\text{CeO}_2$  concentrations (100 and 200 mg/L) suppressed ROS production and enhanced cellular resistance to oxidative stress in *M. arborea*. Related studies on *A. thaliana* germinated and grown in  $n\text{CeO}_2$  and  $n\text{In}_2\text{O}_3$  (0–1,000 ppm) for 25 days revealed that only 1,000 ppm  $n\text{CeO}_2$  induced lipid peroxidation by 2.5-fold increase relative to the control, while  $n\text{In}_2\text{O}_3$  did not cause lipid peroxidation at all (Ma et al. 2010). It is possible that the increase in ROS generation, as indicated by elevated anthocyanin content, caused the lipid peroxidation in *A. thaliana* at 1,000 ppm  $n\text{CeO}_2$ .

Fenoglio et al. (2009) evaluated the ability of rutile or anatase  $n\text{TiO}_2$  to produce free radicals ( $\text{O}_2^{\bullet-}$ ,  $\text{HO}^\bullet$ ,  $\text{CO}_2^{\bullet-}$ ) and found that both polymorphs generated radicals in light and dark conditions. These researchers also reported that the ability of  $n\text{TiO}_2$  to generate ROS was independent of its size. The impact of  $n\text{TiO}_2$ -A on oxidative stress and damage in spinach chloroplasts under UV-B radiation ( $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was investigated by Lei et al. (2008). The spinach seeds were pre-treated with 0.25 %  $n\text{TiO}_2$ -A (4 h, 10 °C), washed with distilled water and planted in the soil. At four leaf stage, the spinach was sprayed once with  $n\text{TiO}_2$ -A and the chloroplasts were extracted. The chloroplasts were illuminated with UV-B light and the oxidative stress and damage were measured. Results showed that  $n\text{TiO}_2$ -A treatment significantly decreased the accumulation of  $\text{O}_2^{\bullet-}$  and  $\text{H}_2\text{O}_2$ , which resulted in marked reduction of lipid peroxidation, in spinach chloroplasts under UV-B irradiation. This was attributed to the ability of  $\text{Ti}^{4+}/\text{Ti}^{3+}$  to oxidize/reduce  $\text{O}_2^-/\text{O}_2^{\bullet-}$  to  $\text{O}_2/\text{H}_2\text{O}_2$ .  $n\text{TiO}_2$ -A (2, 5, 10 ppm) also caused reduction in electrolyte leakage in the leaves of both cold stress sensitive (ILC 533) and tolerant (Sel 11,439) chickpea (*Cicer arietinum* L.) genotypes under cold stress (4 °C), but the reduction in electrolyte leakage was vaguely attributed to the “increased tolerance mechanisms” induced by  $n\text{TiO}_2$ -A in chickpea (Mohammadi et al. 2013). On the other hand, duckweed (*Lemna minor*) exposed to  $n\text{TiO}_2$ -A (10–2,000 ppm) suspensions for 7 days, did not show lipid peroxidation at  $\leq 200$  ppm treatments but exhibited significant membrane damage at  $\geq 500$  ppm (Song et al. 2012). However, there was no mechanism given on how the  $n\text{TiO}_2$ -A modified the lipid peroxidation in duckweed. Similarly, altered  $n\text{TiO}_2$ -R (5–50 ppm) did not cause lipid peroxidation in *V. faba* even after 48-h exposure (Foltete et al. 2011). The lack of lipid peroxidation was probably due to biologically inert forms of  $n\text{TiO}_2$ -R internalized in the roots.

Nanoparticulate ZnO also possesses photocatalytic activity making it able to generate free radicals (Xia et al. 2008). Studies revealed that the photocatalytic activity and ROS generation by  $n\text{ZnO}$  are related to its morphology: greater exposure of polar faces leads to higher surface oxygen vacancy that could trap electrons and produce free radicals like  $\text{HO}^\bullet$  (Li et al. 2008; McLaren et al. 2009). Phytotoxicity studies on  $n\text{ZnO}$  are inconclusive on whether the NPs or NP-released ions are contributing to the observed toxic responses. For example, ROS production in *Allium cepa* exposed to  $n\text{ZnO}$  were attributed to both NPs and NP-released Zn ions (Kumari et al. 2011). In contrast, oxidative stress in *A. cepa* and buckwheat (*Fagopyrum esculentum*) was attributed to  $n\text{ZnO}$  (Ghodake et al. 2011;

Lee et al. 2013), while that in green algae *Pseudokirchneriella subcapitata* was attributed to dissolved free zinc ions (Lee and An 2013).

Reactive oxygen species generation in tomato roots treated with *n*NiO (0.25, 0.5, 1.0, 1.5, and 2.0 mg/mL) has been investigated by Faisal et al. (2013). The researchers found that *n*NiO caused ROS generation in tomato roots with a very sharp increase observed at higher *n*NiO concentrations (1.0, 1.5 and 2.0 mg/mL). They also found high levels of ROS in the protoplasts extracted from tomato roots. As a consequence, lipid peroxidation in the treated tomato roots was greatly elevated by 39.3–49.5 %, relative to the control. However, it is not clear if ROS generation was induced by *n*NiO or Ni ions since both Ni species were detected in the tomato root cells. It has already been established that Ni ions generates HO<sup>•</sup> radical in plant cells through the Haber-Weiss cycle; however there is no clear mechanism on how *n*NiO induces ROS production in plants. Similarly, studies on *n*CuO are inconclusive on whether ROS generation and oxidative damage in plants was due to NPs or NP-released Cu ions (Shi et al. 2011; Lee et al. 2013; Nair and Chung 2014). For example, an experiment on *n*CuO with appropriate soluble copper control revealed that increased lipid peroxidation in plants could apparently be attributed to *n*CuO because of its limited dissolution in growth media (Shi et al. 2011; Lee et al. 2013). However, some researchers believe that *n*CuO gets dissolved inside the plant releasing Cu ions that may undergo redox reactions between Cu<sup>2+</sup> and Cu<sup>+</sup> and cause oxidative damage (Hoshino et al. 1999; Shi et al. 2011). However, some studies also showed that phytotoxicity could be induced by both *n*CuO and NP-released Cu ions (Dimpka et al. 2013).

*Elodea densa* exposed to *n*CuO (0.025, 0.25, 0.5, 1.0, and 5.0 mg/L) for 3 days also manifested significantly higher lipid peroxidation in the leaves at 0.25, 1.0, and 5.0 mg/L concentrations compared to the control (Nekrasova et al. 2011). The enhanced lipid peroxidation was attributed to the involvement of *n*CuO as polyvalent metals in ROS generation via the Fenton reaction (Fubini et al. 2007). The membrane damage, as measured by K<sup>+</sup> leakage assays, in maize exposed to *n*CuO (10 and 100 mg/L) for 15 days in a hydroponic setup has also been reported (Wang et al. 2012). Results demonstrated that *n*CuO compromised the membrane integrity in roots than the shoots in both 10 and 100 mg/L concentrations, relative to the control, which was obviously due to the direct exposure of roots to the *n*CuO solution. It is interesting to note that the membrane damage at 100 mg/L *n*CuO was concomitant with the significant reduction in water content. The researchers hypothesized that water deficit due to blocking of water channels by *n*CuO lead to the inhibition in respiration rate that resulted in ROS generation and oxidative damage (Wang et al. 2012). *n*Fe<sub>3</sub>O<sub>4</sub> (30 and 100 mg/L) also greatly enhanced the degree of lipid peroxidation in the roots, but not in the shoots, of ryegrass and pumpkin (Wang et al. 2011). The increase in lipid peroxidation was also attributed to *n*Fe<sub>3</sub>O<sub>4</sub> blocking the aquaporins and disturbance in the respiration rate in the root. Similarly, the massive deposition of cobalt (II, III) oxide nanoparticles on root surface caused the oxidative damage in *A. cepa* (Ghodake et al. 2011).

### 1.2.2.2 Other Nanoparticles

Sharma et al. (2012a) determined the effect of 7-day treatment of *nAg* (25–400 ppm) on the  $H_2O_2$  generation and lipid peroxidation in *B. juncea*. Interestingly enough, they found a significant reduction in  $H_2O_2$  accumulation and lipid peroxidation at 25 and 50 ppm *nAg*-treated plants. These researchers hypothesized that *nAg* increased the efficiency of redox reactions, based on the ability of *nAg* to act as electron relay center that improves the efficiency of catalytic activity in redox reactions (Mallick et al. 2006).

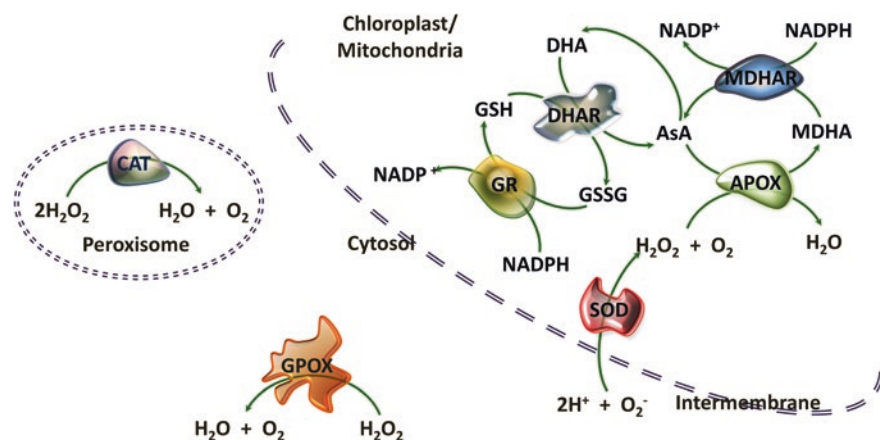
Begum et al. (2011) performed toxicity study in cabbage, tomato, and red spinach exposed to graphene (500, 1000, and 2000 mg/L) for 20 days. Results showed a graphene concentration-dependent increase in  $H_2O_2$  production, cell death and electrolyte leakage in graphene-treated leaves. The negative impact of graphene was attributed to its aggregation on root surface. Studies in rice suspension cells exposed to 20 mg/L sonicated multi-walled carbon nanotubes (S-MWCNTs) revealed a time-dependent increase in ROS content, which reached up to 3.5 times higher than the untreated, and decreased cell viability at increased S-MWCNTs concentrations (20–80 mg/L) (Tan et al. 2009). An increased ROS generation and enhanced degree of membrane damage in tobacco BY-2 cells incubated in 0.01 mg/mL water-soluble carboxyfullerenes for 3 days has also been observed (Liu et al. 2010). Similarly, ROS accumulation and lipid peroxidation were attributed to the association of CNTs with the cell walls of both rice and tobacco BY-2 suspension cells. On the contrary, a significant reduction in ROS concentration and absence of lipid peroxidation in root tips of *A. thaliana* seedlings germinated in agar treated with 0.01 mg/mL water-soluble fullerene malonic acid derivative (FMAD) were reported (Liu et al. 2013). Here, neither cell wall nor membrane damage was observed, which led the researchers to conclude that auxin disruption, abnormal cell division, and microtubule disorganization resulted in reduced mitochondrial activity and lower ROS generation. These findings are in agreement with those reported by Boghossian et al. (2013); they found that fullerenol and SWCNT had no ROS scavenging ability.

### 1.2.3 Antioxidative Defense System

Figure 1.4 displays the different enzymes and low molecular weight antioxidants that comprise the antioxidative defense system in plants. The enzymes include catalase (CAT), guaiacol peroxidase (GPOX), ascorbate peroxidase (APOX), superoxide dismutase (SOD), glutathione reductase (GR), and dehydroascorbate reductase (DHAR). Thiols (GSSG or GSH) and ascorbate are the common low molecular weight antioxidants. As shown in the figure, CAT and GPOX quench both ROS and peroxy radicals while SOD catalyzes the dismutation of  $O_2^-$  to  $H_2O_2$ .

The APOX, DHAR, and GR are involved in a network of redox reactions in the Halliwell-Asada pathway (ascorbate-glutathione cycle) that control ROS. The





Enzymes	NPs with enzyme-like activity
Catalase	CeO <sub>2</sub> , Fe <sub>3</sub> O <sub>4</sub> , Co <sub>3</sub> O <sub>4</sub>
Peroxidase	CeO <sub>2</sub> , Fe <sub>3</sub> O <sub>4</sub> , Co <sub>3</sub> O <sub>4</sub> , CuO, MnO <sub>2</sub> , Au
Superoxide dismutase	CeO <sub>2</sub> , Pt, Fullerene

**Fig. 1.4** *Upper part* The antioxidative enzyme defense system in plants. Reprinted with permission from Rico et al. (2013a). Copyright 2013 American Chemical Society. *Bottom part* Nanoparticles mimicking the activity of natural enzymes (Wei and Wang 2013)

APOX directly reduces the H<sub>2</sub>O<sub>2</sub> generated by SOD into H<sub>2</sub>O. The DHAR regenerates ascorbate that is utilized by APOX for the reduction of H<sub>2</sub>O<sub>2</sub>. The GR generates reduced glutathione that is utilized by DHAR to regenerate ascorbate. Wei and Wang (2013) reviewed the antioxidant ability of nanoparticles, and their mechanisms, that mimic the activity of natural enzymes. They found that various nanoparticles exhibit enzyme-like activities: *n*CeO<sub>2</sub>, *n*Fe<sub>3</sub>O<sub>4</sub>, *n*Co<sub>3</sub>O<sub>4</sub> mimic catalase; *n*CeO<sub>2</sub>, *n*Fe<sub>3</sub>O<sub>4</sub>, *n*Co<sub>3</sub>O<sub>4</sub>, *n*MnO<sub>2</sub>, *n*CuO, and *n*Au exhibit peroxidase activity; *n*CeO<sub>2</sub>, *n*Pt, and fullerene demonstrate superoxide dismutase property. Unfortunately it is difficult, if not impossible, to detect these mimetic activities in experiments using NPs exposure to whole individual plant. Despite the numerous nanophytotoxicity studies showing the disturbances in enzyme activities in plants exposed to NPs, there is no evidence that could correlate the former to the chemical properties of NPs. There is no way to ascertain that the observed changes in enzyme activities were due to the enzyme interactions with the NPs. In fact, studies showed irregular and unpredictable effects of NPs on enzyme activities. For example, *n*TiO<sub>2</sub>-A enhanced the activities of SOD, CAT, APOX, and GPOX

in spinach (Lei et al. 2008) and GPOX, SOD, CAT in *L. minor* (Song et al. 2012), but decreased the GR and APOX activities in *V. faba* (Foltete et al. 2011). This makes it difficult to conclude which NPs affect which enzymes. Earlier reviews have shown that the type, concentration, properties, and exposure media of NPs are the important factors affecting the toxicity responses, including oxidative stress and antioxidative defense system in plants. Further, it is generally assumed that the alterations in enzyme activities in exposed plants are responses to modulations in ROS concentration (Fig. 1.1). Thus, the role of NPs chemical attributes on the modulation of antioxidant defense system in plants is not clear, and a topic needed to be explored.

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# Chapter 2

## Role of Nanoparticles in Plants

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**Abstract** Nanotechnology opens a large scope of novel application in the fields of biotechnology and agricultural industries, because nanoparticles (NPs) have unique physicochemical properties, i.e., high surface area, high reactivity, tunable pore size, and particle morphology. Nanoparticles can serve as “magic bullets”, containing herbicides, nano-pesticide fertilizers, or genes, which target specific cellular organelles in plant to release their content. Despite the plenty of information available on the toxicity of nanoparticles to plant system, few studies have been conducted on mechanisms, by which nanoparticles exert their effect on plant growth and development. Therefore, the present review highlights the key role of nanoparticles in plants. Moreover, nanoscience contributes new ideas leading us to understand the suitable mode of action of nanoparticles in plants. The appropriate elucidation of physiological, biochemical, and molecular mechanism of nanoparticles in plant leads to better plant growth and development.

**Keywords** Plant nutrition · Plant growth and development · Nanoparticles · Photosynthesis

### 2.1 Introduction

Nanotechnology, a new emerging and fascinating field of science, permits advanced research in many areas, and nanotechnological discoveries could open up novel applications in the field of biotechnology and agriculture. In the field of electronics, energy, medicine, and life sciences, nanotechnology offers an expanding research, such as reproductive science and technology, conversion of agricultural and food

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