Chapter 6
Effect of Nanoparticles on Plants with Regard to Physiological Attributes

M. Sheikh Mohamed and D. Sakthi Kumar

Abstract The growth parameters of plants are influenced by various biotic and abiotic factors. The increased interference of humans with the environment has led to heightened concern over such activities on the living systems, including plants. With tremendous progress being made in the field of engineering, manufacturing, construction, etc., onus has shifted to the possible effects of such developments on the ecosystem. Nanotechnology has emerged as an indispensable tool for the future, with its reach spanning across diverse domains. Such a rapid advance has resulted in the exodus of various types of nanomaterials into the environment. Thus, it becomes essential to understand the imminent effects, either advantageous or deleterious, of these nanomaterials on the living subjects advertently or inadvertently exposed to them. Numerous studies have focused on the effects of such nanomaterials in the nanoparticulate form on the mammalian system, with increased studies on the plant system as well. Due to the complex nature of uptake and translocation mechanism present in plants, it has been relatively difficult to unanimously devise a general dataset of the effects that nanoparticles (NPs) have on them. Research over the past years has documented mostly toxic effects of the NPs, either during the germination stage or with respect to the shoot–root length, while few others have explored the possibilities of utilizing them as carriers for chemicals as herbicides, pesticides, fertilizers, or in some cases genes. There have been numerous contradictory findings with some reports suggesting growth enhancing effects and others observing retarding effects of similar NPs on similar or different plant species. Such contradictions and lack of conclusive observations has slowed down the impact of nanotechnology in the agriculture industry when compared with the medical scene. This scenario demands a comprehensive calibration of the analysis and interpretation of NP–plant interaction and effects thereof from the

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physiological, biochemical, and photosynthetic level to the molecular level to decisively devise a verdict on the actual effects of nanoparticles on the plant system. This chapter summarizes the research conducted so far in this field and attempts at providing an outlook for the future.

Keywords Nanotechnology · Nanoparticles · Plants · Physiological · Biochemical · Photosynthesis · Toxicity

6.1 Introduction

Nanotechnology has established itself as one of the fastest and biggest research and development fields in recorded history. Although previous technological revolutions as space exploration, semiconductors, and biotechnology have made it big, they were and still are, severely confined to their respective domains and find fewer interest from other disciplines. Nanotechnology, on the other hand, though started off like the aforementioned fields of study and research, limited mostly to the electronics industry, has expanded its horizons of application by the amalgamation of nearly all subjects of science viz., biology, chemistry, physics, etc. The rapid strides of nanotechnology in electronics and manufacturing have recently been paralleled by advances in medical nanotechnology, with an ever-increasing list of scientific publications, patents, and products being commercially released.

Although, this introduction of nanotechnology is highly impressive, like many of its predecessor technologies, it also has been very slow to exert its influence on the agricultural domain. The main reasons include limited research and knowledge base available on the impact of nanomaterials (NMs) on the plant system. Still, researchers world over have tried to elucidate the effects of this technology in plants, both in vitro and in nature. Most of the works have focused on the deleterious effects while a few have shown promising applications in boosting the native functions as growth, yield, and biomass enhancement, improving the photosynthesis conversion efficiency, and as carriers for chemicals as herbicides, pesticides, fertilizers, or genes (Galbraith 2007; Torney et al. 2007; DeRosa et al. 2010; Nair et al. 2010; Lahiani et al. 2013; Kole et al. 2013; Cossins 2014; Giraldo et al. 2014; Siddiqui and Al-Whaibi 2014). Despite all the information available on the toxicity of nanoparticles (NPs) to plant system, the appropriate elucidation of physiological, biochemical, and molecular mechanisms is crucial for the future of this technology in wide-scale agricultural implications (Fig. 6.1).

In this chapter, we discuss on the positive as well as negative impacts of NPs on the plant system, with respect to physiological parameters.

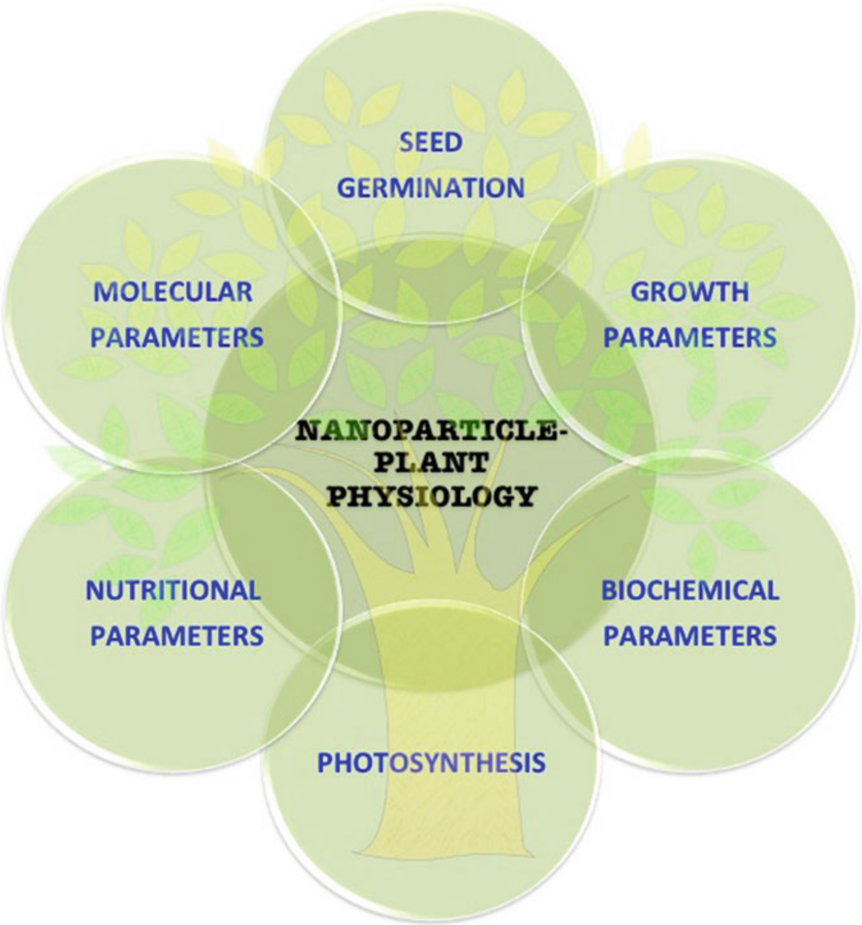


Fig. 6.1 Major parameters to be assessed while investigating the effects of nanoparticles on plant system

6.2 Effects of Nanoparticles on Germination, Growth, and Development

The interaction and subsequent effects of NPs on any biological system, including plants, depend primarily on the inherent physicochemical properties of the NPs, such as size, shape, charge, chemical composition, surface modifications, and reactivity (Ma et al. 2010; Khodakovskaya et al. 2012). Also, the NP–plant relation crucially depends on their concentration and varies from plant to plant. The primary focus of this chapter will be on the recorded role of NPs in seed germination, plant growth and biomass yield, biochemical parameters, and photosynthesis based on the type of NPs administered.

6.2.1 Germination

Seed germination is the most important event of a plant life. The first point of contact between the plant system and NPs has predominantly been through the seeds. The appearance of radicle and plumule mark the initiation of seed germination and seedling growth. Seed germination rate forms the prime dataset for the initial assessment of the effects of various nanomaterials on the subsequent developmental stages of plants. Numerous studies have focused on this aspect with both negative and positive observations. Some examples are provided in Table 6.1 and Figure 6.2.

6.2.1.1 Oxide Nanoparticles

It was reported that silica NPs (SiO_2 NPs) at relatively lower concentrations improved seed germination in tomato (Siddiqui and Al-Whaibi 2014). Suriyaprabha et al. (2012), observed better nutrient availability to maize seeds along with optimal pH and conductivity of the growth medium on supplementation with SiO_2 NPs, which in turn had positive effects on the germination rate. Plants are cosmopolitan in their choice of habitat and have been found to grow even under various biotic and abiotic stresses, which have a significant impact on their physiological features. In this regard, Haghighi et al. (2012), in tomato (*Solanum lycopersicum*), and Siddiqui et al. (2014), in zucchini (*Cucurbita pepo*), found that SiO_2 NPs enhanced seed germination under abiotic (NaCl) stress. Shah and Belozerova (2009) assessed the effects of a range of NPs including silica on lettuce (*Lactuca sativa*) and found that all the NPs had a significant influence on germination. Lu et al. (2002) and Zheng et al. (2005) observed that SiO_2 and titanium dioxide NPs (TiO_2 NPs) positively impact seed germination in soybean (*Glycine max*) and spinach (*Spinacea oleracea*) by increasing nitrate reductase and enhancing the uptake and utilization of water and nutrients.

Beneficial effects of lower concentrations of zinc oxide NPs (ZnO NPs) on seed germination have been observed in a variety of plant species as peanut (*Arachis hypogea*) (Prasad et al. 2012), soybean (Sedghi et al. 2013), wheat (*Triticum aestivum*) (Ramesh et al. 2014), and onion (*Allium cepa*) (Raskar and Laware 2014). ZnO NPs showed differential effects when tested on cucumber (*Cucumis sativus*), alfalfa (*Medicago sativa*), and tomato, with only the former exhibiting enhancement in germination (De la Rosa et al. 2013). The determination of phytotoxicity of metallic NPs and their oxides is relatively complex, primarily due to the potential dissolution of ions released from the NPs and their associated toxicity (Ma et al. 2010). Germination and root growth of zucchini seeds in hydroponic culture augmented with ZnO NPs presented no negative effects (Stampoulis et al. 2009) whereas in the case of rye grass (*Lolium perenne*) and maize (*Zea mays*), the germination was significantly inhibited by nano-Zn (35 nm) and zinc oxide (15–25 nm), respectively (Lin and Xing 2007).

Table 6.1 Effects of different nanoparticles on the germination of seeds [positive effect (+), negative effect (−), no effect (N)]

Nanoparticle	Plant	Effect on germination	Reference
Graphene oxide	Fava bean	+	Anjum et al. (2014)
CNTs	Tomato	+	Morla et al. (2011)
MWCNTs	Barley, soybean maize	+	Lahiani et al. (2013)
ZnO	Peanut	+	Prasad et al. (2012)
Au	Arabidopsis	+	Kumar et al. (2013)
Ag	*Boswellia ovalifoliolata*	+	Savithramma et al. (2012)
TiO_2	Fennel	+	Feizi et al. (2013b)
Se	Tobacco	+	Domokos-Szabolcsy et al. (2012)
TiO_2	Garden sage	+	Feizi et al. (2013a)
SiO_2	Maize	+	Suriyaprabha et al. (2012)
Au	Glory lily	+	Gopinath et al. (2014)
SiO_2	Tomato	+	Siddiqui et al. (2014)
CNTs	Onion, Indian mustard mungbean	+	Ghodake et al. (2010), Mondal et al. (2011)
CNTs	Rice	+	Nair et al. (2010)
TiO_2	Spinach	+	Zheng et al. (2005)
TiO_2	Wheat	+	Feizi et al. (2012)
Si, Pd, Au, Cu	Lettuce	+	Shah and Belozerova (2009)
SiO_2 and TiO_2	Soybean	+	Lu et al. (2002)
Zero-valent Fe	Flax, barley, rye	−	El-Temsah and Joner (2010)
Ag	Rye	−	El-Temsah and Joner (2010)
Ag	Barley	−	El-Temsah and Joner (2010)
Si	Zucchini	−	Stampoulis et al. (2009)
Al	Rye	−	Lin and Xing (2007)
ZnO	Maize	−	Lin and Xing (2007)
CeO_2	Alfalfa, tomato, cucumber, maize, soybean	−	Lopez-Moreno et al. (2010b)
Zero-valent Fe	Flax, red clover white, meadow fescue, barley, rye	N	El-Temsah and Joner (2010)
Al	Radish, rapeseed, lettuce, maize, cucumber	N	Lin and Xing (2007)
Ag	Flax	N	El-Temsah and Joner (2010)
Au	Cucumber, lettuce	+	Barrena et al. (2009)
Si	Zucchini	N	Stampoulis et al. (2009)
Cu	Lettuce	N	Shah and Belozerova (2009)

(continued)

Table 6.1 (continued)

Nanoparticle	Plant	Effect on germination	Reference
Au	Lettuce	N	Shah and Belozerova (2009)
Pd–Al(OH)$_2$	Lettuce	N	Shah and Belozerova (2009)
SiO$_2$	Lettuce	N	Shah and Belozerova (2009)
Al$_2$O$_3$	Radish, rapeseed, rye, lettuce, maize, cucumber	N	Lin and Xing (2007)
r-TiO$_2$	Spinach	+	Zheng et al. (2005)
SiO$_2$ + TiO$_2$	Soybean	+	Lu et al. (2002)
Au/Cu	Lettuce	N	Shah and Belozerova (2009)
MWCNTs	Radish, rapeseed, rye, lettuce, maize, cucumber	N	Lin and Xing (2007)
MWCNTs	Zucchini	N	Lin and Xing (2007)

Several examples exist depicting the positive aspects of TiO$_2$ NPs on plants (Zheng et al. 2005; Hong et al. 2005a; Yang et al. 2007; Gao et al. 2008). TiO$_2$ NPs have been observed to enhance seed germination and promoted radicle and plumule growth of canola (*Brassica napus*) seedlings (Mahmoodzadeh et al. 2013). Zheng et al. (2005) presented enhanced growth in spinach when TiO$_2$ NPs were administered to the seeds. Recently, seed priming has been found to increase the seed vigor and germination synchronization, which has resulted in growth enhancement of many crops under particularly stressful conditions (Carvalho et al. 2011). For example, anatase NPs treatment to parsley seeds increased the germination rate index of the test subject (Dehkourdi and Mosavi 2013). In another case, biogenic anatase NPs were used to increase the seedling vigor and germination percentage of tridax daisy (*Tridax procumbens*) by the possible triggering of antioxidative mechanism in germinating seeds under chilling (Bhati-Kushwaha et al. 2013). Feizi et al. (2013a) verified the germination rate enhancement of common sage (*Salvia officinalis*) while the seeds were exposed to 60 mg L^{-1} of bulk and TiO$_2$ NPs.

6.2.1.2 Carbon Materials

Carbon nanotubes (CNTs), owing to their unique mechanical, electrical, thermal, and chemical properties have found commendable presence in plant science as well. It has been prominently observed that CNTs are able to penetrate the cell wall and membrane of cells, facilitating the enhanced uptake of water and nutrients by forming additional transport channels and also act as delivery systems for certain specialized chemicals to cells. Various studies have demonstrated the ability of multiwalled CNTs (MWCNTs) to positively influence seed germination and plant growth. Villagarcia et al. (2012) and Tiwari et al. (2014) found that MWCNTs induced increased water, Ca, and Fe uptake, which enhanced the seed germination

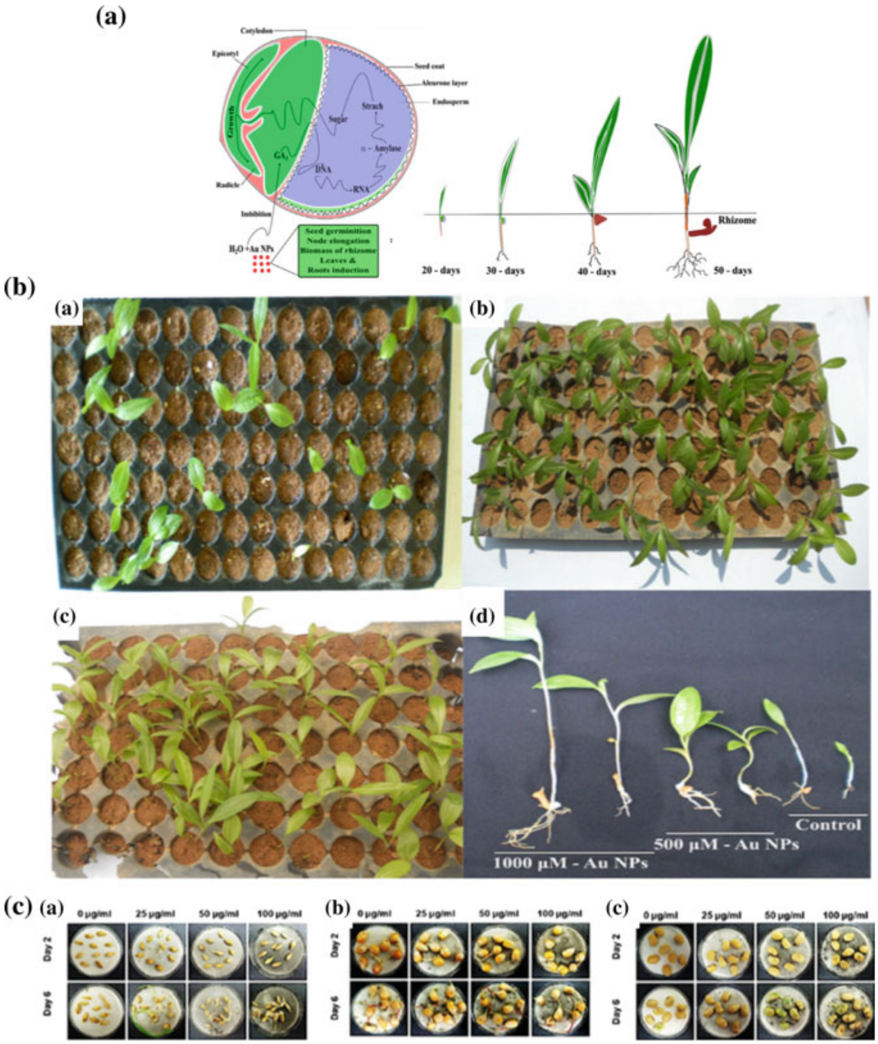


Fig. 6.2 **a** Schematic representation of the effect of Au NP-induced lily seed germination, node elongation, biomass of rhizome, leaf, and root initiation. **b** Effect of Au NPs on lily seed germination: *a* Control, *b* 500 µM Au NPs, *c* 1000 µM Au NPs for a duration of 30 day, *d* Induction of node elongation, biomass of rhizome, leaf, and root initiation of Au NP-treated samples for a duration of 40 day (Gopinath et al. 2014). **c** Germination of crop seeds (soybean, barley, corn) exposed to MWCNTs through the airspray technique. Phenotype of control and MWCNT-coated seeds of *a* barley, *b* corn, and *c* soybean are presented on the second and sixth day after MWCNT spray treatment (Adopted from Lahiani et al. 2013)

and plant growth. Raman spectroscopy and transmission electron microscopy (TEM) revealed the presence of MWCNTs aggregates inside the seed coats of barley (*Hordeum vulgare*), soybean, and maize, supporting the assumption that MWCNTs have the tendency to penetrate the seed coat to facilitate water and nutrient supply to the germinating seeds (Lahiani et al. 2013). Highly maximized germination rate was observed in crop species as tomato, hybrid Bt cotton (*Gossypium hirsutum*), Indian mustard (*Brassica juncea*), urdbean (*Vigna mungo*), and rice (*Oryza sativa*) with MWCNTs treatment (Nair et al. 2010; Gajanan et al. 2010; Morla et al. 2011; Mondal et al. 2011; Nalwade and Neharkar 2013). Other studies have also supported the positive influence of MWCNTs on seed germination and growth of six different crop species (radish (*Raphanus sativus*), rapeseed (*Brassica napus*), rye, lettuce, maize, and cucumber) (Lin and Xing 2007). Surprisingly, the effects of MWCNTs and single-walled CNTs (SWCNTs) have been observed to vary. For example, zucchini plants exposed to MWCNTs did not show any detrimental effects on seed germination and root elongation whereas a marked decrease in the biomass was recorded during further growth in the presence of SWCNTs (Stampoulis et al. 2009). Similarly, Cañas et al. (2008) studied the effects of functionalized and non-functionalized SWCNTs on root length parameters of six crop species (cabbage (*Brassica oleracea*), carrot (*Daucus carota*), cucumber, lettuce, onion, and tomato). Although the effects were concentration and species dependent, the non-functionalized CNTs exhibited higher degree of phytotoxicity when compared to its functionalized counterpart, signifying the important role of NP surface modifications.

6.2.1.3 Metal Nanoparticles

Although not many reports have been recorded with respect to the impact of gold NPs (Au NPs) on plant system, Barrena et al. (2009) in lettuce and cucumber, Arora et al. (2012) in Indian mustard; Savithramma et al. (2012) in *Boswellia ovalifoliolata,* and Gopinath et al. (2014) in lily have reported that Au NPs improve seed germination in the respective plant species.

Krishnaraj et al. (2012) observed that biologically synthesized silver NPs (Ag NPs) showed a significant effect on seed germination of hydroponically grown water hyssop (*Bacopa monneri*). Also, biosynthesized Ag NPs enhanced seed germination and seedling growth in *Boswellia ovaliofoliolata* (Savithramma et al. 2012). However, Yin et al. (2012) reported on the enhanced germination rate of trumpet weed (*E. fistulosum*) alone out of 11 wetland plant species (rye grass, switch grass (*Panicum virgatum*), sallow sedge (*Carex lurida*), broom sedge (*C. scoparia*), fox sedge (*C. vulpinoidea*), fringed sedge (*C. crinita*), trumpet weed, simple pokeweed (*Phytolaca americana*), wool grass (*Scirpus cyperinus*), scarlet lobelia (*Lobelia cardinalis*), and soft rush (*Juncus effusus*) on treatment with Ag NPs.

6.2.2 Growth Parameters

Plant growth is characterized by increase in biomass of the germinated seeds. The length of roots and shoot, number of laterals, number and size of leaves, total biomass, and yield represent the major growth parameters. Many studies on NP–plant interactions have focused on these factors and reports of both enhanced and retarded growth have been documented. Most of the NPs applied to seedlings or plants as such are through the roots, which causes a kind of bias in determining their effects. The main reason behind this speculative assessment is that the movement of NPs through the plant tissues (translocation) has not been clearly understood, although there are a few reports available. Overall, the observed effects are linked to the NP interaction with roots, either promoting or blocking nutrient supply and subsequent translocation to higher tissues. Table 6.2 provides a few examples of the various NPs influencing the growth parameters of plants.

6.2.2.1 Oxide Nanoparticles

SiO_2 NPs were found by Bao-shan et al. (2004) to improve seedling growth and quality, including mean height, root collar diameter, main root length, and the number of lateral roots while affecting the synthesis of chlorophyll in Changbai larch (*Larix olgensis*) seedlings. Wang et al. (2014) treated rice plants with bare quantum dots (QDs) and silica-coated QDs and found that the latter significantly promoted root growth.

Jaberzadeh et al. (2013) documented the growth enhancement in wheat plants on exposure to TiO_2 NPs under water-deficit stress. Zheng et al. (2005) described the enhanced growth performance of spinach when TiO_2 NPs were sprayed onto the leaves. Nano-anatase treatment to parsley (*Petroselinum crispum*) seeds had a positive effect on the root/shoot length, fresh weight, vigor index, and chlorophyll content (Dehkourdi and Mosavi 2013).

Juhel et al. (2011) evaluated the effects of alumina NPs on the growth, morphology, and photosynthesis of the aquatic plant duck weed (*Lemna gibba*) and concluded that aluminum oxide NPs (alumina and Al_2O_3 NPs) enhanced the growth of duck weed significantly with an evident increase in the biomass, which in turn was explained to be proportional to the morphological adjustments of the plant in response to alumina NPs exposure, such as increase in root length, number of fronds per colony, and photosynthetic efficiency.

Clément et al. (2013) characterized the phytotoxicity of the Ti NPs (crystal anatase or rutile) in daphnia and algae, rotifers, and plants as model organisms. TiO_2 NPs with anatase crystal structure were toxic in all of the tests at higher concentrations, but due to their antimicrobial properties, a significant growth of the roots was observed. As the rutile form exhibits lipophilicity, the TiO_2 NPs produce larger aggregates in aqueous medium, resulting in reduced effects on biological organisms and a lower toxicity compared with anatase form.

Table 6.2 Effect of nanoparticles on the overall growth parameters of plants [positive effect (+), negative effect (−), no effect (N)]

Nanoparticle	Plant	Growth parameters	Reference
CNTs	Alfalfa, wheat	Root elongation (+)	Miralles et al. (2012)
SWCNTs	Onion, cucumber	Root elongation (+)	Cañas et al. (2008)
MWCNTs	Wheat	Root growth (+)	Wang et al. (2012)
ZnO	Peanut	Root growth (+)	Prasad et al. (2012)
Au	Arabidopsis	Root length (+)	Kumar et al. (2013)
Ag	Common bean, maize	Root length (+)	Salama (2012)
TiO_2	Arabidopsis	Root length (+)	Lee et al. (2010)
Aluminum Oxide	Arabidopsis	Root length (+)	Lee et al. (2010)
Alumina	Duckweed	Root length root growth (+)	Juhel et al. (2011)
Zero-valent Iron Oxide	Arabidopsis	Root elongation (+)	Kim et al. (2014)
Co_3O_4	Radish	Root elongation (+)	Wu et al. (2012)
Ag	Tomato	Root growth (−)	Song et al. (2013)
TiO_2	Tomato	Root length (N)	Song et al. (2013)
Ni	Tomato	Root growth (−)	Faisal et al. (2013)
ZnO	Cluster bean	Root growth (+)	Raliya and Tarafdar (2013)
CNTs	Wheat	Root growth (+)	Wang et al. (2012)
TiO_2	Wheat	Root elongation (+)	Larue et al. (2012)
Al_2O_3	Arabidopsis	Root growth and elongation (N)	Lee et al. (2010)

(continued)

Table 6.2 (continued)

Nanoparticle	Plant	Growth parameters	Reference
Al	Radish, rapeseed	Root growth (+)	Lin and Xing (2007)
CeO_2	Maize, alfalfa, soybean	Root growth (+)	Lopez-Moreno et al. (2010b)
ZnO	Soybean	Root growth (+)	Lopez-Moreno et al. (2010a)
MWCNTs	Rye	Root length (+)	Lin and Xing (2007)
SWCNTs	Onion, cucumber	Root length (+)	Cañas et al. (2008)
Cu	Wheat	Root (−)	Lee et al. (2008)
Al	Rye	Root length (−)	Lin and Xing (2007)
Al	Maize, lettuce	Root length (−)	Lin and Xing (2007)
Zn	Radish, rapeseed, rye, lettuce, maize, cucumber	Root length (−)	Lin and Xing (2007)
ZnO	Rye	Root tips (−); root cap (−)	Lin and Xing (2007)
ZnO	Radish, rapeseed, rye, lettuce, maize, cucumber	Root growth (−)	Lin and Xing (2007)
ZnO	Maize	Root growth (−)	Stampoulis et al. (2009)
CeO_2	Maize, tomato, alfalfa	Root growth (−)	Lopez-Moreno et al. (2010b)
Al_2O_3	Maize, cucumber, carrot, cabbage	Root growth (−); root length (−)	Yang and Watts (2005); Lin and Xing (2007)
CNTs	Tomato	Root reduction	Cañas et al. (2008)
CNTs	Lettuce	Root length (−)	Cañas et al. (2008)
MWCNTs	Lettuce	Root length (−)	Lin and Xing (2007)
Au	Arabidopsis	Shoot length (+)	Kumar et al. (2013)
Ag	Bean, maize	Shoot length (+)	Salama (2012)
Ag	Rice	Shoot growth (−)	Mirzajani et al. (2013)
ZnO	Cluster bean	Shoot growth (+)	Raliya and Tarafdar (2013)

(continued)

Table 6.2 (continued)

Nanoparticle	Plant	Growth parameters	Reference
CeO_2	Maize, alfalfa, soybean	Shoot growth (+)	Lopez-Moreno et al. (2010b)
$SiO_2 + TiO_2$	Soybean	Shoot growth (+)	Lu et al. (2002)
Ag	Barley, flax, rye	Shoot length (−)	El-Temsah and Joner (2010)
Cu	Mungbean	Shoot growth (−)	Lee et al. (2008)
CeO_2	Alfalfa, tomato, cucumber, maize	Shoot growth (−)	Lopez-Moreno et al. (2010b)
CNTs	Tomato	Seedling growth (+)	Morla et al. (2011)
wsCNTs	Chickpea	Growth rate (+)	Tripathi et al. (2011)
MWCNTs	Tobacco	Growth rate (+)	Khodakovskaya et al. (2012)
Ag	*Boswellia ovaliofoliolata*	Seedling growth (+)	Savithramma et al. (2012)
SiO_2	Maize	Growth parameters (+)	Yuvakkumar et al. (2011), Suriyaprabha et al. (2012)
TiO_2	Duck weed	Plant growth (+)	Song et al. (2012)
Ag	Mungbean, Sorghum	Plant growth (−)	Lee et al. (2012)
GA-Ag	Rye, switch grass, sallow sedge, broom sedge, fox sedge, fringed sedge, simple pokeweed, wool grass, scarlet lobelia, soft rush	Plant growth (−)	Yin et al. (2012)
Se	Tobacco	Plant growth (+)	Domokos-Szabolcsy et al. (2012)
Alumina	Duck weed	Plant growth (+)	Juhel et al. (2011)
Ag	Duck weed	Plant growth (−)	Gubbins et al. (2011)
CNTs	Indian mustard, mungbean	Seedling growth (+)	Mondal et al. (2011)
TiO_2	Spinach	Plant growth (+)	Yang et al. (2006)
SiO_2 and TiO_2	Soybean	Plant growth (+)	Lu et al. (2002)
Cu	Mungbean, wheat	Seedling growth (−)	Lee et al. (2008)

ZnO NPs were instrumental in significantly improving the plant biomass, shoot and root growth, and root area in cluster bean (*Cyamopsis tetragonoloba*) rhizosphere (Raliya and Tarafdar 2013). Mahajan et al. (2011), employing correlative light and scanning microscope, and inductively coupled plasma/atomic emission spectroscopy, evidently revealed the presence of ZnO NPs in roots of mungbean (*Vigna radiata*) and chickpea (*Cicer arietinum*), which related to promotion of root/shoot length and biomass. Helaly et al. (2014) augmented MS media with nano-ZnO and found increased somatic embryogenesis, shooting, and subsequent regeneration of plantlets. Lin and Xing (2007) found retarded root growth of six higher plant species when treated with 2000 mg L^{-1} nano-Zn or ZnO NPs.

Faisal et al. (2013) investigated the nickel oxide NP (NiO NP)-induced phytotoxicity in the roots of tomato seedlings. Short duration treatment of tomato seeds to NiO NPs resulted in a significant repression of root growth. This anomaly was responsible for an oxidative imbalance, evidenced from the enhancement in antioxidant enzyme levels. An ultrastructure analysis of root cells revealed the translocation of the NiO NPs in the cell cytoplasm, characterizing changes in the structure of the organelles. Also, enhancement in activity of oxidative stress-related enzymes and mitochondrial dysfunction are related to the observed phytotoxicity.

6.2.2.2 Carbon Materials

Wang et al. (2012) recorded significantly enhanced root cell elongation and dehydrogenase activity with oxidized MWCNTs. The improved root and stem growth on MWCNTs exposure may be due to the uptake and accumulation of MWCNTs by roots with their subsequent translocation to leaves (Smirnova et al. 2012). The presence of water-soluble CNTs inside wheat plants was evidenced by Tripathi and Sarkar (2015) with scanning electron and fluorescence microscope. Furthermore, the authors linked this observation to the CNTs-induced root and shoot growth under both light and dark conditions. Interestingly, MWCNTs have been recognized to augment water retention, improve biomass, flowering, and fruit yield, and also enhance medicinal properties of plants (Khodakovskaya et al. 2013; Husen and Siddiqi 2014). A few examples of NPs influencing the yield and biomass of plants are compiled in Table 6.3. However, inhibitory effects of MWCNTs on plant growth have also been reported by many researchers (Begum and Fugetsu 2012; Ikhtiar et al. 2013; Tiwari et al. 2014; Begum et al. 2014). In another study, the uptake, accumulation, and transmission of natural organic matter (NOM)-suspended MWCNTs in rice were reported (Lin et al. 2009). The observations revealed a negative impact of the MWCNTs-NOM on the rice plants with delay in flowering and reduced seed set.

Carbon-based fullerol [$C_{60}(OH)_{20}$] NPs treatment resulted in increases of up to 54 % in biomass yield and 24 % in water content in bitter melon (*Momordica charantia*). A 20 % fruit length, 59 % fruit number, and 70 % fruit weight gain resulted in an overall improvement of up to 128 % in fruit yield (Kole et al. 2013).

Table 6.3 Effects of nanoparticles on the yield and biomass of plants [positive effect (+), negative effect (−), no effect (N)]

Nanoparticle	Plant	Yield/biomass	Reference
MWCNTs	Tomato	Number of flowers (+)	Khodakovskaya et al. (2013)
ZnO	Peanut	Yield (+)	Prasad et al. (2012)
Au	Arabidopsis	Early flowering and yield (+)	Kumar et al. (2013)
Iron oxide	Soybean	Yield (+)	Sheykhbaglou et al. (2010)
SWCNTs	Rice	Flowering (−); yield (−)	Burman et al. (2013)
MWCNTs	Wheat	Vegetative biomass (+)	Wang et al. (2012)
MWCNTs	Maize	biomass (+)	Tiwari et al. (2014)
ZnO	Chickpea	Shoot biomass (+)	Burman et al. (2013)
ZnO	Mungbean	Biomass (+)	Dhoke et al. (2013)
ZnO	Mungbean	Dry weight (+)	Patra et al. (2013)
Ag	Bean, maize	Dry vegetative weight (+)	Salama (2012)
S	Mungbean	Dry weight (+)	Patra et al. (2013)
Alumina	Duck weed	Biomass (+)	Juhel et al. (2011)
Iron oxide	Mungbean	Biomass (+)	Dhoke et al. (2013)
ZnFeCu oxide	Mungbean	Biomass (+)	Dhoke et al. (2013)
CeO_2	Arabidopsis	Biomass (+)	Ma et al. (2013)
CuO	Wheat	Biomass (+)	Dimkpa et al. (2012)
Ti and Ag	Tomato	Biomass (N)	Song et al. (2013)
Alumina	Duck weed	Biomass (+)	Juhel et al. (2011)
Zn	Maize	Biomass (N)	Zhao et al. (2013)
Ti	Bean, wheat, curly dock, pond weed	Biomass (N)	Jacob et al. (2013)
SiO_2	Maize	Biomass (+)	Suriyaprabha et al. (2012)
TiO_2	Spinach	Biomass (+)	Zheng et al. (2005)
TiO_2	Spinach	Dry weight (+)	Jacob et al. (2013)
Ag	Zucchini	Biomass (−)	Stampoulis et al. (2009)
Cu	Zucchini	Biomass (−)	Stampoulis et al. (2009)
ZnO	Rye	Biomass (−)	Lin and Xing (2007)
ZnO	Zucchini	Biomass (−)	Stampoulis et al. (2009)
CeO_2	Alfalfa	Biomass (−)	Lopez-Moreno et al. (2010b)
MWCNTs	Zucchini	Biomass (−)	Stampoulis et al. (2009)

6.2.2.3 Metal Nanoparticles

Au NPs have been seen to increase number of leaves, leaf area, plant height, and chlorophyll content, culminating in better crop yield (Arora et al. 2012; Gopinath et al. 2014).

Ag NPs were witnessed to increase the shoot/root length and leaf area of Indian mustard, common bean (*Phaseolus vulgaris*), and maize (Salama 2012; Sharma et al. 2012). Meanwhile, Gruyer et al. (2013) observed both positive and negative effects of Ag NPs on root elongation, with increment in barley roots, but inhibition in lettuce. Size and shape of the NPs under consideration play a major role in translating their effects on the morphological and physiological aspects of the host plant system. Syu et al. (2014) analyzed the effect of three varying morphologies of Ag NPs on the physiological and molecular response of Arabidopsis (*Arabidopsis thaliana*). They concluded that the decahedral Ag NPs exhibited the highest percentage of root promotion whereas the spherical particles had no effect. Lee et al. (2012) studied the phytotoxic aspects of Ag NPs (5–25 nm) on two edible crops, bean and sorghum in agar and soil cultures. In agar dispersed NPs experiment, bean and sorghum showed concentration-dependent growth inhibition whereas in the soil media fortified with Ag NPs, bean was not significantly affected and sorghum exhibited a slightly reduced growth rate. This study demonstrates the importance of media utilized for the dissolution of NPs on the toxicity of the plants. In the aquatic plant duck weed (used as an environmental toxicity plant indicator), Gubbins et al. (2011) indicated an inhibition of plant growth when plants were exposed to Ag NPs (5 mg L^{-1}) with a size ranging from 20 to 100 nm. Contrasting data obtained by Juhel et al. (2011), working with alumina NPs in duck weed, have shown an increase in the biomass accumulation. Ma et al. (2010) found that at lower concentrations of Ag NPs (1 mg L^{-1}), toxicity could be observed in seedlings of Arabidopsis plants. Studies on seed germination and root growth of hydroponically cultured zucchini plants in solution amended with Ag NPs showed no negative effects except for a decrease in total biomass and transpiration that was recorded on prolonged exposure to Ag NPs (Stampoulis et al. 2009).

Perchloric acid-coated magnetic NPs were tested on germinated maize seeds (Racuciu and Creanga 2009). Slight inhibitory effect was observed with brown spots on leaves at higher concentrations of the ferrofluid possibly due to generation of oxidative stress in leaf cells leading to altered photosynthesis rate and subsequent decreased metabolic activity.

Unmodified alumina NPs (13 nm) retarded the root elongation in maize, cucumber, soybean, carrot, and cabbage (Yang and Watts 2005). On the contrary, phenanthrene (a major constituent of polycyclic aromatic hydrocarbons)-loaded alumina NPs exhibited significantly decreased toxicity, implying on the relevance of appropriate surface modifications, which could facilitate in mitigating the phytotoxicity of NPs.

Copper NPs (Cu NPs) supplemented to agar culture media were tested for seedling growth of mungbean and wheat (Lee et al. 2008). Mungbean exhibited higher sensitivity to Cu NPs than wheat with noticeable inhibition in the growth of seedlings being observed. Similarly, Cu NPs were found to negatively influence the length of emerging roots of zucchini plants and had detrimental effects on the growth (Stampoulis et al. 2009). Jiang et al. (2014), with the wide medical applications of hydroxyapatite (HAP) in mind initiated a study on mungbean plants exposed to HAP NPs to understand the relationship between biocompatibility and biotoxicity of these NPs. The mungbean sprouts growth was inhibited, depending on the amount of HAP NPs that ruptured the cell wall and gained intracellular access, and also the Ca^{2+} concentrations were considered as the primary factors for cellular apoptosis and consequently for the observed inhibitory effect.

6.3 Biochemical Parameters

The biochemical evaluation of any system, including plants gives an approximate overview of the performance of that system. The biochemistry analysis of plants would shed light on the efficiency and extent of metabolic activity, actively taking place inside the subject, which is responsible for all the parameters of growth, development and reproduction and directly corresponds to the overall health of the plant. These parameters are greatly influenced by external biotic and abiotic factors, which include the NPs. Reports have been made on both the positive and negative influence of NPs on the biochemical features (Table 6.4).

6.3.1 Oxide Nanoparticles

It has been recorded that under high saline stress, SiO_2 NPs increase fresh and dry leaf weight, chlorophyll content, and proline accumulation. The reason for such a tolerance of plants to abiotic stress could be attributed to an increase in the accumulation of proline, free amino acids, nutrients, and enhanced activity of antioxidant enzymes due to the presence of SiO_2 NPs (Haghighi et al. 2012; Li et al. 2012; Siddiqui et al. 2012; Kalteh et al. 2014). Raliya and Tarafdar (2013) found that ZnO NPs were instrumental in significantly improving the chlorophyll content and protein synthesis, rhizospheric microbial population, acid phosphatase, alkaline phosphatase, and phytase activity in a cluster bean rhizosphere. ZnO NPs-supplemented MS media induced proline synthesis and increased activity of superoxide dismutase (SOD), catalase (CAT), and peroxidase resulting in heightened tolerance to biotic stress (Helaly et al. 2014). Hernandez-Viezcas et al. (2011) studied the effects of 10 nm ZnO NPs in hydroponic cultures of velvet mesquite at concentrations varying from 500 to 4000 mg L^{-1}. To evaluate NP-induced stress on the plant, specific activity of CAT and ascorbate peroxidase (APX) was performed. The NPs were recorded to increase the specific activity of CAT (in the root, stem, and leaves) and APX (only in the leaves), while no evidence of detrimental

Table 6.4 Biochemical aspects of nanoparticle plant interaction [positive effect (+), negative effect (-), no effect (N)]

Nanoparticle	Plant	Biochemical parameters	Reference
Al_2O_3	Tobacco	MicroRNA expression (+)	Burklew et al. (2012)
ZnO	Velvet mesquite	Levels of CAT and APOX (+)	Hernandez-Viezcas et al. (2011)
MWCNTs	Tomato	Uptake of nutrients (+)	Tiwari et al. (2013)
MWCNTs	Maize	Nutrient transport (+)	Tiwari et al. (2014)
ZnO	Cucumber	Micronutrients (+)	Zhao et al. (2014)
TiO_2	Wheat	Chlorophyll content (+)	Mahmoodzadeh et al. (2013)
TiO_2	Tomato	Net photosynthesis (+); transpiration and water conductance (+)	Qi et al. (2013)
TiO_2	Spinach	Enzymatic activities (+)	Yang et al. (2006)
Fe	Wheat, arabidopsis	Chlorophyll (−);	Larue et al. (2012), Marusenko et al. (2013)
ZnO	Cluster bean	Chlorophyll (+); protein content (+); P-nutrient-metabolizing enzymes (+)	Raliya and Tarafdar (2013)
SiO_2	Maize	Proteins (+); chlorophyll (+); phenols (+)	Suriyaprabha et al. (2012)
TiO_2	Spinach	Photosynthesis (+); nitrogen metabolism (+); oxidative stress (+)	Hong et al. (2005a), Lee et al. (2012), Lei et al. (2007)
SiO_2 and TiO_2	Soybean	Nitrate reductase activity (+)	Lu et al. (2002)
TiO_2	Spinach	RCA mRNA expression (+); protein levels (+);	Gao et al. (2006)
TiO_2	Spinach	N_2 fixation (+)	Linglan et al. (2008)
SiO_2 + TiO_2	Soybean	Nitrate reductase activity (+); water absorption (+); antioxidant potential (+)	Lu et al. (2002)
Ag	Zucchini	Transpiration (−)	Stampoulis et al. (2009)
TiO_2	Maize	Hydraulic conductivity (−); transpiration (−)	Asli and Neumann (2009)

aspects as chlorosis, necrosis, stunting, or wilting, even after 30 days of treatment, was observed, suggesting a significant tolerance level toward ZnO NPs. Kumari et al. (2011) during the evaluation of effects of ZnO NPs using root cells of onion showed that on increasing the ZnO NPs or the ZnO bulk concentrations, higher values for the thiobarbituric acid reactive species (TBARS) were observed. During reactive oxygen species (ROS) formation and release, fatty acid conversion to toxic lipid peroxides occurs, causing disruption of biological membranes (Gratão et al. 2005), facilitating the entry of and damage by NPs and metals, resulting in TBARS formation, which damages the membrane permeability and is predicted to be one the reasons for the observed phytotoxicity.

Effects of CuO NPs were studied in an economically important oil seed crop, Indian mustard. Significant increases in peroxidase enzyme activity and H_2O_2 formation were observed. The lipid peroxidation levels were found to have increased significantly in both the shoots and roots of seedlings. Gene expression studies revealed significant activation of CuZn SOD in roots and shoots while the MnSOD gene levels remained unchanged. Also, the CAT and APX expression levels were not observed to have changed in shoots. However, significant inhibition of CAT and APX was recorded in roots. The SOD enzyme activity also significantly increased in roots and shoots as a result of exposure to 50–500 mg L^{-1} of CuO NPs (Nair and Chung 2015).

Hydroponic cultures of cucumber, aerially treated with nano-ceria powder (CeO_2), displayed increased CAT activity in roots and decreased APX activity in leaves (Hong et al. 2014). TiO_2 NPs act as photocatalysts and are responsible for the induction of an oxidation–reduction reaction (Crabtree 1998). They regulate nitrogen metabolism-related enzymic activity such as glutamate dehydrogenase, nitrate reductase, glutamic–pyruvic transaminase, and glutamine synthase, which assist in the uptake of nitrates and facilitate the conversion of inorganic nitrogen to organic nitrogen in the form of protein and chlorophyll (Yang et al. 2006; Dehkourdi and Mosavi 2013; Mishra et al. 2014). According to the observations made by Hong et al. (2005b), TiO_2 NPs were seen to protect the chloroplasts from excess light by increasing the activity of antioxidant enzymes, such as CAT, peroxidase, and SOD. Nano-anatase TiO_2 has also been found to promote antioxidant stress by decreasing the production of superoxide radicals, malonyldialdehyde content, and hydrogen peroxide and enhancing the activities of SOD, APX, CAT, and guaiacol peroxidase resulting in the increased oxygen evolution rate in spinach chloroplasts under UV-B radiation (Lei et al. 2008). Phytotoxicity in tomato seedlings due to NiO NPs was partially related to the increase in caspase-3-like protease activity, which linked NiO NPs to trigger the intrinsic apoptotic pathway in tomato plants due to the release of the Ni ions (Faisal et al. 2013).

6.3.2 Carbon Materials

MWCNTs have been shown to improve the peroxidase and dehydrogenase activity (Smirnova et al. 2012). It was observed that graphene oxide (GO) exposure did not induce H_2O_2 production, formation of oxidative stress, increase in malondialdehyde content, or altered activities of antioxidant enzymes in Arabidopsis plants. These results along with other observations provided a physiological basis for the safety of GO (Zhao et al. 2015).

6.3.3 Metal Nanoparticles

Shah and Belozerova (2009) demonstrated Au NP-induced toxicity in plants due to markedly arrested aquaporin function, which help in the transportation of wide range of molecules including water. Au NPs treatment improved the chlorophyll and sugar content of test plants resulting in better crop yield (Arora et al. 2012; Gopinath et al. 2014).

Effects of biosynthesized Ag NPs on hydroponic cultures of water hyssop revealed the induction of protein, carbohydrate synthesis, and decreased total phenol contents in addition to reduced CAT and peroxidase activities (Krishnaraj et al. 2012). Ag NPs increased the biochemical attributes (chlorophyll, carbohydrate, and protein contents, antioxidant enzymes) of Indian mustard, common bean, and maize (Salama et al. 2012; Gruyer et al. 2013). Rezvani et al. (2012) found that Ag NP-induced root growth by blocking ethylene signaling in saffron (*Crocus sativus*). Syu et al. (2014) while analyzing the effect of three varying morphologies of Ag NPs on the physiological and molecular response of Arabidopsis concluded that the spherical particles triggered the highest levels of anthocyanin accumulation and Cu/Zn SOD in Arabidopsis seedlings when compared to decahedral NPs, which gave the lowest values. The Ag NPs were also responsible for the regulation of protein accumulations such as protochlorophyllide oxidoreductase, cell-division-cycle kinase 2, and fructose-1,6 bisphosphate aldolase along with activation of the aminocyclopropane-1-carboxylic acid-derived inhibition of root elongation. A proteomic approach (2-DE and NanoLC/FT-ICR MS identification) was employed to study the effects of colloidal suspension of spherical Ag NPs on rice. Results revealed an accumulation of protein precursors, indicative of the dissipation of a proton motive force upon Ag NP administration. The proteins were identified to be involved in oxidative stress tolerance, transcription and protein degradation, calcium ion regulation and signaling, cell division, apoptosis, and cell wall and DNA/RNA/protein direct damage (Mirzajani et al. 2013). The effects of Ag NPs and $AgNO_3$ on mustard (*Brassica nigra*) seed germination were investigated at physiological and molecular levels. Both nanoformulations inhibited lipase activity and soluble and reducing sugar contents along with increased transcription of heme oxygenase-1 (Amooaghaie et al. 2015).

6.4 Role of Nanoparticles in Photosynthesis

Photosynthesis is the most essential and vital physiological process in the plant kingdom. It involves the conversion of light energy to chemical energy in the chloroplasts, specifically using chlorophyll, and storing it in the bonds of sugar, which is later used as the energy currency to regulate various other processes. The only raw materials required for this are light energy, CO_2, and H_2O, which are abundantly available in nature. Still, the conversion efficiency of light to energy by plants remains only 2–4 % (Kirschbaum 2011). This significant deficiency has prompted a large number of researchers world over to either mimic the process of photosynthesis artificially or improve the existing efficiency in planta. NPs tend to interfere and alter the photosynthetic efficiency, photochemical fluorescence, and quantum yield in plants based on their inherent light interaction capabilities (Table 6.5).

Table 6.5 Interaction of nanoparticles with the photosynthetic machinery of plants [positive effect (+), negative effect (−), no effect (N)]

Nanoparticle	Plants	Mechanism involved	Reference
TiO_2	Spinach, tomato	Light absorption (+); quantum yield in PS-II (+)	Mingyu et al. (2007a), Lei et al. (2007)
TiO_2	Long raceme ulm	Light absorption (−)	Gao et al. (2013)
Au	Soybean	Light absorption (+)	Falco et al. (2011)
CeO_2	Alfalfa	Light absorption (−) and photochemical efficacy (−)	Gomez-Garay et al. (2014)
TiO_2	Fava bean	Quantum yield in PS-II (N)	Foltete et al. (2011)
CuO, TiO_2	Duck weed, long raceme ulm	Photochemical fluorescence (+)	Gao et al. (2013)
Ag	Indian mustard	Quantum yield in PS-I I (+)	Sharma et al. (2012)
Au	Soybean	Quantum yield (−)	Falco et al. (2011)
Mn, TiO_2	Spinach, mungbean	Splitting of water (+); evolution of oxygen (+)	Lei et al. (2007)
Mn, TiO_2	Spinach, mungbean	Photophosphorylation in ETC (+)	Lei et al. (2007)
CuO, TiO_2	Long raceme ulm	ETC activity (−)	Gao et al. (2013)
CeO_2	Moringa	ETC activity (+)	Gomez-Garay et al. (2014)
TiO_2	Spinach	Light absorption (+); energy conversion (+); CO_2 assimilation (+)	Baun et al. (2008)
TiO_2	Spinach	Chlorophyll Formation (+); Ribulosebisphosphate carboxylase/oxygenase activity (+); photosynthetic rate (+)	Zheng et al. (2005)
TiO_2	Spinach	Photosynthetic rate (+)	Hong et al. (2005a)
TiO_2	Spinach	Hill reaction and non-cyclic photophosphorylation (+)	Hong et al. (2005a)
TiO_2	Spinach	Rubisco activase expression (+)	Ma et al. (2008)
TiO_2	Spinach	Oxygen evolution (+); Rubisco carboxylation (+); Rubisco activase and photosynthesis (+)	Gao et al. (2006), Zheng et al. (2007), Gao et al. (2008)

6.4.1 Oxide Nanoparticles

A modified mesoporous silica (SBA)–photosystem II (PSII) complex demonstrated active light-driven electron transport from water to quinone molecules as a result of the stable activity of photosynthetic oxygen-evolving reaction (Noji et al. 2011). The PSII–SBA complex was proposed as a potential candidate for the development of photosensors and artificial photosynthetic systems. Siddiqui et al. (2014) and Xie et al. (2012) showed that SiO_2 NPs enhance the photosynthetic rate by improving activity of carbonic anhydrase (supplies CO_2 to Ribulose 1,5-bisphosphate carboxylase-RuBisCo) and synthesis of photosynthetic pigments. SiO_2 NP-enhanced gas exchange and chlorophyll fluorescence parameters, such as net photosynthetic/transpiration rate, stomatal conductance, PSII potential activity, effective photochemical efficiency, actual photochemical efficiency, electron transport rate, and photochemical quench, were reported.

The potential of nano-anatase TiO_2 in enhancing the light-harvesting complex content of plants can be readily compared with TiO_2-quantum dot (QD) solar energy conversion assemblies (Kongkanand et al. 2008). Nano-anatase TiO_2 possess photocatalyzing properties, which helps improve the light absorbance and subsequent conversion to chemical and electrical energy. It is also interesting to note that the TiO_2 NPs were found to protect chloroplasts from aging during long illumination regimes and promoted chlorophyll formation, in addition to stimulating RuBisCo activity and increasing photosynthesis (Hong et al. 2005b, c; Yang et al. 2006). These TiO_2 particles enhance the photosynthetic carbon assimilation by activating RuBisCo (Sharma et al. 2012). Ma et al. (2008) found enhancement of RuBisCo carboxylation with high rate of photosynthetic carbon reaction as a result of nano-anatase-induced marker gene for RuBisCo activase mRNA, enhanced protein levels, and activities of RuBisCo activase. Qi et al. (2013) investigated the exogenous application of TiO_2 NPs on plants and commented on the improved net photosynthetic rate, water conductance, and transpiration rate. Nano-anatase was observed to strongly promote electron transport chain reaction, photoreduction activity of PSII, O_2 evolution, and photophosphorylation of chlorophyll under both visible and ultraviolet light (Lei et al. 2007). Reports also suggest the nitrogen photoreduction to exercise positive effects on the improved growth of TiO_2-treated spinach plants (Yang et al. 2007; Mingyu et al. 2007b). An increase in the light-harvesting complex II (LHC II) content, which promotes energy transfer and oxygen evolution in PS-II, on thylakoid membranes of spinach was observed with the application of anatase NPs (Hong et al. 2005c; Lei et al. 2007). On the contrary, foliar applied anatase-TiO_2 NPs resulted in reduced PSII quantum yield, photochemical quenching and electron transfer rate, and chlorophyll fluorescence, but promoted higher non-photochemical quenching and water loss in long raceme ulm (Gao et al. 2013). Increased water loss due to decreased mesophyll activity and reduced electron transfer rate by blocking the electron transfer from quinone A (Q_A) to quinone B (Q_B) are suggested for the marked reduction in photosynthetic activity.

Fluorescence analysis of CeO_2 NPs (100–400 mg L^{-1})-treated alfalfa revealed a reduction in photochemical efficiency at 100 and 200 mg L^{-1} CeO_2 NP treatments. CeO_2 NPs at 200 mg L^{-1} were found to enhance the fluorescence levels of fully oxidized and completely reduced plastoquinone electron acceptor pool (Q_A), indicating the damage to PSII and the impairment of electron transport system (Gomez-Garay et al. 2014). These observations are contrary to the findings of Boghossian et al. (2013) and Giraldo et al. (2014) where isolated chloroplasts incubated with CeO_2 NPs displayed improved photosynthetic activity due partly to the ROS scavenging ability of CeO_2 NPs, which protected the chloroplasts from ROS damage. Chlorophyll *a* is known to be highly sensitive to photodegradation and many researchers have utilized this phenomenon as an indicator of NPs altering effects on the photosynthetic machinery. For example, Rico et al. (2013) found that CeO_2 NPs severely limited the chlorophyll content in rice. Fe_3O_4 and $CoFe_2O_4$ NPs also showed decreased chlorophyll content in sunflower (*Helianthus annus*) seedlings. When compared to the controls, about 50 % of chlorophyll reduction in Fe_3O_4 and 28 % in $CoFe_2O_4$ treatments were recorded (Ursache-Oprisan et al. 2011). In contrast, Ghafariyan et al. (2013) found superparamagnetic iron oxide NPs (SPIONs) to enhance the chlorophyll content in soybean. Another deleterious effect of NP on chlorophyll *a* fluorescence was observed in duck weed where CuO NPs markedly decreased the quantum yield and inhibited the photosynthetic process (Perreault et al. 2014). These NPs were also responsible for causing major modifications in PSII and decreased conversion of absorbed light energy via PSII e^- transport.

6.4.2 Carbon Materials

Recently, Giraldo et al. (2014) incorporated SWCNTs in isolated chloroplast and found that the photosynthetic activity was enhanced threefold along with relatively increased e^- transport rates. The researchers have augmented plants' ability to harvest more light energy by delivering carbon nanotubes into chloroplast, and also carbon nanotubes could serve as artificial antennae that allow chloroplast to capture wavelengths of light, which is not in their normal range, such as ultraviolet, green, and near infrared.

6.4.3 Metal Nanoparticles

According to Govorov and Carmeli (2007), metal NPs possess the tendency to influence the efficiency of energy conversions in photosynthetic systems. Chlorophyll would bind to Au and Ag NPs, forming a novel hybrid system that is projected to produce ten folds more excited electrons due to plasmon resonance and fast electron-hole separation. Such enhancement modules may assist in the design of future artificial light-harvesting systems. Electron transfer from excited fluorophore to Au or Ag NPs has been reported by numerous researchers (Barazzouk et al. 2005; Nieder et al. 2010; Beyer et al. 2011; Olejnik et al. 2013). The concentration-dependent effects of Au NPs (5–20 nm) on PSII chlorophyll *a* fluorescence quenching in soybean leaves were analyzed by Falco et al. (2011). The absorbance and fluorescence quenching were both found to be enhanced at higher concentration of Au, due to the light-absorbing tendency of Au and higher Au availability for electron transfer, respectively. On the contrary, lowest absorbance was recorded with larger Au NPs while greatest fluorescence quenching was registered with smallest Au NPs. This was due to the higher surface area of small NPs that absorb large amounts of chlorophyll molecules, facilitating better chlorophyll NPs energy transfer. In similar observations, 8-nm Au NPs enhanced quenching of chlorophyll fluorescence in a solution as a result of the enhanced e^- transfer from excited chlorophyll molecules to the metal NPs (Barazzouk et al. 2005). Falco et al. (2011) observed a shift in the fluorescence toward higher wavelength along with quenching of chlorophyll fluorescence in Au NP-treated soybean. Meanwhile, Sharma et al. (2012) documented the Ag NP-enhanced PSII quantum efficiency in Indian mustard.

Pradhan et al. (2013) analyzed the photoreduction activities in isolated chloroplasts of Mn NP-treated mungbean and revealed that the NPs alter the PSII by improving the photophosphorylation activity of electron transport chain (ETC) and by enhancing the H_2O splitting/O_2 evolution.

The influence of tetramethylammonium hydroxide (TMA-OH)-coated magnetic NPs on growth parameters of maize plants revealed an increased chlorophyll *a* level at low ferrofluid concentrations while an inhibited level was observed as the concentrations increased (Racuciu and Creanga 2009). Maize seeds germinated with magnetic fluid were exposed to an electromagnetic field. Post the electromagnetic field exposure, analysis revealed a decrease in essential assimilatory pigments with higher concentration of magnetic fluid solution. This could be attributed to the marginal localized heating due to the electromagnetic energy absorbed by magnetic NPs in plant tissues, which would have affected the redox reactions involved in photosynthesis process (Racuciu et al. 2009). It was also noted that alumina NPs can enhance the electron transfer efficiency of isolated photosynthetic reaction centers (Nadtochenko et al. 2008).

6.5 Other Physiological Parameters

Nano zerovalent iron (nZVI) triggered high plasma membrane H^+-ATPase activity in Arabidopsis, which resulted in a decrease in apoplastic pH, increase in leaf area, and wider stomatal aperture. Gene expression analysis revealed fivefold higher levels of

H^+-ATPase isoform responsible for stomatal opening, AHA2, in plants exposed to nZVI. The researchers demonstrated for the first time that nZVI enhances stomatal opening by inducing the activation of plasma membrane H^+-ATPase, leading to the possibility of increased CO_2 uptake (Kim et al. 2015). TiO_2-NPs' impact on wheat, rapeseed, and Arabidopsis evapotranspiration was evaluated (Larue et al. 2011). Similarly, TiO_2 NPs with diameters ranging from 14 to 655 nm did not impact wheat seed germination, biomass, and transpiration (Larue et al. 2012). The SiO_2 NPs apart from deleteriously affecting the plant height and shoot and root biomass also affected the contents of Cu, Mg in shoots, and Na in roots of transgenic cotton (Le et al. 2014). Nutritional analysis of pods from soybean plants cultivated in farm soil amended with CeO_2 NPs revealed that NPs at 1000 mg kg^{-1} had significantly less Ca but more

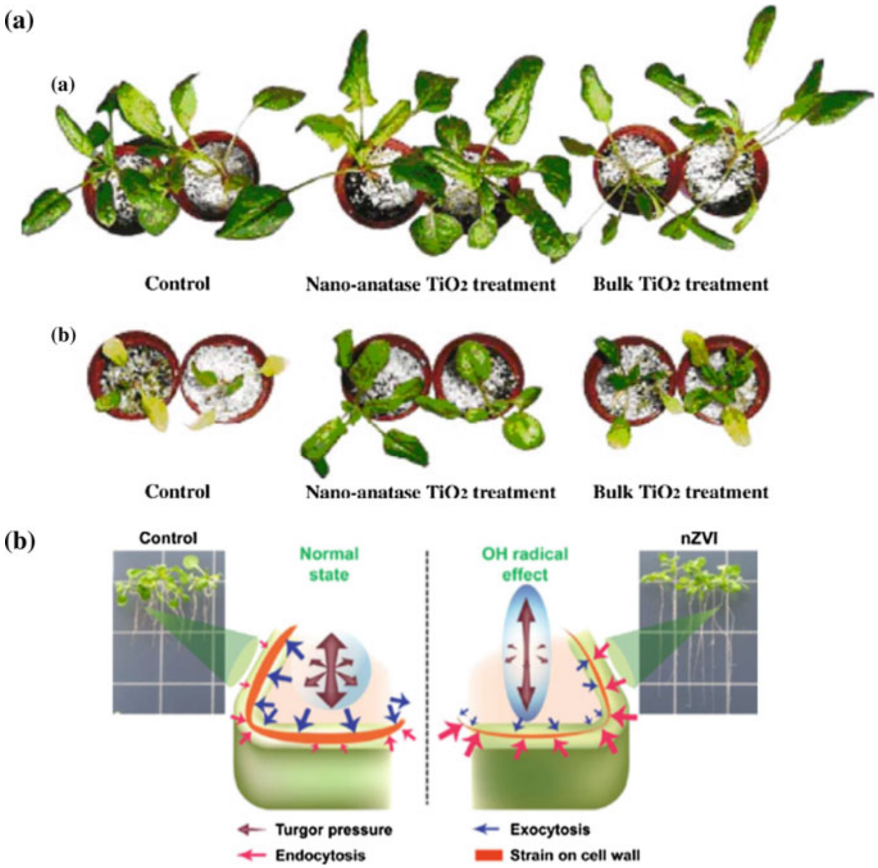
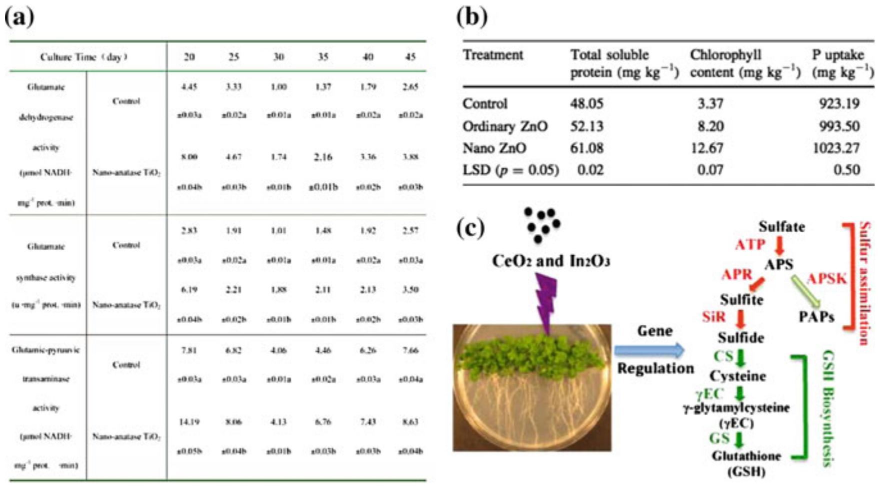


Fig. 6.3 **a** Effect of nano-anatase TiO_2 on growth of spinach. *a* Cultured by Hoagland solution. *b* Cultured by N-deficient Hoagland solution (Yang et al. 2007). **b** Conceptual model on how OH radical-induced cell wall loosening might alter endocytosis in root cells. Endocytosis (*red*) of the nZVI-treated seedlings (*right*) is enhanced compared to that of the control (*left*), and exocytosis (*blue*) is vice versa (Adopted from Kim et al. 2014)

P and Cu, while pods from 100 mg kg^{-1} nZnO had more Zn, Mn, and Cu. ZnO NP-treated plants shared significant correlations among Zn, P, and S in pods with Zn in roots. The data suggested the evident role of CeO_2 NPs and ZnO NPs in altering the nutritional value of soybean (Peralta-Videa et al. 2014). In a similar experiment, CeO_2 NPs effects on the nutritional aspects such as mineral, fatty acid, and amino acid content of wheat were studied. CeO_2 NPs modified S and Mn storage in grains and modified the amino acid composition and increased linolenic acid by up to 6.17 %. The linoleic acid content, however, was decreased by up to 1.63 %, compared to the other treatments (Rico et al. 2014). Khodakovskaya et al. (2012) demonstrated the ability of MWCNTs to enhance tobacco cell culture growth by upregulating the genes for cell divisions (*CycB*), cell wall formation (*NtLRX1*), and water transport (aquaporin, *NNtPIP1*). Lahiani et al. (2013) studied the MWCNTs-regulated gene expression for several water channel proteins in soybean, maize, and barley seed coats. Kumar et al. (2013) reported Au NPs have a significant role in altering microRNAs expression levels, which regulate various morphological, physiological, and metabolic processes in plants. Syu et al. (2014) documented the Ag NP-induced gene expressions such as indoleacetic acid protein 8 (IAA8), dehydration-responsive RD22 and 9-*cis*-epoxycarotenoid dioxygenase (NCED3), involved in various cellular events. Also, Ag NPs were found to negatively influence expression of ACC oxidase 2 and ACC synthase 7, underlining their role as inhibitors of ethylene perception and subsequently interfering with the ethylene biosynthesis in Arabidopsis seedlings (Fig. 6.3).

6.6 Discussion

As discussed earlier, nanotechnology is the only recent technological breakthrough that is revolutionizing every field it is introduced to. With trendsetting models in the electronics industry and unprecedented strides in medical arena, it seems to be only a matter of time when this technology breaks the bounds in the agro-industry. The major restricting force is the limited understanding available on the primary impacts of this technology on plants as such. Although numerous scientific articles have been published on NP–plant interactions, most revolving around the toxicological aspects of NPs, knowledge of their complex relationship with the crucial physiological and biochemical processes impacting a plant system is relatively novice. Plants in their native environment, domesticated or wild, face a number of challenges to survive. Majority of these are environmental factors such as water shortage, nutrient deficiency, alkalinity/acidity of soil, pollution, insects, and pests all of which encompass the biotic and abiotic agents influencing a plants growth and survival (Fig. 6.4).



(a)

Culture Time (day)		20	25	30	35	40	45
Glutamate dehydrogenase activity (μmol NADH·mg^{-1} prot.·min)	Control	4.45 ±0.03a	3.33 ±0.02a	1.00 ±0.01a	1.37 ±0.01a	1.79 ±0.02a	2.65 ±0.02a
	Nano-anatase TiO_2	8.00 ±0.04b	4.67 ±0.03b	1.74 ±0.01b	2.16 ±0.01b	3.36 ±0.02b	3.88 ±0.03b
Glutamate synthase activity (u·mg^{-1} prot.·min)	Control	2.83 ±0.03a	1.91 ±0.02a	1.01 ±0.01a	1.48 ±0.01a	1.92 ±0.02a	2.57 ±0.03a
	Nano-anatase TiO_2	6.19 ±0.04b	2.21 ±0.02b	1.88 ±0.01b	2.11 ±0.01b	2.13 ±0.02b	3.50 ±0.03b
Glutamic-pyruvic transaminase activity (μmol NADH·mg^{-1} prot.·min)	Control	7.81 ±0.03a	6.82 ±0.03a	4.06 ±0.01a	4.46 ±0.02a	6.26 ±0.03a	7.66 ±0.04a
	Nano-anatase TiO_2	14.19 ±0.05b	8.06 ±0.04b	4.13 ±0.01b	6.76 ±0.03b	7.43 ±0.03b	8.63 ±0.04b

(b)

Treatment	Total soluble protein (mg kg^{-1})	Chlorophyll content (mg kg^{-1})	P uptake (mg kg^{-1})
Control	48.05	3.37	923.19
Ordinary ZnO	52.13	8.20	993.50
Nano ZnO	61.08	12.67	1023.27
LSD ($p = 0.05$)	0.02	0.07	0.50

Fig. 6.4 **a** Effects of nano-anatase TiO_2 on the enzyme activities of NH^{4+} assimilation of spinach (Yang et al. 2006). **b** Total soluble protein, chlorophyll content, and P concentration in 6-week-old cluster bean plants (Raliya and Tarafdar 2013). The effects of cerium oxide (CeO_2) and indium oxide (In_2O_3) nanoparticles (NPs) exposure on *Arabidopsis thaliana* (L.) Heynh. were investigated and found to influence both the physiological and molecular level parameters (Adopted from Ma et al. 2013)

This chapter touched on the various major industrially relevant NPs currently under study for various different applications. The primary reason for initiating such studies, NP–plant interaction, was to assess the impact of the accidental release of such NPs into the environment and their potential toxicity to the environmental components including plants. Although much data suggested the toxicity of the NPs, evidenced by either germination inhibition or hindering in the growth and development, surprisingly in some cases, the effects were reverse, with noticeable enhancement of growth characteristics. Of course, the effects did vary according to the type, morphological, and chemical characteristics of the NPs and the type of plants being used (Fig. 6.5).

Initial studies were limited to basic germination and developmental features, which to date remains constant. Very few studies went beyond and analyzed the molecular and biochemical aspects of the effects. Therefore, it still remains an elusive task to concretely link a particular NPs' toxicity/beneficial attribute to a specific trait of the NP or the plant as similar NPs can show different effects on similar plant species in different experimental sets.

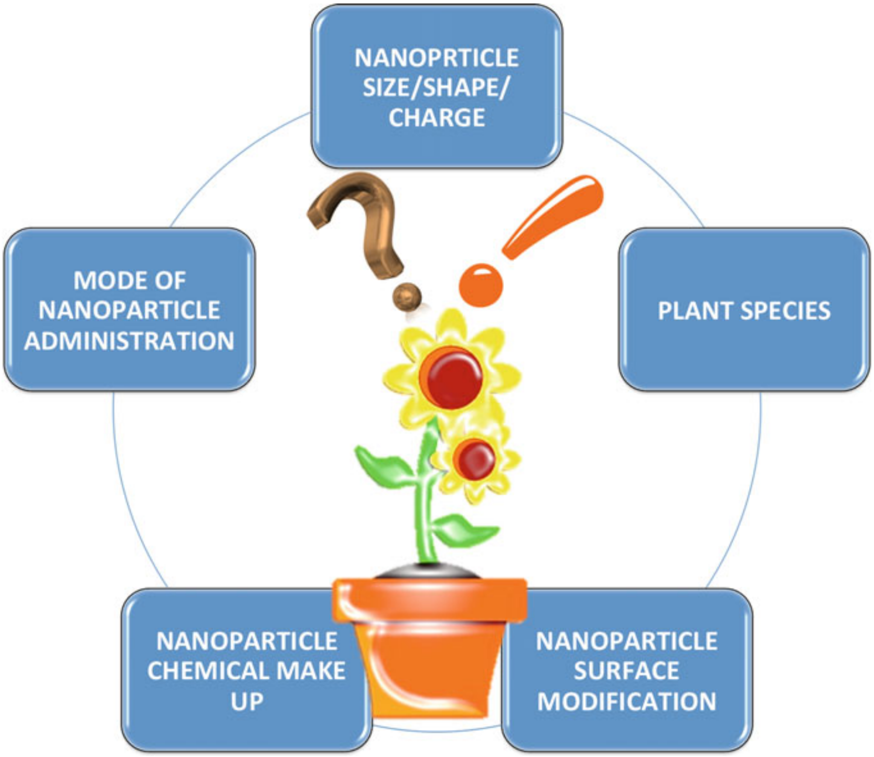


Fig. 6.5 There are multifarious determining factors while studying the effects of nanoparticles on plant system, which have to be cumulatively taken into consideration for a comprehensive understanding of the plant–nanoparticle interactions

6.7 Conclusion

The chapter discloses the use of some of the most important and commercially established NPs on the physiological parameters of various plant subjects. It is clear that the NPs exhibit both beneficial and negative influences. Understanding the chemical and physical processes of plants associated with their growth and development is critical in evaluating the role of NPs in either enhancing or retarding these features. From molecular interactions involved in photosynthesis diffusion of water, minerals, and nutrients to plant development, seasonality, and reproduction need to be thoroughly analyzed prior and post NP application. More comprehensive research needs to be performed to expand knowledge on the alterations induced by NPs on the physiological, biochemical, and molecular mechanisms of plants. Long-term studies need to be designed to assess the NPs role in regulating the physiological processes in plants to construct a database that would be helpful for current and future researchers to progress in the direction of setting a global nano-agro database accessible and useful for all.

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