Chapter 6
Toxicity of Nanomaterials to Plants

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Abstract Nanoparticles have many potential applications, especially in biomedical engineering and agriculture, but the toxicity of nanoparticles to plants has received little attention. Previously, we described an increase in the levels of reactive oxygen species (ROS) in rice (*Oryza sativa*) and *Arabidopsis thaliana* cells after nanoparticle treatments. We found that ROS resulted in programmed cell death and that the nanoparticles caused a dosage-dependent increase in cell death. Since then, accumulating data have indicated that nanomaterials cause toxicity in diverse organisms. Data from our lab and others indicate that we should critically examine the risks of nanoparticles, so that we can safely take advantage of the tremendous potential benefits of this new technology.

Keywords Nanoparticles · Nanomaterials · Plant toxicity · Reactive oxygen species

6.1 Introduction

With recent increases in nanomaterial production and usage in varied applications such as DNA delivery, medicine, and imaging, comes increased opportunities for organisms to be exposed to nanomaterials. Due to their small size and high surface reactivity, nanomaterials can potentially enter into the cell and interact with intracellular structures, which may produce toxicity by diverse mechanisms. Nanoparticles can inhibit the seed germination, reduce seedling, shoot and root growth, delay flowering, and decrease yield (El-Temsah and Joner 2012; Lee et al. 2008; Lin et al. 2009b). Additionally, nanomaterials can cause chromatin

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M.H. Siddiqui et al. (eds.), *Nanotechnology and Plant Sciences*,
DOI 10.1007/978-3-319-14502-0_6

condensation, arrest mitosis, disturb metaphase, break cell walls, and specifically inhibit gene expression (Lin et al. 2009a, b; Kaveh et al. 2013; Kumari et al. 2009). This chapter will review current advances in the study of phytotoxicity of different nanomaterials and speculate on the mechanism of phytotoxicity.

6.2 Current Advances in Phytotoxicity of Nanoparticles

The phytotoxicity of nanomaterials, including carbon-based and metal-based types, is an emerging field and most studies have examined germination, cell cultures, and genetic effects. With the aid of detection techniques such as ion-coupled plasma-mass spectroscopy (ICE-MS), photothermal, and photoacoustic analysis (Khodakovskaya et al. 2011), and Raman spectroscopy, researchers have revealed the phytotoxicity of nanomaterials or nanoparticles.

6.2.1 Toxicity of Nanoparticles

Nanomaterials can be classified as carbon-based or metal-based (Maynard et al. 2011). The chemical characteristics and particle sizes differ between these two types of nanomaterials and thus, they have different negative and positive effects on plants (Table 6.1).

6.2.1.1 Carbon-Based Nanomaterials

Carbon-based nanomaterials include fullerene and carbon nanotubes, which can be single-walled carbon nanotubes and multi-walled carbon nanotubes. According to recent reports, carbon nanotubes and fullerene can cause damage to plants (Begum and Fugetsu 2012; Chen et al. 2010; Liu et al. 2010; Shen et al. 2010). Blossoming of rice plants incubated with C_{70} fullerene was delayed by at least 1 month and their seed-setting rate was reduced by 4.6 % compared to the controls (Lin et al. 2009b). Also, water-soluble fullerene inhibits plant growth and causes shortening of seedling roots and loss of gravitropism (Liu et al. 2010). These adverse effects may be caused by auxin disruption, abnormal cell division, and microtubule disorganization. Shen et al. (2010) found that certain amounts of single-walled carbon nanotubes can induce the production of reactive oxygen species (ROS), which eventually leads to programmed cell death, in *Arabidopsis* leaves, and protoplasts. In red spinach (*Amaranthus tricolor* L), phytotoxicity of multi-walled carbon nanotubes causes growth inhibition and cell death; multi-walled carbon nanotubes also cause ROS production and hypersensitive response-type necrotic lesions of leaf cells and tissues (Begum and Fugetsu 2012). Although plant cells and mammalian cells have different structures, such as the thick and rigid plant cell wall, chloroplasts and large

Table 6.1 The effect of nanomaterials on plants

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
SWCNT (FITC)		Tabacco BY-2 cells		Standard media	No apparent cell death	Liu et al. (2009)
SWCNT	1.19	Rice	400	1/2 MS	Delayed flowering, decreased yield	Lin et al. (2009b)
	1–2	Rice protoplasts	25	W5 media	Decreased viability	Shen et al. (2010)
			250	Injection leaf	Induced chromatin condensation	Shen et al. (2010)
	1–2	Arabidopsis	25	W5 media	Decreased viability	Shen et al. (2010)
		Protoplasts	250	Injection leaf	Induced chromatin condensation	Shen et al. (2010)
MWCNT		Zucchini	1,000	25 % Hoagland solution	Reduced biomass (38 %)	Stampoulis et al. (2009)
		Lettuce	2,000	Aqueous suspension	Reduced root length	Lin and Xing (2007)
	10–30	Rice	20, 40, 80	MS medium	Chromatin condensed inside the cytoplasm and cell death, plasma membrane detachment from cell wall, cell shrinkage	Tan et al. (2009)
	10–35	Tomato	50	MS medium	Upregulation of the stress-related genes	Khodakovskaya et al. (2011)
	11	Red spinach	125, 250, 500, 1000	Modified Hoagland medium	Exhibited growth inhibition, cell death	Begum and Fugetsu (2012)
	20	Tobacco cells	0.1, 5, 100, 500	MS medium	Induced 55–64 % increase of tobacco cell growth over control	Khodakovskaya et al. (2011)
Fullerene C_{60}		Arabidopsis		MS medium	Stress response of gene expression	Landa et al. (2012)
$C_{60}(OH)_{20}$	1.12–1.74 15.69–24.36	*Allium cepa* cell	10 110	Aqueous suspension	Plant cell damage	Chen et al. (2010)
$C_{70}(C(COOH)_2)_4$		Arabidopsis	100	1/2 MS agar	Retarded roots with shortened length and loss of root gravitropism	Liu et al. (2010)
Ag	<100	*Cucurbita pepo*	0, 100, 500	25 % Hoagland's solution	Reduced biomass and transpiration by 66–84 % when compared with bulk Ag	Musante and White (2012)

(continued)

Table 6.1 (continued)

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
Ag	<100	Onion	100	Aqueous suspension	Decreased mitosis; disturbed metaphase; sticky chromosome; cell wall broken	Kumari et al. (2009)
Ag (PVP)	<100	Castor	100, 200, 500, 1000, 2000	Aqueous suspension	No effect on seed germination, root growth, shoot growth	Yasur and Rani (2013)
Ag (PVP-coated)	20	Arabidopsis	1, 2.5, 5, 10, 20	1/2 MS	Biomass declined, upregulated genes associated with the response to metal and oxidative stress, downregulated genes associated with the response to pathogen and hormonal stimuli	Kaveh et al. (2013)
Ag (citrate-coated)	11.0 ± 0.7	Maize	73.4	Aqueous suspension	Inhibition of germination	Pokhrel and Dubey (2013)
		Cabbage	73.4	Aqueous suspension	Inhibition of early development	Pokhrel and Dubey (2013)
	20, 40, 80	Arabidopsis	66.84–534.72 × 10^{-3}	1/4-strength Hoagland media	Less than 80 nm, are phytotoxic to root	Geisler-Lee et al. (2013)
Ag (carbon-coated)	25	Arabidopsis	0.01–100	1/4 strength Hoagland solution	Stimulated root elongation, fresh weight, and evapotranspiration of both plants at a narrow range of sublethal concentrations	Wang et al. (2013)
		Poplar	0.01–100	1/4 strength Hoagland solution	Stimulated root elongation, fresh weight, and evapotranspiration of both plants at a narrow range of sublethal concentrations	Wang et al. (2013)
Ag (PEG-coated)	5, 10	Arabidopsis	0.01–1	1/4 strength Hoagland solution	Stimulated root elongation, fresh weight, and evapotranspiration of both plants at a narrow range of sublethal concentrations	Wang et al. (2013)

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Table 6.1 (continued)

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
		Poplar	0.01–1	1/4 strength Hoagland solution	Stimulated root elongation, fresh weight, and evapotranspiration of both plants at a narrow range of sublethal concentrations	
Cu		Mungbean	<200	Agar culture media	Reduced seedling growth	Lee et al. (2008)
		Mungbean	800	Agar culture media	Reduced shoot growth	Lee et al. (2008)
		Wheat	<200	Agar culture media	Reduced root and seedling growth	Lee et al. (2008)
	50	Zucchini	1,000	1/4 Hoagland solution	Reduced biomass (90 %)	Stampoulis et al. (2009)
	50	Zucchini	1,000	Aqueous suspension	Reduced root growth	Stampoulis et al. (2009)
	<50	*Cucurbita pepo*	100, 500	1/4 Hoagland solution	Reduced growth and transpiration by 60–70 %	Musante and White (2012)
CuO	<100	Radish	10, 100, 500, 1000	NP suspension	Oxidative damage to DNA, inhibited root growth	Atha et al. (2012)
		Perennial Ryegrass	10, 100, 500, 1,000	NP suspension	Oxidative damage to DNA, inhibited root development	Atha et al. (2012)
Si	14	Arabidopsis	250, 1000	Hoagland's No. 2 Basalsalt mixture	No observed effect	Slomberg and Schoenfisch (2012)
	50	Arabidopsis	250, 1000	Hoagland's No. 2 Basal salt mixture	Reduced rosette diameter, biomass, and stem length	Slomberg and Schoenfisch (2012)
	200	Arabidopsis	250, 1000	Hoagland's No. 2 Basal salt mixture	Reduced rosette diameter, biomass, and stem length	Slomberg and Schoenfisch (2012)
	10	Zucchini	1,000	Aqueous suspension	Completely in inhibited germination	Stampoulis et al. (2009)

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Table 6.1 (continued)

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
SiO_2	42.8 ± 3.9	Arabidopsis	400, 2000, 4000	1/2 MS	Increased root length at 400 mg/L, but reduced root length at 2,000 and 4,000 mg/L	Lee et al. (2010)
Fe_3O_4	<50	Arabidopsis	400, 2000, 4000	1/2 MS	Reduced root length	Lee et al. (2010)
Fe_2O_3	10.2 ± 2.6	Mycorrhizal Clover	0.032, 0.32, 3.2 mg/kg	Sand culture (with Arbuscular mycorrhizal fungi)	Reduced biomass	Feng et al. (2013)
					Reduced he biomass	Feng et al. (2013)
Au(+)	2	Rice	1.6 for 5 days	Water suspension	None at this level of AuNP	Koelmel et al. (2013)
			0.14 for 3 months	Major nutrients aqueous suspension	None at this level of AuNP	Koelmel et al. (2013)
Au(–)	3	Rice	1.6 for 5 days	Water suspension	None at this level of AuNP	Koelmel et al. (2013)
			0.14 for 3 months	Major nutrients aqueous suspension	None at this level of AuNP	Koelmel et al. (2013)
Au(0)	4	Rice	1.6 for 5 days	Water suspension	None at this level of AuNP	Koelmel et al. (2013)
			0.14 for 3 months	Major nutrients aqueous suspension	None at this level of AuNP	Koelmel et al. (2013)
Au(–)	3.5	Tobacco	48	1/2 Hoagland's solution	Necrotic lesions observed by 14 days	Sabo-Attwood et al. (2012)
	18	Tobacco	76	1/2 Hoagland's solution	None outward signs of stress	Sabo-Attwood et al. (2012)

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Table 6.1 (continued)

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
NiO	23.34	Tomato	25, 50, 100, 250, 500, 1000, 1500, 2000	Aqueous suspension	Reduced root length and catalase (CAT), glutathione (GSH), superoxide dismutase (SOD) and lipid peroxidation (LPO) exhibit higher activities	(Faisal et al. 2013)
Pd entrapped in $Al(OH)_3$ matrix		Lettuce	0.013–0.06 %(w/w) $Al(OH)_3$ matrix	Aqueous suspension	No effect on germination	Shah and Belozerova (2009)
Al		Ryegrass	2,000	Aqueous suspension	Decreased root length	Lin and Xing (2007)
		Corn, lettuce	2,000	Aqueous suspension	Reduced germination and root length	Lin and Xing (2007)
Zn		Radish, rape, ryegrass, lettuce, corn, cucumber	2,000	Aqueous suspension	Highly reduced root growth	Lin and Xing (2007)
ZnO	9–37 (Mean: 19 ± 7)	Ryegrass	1,000	Hoagland solution	Reduced biomass, shrank root tips, epidermis and root cap broken, highly vacuolated and collapsed cortical cells	Lin and Xing (2008)
		Corn	2,000	Aqueous suspension	Reduced germination	Lin and Xing (2007)
		Radish, rape, ryegrass, lettuce, corn, cucumber	2,000	Aqueous suspension	Highly reduced root growth	Lin and Xing (2007)
	5	Zucchini	1,000	25 % Hoagland solution	Reduced biomass (78–90 %)	Stampoulis et al. (2009)
	8	Soybean	2000, 4000	Aqueous suspension	Decreased root growth	Lopez-Moreno et al. (2010)
	11.0 ± 0.7	Maize	0.01–1,000	Aqueous suspension	Did not inhibit seed germination	Pokhrel and Dubey (2013)
		Cabbage	0.01–1,000	Aqueous suspension	Dose-dependent inhibition of germination	Pokhrel and Dubey (2013)

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Table 6.1 (continued)

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
	<100	Arabidopsis	100	MS media	Stress response of gene	Landa et al. (2012)
	44.4 ± 6.7	Arabidopsis	400, 2000, 4000	1/2 MS	Heavily inhibited seed germination, reduced root length and leaf number	Lee et al. (2010)
	8	Soybean	500, 1000, 2000, 4000	Hoagland's solution	No effect on germination, increased root length at 500 mg/L (30 % over control) and decreased root length at 4,000 mg/L (40 % shorter than control)	Lopez-Moreno et al. (2010)
	44.46 ± 4.84	*Fagopyrum* Esculentum	1, 5, 100, 1000, and 2000	1/2 strength Hoaland's solution	Reduced biomass	Fan et al. (2014)
CeO_2	7	Alfalfa	1000, 2000		Slightly reduced shoot growth	Lopez-Moreno et al. (2010)
		Tomato	2,000	Aqueous suspension	Reduced shoot growth (30 %)	Lopez-Moreno et al. (2010)
		Cucumber	2,000	Aqueous suspension	Reduced shoot growth (20 %)	Lopez-Moreno et al. (2010)
		Maize	500, 1000, 2000	Aqueous suspension	Reduced shoot growth (30 %)	Lopez-Moreno et al. (2010)
		Alfalfa	500	Aqueous suspension	Significantly reduced biomass	Lopez-Moreno et al. (2010)
		Maize	500–2,000	Aqueous suspension	Reduced germination	Lopez-Moreno et al. 2010)
		Maize	4,000	Aqueous suspension	Reduced root growth	Lopez-Moreno et al. (2010)
		Tomato, cucumber	2,000	Aqueous suspension	Reduced germination	Lopez-Moreno et al. (2010)

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Table 6.1 (continued)

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
		Tomato	1,000–2,000	Aqueous suspension	Reduced root growth	Lopez-Moreno et al. (2010)
		Alfalfa	200–4,000	Aqueous suspension	Reduced root growth	Lopez-Moreno et al. (2010)
		Soybean	2,000	Aqueous suspension	Reduced germination	Lopez-Moreno et al. (2010)
		Alfalfa	4,000	NP suspensions	Reduced root growth	Lopez-Moreno et al. (2010)
		Corn	4,000	NP suspensions	Germination significantly reduced, root growth was prompted	Lopez-Moreno et al. (2010)
		Cucumber	4,000	NP suspensions	Germination significantly reduced 30 % at 2,000 mg/L, root growth was prompted	Lopez-Moreno et al. (2010)
		Tomato	4,000	NP suspensions	Germination significantly reduced 20 % at 2,000 mg/l, root growth was prompted	Lopez-Moreno et al. (2010)
		Cilantro	62.5–500 mg/kg	Organic potting soil	Large roots, longer shots	Morales et al. (2013)
		Arabidopsis	250	1/2 MS medium	Increased plant biomass	Huang et al. (2012)
			500–2,000	1/2 MS medium	Decreased plant growth by up to 85 %	Huang et al. (2012)
			1,000–2,000	1/2 MS medium	Reduced chlorophyll production by 60 and 85 %	Huang et al. (2012)
CeO_2 (uncoated)	17–100	Wheat	100	10 % Hoagland	No phenotypic response	Schwabe et al. (2013)

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Table 6.1 (continued)

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
	17–100	Pumpkin	100	20 % Hoagland medium	No phenotypic response	Schwabe et al. (2013)
Al_2O_3	13	Maize, cucumber, carrots, cabbage	2,000	Aqueous suspension	Reduced root growth	yang and Watts (2005)
		Corn	2,000	Aqueous suspension	Reduced root length	Lin and Xing (2007)
	~150	Arabidopsis	400, 2000, 4000	1/2 MS	Positive influence on root elongation	Lee et al. (2010)
	<50	Tobacco BY-2 cells	0.01-0.1	Liquid MS medium	Diminished cell viability, damaged plasma membrane, reduction in the activities of both dehydrogenase and oxidoreductase	Poborilova et al. (2013)
	8	Tomato	104, 315, 1750	Suspension (35:65CNT:poly-3-aminobenzoic acid)	Root reduction	Canas et al. (2008)
TiO_2	30	Maize	30, 1000	10 % strength Hoagland solution	Inhibited leaf growth, and transpiration	Asli and Neumann (2009)
	<150	Arabidopsis	100	MS medium	Little effect on gene expression	Landa et al. (2012)
		Arabidopsis	1×10^6	Soil	Increased green content	Lenaghan et al. (2013)
		Algae	1×10^6	Soil	Decreased green content	Lenaghan et al. (2013)
	35	Garden peas seed	100, 250, 500, 750, 1000	Suspension	No effect on the germination of peas grown, and the gross root structure	Fan et al. (2014)
	35	Garden peas	250, 500, 750	FP medium	Decreased the number of secondary lateral roots	Fan et al. (2014)
	5	Arabidopsis	0.25 %	Sprayed with suspension	Increased harvesting light energy	Ze et al. (2011)

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Table 6.1 (continued)

Nanomaterial	Particle size (nm)	Plant	Concentration (mg/L)	Growth condition	Effects	References
TiO_2 (colloidal)	5	Spinach	0.25 %	Aqueous suspension	Promoted antioxidant stress of spinach chloroplasts	Lei et al. (2008)
TiO_2 (Alizarin red S)	2.8 ± 1.4	Arabidopsis	20 μM	1/2 MS medium	Caused reorganization and elimination of microtubules	Wang et al. (2011)
TiO_2 (Alizarin red S)	2.8 ± 1.4	Arabidopsis	5 μM	Aqueous solution	Do not impose an excessive oxidative stress on the root tissues	Kurepa et al. (2010)
In_2O_3	20–70	Arabidopsis	25–2,000		No effect on biomass, 15 % on root elongation	Ma et al. (2013)
Quantum Dot	6.3 + 0.7	Arabidopsis	5.8 nM	1/4 strength	The ratio of reduced glutathione levels (GSH)	Navarro et al. (2012)
CdSe				Hoagland's solution	Relative to the oxidized glutathione (GSSG) in plants decreased	
MPA-linked CdSe QDs		Rice		Aqueous solution	Germination was arrested with quantum dots	Nair et al. (2011)
QD-COOH		WTK1 cells	25, 50, 100, 200, 400	Aqueous solution	Caused DNA damage	Hoshino et al. (2004)
QD-NH_2		WTK1 cells	50, 100, 200, 400	Aqueous solution	No significant effect	Hoshino et al. (2004)
QD-OH		WTK1 cells	50, 100, 200, 400	Aqueous solution	No significant effect	Hoshino et al. (2004)

central vacuoles, they show similar responses to fullerene (Chen et al. 2010). The bioaccumulation of dichlorodiphenyldichloroethylene, a persistent and estrogenic pollutant, in some food crops such as zucchini, soybean, and tomato, increased in the presence of C_{60} fullerene (De La Torre-Roche et al. 2012). Additional work investigated and found carbon nanotubes can penetrate the cell membrane of plant protoplasts (Serag et al. 2011a). Moreover, researchers (Avanasi et al. 2014) have measured plant uptake of C_{60} fullerene and found that ^{14}C-labeled C_{60} can be slowly absorbed by plants and will likely persist in soil for a long period.

6.2.1.2 Metal-Based Nanomaterials

Metal-based nanoparticles hold an important place due to their application prospect in the group of nanoparticles, and include metal oxide and metallic nanoparticles. Accumulating data indicate that metal-based nanoparticles usually inhibit development and can cause genetic damage in plants.

Atha et al. (2012) found that CuO nanoparticles can cause oxidative DNA damage in terrestrial plants such as radish, annual ryegrass, and perennial ryegrass. Landa et al. (2012) used microarray analysis to demonstrate that exposure of *Arabidopsis* to TiO_2, ZnO, and fullerene leads to distinct changes in the expression of stress genes. Also, the genes upregulated in response to nanoparticle treatments were primarily associated with the response to metals and oxidative stress, while downregulated genes were mainly involved in cell organization and biogenesis, indicating that phytotoxicity is highly dependent on the type of nanoparticle (Atha et al. 2012; Landa et al. 2012). Another report investigated the effects of cerium oxide (CeO_2) exposure on wheat and pumpkin by using hydroponic plant culture. CeO_2 nanoparticles have only minor effects and no growth reduction or toxic response was observed (Schwabe et al. 2013), but catalase and ascorbate peroxidase activity significantly increased.

Metallic nanoparticles release ionic salts, and the nanoparticles and ionic salts have similar effects; this prevents us from discerning whether metallic nanoparticles exert particle-specific toxicity. Ag nanoparticle toxicity in *Arabidopsis* is size and concentration dependent. Ag nanoparticles can be apoplastically transported in the cell wall and aggregate at plasmodesmata (Geisler-Lee et al. 2013). Research on *Lemna gibba* showed that the intracellular uptake of Ag directly involved Ag nanoparticles, and the induced oxidative stress was highly related to Ag accumulation inside plant cells (Geisler-Lee et al. 2013). Pokhrel and Dubey's observation of a 'tunneling-like effect' upon treatment with ZnO nanoparticles and varied metaxylem frequency with exposure to nanoparticles or free ions suggest that potential risks of metal nanoparticles, including their free ions, may affect the growth and development of agriculturally important plants such as maize and cabbage (Pokhrel and Dubey 2013). Research on Au nanoparticles shows that they are toxic to rice and can accumulate in the above-ground organs of the plant (Koelmel et al. 2013). Although currently we cannot eliminate the toxic response of ionic salts, the crucial effect of free ions released by metal-based nanoparticle should not be ignored.

6.2.1.3 Quantum Dots

Quantum Dots (QDs), which are often described as "artificial atoms," are one of the first nanotechnologies to be widely used in the biological sciences and are anticipated to eventually find applications in a number of commercial consumer and clinical products (Klimov 2007; Valizadeh et al. 2012). QDs exhibit unique luminescence characteristics and electronic properties, such as wide and continuous absorption spectra, narrow emission spectra, and high light stability (Bruchez et al. 1998). QDs demonstrate great potential for labeling cells, tracking particles, and harvesting solar energy. Nonetheless, before we take advantage of this new material, we should evaluate the risk of its toxicity to organisms. Nair et al. (2011) placed rice seeds in CdSe QDs and observed that the QDs inhibited germination. QDs can cause DNA damage and suppress the proliferation of cells in culture (Hoshino et al. 2004). Cell damage and even cell death can be induced by mercapto-undecanoic acid QDs (Shiohara et al. 2004). Although the exposure of single-walled carbon nanotubules to plants induced positive effects, the addition of QDs to the nanotubules dramatically changed the viability of the tomato plants by significantly accelerating leaf senescence and inhibiting root formation (Alimohammadi et al. 2011). Moreover, *Arabidopsis* root exposure to QDs could induce oxidative stress, as revealed by changes in the GSH/GSSG ratio (Navarro et al. 2012). Also, observation of QD transport and fate in soil, plants, and insects indicates that QDs may be transported across the environment (Al-Salim et al. 2011).

Unlike carbon-based nanomaterials, metal-based nanomaterials include many more types, and have more complex physical characteristics, which may lead to more complicated interactions between nanomaterials and plants. Given that metal-based nanomaterials have tremendous applications in industry, we should critically examine the toxicity of nanomaterials toward not only animals, but also plants.

6.2.2 Positive Effects of Nanoparticles

Although the phytotoxicity of nanomaterials in plants has been intensively reported, some studies of nanomaterials indicate that their phytotoxicity has limits, and some nanomaterials also can facilitate the growth and development of plants. For example, carbon nanotubes can enhance water uptake and growth in gram plants (*Cicer arietinum*) (Tripathi et al. 2011), and 500–4,000 mg/L ZnO can promote the root growth of soybean (Oberdorster 2010). A stimulatory effect was observed on root elongation, fresh weight, and evapotranspiration of both *Arabidopsis* and poplar at a narrow range of sublethal concentrations of Ag nanoparticles coated with both carbon and polyethylene glycol (Wang et al. 2013). Also, SiO_2 nanoparticles can increase root length at 400 mg/L (Lee et al. 2010) and CeO_2 nanoparticle suspensions promoted the root growth of corn and cucumber, even though the germination rate declined (Lopez-Moreno et al. 2010). Cilantro grown in organic potting soil had longer shoots and larger roots with CeO_2 nanoparticles (Morales et al. 2013). *Arabidopsis* grown in MS media containing Al_2O_3 nanoparticles had longer roots (Lee et al. 2010) and TiO_2 nanoparticles can also increase the chlorophyll content of *Arabidopsis* (Lenaghan et al. 2013).

Khodakovskaya et al. (2011) discover that multi-walled carbon nanotubes can upregulate stress-related genes such as pathogen defense genes and water-channel genes. And their further research suggested that these nanotubes can enhance the growth of cultured tobacco cells by the activation of water channels and major gene regulators of cell division and extension, such as *NtPIP1, CycB*, and *NtLRX1* (Khodakovskaya et al. 2012). Researchers suggested the mechanism of the positive effect is the photocatalyzed character of nanomaterials and upregulation of water-channel protein in plants exposed to carbon nanomaterials (Ze et al. 2011). Recently, Giraldo et al. (2014) exploited the interaction between plant organelles and nonbiological nanostructures to augment photosynthesis and biochemical sensing in plants. This plant nanobionics approach can enhance the efficiency of photosynthesis and has the potential to detect real-time nitric oxide in chloroplast and leaves. The increase in absorption spectrum and electron transport rates caused by carbon nanotubes contributed to the enhancement of photosynthesis (Lee et al. 2010). Although several studies support the idea that electron transfer between carbon-based nanomaterials can increase photosynthesis, the specific mechanisms of various nanomaterials need further research (Boghossian et al. 2011; Giraldo et al. 2014; Ham et al. 2010).

6.2.3 Factors Affecting the Toxicity of Nanoparticles

It is difficult to determine the specific mechanism of phytotoxicity, because of uncertainty in the elements contributing to toxicity. Oberdorster (2010) compared nanoparticles with bulk particles, and concluded that twenty-two aspects could alter their biological effects. Several crucial factors affect the phytotoxicity of nanomaterials: the concentration of nanomaterials, the size and specific area of the particles, the physicochemical properties and stability of the particles, the species of plant and their developmental stage, the growth media, and the solution of the nanomaterials, etc.

Toxicity assessment of CeO_2 nanoparticles in cilantro grown in organic soil showed that the activity of catalyze and ascorbate peroxidase increased significantly only at a concentration 125 mg/kg (Morales et al. 2013). Four edible plants, including alfalfa, corn, cucumber, and tomato, show differential responses to CeO_2 nanoparticles (Lopez-Moreno et al. 2010). Treatment with 2,000 mg/L CeO_2 nanoparticles reduced the germination rate of corn, cucumber, and tomato, but did not cause significant reduction for alfalfa. Also, the root and stem growth of these four plants was differentially inhibited by CeO_2 nanoparticles. Although cucumber germination was not strongly affected by CeO_2 nanoparticles, its root and stem growth were significantly inhibited.

In addition, surface modification can change the cellular interactions of nanoparticles and modify their mechanism of toxicity. The toxic effects of CuO nanoparticles were found to be mainly driven by the solubilization of particles into toxic metal ions, while polymer coating of CuO nanoparticles changed the mechanism of nanoparticle toxicity to *L. gibba*, resulting in a more important contribution of ROS formation and decreasing plant growth even at a low concentration (Perreault et al. 2014). Surface modification of QDs changed their physicochemical properties. In addition, the cytotoxicity of QDs depends on their surface molecules. The properties of QDs are not related to those of the QD-core materials but to molecules covering the surface of QDs (Shiohara et al. 2004). According to the research on phytotoxicity of Si nanoparticles, the phytotoxic effect of Si nanoparticles is pH-dependent (Geisler-Lee et al. 2013). Furthermore, leaf necrosis caused by Au nanoparticles depends on particle size; 18 nm Au nanoparticles do not induce necrosis, but 3.5 nm Au nanoparticles do (Sabo-Attwood et al. 2012).

6.3 Mechanism of Phytotoxicity

The phytotoxicity of nanoparticles has been well documented, but their mechanism of phytotoxicity remains unclear. Dietz and Herth (2011) clearly put forward five models for the interaction between nanomaterials and organisms: (1) metal ions released by nanoparticles in solution produce a chemical effect; (2) the hard, spherical particles produce mechanical effects; (3) the nanoparticle surface produces catalytic effects; (4) the nanoparticle surface binds proteins, either by non-covalent or covalent mechanisms or causes oxidative effects; and (5) the nanoparticles change the chemical environment, especially the pH. According to the recent research, the mechanism of phytotoxicity may include elements of the five models mentioned above and other effects. The influence of nanoparticles on microorganisms can also play an important role in the environment.

6.3.1 Uptake of Nanomaterials

Assessing the toxicity and safety of nanoparticles requires an understanding of the uptake of nanoparticles. Most research has focused on determining the nature of the phytotoxicity of nanoparticles, but quantitative methods for measuring nanoparticles in plant tissues have not been established. Therefore, research on the uptake of nanoparticles by plants has not reached a conclusive verdict. According to the limiting pore sizes in cell walls (Carpita et al. 1979), nanoparticles smaller than 5 nm may have the capacity to traverse the intact cell wall efficiently. Carbon-based nanomaterials, such as fullerene C_{60} and fullerol, accumulate in plants, and most metal-based nanoparticles can be absorbed by plants and get accumulated in plant tissues (Rico et al. 2011).

Hitherto, several methods have been used directly to observe nanoparticles in plants, such as optical emission spectroscopy, X-ray absorption spectroscopy, Alizarin red S labeling, X-ray fluorescence, transmission electron microscopy (TEM), and fluorescein isothiocynate (FITC) labeling (Lin et al. 2009b; Lopez-Moreno et al. 2010; Kurepa et al. 2010; Serag et al. 2011a). Protoplast systems can also be useful to detect nanoparticles (Shen et al. 2010). For instance, Serag et al. (2011a) suggested that carbon nanotubes can traverse the plant cell membrane via a direct penetration mechanism, rather than endocytosis. However, other reports support the idea that endocytosis plays an essential role in the uptake of nanoparticles. Liu et al. (2009) pretreated the cells with wortmannin, an inhibitor of plant cell endocytosis, and the cellular fluorescence of single-walled carbon nanotubes stained with FITC decreased significantly, which implies that endocytosis functions as a main pathway for carbon nanotubes to enter the plant cell. Furthermore, end caps or carbon shells at the tips or nanotubes can facilitate the endocytosis of nanomaterials (Shi et al. 2011).

Additionally, using inhibitors such as probenecid (an inhibitor of carrier-mediated transport) and Exo1 (an inhibitor of ADP ribosylation factors) or changing the pH of the media can facilitate or inhibit the translocation and uptake of single-walled carbon nanotubes in *Catharanthus roseus*. Consequently, the trafficking of carbon nanotubes through the subcellular membranes of the plant cell involves a carrier-mediated transport (Serag et al. 2011b). Interestingly, Giraldo et al. (2014) reported that single-walled carbon nanotubes can passively transport and irreversibly localize in the lipid envelope of extracted plant chloroplasts, where they promote photosynthetic activity and enhance maximum electron transport rates (Giraldo et al. 2014). Taken together, the mechanism of the uptake of nanomaterials depends on the specific characteristics of the material; therefore, multiple mechanisms may be involved.

6.3.2 Metal Nanoparticles

Metal-based nanoparticles can release metal ions during exposure. Heavy metal ions, including redox active, (e.g., Cu, Fe) and non-redox active, (e.g., Cd, Ni) types, can induce ROS or perturb the redox balance in cells, thereby contributing to cell damage (Sharma and Dietz 2009). Many metals, such as Ag, Au, Fe, and Co, catalyze chemical reactions, especially reduction–oxidation reactions. Nanoparticles entering into cells can release metal ions that may alter proteins. Mechanical effects depend on the size, instead of the chemical properties of the particle (Dietz and Herth 2011). For example, the high concentration adsorption of hydrophobic nanoparticles onto the plant cell wall and their retention within the cell wall can cause cell damage and nanoparticles may clog pores on the cell wall, which can inhibit water uptake or cause physical damage to the cell wall (Chen et al. 2010). The ability to pass through the cell wall might not be a prerequisite for causing oxidative stress and toxicity. Some researchers suggest that despite nanomaterials' inability to pass through the cell wall of plants, they can cause oxidative stress, and eventually lead to chromosome condensation (Shen et al. 2010). Similarly, CuO nanoparticles can also cause oxidative damage to plant DNA and can be detected in plant cells (Atha et al. 2012).

Surface effects have engaged a great deal of attention in the field of nanotoxicology. Particles with an oxic surface often form a layer of OH^- groups at the surface; these negatively-charged groups attract positively-charged side groups of proteins (Dietz and Herth 2011). Rice plants hydroponically exposed to positively, neutrally, and negatively-charged Au nanoparticles bioaccumulated Au nanoparticles and the organ level distribution depended on the surface charge of the nanoparticles, with negatively-charged, more toxic Au nanoparticles accumulating the most in above-ground organs (Koelmel et al. 2013). The cytotoxicity of QDs also depends on their surface molecules (Begum and Fugetsu 2012).

Experiments based on cell culture in Murashige and Skoog medium or Hoalgand's aqueous medium, or protoplast systems are not sufficient to provide information on the real toxicity of nanoparticles. The influence of nanoparticles on plants systems is more complicated than expected in soil-grown plants. Nanoparticles can affect microorganisms in the soil and then indirectly affect the growth of plants. For example, two metal nanoparticles affect arbuscular mycorrhizal fungi and therefore substantially alter plant growth (Feng et al. 2013). There are few reports of the interactions among plants, nanoparticles, and microorganisms, and further research can help us to identify actual risks of the utilization of nanoparticles.

6.3.3 Reactive Oxygen Species

Previously, we described an increase in levels of ROS in rice and *Arabidopsis* cells after nanoparticle treatments (Shen et al. 2010). We found that the stress of ROS resulted in programmed cell death and the effect of nanoparticles on cells was dosage-dependent. Similar to many abiotic and biotic stressors, the most common general stress symptom of nanoparticle toxicity appears to be the development of oxidative stress by enhanced production of ROS and peroxidative processes (Oberdorster et al. 2007). Hitherto, ROS is one of the crucial biomarkers of nanoparticle toxicity and can be measured by the direct quantification of ROS or by activity of anti enzymes such as superoxide dismutase (SOD), or catalase (CAT) (Begum and Fugetsu 2012; Faisal et al. 2013; Lin et al. 2012; Oukarroum et al. 2012; Perreault et al. 2014; Shaw and Hossain 2013; Thwala et al. 2013).

The work of Poborilova et al. (2013) in suspension cultures of tobacco BY-2 cells, showed that Al_2O_3 nanoparticles exhibited toxicity that was only connected with the generation of reactive oxygen and nitrogen in both concentration- and time-dependent ways. The protection against carbon nanotube-induced toxicity by the addition of ascorbic acid supports the idea that carbon nanotubes principally promote ROS generation (Begum and Fugetsu 2012). Ag nanoparticles cause strong decreases in chlorophyll content and viable algal cells, and also increased ROS formation and lipid peroxidation in green algae (Oukarroum et al. 2012). Nano-CuO treatment causes oxidative damages to rice seedlings, as evident from high activity of ROS-scavenging antioxidant enzymes (Shaw and Hossain 2013).

A plausible mechanism of NiO nanoparticle-induced cellular toxicity in tomato root cells was put forward by Faisal et al. (2013). NiO-nanoparticles can traverse the cell wall and enter into the tomato cell and ROS are plausibly produced by surface effect and metal ions. After ROS are produced, they can translocate as a signal to the nucleus and mitochondria. ROS then cause peroxidation of MAPKs or PARP and perturb the balance of antioxidant defenses. These eventually lead to the cell death of tomato cells (Sharma and Dietz 2009). Our observations using cerium chloride detected by TEM also confirm that ROS production induced by nanoparticles on cell wall and plasma membrane directly contribute to downstream cell death (Shen et al. 2010). However, further studies should be done to search for general mechanisms of toxicity of nanoparticle-induced ROS production.

6.4 Summary

The application of nanomaterials in agricultural fields is currently limited. Recent reports (Feng et al. 2013; Landa et al. 2012; Shen et al. 2010) demonstrate the potential of nanomaterial treatments to increase defenses against pathogen attack. Nanomaterials can upregulate the expression of biotic stress genes and directly affect certain pathogens (Elumalai and Vinothkumar 2013). Moreover, some results demonstrate that spinach leaves remained green after treatment with nano TiO_2, even under culture in N-deficient conditions (Yang et al. 2007). Despite increasing excitement based on such examples, we must critically examine the risks of nanoparticles and then take advantage of the tremendous potential benefits of this new technology. For example, reduction of shoot growth is a commonplace phenomenon, even though the aerial organs of the plant do not directly touch the material (Slomberg and Schoenfisch 2012). How can nanomaterials affect the aerial organs of the plant? There are three speculations: first, nanomaterials can directly influence the growth of roots, and then influence the growth of the whole plant, including the shoot or rosette. Second, when the nanomaterials contact roots, an abiotic/biotic stress signal is produced and this signal may alter the aerial organs. Third, nanomaterials can cross the cell wall of root cells and move inside the plant, which may eventually influence the aerial organs. Since the aerial organs of plants provide important food sources for human beings, we need further research to evaluate the toxicity caused by nanomaterials.

So far, although nanotoxicology has attracted more attention and recent research achievements have improved our understanding of the mechanism of phytotoxicity, the following issues still puzzle us: (1) the effect of metal ions released by metal-based nanomaterials cannot be eliminated when we test phytotoxicity,

(2) The mechanism of transportation of nanomaterials through the cell wall remains unclear, and (3) The role of ROS in the interaction between nanomaterials and plants, whether as a signal or a direct cause of damage, also remains unclear.

Finally, the trend of the usage of nanomaterials cannot be halted because of their great potential applications and improvements in efficiency of both protein and DNA delivery to plant cells. However, due to their small size, nanoparticles can enter our life cycle and affect important elements in our environment, such as the atmosphere, aquatic environment, and soil. Plants are widespread and form the foundation of the food chain; therefore, the effects of nanoparticles on plant life cycle should be assessed. Further research on the phytotoxicity of nanomaterials can help us avoid the risk of damaging our environment.

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