

Chapter 8

Uptake, Translocation, Accumulation, Transformation, and Generational Transmission of Nanoparticles in Plants

Pradeep Kumar Shukla, Pragati Misra and Chittaranjan Kole

Abstract The field of plant nanotechnology has recently been up-surged into a new epoch of discovery to dissect the intricate processes and mechanisms for better understanding of plant's functional biology in response to nanoparticle exposure. This chapter reviews the current scenario of pathways, mechanisms, and patterns of uptake, translocation, accumulation, transformation, and generational transmission of nanoparticles in plants. Experimental data support that symplastic route is the dominant and highly regulated pathway for transporting NPs within plants and facilitated by a vast array of carrier proteins, aquaporins, interconnected ion channels, endocytosed pathway, or novel pores for the entry of nanoparticles. Xylem being the most preferred plant tissue along with phloem and stomatal opening for absorption and transportation of nanoparticles. Engineered and carbon-based nanoparticles have shown different responses for their transport and utilization in different plants. Engineered nanomaterials are translocated and accumulated differentially within stems, leaves, trichomes, petioles, and fruits of different plants. At subcellular locations, engineered nanomaterials are accumulated in cell walls, cytoplasm, seldom plastids, nuclei, and small vesicles. Carbon-based

P.K. Shukla (✉)

Department of Biological Sciences, School of Basic Sciences,
Sam Higginbottom Institute of Agriculture, Technology and Sciences,
Allahabad 211007, UP, India
e-mail: pradeepshuklak@yahoo.co.in

P. Misra

Department of Molecular and Cellular Engineering, Jacob School of Biotechnology
and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences,
Allahabad 211007, UP, India
e-mail: pragati.misra@shiats.edu.in

C. Kole

Bidhan Chandra Agricultural University, Mohanpur 741252, West Bengal, India
e-mail: ckole2012@gmail.com

Present Address:

C. Kole

Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute
of Agriculture, Technology and Sciences, Allahabad 211007, India

nanomaterials have shown superior prospective for internalization. Uptake, accumulation, and generational transmission of NOM-suspended carbon nanoparticles in rice plants have been reported. Uptake and biodistribution of fullerol was confirmed almost in all plant organs including petioles, leaves, flowers, and fruits in bitter melon. Carbon nanotubes have shown the possibilities for effective penetration into seed coat. Single-walled carbon nanotubes have shown their capability to penetrate chloroplasts and accumulate on thylakoids and stroma in spinach, whereas, multi-walled carbon nanotubes were observed in the seeds and root systems of the developed tomato seedlings. It is certain that not a single transportation mechanism, but a diverse array of multiple mechanisms at physiological, biochemical, and molecular levels are involved for penetration, acquisition, and in planta trafficking of nanoparticles. The goal of this chapter is to put individual experimental efforts back together to unveil the possible enigmas of mechanisms of internalization of nanoparticles, pathways of their movement, and patterns of accumulation and their generational transmission.

Keywords Nanoparticles • Engineered nanomaterials • Carbon-based nanomaterials • Uptake-mechanism • Translocation-pattern • Generational transmission

8.1 Introduction

The chemistry of the Earth is unimaginably complex mostly due to a splendid tangled web of interdependencies of living and lifeless components that include a vast, diverse, and global array of naturally occurring nanomaterials (Wiesner et al. 2011).

To understand the potential benefits of applying nanotechnology to agriculture, the primary step should be to analyze penetration, transport, interaction, and possible significant roles of nanoparticles (NPs) in plants (Lee et al. 2008). Uptake, translocation, and accumulation of NPs may depend on the plant species and the size, kinds, chemical composition, and stability of the NPs (Rico et al. 2011).

The impact of natural, engineered, and incidental nanomaterials (NMs) on higher plants and their beneficial and harmful effects in different plant systems at the physiological, biochemical, and genetic levels has recently been examined and documented in the literatures (Yang and Watts 2005; Zheng et al. 2005; Lin and Xing 2007; Torney et al. 2007; Lei et al. 2008; Zhu et al. 2008; Lin et al. 2009; Ma et al. 2010; Rico et al. 2011; Miralles et al. 2012a; Bhattacharya et al. 2012; Prasad et al. 2012; Remedios et al. 2012; Kole et al. 2013; Azimi et al. 2014; Rad et al. 2014; Shyla and Natarajan 2014; Chutipajit 2015; Cicek and Nadaroglu 2015; Roohizadeh et al. 2015; Ebbs et al. 2016). These reports explain the effect of different nanomaterials, alone or in combination, on diverse types of plants/vegetation at different growth and developmental stages, but the vital questions regarding the uptake, accumulation, translocation, and transmission of nanomaterials in plant cells and tissues are still unsolved (Navarro et al. 2008). The cell wall of plants, algae, and fungi is the primary site for the interaction and a

barrier for the entrance of engineered nanoparticles (ENPs). Mechanisms allowing ENPs to penetrate through cell walls and membranes are yet to be well understood. Inside cells, ENPs might directly elicit alterations of membranes and other cell structures and molecules, as well as protective mechanisms (Navarro et al. 2008). The cell wall of plants prevents the entrance of different kinds of elements into cells, and the NPs having a lesser diameter than the pores of cell wall can, therefore, easily cross the pores and can penetrate inside the cell. Nanoparticles can also utilize stomata and/or also the base of hairs for entry into the leaves' surface, and are then transported to different organs of the plant (Nair et al. 2010). Among the carbon-based NPs, only the fullerene C₇₀ and fullerols have been reported to get readily accumulated in plants. Conversely, most of the metal-based NPs were found to be taken up and accumulated in plants, although some conflicting data exists (Rico et al. 2011).

Engineered nanomaterials (ENMs) can play pivotal roles to regulate photosynthetic processes, oxidative stress, antioxidative enzyme activity, radical scavenging ability, gene expression, and macromolecular (DNA, protein, carbohydrates, fatty acid, lignin) modification within edible plants (Rico et al. 2011). The absorption of minerals by the plant is nonselective; some of these metal ions (in conjunction with anions) may be toxic beyond the tolerance limit of the plant. After the absorption, NPs are subsequently translocated and finally accumulated in different parts of the plants establishing complex with carrier proteins. Selection criterion of a particular NP by a specific plant species while rejecting other NPs remains unclear. If NPs are larger than the diameter of pores present in the root hairs, they tend to accumulate at the surface, and if NPs are smaller, they get absorbed and transported to other parts of the plants. Some NPs are accumulated in extracellular space, while others are inside the cell (Husen and Siddiqui 2014). The understanding of ionic metal transport in plants may not accurately predict ENPs' transport mechanism (Ebbs et al. 2016). The present review provides a basic platform to understand the possible mechanisms of NP uptake, transport, internalization, and their generational and transgenerational transmission.

8.2 Physiological Aspects of Possible Mechanisms of Uptake, Transport, and Accumulation of Nanoparticles in Plants

Uptake and transport of NPs are integral to their successful functioning in the plant systems. Experimental data are very limited, and many proposed mechanisms are under intense debate to explain uptake, transport, and accumulation of NPs. Accumulation and transformation of NPs in plant cells and tissues suggest a possible mechanism for NP penetration (Lin et al. 2009). Proper understanding of mobilization/remobilization mechanisms of nutrient elements in particulate forms and their conversion into plant operational forms in planta could provide a

promising pathway for micronutrient transport as NPs or the packaging of nutrients in general, in NP encapsulations, which are also capable of being taken up intact by plants (DeRosa et al. 2010; Gogos et al. 2012; Zhang et al. 2012). The uptake and distribution of metal ion/metal itself in the plant is a matter of debate and challenge to the scientific community. It is not clear whether nanocrystals are formed outside the plants and then transported through the membrane into various parts or the NPs are formed within the plant by the reduction of the metal salt (Husen and Siddiqui 2014).

Particulate forms of mineral nutrients could be mobilized and remobilized via the xylem and phloem, respectively (Wang et al. 2012a, b, 2013a, b, c). It is now well-known that plasma membrane (via apoplast) and plasmodesmal (symplastic) transport mechanisms both play central roles in nutrient internalization along with the water. Water, from the soil, can be absorbed by the roots and then can move radially across into xylem tracheary elements. Subsequently, xylem structures are important determinants to regulate the speed of water transport, and different xylem structures may validate diverse uptake kinetics of NPs (Fig. 8.1) (Ma et al. 2010; Rico et al. 2011; Mishra et al. 2014). Root also absorbs water-dissolved minerals, and these metal salts ascend in ionic form and subsequently are reduced to elemental form as NPs. Water movement through the root apoplast is driven only by pressure gradients, while transport across a membrane-delimited pathway implicates capillary

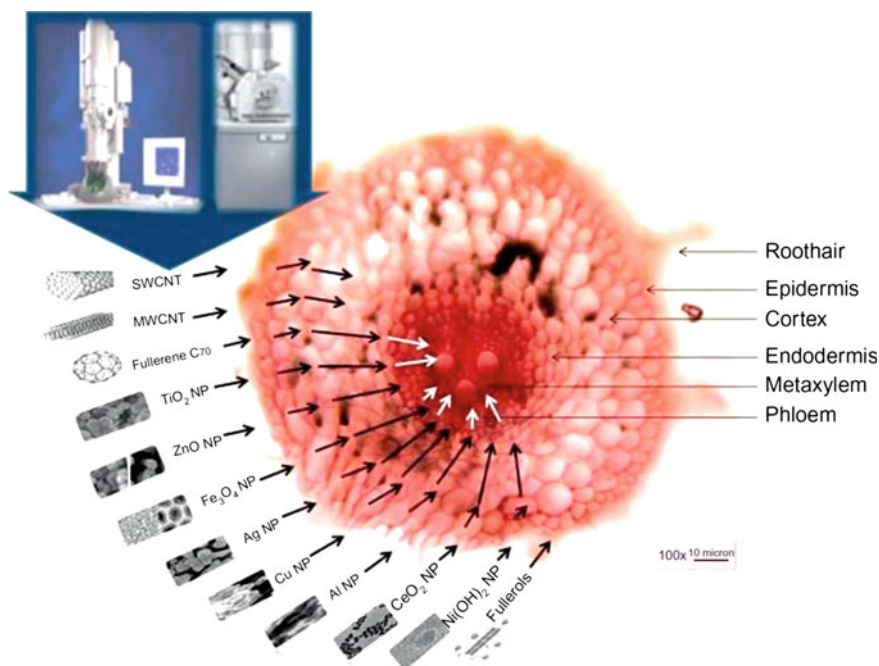


Fig. 8.1 Schematic representation showing uptake of metal-based NPs and carbon-based NPs by root system. Root anatomical structures represent internalization, cellular translocation, and cellular localization of different NPs in root zones [adapted from Mishra et al. (2014)]

action, osmotic pressure, and osmotic gradients (Gardea-Torresdey et al. 2005; Patrick et al. 2015). The transportation of NPs is supposed to pass through the epidermis and cortex and finally to stele of the plant (Shankar et al. 2003).

The stele is the central part of the root containing the pith (if present), vascular tissue, and pericycle and occurs on the inside of the endodermis. At strategic locations (endodermis and sometimes exodermis), the root apoplast is blocked by casparian bands composed of lignin deposited in cell walls. Therefore, it is prerequisite for nutrients to penetrate plasma membranes of each endodermal cell and transport to the stele through the symplasm or transcellularly, or after effluxed from endodermal cells via the apoplast. Some endodermal and exodermal cells have a conspicuous absence of lignin and suberin lamellae and are referred to as passage cells (Fig. 8.2) (Patrick et al. 2015). Also, mobilization of NPs is known to be very prompt, ensuring participation of phloem transport and confirming the nutrient availability to all parts of the plant. Further, the presence of NPs was confirmed in extracellular space and within some cells in the *Cucurbita* plants (Gonzalez-Melendi et al. 2008). The results obtained from both lower and higher plants demonstrate that ion uptake is characterized by (White 2012): (1) Selectivity: Certain mineral elements are taken up preferentially, while others are discriminated against or almost excluded; (2) accumulation: The concentration of elements can be much higher in cell sap than in the external solution; and (3) genotype: There are distinct differences between plant species in their ion uptake characteristics.

ENMs may diffuse in the space between the cell wall and plasma membrane (through porous cell walls): a route well-known as the apoplastic pathway (Lin et al. 2009). Transmission electron microscopy (TEM) images of root cross sections confirmed the presence of NPs in the apoplast, cytoplasm, and nuclei of the endodermal cells in ryegrass (*Lolium perenne*) (Lin and Xing 2008). Through the apoplast, particles may directly reach the endodermis without crossing the edge of epidermal and cortical cells. However, aggregates often accumulate in the endodermis as a result of the significant apoplast barrier imposed by the waxy casparian strip (Larue et al. 2012a; Zhao et al. 2012b; Patrick et al. 2015). For efficient translocation, ENMs in apoplastic flow must eventually unify into the symplasm so as to penetrate into vascular system (Deng et al. 2014).

The symplastic route is hypothesized to be the more important and highly regulated pathway for transporting ENMs within crops. It has been hypothesized that cellular penetration and trafficking of ENMs could be accomplished by binding to a vast array of carrier proteins, through appropriate aquaporins, via interconnected ion channels, via endocytosed pathway, or by crafting novel pores (carbon nanotubes) (Rico et al. 2011; Patrick et al. 2015). Depending upon genotype and environmental conditions, 30–80 % of water flow across roots occurs via the cell-to-cell pathway (symplasmic plus transcellular) (Patrick et al. 2015). Superior expression of aquaporin proteins and upregulation of water channel genes were found to support possible passive uptake mechanisms (Khodakovskaya et al. 2012). Endocytosis has been proved, through the use of temperature control and the addition of wortmannin (an endocytosis inhibiting agent), as one of the possible transport mechanisms (Onelli et al. 2008; Liu et al. 2009; Iversen et al. 2012; Miralles et al. 2012b).

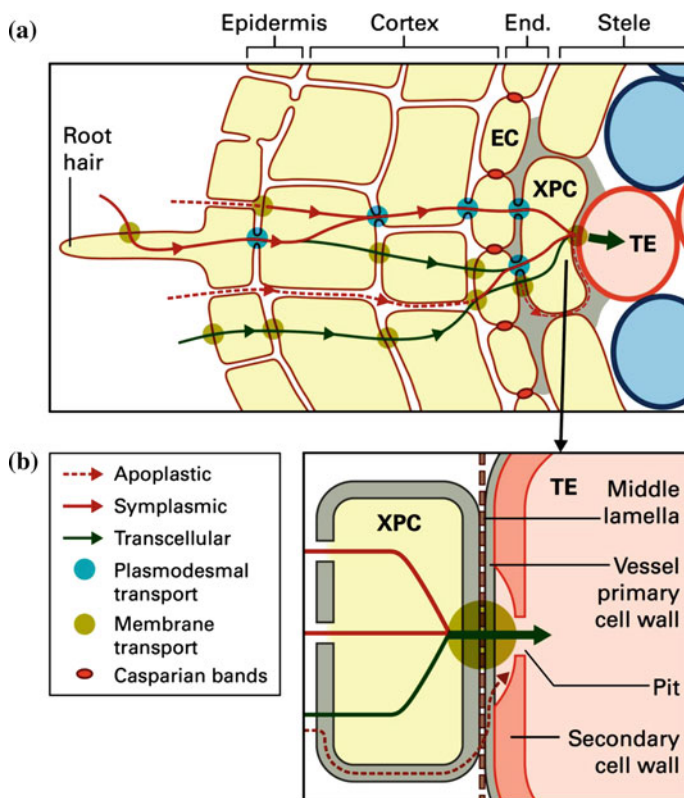


Fig. 8.2 Possible uptake routes of nanoparticle transport in plant system through xylem (including apoplastic, symplasmic, transcellular, plasmodesmal transport, membrane transport, and casparian band). **a** Diagrammatic representation of transverse cross section of a root showing arrangement of root hair, epidermis, cortex, endodermis (End.), and stele. Nutrient and water transport routes across the root are indicated as well as key transport steps through plasmodesmata and plasma membranes. At strategic locations, the root apoplast is blocked by casparian bands composed of lignin deposited in cell walls. Some endodermal cells (ECs) have a conspicuous absence of lignin and suberin lamellae and are referred to as passage cells. **b** Diagram showing possible clues for loading xylem tracheary elements (TE) by Xylem parenchyma cells (XPCs) through half-bordered pits; this process is facilitated by specific membrane transporters [adapted from Patrick et al. (2015)]

Chang and colleagues advocated the involvement of an energy-independent route for mesoporous silica nanoparticles (MSNs) uptake and further proposed possible routes of MSNs uptake by *Arabidopsis* (*Arabidopsis thaliana*) roots. The MSNs, which are in contact with the cell membrane, can be internalized via endocytosis (scheme A) and remain in internal vesicles inside the cells. However, most MSNs cross the plasma membrane directly (scheme B), and then, the particles endure in the cytoplasm or translocate to other organelles (e.g., plastids or nuclei) (scheme C), which is a specific benefit for cargo delivery (Fig. 8.3) (Chang et al. 2013).

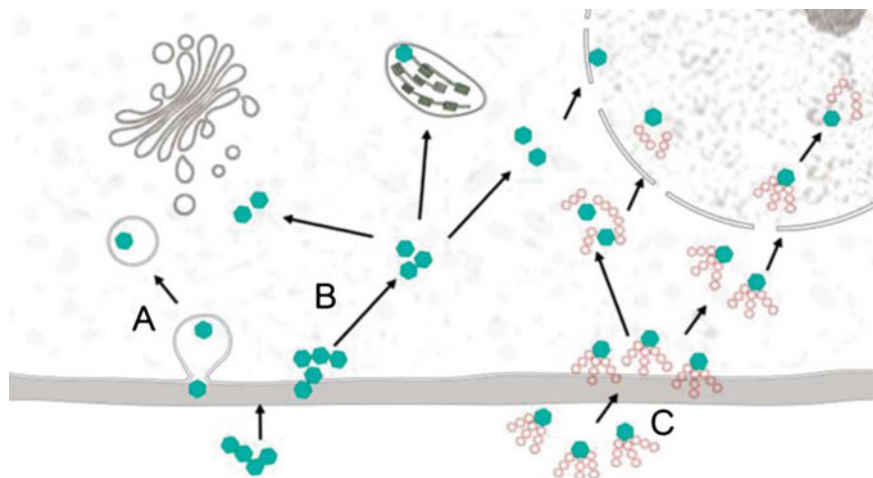


Fig. 8.3 Schematic representation showing possible pathways and localizations of TMAPS/F-MSNs after penetration into the Arabidopsis root system. *Scheme A*—internalization of TMAPS/F-MSNs through endocytosis; *Scheme B*—direct penetration of TMAPS/F-MSNs through plasma membrane as a primary route (after crossing plasma membrane, TMAPS/F-MSNs may localize into cytoplasm or may penetrate organelles, e.g., plastids and the nucleus); *Scheme C*—DNA complexed with TMAPS/F-MSN may internalize into the plant cell and then transported to the nucleus [adapted from Chang et al. (2013)]

Interaction of ENMs with plant cell membranes could alter depending on hydrophobic/hydrophilic nature. Hydrophilic nanomaterials tend to adsorb on bilayer membrane surface, and further, they can bind to intracellular vesicles, while hydrophobic NMs favors to embed into the hydrophobic core of the membrane without resulting in any membrane injury and leakage (Li et al. 2008; Stark 2011). ENMs in the cytoplasm may be embedded by certain proteins or with some specific biomolecules that form a corona (Nel et al. 2009). ENM-containing endosomes or ENM–protein complex (typically with a diameter of 20–50 nm) could undergo effective mobilization to neighboring cells via plasmodesmata. Ultrasmall TiO₂ NPs have been found to disorder structural integrity of microtubular networks of plasmodesmata in Arabidopsis (Wang et al. 2011; Larue et al. 2012a). Moreover, Rab proteins were hypothesized to serve as master regulators for intracellular trafficking of ENMs to specific zones near plasmodesmatal connections (Cifuentes et al. 2010). As a result, ENMs transportation may be regulated through the customary and harmonized action of membranous organelles, diverse array of transport proteins, and interconnected complex trans-walled channels. Because the symplastic flow is inhabited with a diversity and high volume of materials, this pathway shows the possibilities to be highly stringent and well-organized for inter- and intracellular transportation of ENMs through endodermis and into stele and subsequent vascular tissues (Deng et al. 2014).

NPs can also adapt some novel mechanisms to enter plant cells. NPs can penetrate plant root system and/or other plant tissues through selective ion channels, binding to carrier proteins, via aquaporins (water conducting channels), through endocytosed pathway, sometimes also forming new apertures (preferentially for carbon nanotubes, CNTs) or by attaching to organic compounds in the environmental media. NPs exhibit higher surface area to mass ratio in comparison with the bulk metals; therefore, they possess superior reactivity with living and nonliving surroundings. The NPs may be complexed with large numbers of specific and nonspecific membrane transporter proteins or wide varieties of chemicals in root exudates and, subsequently, be transported to the plants (Watanabe et al. 2008; Kurepa et al. 2010). Most of the metal-based NPs (MB NPs) that have been reported as taken up by plants include elements for which ion transporters have been identified (Hall and Williams 2003). NPs can accumulate inside the plant root and/or shoot tissues as intact particles (Gardea-Torresdey et al. 2014; Antisari et al. 2015). Some organic molecules released from root tips may transform metal salts into NPs via reduction, and then, these NPs are transported into plant system (Gardea-Torresdey et al. 2002; Sharma et al. 2007). Size exclusion limits and lateral heterogeneity studies of stomatal foliar uptake pathway for aqueous solutes and water-suspended NPs have suggested that the stomatal pathway differs fundamentally from the cuticular foliar uptake pathway (Eichert et al. 2008). Entry of NPs into the plant was confirmed through leaf surface. Special structural features such as trichome and hypodermis in a leaf of murici (*Byrsonima sericea*) and araçá (*Psidium guineense*) probably formed a barrier, reducing the penetration of metal ions into the mesophyll as observed by the lower iron leaf content and iron accumulation in trichomes (Da silva et al. 2006). Ion transporters specific to cell membrane have been reported for efficient uptake of NPs in the plants (Hall and Williams 2003). Adsorption and aggregation of the NPs were confirmed by scanning electron microscopy (SEM) analysis on the root surface of ryegrass (Lin and Xing 2008).

The genetic response of plants in the presence of NPs is also a topic of discussion. Differences in xylem anatomical structures may lead to different internalization route of NPs into vasculature of plant systems. Solutes may follow either apoplastic or symplastic mode of internalization or sometimes through plasmodesmata connection for entry into vascular tissues. More studies are required in the field to confirm this hypothetical view (Singh et al. 2015). Wheat (*Triticum aestivum*), maize (*Zea mays*), spinach (*Spinacia oleracea*), zucchini (*Cucurbita pepo*), rapeseed (*Brassica napus*), and some desert plants showed their differential ability for metal-based NPs to penetrate seeds without affecting germination (Răcuciu and Creangă 2009; Stampoulis et al. 2009; De la rosa et al. 2011; Pokhrel and Dubey 2013; Kouhi et al. 2014; Taran et al. 2014; Chichiricò and Poma 2015).

Till now, symplastic transport is the most accepted pathway for NP uptake. Some studies also explained the apoplastic mode of transport, whereas some other studies have explained a diverse array of involvement of plasmodesmata, carrier proteins, aquaporins, ion channels, and endocytosis. While xylem being the most preferred plant tissue along with the phloem and stomatal opening plays a

significant role in absorption and transport processes, a well-defined mechanism of NP uptake and transport is still under question and need to be explored.

8.3 Uptake, Translocation, Accumulation, and Transformation of Engineered Nanomaterials in Plants

The in planta uptake and internalization of ENMs is a dynamic phenomenon and may depend on exposure conditions, chemical properties of ENMs (including surface charge, particle size, hydrophobic/hydrophilic nature, aggregation state, and protein/biomolecule adsorption), and crop species (Nedosekin et al. 2011). Concentration and charge of the NP are important determinants for uptake of NP in roots and translocation to above ground plant tissue such as leaves and stems (Burke et al. 2014). All NPs, once released into the environment, undergo dramatic and complex transformations through interactions with various chemicals and other factors (e.g., UV light, interaction with (in) organic ligands, redox reactions, bio-transformations, and aggregation) (Wiesner et al. 2011). ENMs have been shown to translocate and accumulate differentially within stems, leaves, petioles, and fruits of different crops. Direct imaging or whole-plant mapping confirms possible evidences for uptake via roots, translocation through vasculature, and aggregation of ENMs in different plant parts. Based on the experimental observations, the following patterns for ENMs uptake and translocation are evident (Deng et al. 2014): (1) In shoots, transpiration flow pattern and/or the leaf architecture plausibly regulate translocation of ENMs and their accumulation near or within vasculature (Ghafariyan et al. 2013); (2) long-distance transport of ENMs is size/dimension reliant, i.e., smaller aggregates or individual ENPs showing selective advantage and greater efficiency for long-distance transportation (viz. root system to subapical tissues), as compared to larger aggregates from ENMs of same type; (3) accumulation of ENMs (expressed as amount per dry weight tissue) in leaf is higher than that of stems; and (4) some particular sites of distribution of ENMs (away from vascular transport), e.g., leaf periphery and trichomes, may be associated with detoxifying pathways (Cifuentes et al. 2010).

8.3.1 Metal-Based Nanoparticles

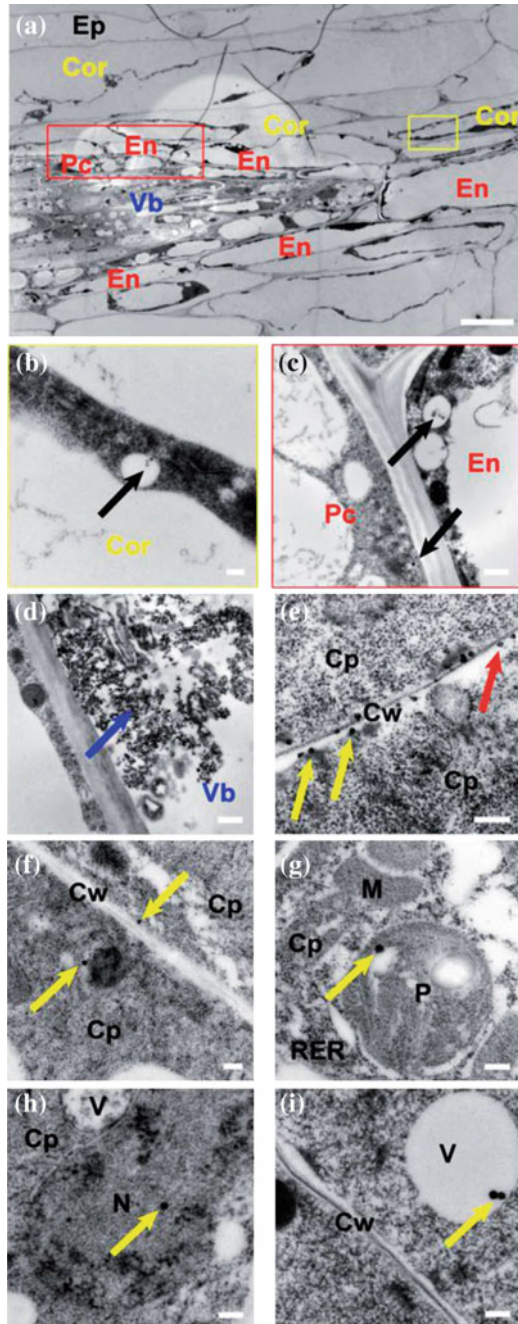
8.3.1.1 Silica-Based Nanoparticles

Chang and colleagues showed the delivery of DNA using 100-nm mesoporous silica nanoparticles (MSNs) to the cortical cells and endodermis of intact roots of *Arabidopsis*. The localization and subcellular distribution of MSNs in the roots

were examined through transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). The results showed that TMAPS/F-MSNs (*N*-trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride labeled MSNs) were present in cortical cells, endodermal cells, pericycle, and vasculature of the root. Subcellular distribution studies of MSNs in root cells showed that MSNs were accumulated in cell walls, cytoplasm, seldom plastids, nuclei, and small vesicles. In addition, the accumulation of some MSNs in vesicles advocates that endocytosis might be one of the uptake routes. Most importantly, the occurrence of MSNs at the cell nucleus notifies that the nanoparticles could penetrate the nuclear envelope or nuclear pore (Fig. 8.4) (Chang et al. 2013).

Uptake and cellular distribution of fluorescently labeled MSNs, with size of 20-nm harboring integrated pores with an estimated diameter of 2.58 nm, were investigated in four plant species, viz. lupin (*Lupinus albus*), wheat (*Triticum aestivum*), maize (*Zea mays*), and Arabidopsis. The results obtained from the study revealed that MSNs transported into the roots via symplastic and apoplastic pathways and further destined to the aerial parts of the plants including the stems and leaves through the conducting tissues of the xylem. The results also confirmed that MSNs sufficiently penetrated the cell wall, entered the endodermis and intercellular spaces and to the vascular tissue, and finally transported to the aerial parts of the plants. Moreover, when MSNs were taken up by the protoplasts, the accumulation of MSNs was also observed in the chloroplast. It was also hypothesized that the translocation and broader internalization of MSNs in plants will facilitate them to be utilized as a novel delivery means for the transportation of different sized biomolecules into plants (Sun et al. 2014). Uptake, transport, and distribution of SiO₂ NPs were also examined in Bt-transgenic cotton (*Gossypium* spp.). Results, as revealed by TEM analysis, confirmed the presence of SiO₂ NPs in the xylem sap. Also, SiO₂ NPs were transported from roots to shoots via xylem sap in both nontransgenic and Bt-transgenic cotton. The presence of dark dots (particles) in the endodermal region and vascular cylinder (under 2000 mg L⁻¹ SiO₂ NPs treatment) was confirmed in both Bt-transgenic cotton and nontransgenic cotton. The presence of SiO₂ NPs was more prominent on the root outer epidermis, whereas only a few were located in intercellular spaces. These results exemplified that most of SiO₂ NPs were adhered on root surface and only a very small amount of NPs could succeeded to penetrate roots. Moreover, Si content in the Bt-transgenic roots was higher than nontransgenic when treated with 2000 mg L⁻¹ SiO₂ NPs, suggesting that SiO₂ NPs have great potential to penetrate into the root of Bt-transgenic cotton as compared to nontransgenic cotton (Le et al. 2014). An attempt was made to use MSNs as carriers to deliver Cre recombinase protein (immobilized on gold-plated MSNs) into maize cells (Martin-Ortigosa et al. 2014). SiO₂ NPs, at concentration range 10–1000 mg/l, showed accumulation and aggregation on the root surface of pear plant. Aggregation was very prominent at higher concentrations (500 and 1000 mg/l) of NPs, whereas an insignificant amount of particles were found to be attached to the roots for NSiO₂ at 10 and 100 mg/l (Zarafshar et al. 2015).

Fig. 8.4 Transmission electron microscopy of Arabidopsis root tissue confirming distribution of MSN. **a** Root section showing organization of tissues from the epidermal cells to the vascular bundle; **b** The presence of MSNs (black arrow) in the cortical cell (Cor) as seen in an enlarged view of the yellow box in **a**; **c** The presence of MSNs (black arrow) in the endodermal (En) and pericycle (Pc) cells in an enlarged view of the red box in **a**; **d** The presence of MSNs (blue arrow) in the vascular bundle (Vb); **e** localization of MSNs in the cell wall (Cw) (red arrow) or penetration through the plasma membrane (entered the cell) (yellow arrows); **f-h** MSNs accumulation (yellow arrow) in the cytoplasm (Cp) (f) or in the plastid (P) (g); (h) nucleus (N) after penetrating cell wall; **i** MSNs accumulation in vesicles (V) (yellow arrows). Scale bars **a** 20 mm; **b** 200 nm; **c** and **d** 500 nm; **e-i** 200 nm. Ep epidermis; Cor cortex; En endodermis; Pc pericycle; Vb vascular bundle; Cp cytoplasm; Cw cell wall; P plastid; M mitochondrion; N nucleus; V vacuole; RER rough endoplasmic reticulum [adapted from Chang et al. (2013)]



8.3.1.2 Titanium-Based Nanoparticles

Electron and X-ray fluorescence microscopy studies established penetrating ability of TiO₂ nanoconjugates (2.8 ± 1.4 nm) on seedlings of *Arabidopsis* grown on agar medium. The results confirmed distribution of TiO₂ nanoconjugates into the epidermis and underlying palisade tissue, signifying stomatal contribution and involvement of endocytotic vesicles in the internalization process. Further, mass spectroscopy and electron microscopy analysis evidenced the foliar uptake following aerial treatments. However, TiO₂ nanoconjugates smaller than 5 nm remained stuck to the seed mucilage and failed to penetrate, while TiO₂ nanoconjugates of 2.8 ± 1.4 nm in diameter succeeded in root cell penetration up to inside vacuoles and the nucleus (Kurepa et al. 2010; Chichiriccò and Poma 2015). Kurepa et al. (2010) also revealed that roots of *Arabidopsis* bounded by pectin hydrogel capsule formed by mucilaginous root exudates, which may play miraculous role either by hindering or by enabling the entry of the TiO₂ nanoconjugates with Alizarin red S or sucrose (Rico et al. 2011). Leaf penetration by TiO₂ NPs in wheat and rapeseed (*Brassica napus*) was also evidenced (Larue et al. 2012b). TiO₂ NPs differing in size and concentration could show differential responses for seed growth and germination because the small particles can easily enter the cell wall pores of the plant and transport to various other parts (Lu et al. 2002). Doping TiO₂ NPs with N could affect plant translocation of NPs to above ground plant tissue (Burke et al. 2014).

8.3.1.3 Zinc-Based Nanoparticles

ZnO NPs have been found to associate with highly vacuolated and collapsed cortical cells along with the shrinking and partial death of the vascular cells (Lin and Xing 2008). The ZnO NPs were absorbed by the plant roots and circulated equivalently throughout the plant tissues. But All ENPs may not be similarly operative for all crops. Unlike CeO₂ NPs, ZnO NPs were found to be translocated into above ground plant tissue, suggesting that uptake and translocation are dependent on NP type (Priestera et al. 2012).

Uptake and accumulation of ZnO NPs (8 nm) were investigated in soybean (*Glycine max*) seedlings at the range of 500–4000 mg L⁻¹. The uptake of Zn NPs by the soybean seedlings was significantly higher at 500 mg L⁻¹ than the concentrations at 1000 mg L⁻¹ and above. This may be because at lower concentration (500 mg L⁻¹), the NPs have lesser aggregation, whereas at high concentrations (1000–4000 mg L⁻¹), the probability of agglomerates formation is proposed. Passage of oversized agglomerates through the cell pore walls, therefore, becomes problematic. This ultimately reduces uptake and accumulation in case of ZnO NPs as understood from the results (Lopez-Moreno et al. 2010a). ZnO NPs were absorbed as Zn²⁺ oxidation state by hydroponically grown soybean plants. Later, it was hypothesized that ZnO NPs transformed in Zn²⁺ oxidation state at the root surface (Lopez-Moreno et al. 2010a). Similar results were also obtained by Dimkpa et al. (2013) and Wang et al. (2013a, b, c).

Scanning electron microscopy and energy dispersive analysis of X-rays (SEM-EDAX) showed Zn uptake by the peanut (*Arachis hypogea*) seeds treated with nanoscale ZnO. Thin sections of the peanut embryo were analyzed by SEM. Although, an expected, low Zn concentration in peanut seeds was observed in EDAX spectra, EDAX images confirmed that the regions showing higher C and N concentrations also exhibited high accumulation of Zn in the seeds treated with nanoscale ZnO. The postharvest leaf and kernel samples were analyzed using Atomic Absorption Spectrophotometer (AAS) to estimate the zinc content (Prasad et al. 2012).

8.3.1.4 Copper-Based Nanoparticles

CuO NPs were transported to the shoots and translocated back to the roots via phloem (Shankar et al. 2003). CuO NPs were taken up by maize and wheat in the particulate form (Dimkpa et al. 2012, 2013; Wang et al. 2012a, b). Uptake and translocation of Cu NPs in mung bean (*Vigna radiata*) and wheat in agar growth medium were evaluated. The results showed that the Cu NPs were able to cross the cell membrane and agglomerate in the cells. A significant relationship between the bioaccumulated NPs in plant tissues and growth media was also established. It was also noticed that mung bean was more sensitive than wheat to toxicity of Cu NPs probably due to root anatomical differences (Lee et al. 2008; Rico et al. 2011).

Copper NPs exhibited greater ability for uptake in shoots than copper bulk particles (BPs). Results revealed that total uptake into the shoots was approximately three times greater for the NPs. Scanning transmission electron microscopy (STEM) images of radish (*Raphanus sativus*) shoot samples did not reveal any significant evidence of electron-dense deposits, and energy dispersive spectroscopy (EDS) analysis did not reveal specific elemental signals for Cu in either control samples or samples exposed to 500 mg/L NPs (Atha et al. 2012).

8.3.1.5 Silver-Based Nanoparticles

Uptake and distribution of silver nanoparticles (SNPs) were investigated in Indian mustard (*Brassica juncea*) and alfalfa (*Medicago sativa*). Alfalfa, in contrast to Indian mustard, showed better uptake with a parallel upsurge in the metal concentration and exposure time (Harris and Bali 2008). In another study, Ag NPs did not seem to accumulate Ag in any form in Indian mustard plants (Haverkamp and Marshall 2009). The silver NPs were found to be located in the nucleus (Monica and Cremonini 2009). The seeds of *Boswellia ovalifoliolata*—an endemic and globally threatened medicinal tree species placed in MS medium containing SNPs, showed 90 % germination, in contrast to 70 % germination without SNPs. It was proposed that SNPs can penetrate through seed coat and may stimulate the embryo for germination (Savithramma et al. 2012). Uptake and the internalization of silver NPs and its bulk counterpart were first time compared in zucchini (*Cucurbita pepo*)

plants. Plants exposed to 10–1000 mg L⁻¹ Ag NPs exhibited 4.7 times higher Ag concentration in the shoots than those treated with bulk Ag powder at similar concentrations (Stampoulis et al. 2009).

The leaves of lettuce (*Lactuca sativa*) plant, when sprayed with the salt AgNO₃ and with Ag-NPs, which were both round (38.6 nm in diameter) and nonround (38.2 nm × 57.8 nm), and had hydrodynamic diameters of 47.9 nm ± 29.2 nm, evidenced by the cuticular and stomatal uptake of NPs and translocation into the vascular tissue. Translocation pathways seemed to be both apoplastic and symplastic. Transformation cycles within the plant involving the binding of Ag⁺ ions to thiol groups and the conversion of Ag⁺ ions in Ag-NPs, starting from the dissolution of both the salt AgNO₃ and Ag-NPs were also proposed (Larue et al. 2014). Accumulation of Ag NPs was found to be accumulated in vacuoles of root cell. Deposition of both individual and the aggregate particle was observed inside the cell wall, indicating the penetration of Ag particle inside the cells. Spherical Ag NPs with a diameter of 20 nm were observed inside the plant cell. Regarding transportation of smaller particles inside the cells, it was hypothesized that cell wall thickness (about 5–20 nm) may respond as natural molecular sieves, allowing transport of smaller nanoparticles through larger pores to enter in the protoplasm (Figs. 8.5 and 8.6) (Mazumdar 2014).

8.3.1.6 Cerium-Based Nanoparticles

Seedlings of soybean, alfalfa, maize, and tomato (*Solanum lycopersicum*) exhibited Ce accumulation in tissues with the increased external concentration of CeO₂ NPs (7 nm) (Lopez-Moreno et al. 2010a, b). This differential accumulation could be the result of variances in root microstructures and the physical and chemical interfaces between the NPs and diverse variety of the root exudates in the rhizosphere.

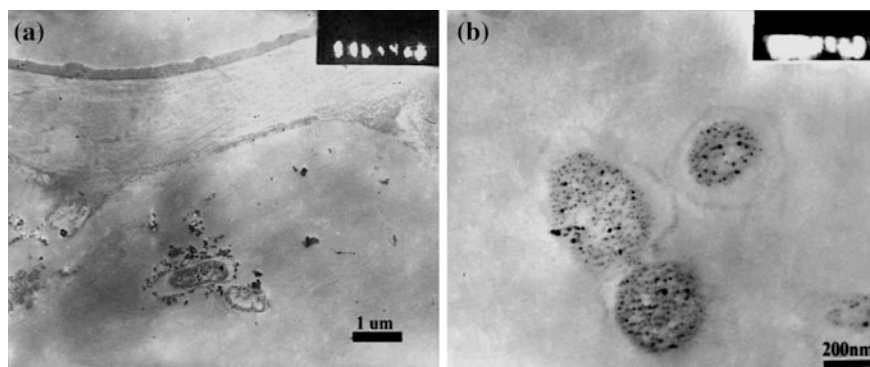


Fig. 8.5 TEM images showing ultrastructures of *V. radiata* roots treated with Ag NPs at a concentration of 1000 μg/mL. **a** Accumulation of AgNPs inside the cell and **b** accumulation of AgNPs inside vacuoles [adapted from Mazumdar (2014)]

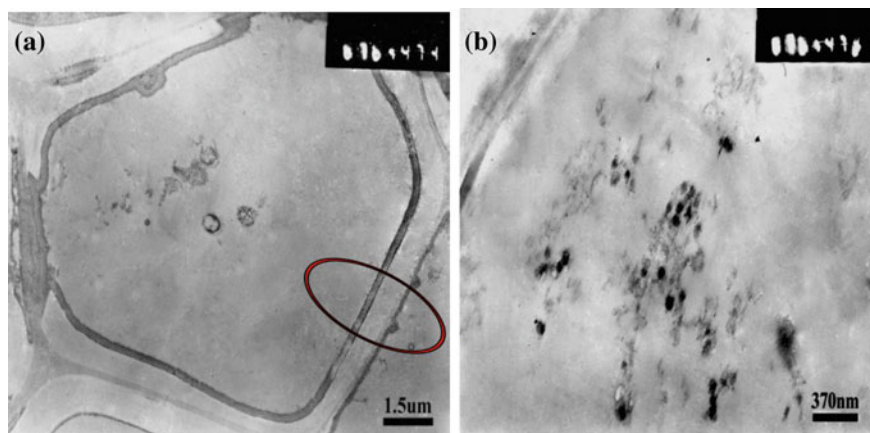


Fig. 8.6 TEM images showing ultrastructures of *B. campestris* roots treated with Ag NPs at a concentration of 1000 $\mu\text{g}/\text{mL}$. **a** Accumulation of AgNPs inside whole cell, **b** enlarged view (encircled) of image **a** showing accumulation of AgNPs in plasmodesmata and cell wall [adapted from Mazumdar (2014)]

Aerosol or suspension of CeO_2 NPs was absorbed by the corn leaves but did not translocate to new leaves. Application of NPs along with the irrigation water did not evidence for any detectable translocation of the NPs within the plant (Birbaum et al. 2010). Soybean plants also exhibited uptake and accumulation of CeO_2 NPs and did not show biotransformation (Lopez-Moreno et al. 2010a). CeO_2 -NPs exhibited primary diameter of $8 \text{ nm} \pm 1 \text{ nm}$ and hydrodynamic diameter of $1373 \text{ nm} \pm 32 \text{ nm}$, internalized by the roots and translocated to the shoots when added to the soil where maize plants were growing. The translocation pathway was proposed to be apoplastic. The studies also pointed out that the mobility of NPs and NP accumulation in the roots and translocation to shoots were favorably influenced by an organic substance in the soil and alginates, respectively (Zhao et al. 2012a, b, 2014). Unexpectedly, soybean plants, treated with nano- CeO_2 , showed reduced leaf counts irrespective of its concentration. Even the lowest concentration of nano- CeO_2 showed growth retardation in the harvested plant (Priestera et al. 2012; Husen and Siddiqi 2014). Tomato plants were treated with low concentrations of CeO_2 NPs (10 mg/L) to investigate its effect on seed quality and the development of second-generation seedlings. These NPs in fact slightly improved the growth of the plant (first-generation seedlings) but, at the same time, weakened the capacity to respond to the fertilization effect of the CeO_2 NPs. The accumulation of CeO_2 NPs in plant seeds and fruit tissues suggested that they have a high impact that can influence subsequent generations. These results demonstrate that although the instant results are positive, there is the need to evaluate the long-term, multigenerational impact of NPs on plants. The results showed that the benefits obtained in the first generation, as illustrated in the previous works, were not persistent in seedlings of the second generation. This study provided, probably, the first evidence

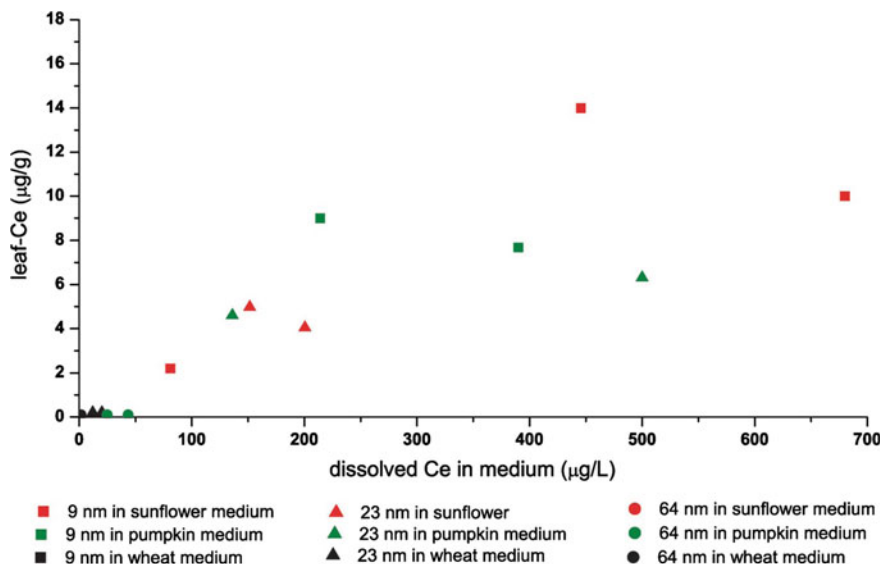


Fig. 8.7 Graphical representation of concentration ($\mu\text{g/L}$) of dissolved Ce in plant-growth medium (x-axis) against Ce concentration ($\mu\text{g/g}$) inside the leaves of the plants (sunflower, pumpkin, and wheat) grown on that medium (y-axis) at the end of the experiment [adapted from Schwabe et al. (2015)]

of the transgenerational impact of CeO_2 NPs on the development and growth of tomato plants (Wang et al. 2013d).

An investigation regarding uptake of differently sized CeO_2 NPs by three crop plants including pumpkin (*Cucurbita maxima*), wheat, and sunflower (*Helianthus annuus*) revealed that Ce NPs larger than 20 nm did not translocate from roots to shoots. Ce uptake was particularly high for particles smaller than 10 nm due to their greater dissolution rates (Fig. 8.7). Experiments with Zr/CeO_x NP revealed that Ce NP was not the solitary, but to a significant degree, dissolved Ce(III) ions, were also adequate forms of NPs for uptake. The study highlighted that dissolution of CeO_2 NPs in soil solution was significantly influenced by plant root activity and that uptake of dissolved Ce(III) trailed by reprecipitation needs to be considered as an important pathway to explain CeO_2 NPs uptake by plants. Further, NP-root-exposure studies confirmed that translocation of Ce was species-dependent. Sunflower had a high affinity for Ce-ion accumulation inside the leaves when Ce was supplied as dissolved ions (Fig. 8.8), while no significant difference between pumpkin and wheat were observed (Schwabe et al. 2015).

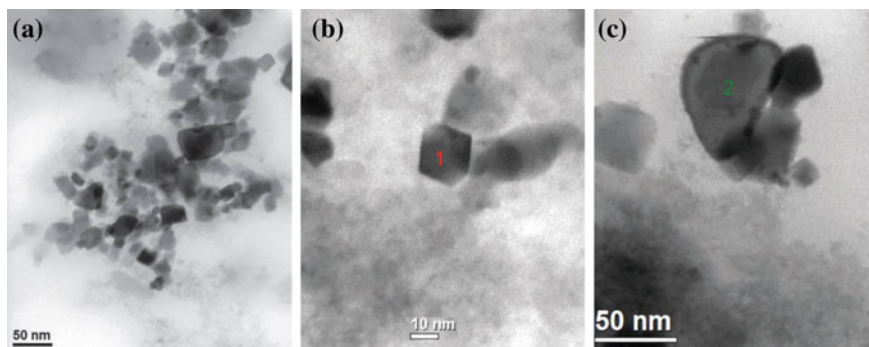


Fig. 8.8 STEM-EDX analysis of NPs extracted from Zr/CeO_x-treated sunflower leaves. **a** Bright-field STEM image showing occurrence of NPs in sunflower leaves; **b** STEM image confirming the presence of rhombic NP in higher magnification. **c** STEM image of round-shaped NPs [adapted from Schwabe et al. (2015)]

8.3.1.7 Iron-Based Nanoparticles

The influence of magnetic nanoparticles coated with tetramethylammonium hydroxide was analyzed on the growth of maize plants in early ontogenetic stages (Răcuciu and Creangă 2007). Magnetite (Fe₃O₄ NPs, with 20 nm diameter) NPs uptake was analyzed using a vibrating sample magnetometer by pumpkin seedlings grown under hydroponic conditions. The results confirmed that signals of magnetic NPs were detected in roots, stems, and leaves of edible pumpkin plants but no uptake occurred in Fe₃O₄ NPs-treated lima bean (*Phaseolus limensis*) plants. It was, therefore, proposed that uptake of Fe₃O₄ NPs also depends on the plant species (Zhu et al. 2008). Epidermal cells of leaf petioles of living pumpkin plants accumulated carbon-coated Fe NPs. Results also showed that accumulation site (epidermal cells) was closer to the application site, whereas no NPs were noticed in the cells located distant from the application points or near the xylem (Corredor et al. 2009). ENMs were detected in shoots within a period of 24 h, when sunflower, tomato, pea (*Pisum sativum*), and wheat plant were exposed to the carbon-coated magnetic NPs (Cifuentes et al. 2010). Application of Fe₃O₄ NPs increased the translocation of Fe to leaf tissue, and positively charged Fe₃O₄ NPs caused a reduction in root colonizing rhizobia (Burke et al. 2015). Rapid accumulation of engineered iron NPs in leaves of aquatic plant, Brazilian waterweed (*Egeria densa*), was confirmed using electron spin resonance, two photon, and confocal microscopy (Spori et al. 2014).

8.3.1.8 Nickel-Based Nanoparticles

Uptake and translocation of Ni(OH)₂ NPs (8.7 nm) in mesquite (*Prosopis* sp.) were investigated. The X-ray absorption near edge structure (XANES) spectra confirmed

that uncoated Ni(OH)₂ NPs were observed in roots and shoots of plants, while citrate-coated NPs showed Ni NPs only in roots (Parsons et al. 2010).

8.3.1.9 Aluminum-Based Nanoparticles

Red kidney beans (*Phaseolus vulgaris*) and ryegrass were treated with nanoscale aluminum (Al) particles (1–100 nm) for uptake analysis. No significant variation in Al concentration in the red kidney beans was observed due to Al NPs treatment compared to untreated control, whereas in ryegrass leaves, 2.5-fold increase in aluminum concentration was noticed. No negative effect due to Al NPs treatment was observed on the growth of red kidney beans and ryegrass in the tested concentration range (Doshi et al. 2008).

8.3.1.10 Other Metal-Based Nanoparticles

Plants when exposed to Fe and Mn also exhibited incidence of particulate Fe oxide and Mn (Ghafariyan et al. 2013; Pradhan et al. 2013). In a similar fashion, MgO NPs were observed in roots, when applied via foliar application (Wang et al. 2013a, b, c). Notably, the same crop showed differential absorption pattern for different nutrient elements provided in particulate form through the root, and it was also evident where wheat showed differential pattern for CuO versus ZnO NPs, confirming Cu existence in wheat shoot mainly as CuO particles and a lower amount of dissolved forms, and Zn as Zn phosphate (Dimkpa et al. 2012, 2013). Development and growth processes of the mung bean plant were prominently affected by foliar spray of the NP suspensions of ZnO, FeO, and ZnFeCu-oxide. Enhancements in root and shoot length as well as accumulation of biomass were recorded for NPs-treated plant as compared to the nontreated plants. The maximum enhancement was found at 50 ppm ZnFeCu-oxide followed by 50 ppm FeO and least for 20 ppm ZnO depending on their chemical composition, size, and surface energy (Dhoke et al. 2013).

Alfalfa seedlings, when exposed to Au(III) and Ag(I) ions through agar solid growth media, got reduced and accumulated as Au and Ag NPs (Gardea-Torresdey et al. 2002, 2003). Similar observations, regarding accretion and biotransformation of Ag(I) and Pt(II) ions into Ag and Pt NPs, were also recorded in alfalfa and Indian mustard (*Brassica juncea*) seedlings. TEM images confirmed accumulation of Pt NPs ranging between 3 and 100 nm with different morphologies in roots of alfalfa (Harris and Bali 2008; Bali et al. 2010). Experimental evidence suggested that gold NPs were able to translocate and accumulate in the soybean plants after seed inoculation (Falco et al. 2011; Maharramov et al. 2015).

8.3.2 Carbon-Based Nanoparticles

Carbon-based nanomaterials (CNMs) have shown superior prospective for internalization through leaves' surface and further translocation to the root system of the plant. However, their foliar uptake is not well recognized. Further, CNMs are not considered potential contaminants in the liquid phase (Ke and Qiao 2007; Deng et al. 2014). Conversely, the hydrophobicity of NMs can be obviated through their interaction with natural organic matter (NOM), when discharged into the environment (Hyung et al. 2007). Uptake and translocation of CNMs to aerial parts were provided by many researchers (Lin and Xing 2007; Cañas et al. 2008; Khodakovskaya and Biris 2009; Lin et al. 2009; Nedosekin et al. 2011; Smirnova et al. 2011; Bhattacharya et al. 2012; Kole et al. 2013; Cicek and Nadaroglu 2015). The first evidence on the uptake, accumulation, and generational transmission of NOM-suspended carbon NPs in rice plants was provided by Lin et al. (2009). The potential impact of nanomaterial exposure on plant development and genetic consequences through plant–nanomaterial interactions was documented by these authors. The abundance of NOM (a heterogeneous mixture of proteins, lipids, amino acids, and peptides that are derived from decomposed animals and plants) in natural soil and water sources permits its interaction with NPs to provoke water solubility and kinesis in the environment (Davies et al. 1997; Ke and Lamm 2011). An *in vivo* flow cytometry analysis in tomato stems showed that the average velocity of quantum dot–carbon nanotube conjugates was approximately 0.2 mm/s (Nedosekin et al. 2011). Kole et al. (2013) investigated uptake and biodistribution of a fullerene derivative $C_{60}(OH)_{20}$, or “fullerol” in bitter melon (*Momordica charantia*). Uptake, translocation, accumulation, transformation, and generational transmission of carbon-based NPs are scantily examined; some of them are discussed below.

8.3.2.1 Fullerene Nanoparticles

Probably for the first time, Lin et al. (2009) investigated the uptake, accumulation, and generational transmission of NOM-suspended fullerene in rice plants. Suspensions of fullerene C_{70} and multiwalled carbon nanotubes (MWNTs) in NOM solution at a concentration of 100 mg L^{-1} in Milli-Q water were prepared. Dynamic uptake, compartment distribution, and transformation of fullerene C_{70} in rice plants were characterized, and transgenerational transmission of C_{70} particles to the next progeny through seeds was detected. Results showed that distribution of C_{70} particle was not reliant on concentration. The prevalent C_{70} particles were dominant in the roots as well as on the stems and leaves of the 2-week-old plants (Fig. 8.9). However, C_{70} particle was predominantly present in or near the stems' vasculature systems, lesser in the leaves in the mature (6-month-old) plants, and least in the seeds due to the multiplied uptake rates, therefore reducing the amount of translocated NPs. However, no C_{70} aggregates were found in the epidermis, plausibly due to a greater distance from the vascular system. Furthermore, no C_{70}

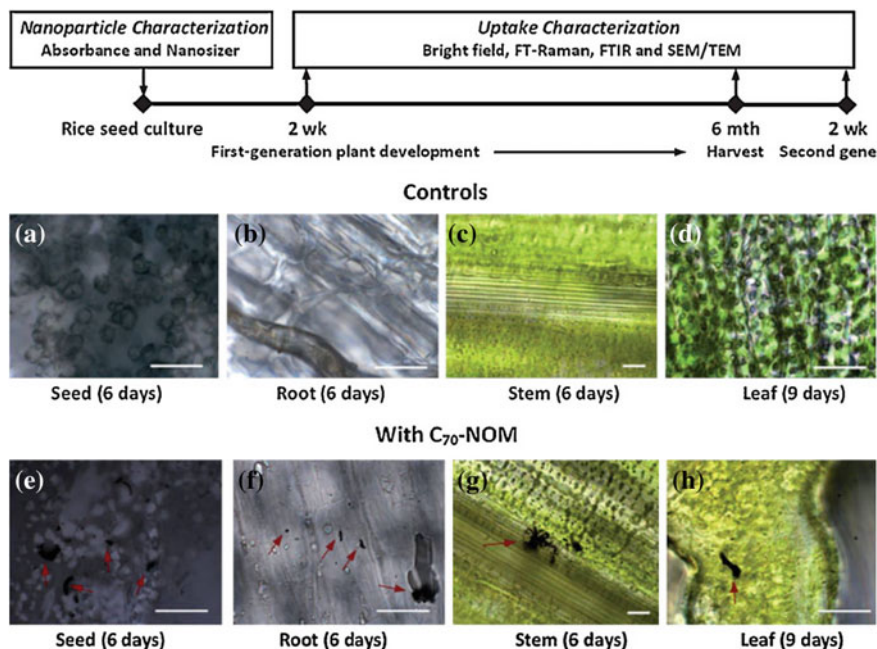


Fig. 8.9 Schematic representations of experimental details and bright-field imaging of C_{70} uptake by 1-week-old rice plants. *First row*—experiment scheme. *Second row*—bright-field images of controls seeds (a), root (b), stem (c), leaf (d). *Third row*—aggregates of NPs (as shown by arrows) observed in seeds (e), root (f), stem (g), leaf (h) treated with C_{70} -NOM. The scale bars are 20 μ m for all images [adapted from Lin et al. (2009)]

was found left in the roots of the mature plants, indicating robust transport of NMs from the roots to the aerial parts of the plant. It was hypothesized in the previous studies that penetration of C_{70} nanoparticles may ensue via osmotic pressure, capillary forces and pores on cell walls (≈ 3.5 – 5 nm) (Carpita et al. 1979) or through intercellular plasmodesmata (≈ 50 – 60 nm at midpoint) (Smith 1978) or via the highly regulated symplastic route. NPs' small dimension and self-assembly and from the NP interactions with plant organelles and the NOM are important factors for the integration of NPs by plant species. Interestingly, though much less frequently, C_{70} NPs were also marked in the leaf tissues of the second-generation plants grown without the addition of NMs (Fig. 8.10) (Lin et al. 2009).

Uptake and accumulation of two fullerene derivatives (i.e., a $C_{60}(\text{OH})_{20}$ molecule a supramolecular assembly of C_{70} -NOM) were investigated in onion (*Allium cepa*) plants. To avoid the structural complexity of NOM, a Temple Northeastern-Birmingham (TNB) model (Davies et al. 1997), expressing a monomer of the humic substance in NOM, was used in present exploration. TNB monomers were attached to the surfaces of the C_{70} molecules through hydrophobic interaction. Interaction of NPs with onion plant cells was dependent on particle size and surface properties. When plant cells were exposed a higher concentration of $C_{60}(\text{OH})_{20}$ NPs

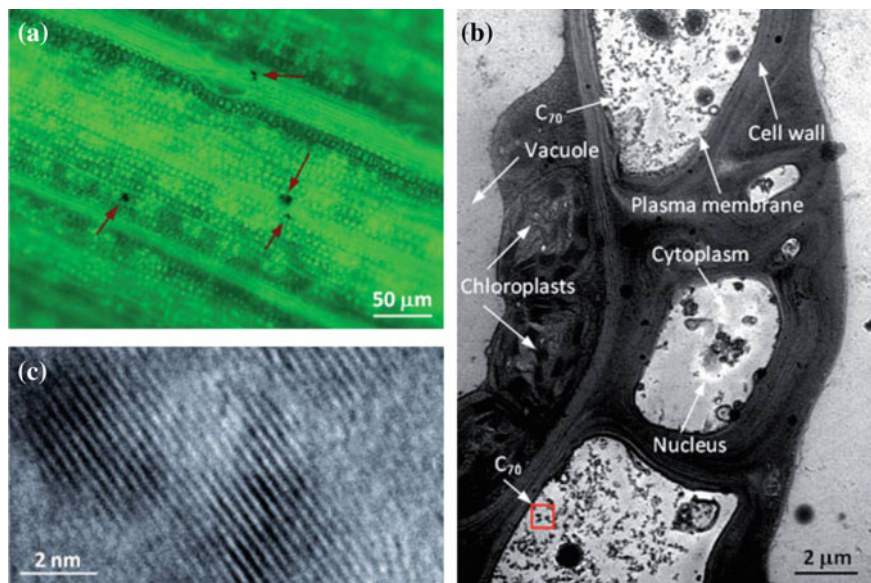


Fig. 8.10 Experimental evidences of generational transmission of C₇₀ NPs. **a** Bright-field image showing aggregation of C₇₀ (indicated by *arrows*) appeared mostly in or near the vascular system of the leaf in second-generation rice plant. **b** TEM image of C₇₀ particles in the leaf cells (plant cell walls and other organelles) of a 2-week-old rice plant. **c** Enlarged view of TEM image of the C₇₀ particles in **(b)** (*red square*). FFT analysis of the TEM image confirmed the lattice spacing of the C₇₀ particles to be 0.257 nm [adapted from Lin et al. (2009)]

(i.e., 70 mg L⁻¹), a steady upsurge in cellular damage was observed. It was hypothesized that the presence of a thick, rigid, and porous cell wall acts as a barrier for large and hydrophobic NPs and their aggregates while imposing slight interference to the translocation of hydrophilic NPs (Fig. 8.11) (Chen et al. 2010; Ke and Lamm 2011).

8.3.2.2 Fullerol Nanoparticles

Uptake, biodistribution, and accumulation of fullerol (a fullerene derivative) were examined in bitter melon (*Momordica charantia*) through bright-field imaging (BFI) and Fourier-transformed infrared (FTIR) spectroscopy by Kole et al. (2013). Seeds were treated with five stock concentrations (0.943, 4.72, 9.43, 10.88, and 47.2 nM) of fullerol, C₆₀(OH)₂₀, nanoparticles, referred to as C₁, C₂, C₃, C₄, and C₅, respectively, and C₀ was controlled without fullerol, C₆₀(OH)₂₀, nanoparticles. Black aggregates observed through FTIR analysis confirmed biodistribution of fullerols almost in all plant organs including petioles, leaves, flowers, and fruits (Fig. 8.12). The result had confirmed that most of the stem and fruit samples (excluding C₀ and C₁) exhibited distinct FTIR peaks common to fullerols across the 1500–1700 cm⁻¹

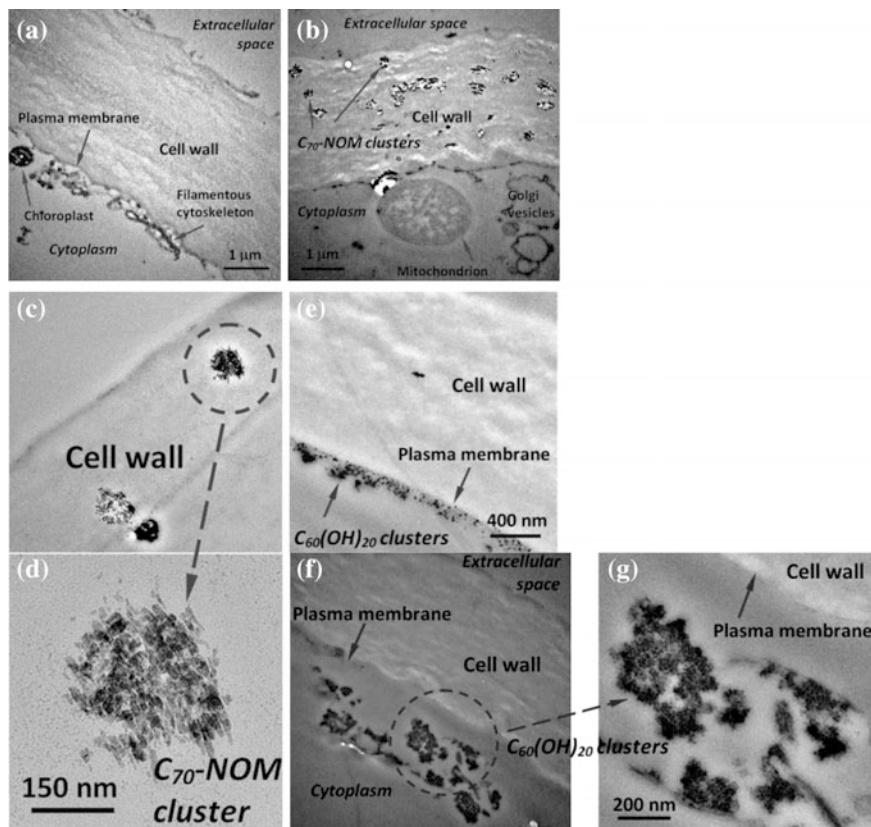


Fig. 8.11 Transmission electron microscopy of *Allium cepa* plant cells showing uptake and translocation of carbon nanoparticle. **a** TEM imaging of control plants showing plant cell wall and plasma membrane. **b–d** Aggregation of C_{70} -NOM at plant cell wall showing C_{70} -NOM clusters ranging 50–400 nm, when exposed to C_{70} -NOM concentration at 50 mg L^{-1} . **d** Enlarged image of a C_{70} -NOM cluster as encircled in **c**. **e–g** Translocation of $C_{60}(\text{OH})_{20}$ across plant cell walls. **e** Accumulation of $C_{60}(\text{OH})_{20}$ clusters at cell wall and a plasma membrane interface. **f** Localization of C_{70} -NOM clusters in intracellular space. **g** Magnified view of C_{70} -NOM clusters in intracellular space as encircled in **f** [adapted from Chen et al. (2010)]

spectral region, suggesting the presence of fullerols in the samples, whereas fullerol-like IR peaks were found absent in sample C_0 , obviously reflecting the absence of the NM. Intense FTIR signal for fullerols was observed only in the fruits from C_3 and C_5 samples. This result was predictable as the C_5 seeds were treated at the highest fullerol concentration. Authors proposed that the foremost mechanisms for the fullerol uptake could be through the diverse array of (1) transpirational stream generated through rapid water evaporation from the aerial plant parts especially leaves, (2) in planta NPs concentration gradient, or (3) hydrophobic interface between the NPs and the waxy coatings among plant cells (Fig. 8.13) (Kole et al. 2013).

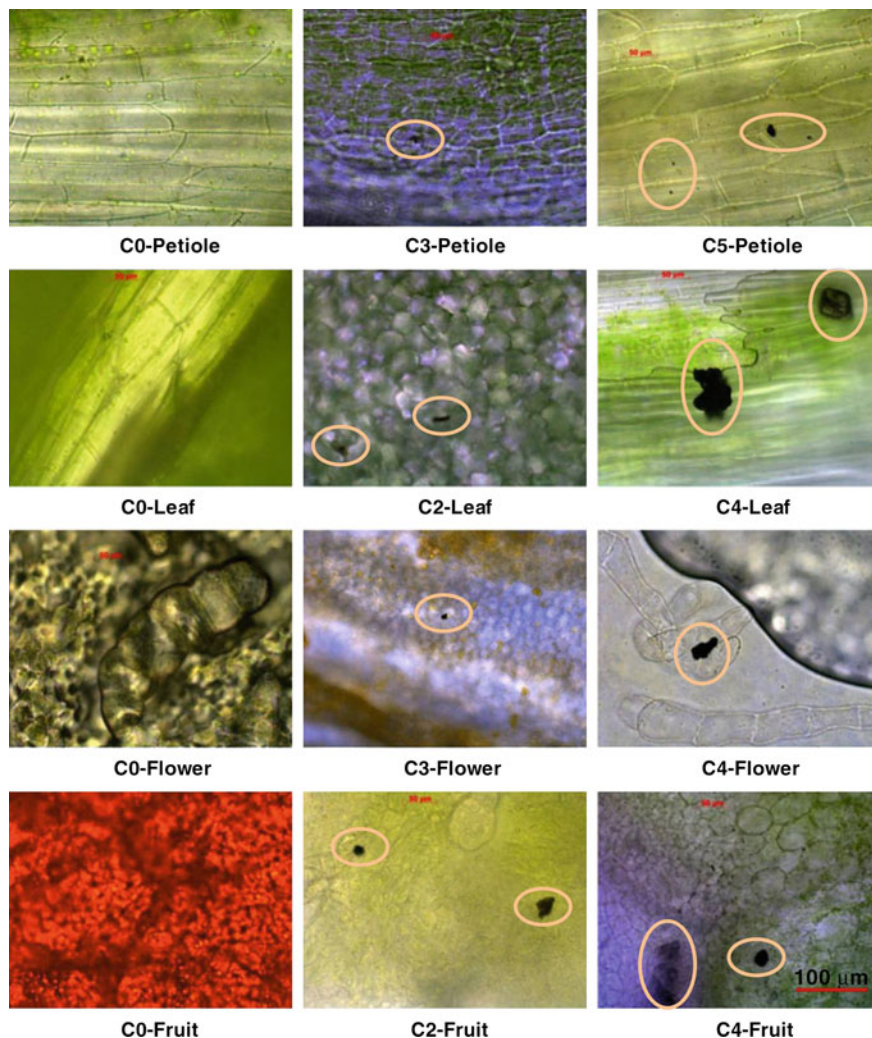
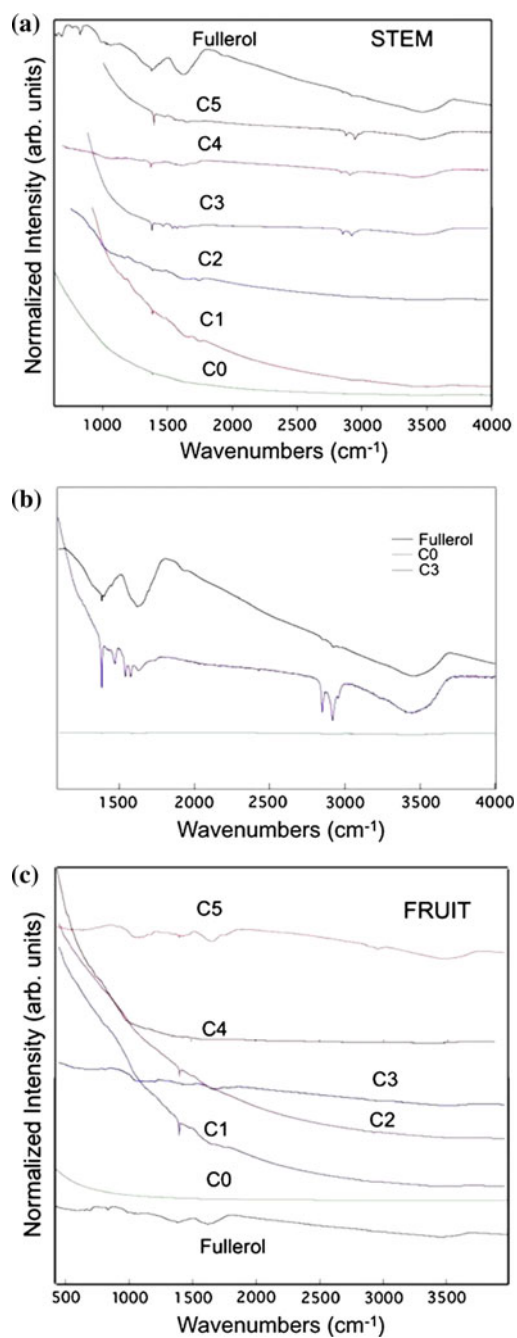


Fig. 8.12 Fullerol biodistribution in different plant organs including petioles, leaves, flowers, and fruits of bitter melon. The *circles* highlight black aggregates which were later confirmed by FTIR as fullerenes. Fullerol, $C_{60}(OH)_{20}$, and nanoparticles (BuckyUSA) were dissolved in Milli-Q water (pH 6.5) to prepare five stock concentrations (0.943, 4.72, 9.43, 10.88, and 47.2 nM), referred to C1, C2, C3, C4, and C5, respectively, whereas C0 is control without fullerol nanoparticles [adapted from Kole et al. (2013)]

8.3.2.3 Single-Walled Carbon Nanotubes

Single-walled carbon nanotubes (SWNTs) are capable to transverse across the plant cell wall and cell membrane as well (Liu et al. 2009). NPs act as smart treatment delivery systems for plant systems (Gonzalez-Melendi et al. 2008). Due to a



◀ **Fig. 8.13** Fourier-transformed infrared spectroscopy of fullerols in different plant organs of bitter melon. **a** FTIR data for stem samples. C1–C5 samples exhibit clear FTIR signatures for fullerol. All the spectra were counterpoise for precision. **b** Fullerol peaks ($\sim 1580\text{--}1640\text{ cm}^{-1}$) of scaled and expanded view of C3 sample. **c** FTIR data for fruit samples. C1–C5 samples display precise fullerol signatures. All the spectra were counterpoise for clarity. Sample C5 shows very distinct features similar to fullerols due to preliminary incubation of seeds in highest fullerol concentration [adapted from Kole et al. (2013)]

thickness of seed coats, penetration of NPs into seeds could be difficult compared to plant cell walls and membranes (Srinivasan and Saraswathi 2010). But carbon nanotubes may effectively penetrate seed coat possibly due to an enlarged water uptake (Khodakovskaya and Biris 2009; Ganguly et al. 2014). The low surface friction of CNTs facilitates the flow of organic substances into the cytoplasm (Whitby and Quirke 2007).

Confocal microscopy studies confirmed that SWCNTs (length $<500\text{ nm}$) bound noncovalently to fluorescein isothiocyanate (FITC) dye, followed a penetration pathway via endocytosis in suspension cultures of intact tobacco (*Nicotiana tabacum*) cells line BY-2 (Liu et al. 2009). A similar approach of membrane penetration via endocytosis was also reported in in vitro cultures of rice and Arabidopsis cells (enzymatically treated for removing walls) (Shen et al. 2010).

By applying advanced methods, SWCNTs have shown their capability to penetrate chloroplasts and accumulate on thylakoids and stroma in spinach (*Spinacea oleracea*) leaves. Further, it was also found that once penetrated in the membranes of spinach chloroplasts, SWCNTs improved the flow of electrons and photosynthetic activity, thereby exciting action on the uptake of light with near-infrared wavelengths. Moreover, SWCNTs were noticed to be sensitive to nitric oxides (NO_x), and therefore, plants-harboring nanotubes could be used as indicators for NO_x (Giraldo et al. 2014). Apical meristem at the base of tomato roots (where root elongation occurs) showed a high accumulation of CNTs (Cañas et al. 2008). SWCNTs were found accumulated on the peripheral surface of the main root and also in secondary roots in the form of nanotube sheets (Tan and Fugetsu 2007).

Tomato seeds inoculated in CNTs-complemented agar medium showed the presence of CNTs inside the seeds escorted with higher moisture percentage (Srinivasan and Saraswathi 2010). Nanotubes also served as potential nanotransporters to deliver DNA and small dye molecules into intact plant cells (Lin et al. 2009; Savithramma et al. 2012). Further, endocytotic method of the nanotubes penetration was reported in the nucleus, plastids, and vacuoles, and nanotubes also induced organelle recycling in tobacco and periwinkle (*Catharanthus roseus*) plants (Serag et al. 2011a, b, 2012a, b; Chichiriccò and Poma 2015).

8.3.2.4 Multi-walled Carbon Nanotubes

Multi-walled carbon nanotubes (MWCNTs) were observed in the seeds and root systems of the developed tomato seedlings (Khodakovskaya and Biris 2009),

whereas the cell walls of rice cell suspension limited the access of the MWCNTs into the cellular cytoplasm (Tan and Fugetsu 2007). At high concentrations, MWNTs showed higher affinity for the epidermis and the waxy casparian strips of the roots, and consequently, MWNTs adsorbed to the plant root surfaces. Interestingly, at higher concentration, MWNTs aggregated at root surface caused a blockage at the plant roots and root hairs, thereby impeding the uptake of water, nutrients, and NOM, as well as plant development. Delayed flowering and reduction in seed setting was observed in the rice plants nurtured with MWNT–NOM (400 mg/L), compared to the controls or the NOM-fed plants (Bhattacharya et al. 2012). MWCNTs showed insignificant particle uptake and translocation, whereas it enhanced germination and root elongation in alfalfa and wheat (Miralles et al. 2012a). Investigation of potential effects of oxidized multi-walled carbon nanotubes (o-MWCNTs) (differing in length ranging from 50 and 630 nm) on wheat physiology and development revealed that enhanced root growth and higher plant biomass were observed in the plants exposed to o-MWCNT compared to the control (Han et al. 2012; Cicek and Nadaroglu 2015).

8.4 Prospects of Transgenerational Transmission of Nanoparticles: Possible Clues

Nanoparticles, being miniature in size, can penetrate easily into plant cells, interrelate with biomolecules, and may not hold as the promise for transgenerational transmission. Plant cell–NP interaction could regulate plant gene expression and related metabolic pathways. However, the transgenerational transmission of NP and their associated genetic, physiological, biochemical, and molecular avenues still have a huge gap. Following are some studies, showing some clues for transgenerational transmission of the NP.

A mesoporous silica nanoparticle system was pragmatic to transport DNA and chemicals into isolated plant cells (protoplasts from tobacco culture) and intact leaves (young maize embryos), (Torney et al. 2007). Although biocompatible, ZnO NPs can influence the genetic material of terrestrial plants. The presence of new bands may reveal a change in the priming sites leading to new annealing events. Also, large deletions and homologous recombination could lead to the appearance of new bands. High concentrations of CeO₂ NPs and Zn ions released from NPs can alter redox chemistry, thereby increasing oxidative stress leading to DNA damage that affects random amplified polymorphic DNA (RAPD) profiles. RAPD profile of soybean DNA revealed the presence of four new bands at 2000 mg L⁻¹ CeO₂ NPs and three new bands at 4000 mg L⁻¹ CeO₂ NPs. RAPD profiles confirmed that both ZnO and CeO₂ NPs influence the integrity of the DNA, but CeO₂ NPs caused the highest effect on the genetic stability of soybean plants (Atienzar and Jha 2006;

Singh et al. 2009; Lopez-Moreno et al. 2010a). ZnO NPs interfered with the development of mitosis and inhibited mitotic division in onion (*Allium cepa*). It was hypothesized that inhibition of DNA synthesis at S-phase or an arrest at the G₂ phase of the cell cycle could be the reasons for this cytotoxic effect (Duan and Wang 1995; Borboa and De la Torre 1996; Sudhakar et al. 2001). ZnO NPs also led to an upsurge of chromosomal aberrations (Shaymurat et al. 2011). High-resolution gas chromatography/isotope dilution mass spectrometry (GC/IDMS) confirmed CuO NP-induced accumulation of multiple DNA lesions in three plant systems including radish, perennial ryegrass (*Lolium perenne*), and annual ryegrass (*Lolium rigidum*) (Dizdaroglu 1985; Jaruga et al. 2008). CuO NPs can significantly affect formation and accumulation of DNA base lesions in radish seedlings compared to DNA damage in grassland plants (perennial and annual ryegrass). Moreover, DNA damage in all three plant systems was dependent on exposure time and dose of NP (Atha et al. 2012).

Chromosomal aberrations, micronuclei, and DNA damage were observed in root-tip meristematic cells of onion and maize when exposed to silver NPs, zinc oxide NPs, and coated magnetic NPs of ferrofluid. It was further recommended that NMs could penetrate plant system and may interact with intracellular components causing destruction to cell division (Răcuciu and Creangă 2007; Kumari et al. 2009, 2011, 2012; Patlolla 2013). Effect of NPs (ZnO and TiO₂) on the plant regeneration frequency was investigated for the study of plant line improvement and genetic transformation in the future (Chutipaijit 2015). Nano-CeO₂ may impact the second-generation seedlings growth establishing transgenerational effect. The experimental finding revealed higher Ce accumulation in the fruit from 200 mg/L nCeO₂ treatment, indicating potential transgenerational effects (Wang et al. 2013d; Hong et al. 2015). It was hypothesized that the ferrophase might penetrate the nuclear membrane, and magnetic fluids can target the extra nuclear DNA preferably the plastome. The magnetic NPs can influence chromosomal aberrations and perturbation of the proliferative capacity (Răcuciu and Creangă 2009).

Cellular “injection” with carbon nanofibers containing foreign DNA has been used to modify genetically golden rice (AZoNano.com 2014). Nanoparticles, nanofibers, and nanocapsules are capable of carrying foreign DNA and gene-modifying chemicals. This virtue has established nanobiotechnology as a novel industry with new tools to modify genes and even produce new organisms (Torney et al. 2007). It is easier for coated nanoparticles to penetrate cell wall, where the genes might be inserted at factual target site and after that activated in a precise and controlled manner, without any toxic side or after effects. This procedure has already been realistic to introduce DNA successfully to plants, including tobacco and maize plants (Galbraith 2007; Park et al. 2008; Kovalchuk et al. 2012; Sekhon 2014).

8.5 Conclusion and Future Perspectives

In almost all studies, patterns of in planta uptake, translocation, accumulation, and transformation of NPs are poorly understood and only limited data and experimental evidences are available to explain uptake, translocation, and transmission of NPs. However, it is clear that the size of NPs appears to be the critical factor for uptake, accumulation, and further translocation and transgenerational transmission. As the concentration of metal-based NPs or carbon-based NPs increases, the growth increases and reaches an optimum value after which constant or retardation in growth occurs, indicating consequences of NPs accumulation. A NP may either adopt some common routes such as symplastic, apoplastic, or plasmodesmata pathway for transmission to a different part of plant system or may travel through some novel mechanism such as by binding to a diverse array of carrier proteins, through appropriate aquaporins, through interconnected ion channels, via endocytosed pathway, by creating new pores, or by binding to organic chemicals. But, the exact mechanism regarding uptake, translocation, and accumulation of NPs is yet not confirmed. Followings are some possible criteria which could outline “nature of mechanism” for NP uptake, translocation, and accumulation.

1. **Selectivity:** Structure and biochemistry of plasma membrane are important determining features for NP internalization. Membranes harbor specific ion channels for selective ions; therefore, it would be interesting to know that how NPs reacts with the plasma membrane.
2. **Size and charge of NPs:** Nanosized particles have greater degree of freedom for movement; hence, absorption and trafficking of NPs may be reliant on size of the particles, and in some cases, particle charge and hydrophobic/hydrophilic nature of NPs may also determine absorption and trafficking of NPs.
3. **Aquaporins:** Since water plays the crucial role in nutrient internalization. Therefore, NPs-water channel (aquaporins) interaction would of a greater concern and can play a central role in the transmission of the NPs.
4. **Essentiality versus nonessentiality:** Nature and physiology of plant system for essential mineral NPs and nonessential mineral NPs may be important determinants to regulate uptake and trafficking of NPs.
5. **Xylem anatomy:** Variations in xylem structures may authenticate different uptake kinetics of NPs and therefore, influence translocation of NPs in plant system.
6. **NP-plant interaction:** NPs uptake and transmission may also be dependent on plant species; i.e, the same NP may behave differentially with different plants having different genetic backgrounds.

Further research needs to address questions about the mobilization/remobilization mechanisms of NPs and their conversion into operational forms in planta, thereby providing a promising pathway for NPs transmission. It is, therefore, a challenge for scientific community to solve physiological, molecular, and genetic mechanisms for NPs' internalization and trafficking. Furthermore, a holistic view of mitigating the adverse effects of NMs on plant development also needs to be answered.

References

- Antisari LV, Carbone S, Gatti A, Vianello G, Nannipieri P (2015) Uptake and translocation of metals and nutrients in tomato grown in soil polluted with metal oxide (CeO_2 , Fe_3O_4 , SnO_2 , TiO_2) or metallic (Ag, Co, Ni) engineered nano particles. *Environ Sci Pollut Res* 22:1841–1853
- Atha DH, Wang H, Petersen EJ, Cleveland D, Holbrook RD, Jaruga P, Dizdaroglu M, Xing B, Nelson BC (2012) Copper oxide nano particle-mediated DNA damage in terrestrial plant models. *Environ Sci Technol* 46:1819–1827
- Atienzar FA, Jha AN (2006) The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutat Res* 613:76–102
- Azimi R, Borzelabad MJ, Feizi H, Azimi A (2014) Interaction of SiO_2 nano particles with seed pre chilling on germination and early seedling growth of tall wheatgrass (*Agropyron elongatum* L.). *Polish J Chem Technol* 16(3):25–29
- AZoNano.com (2013) Nanofibers to be used in drug delivery, gene therapy, crop engineering and environmental monitoring [webpage on the Internet]. AZoM.com Pty. Ltd, Manchester, UK. Updated 11 June 2013. Available from: <http://www.azonano.com/article.aspx?ArticleID=114>. Accessed 19 April 2014
- Bali R, Siegel R, Harris AT (2010) Biogenic Pt uptake and nano particle formation in *Medicago sativa* and *Brassica juncea*. *J Nanopart Res* 12:3087–3095
- Bhattacharya P, Salonen E, Ke PC (2012) Transformation of engineered nanostructures in the natural environment. In: Barnard AS, Guo H (eds) *Nature's Nano Structures*. Pan Stanford Publishing Pte. Ltd, Temasek Boulevard, Singapore, pp 509–536
- Birbaum K, Brogiolli R, Schellenberg M, Martinoia E, Stark WJ, Gunther D, Limbach L (2010) No evidence for cerium dioxide nano particle translocation in maize plants. *Environ Sci Technol* 44(22):8418–8423
- Borboa L, De la Torre C (1996) The genotoxicity of Zn(II) and Cd(II) in *Allium cepa* root meristematic cells. *New Phytol* 134:481–486
- Burke DJ, Zhu S, Pablico-Lansigan MP, Hewins CR, Samia ACS (2014) Titanium oxide nano particle effects on the composition of soil microbial communities and plant performance. *Biol Fertil Soils* 50:1169–1173
- Burke DJ, Nicole PN, Shu F, Situ SF, Abenojar EC, Porche M, Kraj P, Lakliang Y, Samia ACS (2015) Iron oxide and titanium dioxide nano particle effects on plant performance and root associated microbes. *Int J Mol Sci* 16:23630–23650
- Cañas JE, Long M, Nations S, Vadan R, Dai L, Luo M, Ambikapathi R, Lee EH, Olszyk D (2008) Effects of functionalized and non-functionalized single-walled carbon-nano tubes on root elongation of select crop species. *Nanomat Environ* 27:1922–1931
- Carpita N, Sabularse D, Montezinos D, Delmer DP (1979) Determination of the pore size of cell walls of living plant cells. *Science* 205(4411):1144–1147
- Chang F-P, Kuang L-Y, Huang C-A, Jane W-N, Hung Y, Yue-ie CH, Mou C-Y (2013) A simple plant gene delivery system using mesoporous silica nanoparticles as carriers. *J Mater Chem B* 1:5279–5287
- Chen R, Ratnikova TA, Stone MB, Lin S, Lard M, Huang G, Hudson JS, Ke PC (2010) Differential uptake of carbon nano particles by plant and mammalian cells. *Small* 6:612–617
- Chichiriccò G, Poma A (2015) Penetration and toxicity of nano materials in higher plants. *Nanomaterials* 5:851–873
- Chutipaijit S (2015) Establishment of condition and nano particle factors influencing plant regeneration from aromatic rice (*Oryza sativa*). *Int J Agric Biol* 17:1049–1054
- Cicek S, Nadaroglu H (2015) The use of nanotechnology in the agriculture. *Adv Nano Res* 3(4):207–223
- Cifuentes Z, Custardoy L, de la Fuente JM, Marquina C, Ibarra MR, Rubiales D, Pérez-de-Luque A (2010) Absorption and translocation to the aerial part of magnetic carbon-coated nano particles through the root of different crop plants. *J Nanobiotechnol* 8(26):1–8

- Corredor E, Testillano PS, Coronado MJ, Gozalez-Melendi P, Fernandez-Pacheco R, Marquina C, Ibarra MR, de la Fuente JM, Rubiales D, Perez de Luque A, Risueno MC (2009) Nano particle penetration and transport in living pumpkin plants: in situ subcellular identification. *BMC Plant Biol*. doi:[10.1186/1471-2229-9-45](https://doi.org/10.1186/1471-2229-9-45)
- Da silva LC, Oliva MA, Azevedo AA, De Araujo MJ (2006) Response of resting a plant species to pollution from an iron palletization factory. *Water Air Soil Pollut* 75:241–256
- Davies G, Fataftah A, Cherkasskiy A, Ghabbour EA, Radwan A, Jansen SA, Kolla S, Paciolla MD, Buermann W, Balasubramanian M, Budnick J, Xing B (1997) Tight metal binding by humic acids and its role in biomineralization. *J Chem Soc-Dalton Transact* 21:4047–4060
- De la Rosa G, Lopez-Moreno ML, Hernandez-Viescaz J, Montes MO, Peralta-Videa JR, Gardea-Torresdey JL (2011) Toxicity and biotransformation of ZnO nano particles in the desert plants *Prosopis juliflora-velutina*, *Salsola tragus* and *Parkinsonia florida*. *Int J Nanotechnol* 8:492–506
- Deng Y, White JC, Xing B (2014) Interactions between engineered nano materials and agricultural crops: implications for food safety. *J Zhejiang Univ SCI A (Appl Phys Eng)* 15(8):552–572
- DeRosa MC, Monreal C, Schnitzer M, Walsh R, Sultan Y (2010) Nanotechnology in fertilizers. *Nat Nanotechnol* 5:91–94
- Dhoke SK, Mahajan P, Kamble R, Khanna A (2013) Effect of nano particles suspension on the growth of mung (*Vigna radiata*) seedlings by foliar spray method. *Nanotechnol Dev* (3)1:1–5
- Dimkpa CO, McLean JE, Latta DE, Manangón E, Britt DW, Johnson WP, Boyanov MI, Anderson AJ (2012) CuO and ZnO nano particles: phytotoxicity, metal speciation and induction of oxidative stress in sand-grown wheat. *J Nanopart Res* 14:1125
- Dimkpa CO, Latta DE, McLean JE, Britt DW, Boyanov MI, Anderson AJ (2013) Fate of CuO and ZnO nano and micro particles in the plant environment. *Environ Sci Technol* 47:4734–4742
- Dizdaroğlu M (1985) Application of capillary gas-chromatography mass-spectrometry to chemical characterization of radiation-induced base damage of DNA: implications for assessing DNA-repair processes. *Ann Biochem* 144(2):593–603
- Doshi R, Braida W, Christodoulatos C, Wazne M, O'Connor G (2008) Nano-aluminum: transport through sand columns and environmental effects on plants and soil communities. *Environ Res* 106:296–303
- Duan CQ, Wang HX (1995) Cytogenetical toxic effects of heavy metals on *Vicia faba* and inquires into the *Vicia*-micronucleus. *Acta Bot Sin* 37:14–24
- Ebbs SD, Bradfield SJ, Kumar P, White JC, Musante C, Mac X (2016) Accumulation of zinc, copper, or cerium in carrot (*Daucus carota*) exposed to metal oxide nano particles and metal ions. *Environ Sci Nano* (Advance Article). doi:[10.1039/C5EN00161G](https://doi.org/10.1039/C5EN00161G)
- Eichert T, Kurtz A, Steiner U, Goldbach HE (2008) Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water suspended nano particles. *Physiol Planta* 134:151–160
- Falco WF, Botero ER, Falcão EA, Santiag EF, Bagnato VS, Caires ARL (2011) In vivo observation of chlorophyll fluorescence quenching induced by gold nano particles. *J Photochem Photobiol A Chem* 225:65–71
- Galbraith DW (2007) Nano biotechnology: silica breaks through in plants. *Nat Nanotechnol* 5:272–273
- Ganguly S, Das S, Dastidar SG (2014) Effect of zinc sulphide nano particles on germination of seeds of *Vigna radiata* and their subsequent acceleration of growth in presence of the nano particles. *Euro J Biomed Pharma Sci* 1(2):273–280
- Gardea-Torresdey JL, Parsons JG, Gomez E, Peralta-Videa J, Troiani HE, Santiago P, Yacaman MJ (2002) Formation and growth of Au nano particles inside live alfalfa plants. *Nano lett* 2:397–401
- Gardea-Torresdey JL, Gomez E, Peralta-Videa J, Parsons JG, Troiani HE, Yacaman MJ (2003) Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. *Langmuir* 19(4):1357–1361

- Gardea-Torresdey J, Rodriguez E, Parsons JG, Peralta-Videa JR, Meitzner G, Cruz-Jimenez G (2005) Use of ICP and XAS to determine the enhancement of gold phyto extraction by *Chilopsis linearis* using thiocyanate as a complexing agent. *Ann Bioanal Chem* 382:347–352
- Gardea-Torresdey JL, Rico CM, White JC (2014) Trophic transfer, transformation, and impact of engineered nanomaterials in terrestrial environments. *Environ Sci Technol* 48:2526–2540
- Ghafariyan MH, Malakouti MJ, Dadpour MR, Stroeve P, Mahmoudi M (2013) Effects of magnetite nano particles on soybean chlorophyll. *Environ Sci Technol* 47:10645–10652
- Giraldo JP, Landry MP, Faltermeier SM, Mc Nicholas TP, Iverson NM, Boghossian AA, Reuel NF, Hilmer AJ, Sen F, Brew JA (2014) Plant nano bionics approach to augment photosynthesis and biochemical sensing. *Nat Mater* 13:400–408
- Gogos A, Knauer K, Bucheli TD (2012) Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. *J Agric Food Chem* 60:9781–9792
- Gonzalez-Melendi P, Fernandez Pacheco R, Coronado MJ, Corredor E, Testillano PS, Risueno MC, Marquina C, Ibarra MR, Rubiales D, Perez-De-Luque A (2008) Nanoparticles as smart treatment-delivery systems in plants: assessment of different techniques of microscopy for their visualization in plant tissues. *Ann Bot* 101:187–195
- Hall JL, Williams LE (2003) Transition metal transporters in plants. *J Exp Bot* 54:2601–2613
- Han H, Wang X, Liu X, Gu X, Chen K, Lu D (2012) Multi-walled carbon nano tubes can enhance root elongation of wheat (*Triticum aestivum*) plants. *J Nanopart Res* 14:841–851
- Harris AT, Bali R (2008) On the formation and extent of uptake of silver nano particles by live plants. *J Nanopart Res* 10:691–695
- Haverkamp RG, Marshall AT (2009) The mechanism of metal nano particle formation in plants: limits on accumulation. *J Nanopart Res* 11:1453–1463
- Hong J, Wang L, Sun Y, Zhao L, Niu G, Tan W, Rico CM, Peralta-Videa JR, Gardea-Torresdey JL (2015) Foliar applied nanoscale and microscale CeO₂ and CuO alter cucumber (*Cucumis sativus*) fruit quality. *Sci Total Environ*. doi:10.1016/j.scitotenv.2015.08.029
- Husen A, Siddiqi KS (2014) Phytosynthesis of nanoparticles: concept, controversy and application. *Nanoscale Res Lett* 9:229
- Hyung H, Fortner JD, Hughes JB, Kim JH (2007) Natural organic matter stabilizes carbon nanotubes in the aqueous phase. *Environ Sci Technol* 41(1):179–184
- Iversen TG, Frerker N, Sandvig K (2012) Uptake of ricin B-quantum dot nanoparticles by a micro pinocytosis like mechanism. *J Nanobiotechnol* 10:33
- Jaruga P, Kirkali G, Dizdaroglu M (2008) Measurement of formamido pyrimidines in DNA. *Free Radical Biol Med* 45:1601–1609
- Ke PC, Lamm MH (2011) A biophysical perspective of understanding nano particles at large. *Phys Chem Chem Phys* 13:7273–7283
- Ke PC, Qiao R (2007) Carbon nano materials in biological systems. *J Phys Condens Matt* 19 (37):373101. doi:10.1088/0953-8984/19/37/373101
- Khodakovskaya MV, Biris AS (2009) Method of using carbon nanotubes to affect seed germination and plant growth. WO 2011059507 A1—patent application
- Khodakovskaya MV, de Silva K, Biris AS, Dervishi E, Villagarcia H (2012) Carbon nano tubes induce growth enhancement of tobacco cells. *ACS Nano* 6:2128–2135
- Kole C, Kole P, Randunu KM, Choudhary P, Podila R, Ke PC, Rao AM, Marcus RK (2013) Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon (*Momordica charantia*). *BMC Biotechnol* 13:37
- Kouhi SM, Lahouti M, Ganjeali A, Entezari MH (2014) Comparative phytotoxicity of ZnO nano particles, ZnO micro particles, and Zn²⁺ on rapeseed (*Brassica napus* L.): investigating a wide range of concentrations. *Toxicol Environ Chem* 96:861–868
- Kovalchuk I, Ziemienowicz A, Eudes F, inventors Plantbiosis Ltd., assignee (2012) T-DNA/protein nano-complexes for plant transformation. United States patent US 20120070900 A1, 22 Mar 2012
- Kumari M, Mukherjee A, Chandrasekaran N (2009) Genotoxicity of silver nano particles in *Allium cepa*. *Sci Total Environ* 407:5243–5246

- Kumari M, Khan SS, Pakrashi S, Mukherjee A, Chandrasekaran N (2011) Cytogenetic and genotoxic effects of zinc oxide nano particles on root cells of *Allium cepa*. *J Hazard Mater* 190:613–621
- Kumari M, Ernest V, Mukherjee A, Chandrasekaran N (2012) In vivo nano toxicity assays in plant models. *Meth Mol Biol* 926:399–410
- Kurepa J, Paunesku T, Vogt S, Arora H, Rabatic BM, Lu J, Wanzer MB, Woloschak GE, Smalle JA (2010) Uptake and distribution of ultrasmall anatase TiO₂ alizarin red S nano conjugates in *Arabidopsis thaliana*. *Nano Lett* 10:2296–2302
- Larue C, Laurette J, Herlin-Boime N, Khodja H, Fayard B, Flank AM, Brisset F, Carriere M (2012a) Accumulation, translocation and impact of TiO₂ nanoparticles in wheat (*Triticum aestivum* spp.) Influence of diameter and crystal phase. *Sci Total Environ* 431:197–208
- Larue C, Veronesi G, Flank AM, Surble S, Herlin-Boime N, Carriere M (2012b) Comparative uptake and impact of TiO₂ nano particles in wheat and rapeseed. *J Toxicol Environ Health A* 75:722–734
- Larue C, Castillo-Michel H, Sobanska S, Cécillon L, Bureau S, Barthès V, Ouerdane L, Carrière M, Sarret G (2014) Foliar exposure of the crop *Lactuca sativa* to silver nano particles: evidence for internalization and changes in Ag speciation. *J Hazard Mater* 261:98–106
- Le VN, Rui Y, Gui X, Li X, Liu S, Han Y (2014) Uptake, transport, distribution and Bio-effects of SiO₂ nanoparticles in Bt-transgenic cotton. *J Nanobiotechnol* 12:50
- Lee WM, An YJ, Yoon H, Kwbon HS (2008) Toxicity and bioavailability of copper nano particles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): plant agar test for water-insoluble nanoparticles. *Environ Toxicol chem* 27:1915–1921
- Lei Z, Mingyu S, Xiao W (2008) Antioxidant stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. *Biol Trace Elem Res* 121:69–79
- Li Y, Chen X, Gu N (2008) Computational investigation of interaction between nano particles and membranes: hydrophobic/hydrophilic effect. *J Phys Chem B* 112(51):16647–16653
- Lin D, Xing B (2007) Phytotoxicity of nano particles: inhibition of seed germination and root growth. *Environ Pollut* 150:243–250
- Lin D, Xing B (2008) Root uptake and phytotoxicity of ZnO nano particles. *Environ Sci Technol* 42:5580–5585
- Lin S, Reppert J, Hu Q, Hudson JS, Reid ML, Ratnikova TA, Rao AM, Luo H, Ke PC (2009) Uptake, translocation, and transmission of carbon nano materials in rice plants. *Small* 5:1128–1132
- Liu Q, Chen B, Wang Q, Shi X, Xiao Z, Lin J, Fang X (2009) Carbon nano tubes as molecular transporters for walled plant cells. *Nano Lett* 9:1007–1010
- Lopez-Moreno ML, De La Rosa G, Hernandez-Viezcas JA, Castillo-Michel H, Botez CE, Peralta-Videa JR, Gardea-Torresdey JL (2010a) Evidence of the differential biotransformation and genotoxicity of ZnO and CeO₂ nanoparticles on soybean (*Glycine max*) plants. *Environ Sci Technol* 44:7315–7320
- Lopez-Moreno ML, De La Rosa G, Hernandez-Viezcas JA, Peralta-Videa JR, Gardea-Torresdey JL (2010b) X-ray absorption spectroscopy (XAS) corroboration of the uptake and storage of CeO₂ nanoparticles and assessment of their differential toxicity in four edible plant species. *J Agric Food Chem* 58:3689–3693
- Lu CM, Zhang CY, Wen JQ, Wu GR, Tao MX (2002) Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. *Soybean Sci* 21:168–172
- Ma X, Geiser-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nano particles (ENPs) and plants: phytotoxicity, uptake and accumulation. *Sci Total Environ* 408:3053–3061
- Maharramov AM, Ahmadov IS, Ramazanov MA, Aliyeva SQ, Ramazanli VN (2015) Fluorescence emission spectrum of elodea leaves exposed to nano particles. *J Biomater Nanobiotechnol* 6:135–143
- Martin-Ortigosa S, Peterson DJ, Valenstein JS, Lin VS-Y, Trewyn BG, Lyznik LA, Wang K (2014) Mesoporous silica nanoparticle-mediated intracellular Cre protein delivery for maize genome editing via *loxP* site excision. *Plant Physiol* 164:537–547

- Mazumdar H (2014) The impact of silver nano particles on plant biomass and chlorophyll content. Research inventory Int J Eng Sci 4(7):12–20
- Miralles P, Johnson E, Church TL, Harris AT (2012a) Multiwalled carbon nanotubes in alfalfa and wheat: toxicology and uptake. J Roy Soc Interf 9(77):3514–3527
- Miralles P, Church TL, Harris AT (2012b) Toxicity, uptake, and translocation of engineered nano materials in vascular plants. Environ Sci Technol 46(17):9224–9239
- Mishra V, Mishra RK, Dikshit A, Pandey AC (2014) Interactions of nanoparticles with plants: an emerging prospective in the agriculture industry. In: Ahmad P, Rasool S (eds) Emerging technologies and management of crop stress tolerance: biological techniques, vol 1. Elsevier Academic Press, New York, pp 159–180
- Monica RC, Cremonini R (2009) Nanoparticles and higher plants. Caryologia 62:161–165
- Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS (2010) Nano particulate material delivery to plants. Plant Sci 179:154–163
- Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao AJ, Quigg A, Santschi PH, Sigg L (2008) Environmental behavior and ecotoxicity of engineered nano particles to algae, plants, and fungi. Ecotoxicology 17:372–386
- Nedosekin DA, Khodakovskaya MV, Biris AS, Wang D, Xu Y, Villagarcia H, Galanzha EI, Zharov VP (2011) In vivo plant flow cytometry: a first proof-of concept. Cytometry A 79(10):855–865
- Nel AE, Madler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, Klaessig F, Castranova V, Thompson M (2009) Understanding biophysicochemical interactions at the nano-bio interface. Nat Mater 8(7):543–557
- Onelli E, Prescianotto-Baschong C, Caccianiga M, Alessandra M (2008) Clathrin-dependent and independent endocytic pathways in tobacco protoplasts revealed by labelling with charged nanogold. J Exp Bot 59(11):3051–3068
- Park IY, Kim IY, Yoo MK, Choi YJ, Cho MH, Cho CS (2008) Mannosylated polyethylenimine coupled mesoporous silica nanoparticles for receptor-mediated gene delivery. Int J Pharm 359:280–287
- Parsons JG, Lopez ML, Gonzalez CM, Peralta-Videa JR, Gardea-Torresdey JL (2010) Toxicity and biotransformation of uncoated and coated nickel hydroxide nanoparticles on mesquite plants. Environ Toxicol Chem 29:1146–1154
- Patlolla AK (2013) Environmental toxicity monitoring of nanomaterials using *Vicia faba* GENE-TOX assay. J Nanomed Nanotechnol 4:e129. doi:10.4172/2157-7439.1000e129
- Patrick JW, Tyerman SD, Bel AJE (2015) Long-distance transport. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants, 2nd edn. Wiley, West Sussex, pp 658–710
- Pokhrel LR, Dubey B (2013) Evaluation of developmental responses of two crop plants exposed to silver and zinc oxide nanoparticles. Sci Total Environ 452–453:321–332
- Pradhan S, Patra P, Das S, Chandra S, Mitra S, Dey KK, Akbar S, Palit P, Goswami A (2013) Photochemical modulation of biosafe manganese nano particles on *Vigna radiata*: a detailed molecular, biochemical, and biophysical study. Environ Sci Technol 47:13122–13131
- Prasad TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Sreeprasad TS, Sajanlal PR, Pradeep T (2012) Effect of nano scale zinc oxide particles on the germination, growth and yield of peanut. J Plant Nutri 35(6):905–927
- Priestera JH, Gea Y, Mielkea RE, Horsta AM, Moritzb SC, Espinosae K, Gelbf J, Walkerg SL, Nisbetb RM, Ani YJ, Schimelb JP, Palmere RG, Hernandez-Viezcas JA, Zhaoc L, Gardea-Torresdey JL, Holdena PA (2012) Soybean susceptibility to manufactured nano materials with evidence for food quality and oil fertility interruption. Proc Natl Acad Sci USA 109:14734–14735
- Răuciu M, Creangă D (2007) TMA-OH coated magnetic nanoparticles internalized in vegetal tissues. Rom J Phys 52:395–402
- Răuciu M, Creangă D (2009) Cytogenetical changes induced by β -cyclodextrin coated nanoparticles in plant seeds. Rom J Phys 54:125–131

- Rad JS, Karimi J, Mohsenzadeh S, Rad MS, Moradgholi J (2014) Evaluating SiO₂ Nano particles effects on developmental characteristic and photosynthetic pigment contents of *Zea mays* L. Bull Environ Pharmacol Life Sci 3:194–201
- Remedios C, Rosario F, Bastos V (2012) Environmental nano particles interactions with plants: morphological, physiological, and genotoxic aspects. J Bot 1–8 doi:10.1155/2012/751686
- Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL (2011) Interaction of nano particles with edible plants and their possible implications in the food chain. J Agric Food Chem 59:3485–3498
- Roohizadeh G, Majd A, Arbabian S (2015) The effect of sodium silicate and silica nano particles on seed germination and growth in the *Vicia faba* L. Trop Plant Res 2(2):85–89
- Savithamma N, Ankanna S, Bhumi G (2012) Effect of nano particles on seed germination and seedling growth of *Boswellia ovalifoliolata*—an endemic and endangered medicinal tree. Taxon Nano Vision (1, 2 & 3):61–68
- Schwabe F, Tanner S, Schulin R, Rotzetter A, Stark W, Quadt A, Nowack B (2015) Dissolved cerium contributes to uptake of Ce in the presence of differently sized CeO₂-nanoparticles by three crop plants. Metallomics 7:466–477
- Sekhon BS (2014) Nanotechnology in agri-food production: an overview. Nanotechnol Sci Appl 7:31–53
- Serag MF, Kaji N, Gaillard C, Okamoto Y, Terasaka K, Jabasini M, Tokeshi M, Misukami H, Bianco A, Baba Y (2011a) A functional platform for controlled subcellular distribution of carbon nanotubes. ACS Nano 5:9264–9270
- Serag MF, Kaji N, Venturelli E, Okamoto Y, Terasaka K, Tokeshi M, Mizukami H, Ugent KB, Bianco Baba Y (2011b) Trafficking and subcellular localization of multiwalled carbon nanotubes in plant cells. ACS Nano 5:493–499
- Serag MF, Braeckmans K, Habuchi S, Kaji N, Bianco A, Baba Y (2012a) Spatiotemporal visualization of subcellular dynamics of carbon nano tubes. Nano Lett 12:6145–6151
- Serag MF, Kaji N, Tokeshi M, Baba Y (2012b) Introducing carbon nano tubes into living walled plant cells through cellulase-induced nanoholes. RSC Adv 2:398–400
- Shankar SS, Ahmad A, Sastry M (2003) Geranium leaf assisted biosynthesis of silver nano particles. Biotechnol Prog 19(1627–1631):44
- Sharma NC, Sahi SV, Nath S, Parsons JG, Gardea-Torresdey JL, Pal T (2007) Synthesis of plant-mediated gold nano particles and catalytic role of biomatrix-embedded nano materials. Environ Sci Technol 41:5137–5142
- Shaymurat T, Gu J, Xu C, Yang Z, Zhao Q, Liu Y, Liu Y (2011) Phytotoxic and genotoxic effects of ZnO nano particles on garlic (*Allium sativum* L.): a morphological study. Nanotoxicology 6 (3):241–248
- Shen CX, Zhang QF, Li J, Bi FC, Yao N (2010) Induction of programmed cell death in *Arabidopsis* and rice by single wall carbon nanotubes. Am J Bot 97:1602–1609
- Shyla KK, Natarajan N (2014) Customizing Zinc oxide, silver and titanium dioxide nano particles for enhancing groundnut seed quality. Ind J Sci Technol 17:1376–1381
- Singh N, Manshian B, Jenkins GJ, Griffiths SM, Williams PM, Maffei TG, Wright CJ, Doak SH (2009) Nano geno toxicology: the DNA damaging potential of engineered nano materials. Biomaterials 30(23–24):3891–3914
- Singh A, Singh NB, Hussain I, Singh H, Singh SC (2015) Plant-nanoparticle interaction: an approach to improve agricultural practices and plant productivity. Int J Pharmaceut Sci Inven 4 (8):25–40
- Smirnova EA, Gusev AA, Zaitseva ON, Lazareva EM, Onishchenko GE, Kuznetsova EV, Tkachev AG, Feofanov AV, Kirpichnikov MP (2011) Multi-walled carbon nano tubes penetrate into plant cells and affect the growth of *Onobrychis arenaria* seedlings. Acta Nat 3:99–106
- Smith H (ed) (1978) The molecular biology of plant cells. University of California Press, Berkeley
- Spori CL, Prigent G, Schaer M, Crittin M, Matus P, Laroche T, Sikora B, Kaminska I, Fronc K, Elbaum D, Digigow R, Fink A, Ahmadov I, Khalilov R, Ramazanov M, Forró L, Sienkiewicz A (2014) Uptake and biomagnification of multifunctional magnetic and NIR

- sensitive nano particles by aquatic plants: electron spin resonance, two photon and confocal microscopy studies. In: Proceedings of the Nano-Tera Annual Plenary Meeting, Lausanne, Switzerland, 19–20 May 2014, p 82
- Srinivasan C, Saraswathi R (2010) Nano-agriculture-carbon nano tubes enhance tomato seed germination and plant growth. *Curr Sci* 99:274–275
- Stampoulis D, Sinha SK, White JC (2009) Assay-dependent phytotoxicity of nano particles to plants. *Environ Sci Technol* 43:9473–9479
- Stark WJ (2011) Nano particles in biological systems. *Angew Chem Int Ed Engl* 50(6):1242–1258
- Sudhakar R, Gowda N, Venu G (2001) Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. *Cytologia* 66:235–239
- Sun D, Hussain HI, Yi Z, Siegele R, Cresswell T, Kong L, Cahill DM (2014) Uptake and cellular distribution, in four plant species, of fluorescently labeled mesoporous silica nanoparticles. *Plant Cell Rep* 33:1389–1402
- Tan XM, Fugetsu B (2007) Multi-walled carbon nano tubes interact with cultured rice cells: evidence of a self-defense response. *J Biomed Nanotechnol* 3:285–288
- Taran N, Batsmanova L, Konotop Y, Okanenko A (2014) A redistribution of elements of metals in plant tissues under treatment by non-ionic colloidal solution of biogenic metal nano particles. *Nanoscale Res* 9:354–357
- Torney F, Trewyn BG, Lin VS, Wang K (2007) Mesoporous silica nano particles deliver DNA and chemicals into plants. *Nat Nanotechnol* 2:295–300
- Wang H, Kou X, Pei Z, Xiao JQ, Shan X, Xing B (2011) Physiological effects of magnetite (Fe_3O_4) nanoparticles on perennial ryegrass (*Lolium perenne* L.) and pumpkin (*Cucurbita mixta*) plants. *Nanotoxicology* 5(1):30–42
- Wang J, Mao H, Zhao H, Huang D, Wang Z (2012a) Different increases in maize and wheat grain zinc concentrations caused by soil and foliar applications of zinc in Loess Plateau China. *Field Crops Res* 135:89–96
- Wang Z, Xie X, Zhao J, Liu X, Feng W, White JC, Xing B (2012b) Xylem- and phloem-based transport of CuO nano particles in maize (*Zea mays* L.). *Environ Sci Technol* 46:4434–4441
- Wang J, Koo Y, Alexander A, Yang Y, Westerhof S, Zhang QB, Schnoor JL, Colvin VL, Braam J, Alvarez PJJ (2013a) Phytostimulation of poplars and *Arabidopsis* exposed to silver nanoparticles and Ag^+ at sublethal concentrations. *Environ Sci Technol* 47:5442–5449
- Wang P, Menzies NW, Lombi E, McKenna BA, Johannessen B, Glover CJ, Kappen P, Kopittke PM (2013b) Fate of ZnO nano particles in soils and Cowpea (*Vigna unguiculata*). *Environ Sci Technol* 47:13822–13830
- Wang Q, Ebbs SD, Chen Y, Ma X (2013c) Trans-generational impact of cerium oxide nano particles on tomato plants. *Metallomics* 5(6):753–759
- Wang WN, Tarafdar JC, Biswas P (2013d) Nano particle synthesis and delivery by an aerosol route for watermelon plant foliar uptake. *J Nanopart Res* 15:1417
- Watanabe T, Misawa S, Hiradate S, Osaki M (2008) Root mucilage enhances aluminum accumulation in *Melastoma malabathricum*, an aluminum accumulator. *Plant Signal Behav* 3:603–605
- Whitby M, Quirke N (2007) Fluid flow in carbon nano tubes and nano pipes. *Nat Nanotechnol* 2:87–94
- White PJ (2012) Ion uptake mechanisms of individual cells and roots: short-distance transport. In: Marschner P (ed) *Marschner's mineral nutrition of higher plants*. Elsevier, London, pp 7–47
- Wiesner MR, Lowry GV, Casman E, Bertsch PM, Matson CW, Di Giulio RT, Liu J, Hochella MF Jr (2011) Meditations on the ubiquity and mutability of nano-sized materials in the environment. *ACS Nano* 5(11):8466–8470
- Yang L, Watts DJ (2005) Particle surface characteristics may play an important role in phytotoxicity of alumina nano particles. *Toxicol Lett* 158:122–132
- Zarafshar M, Akbarinia M, Askari H, Hosseini SM, Rahaie M, Struve D (2015) Toxicity assessment of SiO_2 nanoparticles to pear seedlings. *Int J Nanosci Nanotechnol* 11(1):13–22
- Zhang M, Ellis EA, Cisneros-Zevallos L, Akbulut M (2012) Uptake and translocation of polymeric nano particulate drug delivery systems into ryegrass. *RSC Advances* 2:9679–9686

- Zhao L, Peralta-Videa JR, Varela-Ramirez A, Castillo-Michel H, Li C, Zhang J, Aguilera RJ, Keller AA, Gardea-Torresdey JL (2012a) Effect of surface coating and organic matter on the uptake of CeO₂-NPs by corn plants grown in soil: insight into the uptake mechanism. *J Hazard Mater* 225–226:131–138
- Zhao L, Peralta-Videa JR, Ren M, Varela-Ramirez A, Li C, Hernandez-Viezcas JA, Aguilera RJ, Gardea-Torresdey JL (2012b) Transport of Zn in a sandy loam soil treated with ZnO NPs and uptake by corn plants: electron microprobe and confocal microscopy studies. *Chem Eng J* 184:1–8
- Zhao L, Peralta-Videa JR, Peng B, Bandyopadhyay S, Corral-Diaz B, Osuna-Avila P, Montes MO, Keller AA, Gardea-Torresdey JL (2014) Alginate modifies the physiological impact of CeO₂ nano particles in corn seedlings cultivated in soil. *J Environ Sci* 26:382–389
- Zheng L, Hong F, Lu S, Liu C (2005) Effect of nano-TiO₂ on strength of naturally aged seeds and growth of spinach. *Biol Trace Elem Res* 104:83–92
- Zhu H, Han J, Xiao JQ, Jin Y (2008) Uptake, translocation, and accumulation of manufactured iron oxide by pumpkin plants. *J Environ Monit* 10:713–717