Chapter 10
Carbon Nanotubes and Modern Nanoagriculture

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Abstract Since their discovery, carbon nanotubes have been prominent members of the nanomaterial family. Owing to their extraordinary physical, chemical, and mechanical properties, carbon nanotubes have been proven to be a useful tool in the field of plant science. They were frequently perceived to bring about valuable biotechnological and agricultural applications that still remain beyond experimental realization. An increasing number of studies have demonstrated the ability of carbon nanotubes to traverse different plant cell barriers. These studies, also, assessed the toxicity and environmental impacts of these nanomaterials. The knowledge provided by these studies is of practical and fundamental importance for diverse applications including intracellular labeling and imaging, genetic transformation, and for enhancing our knowledge of plant cell biology. Although different types of nanoparticles have been found to activate physiological processes in plants, carbon nanotubes received particular interest. Following addition to germination medium, carbon nanotubes enhanced root growth and elongation of some plants such as onion, cucumber and rye-grass. They, also, modulated the expression of some genes that are essential for cell division and plant development. In addition, multi-walled carbon nanotubes were evidenced to penetrate thick seed coats, stimulate germination, and to enhance growth of young tomato seedlings. Multi-walled carbon nanotubes can penetrate deeply into the root system and further distribute into the leaves and the fruits. In recent studies, carbon nanotubes were reported to be chemically entrapped into the structure of plant tracheary

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elements. This should activate studies in the fields of plant defense and wood engineering. Although, all of these effects on plant physiology and plant developmental biology have not been fully understood, the valuable findings promises more research activity in the near future toward complete scientific understanding of the behavior of carbon nanotubes in plants. This chapter focuses on the impact of carbon nanotubes on plants and the potential use of these unique nanomaterials in crop management and plant biotechnology.

Keywords Carbon nanotubes · Modern nanoagriculture · Crop plants

10.1 Introduction

Carbon nanomaterials are experiencing renewed interest as motivated by their interesting size, shape, and structure, as well as attractive, unique physical properties (Chen et al. 2003; Kam et al. 2004; Bianco et al. 2005; Cherukuri et al. 2004). A wide variety of carbon based nanomaterials such as fullerene, fullerene cages, single-walled carbon nanotubes, multi-walled carbon nanotubes, and cup-stacked carbon nanotubes have been engineered with high precision and quality. Carbon nanotubes are rolled up seamless cylinders of graphene sheets (Iijima 1991; Dai 2002; Dresselhaus and Dai 2004; Golberg et al. 2008). Single-walled carbon nanotubes are composed of one rolled sheet of graphene, while multi-walled carbon nanotubes are composed of several sheets of graphenes rolled up into concentric cylinders. Single-walled carbon nanotubes have a diameter close to 1 nm, with a tube length that can reach few micrometers, while the diameter of multi-walled carbon nanotubes can attain several tenths of nanometers. In recent years, efforts have been dedicated to unveil the biological application of carbon nanotubes because of their unique physicochemical properties (Singh et al. 2005; Gao et al. 2006; Kam et al. 2005, 2006). Work in this area is motivated both by the hope that carbon nanotubes will have useful applications in biology and by the concern that they may exert harmful effects on organisms. A considerable volume of the literature has appeared in the last decade on the application of carbon nanotubes in biological and medical sciences. Indeed, many valuable applications have emerged which admirably enriched the bioscience field (Kam et al. 2005; Bianco et al. 2005, 2011; Kostarelos et al. 2007; Pantarotto et al. 2004; Mènard-Moyon et al. 2010; Lacerda et al. 2006, 2012; Ali-Boucetta 2011).

During the past few years, there has been extensive interest in applying nanoparticles to plants for agricultural and crop management (Nanotechnology in Agriculture and Food 2006; Toreney et al. 2007; Khodakaovskaya et al. 2009, 2012; Serag et al. 2011a, 2012a; Husen and Siddiqi 2014). Indeed, the application of carbon nanomaterials in crop management becomes more pressing in the context of the increasing population and depleting resources. Researchers have demonstrated that the exposure of carbon nanotubes to plant seeds can increase the germination percentage and can enhance the growth of seedlings. These findings could result in significant developments of the production of valuable crops such

as maize and tomato, by taking advantage of the enhancement in the biomass of the plants. However, conflicting reports on the safety of carbon nanotubes have been outlined. These controversial findings require clarification to avoid confusion to the public (Liu et al. 2007, 2008; Singh et al. 2006; Lacerda et al. 2008).

In this chapter, we first shed light on the uptake and distribution of carbon nanotubes in plant cells, and then we give specific examples illustrating the application of carbon nanotubes in agricultural biotechnology. Specifically, we discuss the capability of carbon nanotubes to promote crops yield and reduce the pesticides uptake. Finally, we discuss the environmental and toxicity impacts of carbon nanotubes and further shed light on the future applications of carbon nanotubes in agriculture.

10.2 Plant Cell Uptake, Distribution, and Elimination of Carbon Nanotubes

The cell wall—a multilayered structure surrounding the cell—is unique to the plant kingdom, so it is not surprising that plants grow with defined shape, strength, and rigidity. The plant cell wall is a composite material built from a meshwork of hard cellulose fibers imbedded in a matrix of sugar polymers known as glycans. The plant cell wall represents a formidable barrier to parasites, bacteria, and macromolecules (McNeil et al. 1984). The ability to overcome this barrier has turned out to be beneficial for wide variety of biotechnology application including genetic manipulation and germplasm production (Evans 1983). The plant cell wall has narrow pores of average diameter of 5 nm. These pores allow the passage of solutes while limiting the diffusion of larger particles and macromolecules including some enzymes (Meiners et al. 1991). To tackle this barrier, enzymatic digestion was successfully employed. Enzymatic digestion of the cellulose meshwork via cellulose generates fragile protoplasts—plant cells appearing as spheroplasts after removal of their cell wall—that are easily damaged either physically or chemically. Also, the osmolality of the surrounding medium should be adjusted carefully to prevent cellular burst.

Several strategies have been employed to help carbon nanotubes to enter through the plant cell wall and plant cell membrane. These strategies are largely dependent on the diameter of carbon nanotubes with respect to the diameter of the plant cell wall pores:

1. Since the diameter of single-walled carbon nanotubes (Fig. 10.1) is 1–2 nm which is smaller than the diameter of the plant cell wall pores (5 nm), they were perceived to spontaneously leak into the apoplast (the fluidic space sandwiched between the plant cell wall and the plasma membrane). In order for this spontaneous leakage to be successful, the single-walled carbon nanotubes have to be shortened to a comparable size. Chemical methods such as ultrasonic-assisted chemical oxidative cutting are commonly used to break the carbon nanotubes into small pieces and, simultaneously, introduce carboxylic groups at their tips and walls to enhance their water solubility (Nakayama-Ratchford et al. 2007).

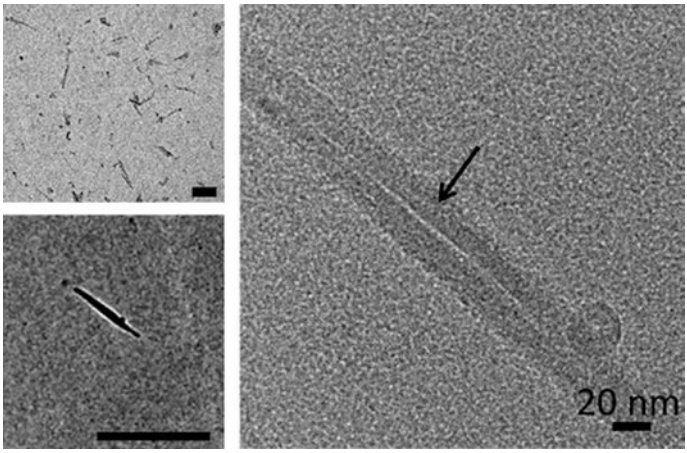

Fig. 10.1 Transmission electron micrograph of single-walled and multi-walled carbon nanotubes. *Top left* Single-walled carbon nanotubes. *Scale bar* 100 nm. *Bottom left* Multi-walled carbon nanotubes. *Scale bar* 500 nm. *Right* Zoomed image of multi-walled carbon nanotubes. The *arrow* indicates the thick wall of the nanotubes. Reprinted and adapted with permission from (Serag et al. 2011a, b). Copyright (2011) American Chemical Society

2. Generation of protoplasts was conceived to tackle the robust barrier of the cell by complete removal of the cell walls. Carbon nanotubes of diameters as wide as tens of nanometers (Fig. 10.1) can be made to penetrate the cell via simple interfacing after cell wall removal. Multi-walled carbon nanotubes were observed to penetrate the cell membrane of *Catharanthus roseus* protoplasts as nanoneedles similar to what have been previously shown for mammalian cells (Pantarotto et al. 2004; Lacerda et al. 2012).
3. Wide-diameter carbon nanotubes have been introduced into walled plant cells via local hydrolysis of the cellulosic cell wall. Cellulose molecules immobilized on the tips and walls of cup-stacked carbon nanotubes generated local lesions in the cell wall, through which the uptake of carbon nanotubes was facilitated.

10.2.1 Cellular Uptake, Distribution, and Exocytosis of Single-Walled Carbon Nanotubes

Single-walled carbon nanotubes have shown a unique ability to leak through the cell wall pores of both *Nicotiana tobacum* and *C. roseus* cells (Serag et al. 2011a, 2013). The first evidence on the internalization of single-walled carbon has been shown for *N. tobacum* in 2009 (Liu et al. 2009). *Nicotiana tobacum* showed temperature-dependent uptake of single-walled carbon nanotubes which suggested that the cell membrane internalization occurred via endocytosis (Fig. 10.2).

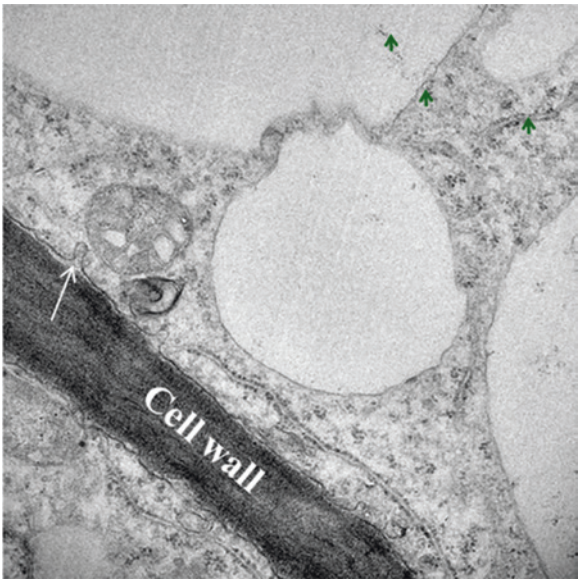

Fig. 10.2 Transmission electron micrograph showing a cytoplasmic view of *Catharanthus roseus* cell. The *white arrow* indicates invagination of plasmalemma leading to formation of small endocytosis organelle. *Scale bar* 500 nm. Reprinted and adapted with permission from (Serag et al. 2011a). Copyright (2011) American Chemical Society

Moreover, wortmanin—an endocytosis inhibitor—hampered the cell uptake and this provided conclusive evidence on the endocytosis-mediated internalization of single-walled carbon nanotubes. The capacity of *N. tobacum* cells to internalize single-walled carbon nanotubes was used to effectively co-transport molecular cargoes into the cellular compartments. Fluorescein isothiocyanate either free or conjugated to single-stranded DNA were successfully introduced into *N. tobacum* cells using single-walled carbon nanotubes. Interestingly, surface modification of single-walled carbon nanotubes by these molecular constructs has directed them to different cellular compartments. Single-walled carbon nanotubes with free fluorescein isothiocyanate were mainly internalized into the cell vacuole, while nanotubes wrapped with fluorescein isothiocyanate linked to single-stranded DNA were sequestered into the cytoplasm (Liu et al. 2009). The mechanism by which different payloads target different cellular compartments is still unclear. Nevertheless, it has been confirmed that single-walled carbon nanotubes follow the same mechanism by which free fluorescein isothiocyanates is translocated from the cytoplasm to the vacuole. After internalization, free fluorescein isothiocyanate anions were evidenced to move from the cytoplasm to the vacuole via protein carriers distributed through the tonoplast (the vacuolar membrane) (Oparka 1991). This carrier mediated transport is inhibited by the uricosuric drug probenecid, causing fluorescein isothiocyanate to exclusively accumulate in the cytoplasm. Single-walled carbon nanotubes conjugated with fluorescein isothiocyanate showed the same translocation/accumulation mechanisms after plant cell interfacing. Furthermore, washing-out probenecid caused the conjugates to redistribute from the cytoplasm to the vacuolar compartment. This indicated that single-walled

carbon nanotubes had no effect on the distribution mechanism of fluorescein isothiocyanate in the plant cell (Khodakovskaya et al. 2012). This observation is not surprising because the drastic sonication-assisted oxidation of single-walled carbon nanotubes usually downsizes the carbon nanotubes to a comparable size to the fluorescein isothiocyanate molecule. Therefore, the small size of fluorescein isothiocyanate conjugates and the high loading density on the surface of carbon nanotubes compared with the loading density of the single-stranded DNA-fluorescein isothiocyanate conjugate was hypothesized to favor the delivery of the former conjugate into the vacuoles via the protein carriers and to inhibit the vacuolar delivery of the later conjugates. These observations might explain the organelles-targeted delivery of carbon nanotubes.

The exclusive cytoplasmic accumulation of carbon nanotubes with stacked fluorescein isothiocyanate molecules in presence of probenecid increased their chances to enter the cell nucleus. Fluorescence recovery after photobleaching[1] revealed that single-walled carbon nanotubes conjugated with fluorescein isothiocyanate accumulated inside the nucleus of *C. roseus* cells. The positively charged nuclear proteins assisted the liberation of fluorescein isothiocyanate molecules in the nucleoplasm where they further accumulated inside the nucleolus.

Using raster scan image correlation spectroscopy,[2] it was further revealed that single-walled carbon nanotubes induce autophagy in *C. roseus* cells. Raster scan image correlation spectroscopy was used to generate quantitative spatial maps of single-walled carbon nanotubes dynamics inside different cell compartments. Raster scan image correlation spectroscopy revealed that the diffusion coefficient of single-walled carbon nanotubes conjugated with fluorescein isothiocyanate

[1] FRAP (Fluorescence recovery after photobleaching) is an optical technique used to quantify the lateral mobility of molecules inside the cells. The experimental setup comprises a microscope, a laser light source and a fluorescent probe (e.g. fluorescein isothiocyanate molecule) coupled to the molecule of interest (single-walled carbon nanotubes). Several images of the fluorescent probe were acquired to determine its initial fluorescence intensity, and then a bright laser illumination are focused for a short time on the region of interest to photobleach the fluorescence of the probe. Finally, another series of images are acquired using low level of illumination to track the gradual recovery of the fluorescence in the bleached area. This should give information about the lateral diffusional mobility of the molecules of interest.

[2] Raster scan image correlation spectroscopy (RICS) is an optical technique to measure the spatiotemporal distribution of fluorescent probes inside the cell. RICS is considered a merge of the conventional fluorescence correlation spectroscopy (FCS) and image correlation spectroscopy (ICS) technologies. FCS is an optical method that monitors the spontaneous fluctuations of fluorescence intensity collected from fluorophores in a small, open excitation laser beam volume. FCS can measure fast diffusion with only temporal information. ICS monitors temporal fluctuations at every point in a stack of 2-D images. ICS can measure very slow diffusion with spatial information. RICS is used to measure molecular dynamics from fluorescence confocal images such as binding and diffusion. It can be used to efficiently measure a wide range of diffusion coefficients ranging between 0.1 and 1,000 $\mu m^2 s^{-1}$.

inside *C. roseus* cell vacuoles was close to the diffusion coefficient in the cytoplasm. This indicated that parts of the cytoplasm leaked inside the vacuoles through autophagy (Autophagy ('self-eating'), is an ubiquitous stress response in eukaryotic organisms that targets damaged organelles for vacuolar degradation) (Serag et al. 2013; Minibayeva et al. 2012). High-resolution transmission electron microscopy further revealed that Golgi apparatus was involved in this stress response (Fig. 10.3) (Serag et al. 2012c).

The plant cell has a unique ability to limit the cytoplasmic localization of single-walled carbon nanotubes to minimize their toxicity. Laser scanning confocal microscopy showed that the plant cell rapidly distributes the internalized single-walled carbon nanotubes into the cell vacuole. Following this vacuolar redistribution, single-walled carbon nanotubes migrate toward the cell membrane via a vesicle-mediated transport pathway. The vesicles, filled with the nanotubes, shuttle between the tonoplast (the vacuolar membrane) and the plasma membrane. These shuttling vesicles, then, fuse with the plasma membrane expelling the nanotubes outside the cell (Fig. 10.4) (Serag et al. 2011a). Adding inhibitors of the vesicle-mediated transport to the cell medium (e.g., Exo1) resulted in trapping of the nanotubes inside the vesicles. This suggested the involvement of the vesicle-mediated transport in the exocytosis process of single-walled carbon nanotubes (Serag et al. 2011a).

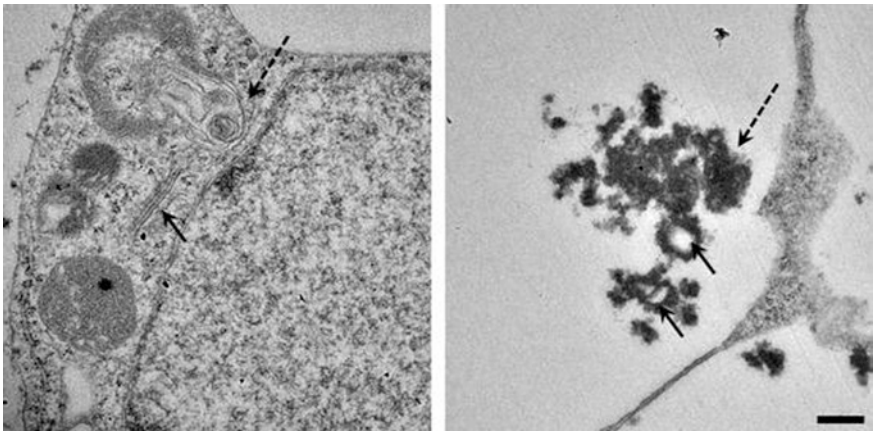

Fig. 10.3 High-resolution transmission electron microscopy imaging of the autophagy phenomenon. *Left* Cytoplasmic view of *C. roseus* cell incubated with single-walled carbon nanotubes. The *solid arrow* indicates an intact Golgi body, while the *dashed arrow* indicates a damaged one. *Right* Vacuolar view of *C. roseus* cell incubated with single-walled carbon nanotubes. The *arrows* indicate damaged Golgi-associated vesicles. The *dashed arrow* indicates extensive aggregates of single-walled carbon nanotubes due to the high salt concentration inside the cell vacuole. Reprinted and adapted with permission from (Serag et al. 2012b). Copyright (2012) American Chemical Society

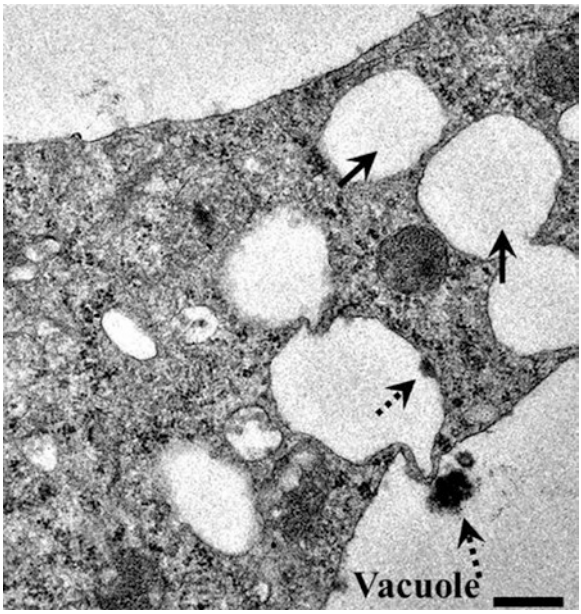

Fig. 10.4 Transmission electron micrograph showing a cytoplasmic view of *C. roseus* cell. The *black dashed arrows* indicate aggregates of single-walled carbon nanotubes associated with a vesicular membrane or inside vacuoles during vesicle-mediated transport. The *solid arrows* indicate large trafficking vesicles. *Scale bar* 500 nm. Reprinted and adapted with permission from (Serag et al. 2011b). Copyright (2011) American Chemical Society

10.2.2 Cellular Uptake, Distribution, and Exocytosis of Multi-walled Carbon Nanotubes

Unlike single-walled carbon nanotubes, the multi-walled nanotubes physically penetrated the cell membrane of *C. roseus* protoplasts through a nonendosomal route. Using high-resolution transmission electron microscopy, multi-walled carbon nanotubes were observed to penetrate the subcellular membranes as nanoneedles. Electron microscopy further revealed that the uptake of multi-walled carbon nanotubes are poorly associated with the endosomal organelles. Although the uptake of multiwalled carbon nanotubes occurs through an energy-independent route, increasing the concentration of nanotubes in the cell medium resulted in a decrease in the rate of the normal endocytosis function of the cell. This phenomenon was attributed to a carbon nanotubes-dependent increase in the medium tonicity which resulted in temporary inhibition of the endosomal cycle. This inhibition was proposed to promote the direct penetration of multi-walled carbon nanotubes into the cell rather than the endosomal route (Serag et al. 2011a).

The direct penetration of multi-walled carbon nanotubes assisted their translocation to most of the cell organelles. High-resolution transmission electron microscopy identified vacuoles, plastids and nucleus as the primary locations of nanotubes accumulations (Figs. 10.5 and 10.6). Following the uptake, multi-walled carbon nanotubes penetrated deeply into cell nucleus and localized in the peri-nuclear region. The average lengths of multi-walled carbon nanotubes distributed into different cell organelles were around 100 nm. Multi-walled carbon

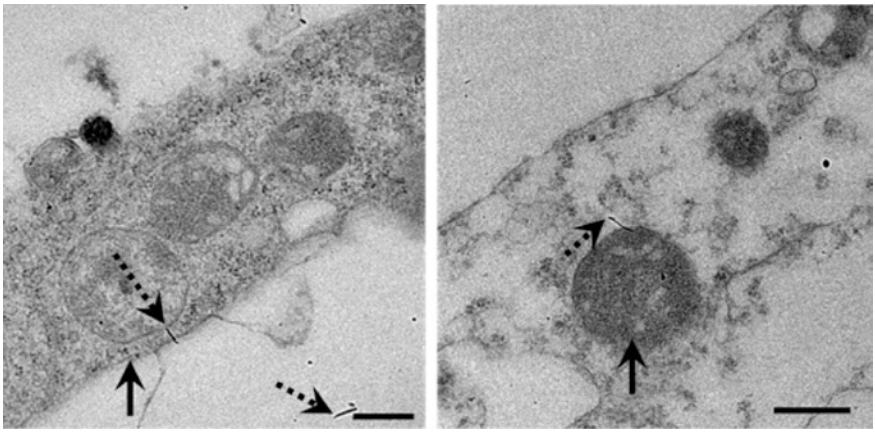

Fig. 10.5 Transmission electron micrograph showing cytoplasmic views of *C. roseus* cell. *Left* The micrograph shows a multi-walled carbon nanotubes penetrating the vacuolar membrane. *Dashed arrows* indicate multi-walled carbon nanotubes. The *solid arrow* indicates the vacuolar membrane (tonoplast). *Right* The *dashed arrow* indicates a multi-walled carbon nanotube penetrating the mitochondrial membrane. The *solid arrow* indicates a mitochondrion. *Scale bars* 500 nm. Reprinted and adapted with permission from (Serag et al. 2011b). Copyright (2011) American Chemical Society

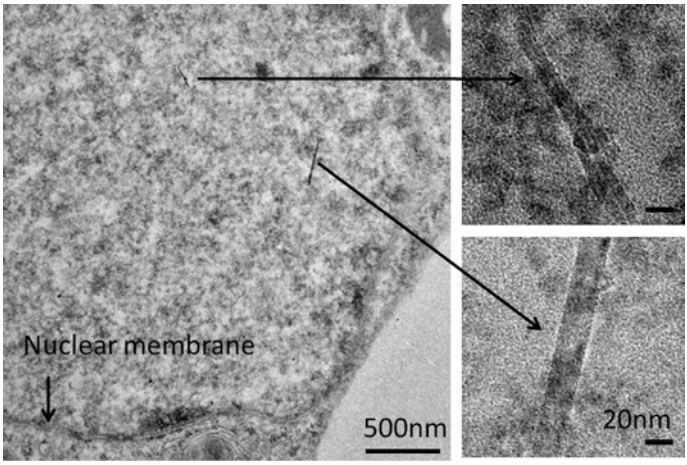

Fig. 10.6 Transmission electron micrographs showing carbon nanotubes accumulation inside the nucleus of *C. roseus*. *Left* The micrograph shows multi-walled carbon nanotubes inside the nuclear matrix. *Right* The corresponding zoomed-in images of the nucleus. Reprinted and adapted with permission from (Serag et al. 2011b). Copyright (2011) American Chemical Society

nanotubes longer than 100 nm were accumulated in most of the organelles of *C. roseus* cells, while short tubes (30–100 nm) accumulated inside vacuoles, plastids and the nucleus. Endoplasmic reticulum and mitochondria were resistant to the penetration of short multi-walled carbon nanotubes (Fig. 10.5). This suggested a

size-dependent distribution of multi-walled carbon nanotubes in *C. roseus* cells. This size-dependent distribution was proposed to offer a strategy of organelles-targeting via multi-walled carbon nanotubes.

10.2.3 Cellular Uptake and Fate of Cup-Stacked Carbon Nanotubes

Cup-stacked carbon nanotubes consist of closely-packed truncated graphene buckets. This unique structure differentiates them from the seamless cylinders structure characteristic of both single and multi-walled carbon nanotubes (Endo et al. 2002; Kim et al. 2004; Hasobe et al. 2006; Saito et al. 2006). This truncated conical structure provides wide room of reactive edges in the outer as well as inner surfaces and hence permits efficient functionalization. The average diameter of these nano-buckets range between 60 and 100 nm (Serag et al. 2012a).

A simple strategy has been proposed to deliver the cup-stacked carbon nanotubes into the walled plant cells. Cellulase enzyme that has the ability to hydrolyze the cellulosic wall surrounding the plant cell was immobilized on the tips of the cup-stacked carbon nanotubes. Cellulase subunits are tadpole-like protein structures with 21.5 nm size (Pilz et al. 1990). Therefore, the wide diameter cup-stacked carbon nanotubes are suited to carry this large protein structure. To introduce the cup-stacked carbon nanotubes into the plant cell, cellulose enzyme was covalently attached to the tips and walls of the cup-stacked carbon nanotubes. The immobilized cellulose induced tiny openings in the plant cell wall, through which the cup-stacked carbon nanotubes penetrated the cell. Indeed, this strategy bypassed the complete removal of the cell wall that negatively affects cell viability and plant regeneration (Serag et al. 2011a).

The cellular fate of cup-stacked carbon nanotubes has been elucidated using *Arabidopsis thaliana* cells. Cup-stacked carbon nanotubes were observed to participate in the cell transdifferentiation process into tracheary cells.[3] The presence of cup-stacked carbon nanotubes inside *A. Thaliana* cells has promoted their attachment to the cell microtubules. This was followed by oxidative cross-linking of monolignol (precursors of lignin biosynthesis) to the surface of the nanotubes leading to their deposition in the structure of the tracheary cells' walls (Serag et al. 2012a).

[3] Tracheary elements are lignified structures associated with xylem tissues in the plants. They are highly specialized for moving water and solutes from the root to the shoot system. In cell culture, the cells of some plants transdifferentiate into tracheary cells by the effect of phytohormones present in the cell medium. The transdifferentiation process begins with the deposition of secondary cell wall consisting of cellulose, lignin and hemicellulose. The transdifferentiation ends with a process called "programmed cell death" where all of the cells contents are removed leaving the cell empty.

10.3 Carbon Nanotechnology for Crops Biotechnology

The development of nanotechnology-based applications in agriculture is essential to tackle many difficulties that farming and food industry face across the world. The challenge has always been how to increase the agricultural output, detect plant diseases at appropriate timing, and efficiently advance the treatment. The process of maximizing the agricultural output requires improving the ability of plants to absorb nutrients from the soil. In this section, several examples are presented to illustrate the capability of carbon nanotubes to improve the agriculture output of edible plants.

10.3.1 Tomato

Compared with the plant cell wall and cell membrane, the thick seed coat is expected to hamper the penetration of carbon nanotubes into the embryo. However, researchers elucidated that multi-walled carbon nanotubes have the ability to penetrate the thick seed coat of tomato plant and increase seed germination and plant growth (Khodakaovskaya et al. 2009). Adding multi-walled carbon nanotubes at concentrations 10, 20, and 40 μg/ml to the nutrient medium significantly shortened the period of germination compared to the control sample lacking the multi-walled carbon nanotubes. The tomato plantlets germinated in presence of the multi-walled carbon nanotubes showed an increase in the biomass and, also, possessed well-developed stems compared with the control. The length of the rootlets appeared the same in both cases. Overall, tomato plants were shown to grow up to two times faster than the control. This was attributed to the increased number of pores in the seed coat that the multi-walled carbon nanotubes generate upon their penetration. These pores were hypothesized to provide better water and nutrient permeation (Khodakaovskaya et al. 2009, 2013).

Researchers also demonstrated the effect of multi-walled carbon nanotubes on the tomato plant in all stages of plant developments (Nair et al. 2012). In addition to the double height attained after treatment with multi-walled carbon nanotubes, the number of flowers was also doubled compared with the control. The mechanisms of all of these effects remain to be investigated.

The effect of single-walled carbon nanotubes on root elongation has been studied (Canas et al. 2008). Pristine single-walled carbon nanotubes negatively affected root elongation in tomato at 24 and 48 h incubation times. This inhibitory effect was attributed to an enhanced accumulation of the single-walled carbon nanotubes around the base of the apical meristem of tomato roots. Since the apical meristem is located in the zone of cell division, root elongation was inhibited.

10.3.2 Rice

The effect of carbon nanomaterials on the germination of rice seeds has been demonstrated (Nair et al. 2012). Particularly significant, carbon nanotubes were found to increase the water contents of the seeds during germination. The carbon nanotubes-treated seeds showed healthy and well-developed roots and shoots compared with control seedlings.

Experiments done on cell suspension of rice cells with multi-walled carbon nanotubes, however, showed various toxic effects. Primarily, multi-walled carbon nanotubes elicited the release of reactive oxygen species with consequent increase in the dead cells. Multi-walled carbon nanotubes also suppressed the activity of superoxide dismutase and decreased chlorophyll contents. These changes have been accounted for the natural self-defense of the plant to avoid affecting other cells (Tan et al. 2009).

Second generation rice plants grown from plant seeds initially germinated in nutrient media containing single-walled carbon nanotubes and multi-walled carbon nanotubes and, then, harvested after 6 months showed residual carbon nanotubes in their tissues. The accumulated carbon nanotubes in the seed resulted in 1 month delay in the germination of the seeds. The seed had more residual carbon nanotubes followed by root, stem, and then leaves. This indicated that carbon nanotubes were transmitted to the next generation of the plant and resulted in adverse effects (Tan et al. 2009).

10.3.3 Maize

Maize seedlings grown in agar nutrient media and treated with different concentration of multi-walled carbon nanotubes had dramatic effect on the growth. Pristine multi-walled carbon nanotubes increased the growth of plant rootlets. The water imbibition rate was found to be higher in the presence of the multi-walled carbon nanotubes. Furthermore, ionic nutrient transport showed dramatic enhancement that could be facilitated by water inflow. Higher concentrations of multi-walled carbon nanotubes, however, had less dramatic effect. These findings provided a clue for the utilization of multi-walled carbon nanotubes for optimizing water transport and increasing the biomass yield of maize (Tiwari et al. 2013).

10.3.4 Mustard

The rate of germination and growth of *Brassica juncea* has been evaluated in presence of pristine multi-walled carbon nanotubes and oxidized multi-walled carbon nanotubes. Researchers found that oxidized multi-walled carbon nanotubes

increased the moisture contents of seeds and enhanced the water absorption machinery of root tissues. The also had a positive effect on the seed germination rate. These effects were observed at very low concentration of the nanotubes (2.3×10^{-3} mg/mL). The enhancement of the growth rate could be obtained by soaking the seed in a solution of the oxidized multi-walled carbon nanotubes instead of adding them to the germination and growth media (Mondal et al. 2011). The mechanism of water influx into the seed in presence of carbon nanotubes is still under intensive research. It was shown that immersing the plant tissues in a solution of carbon nanomaterials increased its electrical conductance (Zheng et al. 2005). This effect was suggested to affect the plant aquaporins (specialized channels that regulate water and salts influx by selectively allow water molecules to flow into the plant tissues to maintain equilibrium). Furthermore, it has been reported that multi-walled carbon nanotubes activates the expression of the aquaporin gene (*LeAqp2*) (Zheng et al. 2005).

High concentration of the oxidized multi-walled carbon nanotubes (exceeding 46×10^{-3} mg/mL) had harmful effect to the plant growth and the biomass production. It was concluded that the rate of growth was concentration dependent. Researchers, also, found that the nanotubes migrate through the plant vascular tissues through the plant. These significant effects were proposed to be useful in horticulture, agriculture, and biofuel production (Mondal et al. 2011).

10.3.5 Onobrychis

Multi-walled carbon nanotubes have been shown to enhance the growth of *Onobrychis arenaria* plant and stimulate the peroxidase activity as well. The translocation of multi-walled carbon nanotubes to leaves and roots was also reported for *Onobrychis*. The increase in the peroxidase activity was associated with the oxidative stress, a characteristic effect of carbon nanomaterials on plant cells. Researchers have shown that multi-walled carbon nanotubes accumulated at the root surface and penetrated the epidermal cells. This penetration resulted in an injury associated elevation of peroxidase activity. Transmission electron microscopy images have supported the accumulation of multi-walled carbon nanotubes in different plant tissues (Smirnova et al. 2012).

10.3.6 Wheat

Wheat germination exhibited significant enhancement in presence of multi-walled carbon nanotubes. After treating wheat with Fe_3O_4-functionalized multi-walled carbon nanotubes, they were detected in the epidermis of the root. With the aid of Raman spectroscopy, it was elucidated that the functionalized carbon nanotubes adsorbed onto the root surface without affecting the plant development or

root-tissue morphology. Researchers detected functional groups such as carboxylic acids and isothiocyanates at the sites of carbon nanotubes adsorption. This might be a consequence of the chemical binding of Fe_3O_4-functionalized multi-walled carbon nanotubes to the plant tissues (Miralles et al. 2012).

In the same study, (Miralles et al. 2012) the researchers have compared the effect of multiwalled carbon nanotubes on the germination rate of wheat and alfalfa plants. Multi-walled carbon nanotubes enhanced wheat germination rate compared with that of alfalfa. This has been accounted for the ease of nanotubes penetrability in the case of Wheat. Wheat seed is relatively softer that than of alfalfa, therefore, the uptake of carbon nanotubes was conceived to be much more facilitated.

10.3.7 Cucumber, Onion, Lettuce, Carrot, and Cabbage

In a pilot study, the effect of single-walled carbon nanotubes on root elongation of cucumber, onion, lettuce, carrot, and cabbage was explored (Canas et al. 2008). Interestingly, different crops showed different responses to single-walled carbon nanotubes. Cucumber and onion were the only crops that responded positively to single-walled carbon nanotubes where root elongation was enhanced at concentrations more than 100 mg/L. Root elongation of cabbage and carrot was unaffected by single-walled carbon nanotubes while the root elongation in only one species, lettuce, was inhibited after 48 h. Alteration of surface chemistry of the root was hypothesized to be a major factor that contribute to these effects. For example, single-walled carbon nanotubes may affect microbial–root interaction in the rhizosphere and may cause microbial toxicity.

Cucumber was chosen as a model plant to explore the root uptake. After exposure to single-walled carbon nanotubes for 48 h, the predominant mechanism of the uptake was energy-independent passive uptake where no sign of active uptake has been detected.

10.4 Carbon Nanotubes and Pesticides

The effect of multi-walled carbon nanotubes on the residues of pesticides in a number of plants was studied (De La Torre-Roche et al. 2013). It was evidenced that pesticide residues in zucchini, corn, tomato and soybean was reduced in presence of multi-walled carbon nanotubes. For example, chlordane and DDT accumulation in different parts of the above plants has been reduced in a dose-dependent fashion by the presence of multi-walled carbon nanotubes. Although this effect has been demonstrated for different species, the underlying mechanisms and implications for food safety should receive rigorous investigations.

10.5 Environmental and Toxicity Impact of Carbon Nanotubes

An increasing number of studies outlined the environmental impacts and safety profile of carbon nanomaterials (Lin and Xing 2007; Schwab et al. 2011; Khodakovskaya et al. 2011; Oleszczuk et al. 2011; Wei et al. 2010; Shen et al. 2010; Stampoulis et al. 2009). It was revealed that carbon nanotubes functionalization dramatically reduces their toxic effects (Sayes et al. 2006; Kostarelos et al. 2009). In several studies, functionalized carbon nanotubes have not shown toxic effects on plant cell (Serag et al. 2011a, 2012b). However, in other studies pristine carbon nanotubes were reported to induce various toxic effects. For example, single-walled carbon nanotubes induced toxic effects in rice and *Arabidopsis* including cell aggregation, chromatin condensation, plasma membrane deposition, and H_2O_2 accumulation. Twenty-five mg/mL of single-walled carbon nanotubes was sufficient to cause death to 25 % of cultured protoplasts within 6 h. This toxic effect was due to oxidative stress that led to apoptosis (Shen et al. 2010). With a similar mechanism, pristine multi-walled carbon nanotubes were reported to reduce the biomass of *Cucurbita Pepo* plants in hydroponic cultures indicating a high degree of toxicity.

10.6 Future of Nanoagriculture Technologies Based on Carbon Nanotubes

The emerging science of nanoagriculture continues to rapidly advance and promises valuable technologies for enhancing the agricultural output. In a recent study (Giraldo et al. 2014), it has been shown that single-walled carbon nanotubes passively transport and target the lipid envelope of extracted plant chloroplasts. Upon their accumulation, they enhance the electron transport rate and the photosynthetic activity over three times compared with that of the control. The semiconducting single-walled carbon nanotubes were shown to increase chloroplast carbon capture by promoting chloroplast solar energy utilization. Furthermore, single-walled carbon nanotubes were shown to enable near-infrared fluorescence monitoring of nitric oxide. This property could open new avenue toward development of plants as photonic chemical sensors. For example, single-walled carbon nanotubes real-time sensing of nitric oxide in leaves could be extended to detect pesticides, herbicides, and environmental pollutants.

Several studies have focused on the interface of nanotechnology and plant biology (Serag et al. 2011a, 2012b). Carbon nanotubes were able to traffic through the plant cell wall and most of the subcellular organelles. The subcellular distribution of carbon nanotubes was strongly dependent on their lengths and the nature of the functional tag adsorbed or covalently linked to their surfaces. The demonstrated ability of preferential accumulation is of particular importance for plant biotechnology and agriculture where biomolecules could be co-delivered to specific subcellular organelles.

The incorporation of carbon nanotubes in the structure of tracheary elements (Serag et al. 2012b) could prove useful for wood bioengineering. It was conceived that plant woods can be reinforced with carbon nanotubes fibers to impart novel characteristics to the wood such as more strength (Serag et al. 2013).

Carbon nanotubes could be regarded as a potential nano-vector to transfect plant cells with genes of interest. Single-stranded DNA molecules wrapped around SWCNTs were able to target the cytoplasm of walled plant cells. This property could be used to introduce RNA pieces into the nucleus to activate or silence the genes of interest. Similarly, protoplast could be a target for delivering larger DNA molecules such as the delivery of plasmids into the plant cell genome.

In conclusion, carbon nanotechnology has the potential to enable advanced application in agriculture. Starting from increased crops yield passing by organelles-targeted gene delivery and ending with wood and chloroplasts engineering, in the following years carbon nanotubes are expected to show more opportunities in the agricultural field that remained beyond technical realization.

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