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RESEARCH PAPER

Multi-walled carbon nanotubes can enhance root elongation of wheat (*Triticum aestivum*) plants

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Abstract The potential effects of oxidized multi-walled carbon nanotubes (o-MWCNTs) with a length ranging from 50 to 630 nm on the development and physiology of wheat plants were evaluated by examining their effects on seed germination, root elongation, stem length, and vegetative biomass at a concentration ranging from 10 to 160 μg/mL in the plant. Results indicated that after 7 days of exposure to the o-MWCNTs medium, faster root growth and higher vegetative biomass were observed, but seed germination and stem length did not show any difference as compared with controls. Moreover, a physiological study was conducted at cellular level using a traditional physiological approach to evidence the possible alterations in morphology, the cell length of root zone, and the dehydrogenase activity of seedlings. Transmission electron microscopy images revealed that o-MWCNTs could penetrate the cell wall and enter the cytoplasm after being taken up by roots. The cell length of root zone for the seedlings germinated and grown in the o-MWCNTs (80 μg/mL) medium increased by 1.4-fold and a significant concentration-dependent increase in the dehydrogenase activity for the o-MWCNT-treated wheat seedlings was detected. These findings suggest that o-MWCNTs can significantly promote cell elongation in the root system and increase the dehydrogenase activity, resulting in faster root growth and higher biomass production.

Keywords MWCNTs · Wheat · Root length · Vegetative biomass · Cell morphology · Nanotechnology · Agriculture

Introduction

Carbon-based materials such as carbon nanotubes (CNTs), fullerene, and graphene are one of the most fascinating nanomaterials with unique mechanical, electrical, physical, and chemical properties, and in recent years have aroused widespread interest among researchers (Pillai et al. 2010; Wild and Jones 2009; Jiang et al. 2011). The potential use of carbon-based materials has been actively explored in some important fields like biomedicine, biosensors, and tissue engineering (Baby et al. 2011; Tan et al. 2009), but the exploration of their extensive application in agriculture and plant science has just been initiated (Nair et al. 2010; Kurepa et al. 2010). Recently, it has been found that CNTs at relatively low doses can penetrate even

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thick seed coats, stimulate germination, and activate enhanced growth of tomato plants (Khodakovskaya et al. 2009). Moreover, CNTs can also work as an effective nanocargo to deliver DNA and small molecules into tobacco cells (Liu et al. 2009a, b). There are also reports about multiple nano-technological applications of CNTs in agriculture, from agrochemical treatments to genetic transformation for improved plant disease resistance, efficient nutrient utilization, and enhanced plant growth (Liu et al. 2010; Nair et al. 2010). Nevertheless, before the development of their application in agriculture such as the "smart delivery system", the effects and behavior of carbon-based materials on plant growth and development should be properly identified.

Recently, some reports have been published about the impact of carbon-based materials on plants, focusing on several negative effects, but recent experimental results are inconsistent with the previous reports. It is reported that single-walled carbon nanotubes (SWCNTs) significantly retard root elongation of tomato, cabbage, carrot, and lettuce in 24–48 h (Canas et al. 2008), but stimulate Arabidopsis mesophyll cell growth at a concentration of 50 μg/mL culture medium (Yuan et al. 2011). Multi-walled carbon nanotubes (MWCNTs) have been found to increase reactive oxygen species (ROS) and decrease cell viability of rice plants (Tan et al. 2009). However, the latest research indicates that MWCNTs can increase the growth rate of gram plant in every part (Shweta et al. 2011).

From the above, we can see that although the possible effects of carbon-based materials have raised considerable attention, certain impacts of carbon-based material on plants are still in debate. Additionally, the water solubility of CNTs is a key factor when considering their behavior in biological systems (Rafeeqi and Kaul 2011). To better understand the effects of CNTs on plant physiology and plant development, the water solubility of CNTs must be considered, and its biocompatible role in biological systems should be addressed first before any agricultural application is investigated. Therefore, it is still very necessary to continue the exploration and identification of the effects of CNTs on plant growth and physiology.

In this study, MWCNTs after oxidization with hydrochloric acid were used to evaluate their potential effects on an agriculture plant, wheat (*Triticum aestivum*), one of the staple food crops for nearly half the world's population. The evaluation was focused on the effects of o-MWCNTs on the development and physiology of wheat plants in seed germination, root elongation, stem length, and vegetative biomass as well as the morphology, the cell length, and the dehydrogenase activity of seedlings. Results from this research may help to reveal the effect and behavior of o-MWCNTs in living plants and further their application in the field or laboratory.

Experimental section

Materials

MWCNTs were purchased from Sigma-Aldrich (purity: 99 %, OD × Length 6–13 nm × 2.5–20 μm). 2,3,5-triphenyltetrazolium chloride (TTC) was obtained from Beijing Chemical Reagents Company (China). The wheat seeds were purchased from a local seed company.

Instruments

The morphology of o-MWCNTs was inspected and obtained by TEM (Hitachi H-7650, Japan), and the morphological images of root tissues were obtained using Tecnai G20 microscopy (FEI, Czech). The Raman spectra of MWCNTs and cell images of elongation zone in root system were obtained using inVia Raman spectrometer (Renishaw, UK) equipped with a confocal microscope (Leica, DM LM/P/11888500, Germany). The excitation source was a He–Ne laser (633 nm), focused with a 50× long focal length objective microscope onto a spot size of approximately 2 μm and providing a power of 2 mW on the sample. Fourier transform infrared spectra (FT-IR) were collected on a Nicolet Avatar-330 spectrometer (Thermo Nicolet, US) with 4 cm^{-1} resolution using the KBr pellet technique. Particle size distribution of MWCNTs and o-MWCNTs were measured using a Zetasizer Nano ZS90 DLS system (Malvern, England).

o-MWCNTs preparation

Stable aqueous suspensions of purified, shortened, and functionalized nanotubes were obtained by oxidation and sonication, which were treated by following the

standard procedure described in the literature (Liu et al. 2009a, b). Briefly, 20 mg MWCNTs were added to a 100 mL 3:1 concentrated H_2SO_4:HNO_3 solution. Then the mixture was ultrasonicated for 8 h, followed by centrifugation to remove larger unreacted impurities from the solution, a thorough rinse with pure water and re-suspended in water by sonication for 20 min.

Plant materials and growth conditions

The effects of o-MWCNTs on seed germination and wheat seedlings growth were determined as follows. Seeds were pregerminated and those with a similar size were selected (Stampoulis et al. 2009). Seeds were immersed in a 10 % sodium hypochlorite solution for 10 min and rinsed three times with deionized water to ensure surface sterility. Next, the seeds were soaked in deionized water or o-MWCNTs suspension solution for about 5 h. One piece of filter paper was put into each 100 × 100 mm Petri dish, and 5 mL of o-MWCNTs suspension (10, 20, 40, 80, and 160 μg/mL) was added. After that, the seeds were transferred onto the filter paper, with 10 seeds per dish and 1 cm or a larger distance between each seed (Lin and Xing 2007). Petri dishes were covered and sealed with tape, and the seedlings were placed in a growth room maintained at 23 ± 1 °C in the dark. 5 mL of o-MWCNTs suspension were added every 3 days, and 5 mL water was added on the other days. The seed germination was recorded when nearly 65 % of control roots were 5-mm long or more (Wang et al. 2001; Lopez-Moreno et al. 2010). The 4- or 7-day-old seedlings were washed, and the root elongation and the vegetative biomass were measured.

Root growth rate was defined as the daily growth in root length (Lagrimini et al. 1997). The root lengths of the seedlings in the o-MWCNTs (80 μg/mL) medium were employed to determine the root growth rate every 24 h and successively for 5 days. The root growth rate was calculated as follows: (final length − initial length)/time (day).

Morphological observation by TEM

The root tissues were further observed by TEM to examine whether o-MWCNTs entered the plant cells. When 7-day-old seedlings incubated in o-MWCNTs (80 μg/mL) were obtained, their clean root samples were cut open, prefixed in 3.5 % glutaraldehyde, washed with 0.1 mol/L pH 7.0 phosphate buffers, and postfixed in 1.0 % osmium tetroxide. After fixation, the tissues were dehydrated in an ascending ethanol series, and embedded in Spurr's resin. Finally, the thin sections were excised from the embedded samples using an ultramicrotome equipped with a diamond knife, and the ultrathin sections were mounted on copper grids for TEM examination (Lin et al. 2009a, b; Lin and Xing 2008).

Microscopy observations

Wheat seeds were placed in water (control) and water supplemented with o-MWCNTs (80 μg/mL). Then the 7-day-old seedlings were observed under a microscope, and the mean value of the cell length was determined by measuring at least 200 cells in the root elongation zone from six independent seedlings (Filippo et al. 2006), where the samples were prepared as follows: fresh wheat roots were first thoroughly washed with deionized water and then the primary roots were excised at 10–20 mm from root tips and fixed immediately in a freshly prepared mixture of absolute ethyl alcohol and glacial acetic acid (3:1, v/v) for 24 h before being stored under refrigeration in 70 % ethyl alcohol as preservative solution for further use in cytological studies (Lin and Xing 2008).

Dehydrogenase activity assays

Dehydrogenase activity was tested using TTC reduction to triphenylformazan (TPF) (Li 2000; Liu et al. 2008). The surface of white young roots was washed and dried with blotting paper, and their fresh weights were measured. The roots were first placed in tubes filled with 0.4 % TTC and phosphate buffers (0.06 mol/L, pH 7.0), and then were incubated at 37 °C for up to 3 h followed by the addition of 1 mol/L sulfuric acid into the tubes, and the extraction with TPF. The optical density (OD) values of TPF were recorded at 485 nm, and were used to calculate the equivalent TPF concentrations with which the dehydrogenase activity for each fresh root weight was calculated as follows:

Dehydrogenase activity (TPF μg g^{-1} FW h^{-1}) = TPF reduction (μg)/fresh weight (g)/time (h).

Statistical analysis

Each treatment was performed in four replicates and arranged in a completely random design. The data for

all figures were represented as mean values ± SE (standard errors). The statistical analysis of experimental data was conducted using SAS 8.1 software, and the statistical significance was determined by the p value <0.05 (or <0.01) in a Student's t test.

Results and discussion

Characterization of o-MWCNTs

Currently, the aggregation and agglomeration of CNTs are a major obstacle to their practical applications (Lee et al. 2011; Kurval et al. 2011), but it has been found that o-MWCNTs in concentrated acid can render the CNTs solubility either in aqueous or organic phase by attaching carboxylic groups directly to the CNTs (Lee et al. 2011; Liu et al. 2009a, b). o-MWCNTs were obtained by being immersed in concentrated acid and sonicated for 8 h. The morphologies and structures of the MWCNTs and o-MWCNTs were characterized with Raman spectra, FT-IR spectra, and TEM, respectively.

The Raman spectra of the MWCNTs functionalized by concentrated acid are shown in Fig. 1a. The typical characteristic bands for MWCNTs are the G band and the D band, while the G band between 1500 and 1605 cm^{-1} is related to the vibration of sp^2-bonded carbon atoms in a two-dimensional hexagonal lattice, while the D band around 1310 and 1350 cm^{-1} is associated with the vibration of disordered sp^2-bonded carbon atoms (Qiu et al. 2008). The ratio of the intensity of D and G bands (I_D/I_G) can be taken as a measure of the degree of chemical functionalization. As indicated in Fig. 1a, the relative intensities of the G and the D bands (I_D/I_G) of o-MWCNTs and pristine MWCNTs were found to be 1.48 and 1.42, respectively. The value of I_D/I_G increased after acid functionalization, indicating the occurrence of a grafting reaction and the presence of a higher degree of defects for o-MWCNTs (Saleh et al. 2008).

FT-IR spectroscopy can identify organic functional groups on a MWCNTs' surface by measuring characteristic vibrational modes, and the FT-IR spectra were used to further characterize the o-MWCNTs and pristine MWCNTs. The peaks at 1730 and 1220 cm^{-1} corresponded to C=O and C–O stretching (Fig. 1b), respectively, while no signals were observed at 1730 and 1220 cm^{-1} peaks in pristine MWCNTs, indicating the successful introduction of carboxylic groups on the surface of MWCNTs during nitric acid oxidation (Wepasnick et al. 2010; Pillai et al. 2010).

We observed that pristine MWCNTs are insoluble in water, but the o-MWCNTs can be dispersed to form a homogeneous black solution, which was found to be stable after being stored for 7 days. (Figure 1a–c in Supplementary material). The morphologies of MWCNTs before and after oxidation were investigated by TEM. The images revealed that the o-MWCNTs are shorter than pristine MWCNTs (Figure 1d, e in Supplementary material). The solubility and stability of MWCNTs were examined through dynamic light scattering (Figure 1f–h Supplementary material), where the MWCNTs and o-MWCNTs were present as tube bundles with the length ranging from 100 to 5500 nm and 50 to 630 nm, respectively, indicating that

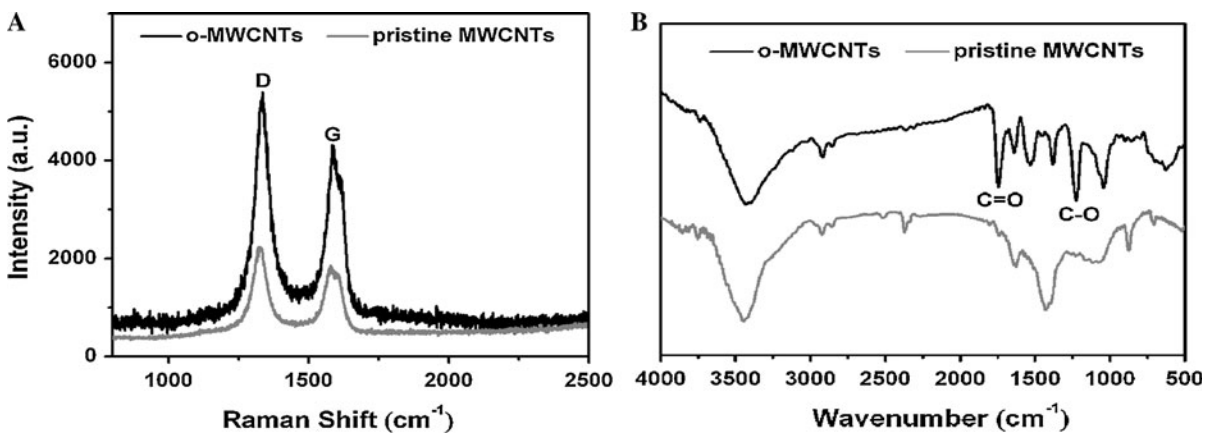

Fig. 1 **a** Raman and **b** FT-IR spectra of pristine MWCNTs and o-MWCNTs

o-MWCNTs are shorter than MWCNTs. This result has confirmed the TEM observation that oxidation induces significant shortening of the MWCNTs. After being stored for 7 days, the length distribution of o-MWCNTs ranges from 50 to 820 nm, suggesting that the stored o-MWCNTs solution is relatively stable (Lin et al. 2009a, b; Saleh et al. 2008).

Effects of o-MWCNTs on root elongation and wheat seedlings growth

To test whether o-MWCNTs could affect root elongation and wheat seedling growth, sterile seeds were placed on Petri dishes supplemented with different concentrations of o-MWCNTs suspension (10, 20, 40, 80, and 160 μg/mL), while water was for control experiments. The relative root length of seeds that were grown on o-MWCNTs suspensions were enhanced by 50 % on the third day and 32 % on the 7th day (Fig. 2a). The effect was especially pronounced at concentrations of 40, 80, and 160 μg/mL of o-MWCNTs. As demonstrated in Fig. 2b, the roots are obviously longer in seedlings treated with o-MWCNTs than in the controls on the 4th and 7th day after germination.

The difference in root elongation between control and o-MWCNT-treated plants was further investigated by examination of the root growth rate. The root growth rate for young wheat seedlings (<5 days after germination) was observed by direct measurement of the root length over time (Fig. 2c). The root elongation rate per day was approximately constant at about

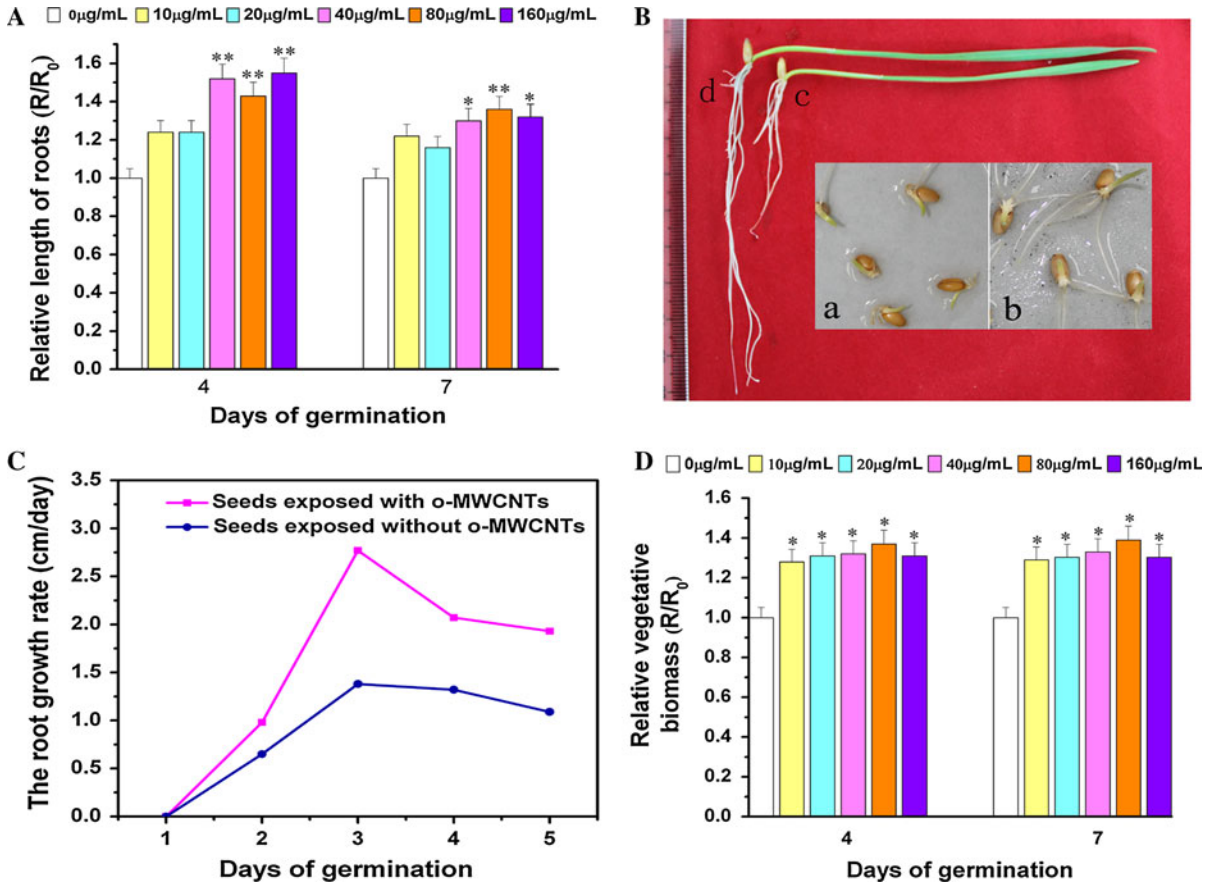

Fig. 2 Seeds with and without exposure to o-MWCNTs. **a** Relative root length of wheat seedlings grown on media with and without o-MWCNTs for 1 week. **b** Phenotypes of wheat seeds incubated for 4 days on media (*a*) without or (*b*) with o-MWCNTs and phenotypes of 7-day-old wheat seedlings grown on media (*c*) without and (*d*) with o-MWCNTs. **c** The growth rate of roots at a concentration of 80 μg/mL o-MWCNTs. **d** Vegetative biomass of wheat seedlings grown on media with and without o-MWCNTs. Results are shown as mean values ± SE of measurements of 10 plants per condition. Where appropriate, statistical significance is indicated: $*p < 0.05$; $**p < 0.01$

0.7 cm per day for control plants throughout the observation period, while the root growth rate of seedlings exposed to o-MWCNTs (80 μg/mL) on the second and the third day after germination increased significantly to 2 and 1.3 cm per day, respectively. When compared with controls, the root growth rate remained higher but the difference was not statistically significant on the 4th and 5th day after germination.

The effects of o-MWCNTs were also investigated on the vegetative biomass of seedlings and it was found that wheat seedlings germinated and developed on media with different concentrations of o-MWCNTs (10, 20, 40, 80, and 160 μg/mL) exhibited a significant increase in vegetative biomass (Fig. 2d). The total biomass of plant vegetation (leaves, stems, and roots) of the seedlings germinated and grown on o-MWCNTs media increased approximately by 30–40 % compared with seedlings grown in water.

Seed germination was not significantly affected by o-MWCNTs, despite a higher germination percentage for seeds treated with nanoparticles in 24 and 48 h (Figure 2a, b in Supplementary material). The o-MWCNTs-exposed wheat seedlings presented a similar stem length when compared with the control, and despite longer stems of wheat seedlings at o-MWCNTs concentrations of 20 and 40 μg/mL, the difference was not as significant as that of roots (Figure 2c, d in Supplementary material).

From our analyses, it can be concluded that o-MWCNTs, after a short-term exposure to wheat plants and under our experimental conditions, stimulated root growth and increased vegetative biomass, but did not induce any significant change in seed germination and stem length as compared with controls.

Our results contradict several prior studies on rice cells and Arabidopsis T87 cells in that MWCNTs had toxic effects on the cells of rice and Arabidopsis. The observed negative effects on cells seemed to be linked to the increase of reactive oxygen species (ROS) and the decrease of cell viability (Tan et al. 2009; Lin et al. 2009a, b). According to other previous studies, MWCNTs, after a short-term exposure to six higher plant species (radish, rape, ryegrass, lettuce, corn, and cucumber), did not induce any significant change in seed germination and root growth (Lin and Xing 2007). As for the positive effects of MWCNTs on plant growth and development, our results have confirmed the previous reports that MWCNT-treated gram increases the growth rate in every part of the plant (Shweta et al. 2011), and that MWCNTs can increase the germination percentage and enhance the growth of seedlings of tomato plants (Khodakovskaya et al. 2009). Therefore, the effects of CNTs on the plant growth and development are dependent upon plant species, the applied concentrations, the specific conditions of experiments, and the impact of surface properties (Khodakovskaya et al. 2009; Castiglione et al. 2010).

Cell elongation is the primary and obligatory growth process of root growth (Antipova et al. 2003); and dehydrogenase activity is an indicator of root vitality and can be responsible for the water uptake and higher biomass production (Taniguchi et al. 2008). Based on our observations, it can be deduced that o-MWCNTs, after being taken up by the roots, play an active role in root elongation and dehydrogenase activity probably by regulating the water uptake of wheat seedlings. To better characterize the potential impact of o-MWCNTs on our systems, physiological analysis was performed as a reliable method to test the effects of activation of o-MWCNTs on root elongation and vegetative biomass of wheat seedlings, base on the detection of morphology, the cell length of root zone, and the activity of dehydrogenase of seedling after treatment with o-MWCNTs.

Morphological observations

To better understand the effects of activation of o-MWCNTs on root elongation and vegetative biomass of wheat seedlings, we employed TEM to examine the presence of o-MWCNTs inside roots exposed to o-MWCNTs and compared them with controls, because the TEM imaging can help increase the understanding of how o-MWCNTs enter cells, where they migrate, and their fate after uptake (Tan et al. 2009). For this experiment, 80 μg/mL of o-MWCNTs was selected to culture seedlings. After 7 days of o-MWCNTs exposure, it was found that o-MWCNTs could traverse the cell wall and enter the cellular cytoplasm. As shown in Fig. 3b, the morphologies of several o-MWCNTs are clearly distributed in the cytoplasm, but o-MWCNTs are completely missing in the images of the control samples (Fig. 3a). These findings suggest that o-MWCNTs can penetrate cell membranes after being taken up by roots of wheat seedlings.

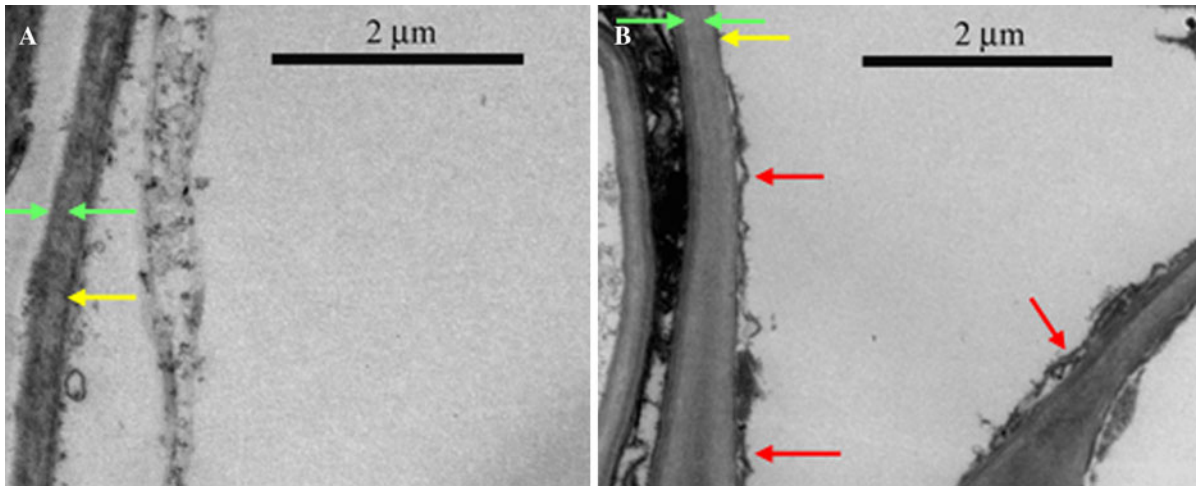

Fig. 3 TEM images of the root system of 7-day-old wheat seedlings grown on media **a** without o-MWCNTs and **b** with o-MWCNTs. *Red arrows* indicate o-MWCNTs; *yellow arrows* indicate the membrane; and the *green arrow* indicates cell wall. (Color figure online)

However, the o-MWCNTs were not observed in the stems or leaves when parts of the grown plants were further analyzed by TEM (data not shown), which indicates that the o-MWCNTs can hardly be translocated to these parts of wheat seedlings. Our results agree with Khodakovskaya et al. (2009), in that the CNTs could penetrate the root systems of tomato seedlings, but were not present in stems and leaves.

o-MWCNTs can promote cell elongation in root elongation zone

As cell elongation is a very important event in root growth (Antipova et al. 2003), we further investigated the growth of the seedling roots at the cellular level. 7-day-old seedling roots were directly imaged under a microscope with a maximum magnification of 400-fold, and the length of cells in the elongation zone was found to have increased significantly in wheat seedlings germinated and developed on o-MWCNTs media. The representative images of cell dimensions are shown in Fig. 4, and the length of root cells of the seedlings treated with o-MWCNTs (Fig. 4b) increased by 1.4-fold compared to controls (Fig. 4a). This result demonstrates that the increase in root elongation observed in the seedlings exposed to o-MWCNTs could be attributed to the promotion of cell elongation in the elongation zone created by o-MWCNTs.

To the best of our knowledge, only two research groups have, till now, published studies on the effects of carbon-based materials on plant physiology at cellular level. Liu et al. (2010) revealed that phytotoxic effects of fullerene would cause the retarded root growth in cellular morphology, and Khodakovskaya et al. (2011) investigated the uptake and translocation of MWCNTs in roots, leaves, and fruits down to the cell level. However, based on the information available, the mechanism of the effect of o-MWCNTs on cell elongation in the root zone is not clear yet. It is possible that o-MWCNTs can affect hormone distribution or microtubule organization (Liu et al. 2010). Therefore, further studies at the cellular level are needed to fully understand the complex interaction between o-MWCNTs and plants.

o-MWCNTs can improve the dehydrogenase activity of wheat plants

Dehydrogenase activity is a comprehensive assessment index that reflects the metabolic activity level and the ability of roots to absorb nutrients and water (Taniguchi et al. 2008). Here, the dehydrogenase activity of root was measured in the following way. Roots were exposed to different concentrations of o-MWCNTs, and the percentage of viability was calculated considering the OD values at 485 nm after 7 days of incubation. Figure 5 shows the typical data for the roots exposed to different concentrations of o-MWCNTs. In the presence of o-MWCNTs, a concentration-dependent increase in the activity of

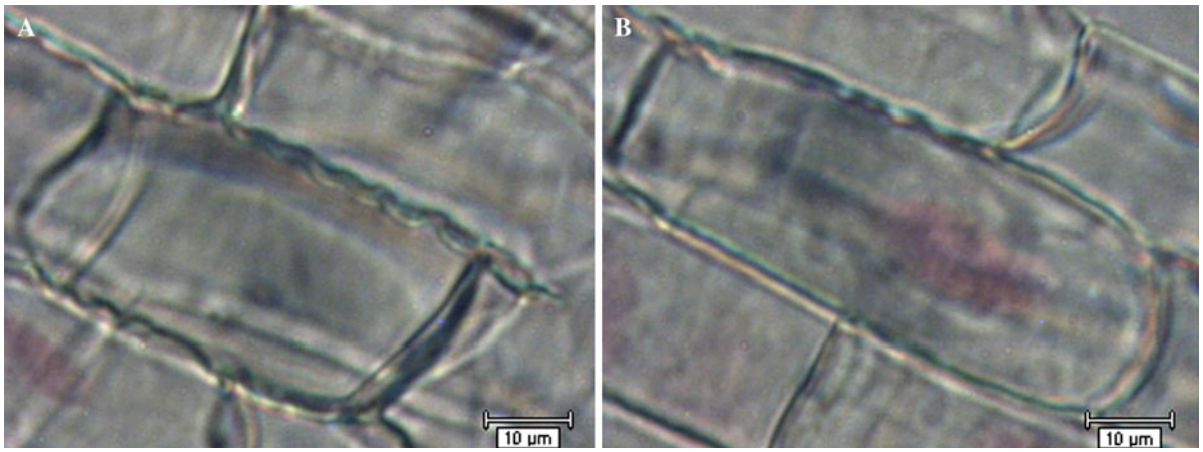

Fig. 4 Microscopy images of the elongation zone in the root of the seedlings grown on media **a** without and **b** with 80 μg/mL o-MWCNTs

dehydrogenase was noted. The result suggests that o-MWCNTs can significantly enhance root dehydrogenase activity, which in turn enhances the ability of water uptake of the seedlings. This finding has confirmed in part some previous studies that SWCNT, after being aligned along and attached to the root surface or an inner portion of root, can enhance the capillary action of water absorption, which leads to the faster growth in gram plants (Shweta et al. 2011), and that CNTs can increase the germination percentage and enhance the growth of seedlings because it can support water uptake inside seeds by creating new pores for water permeation by penetrating seed coat (Khodakovskaya et al. 2009). Therefore, CNTs can act as molecular channels for water (Liu et al. 2009a, b), supporting water uptake and enhancing plant growth. Based on our results, it can be concluded that the improved root dehydrogenase activity induced by o-MWCNTs can enhance the seedlings ability of water uptake.

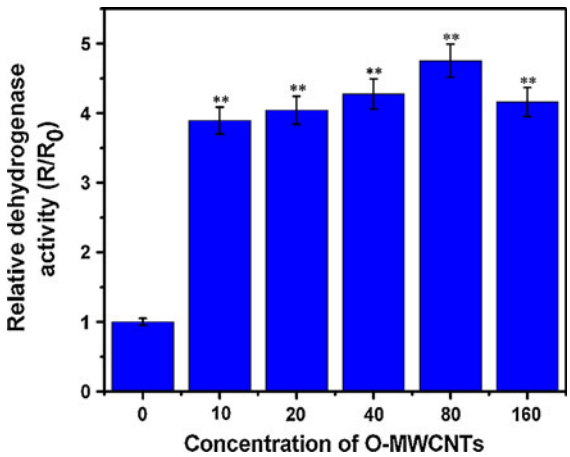

Fig. 5 Dose-dependent effects of o-MWCNTs on dehydrogenase activity

However, the mechanism by which o-MWCNTs increase the activity of root dehydrogenase is not clear yet. One possible explanation is that o-MWCNTs may promote the dehydrogenase electron-transfer reaction. According to previous studies, the purification of CNTs with oxidizing acids such as nitric acid creates surface acid sites that mainly contain carboxylic groups on both the internal and the external surfaces of the o-MWCNTs (Chen et al. 2001). The high surface area created by abundant acidic sites can absorb a wide range of active molecules, including protein, amino acids (Rafeeqi and Kaul 2011), peptides, and nucleic acids as well as other enzymes such as lysozyme (Merli et al. 2011), horseradish peroxidase (Yu et al. 2003), and glucose oxidase (Guiseppi-Elie et al. 2002), making them more biocompatible and retaining the bioactivity of active molecules. It is also found that the surface-modified MWCNTs can induce the increase of electrical conductivities (Lee et al. 2011), and that CNTs exhibit a strong and stable electrocatalytic response toward glucose and NADH due to their high surface area and good electron-transfer rate (Baby et al. 2011; Musameh et al. 2002; Ye et al. 2011). Furthermore, Britto and co-workers used density-functional theory calculation and molecular dynamics simulations to study the microscopic mechanism of electron transfer on nanotubes, and found that

the electrocatalytic activation of oxygen reduction resulted from the presence of topological defects in the lattices, which would act as electrophilic reaction sites and provide higher local electron density (Britto et al. 1999). Similarly, in our experiment, the relatively higher water content of plants could promote molecular mobility such as dehydrogenase in root cells (Zeng et al. 2010). On the other hand, the o-MWCNTs could absorb dehydrogenase to the sidewall and provide higher local electron density, thus effectively promoting the charge transfer in the process of oxygen reduction reaction, which leads to the increase of dehydrogenase activation.

Conclusion

In summary, the effects of o-MWCNTs on wheat plants were investigated in detail. It was experimentally observed in this study that the o-MWCNTs can significantly enhance the root growth and the vegetable biomass in the seedling stage, and it can be concluded temporarily that o-MWCNTs promote cell elongation in the root system and improve the activity of root dehydrogenase after being taken up by wheat plants, which could lead to faster root growth and higher biomass production. This study is important not only from the perspective of the application of nanoparticles in agricultural sciences, but also for the new insights presented to advance our understanding of the interaction mechanism between nanomaterials and plants. Further studies involving interdisciplinary approaches are required to reveal the exact mechanisms and the ultimate influences of such interaction at different stages of plant growth such as flowering and fruit ripening.

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