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Interaction of carbon nanohorns with plants: Uptake and biological effects

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A B S T R A C T

Single-walled carbon nanohorns (SWCNHs) are a unique carbon-based nanomaterial with promising application in different fields including, medicine, genetic engineering and horticulture. Here, we investigated the biological response of six crop species (barley, corn, rice, soybean, switchgrass, tomato) and tobacco cell culture to the exposure of SWCNHs. We found that SWCNHs can activate seed germination of selected crops and enhance growth of different organs of corn, tomato, rice and soybean. At cellular level, growth of tobacco cells was increased in response to exposure of SWCNHs (78% increase compared to control). Uptake of SWCNHs by exposed crops and tobacco cells was confirmed by transmission electron microscopy (TEM) and quantified by microwave induced heating (MIH) technique. At genetic level, SWCNHs were able to affect expression of a number of tomato genes that are involved in stress responses, cellular responses and metabolic processes. We have concluded that SWCNHs can be used as plant growth regulators and have the potential for plant-related applications.

1. Introduction

Carbonaceous nanostructures are new materials that have wide range of applications including electronics [1,2], medicine [3–5], aerospace and agriculture [6]. Among carbon-based nanomaterial, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) have attracted plant biologists due to their positive effect on growth of different crops [7–9] and their potential use as smart delivery systems in plants [10–13]. Single-walled carbon nanohorns (SWCNHs) are another promising carbonaceous nanosized material with distinctive characteristics. Unlike carbon nanotubes, nanohorns are uniform in size and can be well dispersed in solvents [14–17]. Moreover, they can be synthesized in large quantities without any catalyst [17,18]. The metal contamination in different carbon-based nanomaterials including, graphene [19], carbon nanotubes [20], fullerenes [21], nanodiamonds [22], and nanofibers [23] can modify the response of plant. Therefore, the purity of the nanomaterial remains an essential factor to guarantee reproducible biological effects.

SWCNHs are single graphitic tubules with diameter of 2–5 nm and lengths of 40–50 nm [24,25]. Three different types of SWCNHs have been observed including ‘dahlia-like’,

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'bud-like' and 'seed-like' [25]. SWCNHs exist in spherical aggregate with a diameter of 50-100 nm (Fig. 1A). The cylindrical inner nanospace and interstitial channels are among the features that distinguish nanohorns from other carbonaceous nanomaterials [17] (Fig. 1A). In fact, the inner nanospace is large enough to store a large amount of molecules of interest. For this reason, SWCNHs are a good candidate for gas storage [26–28], molecules adsorption [29,30] and drug delivery [31–33] However, concerns about the toxicity and other hazards caused by occupational exposure to this new material remain active [15,34].

Toxicological studies on two bacterial organisms including, *Salmonella typhimurium* and *Escherichia coli* strains showed that SWCNHs did not have either growth inhibitory effect or an increase in the number of revertant [15]. Moreover, *in vivo* toxicological studies on rabbits, guinea pigs and rats have been conducted to test skin primary irritation, eye irritation, skin sensitization, peroral administration and intratracheal instillation [15]. No abnormalities were shown in the tested animals, in either the skin or eye irritation tests. Furthermore, the oral administration of 2000 mg/kg body weight of SWCNHs was not sufficient to illustrate any sign of toxicity even after the 2-week test period [15]. Recent histological studies have shown that SWCNHs can be accumulated in liver and spleen with no symptoms of hematological or immunological toxicity [34]. All the initial reports on SWCNHs toxicity approve the safety of this nanomaterial. However, the numerous applications of SWCNHs can facilitate their release to the environment and, therefore, the impact of SWCNHs on a larger number of organisms should be evaluated.

Unique properties of SWCNHs including the presence of inner nanospace are making them attractive candidates for use as 'smart' delivery systems in a range of plant-related applications. For example, SWCNHs can be used for delivery of DNA, proteins and chemicals including herbicides. However, in order to initiate such investigations, assessment of plant response to direct exposure of SWCNHs to plants and plant cells has to be performed. Recently, we demonstrated that two types of carbon nanotubes (MWCNTs and SWCNTs) could penetrate seed coats, activate seed germination, and plant growth in low doses [8,9,35]. Here, we describe the consequences of exposure of SWCNHs to different plant tissues including seeds, whole plants and plant cell culture. The uptake of SWCNHs was detected using TEM and quantified using MIH technique [36]. The genes that were affected by application of SWCNHs to tomato seeds and seedlings were identified by microarray analysis (Affymetrix platform).

2. Experimental methods

2.1. *Synthesis and Characterization of SWCNHs*

Single wall carbon nanohorns (SWCNHs) were synthesized using high power laser vaporization setup as described early [37,38]. Briefly, the experimental setup includes a quartz tube reactor mounted inside a tube furnace operating at 1150 °C with flowing Argon (flow rate ~4600 sscm) at atmospheric pressure. The Nd: YAG laser light ($\lambda = 1.064\ \mu m$, 20 ms pulse width, 90 J/pulse, 5 Hz repetition rate) was used to vaporize a carbon target located in the center of the quartz tube reactor. Using this approach, we are able to produce relatively large amounts of long SWCNHs with the production rates of 10 g/h. SWCNHs were characterized using scanning and

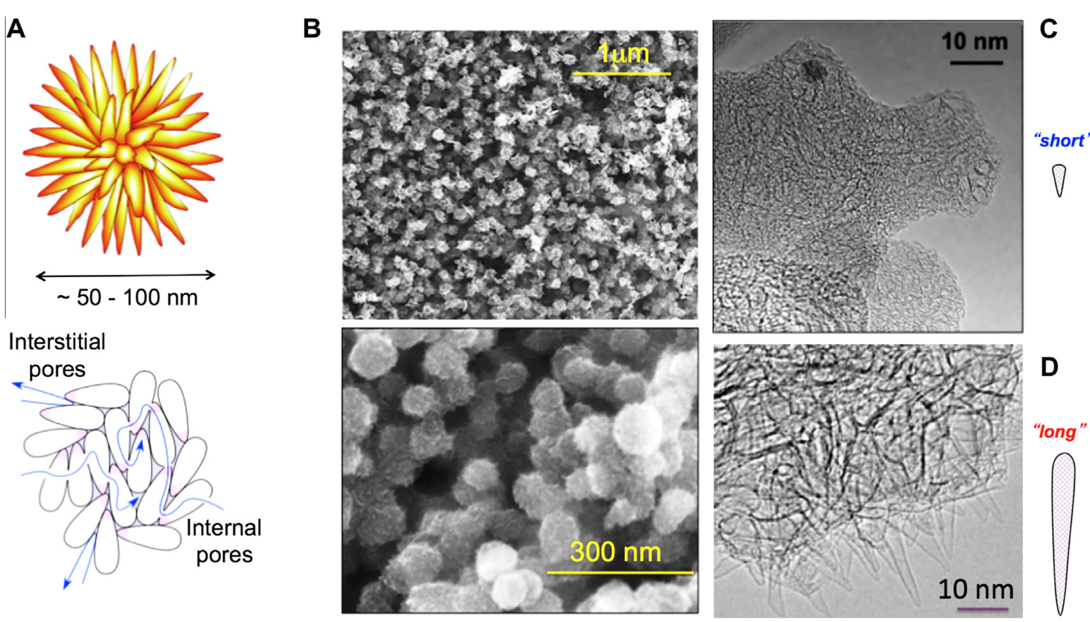

Fig. 1 – (A) Schematic of general morphology of a SWCNH aggregate showing internal and interstitial pores (bottom). (B) SEM images of SWCNH aggregates both in close-view (bottom) and in far-view (top) showing the mesoporous agglomerate of SWCNH aggregates. High-resolution TEM images of SWCNH aggregates, and schematic of individual (C) "short" and (D) "long" SWCNH units, some of which protrude from the aggregate. (A color version of this figure can be viewed online.)

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