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Mechanisms underlying toxicity and stimulatory role of single-walled carbon nanotubes in *Hyoscyamus niger* during drought stress simulated by polyethylene glycol

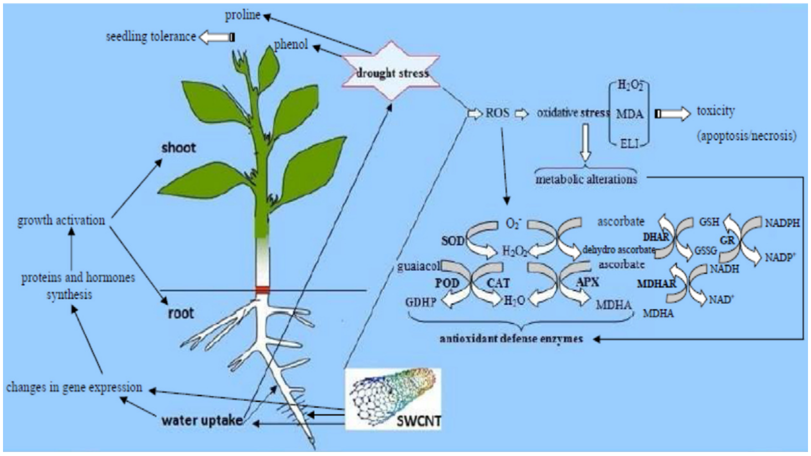
Mehrnaz Hatami[a,*], Javad Hadian[b], Mansour Ghorbanpour[a,**]

[a] *Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, 38156-8-8349, Arak, Iran*
[b] *Medicinal Plants and Drug Research Institute, Shahid Beheshti University, G.C., Evin, 1483963113 Tehran, Iran*

HIGHLIGHTS

- Drought stress showed reduction in germination, vigor and early growth indices in *H. niger*.
- Low dosage of SWCNTs alleviated the drought stress (up to moderate levels only).
- Higher concentrations of SWCNTs improved cellular injury indices.
- SWCNTs dose-dependent modulations of the antioxidant defense enzymes were observed.
- SWCNTs can induce tolerance in *H. niger* seedlings via biosynthesis of specific metabolites.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, seeds of *Hyoscyamus niger* were exposed to different concentrations (50–800 $\mu g\,mL^{-1}$) of single-walled carbon nanotubes (SWCNTs) under different levels of drought stress (0.5–1.5 MPa) for 14 days. Germinated seeds were subsequently allowed to grow in the same culture media for 7 more days to test the further response of the seedlings in terms of biochemical changes to the employed treatments. Seeds subjected to drought showed reduction in germination percentage, vigor and lengths of roots and shoots. However, inclusion of SWCNTs at the two lowest concentrations significantly alleviated the drought stress (up to moderate levels only)-induced reduction in germination and growth attributes. This happened due to increased water uptake, up-regulation of mechanisms involved in starch hydrolysis, and reduction in oxidative injury indices including H_2O_2, malondialdehyde contents and electrolyte leakage. The improved plant performance under PEG-induced drought stress was a consequence of changes in the expression of various antioxidant enzymes including SOD, POD, CAT, and APX, and also biosynthesis of proteins, phenolics, and specific metabolites such as proline. Results demonstrate that treatment by low concentrations of SWCNTs can induce tolerance in seedlings against low to moderate levels of drought through enhancing water uptake and activating plant defense system.

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1. Introduction

Carbonaceous nanomaterials are the most abundant engineered nanomaterials and primarily consist of graphene, fullerene C_{70}, fullerol ($C_{60}(OH)_{20}$), and carbon nanotubes including single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) [1].

The unique physico-chemical, electronic, and mechanical properties of carbon nanotubes have raised their industrial production, and provided a broad range of applications in biotechnology, medicine, pharmaceuticals, agriculture, cosmetics and renewable energy technologies [2]. The widespread production and application of nanotubes increase their possible release into the environment (via recycling or waste). Besides, due to their filamentous structure, carbon nanotubes are compared with asbestos [3], which further raises concerns about their potential threats to living organisms of ecosystems.

Plants are an important part of ecological systems and may serve as a potential route for the uptake, distribution and accumulation of engineered nanomaterials into the food chains and biological cycles [4], and considered as a main group of the biocenosis that encounter the released nanomaterials. Therefore, understanding plant response to nanotubes exposure is an important issue and could open new frontiers in crop production and in agriculture where successive innovation is extremely needed due to increasing global food security and climate change challenges [5]. However, the existing literature exhibits varied effects of nanotubes exposure on seed germination and physiological processes of different plant species, ranging from growth stimulation to acute toxicity.

According to Canas et al. [6] non-functionalized SWCNTs retarded the root elongation in tomato (*Lycopersicon esculentum*) and stimulated root growth in onion (*Allium cepa*) and cucumber (*Cucumis sativus*), while functionalized SWCNTs [poly-3-aminobenzenesulfonic acid (65%): SWCNTs (35%) w/w] inhibited root elongation in lettuce (*Lactuca sativa*). However, cabbage (*Brassica oleracea*) and carrot (*Daucus carota*) were unaffected by either type of the SWCNTs tested. Lahiani et al. [7] studied the effect of MWCNTs (diameter 14–40 nm) on barley (*Hordeum vulgare*), soybean (*Glycine max*) and corn (*Zea mays*) at exposures of 50, 100, and 200 μg/mL for 10–11 days. They reported enhanced rates of seed germination in all the crops tested. However, exposure to 100 and 200 μg/mL MWCNTs significantly improved the germination rate as compared to the lowest concentration. Moreover, Tiwari et al. [8] reported 43% increase in plant fresh biomass in corn (*Zea mays*) exposed to 60 mg/L MWCNTs (6–9 nm) for 7 days as compared to control. The authors reported enhanced water content of the root upon exposure to MWCNTs, and found that fresh weight followed the trends of the water content. According to Khodakovskaya et al. [9] exposure of tomato (*Lycopersicom esculentum*) plants to SWCNTs or MWCNTs promoted the seedling growth and activated the expression of many stress-responsive genes.

Conversely, some studies have shown that engineered nanomaterials can effectively penetrate plant cells and trigger oxidative stress responses through generation of reactive oxygen species (ROS), and consequently interfere with different metabolic pathways in the cell by attacking membranes, lipids, DNA and proteins [10]. Shen et al. [11] exposed rice (*Oryza sativa*) and *Arabidopsis thaliana* leaf protoplasts to SWCNTs, and observed adverse cellular responses such as plasma membrane deposition, H_2O_2 accumulation and 25% cell death in 6 h. They suggested that the nanoscale feature of the particles acts as a key factor for toxicity development. To scavenge the overproduced ROS, plants employ specific mechanisms including activation of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), and non-enzymatic antioxidants such as carotenoids, glutathione, ascorbic acid, alpha-tocopherol, and proline [12].

Despite rising the number of researches over the past decade regarding nanomaterials application in plant sciences, few studies have been carried out on nanomaterials function under environmental factors or constraints, particularly during the full life cycle of a plant. Recently, Yang et al. [13] investigated the influence of environmental factors including sunlight and dissolved organic matter on the toxicity of TiO_2 NPs to zebrafish (*Danio rerio*), a widely used organism for nano-toxicological studies, and found that toxicity of TiO_2 NPs was significantly increased in the presence of humic acid under non-simulated sunlight conditions compared to the TiO_2 NPs exposure only. Siddiqui et al. [14] reported the role of nano-silicon dioxide (SiO_2 NPs, 12 nm) exposure (15 days at 1.5, 3.0, 4.5, 6 and 7.5 g/L) on seed germination and biochemical attributes in *Cucurbita pepo* under salinity stress conditions. The authors reported improved seed germination and growth parameters through reduction of cellular injury indices, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and electrolyte leakage index (ELI), and induction of different antioxidant enzymes.

To the best of our knowledge, no previous research has been carried out concerning the impacts of nanotubes exposure to terrestrial plants under different levels of drought stress, and the mechanisms behind positive or negative plant responses under such conditions are still unknown. Drought stress is one of the most significant environmental factors causing drastic changes in germination, growth and metabolism of plants [15]. According to a prospects report, the number of countries facing extremely high water deficit stress will rise to 54 in 2050 [16]. This type of abiotic stress may disturb the redox homeostasis and cause oxidative stress in plant tissues, leading to decreases in photosynthetic electron chain and increases in ROS production such as singlet oxygen ($^1O_2$), superoxide (O^{2-}), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) [17]. However, up-regulation of antioxidant compounds and biosynthesis of specific metabolites can improve plant performance in drought-prone environments.

To the best of our knowledge, no previous research has been carried out concerning the impacts of nanotubes exposure on terrestrial plants (which may occur deliberately or accidentally) under different levels of drought stress, and the mechanisms underpinning any possible positive or negative responses are still unknown. The role of nanomaterials in plant growth, development and tolerance against abiotic stresses is ambiguous and controversial. Drought is one of the most significant environmental stresses responsible for the failure of plant regeneration in both natural and agricultural ecosystems. The negative consequences of drought is linked to its potential to disturb the redox homeostasis and cause oxidative stress in plant tissues, leading to decreases in photosynthetic electron chain and increases in ROS production. With respect to seed germination, water is a basic requirement and it is essential for enzyme activation, breakdown, translocation, and use of reserve storage materials. The low water potential leads to lower germination percentage and lower velocity of germination. Feasible approaches to improve seed germination under water scarcity are crucial, considering the ever-increasing occurrence of drought worldwide. Thus, in the present article, an attempt is made to shed light on the role of SWCNTs under one of the most important abiotic stress conditions i.e. drought.

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* Corresponding author.
** Corresponding author at: Shahid Beheshti Street, Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak, Markazi Province, Iran.
E-mail addresses: m-hatami@araku.ac.ir (M. Hatami), m-ghorbanpour@araku.ac.ir (M. Ghorbanpour).

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