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Sulfonated graphene-induced hormesis is mediated through oxidative stress in the roots of maize seedlings

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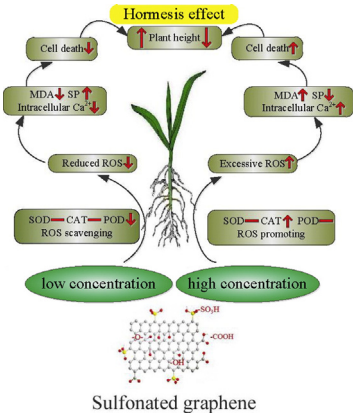
HIGHLIGHTS

- SG induced a hormesis effect on plant height of maize seedlings.
- The hormesis effect was mediated by oxidative stress in roots of maize seedlings.
- Low concentration of SG could scavenge ROS in roots and improve maize health state.
- High dose of SG promoted the generation of ROS and led to cell death in roots.
- Low concentration of SG altered root morphology of maize seedlings

GRAPHICAL ABSTRACT



Sulfonated graphene

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ABSTRACT

The present study investigated the impact of sulfonated graphene (SG) on the growth of maize seedlings at a concentration range of 0–500 mg L^{-1}. Stress-related parameters including reactive oxygen species (ROS), intracellular Ca^{2+}, antioxidant enzyme activities, lipid peroxidation, membrane leakage, cell death and root morphology were examined to reveal the potential mechanisms. The results indicate that SG induced a hormesis effect on plant height, i.e., low-concentration (50 mg L^{-1}) stimulation and high-concentration (500 mg L^{-1}) inhibition. The hormesis effect of SG on plant height was directly correlated with ROS levels in roots. A low concentration (50 mg L^{-1}) of SG promoted ROS scavenging, alleviated oxidative stress, enhanced the soluble protein (SP) content, and decreased intracellular Ca^{2+} and cell death in the roots. At a higher concentration (500 mg L^{-1}), SG stimulated the generation of ROS in the roots, decreased SP content in the leaves, increased antioxidant enzyme activities, intracellular Ca^{2+}, electrolyte leakage and cell death in the roots, and increased the malondialdehyde (MDA) content in both roots and leaves. Different changes were observed for root morphology at SG concentrations of 50 and 500 mg L^{-1}, and a larger amount of SG was deposited onto the root surface at a concentration of 500 mg L^{-1} compared with 50 mg L^{-1}.

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1. Introduction

Graphene, as a novel carbon nanomaterial, has increasingly attracted much interest in both research and application since its discovery (Novoselov et al., 2012). Based on the difference of functional group, graphene encompasses various forms including pristine graphene, graphene oxide, sulfonated graphene and fluorographene and so on. The unique physiochemical properties and large surface area have opened up a variety of application possibilities for graphene in multiple fields, including chemistry (Georgakilas et al., 2012), biomedicine (Byun, 2015), environmental protection (Jiang et al., 2015) and even agriculture (Huang et al., 2015). As the production and application of this nanomaterial are gradually increasing, graphene would inevitably be released into the environment. Thus, there is an urgent need for an improved understanding of the potential impact on ecological and environmental safety.

Currently, most toxicological studies have focused on human health (Liao et al., 2011), bacteria (Akhavan and Ghaderi, 2010), microbial communities (Ren et al., 2015), and other animals (Chen et al., 2015; Liang et al., 2015). However, studies concerning graphene phytotoxicity remain scarce, despite the gradually increasing concerns of scientists. The impact of graphene on plants is dependent on plant species, growth stages and duration of exposure. In a range of 500–2000 mg L^{-1}, graphene significantly inhibited plant growth and biomass, and tomato seedlings were somewhat more sensitive to the detrimental effects of graphene than cabbage or red spinach seedlings (Begurn et al., 2011). Moreover, graphene oxide was toxic to green algae *Raphidocelis subcapitata* at concentrations of 20–100 mg L^{-1}, indicated by a decrease of algal density and autofluorescence (Nogueira et al., 2015). However, graphene oxide had no effect on the germination, seed development, or shoot and root development of *Arabidopsis* seedlings, and did not affect flowering time at concentrations of 10–1000 mg L^{-1} (Zhao et al., 2015). In addition, similar to other nanomaterials, the characteristics and structures of graphene, including its nanoparticle size, chemical composition, surface structure, solubility, shape and aggregation, play an important role in the toxicity on plants. For example, Hu and Zhou (2014) demonstrated that a novel hydrated graphene ribbon (HGR) promoted the germination of aged wheat seeds by 15%, whereas graphene and graphene oxide inhibited germination and root elongation. Thus, the potential effects and mechanisms of graphene phytotoxicity remain in part contradictory and deserve further investigation.

Reactive oxygen species (ROS) generation and oxidative stress have been regarded as the primary mechanisms by which graphene and other nanoparticles affect plant growth and development. ROS are not simply by-products of metabolism but also function as signaling molecules (Foyer et al., 1997). Relatively low levels of ROS can facilitate the signaling responses of plants (Apel and Hirt, 2004). However, excessive ROS accumulation might lead to phytotoxicity, including cell membrane, mitochondria, and DNA damage (Mur et al., 2006), which could ultimately impact the whole organism in terms of development, reproduction, and viability. Therefore, the controlled modulation of ROS levels in plants is extremely important. It has been reported that low levels of some carbon nanotubes and metal nanoparticles could scavenge ROS in plants or animals (Galano, 2010; Zhang et al., 2012), alleviating oxidative stress and promoting plant growth. Whereas most studies have demonstrated that higher concentrations of nanoparticles could generate ROS in plants or animals (Hu et al., 2014b), disrupt antioxidant enzymes and eventually lead to cell death (Begum et al., 2014). Furthermore, nanoparticles might deposit and agglomerate on plant roots, and nanoparticles closely attached to root cell surfaces would penetrate and disrupt the cell wall/membrane and interact with biomolecules, thereby presenting toxicity (Hu et al., 2015; Long et al., 2012).

The present study was designed to (i) investigate the impact of sulfonated graphene (SG) on the growth of maize seedlings under different concentrations and (ii) explore the potential mechanisms underlying this effect. The key biological responses induced by nanoparticles were evaluated using a series of biological assays, including ROS generation, intracellular Ca^{2+} concentration in the roots, antioxidant enzyme activities, MDA and SP contents, cell death and morphological changes of the roots. SG nanosheets were investigated because these materials have high dispersibility in aqueous solution, likely resulting in a stronger interaction between SG and plants. To our knowledge, the present study provides the first experimental demonstration that graphene exhibits a hormesis effect on plants.

2. Materials and methods

2.1. SG characterization and culture solution preparation

SG was obtained from Suzhou Graphene-Tech Co. Ltd. (Suzhou, Jiangsu province, China). The morphology of SG was examined using scanning electron microscopy (SEM, S-3400N II, Hitachi, Japan) and transmission electron microscopy (TEM, JEM-200CX, JEOL, Japan). The surface elemental contents were analyzed via X-ray photoelectron spectroscopy (XPS, PHI 5000 VersaProbe, ULVAC-PHI, Japan). A known amount of SG was dispersed in modified Hoagland's nutrient solution (Hoagland and Arnon, 1950) using sonication (100 W, 40 kHz, 25 °C) for 15 min to prepare stock solution. The working solutions (0, 10, 50, 100, 200, and 500 mg L^{-1}) in the maize culture experiments were prepared by serial dilution of the stock solution with modified Hoagland's nutrient solution. An aqueous solution of sodium hydroxide (0.1 M) was used to neutralize the working solutions to pH 6.3–6.5. The size distribution of SG in Hoagland's nutrient solution (500 mg L^{-1}) before and after maize culture experiment was analyzed using dynamic light scattering machine equipped with a 30 mW, 657 nm laser (ZetaPALS, Brookhaven Instruments Corporation).

2.2. Maize growth and exposure

Maize (*Zea mays*) seeds were purchased from Shenzhou Seed Company in Nanjing Agriculture University (Nanjing, China). The seeds were surface-sterilized by a 10-min soak in hydrogen peroxide (3%), followed by rinsing three times with deionized water. Large, plump seeds were selected and placed on double-layer filter paper moistened with deionized water in glass Petri dishes for germination. The Petri dishes were placed in a growth chamber in the dark at 25 °C for 2 days. Deionized water was added daily to keep the filter paper dampened. After germination, five uniformly germinated seeds were transferred to each glass pot with a basket for fixing the seeds. The pots were filled with 50 mL of working solution with different concentrations of SG, representative of the concentrations used in previous studies. All exposure pots were wrapped with aluminum foil to protect the working solutions from light. The seedlings were cultured in a growth chamber with a 16/8 h day/night photoperiod at 25 °C, 75% relative humidity and a 150–180 μmol m^{-2} s^{-1} light intensity. Deionized water was injected into the pots daily to compensate for the evapotranspiration loss. After 10 days of exposure, the plants were thoroughly washed with deionized water, dried with absorbent paper to remove the surface water, and separated into roots and shoots. Plant height (physical height of the plant) as well as root and shoot fresh weights were recorded. The pH of the hydroponic solution was measured and remained unchanged during the entire culture process.

2.3. Measurement of antioxidant enzyme activities, soluble protein (SP) and malondialdehyde (MDA) contents

The leaves (0.7 g) and roots (1.0 g) were rapidly frozen in liquid nitrogen and subsequently homogenized in normal saline at three times the fresh weight. The entire process was performed in an ice bath for 20 min. Subsequently, the homogenate was centrifuged at 4000 rpm for 20 min at 4 °C, and the supernatant was used for enzyme activity

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