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Reduction of arsenic toxicity in two rice cultivar seedlings by different nanoparticles

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ABSTRACT

In this study, we investigated arsenic uptake and enzymatic activities in rice seedlings after the addition of nanoparticles. Hydroponic experiments were conducted to investigate the effects of different nanomaterials (high-quality graphene oxide, multilayer graphene oxide, 20 nm hydroxyapatite (HA_{20}), 40 nm hydroxyapatite (HA_{40}), nano-Fe_3O_4 (nFe_3O_4) and nano-zerovalent iron [nFe]) on the biomass, arsenic uptake, and enzyme activities in seedlings of the rice cultivars T705 and X24. Compared with the control, the addition of different nanomaterials increased seedling growth, with X24 rice growing better than T705 rice. Nanomaterials effectively reduced arsenic uptake in T705 rice seedlings under low and high arsenic concentrations; however, they were only effective at lower arsenic concentrations in X24 seedlings. nFe_3O_4 and nFe performed better than other nanomaterials in preventing arsenic from being transported to the aboveground parts of the rice seedlings. Different nanomaterials obviously influenced enzyme activities in the T705 seedlings at low arsenic concentrations ($\leq$ 0.8 mg L^{-1}). High-quality and multilayer graphene oxide decreased enzyme activities in the aboveground parts of the T705 seedlings, whereas, HA_{20} and HA_{40} increased the enzyme activities. nFe_3O_4 and nFe also reduced the effect of antioxidants in the aboveground parts of the T705 seedlings. Nanomaterials effectively reduced the arsenic uptake of T705 and X24 rice seedlings at low arsenic concentrations.

1. Introduction

Arsenic is a soil pollutant released by rock weathering, mining, burning fossil fuels and arsenic-containing pesticides that have a strong toxic effect on animals and plants. Statistics show that 52,000 to 112,000 t of arsenic are added to the soil every year due to human manufacturing and use (Nriagu and Pacyna, 1988; Shibayama et al., 2010). At least 150 million people worldwide are at risk for endemic arsenic poisoning, with most of that population living in Asia; thus, arsenic pollution has received worldwide attention (Brammer and Ravenscroft, 2009). Arsenic pollution is a serious threat to food safety. Previously, inorganic arsenic was found in 34 rice cultivars in the Hunan province of China. The physiological characteristics and flood-cultivation technique used to irrigate rice confer a strong ability to enrich arsenic from the soil (Lei et al., 2013). Arsenic affects the growth and photosynthetic physiology of rice; seedlings under arsenic stress often present root dysplasia, short root lengths and leaves that fade and become yellow. Some studies (Shri et al., 2009) have shown that rice seedlings were affected by trivalent arsenic, which caused white and yellow leaves to develop. Because rice is the largest food crop in China and a staple for over 60% of the country's population, the annual consumption of rice accounts for approximately 55% of the total grain consumption. Excessive arsenic can interfere with the normal metabolism of cells, respiration and oxidation processes, and cause cellular disease. Arsenic can also directly damage the arterial and capillary walls, and be used in the vasoconstriction center, resulting in increased vascular permeability, decrease blood volume and aggravated organ damage. Arsenic trioxide has effects on the eyes, upper respiratory tract and skin (Chen et al., 1986; Smith et al., 2000). Therefore, research on the effects of arsenic on rice is particularly important.

Nanomaterial production has increased rapidly due to their widespread use in synthesizing important raw materials, as biosensors, in the food industry, and for sterilization and wastewater treatment (Nel et al., 2006). Nanomaterials have several properties, including small-size effects, quantum effects, surface effects and interface effects, which macroscopic materials lack (Nirmal et al., 2017). Nanomaterials show

Abbreviations: As, arsenic; CAT, catalase; CK, control experiment; GSH, glutathione; HA_{20}, 20-nm hydroxyapatite; HA_{40}, 40-nm hydroxyapatite; HGO, high-quality graphene oxide; MDA, malondialdehyde; MGO, multilayer graphene oxide; nFe, nano-zerovalent iron; nFe_3O_4, nano-Fe_3O_4; POD, peroxidase; SOD, superoxide dismutase

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great potential for use in soil and water pollution treatment because of their high specific surface areas and unsaturation. Nanomaterials can efficiently adsorb heavy metal ions in water solutions. For example, carbon nanotubes can effectively reduce the concentrations of Cd^{2+} and Cu^{2+} in polluted water (Sharma et al., 2009). Metal oxide nanomaterials have a high surface area and a high surface activity, which can be employed to treat heavy metals pollution. Most transition metal oxides, such as nano-Fe_2O_3 (nFe_2O_3), are commonly used as adsorbents. The capacity of nFe_2O_3 to adsorb uranium correlated positively with its particle size, and its large surface and negative potential, enabled nFe_2O_3 to effectively adsorb many metal ions (Zeng et al., 2009). Due to the large scale of nanometer-sized particle release into the environment, there is a high chance that they will coexist with heavy metal ions such as arsenic. Nanomaterials can adsorb arsenic to reduce the uptake of arsenic by plants; however, they also have some bio-toxic properties that may counteract this protective effect. For example, Huang et al. (2017) found that NHAP-Cd particles in the growth medium can be transported to rice seedlings and cause toxicity.

The uptake and transport of heavy metals by plants depend on the physicochemical properties of nanoparticles. Plants can uptake heavy metals within nanostructures and from the culture medium (Liu et al., 2015). Plants produce a wide range of enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids (El-Kemary et al., 2016). It is generally believed that the uptake of heavy metals such as arsenic is positively correlated with the concentration of nanomaterials (Castro-Bugallo et al., 2014). A cell wall is its first line of defense against invasion from external toxins but only nanoparticles are small enough to freely diffuse through the cell wall to enter a cell. However, a previous study demonstrated that nanoparticles can cause cells to produce walls with larger gaps that enable larger nanoparticles to enter (Navarro et al., 2008).

Previous studies on the toxicity and accumulation of arsenic in rice were not comprehensive. Therefore, it is necessary to carefully evaluate the safety of this important food crop, which could be influenced by the toxicity of arsenic and the coexistence of several common nanoparticles. In this study, we examined six nanoparticles: high-quality graphene oxide (HGO), multilayer graphene oxide (MGO), 20 nm hydroxyapatite (HA_{20}), 40 nm hydroxyapatite (HA_{40}), nano-Fe_3O_4 (nFe_3O_4) and nano-zerovalent iron (nFe) with the aim of studying the effects of (1) arsenic on the growth of rice seedlings, and (2) added nanoparticles on arsenic uptake and enzyme activities in rice seedlings. This study was designed to clearly and definitively demonstrate the influence of nanomaterials on arsenic uptake by rice seedlings, which can, in turn, provide reasonable evidence for their potential applications in remediating arsenic pollution.

2. Materials and methods

2.1. Materials

The HGO, MGO, HA_{20}, HA_{40}, nFe_3O_4, and nFe nanomaterials were used in this study and supplied by Jining Lite Nano Technology Co., Ltd. and Beijing Dekedaojin Technology Co., Ltd. The physical and chemical properties of these nanomaterials are shown in Table S1.

A hydroponic experiment was carried out on seeds from the T705 and X24 rice cultivars, both were obtained from Hunan Longping Seed Industry Co., Ltd.

2.2. Plant cultivation

Large, plump rice seeds were selected, soaked in 30% hydrogen peroxide for 15 min, cleaned thoroughly with distilled water, and then placed evenly into nursery seedling plates with deionized water just to soak the seeds (Ye et al., 2012). They were sprouted in the dark in a constant-temperature incubator set at 25 °C. After 48 h, they were transferred to an artificial-climate chamber (temperature: 25 °C, air humidity: 60%, illumination time: 18 h d^{-1}, pan evaporation rate: 30%·d^{-1}) and watered regularly to keep them moist. After 10 d, the seeds were transferred from the nursery seedling plates into hydroponic boxes (340 mm × 270 mm × 130 mm, with an 80-holes PE porous plate) and 1/10 Hoagland nutrient solution (pH maintained at 5.5 with 0.1 mol L^{-1} KOH or HCl). The nutrient solution was refreshed every three days, as reported previously (Dubey et al., 2016; Huang et al., 2017; Xie et al., 2016).

2.3. Preparation of the nano-suspensions

Arsenic trioxide (As[III]) and 500 mL 1/10 Hoagland nutrient solution was added to 500-mL polyvinyl chloride (PVC) cylindrical seedling boxes containing 0.1 g of one of the six nanomaterials at arsenic concentrations of 0, 0.8, 1.6, 3.2 and 4.0 mg L^{-1}. The control solution only contained 500 mL 1/10 Hoagland nutrient solution. The nanoparticles were suspended in an ice bath for 30 min in a CNC Ultrasonic Cleaning Device (Kunshan Ultrasonic Instrument Co. Ltd, China).

2.4. Rice seedling-exposure process

After growing for approximately 18 d in the nutrient solution, T705 and X24 seedlings of a consistent size were rinsed with deionized water and transferred to the 500-mL PVC cylindrical seedling boxes in which the nano-suspensions had been prepared. Twelve seedlings were placed in each box. The solutions were suspended in an ice bath with the CNC Ultrasonic Cleaning Device for 30 min after every 12 h. The seedlings were cultivated in the artificial-climate chamber for five days, and each experiment was performed in quadruplicate. To prevent the nanoparticles from aggregating, the nano-suspension samples were replaced every two days (Abdel-Haliem et al., 2017; Morsy et al., 2014; Shaw and Hossain, 2013). In the whole process, seedling-exposure process was repeated in three independent experiments, each performed with four samples.

2.5. Determination of the biomass of rice

After treatment, the root systems of the rice seedlings were washed gently with deionized water, soaked with 20 mmol L^{-1} EDTA-2Na for approximately 15 min to remove the surface-adsorbed nanoparticles and heavy metal ions, and then cleaned with deionized water. Approximately half of the seedlings were dissected into the root and aboveground parts (stems and leaves) sterilized at 105 °C for 15 min, and then dried at 75 °C to a constant weight (Carbonell-Barrachina et al., 2009). The remaining portion of the rice seedlings was frozen at −20 °C to determination bioenzyme activities.

2.6. Determination of arsenic content in rice

The roots and aboveground sections that had been dried to a constant weight were divided into chips, and 0.25 g of each was weighted into Teflon elimination tubes. Then, 7 mL of nitric acid was added and the tubes were covered and placed in a fume hood for 12 h. Afterwards, they were placed in an electrothermal digester (ED54, Lepertyco, United States of America [USA]) at 110 °C for 4 h to boil until dissipation. The covers were then removed to cool the solutions to room temperature (~25 °C). The volume of each sample was adjusted to 25 mL using deionized water. The arsenic contents of each sample were determined with an atomic fluorescence spectrum analyzer (AFS-9760, Beijing Haiguang Instrument Co., Ltd., China).

2.7. Determination of enzyme activities

The frozen rice seedlings were divided into roots and stems, and cleaned with deionized water, after cleaning filter paper was used to