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Nitric oxide ameliorates zinc oxide nanoparticles-induced phytotoxicity in rice seedlings

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HIGHLIGHTS

- NO can alleviate ZnO NPs-induced growth inhibition of rice seedlings.
- Zn concentration in ZnO NPs-treated rice seedlings was decreased by NO.
- NO alleviates ZnO NPs-induced oxidative stress by mediating antioxidant system.

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ABSTRACT

Nitric oxide (NO) has been found to function in enhancing plant tolerance to various environmental stresses. However, role of NO in relieving zinc oxide nanoparticles (ZnO NPs)-induced phytotoxicity remains unknown. Here, sodium nitroprusside (SNP, a NO donor) was used to investigate the possible roles and the regulatory mechanisms of NO in counteracting ZnO NPs toxicity in rice seedlings. Our results showed that 10 μM SNP significantly inhibited the appearance of ZnO NP toxicity symptoms. SNP addition significantly reduced Zn accumulation, reactive oxygen species production and lipid peroxidation caused by ZnO NPs. The protective role of SNP in reducing ZnO NPs-induced oxidative damage is closely related to NO-mediated antioxidant system. A decrease in superoxide dismutase activity, as well as an increase in reduced glutathione content and peroxidase, catalase and ascorbate peroxidase activity was observed under SNP and ZnO NPs combined treatments, compared to ZnO NPs treatment alone. The relative transcript abundance of corresponding antioxidant genes exhibited a similar change. The role of NO in enhancing ZnO NPs tolerance was further confirmed by genetic analysis using a NO excess mutant (*noe1*) and an *OsNOA1*-silenced plant (*noa1*) of rice. Together, this study provides the first evidence indicating that NO functions in ameliorating ZnO NPs-induced phytotoxicity.

1. Introduction

Nanoparticles (NPs) are ultrafine particles that typically have at least one dimension less than 100 nm in size [1]. With the industrial development at the nanoscale, some metal oxide NPs such as zinc oxide (ZnO), titanium dioxide (TiO_2), copper oxide (CuO) and cerium oxide (CeO_2), are widely applied in market goods. Among them, due to unique electronic, optical, dermatological, and antibacterial properties, ZnO NPs are used in various commercial products including batteries, pigments, catalysts, semiconductors, cosmetics, drug carriers, etc. [2]. The production, use and disposal of a large number of ZnO NPs will inevitably increase their release

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into the environment and has become a serious threat to biological systems including plants [3].

Phytotoxicity of ZnO NPs on seed germination and root development has recently been studied in lettuce, radish, and cucumber [4]. ZnO NPs also seriously inhibited wheat growth under field conditions [5] and caused genotoxicity to broad bean [6]. A limited number of studies on ZnO NPs ecotoxicity suggested several mechanisms of action. First, the release of Zn^{2+} from ZnO NPs in exposure media may be a possible cause for phytotoxicity [3]. Second, ZnO NPs may directly disrupt membranes or DNA [2]. Most importantly, ZnO NPs promote the generation of reactive oxygen species (ROS), that is superoxide radical ($O_2^{\bullet -}$) release and hydrogen peroxide (H_2O_2) production, in the absence of photochemical energy [7]. Excessive generation of ROS can induce lipid membrane peroxidation and cellular damage, which has been suggested as one of the primary reasons contributing to nanotoxicity in general [3]. ZnO NPs-induced toxicity via ROS has been extensively demonstrated and clarified in the previous studies [8]. These studies expanded and deepened our knowledge on phytotoxicity of ZnO NPs. However, to date, no information concerning how to ameliorate ZnO NPs-induced phytotoxicity is available.

Nitric oxide (NO), an important signaling molecule, mediates various plant physiological and developmental processes, including stomatal closure, flowering, root formation, etc. [9,10]. NO also plays a critical role in enhancing plant tolerance to environmental stresses such as salt, drought, chilling and heavy metals [11]. An important mechanism by which NO protects plants against environmental stresses is to eliminate excessive intracellular ROS by increasing the content of antioxidants, as well as regulating antioxidant enzyme activity [11,12]. For instance, Laspina et al. [13] reported that exogenous NO alleviated cadmium-induced oxidative stress in rice and sunflower leaves by increasing the contents of low-molecular-weight antioxidants including ascorbate and reduced glutathione (GSH). Our recent study also found that NO effectively activated antioxidant enzymes and mitigated oxidative damage caused by salt in a mangrove species, *Aegiceras corniculatum* [14]. Therefore, it is logical to hypothesize that NO could ameliorate phytotoxicity caused by ZnO NPs in plants. If this is the case, the detoxication mechanism of NO may be related to antioxidant system.

In the light of these questions, we analyzed the effects of sodium nitroprusside (SNP, a widely used NO donor) on growth (e.g., root length, shoot height, biomass and total chlorophyll (Chl) content), Zn accumulation, ROS production, lipid peroxidation, antioxidant enzyme activity and gene transcript abundance in ZnO NPs-treated rice seedlings in the present study. By employing a NO excess mutant (*noe1*) and an *OsNOA1*-silenced plant (*noa1*) of rice, genetic evidences were provided to further confirm the role of NO in mediating ZnO NPs-induced phytotoxicity in rice. The objective of this study is to investigate whether and how NO functions in ameliorating phytotoxicity caused by ZnO NPs in plants. This study might provide novel and useful information to nanotoxicity studies in plants.

2. Materials and methods

2.1. Characterization of ZnO NPs

ZnO NPs were purchased from DK Nano Technology Co., Ltd., Beijing, China, with a purity of 99.5%, particle size of 30 nm. The morphology of ZnO NPs was examined using scanning electron microscopy (SEM, S-4800, Hitachi, Ltd., Japan) and transmission electron microscopy (TEM, H-7650, Hitachi Ltd., Japan). The image and size distribution of ZnO NPs are shown in Fig. S1, with a size ranging between 10–70 nm and a mean size of 28 ± 4 nm. The mean size was almost the same as that claimed (30 nm) by the producer.

2.2. Plant material and treatments

Rice seeds (*Oryza sativa* L.) were first sterilized in 5% sodium hypochlorite solution for 15 min, followed by rinsing thoroughly with sterilized water and germinated on moist filter paper at 35 °C for 48 h. The germinated seeds were transferred to a hydroponic culture containing Kimura B nutrient solution as described previously [15]. Seedlings were grown in an environmentally-controlled growth chamber with a 14 h/27 °C day and a 10 h/25 °C night regime, a light intensity of 400 μmol m^{-2} s^{-1} photosynthetically active radiation, and relative humidity of about 70%. Three-day-old seedlings of uniform size were selected and used in the following experiments.

Rice (cv. Jiafuzhan) seedlings were divided into the following three experimental groups. In the first group, to study the phytotoxicity of ZnO NPs, the seedlings were transferred to ZnO NPs suspensions prepared in Kimura B nutrient solution as described previously [3]. In brief, the desired ZnO NPs mixtures with concentration of 0, 50, 100, 250, 500 and 1000 mg L^{-1} were stirred for 5 min and later sonicated by water bath ultrasonic treatment (25 °C, 100 W, 40 kHz) for 1 h. In the second group, rice seedlings were transferred to ZnO NPs suspensions containing different concentrations of SNP (0, 5, 10, 25, 50 and 100 μM) to select an appropriate concentration of SNP. In the third group, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) was used as a NO scavenger and $K_3Fe(CN)_6$ as additional control for SNP decomposition. The third group consisted of a control (CK, only Kimura B nutrient solution), 250 mg L^{-1} ZnO NPs (N), 10 μM SNP (S), 250 mg L^{-1} ZnO NPs + 10 μM SNP (N + S), 250 mg L^{-1} ZnO NPs + 10 μM $K_3Fe(CN)_6$ (N + CN), 100 μM cPTIO (C), 250 mg L^{-1} ZnO NPs + 100 μM cPTIO (N + C), 250 mg L^{-1} ZnO NPs + 10 μM SNP + 100 μM cPTIO (N + S + C).

To verify the role of NO in ZnO NPs tolerance at the genetic level, a NO excess mutant (*noe1*), an *OsNOA1*-silenced plant (*noa1*) of rice and their corresponding wild-type rice (cv. Nipponbare and Zhonghua 11) were exposed to 0 or 250 mg L^{-1} ZnO NPs. The *NOE*1 mutant obtained from a large T-DNA-tagged population by genetic screening accumulated more NO than its wild-type plants [16]. The *noa*1 mutant with defect in NO synthesis-associated protein1 (NOA1) was generated via RNA interference [15]. The reduced NO production has been observed in *noa1* mutant plants [16,17].

All the treatment solutions were freshly prepared before each experiment and adjusted to pH 5.8. According to the method of Lin and Xing [3], the treatment solutions were stirred three times per day with an 8 h interval, and were renewed every day to maintain a constant ZnO NPs concentration. After various treatments for 3 days, fresh roots and shoots of rice seedlings were collected for further measurements.

2.3. Plant growth measurement

Root length and shoot height were photographed and measured using Image J 1.4 software (Wayne Rasband National Institute of Health, Bethesda, MD, USA). Biomass was measured after drying at 70 °C for 48 h and was recorded by weighing individual seedling. Total chlorophyll (Chl) content in rice shoot was determined according to the method of Yang et al. [15].

2.4. TEM observation

Fresh roots and shoots samples were cut into 0.5×1.0 mm pieces and prefixed in 2.5% glutaraldehyde for 4 h. After washing with phosphate buffer solution (PBS, 0.1 M, pH 7.0), the samples