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Nitric oxide induces rice tolerance to excessive nickel by regulating nickel uptake, reactive oxygen species detoxification and defense-related gene expression

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HIGHLIGHTS

- Nickel (Ni) and nitric oxide (NO) interaction was examined in rice seedlings.
- Ni-induced toxic effects reflected in growth performance of rice seedlings.
- NO reduced Ni uptake and reversed growth defects of rice plants under Ni stress.
- NO-triggered antioxidant capacity alleviated oxidative damage under Ni-stress.

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ABSTRACT

Soil contamination with nickel (Ni) is a persistent threat to crop production worldwide. The present study examined the putative roles of nitric oxide (NO) in improving Ni-tolerance in rice. Our findings showed that application of exogenous sodium nitroprusside (SNP), a NO donor, significantly improved the growth performance of rice seedlings when grown under excessive Ni. The enhanced Ni-tolerance of rice prompted by SNP could be ascribed to its ability to regulate Ni uptake, decrease Ni-induced oxidative stress as evidenced by reduced levels of hydrogen peroxide, malondialdehyde, and electrolyte leakage in Ni-stressed plants. The positive roles of NO against Ni-toxicity also reflected through its protective effects on photosynthetic pigments, soluble proteins and proline. SNP also boosted antioxidant capacity in Ni-stressed plants by maintaining increased levels of ascorbate, enhanced activities of ROS-detoxifying enzymes, particularly peroxidase (POD) and catalase (CAT) in both roots and shoots compared with Ni-stressed alone plants. Moreover, SNP treatment also upregulated the transcript levels of *CAT, POD, ascorbate peroxidase*, *glutathione reductase* and *superoxide dismutase* genes in shoots under Ni-stress. Using different sulfide compounds and NO scavenger cPTIO, we also provided evidence that NO, rather than other byproducts of SNP, contributed to the improved performance of rice seedlings under Ni-stress. Collectively, our results conclude that exogenous SNP-mediated modulation of endogenous NO enhanced rice tolerance to Ni–stress by restricting Ni accumulation, maintaining photosynthetic performance and reducing oxidative damage through improved antioxidant system, thereby suggesting NO as an effective stress regulator in mitigating Ni-toxicity in economically important rice, and perhaps in other crop plants.

1. Introduction

Rapid industrialization significantly contributes to environ-

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mental pollution by releasing considerable amounts of toxic metals, including nickel (Ni) to the environment. Ni is released into the environment from various anthropogenic sources, such as metal mining, smelting, fossil fuel burning, vehicle emissions, disposal of household, municipal and industrial wastes, fertilizer application and organic manure (Chen et al., 2009). Ni content in normal soils ranges from 10 to 1000 mg kg^{-1}. However, in contaminated soils, the range of Ni concentrations may reach 200–26,000 mg kg^{-1}, as compared with normal soils (Sreekanth et al., 2013; Yusuf et al., 2011). Ni has been found as a key pollutant in farmlands of central and south China, with an average Ni concentration of 226.30 mg kg^{-1} and maximum of 1000 mg kg^{-1} in the farmlands of Jinchang City in Gansu Province (Ding et al., 2008), which is higher than the background value of Ni concentration, that is 40 mg kg^{-1} according to Chinese Soil Environmental Quality Standards. Thus, Ni accumulation and bioavailability to plants has recently gained much attentions due to their potential health risks and food safety problems (Yusuf et al., 2011).

Ni has been designated as essential micronutrient because plants can't complete their growth cycle without sufficient amount of this metal (0.01–10 μg g^{-1} dry wt.) (Gratao et al., 2008; Soares et al., 2016). Ni has been recognized as a constituent of various enzymes, including glyoxalases, peptide deformylases, methyl-CoM reductase and a few superoxide dismutases (SODs) and hydrogenases (Chen et al., 2009). Ni is also a part of the active site of the urease (Kutman et al., 2014), which help to catalyzes the hydrolysis of urea to ammonia and bicarbonate (Polacco et al., 2013). However, Ni at high concentration can cause serious toxic effects on plants, including inhibition of seed germination, reduction of plant growth and yield (Shukla and Gopal, 2009), induction of leaf chlorosis, necrosis and wilting (Mosa et al., 2016), and peroxidation of lipid (Rizwan et al., 2017). Besides these negative effects, Ni stress can lead to a higher production of reactive oxygen species (ROS) by interrupting redox homeostasis of plant cell (Gajewska and Skłodowska, 2008; Israr et al., 2011), causing damage to essential cellular components, such as membrane lipids, proteins, enzymes, pigments and nucleic acids (Sharma and Dubey, 2007). However, plants possess array of mechanisms to counteract ROS damage i.e. complex antioxidant defense system which comprised of enzymatic antioxidants, such as SOD, peroxidase (POD), catalase (CAT) and non-enzymatic antioxidants, including ascorbate (AsA) and glutathione (GSH). These antioxidant compounds act in concert to detoxify excessive ROS under stress conditions (Verma and Dubey, 2003).

To improve plant's adaptability, signal molecules could be exogenously used as a potential tool under environmental stress condition. Among various signal molecules, nitric oxide (NO) is a notable biological messenger in plant tissues to regulate numerous growth and development processes. There are plenty of information that confirmed the functional roles of NO in plant physiological processes, like seed dormancy and germination, root development, photosynthesis, stomata closure, regulation of pollen tube growth, flowering, fruit ripening, cell death, plant metabolism and interaction with plant hormones under abiotic and biotic factors (Ahmad et al., 2016; Saxena and Shekhawat, 2013). Several previous studies have been reported that addition of exogenous NO enhanced plant tolerance to oxidative stress induced by salinity (Mostofa et al., 2015), excess light (Xu et al., 2013), drought (Fancy et al., 2017), heat stress (Uchida et al., 2002), cold stress (Sehrawat and Deswal, 2014), ozone toxicity (Ahlfors et al., 2009), herbicides treatment (Ferreira et al., 2010), mercury and cadmium toxicity (Chen et al., 2015; Wang et al., 2015). In spite of the availability of abundant literature about NO functioning as a signaling molecule in stimulating multiple defense responses against environmental stresses in plants, the interactions of NO with Ni and how NO modulates the physiological, biochemical and molecular changes under Ni stress in an economically important crop like rice are still elusive. In the current study, we aimed to investigate the effects of NO donor sodium nitroprusside (SNP) on growth and physio-biochemical processes in rice to know whether NO could alleviate Ni-induced growth inhibition and oxidative stress and (2) whether these effects could be linked to the activities of major antioxidant enzymes and related gene expressions in rice plants under excessive Ni stress. To this end, a combined physiological, biochemical and molecular approach was used to determine NO induced Ni-stress tolerance by assessing various key parameters: plant growth and biomass, Ni uptake, the contents of photosynthetic pigments, soluble protein and proline (Pro), malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and electrolyte leakage (EL). In addition to this, the impact of exogenous application of NO on transcript levels of representative antioxidant enzyme-encoding genes as well as antioxidant enzymes activities in Ni-challenged rice plants were also examined.

2. Materials and methods

2.1. Plant material and growth conditions

Rice (*Oryza sativa* L., cv. yangliangyou 6) seeds were surface sterilized in 10% H_2O_2 for 10 min, followed by repeated washing with distilled water (dH_2O) thoroughly, and soaked in dH_2O for 24 h. After this, the seeds were placed on plastic nets floating on dH_2O and kept in an incubator under dark for seed germination at 28 ± 2 °C for 4 days. Thereafter, uniform sized seedlings were selected and transferred in plastic pots containing 0.5 strength Hoagland nutrient solution and grown in growth chamber under photon density; 820 mmol m^{-2} s^{-1}, temperature; 28 ± 2 °C and relative humidity; 65–70% for 19 days. Thereafter, the root and shoot samples from control and were harvested and different parameters were analyzed.

2.2. Nitric oxide and Ni treatments

After 10 days of sowing, Ni as $NiSO_4 \cdot 6H_20$ and NO as sodium nitroprusside (SNP) was added to full strength Hoagland nutrient solution. In this experiment, 9 combinations of Ni and SNP were made i.e. (1) control (CK), (2) 100 μM SNP alone, (3) 200 μM SNP alone, (4) 50 μM Ni alone, (5) 200 μM Ni alone, (6) 100 μM SNP + 50 μM Ni, (7) 100 μM SNP + 200 μM Ni, (8) 200 μM SNP + 50 μM Ni, (9) 200 μM SNP + 200 μM Ni. The concentration of Ni was selected on the basis of our previous study, in which several lower and higher levels of Ni were used, i.e., 10, 50, 100 and 200 μM of $NiSO_4 \cdot 6H_20$. Ni at the concentration of 50 μM showed a little damage and 200 μM Ni imposed significant damage to plant growth (Rizwan et al., 2017). The concentrations of SNP (100 and 200 μM) were selected on the basis of literature present. Plants exhibited an optimum response to treatments with 100 and 200 μM SNP concentrations under various abiotic stress conditions (Singh et al., 2016; Mostofa et al., 2014: Namdjoyan and Kermanian, 2013; Kazemi et al., 2010). In the current trial, SNP was selected as NO donor as because it provides a persistent pattern of NO release compared with other donors (Mur et al., 2013). SNP may also produce other residual products besides NO such as sodium cyanide, ferrocyanide, ferricyanide, sodium nitrite and sodium nitrate (Singh et al., 2013). To investigate the possible roles of these byproducts in the alleviation of Ni toxicity in rice. We applied a series of chemicals such as, $Na_4Fe(CN)_6$ (SF), $K_3Fe(CN)_6$ (KF), $NaNO_2$ (SN), $NaNO_3$ (SNT) as an additional control of SNP decomposition. The second group consisted of control (CK, Hoagland nutrient solution only), 200 μM SNP (SNP), 200 μM Ni (Ni), 200 μM