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Alleviation of nickel toxicity in finger millet (*Eleusine coracana* L.) germinating seedlings by exogenous application of salicylic acid and nitric oxide

*Kasi Viswanath Kotapati, Bhagath Kumar Palaka, Dinakara Rao Ampasala**

Centre for Bioinformatics, School of Life Sciences, Pondicherry University, Puducherry 605014, India

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ABSTRACT

This study investigated the effect of salicylic acid (SA) and sodium nitroprusside (SNP; NO donor) on nickel (Ni) toxicity in germinating finger millet seedlings. Fourteen-day-old finger millet plants were subjected to 0.5 mmol L^{-1} Ni overload and treated with 0.2 mmol L^{-1} salicylic acid and 0.2 mmol L^{-1} sodium nitroprusside to lessen the toxic effect of Ni. The Ni overload led to high accumulation in the roots of growing plants compared to shoots, causing oxidative stress. It further reduced root and shoot length, dry mass, total chlorophyll, and mineral content. Exogenous addition of either 0.2 mmol L^{-1} SA or 0.2 mmol L^{-1} SNP reduced the toxic effect of Ni, and supplementation with both SA and SNP significantly reduced the toxic effect of Ni and increased root and shoot length, chlorophyll content, dry mass, and mineral concentration in Ni-treated plants. The results show that oxidative stress can be triggered in finger millet plants by Ni stress by induction of lipoxygenase activity, increase in levels of proline, $O_2^{\bullet-}$ radical, MDA, and H_2O_2, and reduction in the activity of antioxidant enzymes such as CAT, SOD, and APX in shoots and roots. Exogenous application of SA or SNP, specifically the combination of SA + SNP, protects finger millet plants from oxidative stress observed under Ni treatment.

1. Introduction

Degradation of agricultural soils due to heavy metal toxins results from long application of excessive fertilizer, sewage sludge, furnace dust, industrial waste, and inappropriate farming techniques [1,2]. Heavy metals such as Cd, Cu, Zn, Ni, Co, Cr, Pb, and As are toxic to plants and other livestock at concentrations above certain threshold levels. Their contamination of the environment threatens the health of vegetation, wildlife, and human beings [3]. Anthropogenic activities and natural events that can alter the biogeochemical cycles of ecosystems are responsible for heavy metal pollution in soil and water.

Abbreviations: SA, Salicylic acid; SNP, Sodium nitroprusside; Ni, Nickel; SOD, Superoxide dismutase; CAT, Catalase; $O_2^{\bullet-}$, Superoxide anion radical; ROS, Reactive oxygen species; LOX, Lipoxygenase; MDA, Malondialdehyde; TCA, Trichloroacetic acid; TBA, Thiobarbituric acid; POD, Peroxidase; APX, Ascorbate peroxidase; GR, Glutathione reductase; DHAR, Dehydroascorbate reductase; GPX, Glutathione peroxidase.

* *Corresponding author.*
E-mail address: ampasaladr@bicpu.edu.in (D.R. Ampasala).
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Ni is one of the naturally occurring components in soil, plants, and aquatic environments. In plant tissues, Ni is present in minute amounts, at concentrations ranging from 0.01 to 5.00 mg kg^{-1} dry weight [4]. A very low concentration of Ni is essential for the growth of plants such as wheat (*Triticum aestivum* L.), cotton (*Gossypium hirsutum* L.), tomato (*Solanum lycopersicon* L.), potato (*Solanum tuberosum* L.), and other plant species [4,5]. It is also essential for crop yield and provides resistance against some diseases, particularly rust diseases [6,7]. Ni is a key element in urease and is also a constituent of several metalloenzymes such as superoxide dismutase (SOD, EC1.15.1.1), Ni-Fe hydrogenase, methyl-coenzyme M reductase, carbon monoxide dehydrogenase, acetyl coenzyme-A synthase, hydrogenase, and RNase-A [8]. Ni deficiency in plants affects urea metabolism, leading to leaf browning; reduces the scavenging activity of superoxide free radicals; and disturbs nitrogen assimilation and amino acid metabolism [9].

Ni is readily absorbed by plants from soil and nutrient solutions. As a result of anthropogenic activity, high levels of Ni may be observed in plant tissues, causing Ni toxicity. The symptoms of Ni toxicity in plants commonly include reduced seed germination, growth, photosynthesis, and sugar transport; increased chlorosis, necrosis, and wilting; and disruption of metabolic processes [10].

Production of reactive oxygen species (ROS) in abundance is a response of plants to several stress factors. An antioxidative system that comprises antioxidative enzymes and nonenzymatic low-molecular mass antioxidants is required to maintain the balance between generation and degradation of ROS in plants. Many studies have found a decrease in activity of antioxidant enzymes under metal stress [11,12]. Proline is one of the metabolites most commonly generated in plant tissues under stress conditions [13].

Salicylic acid (SA) not only is a signaling molecule in plants, but also increases plant tolerance to biotic and abiotic stresses [14]. Several physiological and biochemical activities in plants are affected by exogenous application of SA and salicylates infers the crucial role of these compounds in plants [15–18]. Increased oxidative stress in the membranes of rice (*Oryza sativa* L.) leaves was observed as a response to increased levels of ROS under heavy metal stress, and was alleviated by exogenous application of SA to rice plants [19].

In plants, nitric oxide (NO) is an essential signaling molecule playing a crucial part in many intracellular and physiological processes [20]. Exogenous supplementation with NO as sodium nitroprusside (SNP) has enhanced tolerance of plants towards heavy metals [21] and salinity [22]. Antioxidative enzymatic systems such as SOD, ascorbate peroxidases (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), and catalase (CAT) are upregulated by NO. It also triggers redox-regulated, defense-related gene expression directly or indirectly to establish plant stress tolerance [23].

High sensitivity to Ni toxicity has been reported in cereals [24]; however, the osmotic adjustment system and capacity to restore the damage caused internally by metal-induced oxidative stress has been little studied in cereals. Finger millet (*Eleusine coracana* L.) is one of the ancient millets in India and Africa, grown since primitive times (2300 BC). The present study was performed to investigate the effect of exogenous application of SA and SNP on germinating finger millet seedlings under Ni stress and also the response of antioxidative enzyme systems under metal stress to SA and SNP.

2. Materials and methods

2.1. Plant material

Finger millet seeds (Sri Chaitanya VR-847) were procured from the agricultural research station at Vizianagaram, Andhra Pradesh, India. Seeds were surface-sterilized using sodium hypochlorite (0.1%, *w/v*) as disinfectant for 10 min and then washed three times with sterile distilled water. They were soaked in sterile distilled water for 12 h at room temperature and were allowed to germinate in sterile Petri plates on wet filter paper at 25 °C for three days. After germination, seedlings of uniform size were transferred to polyethylene pots filled with half-strength Hoagland's hydroponic nutrient solution (Hi media, Mumbai) [25], and were grown in a controlled room at 25 °C under a 16-h photoperiod with an irradiance of 175 μmol m^{-2} s^{-1} for seven days. Each experiment employed three replications and mean values for all the parameters such as dry mass, chlorophyll and proline content, lipoxygenase (LOX) activity levels, lipid peroxidation and $O_2^{\bullet-}$ generation rate were obtained. On average, five plants were taken for quantification of each parameter by simple random sampling.

2.2. Nickel treatment and experimental design

To estimate toxic levels of Ni, preliminary experiments were performed on finger millet seedlings with various Ni concentrations. The concentrations were 0.01, 0.05, 0.10, 0.20, and 0.50 mol L^{-1}. Preliminary results for root and shoot length, dry mass, and chlorophyll content indicated that treatment with 0.5 mol L^{-1} Ni showed more toxicity than the other concentrations (Table S1). Accordingly, the concentration of 0.5 mol L^{-1} Ni was chosen for subsequent experiments. Concentrations of 0.2 mol L^{-1} SA and SNP were selected based on earlier reported data.

Seven-day-old seedlings with uniform size and health were selected and transferred from the half-strength Hoagland nutrient solution to polyethylene pots filled with full-strength Hoagland's hydroponic nutrient solution having pH 6.5. Polyethylene pots with full-strength Hoagland nutrient solution either with or without 0.2 mmol L^{-1} SA or 0.2 mmol L^{-1} SNP or SA + SNP were treated as control plants. Polyethylene pots with full-strength Hoagland nutrient solution supplemented with nickel chloride ($NiCl_2 \cdot 6H_2O$) at a concentration of 0.5 mmol L^{-1} either with or without 0.25 mmol L^{-1} SA, 0.25 mmol L^{-1} SNP, or SA + SNP were treated as test plant samples. Nutrient solutions were replaced every three days and pH was adjusted to 6.5 with 10.0 mmol L^{-1} NaOH. Plants were grown at 25 °C with 16 h photoperiod and irradiance of 175 μmol m^{-2} s^{-1} for 14 days. Plants were harvested at the 14-day-old stage and shoots and roots were separated and repeatedly washed with distilled water. For enzyme determination, fresh plant material was frozen in liquid nitrogen and stored at –70 °C until further use.