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Effect of nickel on growth and biochemical characteristics of wheat (*Triticum aestivum* L.) seedlings

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ABSTRACT

A hydroponic experiment was carried out under controlled conditions to investigate the impact of different levels of nickel (Ni) in the nutrient solution on growth and biochemical characteristics of wheat (*Triticum aestivum* L.) seedlings, including membrane lipid peroxidation (LPO), proline accumulation and superoxide dismutase (SOD). The Ni levels investigated were 0, 25 and 50 μg per litre. A statistically significant increase in LPO was recorded in the seedlings grown at concentrations of 25 or 50 μg Ni per litre, but SOD (1.15.1.11) activity was not significantly affected. Plant height and chlorophyll content were affected significantly in a dose dependent manner. Proline content increased considerably in response to Ni concentration. The results indicate that the increase in the activities of the antioxidant enzymes were not sufficient to protect cell membrane against Ni toxicity.

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1. Introduction

Nickel (Ni), a heavy metal, is an essential micronutrient for plant growth and development [1]. However, it becomes toxic at high concentrations. Excess Ni destroys photosynthesis and membrane functions, inhibits seed germination, plant growth and development, and markedly decreases the yields of plants [2–5]. Soil and water contamination by heavy metals was originally restricted to metalliferous soils but has become a general problem caused by anthropogenic sources including smelting of ore, electroplating and municipal sludge [6,7]. Heavy metals tend to bind sulphydryl groups of enzymes and hence suppress the functioning of essential biological components [8]. They can also lead to ionic imbalances as a result of altered translocation of ions such as Fe^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+} and/or metal substitution.

In the last decades, there has been increasing demand for assessing the ecological risks of soil contamination, using phyto-toxicity tests as important tools for risk assessment and environmental monitoring of heavy metal-polluted soils [9]. Phytotoxicity tests generally use toxicological endpoints such as seedling growth and biomass production [10]. There is increasing interest in using other parameters based on physiological and biochemical biomarkers, such as photosynthesis, chlorophyll fluorescence and enzymatic activities in plant tissues [10–12].

Phytotoxicity from heavy metals is closely related to the production of reactive oxygen species (ROS) in plants [13]. It has been demonstrated that excess Ni and cadmium lead to a significant increases in the content of hydrogen peroxide (H_2O_2) [2] and the membrane lipid peroxidation in a few plant species [14].

In 1996, the US Environmental Protection Agency recommended several plants that have an economic and ecological importance as biomarkers for toxicity assessment in terrestrial and aquatic ecosystems. So in this study we investigated the effects of Ni toxicity on growth parameters, membrane LPO, proline accumulations and some antioxidative enzyme activities (SOD) in tissues of wheat seedlings.

2. Materials and methods

2.1. Plant growth, sample analysis and metal estimation

Seeds of the wheat cultivar Gerek used in this study were obtained from the Central Agricultural Research Institute, Ankara (Turkey). The seeds were surface-sterilized with 10% sodium hypochlorite solution for 10 min, washed and imbibed in distilled water for 1 day. Ten to 15 imbibed seeds were then planted onto plastic cups covered with cheesecloth and containing Hoagland's nutrient solution. The cups were kept for 7 days in a growth chamber at 25 °C and an 8 h dark 16 h light cycle, using a light intensity

Abbreviations: LPO, lipid peroxidation; SOD, superoxide dismutase.

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of 40 mmol m^{-2} s^{-1}. The treatment was started on the 8th day and continued for three days, by replacing the original solution by Hoagland's solutions containing 25 or 50 μg Ni per litre, using $Ni(NO_3)_2$. These Ni concentrations had been earlier established in preliminary experiments. The nutrient solutions were changed every 24 h. After 15 days, the leaves were harvested and either used directly for analysis or frozen in liquid nitrogen followed by storage at −20 °C until further use.

Dried wheat samples were digested with 10 mL of concentrated HNO_3, using a CEM microwave digestion system. After digestion, the volume of each sample was adjusted to 25 mL using double deionized water [15]. Determinations of the Ni concentrations in all samples were carried out using Inductively Coupled Plasma Optical Emission Spectrometry (Varian). The samples were analysed in triplicate.

2.2. Chlorophyll

The amount of chlorophyll in the leaves was determined according to the method described by Knudson et al. [16].

2.3. Lipid peroxidation

Lipid peroxidation (LPO) was determined using the method of Teresa et al. [17] by measuring the amount of TBARS. The leaf tissues (approximately 0.3 g) of control and treated plants were homogenized in 3 mL of 5% trichloroacetic acid (TCA) solution using a cold mortar and pestle. The homogenates were then transferred to fresh tubes and centrifuged at 12,000 × *g* for 15 min at room temperature. Four mL of 0.5% thiobarbituric acid (TBA) in 20% TCA solution (freshly prepared) were then added to 1 mL of the supernatant and incubated at 96 °C for 30 min. The tubes were cooled by transferring them to an ice bath and then centrifuged at 12,000 × *g* for 10 min. The absorbance of the supernatant was recorded at 532 nm and corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. TBARS content was determined by using its extinction coefficient, i.e., 155 mM^{-1} cm^{-1}.

2.4. Proline

The proline content was determined according to the method described by Bates et al. [18]. The leaf tissues (about 0.3 g) of control and treated plants were homogenized by adding 5 mL of 3% sulphosalicylic acid solution. The leaves were homogenized using a cold pestle and mortar. The homogenates were centrifuged at 5000 × *g* for 10 min at 4 °C. For each sample, a glass tube containing 2 mL of acid ninhydrin (0.31 g ninhydrin, 7.5 mL of acetic acid, and 5 mL of 6 M phosphoric acid), 2 mL of 96% acetic acid and 1 mL of 3% sulphosalicylic acid was prepared and supernatant (2 mL) from each homogenate was added to the tubes. The tubes were incubated at 96 °C for 1 h in a hot water bath and after incubation 4 mL of toluene was added to each tube followed by mixing. The absorbance of the pink red upper phase was recorded at 520 nm against a toluene blank. A standard curve for proline in the range of 0.01 μM to 1.5 mM was constructed to determine the proline content of each sample.

2.5. Enzyme and protein

Fresh leaf tissue (1 g) was homogenized in liquid nitrogen using a pestle and mortar and suspended in 3.0 mL of 0.1 M Tris–HCl buffer (pH 7.5) containing 0.5 mM ethylenediaminetetraacetic acid (EDTA) and 1.0% polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 15,000 × *g* for 20 min. The supernatant was used as an enzyme source in all enzyme analysis. The protein content in the leaf extracts was determined according to the Bradford method using bovine serum albumin as a standard [19].

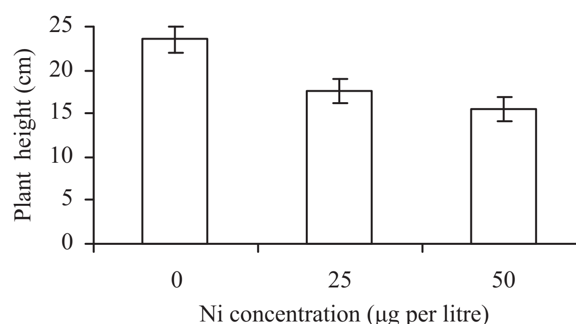

Fig. 1. Plant height of wheat seedlings grown on nutrient solutions varying in nickel concentration.

2.6. Superoxide dismutase (1.15.1.11)

To determine superoxide dismutase (SOD) activity, the assay buffer consisted of 20 mM sodium phosphate (pH 7.5), 0.1 mM EDTA, 10 mM methionin, 0.1 mM p-nitro blue tetrazolium chloride (NBT), 0.005 mM riboflavin and the enzyme extract containing 50 mg protein in a final volume of 3 mL. SOD activity was determined by the inhibition of NBT photo-reduction. A fluorescent lamp was positioned at a distance of 20 cm from the samples for 5 min. One unit of SOD was defined as the amount of enzyme that inhibits NBT photo-reduction by 50% when monitored at 560 nm. A standard curve for SOD in the range of 20–200 ng mL^{-1} was constructed to determine the SOD content in each sample [20].

2.7. Statistical analysis

Statistical significance of the difference between mean values obtained from at least three independent analyses was determined by one-way analysis of variance (ANOVA) at 95% confidence interval using SPSS 10.0 for Windows [21].

3. Results

3.1. Plant growth Ni accumulation

Increasing the Ni concentration in the nutrient solution affected plant height considerably (Fig. 1). Mean seeding height at 50 μg Ni L^{-1} was 15.5 cm against 24 cm in the control (no Ni) (Fig. 1). In this study, the seedlings grown on 50 μg Ni L^{-1} were most affected, but the ones at 25 μg Ni L^{-1} were also significantly different from the control ($p < 0.05$).

The mean values of Ni in the seedling tissues are given in Table 1. The variation in Ni contents was large: 1.12–17.7 μg kg^{-1} for roots and 0.3–12.7 μg kg^{-1} for leaves. The accumulation of Ni in roots was higher than in leaves.

Table 1
Nickel contents (μg g^{-1} DW) in roots and leaves of wheat (*T. aestivum*) seedlings grown at different Ni concentrations of the nutrient solution. Means in the same row, followed by a different letter are statistically different ($p < 0.05$).

	Ni concentration (μg L^{-1})		
	0	25	50
Roots	1.12 ± 0.001^a	7.8 ± 1.4^b	17.7 ± 2.2^c
Leaves	0.3 ± 0.002^a	2.9 ± 0.1^c	12.7 ± 0.05^b

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