Research Article

Ni-inhibited seedling growth in *Zea mays* (maize) involves alterations in associated biochemical processes

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Abstract: An experiment was carried out to investigate the effect of Nickel (Ni) (5–50 μ M) on seedling growth, dry weight, chlorophyll content and carotenoid content of *Zea mays* L. (maize; Var. Aruna 5) grown in petries after 96 h exposure to Ni. The experiment was further extended to investigate the effect of Ni on water soluble protein content and the activities of protease and oxidoreductase enzymes (POD, PPO) in the roots of maize exposed to Ni. The results indicated that Ni inhibits both root and coleoptile length in test plant followed by reduction in chlorophyll and carotenoid contents. Decrease in the activity of proteases and protein content was also observed in test plant. The reduction was observed more in *Z. mays* roots as compared to coleoptile when treated with Ni. Further, significant reduction in chl b was noted more than chl a in the test plant. However, the increased activities of peroxidase (POD) and polyphenol oxidase (PPO) were observed with increase in Ni concentrations in *Z. mays* roots.

Key words: Nickel; Toxicity; enzymes; *Zea mays*; chlorophyll.

Introduction

Nickel (Ni), although considered to be an essential micronutrient for plants (Eskew *et al.*, 1983), becomes toxic at elevated concentrations in most of the plant species. In soil solution this metal occurs as a free ion (Ni^{2+}) and as complexes with inorganic and organic ligands (Kabata-Pendias and Pendias, 1999). It is accessible to plants in these forms. Ni is discharged into the environment (to soil, water) with waste of municipal and industrial sewage in the form of mobile organic chelates (Kabata-Pendias and Pendias, 1999). Studies conducted on higher plants grown in Ni-contaminated soil show reduction of plant growth and disturbance of metabolic and physiological processes, especially of photosynthesis (Mishra and Kar, 1974; Krupa *et al.*, 1993; Gajewska *et al.*, 2006). The influence of Ni on photosynthesis is multidirectional: Ni damages the photosynthetic apparatus on almost every level of its organization. In the present study the experiments were carried out to test the excess concentrations of Ni have negative effect on seedling growth, photosynthetic pigments and biochemical parameters of *Z. mays* seedlings grown hydroponically.

Material and Methods

Plant material: Seeds of maize (*Zea mays*) were procured locally from seed store. Ni was supplied in the form of NiSO_4 (MW=262.84g; procured from Qualigens Fine Chemicals, Mumbai, India). All other chemicals and reagents were of reagent grade and procured from the best available sources including Sigma-Aldrich (USA), Fluka (Switzerland), Acros (Belgium), Hi-Media (India), and Sisco Research Laboratory (India).

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Experimental Setup

Effect of Ni on early growth of maize was studied under laboratory conditions in a dose response manner. Before use, maize seeds were surface sterilized with 0.1% (w/v) sodium hypochlorite solution, washed thoroughly under running tap water and then rinsed with distilled water. Pre-imbibed seeds (for 8 h at 25°C) of test plant were equidistantly placed in 15 cm Petri dishes lined with absorbent cotton overlined with Whatman No. 1 filter circles soaked with 15 ml of respective Ni solutions (5 to 50 μ M). A parallel setup with distilled water served as control. All the treatments were replicated thrice in a completely randomized design. After 96 h, root length, coleoptile length and seedling dry weight of the plant were determined. The seedling dry weight was measured after oven drying the seedling at 80°C for 24 h. Further roots were stored at –80°C for the biochemical estimations.

Preparation of Enzyme Extract

A water-soluble protein was extracted by homogenizing root tissue (50 mg) in 5 ml of pure water. To determine the activities of proteases, peroxidases and polyphenol oxidases crude enzyme extract was prepared by homogenizing root tissue (100 mg) in a pre-chilled pestle and mortar with 10 ml of 0.1 M PO_4^{3-} buffer (pH 7.0). Homogenates were centrifuged at 15,000 g for 25 min at 4°C rotor temperature in a sigma centrifuge. Supernatant thus obtained was used for the biochemical estimations.

Biochemical Estimations

Estimation of Proteins, Proteases, POD and PPO: Protein content of the homogenate was measured spectrophotometrically at 700 nm using Folin-Ciocalteu reagent against a calibration standard of bovine serum albumin (Lowry *et al.*, 1951). Protease activity was estimated using casein (1% w/v, in 0.1 M PO_4^{3-} buffer pH 7.0) as a substrate (Basha and Beevers, 1975) against the tyrosine ($50 \mu\text{g mg}^{-1}$) used as standard. PODs were assayed using 0.2 M hydrogen peroxide as substrate (Batish *et al.*, 2006). The optical density was measured spectrophotometrically at 430 nm for 1 min. Activity of PPOs was determined using pyrocatechol as a substrate (Van Lelyveld and Pretorius, 1973). The increase in absorbance thus obtained was measured at 495 nm and the specific activity of enzyme was expressed as $\text{kat sec}^{-1} \text{mg}^{-1}$ protein.

Chlorophyll and carotenoid content: The fresh and blotted leaves were used for the determination of total chlorophyll and carotenoids. The total chlorophyll content was extracted from the leaves of the test plants in dimethyl sulfoxide (DMSO) as per Hiscox and Israelstam (1979), and its

concentration was determined spectrophotometrically using the equation of Arnon (1949). The chlorophyll content was expressed in terms of dry weight of the tissue as suggested by Rani and Kohli (1991).

Data Analysis

The data were analysed by one-way ANOVA followed by separation of means at $P < 0.05$ applying post hoc Tukey's test. The statistical analysis was performed using SPSS/PC software ver. 16.0 (SPSS Inc., Chicago, IL).

Results

Effect on seedling growth and dry weight

Exposure to Ni caused a significant reduction in root and coleoptile length of *Z. mays* (Table 1). After 96 h, root length of *Z. mays* seedlings declined by 65% ($P \leq 0.05$) and coleoptile length was decreased by 47% at $50 \mu\text{M}$ Ni, compared to the control (Table I). Similar to seedling growth, significant reduction in seedling dry weight was also observed upon Ni exposure, in *Z. mays*. It was declined by 18–57% at 5–50 μM Ni respectively, over the control (Table 1).

Table 1: Effect of Ni^{2+} on the root length (cm) and coleoptile length (cm) of *Z. mays* seedlings measured after 96 h.

Nickel (μM)	Root length (cm)	Coleoptile length (cm)	Seedling dry weight (g / seedling)
0	$14.5 \pm 0.2a$ (0)	$13.3 \pm 0.1a$ (0)	$1.88 \pm 0.001a$ (0) ^a
5	$12.4 \pm 0.2b$ (−14.7)	$12.4 \pm 0.1b$ (−6.8)	$1.54 \pm 0.005b$ (−18.3)
10	$9.0 \pm 0.1c$ (−37.8)	$10.4 \pm 0.1c$ (−22.0)	$1.39 \pm 0.003c$ (−25.9)
25	$7.3 \pm 0.1d$ (−49.3)	$8.7 \pm 0.1d$ (−34.58)	$1.14 \pm 0.004d$ (−39.6)
50	$5.1 \pm 0.1e$ (−65.1)	$7.0 \pm 0.1e$ (−47.1)	$0.80 \pm 0.002e$ (−57.1)

Data presented as mean $\pm$ SE of five independent replicates. Different alphabets within a column represent significant difference at $P \leq 0.05$ applying *post hoc* Tukey's test

Values within parenthesis represent percent decrease over the control

Table 2: Effect of Ni^{2+} on Chlorophyll (*Chl a*, *Chl b* and *total*) and Carotenoid content ($\mu\text{g mg}^{-1}$) in *Z. mays* seedlings measured after 96 h.

Nickel (μM)	Chlorophyll a ($\mu\text{g mg}^{-1}$)	Chlorophyll b ($\mu\text{g mg}^{-1}$)	Total Chlorophyll content ($\mu\text{g mg}^{-1}$)	Carotenoid content ($\mu\text{g mg}^{-1}$)
0	$5.49 \pm 0.01a$ (0)	$6.01 \pm 0.02a$ (0)	$11.27 \pm 0.1a$ (0)	$2.42 \pm 0.01a$ (0)
5	$4.43 \pm 0.06b$ (−19.2)	$5.76 \pm 0.15a$ (−4.2)	$10.02 \pm 0.1b$ (−11.1)	$2.28 \pm 0.04ab$ (−5.7)
10	$3.98 \pm 0.15c$ (−27.5)	$4.32 \pm 0.19b$ (−28.0)	$8.14 \pm 0.3c$ (−27.8)	$2.05 \pm 0.08b$ (−15.2)
25	$3.72 \pm 0.08cd$ (−32.2)	$3.33 \pm 0.09c$ (−44.5)	$6.89 \pm 0.1d$ (−38.9)	$1.79 \pm 0.04c$ (−26.1)
50	$3.49 \pm 0.03d$ (−36.4)	$3.15 \pm 0.04c$ (−47.5)	$6.49 \pm 0.1d$ (−42.4)	$1.45 \pm 0.02d$ (−39.8)

Data presented as mean $\pm$ SE of five independent replicates. Different alphabets within a column represent significant difference at $P \leq 0.05$ applying *post hoc* Tukey's test

Values within parenthesis represent percent decrease over the control

Table 3: Effect of Ni^{2+} on the protein content ($\mu\text{g mg}^{-1}$ f. wt.) and specific activity of proteases ($\mu\text{g h}^{-1} \text{mg}^{-1}$ protein) and oxidoreductase enzymes, Peroxidases, polyphenoloxidases ($\text{kat s}^{-1} \text{mg}^{-1}$ protein) in the roots of *Z. mays* measured after 96 h.

Nickel (μM)	Proteins ($\mu\text{g mg}^{-1}$ f. wt.)	Proteases ($\mu\text{g h}^{-1} \text{mg}^{-1}$ protein)	POD ($\text{kat s}^{-1} \text{mg}^{-1}$ protein)	PPO ($\text{kat s}^{-1} \text{mg}^{-1}$ protein)
0	$38.4 \pm 0.1a$ (0)	$509.8 \pm 3.6a$ (0)	$0.05 \pm 0.01a$ (0)	$0.35 \pm 0.01a$ (0)
5	$31.9 \pm 0.02b$ (−16.8)	$458.7 \pm 2.7b$ (−10.0)	$0.06 \pm 0.03b$ (+29.5)	$0.43 \pm 0.02b$ (+23.1)
10	$30.5 \pm 0.05c$ (−20.6)	$418.2 \pm 2.3c$ (−17.9)	$0.08 \pm 0.01c$ (+67.0)	$0.55 \pm 0.02c$ (+56.2)
25	$25.8 \pm 0.07d$ (−32.7)	$356.9 \pm 3.8d$ (−30.0)	$0.09 \pm 0.02d$ (+90.1)	$0.67 \pm 0.01d$ (+90.9)
50	$21.9 \pm 0.07e$ (−42.8)	$284.9 \pm 5.3e$ (−44.1)	$0.12 \pm 0.02e$ (+136.6)	$0.88 \pm 0.01e$ (+148.8)

Data presented as mean $\pm$ SE of five independent replicates. Different alphabets within a column represent significant difference at $P \leq 0.05$ applying *post hoc* Tukey's test

Values within parenthesis represent percent decrease (−) and increase (+) over control

Effect on chlorophyll and carotenoid content

Chlorophyll content was measured as Chl a, b and total. Significant reduction in chlorophyll content was observed when *Z. mays* seedlings were subjected to various concentrations of Ni. In *Z. mays* declined in chl a noticed by ~ 16–36% at 5–50 μM Ni, respectively, compared to control (Table 2). Likewise, reduction in chl b by 47% and total chl content by 42% at highest concentration (50 μM) of Ni was also reported in test plant (Table 2). Parallel to chlorophyll content, reduction in carotenoid content was also observed in the test plant upon exposure to Ni. In *Z. mays* carotenoid content, declined by 6–38% when subjected with 5–50 μM Ni exposure, respectively, compared to control (Table 2).

Effect on water soluble proteins and proteases

Results obtained from present experiment disclose that upon Ni exposure water soluble protein decreased in the roots of *Z. mays* in a dose response manner. The Protein content in *Z. mays* declined by 17%, 21%, 33% and 43% at 5 μM , 10 μM , 25 μM and 50 μM Ni, respectively, when compared to control. Like proteins, significant reduction in protease activity was also reported in *Z. mays* roots when exposed to Ni. It was reduced by 10–44% at 5–50 μM Ni, when compared to control (Table 3).

Effect on PODs and PPOs

In contrary to above results the activities of PODs and PPOs increased in roots of test plant exposed to different concentrations of Ni when compared to its control value. Peroxidase in *Z. mays* increased by 29% at lowest concentration (5 μM) and it was further increased up to 136% at highest concentration (50 μM). Improved activity of PPOs has also been observed in the roots of *Z. mays* after exposure to Ni. In *Z. mays* PPOs activity increased within the range of 23–148% at 5–50 μM Ni, compared to control (Table 3).

Discussion

Results obtained from present work exposed that Ni toxicity inhibits root and coleoptile length in *Z. mays* followed by reduction in seedling dry wt. in a concentration dependent manner. The inhibitory effect of Ni was greater on root compared to coleoptile in the test plant. These results are in the agreement with past studies. For example, reduction in plumule length, radicle length and dry weight yields under Ni toxicity have observed in radish (Espin *et al.*, 1997), mung (Jagetiya and Aery, 1998), tomato (Kowalczyk *et al.*, 2003) and sunflower (Singh *et al.*, 2004; Ahmad *et al.*, 2009). Substantial reduction of root length and shoot length was also observed in maize (Baccauch *et al.*, 1998, 2001) wheat, (Gajewaska *et al.*, 2006) rice

(Maheshwari and Dubey, 2008) and barley, (Kumar *et al.*, 2012) when exposed to high concentrations of Ni. Similarly, reduction in root length was also reported in garlic (Liu *et al.*, 2009), and pea (Malecka *et al.*, 2008) when exposed to Pb. The main mechanisms behind reduction in root growth are inhibition of cell division and restriction of cell elongation which is in agreement of the findings of Demchenko *et al.*, (2005). It is also assumed that reduction in seedling growth may be associated with the cell wall strengthening by lignification process which was reported in many plants under stressed conditions (Diaz *et al.*, 2001).

Likewise, we observed reduction in total chlorophyll and carotenoid content in the test plant. Similar results has been reported in black gram (Dubey and Pandey, 2011) and radish (Awasthi and Sinha, 2013) when exposed to phytotoxic concentrations of Ni. Gajewska *et al.*, (2006) also reported reduction in chl a, b and total in wheat when treated with 200 μM Ni. Significant decrease in chlorophyll a, b and carotenoid content was also reported in radish when plant was exposed to NiSO_4 (Latif, 2010). In agreement to present results reduction in photosynthetic pigments were also reported in *Ocimum tenuiflorum* (Rai *et al.*, 2004) in Cr stress and *Withania somnifera* (Khatun *et al.*, 2008) in Cu stress. This indicates that in presence of excess metal concentration it disturbs photosynthetic apparatus which further inhibits biosynthesis in higher plants (Prasad and Prasad, 1987).

Decline in protein content obtained in present experiment is in agreement with the results obtained for *Beta vulgaris* in presence of Ni and Cd (Kevresan *et al.*, 1998) and in barley when the plant was exposed to Cu (Demirevska-Kepova *et al.*, 2003). Latif (2010) has also reported sharp reduction in total protein content in radish when treated with NiSO_4 . Parallel to results obtained in present work, reduced protease activity was also observed in rice seedlings (Maheshwari and Dubey, 2008) upon exposure to 400 μM Ni treatment. Shah and Dubey (1997) also reported an inhibition of protease activity in rice seedlings subjected with Pb. Likewise, a decline in protease activity in presence of Cd was also reported in soybean root nodules (Balestrasee *et al.*, 2003). Hence, it is speculated that reduction in protease activity in presence of metal stress declines the proteins synthesis which further declines the protein content in plants.

In contrast to above results, significant induction of oxidoreductase (POD and PPO) enzymes were observed in a dose dependent manner. POD and PPO increased with increase in Ni concentrations.

In support to our findings, significant rise in POD and PPO was also reported in *Raphanus sativus* in response to NiSO_4 (Latif, 2010). Activity of POD has shown a significant increase in response to Ni in *Triticum aestivum* (Gajewska *et al.*, 2006), *Capsicum annum* (Diaz *et al.*, 2001), *Hordeum vulgare* (Simonovicova *et al.*, 2004) and *Brassica juncea* (Alam *et al.*, 2007) which are in agreement with present findings. Batish *et al.* (2008) reported that POD activity increases in response to abiotic stresses. Hence, it is suggested that increased amount of POD and PPO protects plant cells from reactive oxygen species as well as provide inducible plant defense (Batish *et al.*, 2008).

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