IMPROVING EFFECT OF EXOGENOUS NICKEL NITRATE APPLICATION ON PHYSIO-BIOCHEMICAL FEATURES, NITROGEN METABOLISM AND EARLY GROWTH OF RICE

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Abstract. High Ni^{2+} levels are toxic to plant growth; however, being a micro-nutrient, its application and/or presence in low concentration may improve plant growth. Present study investigated the effects of exogenous Ni application on morpho-physiological attributes of two rice cultivars in China i.e., *Yuxiangyouzhan* and *Meixiangzhan 2*. Nickel was applied exogenously to 11 days old seedlings as $Ni(NO_3)_2$ in a solution form in the following concentrations: 0.1 mM (Ni1), 0.2 mM (Ni2) and 0.5 mM (Ni3). The seedlings without Ni^{2+} application were taken as control. Results showed that exogenous Ni^{2+} application improved the early growth of seedlings in terms of seedling length, basal diameter, biomass accumulation and seedling index. Substantial improvements were also observed regarding chl a, chl b, total chl contents and carotenoids in plants under Ni^{2+} application compared to CK. Furthermore, all Ni^{2+} treatments decreased the content of Malondialdehyde (MDA). Among all applied concentrations, Ni2 proved better regarding its promotive effects, however further research is needed to explore the molecular basis of Ni-induced modulations in seedlings of both rice cultivars.
Keywords: *chlorophyll; nickel nitrate; rice; seedling; enzymes in nitrogen metabolism; anti-oxidant enzyme*

Introduction

Rapid industrial development, urbanization and other anthropogenic activities are building pools of heavy metals in soils (Nagajyoti et al., 2010). These heavy metals, at an optimum concentration, act as essential micronutrients for the plants and play a beneficial role for plant growth, development and overall productivity.

Similarly, nickel is also recognized as the double-edged element for plants i.e., toxic at high concentrations whilst promotive at low/trace concentrations. However, long term nickel stress could induce significant inhibitory effects on shoots and roots growth, plant height, number of tillers, 1000 grain weight and paddy yield (Nazir and Asghar et al., 2015). Moreover, nickel (Ni^{2+}) toxicity caused decline in the activities of antioxidants and induced the accumulation of free proline in both leaves and roots in pea plants (Gajewska and Skłodowska, 2005). Exposure of detached leaves of rice seedlings to $NiSO_4$ (1 mM) caused remarkable decline in activity of superoxide

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dismutase (SOD) and enhanced malondialdehyde (MDA) contents, thus caused leaf senescence (Shi and Zhoum 1998). Rice seedlings exposed to high Ni^{2+} concentrations caused substantial reduction in root and shoot growth along with substantial decline in fresh and dry biomass of rice plants (Rizwan and Imtiaz et al., 2017). A decline in the activities of anti-oxidants e.g., superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were noted with the increased Ni^{2+} concentrations in paddy leaves.

On the other hand, nickel is an essential element for high plants with a normal range of 0.01-5.00 mg kg^{-1} dry weight (DW) (Welch, 1981); however, such reports are very few. For instance, application of $NiCl_2$ (1 - 5 μ mol L^{-1}) can effectively promote vegetative growth of rice seedlings (Wang et al., 1999). It also augmented the chlorophyll content, soluble protein, soluble sugars and peroxidase (POD) activity (Wang and Tian et al., 1999). Its involvement as a non-substitution component of urease seems to be the only proof of its beneficial effect in high plants (Gerendás and Polacco et al., 2015). Fishbein et al. (1997) indicated that Ni^{2+} is a necessary component of plant and bacterial urease which has an important role in catalysing the hydrolysis of urea into ammonia and carbon dioxide in plants. Polacco (1997) reported that soybean cells had an absolute requirement for Ni^{2+} while grown with urea as a sole N source in the presence of citrate. Gerendás et al. (2015) also proved that the nickel deficient paddies showed substantial reduction in growth and urea accumulation owing to the lack of urease activity. Ni-induced improvements in seed germination and early growth in rice mimics its stimulatory effects (Das and Kar et al., 1978; Mishra and Kar, 1974). Furthermore, low concentration of Ni^{2+} can promote the activities of peroxidase and ascorbic acid oxidase in alfalfa leaves and can enhance the disease resistance in crops (Theisen and Blincoe, 1984), nonetheless, the promotive effects of Ni^{2+} in high plants still needs further investigation. Present study investigated the effects of exogenous application of Ni^{2+} at lower concentrations on the physio-biochemical attributes and early growth of rice seedlings with the hypothesis that Ni^{2+} being a trace element could improve the early growth of rice if applied at low concentration.

Materials and methods

Experimental details

Pot experiment, between September to October in 2017, was conducted at Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou, (23°09′N, 113°22′E and 11 m from mean sea level) China**.**

The experimental soil in Guangzhou was sandy loam with 25.65% of organic matter content, 1.360% total N, 0.956% total P, and 17.460% total K. Before sowing, seeds of two popular rice cultivars i.e., *Meixiangzhan 2* and *Yuxiangyouzhan* were soaked in water for 24 h at room temperature and germinated under moisture conditions. Geminated seeds were sown in plastic pots (31 cm in diameter and 29 cm in height) and allowed to grow for 10 days then Ni^{2+} in the form of Ni $(NO_3)_2$ was foliar sprayed with three different concentrations i.e., 0.1, 0.2, and 0.5 mM and regarded as Ni1, Ni2 and Ni3, respectively. Pots without Ni^{2+} application were regarded as control (CK) The measurements were repeated in triplicate and averaged.

Thirty days old seedlings collected from each treatment, except those which were used for the determination of seedling growth, were harvested, washed and immediately stored at –80°C till biochemical analyses (*Fig. 1*).

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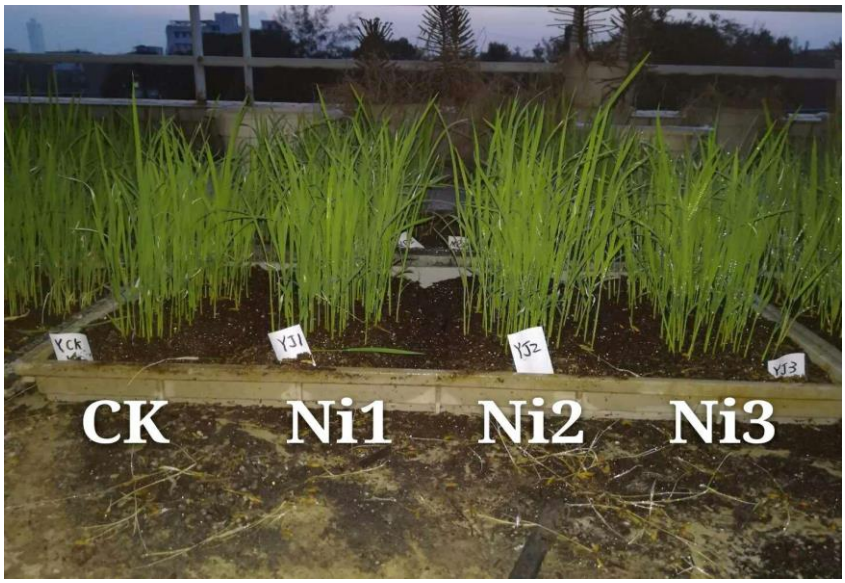

Figure 1. *Experimental site*

Determination of seedling growth

30 days old 10 seedlings from each treatment were randomly harvested for measuring plant height, basal diameter, dry biomass and seedling index. The seedling index was calculated as:

$$\text{Seedling index} = \text{basal diameter} / \text{plant height} \times \text{dry weight}$$

Determination of soluble protein and malondialdehyde (MDA)

Protein contents were estimated according to Bradford (1976) by using G-250 and expressed as ug g^{-1} FW. Malondialdehyde (MDA) content was measured according to the method of Luo and Zhong et al. (2017) with thiobarbituric acid. MDA reacted with thiobarbituric acid (TBA) and the absorbance was read at 532 nm, 600 nm, and 450 nm. The content of MDA was calculated as: MDA content (μmol/L) = 6.45(OD 532 − OD 600) − 0.56OD 450 and final result was expressed as μmol/g FW.

Determination of chlorophyll contents

The fresh leaf samples (0.5 g) were extracted with 95% alcohol and the absorbance was read at 665 nm, 649 nm, 652 nm and 470nm and the chlorophyll contents were estimated according to Arnon (1949).

Determination of anti-oxidants activities

The peroxidase (POD, EC1.11.1.7) activity was measured with the methods of Pan et al. (2013). The reaction solution included enzyme extract (50 μl) containing 1 ml of 0.3% H_2O_2, 0.95 ml of 0.2% guaiacol, and 1 ml of 50 mM l^{-1} sodium phosphate buffer (pH 7.0). The absorbance was read at 470 nm. One POD unit of enzyme activity was defined as the absorbance increase because of guaiacol oxidation by 0.01 (U/g FW). The superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by using nitro

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blue tetrazolium (NBT) according to Pan et al. (2013). enzyme extract (0.05 ml) was added into the reaction mixture containing 1.75 ml of sodium phosphate buffer (pH 7.8), 0.3 ml of 130 mM l $^{-1}$ methionine buffer, 0.3 ml of 750 μmol l $^{-1}$ NBT buffer, 0.3 ml of 100 μmol l $^{-1}$ EDTA-Na 2 buffer and 0.3 ml of 20 μmol l^{-1} lactoflavin. After the reaction, the change in color was measured at 560 nm. One unit of SOD activity is equal to the volume of extract needed to cause 50% inhibition of the color reaction. Catalase (CAT, EC 1.11.1.6) activity was estimated with the methods devised by Aebi (1984). An aliquot of enzyme extract (50 μl) was added to the reaction solution containing 1 ml of 0.3% H_2O_2 and 1.95 ml of sodium phosphate buffer. The absorbance was recorded at 240 nm. One CAT unit of enzyme activity was defined as the absorbance decrease by 0.01 (U/g FW).

Determination of nitrogen metabolism enzymes activities

The activity of nitrate reductase (NR) was measured by using the methods of Sun et al. (2009). One unit of NR activity is defined as $NaNO_2$ μg formed per gram of fresh samples per hour. The activities of glutamic oxaloacetic transaminase (GOT) were measured by using the methods devised by Ebeid et al. (1981). The aminotransferase activity was expressed by pyruvate formed in 30 min. The activities of glutamine synthetase (GS) and glutamine oxoglutarate aminotransferase (GOGAT) were measured according to Lin and Kao (1996). One unit of GS activity is defined as 1 pmol L-glutamate γ-monohydroxamate formed per min. The reaction mixture (1 ml, pH 8.0) contained 80 pmol Tris-HCl buffer, 40 pmol L-glutamic acid, 8 pmol ATF', 24 pmol $MgSO_4$, and 16 pmol NH_2OH. The final pH was 8.0. The reaction was started by addition of the enzyme extract and stopped by adding 2 mL of 2.5% $FeCl_2$ and 5% trichloroacetic acid in 1.5 M HCl after 30 min incubation at 30 °C. One unit of GOGAT activity is defined as a decrease of 1 OD_{340} per min. The reaction was started by adding L-glutamine immediately following the enzyme preparation. The decline in absorbance was recorded at 340 nm.

Statistical analyses

Data were analyzed using statistical software 'Statistix 8.1'(Analytical Software, Tallahassee, FL, USA) while differences amongst means were separated by using least significant difference (LSD) test at 5% probability level. 'Origin 8.1' (OriginLab Co., Northampton, MA, USA) was used for graphical representation.

Results

Seedling growth

No significant difference was noted in seedling length for *Meixiangzhan 2* and basal diameter for both cultivars among all Ni^{2+} treatments and CK, however seedling length was substantially increased under all Ni^{2+} treatments than CK for *Yuxiangyouzhan 2*. The seedling fresh and dry weight were increased by 19.58-32.23% and 23.08-26.93% (for *Yuxiangyouzhan*) as well as 24.65-32.93% and 29.16-46.88% (for *Meixiangzhan 2*), respectively. Furthermore, the highest seedling index i.e., 1.14 ± 0.03 and 0.78 ± 0.05 was recorded in Ni2 for both *Yuxiangyouzhan* and *Meixiangzhan 2* (*Table 1*).

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Table 1. *Effect of Ni^{2+} application on rice seedling quality*

Cultivars	Treatment	Seedling length (cm)	Basal diameter (mm)	Fresh weight (mg)	Dry weight (mg)	Seedling index
Yuxiangyouzhan	CK	15.50±0.13b	1.32±0.01a	55.33±2.64b	10.83±1.09b	0.92±0.09b
	Ni1	17.17±0.67a	1.35±0.03a	67.83±5.13a	13.33±0.45a	1.05±0.04ab
	Ni2	16.10±0.49ab	1.34±0.04a	66.17±4.28ab	13.67±0.39a	1.14±0.03a
	Ni3	16.97±0.65ab	1.32±0.02a	73.17±3.27a	13.75±0.44a	1.07±0.03ab
Meixiangzhan 2	CK	15.40±0.92a	1.095±0.02a	47.60±2.88b	8.00±0.29b	0.57±0.02b
	Ni1	17.28±1.21a	1.10±0.07a	61.40±3.55a	11.75±0.14a	0.75±0.01a
	Ni2	15.85±0.76a	1.14±0.03a	62.80±1.76a	10.83±0.67a	0.78±0.05a
	Ni3	16.27±0.95a	1.10±0.07a	59.33±5.25a	10.33±0.93a	0.70±0.06ab

Data in the table are tested with Statitix 8 by LSD ($P < 0.05$). Values with different small letters in the same line have significant difference

Chlorophyll contents

Exogenous Ni^{2+} application affected the chlorophyll contents significantly. Compared with CK, the contents of chl a were increased by 6.14-20.71% for *Yuxiangyouzhan* and 1.23-8.12% for *Meixiangzhan 2*. The contents of chl b were improved by 10.46-13.5% in Ni2 and Ni3, respectively for *Yuxiangyouzhan* whilst increased by 3.22, 6.66 and 3.79% in Ni1, Ni2 and Ni3 for *Meixiangzhan 2*. Likewise, the total chl contents were increased by 3.97%, 16.33% and 16.96% for *Yuxiangyouzhan* and 2.61%, 7.55% and 2.19% for *Meixiangzhan 2* under Ni1, Ni2 and Ni3, respectively as compared with CK. Furthermore, different nickel applications improved carotenoid contents significantly for both cultivars. For instance, compared with CK, the Ni1, Ni2 and Ni3 resulted in 8.68, 26.40 and 21.88% higher carotenoid contents than CK for *Yuxiangyouzhan* and 3.66, 10.44 and 1.46 higher carotenoid contents for *Meixiangzhan 2*, respectively (*Fig. 2*).

Nitrogen metabolism enzymes

Overall, all Ni^{2+} treatments regulated the activities of key enzymes involved in nitrogen metabolism (*Table 2*). The NR activities were increased by 12.27, 18.13 and 10.56% for *Yuxiangyouzhan* and 8.24, 20.03 and 25.56% higher for *Meixiangzhan 2* in Ni1, Ni2 and Ni3, respectively. The activities of GS in all Ni treatments were found statistically similar ($P>0.05$) for *Yuxiangyouzhan* whereas the highest GS activity was recorded in Ni1 while lowest in Ni3.

Meanwhile, no significant difference was recorded among Ni1, Ni2 and CK for GOGAT activities while lowest GOGAT activity was recorded in Ni3 for *Yuxiangyouzhan*. However, for *Meixiangzhan 2*, 15.12% and 15.15% lower activity of GOGAT was recorded in Ni2 and Ni3 whilst Ni1 remained similar with CK. Compared with control, activities of GOT in Ni1, Ni2 and Ni3 were 1.09, 1.22 and 1.24 fold higher for *Yuxiangyouzhan* while 1.21, 1.16 and 1.02 fold higher for *Meixiangzhan 2*. Moreover, the activities of GPT were improved by 28.74, 51.95 and 29.47% in Ni1, Ni2 and Ni3 for *Yuxiangyouzhan* whilst 49.38, 24.99 and 16.82% for *Meixiangzhan 2.*

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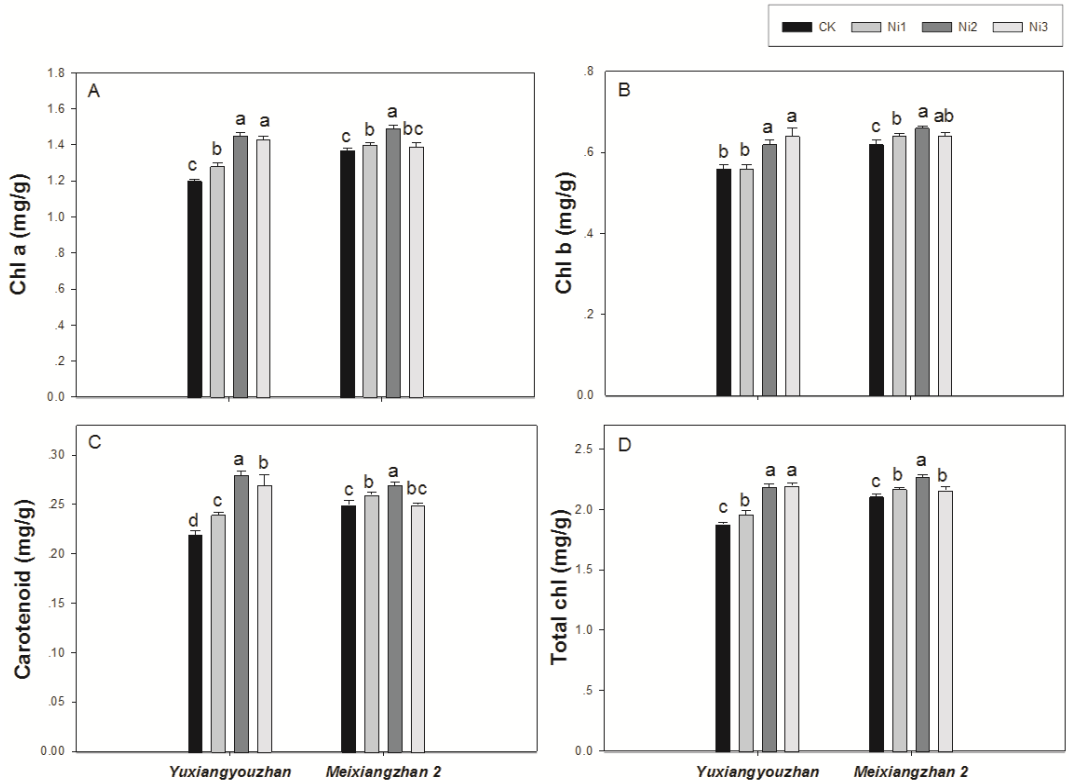

Figure 2. *Effect of Ni^{2+} application on chlorophyll content*

Table 2. *Effect of Ni^{2+} application on nitrogen metabolism*

Cultivar	Treatment	NR activity ($ug \cdot g^{-1}$)	GS activity ($umol \cdot g^{-1} \cdot min^{-1}$)	GOGAT activity ($umol \cdot g^{-1} \cdot min^{-1}$)	GOT activity ($umol \cdot g^{-1} \cdot h^{-1}$)	GPT activity ($umol \cdot g^{-1} \cdot h^{-1}$)
Yuxiangyouzhan	CK	56.74±7.1b	0.36±0.00a	0.30±0.01a	30.59±0.55c	37.36±1.37c
	Ni1	63.71±2.11a	0.34±0.02a	0.34±0.01a	33.58±0.28b	48.10±1.15b
	Ni2	67.03±1.25a	0.37±0.04a	0.32±0.01a	37.18±0.42a	56.77±0.54a
	Ni3	62.74±1.13a	0.37±0.02a	0.24±0.03b	38.00±0.64a	48.37±0.64b
Meixiangzhan 2	CK	58.93±1.23d	0.41±0.02b	0.33±0.01a	34.89±0.59b	42.73±3.00c
	Ni1	63.79±0.68c	0.53±0.06a	0.32±0.01ab	42.36±1.78a	63.83±1.20a
	Ni2	70.74±0.82b	0.38±0.02b	0.28±0.01ab	40.57±0.24a	53.41±0.37b
	Ni3	74.00±1.02a	0.29±0.01c	0.28±0.02b	35.61±0.10b	49.92±0.77

Data in the table are tested with Statitix 8 by LSD ($P < 0.05$). Values with different small letters in the same line have significant difference

MDA contents, osmo-protectants and anti-oxidant responses

Exogenous Ni^{2+} application regulated the anti-oxidative enzymatic activities in terms of SOD, POD and CAT (*Table 3*). Compared with CK, 8.44 and 8.03% higher POD activities were recorded in Ni1 and Ni3 for *Yuxiangyouzhan* while highest POD activity (21.51% higher than CK) was found for *Meixiangzhan 2* in Ni1. The SOD activities were also increasaed by 8.68, 9.51 and 27.73% for *Yuxiangyouzhan* whilst 47.58, 29.66 and 27.73% for *Meixianghzhan 2* in Ni1, Ni2 and Ni3 treatments, respectively. Similarly, compared with CK, the CAT activities increased with 13.48, 30.65 and 11.52% in Ni1, Ni2 and Ni3, respectively for *Yuxiangyouzhan* while increased by 16.89 and 19.37% in Ni1 and Ni3, respectively for *Meixiangzhan 2*.

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The MDA contents were found significantly lower under all Ni^{2+} treatments than CK for both cultivars. The MDA contents were reduced by 21.96, 26.02, and 35.81% for *Yuxiangyouzhan* and 31.43, 21.54, and 13.34% for *Meixiangzhan 2* in Ni1, Ni2, and Ni3 compared to CK. Furthermore, the protein contents were increased by 20.43 and 13.97% in Ni2 and Ni3, respectively for *Yuxiangyouzhan* while it was 31.74% higher in Ni1 for *Meixiangzhan 2* compared with CK.

Table 3. *Effect of Ni^{2+} application on MDA contents, osmo-protectants and anti-oxidant responses*

Cultivar	Treatment	Peroxidase activity ($U \cdot g^{-1} \cdot min^{-1}$)	Super-oxide dismutase activity (U·g-)	Catalase activity ($U \cdot g^{-1} \cdot min^{-1}$)	Malondialdehyde content ($umol \cdot g^{-1}$)	Soluble protein content ($ug \cdot g^{-1}$)
Yuxiangyouzhan	CK	315.94±10.01c	127.61±0.54b	140.42±2.10c	2.52±0.04a	27.14±0.70c
	Ni1	342.60±3.25b	138.69±4.14b	159.35±0.66b	1.97±0.04b	27.32±0.18c
	Ni2	321.634.16c	139.74±3.70b	183.46±1.33a	1.87±0.06b	32.68±0.59a
	Ni3	360.90±0.48a	163.00±0.36a	156.60±1.73b	1.619±0.05c	30.93±0.92b
Meixiangzhan 2	CK	345.07±2.38b	106.49±1.21c	117.45±3.85b	2.35±0.05a	25.66±0.85b
	Ni1	419.28±3.66a	157.16±5.58a	137.29±4.07a	1.61±0.00d	33.81±1.63a
	Ni2	342.77±0.76b	138.08±2.46b	114.13±1.32b	1.84±0.05c	25.87±0.29b
	Ni3	341.32±1.22b	137.78±8.59b	140.20±1.73a	2.04±0.05b	23.90±0.26b

Data in the table are tested with Statitix 8 by LSD ($P < 0.05$). Values with different small letters in the same line have significant difference

Correlation analysis

Significant and positive correlation between seedling index and seedling fresh weight was observed. The same correlation was found among dry weight and activities of both SOD and CAT. Moreover, there also existed a similar correlation between total chlorophyll content and GOT activity (*Table 4*).

Discussion

Recently, the research on plant micro-nutrition has been given a renowned attention due to its significant effects on plant growth, development and overall crop productivity and grain nutritional qualities as well (Taylor and Harrier, 2001). Previously, Ni^{2+} has been recognized as an essential micronutrient for legumes and possibly all high plants and is a necessary constituent of the enzyme 'urease' (Eskew and Welch et al., 1983). In this study, the seedling growth attributes i.e., seedling length, basal diameter, fresh and dry biomass as well asseedling index were substaintially improved by exogenous Ni^{2+} application. For example, remarkable improvement was found in height, fresh weight and dry weight under Ni1 treatment for both cultivars while highest value of seedling index was recorded in Ni2 for *Yuxiangyouzhan* and *Meixiangzhan 2* which are 1.74 and 0.78. The values of the seedling index were calculated considering height, dry weight and basal diameter which all are the result of growth and development. Thus, seedling index represents the growth status of seedlings to a certain extent. In this study, Ni^{2+} treatments enhanced the seedling index and it might have indicated that Ni^{2+} application at low concentration is able to improve or stimulate the growth and development of rice seedlings.

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Table 4. *Relationship among the growth and biochemical parameters*

Index	Seedling index	Height	Basal diameter	Fresh weight	Dry weight	Total chl	Chl a	Chl b	POD	SOD	CAT	NR	GS	GOGAT	GOT	GPT
Height	0.362															
Basal diameter	0.933**	0.163														
Fresh weight	0.782*	0.712*	0.569													
Dry weight	0.918**	0.67	0.741*	0.932**												
Total chl	-0.171	0.106	-0.456	0.261	0.042											
Chl a	-0.077	0.11	-0.364	0.31	0.115	0.990**										
Chl b	-0.357	0.069	-0.62	0.13	-0.119	0.966**	0.921**									
POD	-0.46	0.583	-0.643	-0.023	-0.101	0.319	0.246	0.43								
SOD	0.489	0.805*	0.228	0.842**	0.761*	0.353	0.342	0.329	0.407							
CAT	0.862**	0.403	0.756*	0.63	0.822*	-0.133	-0.043	-0.303	-0.274	0.414						
NR	-0.08	0.168	-0.336	0.311	0.115	0.631	0.615	0.637	0.198	0.287	0.026					
GS	-0.254	0.278	-0.353	-0.139	-0.07	0.208	0.187	0.22	0.688	0.221	-0.228	-0.341				
GOGAT	-0.116	-0.042	-0.029	-0.414	-0.179	-0.427	-0.369	-0.496	0.113	-0.499	0.07	-0.28	0.279			
GOT	-0.153	0.43	-0.464	0.323	0.167	0.839**	0.818*	0.824*	0.672	0.57	-0.153	0.452	0.611	-0.215		
GPT	0.058	0.58	-0.283	0.405	0.375	0.648	0.657	0.591	0.679	0.601	0.201	0.519	0.539	0.078	0.0887**	
Protein	0.497	0.533	0.302	0.475	0.632	0.208	0.252	0.094	0.331	0.627	0.554	-0.208	0.648	0.068	0.494	0.628

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Previously, it was observed that exposure of plants to 10 μM Ni^{2+} lead to a slight increase in fresh mass while 200 μM Ni^{2+} inhibited shoot growth. It also caused a decline in chlorophyll content, an accumulation of proline hence visible symptoms of Ni toxicity. Nickel deficiency disrupted the ureide catabolism in foliage. It also induced accumulation of xanthine, allantoic acid, ureidoglycolate and citrulline while total ureides, urea concentration and urease activity were reduced (Bai and Reilly et al., 2006). Furthermore, an earlier study found that a Ni-binding protein is necessary for urease activity which is encoded by soybean Eu3 gene (Freyermuth and Bacanamwo et al., 2000).

Exogenous Ni^{2+} application also enhanced the chl a, chl b, total chl contents and carotenoids. The results of the present study showed that Ni^{2+} treatments increased the content of chl especially Ni3 and Ni4. Highest total chl content was recorded in Ni2 for *Yuxiangyouzhan* and in Ni3 for *Meixiangzhan 2* while Ni3 had highest content of Chl a for both cultivars. Chl a can have an important role in converting light energy into electrical and chemical energy (Asadov et al., 1995). The increment of photosynthetic pigment implied that nickel treatments could enhance the seedling's photosynthesis thus promoting the carbon metabolism and dry matter accumulation rate.

Furthermore, higher activities of NR, GOT and GPT were noted in Ni^{2+} treatments. Nitrate Reductase (NR), glutamic-oxalacetic transaminase (GOT) and glutamate pyruvic (GPT) are key enzymes for nitrogen metabolism in plants. NR, through its catalytic reaction, not only regulates nitrate reduction but also influences the carbon metabolism of photosynthesis (Jamal and Fazli et al., 2006). Deckard et al. grew six corn (*Zea mays* L.) hybrids in field conditions with supplemental N and irrigation. This experiment proved that there exists a positive relation between nitrate reductase activity and grain protein and yield. The increment of enzymatic activities for nitrogen metabolism mean that nickel treatments could enhance nitrogen metabolism inside the seedling. It will, further, promote the uptake of soil nutrients and increase the nitrogen utilization.

In addition, Ni treatments modulated anti-oxidant responses i.e., POD, SOD and CAT. These results corroborated with Silva et al. (2012) who found that Ni played an important role in plant growth and N uptake in crops supplied with urea in calcareous soils. Ni^{2+} deficiency affects plant growth, plant senescence, nitrogen metabolism, iron uptake and disease resistance. Furthermore, Ni^{2+} is absorbed and redistributed in plants via cation and/or metal-ligand complex transport systems. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) collectively build an enzymatic defense system. They synergistically, defend cell membrane system from getting damaged by superoxide free radical. They also inhibit membrane lipid peroxidation and decrease malondialdehyde (MDA) content, in order to reduce damage to the plant cells (Jebara and Jebara et al., 2005). MDA is one of the most important products of membrane lipid peroxidation which can react with free amino acids, phospholipids and may produce ethylene in cellular membranes (Rakwal and Agrawal et al., 2003). So, in the study of plant resistance, physical and physiological aging is a commonly used indicator. MDA content can be a measure of the membrane lipid peroxidation. Thus, it can be used in indirect determination of membrane system damage and plants resistance. Moreover, soluble protein also has a role in osmo-regulation and maintaining cellular structures and functions while anti-oxidants focus on quenching reactive oxygen species (ROS). For instance, SOD is involved in catalyzing the dismutation of superoxide radical whereas POD and CAT are involved in scavenging H_2O_2 (Sairam and Srivastava,

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2000). So, antioxidant enzyme activity can reflect the resistance of plants to some extent. The improvements in antioxidant enzyme activity and protein content implied that exogenous application of nickel nitrate could have a 'phytosanitary' effect on rice seedlings thus enhancing their resistance.

Conclusion

The exogenous Ni^{2+} application improved seedling growth and development. It also enhanced the activities of key enzymes involved in nitrogen metabolism and anti-oxidant defence enzymes. Among Ni^{2+} treatments, Ni2 proved better than other Ni^{2+} doses for both *Yuxiangyouzhan* and *Meixiangzhan 2*. Even though, application of Ni^{2+} at low concentration could improve the early growth of rice, further investigations are required at physiological and molecular level in order to reveal the exact mechanism of Ni-induced promotive effects in plants. In addition, there are small amount of nitrogen inside nickel nitrate which might affect the result of this study, more research should be carried out with different nickel resources.

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REFERENCES

[1] Aebi, H. (1984): Catalase in vitro. – Methods Enzymol 105: 121-126.
[2] Asadov, A. A., Kotlyarova, N. V., Zulfugarov, I. S. Aliyev, D. A. (1995): Spectral characteristics and orientation of native forms of pigments in the chloroplasts of barley seedlings on continuous and discontinuous illumination. – Biofizika 40(2): 245-251.
[3] Bai, C., Reilly, C. C., Wood, B. W. (2006): Nickel deficiency disrupts metabolism of ureides, amino acids, and organic acids of young pecan foliage. – Plant Physiology 140: 433-43.
[4] Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Analytical Biochemistry 72: 248-254.
[5] Das, P. K., Kar, M., Mishra, D. (1978): Nickel nutrition of plants: I. Effect of nickel on some oxidase activities during rice (Oryza sativa L.) seed germination. – Zeitschrift für Pflanzenphysiologie 90: 225-233.
[6] Ebeid, M., Eder, J., Kutáček, M., Piovarči, A. (1981): Transaminase GOT and GPT activity in extirped sprouts of normal and opaque-2 Maize (Zea mays L.) seedlings. – Biologia Plantarum 23: 345-350.
[7] Eskew, D. L., Welch, R. M., Cary, E. E. (1983): Nickel: an essential micronutrient for legumes and possibly all higher plants. – Science 222: 621-3.
[8] Freyermuth, S. K., Bacanamwo, M., Polacco, J. C. (2000): The soybean Eu3 gene encodes an Ni-binding protein necessary for urease activity. – Plant Journal for Cell Molecular Biology 21: 53.
[9] Gajewska, E., Skłodowska, M. (2005): Antioxidative responses and proline level in leaves and roots of pea plants subjected to nickel stress. – Acta Physiologiae Plantarum 27: 329-340.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1701_357367

[10] Gerendás, J., Zhu, Z., Sattelmacher, B. (1998): Influence of N and Ni supply on nitrogen metabolism and urease activity in rice (Oryza sativa L.). – Journal of Experimental Botany 49: 1545-1554.
[11] Gerendás, J., Polacco, J. C., Freyermuth, S. K., Sattelmacher, B. (2015): Significance of nickel for plant growth and metabolism. – Journal of Plant Nutrition and Soil Science = Zeitschrift fuer Pflanzenernaehrung und Bodenkunde 162: 241-256.
[12] Jamal, A., Fazli, I. S., Ahmad, S., Abdin, M. Z., Yun, S. J. (2006): Effect of nitrogen and sulphur application on nitrate reductase and ATP-sulphurylase activities in soybean. – Korean Journal of Crop Science 51(4): 298-302.
[13] Jebara, S., Jebara, M., Limam, F., Aouani, M. E. (2005): Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (Phaseolus vulgaris) nodules under salt stress. – Journal of Plant Physiology 162: 929-936.
[14] Lin, C. C., Kao, C. H. (1996): Disturbed ammonium assimilation is associated with growth inhibition of roots in rice seedlings caused by NaCl. – Plant Growth Regulation 18: 233-238.
[15] Luo, H., Zhong, Z., Nie, J., Tang, X. (2017): Effects of ultrasound on physiological characters, yield and quality of rice Yuejingsimiao. – China Rice 23(2): 64-67.
[16] Mishra, D., Kar, M. (1974): Nickel in plant growth and metabolism. – Botanical Review 40: 395-452.
[17] Nazir, H., Asghar, H. N., Zahir, Z. A., Akhtar, M. J., Saleem, M. (2015): Judicious use of kinetin to improve growth and yield of rice in nickel contaminated soil. – International Journal of Phytoremediation 18: 651.
[18] Polacco, J. C. (1977): Is nickel a universal component of plant ureases? – Plant Science Letters 10: 249-255.
[19] Rakwal, R., Agrawal, G. K., Kubo, A., Yonekura, M., Tamogami, S., Saji, H., Iwahashi, H. (2003): Defense/stress responses elicited in rice seedlings exposed to the gaseous air pollutant sulfur dioxide. – Environmental, Experimental Botany 49: 223-235.
[20] Rizwan, M., Imtiaz, M., Dai, Z., Mehmood, S., Adeel, M., Liu, J., Tu, S. (2017): Nickel stressed responses of rice in Ni subcellular distribution, antioxidant production, and osmolyte accumulation. – Environmental Science, Pollution Research International 2017: 1-12.
[21] Sairam, R. K., Srivastava, G. C. (2000): Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible wheat genotypes. – Biologia Plantarum 43: 381-386.
[22] Shi, G., Zhou, Q. (1998): Effect of nickel on lipid peroxidation in isolated rice leaves. – Guihaia.
[23] Silva, J. A. T. D., Naeem, M., Idrees, M. (2012): Beneficial and toxic effects of nickel in relation to medicinal and aromatic plants. – Medicinal and Aromatic Plant Science and Biotechnology 6(Special Issue 1): 94-104.
[24] Sun, Y. J., Sun, Y. Y., Xu-Yi, L. I., Guo, X., Jun, M. A. (2009): Relationship of nitrogen utilization and activities of key enzymes involved in nitrogen metabolism in rice under water–nitrogen interaction. – Acta Agronomica Sinica 35: 2055-2063.
[25] Taylor, J., Harrier, L. A. (2001): A comparison of development and mineral nutrition of micropropagated Fragaria x ananassa cv. Elvira (strawberry) when colonised by nine species of arbuscular mycorrhizal fungi. – Applied Soil Ecology 18: 205-215.
[26] Theisen, M. O., Blincoe, C. (1984): Biochemical form of nickel in alfalfa. – Journal of Inorganic Biochemistry 21: 137-146.
[27] Wang, Y., Tian, T., Fu, H. (1999): Effects of nickel on growth of rice seedling. – Journal of Central China Normal University 33: 104-107.
[28] Welch, R. M. (1981): The biological significance of nickel. – Journal of Plant Nutrition 3: 345-356.

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DOI: http://dx.doi.org/10.15666/aeer/1701_357367