American Journal of Biochemistry and Biotechnology

Original Research Paper

The Bioaccumulation and Detoxification Mechanism of Neodymium on the Shoot of Rice Seedlings

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Article history
Received: 22-01-2021
Revised: 05-03-2021
Accepted: 09-03-2021

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Abstract: Neodymium (Nd) potentially pollutes environment for its wide usage in agriculture and industry. In this study, the chemical forms of Nd and effect of Nd on rice shoots were studied after rice seedlings were exposed with 0, 1, 10, 100 and1000 μM Nd. The higher concentration of Nd inhibited shoot growth, but the lower concentration of Nd induced shoot growth. Six different chemical forms of Nd were present in shoot and the major forms of Nd were the insoluble phosphate and oxalate Nd. The content of Nd was further detected in the soluble fractions, organelles and cell walls of rice shoot, respectively. Nd was mainly accumulated in the cell wall of shoot. The higher concentration of Nd decreased the content of Ca, K, Mg, Fe, Mn, Cu and Zn in shoot. Moreover, the activities of antioxidant enzymes and the contents of Reactive Oxygen Species (ROS) were elevated by Nd treatments. Our results showed that the inactive oxalate or phosphate Nd was the major forms of Nd in rice shoot and the efficient sequestration into cell wall was a key detoxification pattern of Nd in the shoot of rice seedlings.With the wide usage of Rare Earth Elements (REEs) in agriculture, the detoxification mechanism of Nd in rice shoot was helpful for assessing the environmental safety of REEs.

Keywords: Neodymium, Uptake, Sequestration, Detoxification, Rice Seedlings

Introduction

Rare Earth Elements (REEs) have been widely used in the industries, high-technology factories, forestry and agriculture (Bergsten-Torralba *et al*., 2020; Gwenzi *et al*., 2018; Jiachen *et al*., 2006; Zhao and Yang, 2019). However, REEs will pollute environment, contaminate the plants and affect the food quality with the wide usage of REEs. The negative effect of REEs on the growth of crops has been observed as the soil surfaces are continuously polluted by REEs (Carpenter *et al*., 2015; Tang *et al*., 2017; Thomas *et al*., 2014).

Neodymium (Nd) is a light REEs existing in REEs Micro-Fertilizerand environment (Chen *et al*., 2019; Gwenzi *et al*., 2018). Nd has been extensively applied in the modern industries for its special chemical characteristics (Gwenzi *et al*., 2018; Kucuker *et al*., 2017; Nunes *et al*., 2017; Zakotnik *et al*., 2016). In the recent years, Nd has been treated as an emerging contaminant of environment (Carpenter *et al*., 2015; Kotelnikova *et al*., 2020; Liu *et al*., 2006; Zhao *et al*., 2019). However, as one of new pollutants in environment, there is still not enough information on the biological risks of Nd.

Reactive Oxygen Species (ROS), including Hydrogen peroxide (H_2O_2), Hydroxyl radical ($OH^{\cdot}$) and superoxide anion ($O_2^{\cdot-}$), are harmful for plants if the higher ROS levels are induced and exceed the detoxification capacity of plant cells. However, a number of enzymatic antioxidants and antioxidases can scavenge ROS in plant cells (Gill and Tuteja, 2010; Gill *et al*., 2015; Qi *et al*., 2018). It has been found that REEs could affect the level of ROS and activities of antioxidases (Liu *et al*., 2016; Sun *et al*., 2019; Zicari *et al*., 2018). Although Nd has become an emerging contaminant of environment, the detoxification mechanism of Nd in plants has not been systematically studied until now. Since rice is one of the most seriously polluted crops by REEs (Jiachen *et al*., 2006), the detoxification mechanism of Nd will be studied in the rice seedlings in this study. Furthermore, the bioaccumulation and effect of Nd on mineral nutrition elements and ROS levels were to be studied in the shoot of rice seedlings.

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Materials and Methods

Plant Materials

The seeds of rice (*Oryza sativa* L., cv. Zhonghua 11) were sterilized with NaClO (2%) and germinated in an artificial climate incubator (26±2°C). The seedlings were cultured in 1/10 Hoagland nutrient solution for 3 days. Then the nutrient solution was added 0, 1, 10, 100 and 1000 µM Nd (by adding $Nd(NO_3)_3$, pH 5.5), respectively. There were 3 replicates for each Nd treatments. Rice seedlings were collected after 10 days and the Fresh Weight (FW) and height of shoots (collected from 20 plants) were analyzed. Then the analysis on rice shoot samples was carried out as Fig. 1.

Tissue Fractionation of Nd and the Different Chemical Forms of Nd Analysis

The shoots samples were homogenized by using extraction buffer (250 mM sucrose, 1.0 m Mdithiothreitol ($C_4H_{10}O_2S_2$,) and 50 mM pH 7.5 Tris-HCl). Three fractions, including cell wall, soluble fraction and organelle-containing fraction, were collected with the previous study (Weigel and Jager, 1980). In addition, the different forms of Nd were extracted with the deionized water, 2% Acetic Acid (HAC), 1 M NaCl, 80% ethanol and 0.6 M HCl, respectively (Zhang *et al*., 2014). Then three fractions and 6 chemical forms of Nd samples were subsequently oven dried at 65°C to dryness and digested with $HNO_3/HClO_4$ (v/v = 3/1). Finally, Nd content was assayed with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent, Japan).

Nd and Mineral Nutrition Elements Analysis

A microwave-assisted digestion procedure was used to digest the shoot samples. Approximately 0.1 g shoot sample (Dry Weight, DW) and 10 cm^3 HNO_3 was added into teflon bombs for digestion. Subsequently, the concentration of Na, Fe, Mn, Mg, Zn, K, Ca, Mo and Cu were detected by an ICP-MS (Agilent, Japan).

Cluster Analysis

The software T Btools 0.665 (https://github.com/CJ-Chen/TBtools) was used to perform cluster analysis and acquire the profiles of Nd abundance. TreeView of hierarchical clustering and the interactive heat-map were further analyzed.

Assay Antioxidant Enzyme Activitiesand the Levels of ROS

The activities of SOD, CAT and POD in rice seedlings were assayed with the previous methods (Goth, 1991; Liu *et al*., 2016; Oyanagui, 1984). Moreover, the contents of H_2O_2, $O_2^{\cdot -}$, MDA and the soluble proteins were assayed according to the methods of previous studies, respectively (Bradford, 1976; Goth, 1991; Heath and Packer, 1968; Liu *et al*., 2016).

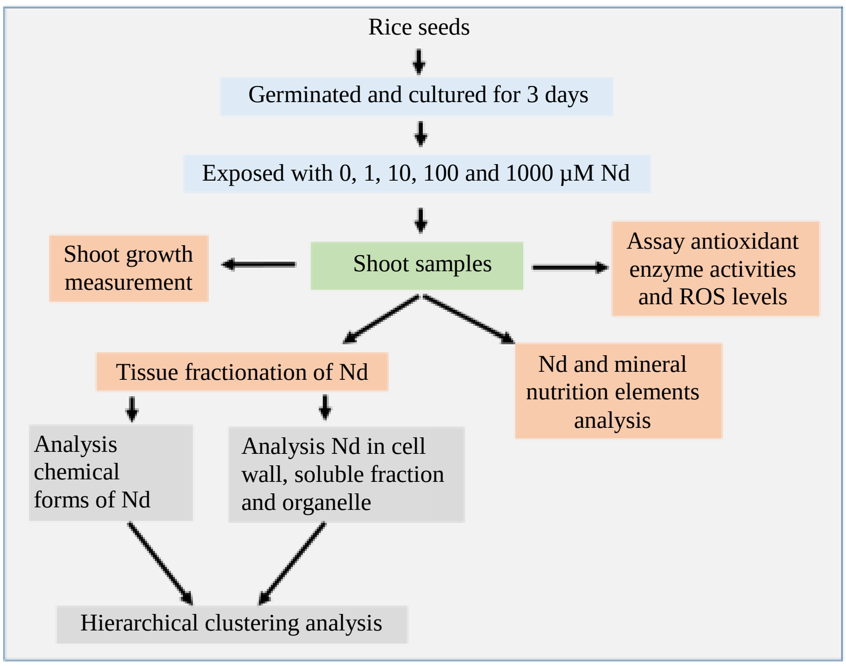

Fig. 1: The flowchart of the experimental procedures on the rice shoot. The seeds of rice were germinated and cultured for 3 days and the rice seedlings were exposed with 0, 1, 10, 100 and 1000 µM Nd for 10 days. Then the shoot samples were used for various of analysis

Statistical Analysis

The data were presented as the mean ± SEM and analyzed with the SPSS statistical software (16.0). The differences between Nd treatment groups were analyzed with one-way ANOVA (Tukey's test) at the significance level of $P<0.05$.

Results

Effect of Nd on the Height and Weight of Shoots

The treatments of 100 and 1000 μM Nd significantly inhibited the height of shoots, but there was not any significant difference when rice was exposed with 1 and 10 μM Nd (Table 1). Moreover, 1 and 10 μM Nd significantly increased, but 100 and 1000 μM Nd significantly decreased the fresh weight of shoots (Table 1).

The Subcellular Distribution of Nd in Shoots

The content of Nd was significantly enhanced in the soluble fractions, organelles and cell walls with the increase of Nd treatments (Fig. 2A). The accumulation of Nd in the soluble fractions and organelles was lower than that in the cell walls when rice was exposed with 10, 100 and 1000 μM Nd (Fig. 2A). In addition, the content of Nd in the soluble fractions was lower than that in the cell organelles (Fig. 2A). The percentage of Nd in the cell walls was elevated (Fig. 2B), while the percentage of Nd in the cell organelles and soluble fractions was decreased with the increase of Nd treatments (Fig. 2B).

The Different Chemical Forms of Nd in Shoots

Six chemical forms of Nd included water-soluble Nd, Nd binding with oxalate acid, inorganic Nd, Nd integrated with pectate and protein, insoluble $NdPO_4$ and $Nd_2(HPO_4)_3$, as well as Nd in residues. Six forms of Nd were enhanced with the increase of Nd treatments in rice shoots (Fig. 3A). Moreover, the content of Nd extracted by HCl and HAC was higher than the other 4 Nd forms (Fig. 3A). The percentage of Nd extracted by HAC was increased, but Nd extracted by HCl was decreased with the increase of Nd treatments in rice seedlings (Fig. 3B).

Hierarchical Clustering Analysis

The cluster map was made to show the chemical forms and intracellular location of Nd abundance in shoots by cluster analysis. The highest Nd accumulation was found in the cell walls (Fig. 4). However, the main forms of Nd were the HCl-and HAc-extractable Nd (Fig. 4).

Effect of Nd on the Content of Mineral Nutrition Elements in Shoots

The content of Ca, K and Mg was significantly decreased by 1000 μM Nd treatment in shoot (Table 2). However, the content of Na was not affected by the different concentration of Nd treatments (Table 2). The level of Fe, Mn and Cu in shoot was significantly decreased by 100 and 1000 μM Nd (Table 2) and Zn was decreased by 10, 100 and 1000 μM Nd (Table 2).

Effect of Nd on the Levels of ROS and SOD, POD and CAT Activities

The treatments of 100 and 1000 μM Nd significantly increased the content of MDA and H_2O_2 in rice shoots (Table 3). At 10, 100 and 1000 μM Nd, the level of $O_2^{\cdot-}$ was significantly increased in the rice shoots (Table 3). The different Nd treatments significantly enhanced CAT and POD activities in shoots (Table 3). The activity of SOD was significantly induced by 10, 100 and 1000 μM Nd in the rice shoots (Table 3).

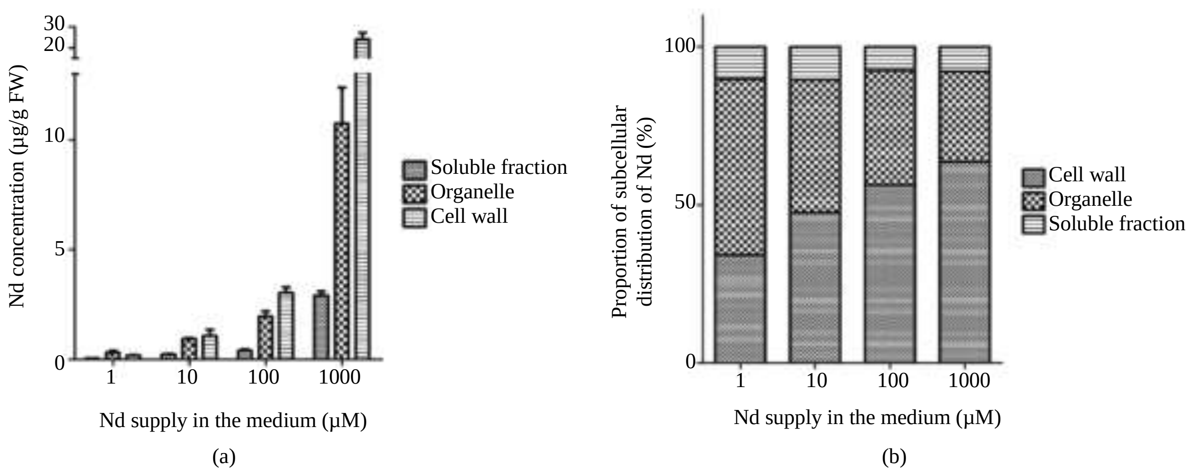

Fig. 2: The concentration and percentage of Nd in different subcellular fractions in the shoots of rice. (A) The concentration of Nd in different subcellular fractions in shoots. (B) The percentage of Nd in different subcellular fractions in shoots. Data are means ± SEM (n = 3). Means followed by the same letter do not differ significantly according to Tukey's test at $P<0.05$

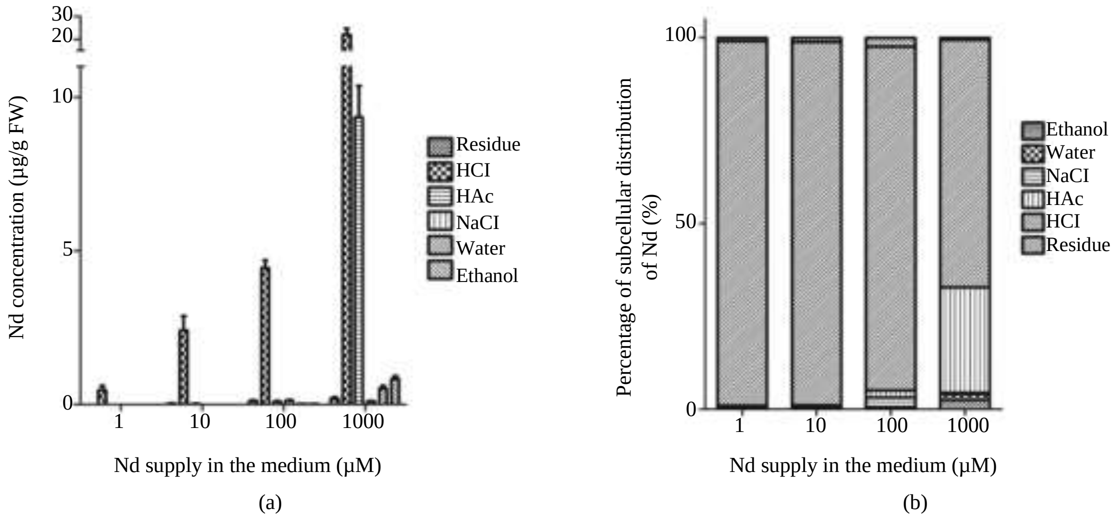

Fig. 3: The concentration and percentage of different chemical forms of Nd in the shoots of rice. (A) The concentration of different chemical forms of Nd in shoots; (B) The percentage of different chemical forms of Nd in shoots. Data are means ± SEM (n = 3). Means followed by the same letter do not differ significantly according to Tukey's test at P<0.05

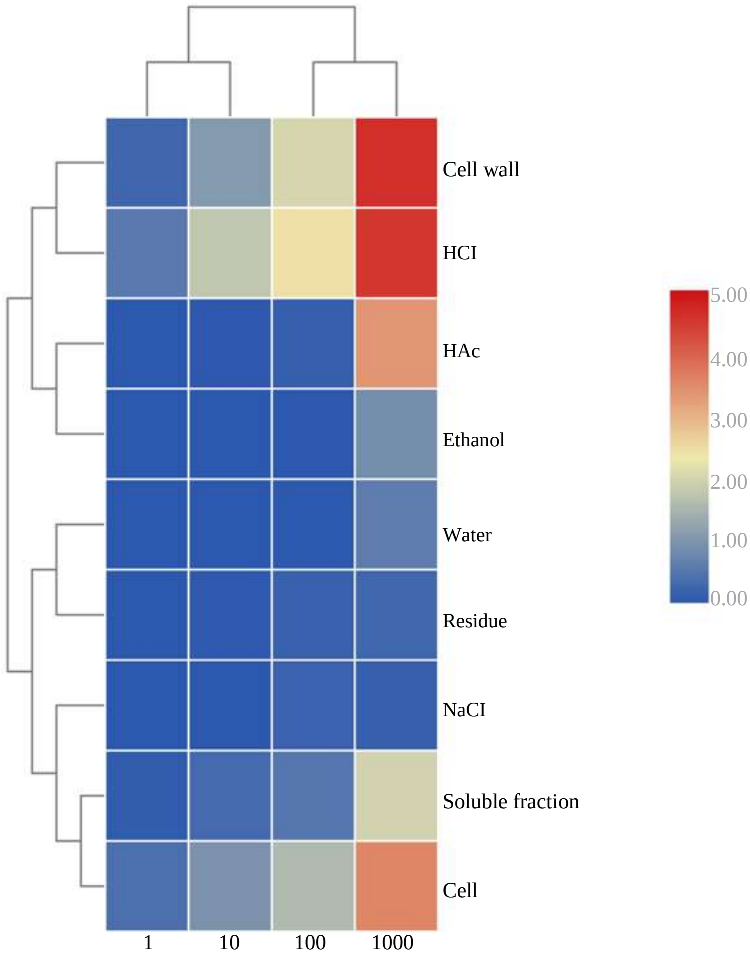

Fig. 4: Cluster map of intracellular location and chemical forms of Nd abundance level in shoots. The color weighting represents normalized levels of each variable from the high (red) to the low (blue)

Table 1: The effect of Nd on shoot height and shoot fresh weight

	Nd concentration (μM)				
Morphological features	0	1	10	100	1000
Shoot height (cm)	16.67±1.30[a]	17.36±0.76[a]	16.95±1.31[a]	13.73±1.47[b]	7.63±0.63[c]
Shoot fresh weight (g/plant)	0.22±0.01[a]	0.25±0.01[b]	0.24±0.02[c]	0.19±0.01[d]	0.09±0.00[e]

Data are means ± SEM (n = 20). Means followed by the same letter do not differ significantly according to Tukey's test at $P<0.05$

Table 2: Effects of Nd on the content of nutrient elements in rice shoots

	Nd concentration in culture medium (μM)				
Element content	Control	1	10	100	1000
K (mg/g DW)	33.43±0.96[a]	34.11±2.35[a]	32.22±2.17[a]	30.61±1.44[a]	24.93±1.18[b]
Ca (mg/g DW)	1.09±0.03[a]	1.04±0.11[a]	0.95±0.21[a]	0.76±0.13[ab]	0.53±0.07[b]
Mg (mg/g DW)	1.09±0.04[a]	1.10±0.05[a]	1.10±0.06[a]	1.04±0.07[ab]	0.90±0.07[b]
Na (mg/g DW)	0.70±0.08[a]	0.74±0.08[a]	0.74±0.03[a]	0.70±0.15[a]	0.82±0.07[a]
Fe(μg/g DW)	318.95±8.43[a]	294.01±4.36[a]	291.74±5.68[a]	242.94±30.25[b]	184.89±13.50[c]
Mn(μg/g DW)	78.40±10.29[a]	78.41±3.11[a]	68.16±5.07[ab]	51.45±6.22[bc]	44.09±4.36[c]
Zn(μg/g DW)	43.49±1.29[a]	41.16±1.61[ab]	37.38±0.80[b]	32.60±1.81[c]	29.64±2.23[c]
Cu(μg/g DW)	9.23±0.49[a]	8.40±0.83[ab]	8.70±0.98[ab]	6.91±0.66[b]	4.32±0.58[c]

Data are means ± SEM (n = 3). Means followed by the same letter do not differ significantly according to Tukey's test at $P<0.05$

Table 3: Effect of Nd on enzymatic activities and the content of ROS in rice shoots

	Nd concentration (μM)				
Parameters	0	1	10	100	1000
CAT (μmol H_2O_2/mg protein/min)	464.09±5.84[a]	691.54±30.14[b]	728.94±34.52[b]	706.08±28.20[b]	578.55±47.25[a]
SOD (units/mg protein)	8.86±0.24[a]	9.72±0.40[a]	11.13±0.75[b]	11.52±0.96[b]	12.19±0.86[b]
POD (units/min/mg protein)	28.23±1.21[a]	39.65±2.55[b]	41.16±3.03[b]	39.45±4.04[b]	55.20±5.75[c]
MDA (nmol/g FW)	17.33±0.36[a]	18.37±1.81[a]	17.42±4.03[a]	27.34±3.09[b]	33.42±3.52[c]
H_2O_2 (μmol/g FW)	0.17±0.02[a]	0.19±0.01[a]	0.18±0.02[a]	0.24±0.02[b]	0.29±0.02[c]
Generating rate of $O_2^{\cdot-}$ (nmol$O_2^{\cdot-}$/min/g FW)	0.18±0.01[a]	0.18±0.02[a]	0.30±0.01[b]	0.28±0.03[b]	0.33±0.02[c]

Data are means ± SEM (n = 3). Means followed by the same letter do not differ significantly according to Tukey's test at $P<0.05$

Discussion

It has been found that REEs could be accumulated in various of plants (Liu *et al*., 2020; Wang and Liu, 2017) and REEs was easily accumulated in the plants that grow in the REEs-enriched soil (Liu *et al*., 2020; Ma *et al*., 2017; Wang *et al*., 2001). In this study, the higher concentration of Nd inhibited the shoot growth, but the lower concentration of Nd had positive effects on the shoot growth of rice. In the previous studies, the growth of *Lemna minor* was enhanced by the lower concentration of cerium (Zicari *et al*., 2018) and the higher concentration of La inhibited plant growth (Agathokleous *et al*., 2018). Our results also showed that the lower concentration of Nd could induce shoot growth, which was consistent with these previous studies (Agathokleous *et al*., 2018; Salgado *et al*., 2020; Zicari *et al*., 2018).

It has been found that the toxic metals mainly exist in the cell wall, by which the toxicity of metal ions was decreased (Li *et al*., 2009; Weng *et al*., 2012). In the cell walls of plants, the negative charge sites were established by forming Cd coordination bonds with proteins, polysaccharides, carboxyl, hydroxyl, etc., (Krzesłowska, 2011; Küpper *et al*., 2000). The accumulation of cerium in cell walls plays a key role in the REEs' tolerance mechanism (Wang and Liu, 2017). In the present study, more Nd ions were deposited in the fraction of cell wall, which formed the first barrier for preventing Nd toxicity.

The toxicity of REEs was closely related to the chemical forms of REEs (Wang and Liu, 2017) and the different distribution patterns of REEs affected the growth of plants (Liu *et al*., 2020; Ma *et al*., 2017; Wang *et al*., 2001). The toxicity of Cu ions could be decreased by the formed bonds with phosphate (Rauser, 1999). In this study, there were more Nd ions bound with the low activity salt oxalate or phosphate in the rice shoots by cluster analysis. It showed that the bonds with salt oxalate or phosphate also play a role in the process of Nd detoxification. In addition, previous study showed that the toxicity of REEs was decreased by the combination with active amino acids and organic salts (Lai *et al*., 2006) and the toxicity of Cu could be decreased by binding with proteins and organic acids in vacuoles (Pilon *et al*., 2006). In this study, the partial Nd exists in the soluble fraction and vacuoles, which could further decrease the toxicity of Nd by binding with proteins and organic acids.

The over-production of ROS are also the toxic effects of REEs on plants (Peng and Zhou, 2009; Wang *et al*., 2009). In this study, the higher concentration of Nd enhanced the production of ROS in shoots, which showed that the oxidative stress was induced by Nd accumulation in rice shoots. Nevertheless, for the activities of CAT, SOD and POD were induced in rice shoots, the oxidative stress could be efficaciously controlled by the increase of anti-oxidative enzyme activities in shoots.

The uptake of mineral elements is important for the growth of plants. In this study, the higher concentration of Nd decreased the content of Ca, K, Mg, Fe, Mn, Cu and Zn in shoot. It showed that Nd affected the uptake of mineral elements in the rice shoots. For the mineral elements play a crucial role for plant growth, the decrease of mineral element content may lead to the growth inhibition of rice seedlings.

Conclusion

In summary, it is found that the higher concentration of Nd inhibited shoot growth, but the lower concentration Nd had positive effects on rice seedlings. Nd was mainly accumulated in the cell wall of rice shoot and Nd affected the level of ROS, the activities of antioxidant enzymes and the uptake of mineral elements in shoot. In addition, the inactive oxalate and phosphate Nd was the major forms of Nd in rice shoot. The efficient sequestration into cell wall was a crucial detoxification pattern of Nd in rice seedlings. With the wide usage of REEs in agriculture and industry, the detoxification mechanism of Nd in rice shoot was helpful for assessing the environmental safety of REEs.

Acknowledgement

This work was supported by Major Basic Research program of Shandong Natural Science Foundation (ZR2020ZD19), Natural Science Foundation of Shandong (ZR2014DM010) and National Natural Science Foundation of China (30900071).

Author’s Contributions

Kailun Shi: Participated in all experiments and coordinated the data-analysis of the manscript.

Chengkun Liu and Keliang Lyu: Participated in all experiments and coordinated to the writing of the manscript.

Jie Chen: Participated in writing of the manuscript.

Chengkun Liu: Critical revision of the article.

Xue Wang: designed the research plan and organized the study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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