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Full Length Article

Interference of Neodymium with the Antioxidative Defense Mechanisms of Jerusalem Artichoke (*Helianthus tuberosus*) Seedlings Grown under Cadmium Stress

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Abstract

Cadmium (Cd) is a serious environmental pollutant constraint to plant production in some regions. As an important rare earth element (REE), neodymium (Nd) plays a positive role in plant growth. However, few researches have reported the effect of Nd on Cd toxicity. Thus, our study was to investigate the role of Nd in alleviating of Cd toxicity induced stress. Our results showed that $CdCl_2$ addition significantly inhibited the growth of Jerusalem artichoke (*Helianthus tuberosus* L.) seedlings, while the inhibition was improved by $NdCl_3$ (10 μ*M* and 20 μ*M*). $CdCl_2$ treatment also increased reactive oxygen species (ROS) and lipid peroxidation measured as malondialdehyde (MDA) concentration and decreased the activities of the antioxidant enzymes. However, $NdCl_3$ (10 μ*M* and 20 μ*M*) could significantly alleviate oxidative damage and increased the activities of antioxidant enzymes in Jerusalem artichoke seedlings exposed to Cd stress. The result suggested that appropriate concentration of $NdCl_3$ could effectively alleviate Cd toxicity is partly related to decreasing oxidative stress and an increase in antioxidant enzyme in *H. tuberosus* seedlings. © 2017 Friends Science Publishers

Keywords: Antioxidant enzymes; Cadmium stress; *Helianthus tuberosus* L; Neodymium

Introduction

Cadmium (Cd) is one of the most toxic heavy metals to plants and has become a serious environmental contaminant due to the use of Cd in various anthropogenic activities such as metalworking industries, power stations and waste incinerators. Cd is easily taken up by roots and transported to other parts of the plant being toxic to living cells at very low concentrations (Gallego *et al*., 2012; Zafar and Javed, 2016). Recent studies have shown that Cd can cause adverse effects on some physiological and biochemical process such as photosynthesis, energy production, lipid metabolism and water relations, thereby leading to diminished plant biomass and economic yield (Sanita and Gabbrielli, 1999; Ali *et al*., 2011; Atta Ullah *et al*., 2016). The toxic effect of Cd on plants is partly related to the increase of reactive oxygen species (ROS) including superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) (Zhang *et al*., 2009; Gill and Tuteja, 2010; Hussain *et al*. 2017). Plants affected by ROS are extremely harmful to proteins, lipids and nucleic acids and bring about their oxidation (Pandey *et al*., 2005). To alleviate oxidative injury plants have developed an antioxidative system through enhancing antioxidant enzyme activities and antioxidants synthesis (Qiu *et al*., 2013; Gowayed *et al*., 2017).

Rare earth elements (REEs) are a special class of metal elements. In recent years, the effects of REEs on physiological responses have been reported in different kinds of plants (Zhang and Chen, 2007; Wang *et al*., 2011). Previous studies have shown that neodymium (Nd), an important light REEs can increase chlorophyll content and photosynthetic rate and stimulate the growth of plant at proper concentrations. Moreover, an appropriate amount of Nd could increase activities of the antioxidative defense system in some plants (Peng *et al*., 2013). However, currently few studies have focused on whether Nd can relieve oxidative damage in plants under environmental stresses.

Jerusalem artichoke (*Helianthus tuberosus* L.) is a C_3 warm-season plant easily grown in semiarid and coastal areas. It is an economically-important crop species, which is used as food for human consumption, forage and feed for animal (Long *et al*., 2008; Huang *et al*., 2012). In recent years, it has been recognized that Jerusalem artichoke is a good source of inuline and fructose and therefore, it has potential applications in a wide variety of industries (Kosaric *et al*., 1984). Previous research has indicated that a high oxidative damage and inhibited antioxidant defense systems of Jerusalem artichoke grown under Cd stress (Tao *et al*., 2007). Up to now, there is no information known

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about the effect of Nd on the oxidative damage in this plant. In the present study, the influence of Nd on the growth and antioxidative defense system in Jerusalem artichoke seedlings under Cd stress was investigated. The aim of this research was to provide a certain reference for understanding the potential mechanism of Nd alleviating damage of Cd stress to plant.

Materials and Methods

Plants and Treatments

Jerusalem artichoke (*Helianthus tuberosus* L.) tubers were collected from Yantai of Shandong province, China. Tubers were cut into small square pieces, sterilized with 75% ethanol (v/v) for 30 min and rinsed drastically with distilled water. Subsequently, tuber slices with buds of Jerusalem artichoke were sprouted in plastic containers covered with 20-mesh quartz sand. Each container culured a single plant and was watered with nutrient solution once a day. The nutrient solutions were prepare as follows(m*M*): H_3BO_3, 1.25; $Ca(NO_3)_2$, 0.5; KNO_3, 0.5; $MgSO_4$, 0.017; $MnSO_4$, 0.1; $NiSO_4$, 0.04; K_2SO_4, 0.034; $CuSO_4$, 0.025; Na_2MoO_4, 0.025; $ZnSO_4$, 0.025; and Fe–EDTA ,10 (Wang *et al*., 2009; Wang *et al*., 2011). The nutrient solution was replaced every two days. After 15 days, uniform size seedlings with one expanding leaf and three fully extended leaves were planted into plastic pots containing quartz sand (each pot contained three plant)) (Monti *et al*., 2005; Xue and Liu, 2008).

Five days later, $CdCl_2$ and $NdCl_3$ were added into nutrient solution making 5 treatments: (1) control (CK), (2) 100 μ*M* $CdCl_2$ alone (Cd), (3) 5 μ*M* $NdCl_3$ +100 μ*M* $CdCl_2$ (5 μ*M*), (3) 10 μ*M* $NdCl_3$+100 μ*M* $CdCl_2$ (10 μ*M*), (4) 20 μ*M* $NdCl_3$ +100 μ*M* $CdCl_2$ (20 μ*M*) and (5) 50 μ*M* $NdCl_3$ +100 μ*M* $CdCl_2$ (50 μ*M*). The treatment of concentrations was according previous research, in which a number of higher and lower levels of Cd were applied. Care was taken to enture that each container received the same volume of the planned solution for every treatment and that there was no water stress. All treatments were carried out in triple. The experiment repeated three times. After 15 days of treatment plants were harvested for biochemical and physiological assay.

Plant Growth Measurement

At harvest, the height of the plants was recorded. Then the plants were partitioned into root, stem and leaves. The dry weight (DW) of root, stem and leaves was determined by drying a known fresh weight of homogenized samples at 60ºC until constant weights were obtained.

Assay of H_2O_2, $O^{\cdot}{}_2^-$ and MDA Levels and SOD, POD and CAT Activities

Leaves collected from plants grown under various conditions were homogenized in cold phosphate buffer (50 m*M*, pH7.8) containing 1% (v/v) polyvinylpyrrolidone, 5 m*M* ethylenediaminetetraacetic acid and 5 m*M* dithiothreitol. The homogenates were centrifuged at 15,000 rpm for 15 min at 4ºC and the supernatant was applied to the analysises of $O_2\cdot^-$, H_2O_2 and MDA levels and antioxidant enzyme activities.

Content of $O_2\cdot^-$ was determined at 530 nm by monitoring the nitrite formation from hydroxylamine in the presence of $O_2\cdot^-$ (Elstner and Heupel, 1976). H_2O_2 content was measured at 390 nm (Wang *et al*., 2009). Lipid peroxidation was assayed by measuring MDA content using the thiobarbituric acid (TBA) reaction and the absorbance was measured at 532 and 600 nm to calculate MDA concentration (Buege and Aust, 1978).

Superoxide dismutase (SOD) activity was assayed by measuring the inhibition in photochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich, 1971). The reaction mixture (3.0 mL) included 20 m*M* phosphate buffer, 13 m*M* methionine, 2 μ*M* μriboflavin, 75 μ*M* NBT, 10 μ*M* EDTA and 0.05 mL enzyme extract. The tubes was illuminated with two 20 W fluorescent for 20 min to initiate the reaction. The absorbance of the reaction mixture was read at 560 nm. One unit of SOD activity represented the amount of enzyme required to cause 50% inhibition the NBT photoreduction rate.

Catalase (CAT) activity was determined based on the procedures described by Durner and Klessing (1996). The reaction mixture (3.0 mL) contained 50 m*M* phosphate buffer (pH 7.8), 0.2 mL enzyme extract and 10 m*M* H_2O_2. The absorbance was read at 240 nm. 1 μ*M* H_2O_2 decomposed in 1 min represented one unit (U) of CAT.

Peroxidase (POD) activity was determined based on the method of Hammerschmidt *et al.* (1982). 2.9 mL of 100 m*M* phosphate buffer (pH 7.0) containing guaiacol (0.05%) and 10 m*M* H_2O_2 was mixed with 0.1 mL of enzyme extract. Activity was determined by the increase per minute in the absorbance at 470 nm. One unit of peroxidase activity was equated to an increase of 0.01 absorbance units per minute.

Statistical Analysis

Each treatment was repeated three times. All measured data were expressed as mean ± SD deviation. SPSS software (SPSS Inc., version 11.5, Chicago, USA) was used to perform statistical analysis. Differences between comparisons with $P<0.05$ were considered as statistical significance.

Results

Plant Growth of Jerusalem artichoke

Table 1 shows the plant height and dry weight of different organs of Jerusalem artichoke seedlings under various

Table 1: Effect of $NdCl_3$ and $CdCl_2$ on growth indexes of *H. tuberosus*

Treatment	Plant height(cm)	Dry weight (g/plant)		
		Leaves	Stems	Roots
CK	21.36±1.84a	0.87±0.11a	1.09±0.11a	0.36±0.05a
Cd	10.23±1.27c	0.38±0.05c	0.61±0.07c	0.19±0.02c
Nd 5	12.17±1.05c	0.41±0.06c	0.67±0.05c	0.20±0.03c
Nd10	15.23±1.96b	0.67±0.10a	0.85±0.06b	0.32±0.05b
Nd 20	16.41±2.12ab	0.62±0.11b	0.98±0.09ab	0.28±0.06b
Nd 50	9.83±1.17d	0.32±0.08d	0.62±0.08d	0.15±0.02d

Each value represents the mean ± SD, n=3. Different letters in each column indicate significant difference at $P<0.05$

conditions. The plant height and dry weight of leaves, stems and roots in 100 μ*M* $CdCl_2$ treatment group were significantly decreased ($P<0.05$) when compared with those of the control group. The application of 5 μ*M* $NdCl_3$ seemed to be no notable effects on the the plant growth compared to 100 μ*M* $CdCl_2$ treatment groups. However, treatment with 10 μ*M* and 20 μM neodymium significantly alleviated ($P<0.05$) $CdCl_2$ induced inhibition of growth. 10 μ*M* $NdCl_3$ had the best effect on the dry weight of leaves and roots and they were increased by 76.32 and 68.42%, respectively as compared with 100 μ*M* $CdCl_2$ treatment groups. In 20 μ*M* neodymium treatment group, plant height and dry weight of stems increased by 62.66 and 60.65% as compared with 100 μ*M* $CdCl_2$-treatment group. However, the application of 50 μM neodymium resulted in a negative effect the growth of Jerusalem artichoke seedlings.

ROS Accumulation and Lipid Peroxide Level

The contents of $O_2\cdot^-$, H_2O_2 and MDA in Jerusalem artichoke seedlings under various conditions are shown in Fig. 1. The contents of $O_2\cdot^-$, H_2O_2 and MDA in 100 μ*M* $CdCl_2$ treatment group were significantly increased ($P<0.05$) as compared with those of the control. In 5 μ*M* $NdCl_3$ treatment groups, the contents of $O_2\cdot^-$, H_2O_2 and MDA was slightly decreased ($P>0.05$) compared with 100 μ*M* $CdCl_2$ treatment groups. Nevertheless, Application of 10 μ*M* and 20 μ*M* $NdCl_3$ significantly reduced ($P<0.05$) the contents of $O_2\cdot^-$, H_2O_2 and MDA. At the same $CdCl_2$ concentration, application of 10 μ*M* $NdCl_3$ reduced the seedling contents of $O_2\cdot^-$, H_2O_2 and MDA by 23.89, 33.83 and 31.52%, respectively. In 20 μ*M* $NdCl_3$ treatment groups, the contents of $O_2\cdot^-$, H_2O_2 and MDA decreased by 11.94, 35.71 and 29.15%, respectively as compared with 100 μ*M* $CdCl_2$ treatment groups. In contrast, application of high concentration of $NdCl_3$ (50 μ*M*) notably increased the contents of H_2O_2, $O_2\cdot^-$ and MDA of the seedlings.

Antioxidant Defense

The analysis of SOD, POD and CAT enzymes activities in different treatments is presented in Fig. 2. The plants exposed to 100 μ*M* $CdCl_2$ appeared significant decrease ($P<0.05$) in SOD activity versus the control. $NdCl_3$ was

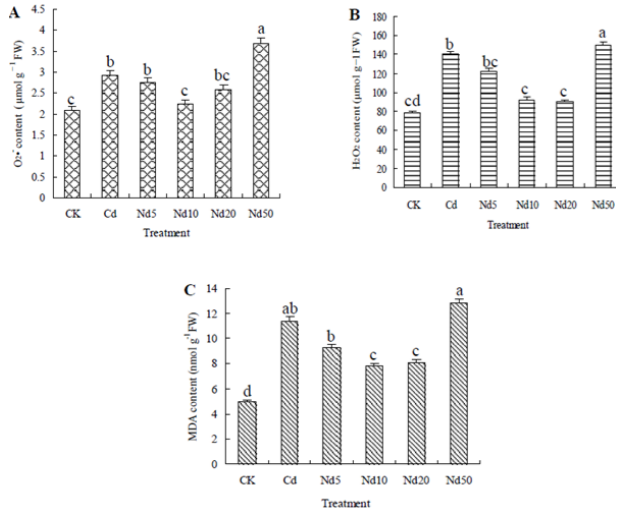

Fig. 1: Effect of $NdCl_3$ and $CdCl_2$ on H_2O_2 (A), $O\cdot_2^-$ (B), and MDA(C) content in *H. tuberosus*

Each value represents the mean ± SD, n=3. Different letters within each graph indicate statistically significant differences at $P<0.05$

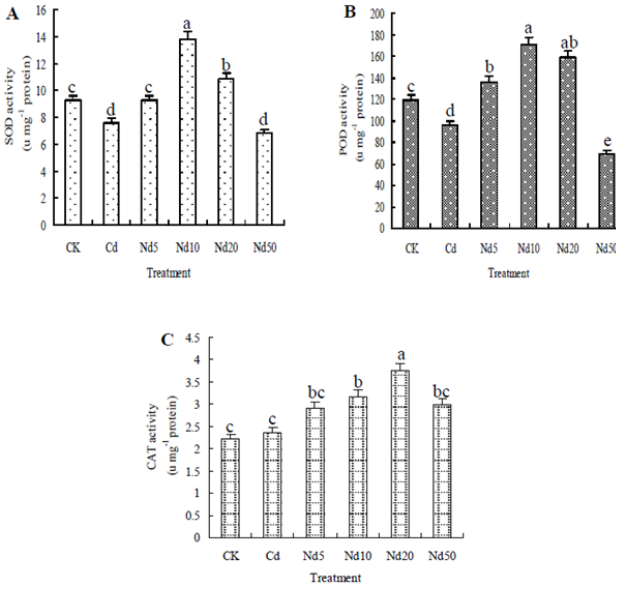

Fig. 2: Effect of $NdCl_3$ and $CdCl_2$ on SOD (A), POD (B), and CAT (C) activities of *H. tuberosus*

Each value represents the mean ± SD, n=3. Different letters within each graph indicate statistically significant differences at $P<0.05$

effective in alleviating Cd inhibited SOD activity, especially at 10 μ*M* and 20 μ*M*. Application of 10 μ*M* and 20 μ*M* $NdCl_3$ significantly increased the activities of SOD by 82.19 and 43.67%, respectively relative to Cd stressed plants. In terms of POD activity, $CdCl_2$ treatment alone could not exert a remarkable effect on POD activity against that of the control. A little increase of POD activity was observed as the plants were treated with 5 μ*M* $NdCl_3$ and the enhancement of POD in 10 μ*M* and 20 μ*M* $NdCl_3$ treatment

groups was significant (P<0.05). However, application of high concentration of $NdCl_3$ (50 μ*M*) significantly decreased (P<0.05) POD activity compared with 100 μ*M* $CdCl_2$ treatment groups. No obvious effects on CAT activity were observed in $CdCl_2$ treatment groups, whereas different $NdCl_3$ concentrations significantly increased (P<0.05) CAT activity compared with the seedling under control and $CdCl_2$ treatments. The 20 μ*M* $NdCl_3$ had the most prominent effect on CAT activity, which was increased by 82.04% as compared with 100 μ*M* $CdCl_2$ treatment groups.

Discussion

The growth inhibition is a response of plants exposure to toxic concentrations of Cd (Wang *et al*., 2007; Gallego *et al*., 2012). Our study revealed that treatment with $CdCl_2$ (100 μ*M*) significantly reduced (P<0.05) the plant height, fresh weight and dry weight of Jerusalem artichoke seedlings. Previous studies have found that the plant growth was suppressed by Cd owing to interfering in a range of metabolic processes, i.e., damage to photosynthetic machinery, decline in root elongation and inhibition of proton pump (Basharat *et al*., 2014). The results from the present study indicate treatment with appropriate concentration $NdCl_3$ could significantly improved (P<0.05) the growth of seedlings grown under Cd stress. It has been reported that suitable concentration $NdCl_3$ can promote the cell proliferation and increases chlorophyll content in plant (Wang *et al*., 2011; Peng *et al*., 2013). We speculate that $NdCl_3$ enhances the photosynthesis and induces the cell proliferation might be associated with its effect on growth of plants in the present stress conditions.

Studies have found that Cd toxicity could increase reactive oxygen species (ROS) and produce oxidative stress in plants, i.e., H_2O_2 and $O_2\cdot^-$ (Wu *et al*., 2014). Overproduction of ROS might cause adverse effect on the growth, metabolism and development of cells through their ability to initiate reaction cascades that result in extensive lipid peroxidation as indicated by higher accumulation of MDA in chloroplasts of plants (Gossett *et al*., 1994). In our current study, treatment with 100 μ*M*-$CdCl_2$ significantly increased (P<0.05) H_2O_2, $O_2\cdot^-$ and MDA content in Jerusalem artichoke seedlings, implying that Cd stress caused increased ROS production, thus conduced to the damage of plasma membrane structure. Previous studies have found that REEs could protect plants against oxidative stress under various adverse conditions (Wang *et al*., 2009; Wang *et al*., 2011). In this experiment, Low concentration of $NdCl_3$ (10 m*M* and 20 μ*M*) decrease production of H_2O_2, $O_2\cdot^-$ and accumulation of MDA induced by Cd stress, suggesting that $NdCl_3$ could directly remove ROS such as H_2O_2 and $O_2\cdot^-$ and protect plasma membrane lipid against oxidative damage.

To protect themselves from oxidative damage in adverse environments, plants have developed complex antioxidant systems consisting of antioxidative enzymes including SOD, CAT and POD and antioxidant compounds (Zhao *et al*., 2008; Sharma and Dietz, 2009). In this paper, the activities of SOD and POD were significantly inhibited (P<0.05) under Cd stress, implying that exposure to high salinity resulted in an imbalance between ROS and their removal in Jerusalem artichoke seedlings. Previous studies have shown that RE could affect the activities of many antioxidative enzymes and antioxidant compounds in plants to alleviate the injury of stress. It was reported that addition of $LaCl_3$ increased the activities of SOD, ascorbate peroxidase (APOX), CAT and glutathione reductase (GR) in *Saussurea involucrata* under salt stress (Xu *et al*., 2008). The treatments of $LaCl_3$, $NdCl_3$ and $CeCl_3$ significantly increase the activities of antioxidative enzyme in maize seedling under cold stress (Wang *et al*., 2011). In this experiment, compared with $CdCl_2$ treatment, addition of $NdCl_3$ (10 μ*M* and 20 μ*M*) could significantly increase (P<0.05) the activities of SOD, CAT and POD in seedling under Cd stress, implying that $NdCl_3$ could remarkedly enhance the capacity of antioxidant system against oxidative damage induced by Cd toxicity in Jerusalem artichoke.

In conclusion, the results from this study demonstrated that appropriate concentration $NdCl_3$ could significantly stimulate the growth of Jerusalem artichoke alleviated the oxidative damage and increase the activities of SOD, POD and CAT enzymes under Cd stress. The future research should be focus on elucidating $NdCl_3$ induced changes of these physiobiochemical processes at molecular and cellular level and discussing whether the $NdCl_3$ has active action in the nonenzymatic reaction.

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