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Polyamines content and physiological and biochemical responses to ladder concentration of nickel stress in *Hydrocharis dubia* (Bl.) Backer leaves

Juan Zhao · Guoxin Shi · Qiuhong Yuan

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Abstract Influence of ladder concentration of nickel (Ni) on the leaves of *Hydrocharis dubia* were studied after 3 days treatment. The accumulation of Ni, the content of polyamines, proline, malondialdehyde (MDA) and soluble protein, as well as the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in the leaves were investigated. The result indicated that the toxicity of Ni manifested in respective aspect of physiological and biochemical characters. Significant increase of Ni concentration in the leaf tissue was observed, which was concentration dependent. Visible symptoms of Ni toxicity: chlorosis and necrosis occurred following the 3rd day. Meantime, treatment with Ni resulted in the increase in the generation rate of $O_2^{\bullet -}$ in the leaves. SOD and CAT activities decreased significantly in response to Ni treatment, it was possibly the reason of accumulation of $O_2^{\bullet -}$. However, a several-fold decrease in POD activities was found. Our results indicated that because of prolonged increases in $O_2^{\bullet -}$ level, oxidative damage, measured as the level of lipid peroxidation, occured in the leaves of Ni treated fronds. The changes of the content of polyamines (PAs) were also investigated in the leaves of *Hydrocharis dubia*. Ni treatment significantly increased the putrescine (Put) level and lowered spermidine (Spd) and spermine (Spm) levels, thereby significantly reducing the ratio of free (Spd + Spm)/Put in leaves, which has been considered as the signal under stress. Although the trend that PS-conjugated PAs and PIS-bound PAs changed the same as free PAs, they changed in more less extent.

Keywords Nickel · Polyamine · Physiology · Stress · *Hydrocharis dubia*

J. Zhao · G. Shi (✉) · Q. Yuan
College of Life Science, Nanjing Normal University, Nanjing, Jiangsu 210046, China
e-mail: gxshi@njnu.edu.cn

J. Zhao
e-mail: zhaojuannj@yahoo.com.cn

Introduction

With increasing heavy metal contamination due to various human and natural activities, ecosystems have been suffered from heavy metals (HMs) pollution. Heavy metal contamination of water body is an especially serious problem due to the application of pesticides in agriculture, discharge of untreated industrial wastes, and mining operations (Alloway 1990; Lou et al. 2004).

Bioavailability and bioaccumulation of various heavy metals in aquatic and wetland ecosystems is being of tremendous significance globally. Even though Ni is a kind of trace essential element for plant metabolism, many of researches have demonstrated that it can also be strongly phytotoxic at high concentrations on most plant species (Baccouch et al. 2001; Gonnelli et al. 2001). Although mechanisms of detrimental impact of Ni on plants are not clearly

understood, a growing body of evidence indicates that phytotoxicity of this metal may be attributed, at least in part, to oxidative stress (Baccouch et al. 2001; Gonnelli et al. 2001). Another mechanism is that Ni may alter cell membranes by peroxidative degradation of polyunsaturated fatty acids, and destroy the microstructure of the cell (Ghaderian et al. 2006).

Aquatic macrophytes take up metals from the water, causing an internal concentration several folds greater than the surroundings (Greger 1999). Many of the aquatic macrophytes were found to be the potential scavengers of heavy metals from aquatic environment and are being used in wastewater renovation systems (Abbasi and Ramasami 1999; Kadlec et al. 2000). *Hydrocharis dubia* (Bl.) Backer is a kind of ordinary aquatic macrophytes which is a clonal weedy species native to Asia. For its strong ability to reproduce, it is a convenient plant material for ecotoxicological investigations.

To protect against oxidative stress, plants developed an antioxidative system consisting of both antioxidative enzymes and non-enzymatic antioxidants. Superoxide dismutase (SOD, EC 1.15.1.1), as the first line of defense against reactive oxygen species (ROS), can catalyze disproportionation of $O_2^{\bullet -}$ to H_2O_2 and O_2. Catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) are responsible for the scavenging of H_2O_2. CAT converts H_2O_2 to H_2O and O_2. Other peroxidases, including guaiacol peroxidase (POD, EC 1.11.1.7), as a key enzyme in biosynthesis of lignin (Gaspar et al. 1991), catalyzing oxidation of many phenolic compounds at the expense of H_2O_2, are also involved in H_2O_2 elimination.

Although antioxidative reactions of plants subjected to Ni have been studied by several authors, there are less reports on the involvement of non-enzymatic antioxidants in the plant response to this heavy metal, including polyamine. Polyamines (PAs) are small, organic polycations found in all eukaryotic cells (Cohen 1998). Common polyamines are ubiquitous aliphatic polycations that have been recognized as modulators of plant growth and development (Evans Malberg 1989), and are also implicated in plant responses to environmental cues (Bouchereau et al. 1999). Putrescine (Put), spermidine (Spd), and spermine (Spm) are the major PAs in plants and they are involved in various processes such as cell proliferation, growth, morphogenesis, differentiation, and programmed cell death (Kumar et al. 1997; Malmberg et al 1998; Serafini-Fracassini et al. 2002). Stress induced expression and activity of arginine decarboxylase is the most commonly accepted feature of polyamine metabolism, a phenomenon that is responsible for the typical accumulation of putrescine observed under stress. In the particular case of salt stress, induction of arginine decarboxylase and accumulation of putrescine seems to be a consequence of the osmotic elicitation due to salt shock (Bouchereau et al. 1999). But the investigation about the changes of PAs under heavy metals was scarce, especially under Ni stress. And the investigation about the activity of polyamine oxidase (PAO) in PAs metabolism under heavy metals stress was also infrequent.

In this work, we used *Hydrocharis dubia* to test the hypothesis that free spermidine and spermine are biochemical indicators of Ni stress response. In order to know their roles in polyamines metabolizability, PS-conjugated and PIS-bound PAs were also measured. Moreover, the antioxidative system in the plant was investigated including the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and the changes in generating rate of $O_2^{\bullet -}$ and the changes of content of MDA.

Materials and methods

Plant material and metal treatment

Hydrocharis dubia was collected from Tai lake in Suzhou, early June, 2006. It was cultured in an aquarium in totally enclosed incubator (Forma 3744, England) at the day/night temperature of 24/18°C. The illumination procedure consisted of 12 h light (70 μM m^{-2} s^{-1}) and 12 h dark, alternately. One week before experiment, the plant material was transferred to 1/20 Hoagland solution (Shi et al. 2003). Similar fronds were treated with Ni (vitriolic nickel) in concentrations 0.5, 1, 2, 3 and 4 mM, respectively, in the culture solution of 2,000 ml glass beakers. All solutions were replaced with fresh solutions everyday. Physiological and biochemical indexes were measured after 3 days.

Analysis of metal accumulation by ICP-ES

The accumulation of nickel was analyzed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-ES, Leeman labs, Prodigy, USA).

Photosynthetic pigment assay

Hydrocharis dubia leaves (weighing 200 mg of the fresh weight) were homogenized on ice with mortar and pestle in cold acetone, the homogenate was then adjusted to 8 ml with cold acetone. The absorbance of pigment extract was measured at wavelength 470, 645, 663 nm with spectrophotometer (Shenguang Instrument, UV 754, China). The content of Chl *a*, Chl *b* was estimated according to the experimental equation (Liu et al. 1999).

Measurement of protective enzyme activity and generating rate of $O_2^{\bullet-}$

Hydrocharis dubia leaves (weighing 500 mg ± 10% of the fresh weight) were homogenized on ice with mortar and pestle in 50 mM phosphate buffer. Solid phase was separated centrifugally at 10,000 r/min for 20 min and the supernatant was analyzed. The SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries (1977). The activity of CAT was measured by assaying hydrogen peroxide forming stable complex with ammonium molybdate (Gyth 1991). The POD activity was determined by using the guaiacol method (Maehly 1955). Generating rate of $O_2^{\bullet-}$ was measured according to the method of hydroxylamine chloride (Wang and Luo 1990).

Measurement for the content of different form polyamines,and the activity of PAO

Plant material (1 g) was homogenized in 4 ml of 6% (v/v) cold perchloric acid (PCA), kept on ice for 1 h, and then centrifuged at 21,000 *g* for 30 min. The pellet was extracted twice with 2 ml 5% PCA and recentrifuged. The three supernatants were pooled and used to determine the levels of free and PS-conjugated PAs, whereas the pellet was used to determine the levels of PIS-bound PAs. The pellet was resuspended in 5% PCA and hydrolyzed for 24 h at 110°C in flame-sealed glass ampoules after being mixed with 12 N HCl (1:1, v/v). The hydrolyzates were filtered, dried at 70°C, and then resuspended in 1 ml of 5% PCA for analysis of PIS-bound PAs. For PS-conjugated PAs, 2 ml of the supernatant were mixed with 2 ml of 12 N HCl and hydrolyzed under the conditions described above. The supernatant, hydrolyzed supernatant and pellet were benzoylated in accordance with the method of Aziz and Larher (1995).

PAO (EC 1.4.3.67) activity was determined by the improved method of Smith (1985). Leaves were homogenized on ice, in 0.05 mmol L^{-1} Na_2HPO_4–$KHPO_4$ buffer (ratio of buffer volume to g fresh weight was 4, pH 7.0); then separated centrifugally at 8,000 rpm for 10 min. The filtrate was used to assay PAO activity. 3.5 ml of the reaction mixture was consisted of 3 ml phosphate buffer (pH 7.0) containing 3 mmol L^{-1} Spd, 0.03 mmol L^{-1} pyridoxal phosphate, and 0.5 ml enzyme extract. The reaction was conducted at 37°C for 60 min, and stopped by adding 0.5 ml 10% TCA. After centrifugation, anthranilic aldehyde at equal volume was added into the supernatant and measured spectropho-tometrically at 435 nm. 1ΔA435 g^{-1} FW h^{-1} was equal to one enzyme activity unit (1 U). Three measures were made independently on three independent samples.

Measurement for the content of MDA, proline and soluble protein

Lipid peroxidation was measured by the level of malondialdehyde (MDA), a product of lipid peroxidation, using a reaction with thiobarbituric acid (TCA) as described by Hodges (Hodges et al. 1999). About 0.5 g leaf segments were homogenized in 10 ml of 10% TCA, and centrifuged at 12,000*g* for 10 min. After that, 2 ml 0.6% thiobarbituric acid (TBA) in 10% TCA was added to an aliquot of 2 ml from the supernatant. The mixture was heated in boiling water for 30 min, and then quickly cooled in an ice bath. After centrifugation at 10,000 *g* for 10 min, the absorbance of the supernatant at 450, 532 and 600 nm was determined.

The free proline content in leaves was estimated according to the method of Bates et al. (1973), and soluble protein content was determined following Bradford (1976), with bovine serum albumin as the standard.

All the experimental values reported in this article are the means of at least three individual experiments. The significant differences were calculated using *t*-test wherever applicable by Excel2003. The coefficients of correlation (*r* value) were calculated by using Origin6.0.

Results

Bioaccumulation of nickel in *Hydrocharis dubia* (Bl.) Backer leaves

Nickel bioaccumulation was observed in *Hydrocharis dubia* (Bl.) Backer leaves, showing concentration dependent characteristics ($r = 0.96366$, $^{*}P < 0.05$) in Table 1. Nickel was accumulated up to 948 μg g^{-1} DW after 3 days in *Hydrocharis dubia* (Bl.) Backer which grew in culture medium containing 0.4 mmol L^{-1} Ni^{2+}. Compared with the control, Ni treatment significantly increased the levels of Zn ($^{**}P < 0.01$), Ca ($^{*}P < 0.05$); and markedly decreased the levels of Cu ($^{*}P < 0.05$) and Na ($^{**}P < 0.01$) in leaves.

The changes of Photosynthetic Pigment content of *Hydrocharis dubia* leaves under Ni stress

It can be seen from Fig. 1 that pigment content of *Hydrocharis dubia* leaves decreased with the increase of the Ni gradient concentrations under Ni stress. But under comparable low-concentration nickel treated (0.5 mM), the content of pigment including chlorophyll and carotenoid was higher than leaves of control, indicating that the photosynthetic pigment was synthesized more in response to low-concentration nickel. However, when the concentration of Ni continued to rise up, the distinct decrease of pigment content was observed. When the concentration of Ni was 4 mM, the content of chlorophyll (chlorophyll a and chlorophyll b) was only 67.6% of the leaves of control.

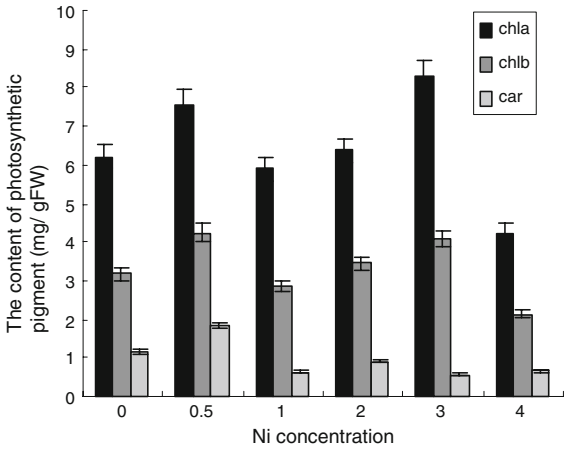

Fig. 1 the content of Photosynthetic pigment in *Hydrocharis dubia* leaves under Ni stress

The activity of antioxidation enzymes of *Hydrocharis dubia* leaves under Ni stress

In Fig. 2a, it could be found that the activity of SOD almost decreased with Ni concentration. When the frond was treated with 4 mM Ni, the SOD activity reached its rock bottom, remarkably decreasing to 27.55% of the control ($^{**}P < 0.01$). The activity of CAT reduced respectively at all concentration, but the activity of CAT at all concentration of Ni stress had not distinct difference (Fig. 2b). The change of POD activity was different from that of SOD and CAT, and it didn't ascend sharply in every concentration of nickel treatment. Its activity changed in little extent irregularly, which was represented when fronds were treated at 2 mM concentration of Ni. However, as the concentration of Ni rose up to 4 mM, the activity began to drop down gradually (Fig. 2c).

Generation rate of $O_2^{\bullet-}$

As shown in Fig. 3, when treated with considerable low-concentration (0.5 mM, 1 mM) nickel, the generation rate of $O_2^{\bullet-}$ of leaves increased unconspicuously, while as the concentration of Ni continued to rise, the generation rate of $O_2^{\bullet-}$ decreased indistinctly, then it rose again. The peak was observed in 4 mM concentration, the generation of $O_2^{\bullet-}$ increasing to 170.7% of the control ($^{**}P < 0.01$). Its trend of change was opposite to the activity of SOD.

The effect of concentration of soluble protein and MDA in *Hydrocharis dubia* leaves under Ni stress

As shown in Fig. 4a, the content of MDA in *Hydrocharis dubia* leaves increased significantly when the plants were exposed to ladder concentration of Ni, and its content increased with the increase of Ni. It was indicated that the accumulation of MDA was closed related with concentration. When fronds were treated in 4 mM Ni, the content of MDA reached its peak which was increasing 23.6% of the control. The soluble protein content of *Hydrocharis*

Table 1 Bioaccumulation of nickel in *Hydrocharis dubia* (Bl.) Backer leaves after 3 days of treatment (μg/g Dw)

	0	0.05 mM	0.1 mM	0.2 mM	0.3 mM	0.4 mM
Ni	ND	152 ± 7.6	491 ± 24.55	572 ± 28.6	868 ± 43.4	948 ± 47.4
Zn	5.42 ± 0.271	8.07 ± 0.4035	10.1 ± 0.505	10.4 ± 0.52	12.0 ± 0.6	13 ± 0.655
Ca	478 ± 23.9	764 ± 38.4	1,080 ± 54	430 ± 21.5	786 ± 39.3	747 ± 37.35
Cu	0.848 ± 0.0424	0.333 ± 0.01665	0.664 ± 0.0332	0.233 ± 0.01165	0.588 ± 0.0294	0.794 ± 0.0397
Na	1,063 ± 53.15	464 ± 23.2	871 ± 43.55	192 ± 9.6	420 ± 21	739 ± 36.95

ND: not detected

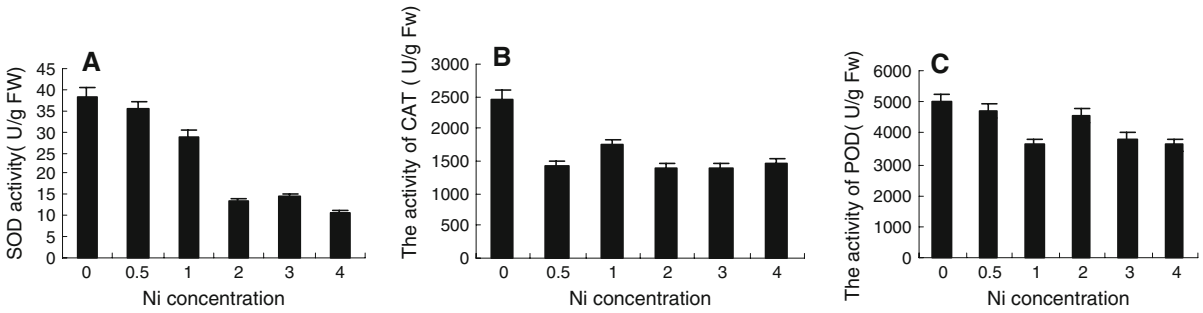

Fig. 2 The activity of antioxidant enzymes in *Hydrocharis dubia* leaves under Ni stress

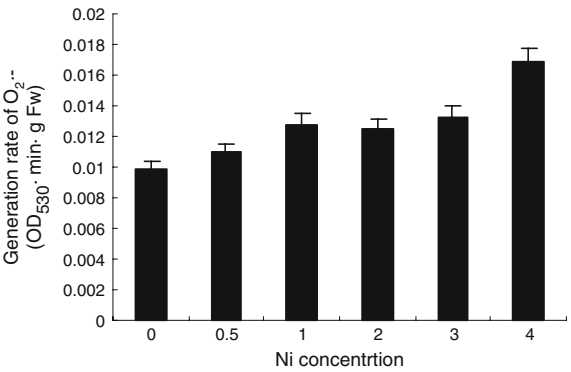

Fig. 3 The generation rate of $O_2^{\bullet -}$ in *Hydrocharis dubia* leaves under Ni stress

dubia leaves from Ni-treated plants was not significantly different from that of the control, but its concentration compared with control decreased respectively, especially the content of soluble protein under 4 mM Ni stress which was only 68% of the control (Fig. 4b).

Proline levels gradually ascend in response to Ni stress, which showed positive correlations ($r = 0.83359$, $^*P < 0.05$). In plants treated with 4 mM Ni, proline levels reached peak as 2.58-fold of the control (Fig. 4c).

The effect of concentration of PAs and the activity of PAO in *Hydrocharis dubia* leaves under Ni stress

In comparison with the control plants, Ni stress induced a massive accumulation of free Put ($^{**}P < 0.01$) and decreasing of free Spd ($^{**}P < 0.01$) and Spm ($^{**}P < 0.01$) in different extent (Fig. 5a) with the increase of the Ni gradient concentrations. When grown in culture medium containing 4 mM Ni, the concentration of free Put reached the peak as 27.46 μmol/g Fw, which was 4.09 times of control plants. The content of free Spd reduced distinctly under the stress of Ni gradient concentrations, and when grown in 4 mM Ni, it was only 1.700 μmol g^{-1} Fw, 31.4% of the control. The same as Spd, the content of Spm reached to 8.71% of the control when grown in 4 mM Ni. For the reason of Spd and Spm sharply decreased under the considerable high concentration of Ni, and highly accumulation of Put, the rate of free (Spd + Spm)/Put decreased sharply (Fig. 5d).

Ni treatment significantly increased the level of PS-conjugated Put compared to the control ($^{**}P < 0.01$). The elevation of PS-conjugated Put level in the leaves by Ni stress was only 3.1-fold greater than the control when folds were cultivated in

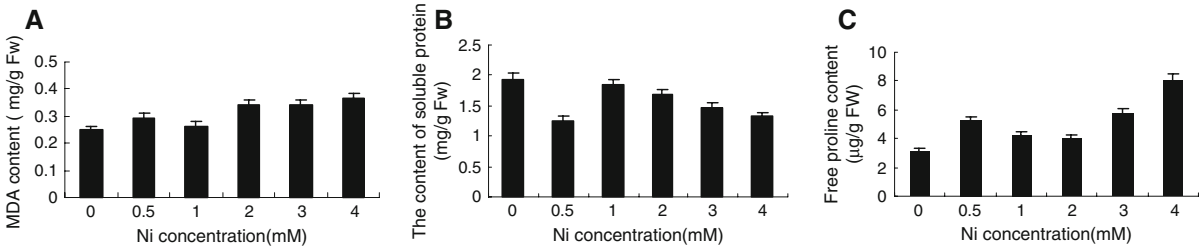

Fig. 4 The content of MDA, proline and soluble protein in *Hydrocharis dubia* leaves under Ni stress

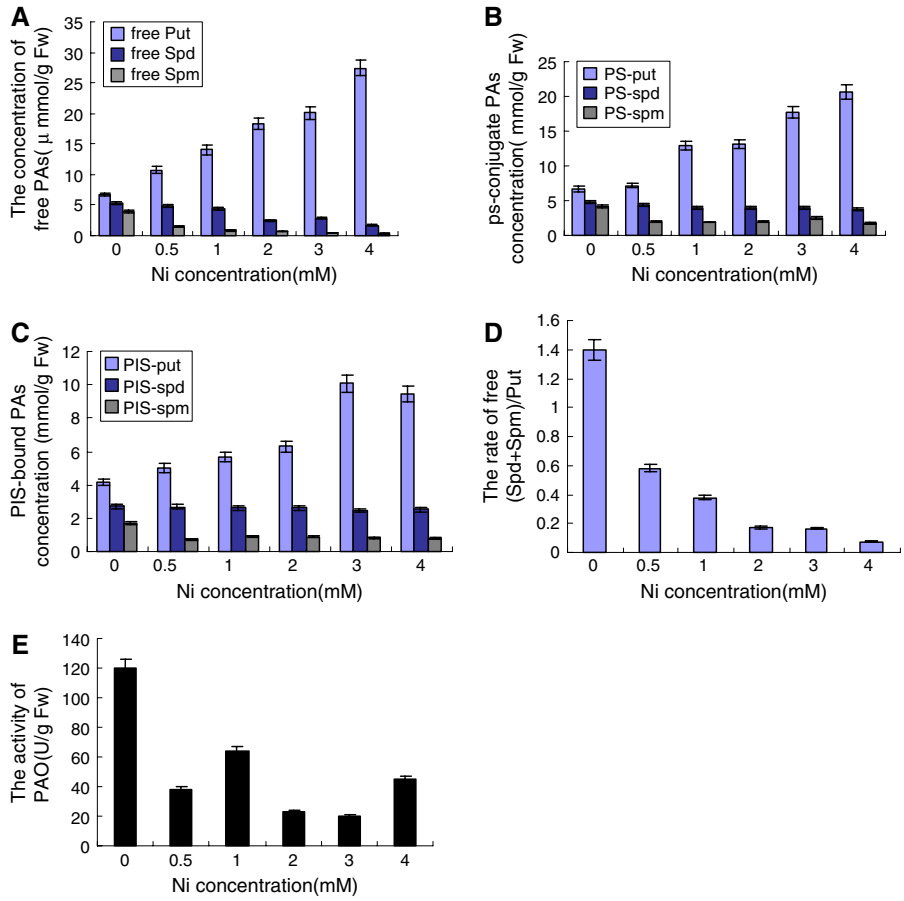

Fig. 5 The content of PAs and the activity of PAO in *Hydrocharis dubia* leaves under Ni stress

4 mM Ni, while the free Put level rose 4.09-fold over the control, indicating a marked difference (Figs. 5a, b). However, the content of PS-conjugated Spd and PS-conjugated Spm was not changed much compared with the control, the minimum of PS-conjugated Spd decreased to 78.89% of the control. And the minimum of PS-conjugated Spm decreased to 43.14% of the control.

The effects of Ni application on PIS-bound PAs were similar to their effects on PS-conjugated PAs in the leaves of *Hydrocharis dubia* (Fig. 5c). The content of PIS-bound Spd ($^{**}P < 0.01$) and Spm ($^{**}P < 0.01$) decreased under Ni stress too, but compared with free PAs they changed much less, indicating the PS-conjugated PAs and PIS-bound PAs were more steady.

As shown in Fig. 5e, the activity of polyamine oxidase(PAO) decreased markedly under gradient concentrations Ni stress compared with the control ($^{**}P < 0.01$). The minimum of PAO appeared in 3 mM Ni, its activity was only 16.67% of the control. That maybe due to Ni destroyed the structure of protein and induced integrant protein decomposed.

Discussion

In the present study, under comparable low-concentration nickel treated (0.5 mM), the content of pigment was higher than leaves of control. The reason is that Ni is a kind of necessary metal for plant growth (Eskew et al. 1983), then Ni may facilitate plant growth under low-concentration of nickel and the photosynthetic pigment was synthesized more. However, treatment of *Hydrocharis dubia* with high concentration Ni resulted in a substantial reduction in frond growth as well as in the appearance of visual symptoms such as chlorosis and necrosis (data not shown), which was induced by that Ni modified a number of physiological processes and particularly chlorophyll degradation. We investigated that when the concentration of Ni rose up to 4 mM, nickel was accumulated up to 948 μg g^{-1} DW and the distinct decrease of pigment content was observed, it was only 67.6% of the leaves of control. Furthermore, Ni treatment significantly increased the levels of Zn ($^{**}P < 0.01$), Ca ($^{*}P < 0.05$). Zn and Ni can replace Mg at the center of chlorophyll molecule, and resulted in inhibit of photosynthesis, so photosynthetic pigment was degradated under higher concentration Ni stress. Besides, Ni reduced chlorophyll by disturbance of chlorophyll biosynthesis or its degradation caused by lipid peroxidation (Davies 1987).

Previous studies indicated that a common feature of both abiotic and biotic stress is the generation of reactive oxygen species (ROS) (Mithufer et al. 2004). Heavy-metal stress affects the normal translocation of electrons, resulting in free-radical production that in turn leads to lipid peroxidation (Atal et al. 1991). Although three protective enzymes harmonize and maintain the stability of membrane system, there is a threshold of enzyme activity and the protective function of SOD, POD and CAT to membrane system is limited (Gu et al. 2002). Our result shows that the protective enzymes of *Hydrocharis dubia* leaves lost their intrinsic balance under treated with nickel. With the increasing of the concentration of nickel, three of the protective enzymes began to drop down, also the ability to restrain the generating rate of $O_2^{\bullet -}$ decreased. Plenty of $O_2^{\bullet -}$ un-eliminated accumulated to high concentration, which induced the damnification of the cell membrane of *Hydrocharis dubia* leaves. The plasma membrane is the biomembrane that encloses the cellular content. It regulates the passage of solutes between the cell and the external environment by selectively absorbing nutrients into the cell against a concentration gradient and preventing the entry of certain solutes present in the environment (Wang et al. 2007).

Cellular PA content changes upon exposure to abiotic stresses (Bouchereau et al. 1999), nevertheless, its physiological relevance remains elusive.

PAs are essential for cellular growth and differentiation (Wallace et al. 2003; Jänne et al. 2004). Deregulation of PA homeostasis may negatively affect cell proliferation and eventually lead to cell death (Wallace et al. 2003; Takao et al. 2006). In the present experiment, Ni increased the Put level and markedly decreased Spd and Spm levels, and these changes were accompanied by the substantial generation of ROS. Although some experiments have shown that Put has the ability to scavenge ROS in vitro (Sharma and Dietz 2006), it should be noted that a mass accumulation of Put is generally considered toxic to plants and eventually leads to apoptotic cell death if its level becomes too high (Panicot et al. 2002; Takao et al. 2006). It has often been suggested that Put and derived PAs (Spd, Spm) may have distinct functions in response to stress. In several plant systems (Drolet et al. 1986; Bar et al. 1996; Benavides et al. 1997) it has been shown that Spd and Spm act as anti-senescent compounds in response to stress, while Put produces either no effects or negative effects, such as depolarization of membranes, potassium leakage, tissue necrosis, and protein loss (Tiburcio et al. 1990). So according to the present experiment, we can draw a conclusion that the decreasing of Spd and Spm, and the increasing of Put, deteriorated characters of Ni stress. Spd and Spm seem to play a key role in preserving the integrity of thylakoid membranes of osmotically stressed oat leaves (Besford et al. 1993), whereas Put

has been reported to cause depolarization of membranes and increase potassium leakage (Tiburcio et al. 1990). Based on these data, the elevation of the free (Spd + Spm)/free Put ratio might be critical the tolerance of stress in plants. That was according with the results by several authors (Bouchereau et al. 1999) about salt and osmotic stress. This result is supported by the significant negative correlation between MDA content and the ratio of free (Spd + Spm)/Put in leaves of *Hydrocharis dubia*, which suggests that decrease of the ratio might be disadvantage to maintaining the structure and function of membranes of *Hydrocharis dubia* under Ni stress. Different results about PAs accumulation in response to stressing agents may be due to plants specific difference and different experimental procedures.

The trend of the change of PIS-bound PAs in response to Ni application was similar to that of PS-conjugated PAs in the leaves of *Hydrocharis dubia* (Fig. 5c), but compared to free PAs they changed gently, indicating the PS-conjugated PAs and PIS-bound PAs were more stable. The three forms of PAs can transform each other, which maybe was the reason that they changed synchronously. In their transformation several kinds of alkaloid come into being in order to detoxification (Suman and James 1997).

We observed drastic and continuous accumulations of Pro or PAs in response to Ni stress, supporting the idea that the synchronous changes are strongly related to cell survival in the long-term salt stress (Giustino et al. 2004). The synchronism in the changes of Pro and PAs also supports the recent suggestion that PAs metabolism might exert a direct or indirect action on Pro biosynthesis (Larher et al. 1998). Some authors (Galston 1997, Santa Cruz et al. 1999) suggested that the regulatory effect could be related to the oxidative stress caused by hydrogen peroxide formed through the oxidation of Put via diamine-oxidase. This regulatory mechanism of PAs on Pro biosynthesis could rely on the well-known relationship between PAs and Pro diverting from glutamate, but other possible indirect actions related to sub-products of the PAs catabolism, such as H_2O_2 and Gamma-aminobutyric acid(GABA), have to be underlined (Galston 1997).

PAO was correlated with PAs metabolization, such as PAs decomposition. Ni stress changed the activity of PAO, which decreased significantly in our study. The decrease of PAO was the dominating reason of the accumulation of Put in *Hydrocharis dubia* leaves. At the same time, Ni stress resulted in significant decreases in Spd and Spm levels in the leaves of *Hydrocharis dubia* concerned with PAs metabolization turbulence. PAO was also concerned with the regulatory mechanism of PAs on Pro biosynthesis and the product of sub-products of the PAs catabolism, such as H_2O_2 and GABA (Galston 1997). But the relationship between PAO and Ni stress require extensive biochemical characterization.

In conclusion, Ni induced the change of polyamines content and physiological and biochemical responses, which indicated it was strongly phytotoxic at high concentrations in *Hydrocharis dubia* leaves. It induced lipid peroxidation, destroyed the structure and functions of membranes, altered the balance of nutrient elements, caused the toxicity of *Hydrocharis dubia.* PAs may be involved in the adaptation of plants to Ni-induced stress.

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