Exogenous abscisic acid alleviates the toxicity of nickel in wheat seedlings

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Abstract. In order to evaluate the effects of exogenous abscisic acid (ABA) in alleviating nickel (Ni) stress in wheat plants. We studied the changes of biochemical and physiological in wheat seedlings exposed to 250 μM Ni with or without different treatments of ABA. Exposed to Ni (250 μM) caused adverse effect on growth of wheat seedlings, which was accompanied by increased the concentrations of superoxide anion（O_2^-） and malondialdehyde (MDA). However, exogenous application of ABA (2.5 and 5 μM) alleviated the Ni-induced inhibition of plant growth, decreased the concentrations of O_2^- and MDA in wheat shoots. Further, application of ABA significantly modulated the activities of antioxidant enzymes and enhanced content of proline and soluble sugar in Ni-stressed wheats, but the application of 20 μM of ABA had no different significantly response for these parameters. The results indicated that application of ABA enhanced the antioxidant defense activities in Ni-stressed wheats, thus alleviating Ni-induced oxidative injury and enhancing Ni tolerance.

1 Introduction

Nickel is a essential plant minerals which is required by plants in order to maintain healthy growth and development but it become extremely toxic in higher concentrations. The accumulation of Ni in plants can cause numerous morphological and physiological changes. At the morphological level, a high concentrations of Ni causes plant growth retardation, chlorosis and wilting [1]. At the physiological level, excess Ni results in an inhibition of chlorophyll synthesis [2], damage to photosynthetic electron transport chain [3], induction of oxidative stress [4], and changes in enzyme activity [5]. Likewise, Ni promotes the accumulation of reactive oxygen species (ROS) causing lipid peroxidation and metabolism imbalance. Plants possess antioxidant systems to scavenge reactive ROS by increasing the activities of key enzymatic and non-enzymatic antioxidants, including superoxide dismutase (SOD), peroxidase (POD) and ascorbic acid [6].

Phytohormones play important roles in the coordination of adaptive responses to environmental stresses. Abscisic acid (ABA) is classified as a sesquiterpene hormone for regulating many plants physiological responses to various abiotic stresses. The level of ABA in plant tissues has been shown to increase upon exposure to environmental stresses such as drought, salt and copper stress [7, 8]. It is reported that exogenous application of ABA influences a range of diverse processes in plants, such as seed germinations, growth and development, photosynthesis, secondary metabolism and gene expression [9,10]. There is also evidence that ABA can ameliorate the damaging effects of chilling and heavy metals [11, 12]. However, to the best of our knowledge, whether exogenous applications of ABA can alleviate Ni-induced oxidative stress in wheat seedlings, has not been reported yet. Therefore, the aim of this study was to examine whether ABA could alleviate the injurious effects of Ni stress and regulate wheat plant growth by regulating the antioxidant enzymes activities and osmolytes involved in stress tolerance.

2 Materials and methods

2.1 Plant growth and treatment

Wheat (*Triticum aestivum* L.) seeds were surface-sterilized with 1 % (w/v) NaClO for 10 min and rinsed thoroughly several times with sterile distilled water, then the seeds were soaked in distilled water for 8 h, the seeds were placed into petri dishes containing three layers gauze moistened with distilled water and germinated for 3 d at 25 ℃ under dark conditions. When the plumule emerged, seedlings were cultivated in a climate-controlled room under the following conditions: 25 ℃, 12/12 h light/dark photoperiod and 65±5 % relative humidity. The seedlings were treated with 0 (control), 50, 100, 250, 500 μM $NiSO_4$ and renews every 2 d. After 4 d, the shoot and root length were measured, and the relevant biomarkers were measured.

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We found that the growth of wheat was clearly inhibited when the concentration of $NiSO_4$ was 250 μM. This dose of $NiSO_4$ was selected for the next phase of the experiment with ABA. The 3 d old seedlings of wheat were pretreated with the ABA (0, 2.5, 5, 10, 20 μM） for 8 h before exposure to 250 μM $NiSO_4$, respectively. The control wheat seedlings were only treated with distilled water. The growth conditions of the wheat seedlings were as described earlier. The treated seedlings were harvested for analysis after 4 d.

2.2 Lipid peroxidation and superoxide anion (O_2^-)

Malondialdehyde (MDA) content was assayed by the method of Thomas et al. [13]. O_2^- was determined as described by Jiang and Zhang [14].

2.3 Proline and soluble sugars

Proline content was measured according to the method of Bates et al. [15]. The contents of soluble sugars was measured with the method described by Chow and Landhausser [16].

2.4 Antioxidant enzyme activities

Fresh samples (0.2 g) were grinded to homogenize in 5.0 mL of 50 mM potassium phosphate buffer (pH 7.8) by an ice–cold mortar and pestle to extract shoot enzymes. The homogenates were centrifuged at 10,000×g for 15 min at 4 ℃,and the supernatants were used for analysing the activity of SOD (EC 1.15.1.1.) [17], POD (EC 1.11.1.7.) [17] and catalase (CAT) (EC 1.11.1.6.) [18].

2.5 Statistical analysis

Statistical analysis was performed using SPSS Version 19.0 software. Data are presented as mean ± standard deviation (SD). Duncan's multiple test were applied to determine the significant difference ($p < 0.05$) among the treatments.

3 Results

As shown in Table 1, compared to the control, there was no significant effects in root and shoot lengths of wheat in 50 and 100 μM Ni treatments. However, the presence of 250 and 500 μM Ni significantly reduced the lengths of root and shoot in wheat seedlings. The lengths of root and shoot decreased by 31.39 and 22.56 % in 250 μM Ni treatments, respectively, relative to the control. The accumulation of MDA was dose dependent in roots treated with 50-500 μM Ni. Meanwhile, we also observed a similar increasing trend in O_2^- contents of roots. However, MDA and O_2^- contents in shoots were no significant effects under 100 μM Ni treatment as compared with the control. In accordance with these results, 250 μM Ni was used as the treatment concentration.

As shown in Table 2, exposure of wheat seedlings to 250 μM Ni significantly decreased root and shoot lengths. However, exogenous application of ABA produced significant changes in plants growth, and only 2.5 and 5μM ABA significantly enhanced root and shoot lengths of wheat seedlings. This effect was even more pronounced for the group with 5 μM ABA; it improved the root and shoot lengths of plants by 27.66 and 19.28 %, respectively, as compared to Ni alone treatment. The results indicated that the alleviative effect by exogenous application of ABA on Ni-induced growth inhibition was more obvious in roots than in shoots. Whereas application with 20 μM ABA reduced significantly shoot growth as compared to Ni alone treatment.

Wheat seedlings were treated with 250 μM Ni for 4 d, the proline level in the shoots increased to 25.85 % of that of the untreated control (Table 2). ABA improved further the level of proline in the shoot of plants in comparison to the control. In contrast, 5 μM ABA had the greatest effect on proline level, which increased by 16.04 % in shoot tissues than that of Ni alone treatment. In addition, the contents of soluble sugar was significantly decreased by Ni treatment as compared to the control. After application of different concentrations ABA, the contents of soluble sugar in shoots were significantly increased by 26.06, 62.29, 18.81 and 21.70 %, respectively, as compared to the treatment of Ni alone (Table 2).

The contents of MDA and O_2^- significantly increased in the shoots under Ni stress, about increased by 79.17 % and 140.21 % than that of control, respectively (Table 2). ABA reduced the accumulation of MDA and O_2^- to varying extents in wheat seedlings. Especially, ABA at 5μM had the greatest effect, and MDA and O_2^- contents decreased by 20.83 and 32.92 % in shoots, respectively, as compared to the treatment of Ni alone (Table 2).

Table1. Effects of Ni on growth and oxidative stress in wheat seedlings. Values show the means of four replicates ± SD. The different lower case represents statistically differences between treatments ($p < 0.05$).

Ni μM	Length (cm)		MDA (nmol g^{-1} FW)		O_2^- (nmol min^{-1} g^{-1} FW)	
	Root	Shoot	Root	Shoot	Root	Shoot
0	7.84 ± 0.50a	8.32 ± 0.47a	5.19 ± 0.96d	5.32 ± 0.91c	3.52 ± 0.46d	3.19 ± 0.61c
50	8.03 ± 0.62a	8.72 ± 0.39a	5.20 ± 0.61d	4.98 ± 0.72c	3.47 ± 0.49d	3.07 ± 0.41c
100	7.22 ± 0.39a	8.11 ± 0.37a	6.98 ± 0.44c	5.98 ± 0.77c	4.74 ± 0.46c	4.31 ± 0.66c
250	5.37 ± 0.35b	6.44 ± 0.46b	10.59 ± 0.93b	9.81 ± 0.42b	7.13 ± 0.58b	7.61 ± 0.59b
500	3.03 ± 0.52c	3.17 ± 0.35c	14.60 ± 0.43a	31.18 ± 0.91a	9.50 ± 0.61a	10.42 ± 1.04a

Table2. Effects of ABA on root and shoot lengths, proline, soluble sugar, malondialdehyde (MDA) and superoxide anion (O_2^-) of wheat seedlings under Ni stress. Values show the means of four replicates ± SD. Different lower case represents statistically significant differences between treatments ($p < 0.05$).

Treatments/ Parameters	Root length (cm)	Shoot length (cm)	Proline (μg g^{-1} FW)	Soluble sugar (mg g^{-1} FW)	MDA(nmol g^{-1} FW)	O_2^- (nmol min^{-1} g^{-1} FW)
Control	8.16 ± 0.54a	8.99 ± 0.54a	17.76 ± 1.10e	21.69 ± 1.04b	5.57 ± 0.99c	3.25 ± 0.42e
Ni（250μM）	5.48 ± 0.42c	6.49 ± 0.43c	22.35 ± 0.54cd	15.25 ± 1.25d	9.98 ± 0.55a	7.81 ± 0.33a
Ni + ABA (2.5)	6.40 ± 0.49b	7.25 ± 0.19b	24.55 ± 0.66ab	19.22 ± 1.19c	7.96 ± 0.26b	5.45 ± 0.31d
Ni + ABA (5)	6.99 ± 0.61b	7.75 ± 0.33b	25.93 ± 1.06a	24.74 ± 1.22a	7.89 ± 0.89b	5.24 ± 0.37d
Ni + ABA (10)	5.38 ± 0.32c	6.33 ± 0.54c	23.19 ± 0.37bc	18.12 ± 1.29c	9.59 ± 0.73a	6.13 ± 0.16c
Ni + ABA (20)	5.18 ± 0.27c	5.29 ± 0.23d	21.16 ± 0.71d	18.55 ± 0.77c	10.30 ± 0.93a	7.45 ± 0.29a

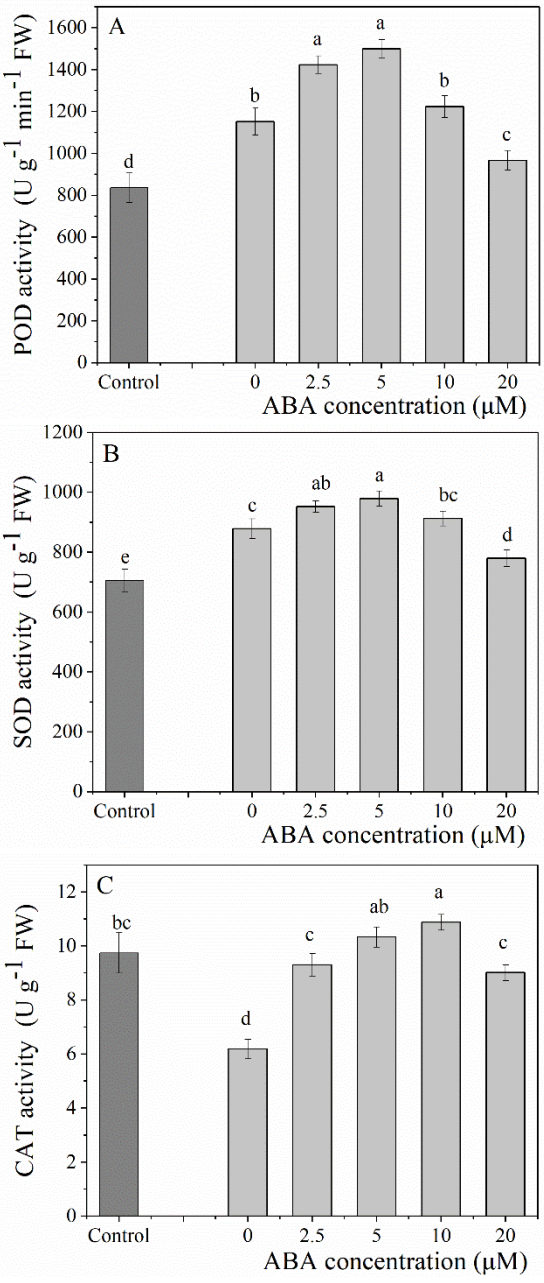

Figure 1. Effects of ABA on POD (A), SOD (B), CAT (C) in shoots of wheat seedlings under Ni stress. Bars (n = 4) showing the different letters are significantly different at $p < 0.05$ as determined by Duncan analyze.

The activities of SOD and POD were found to be increased under Ni toxicity. In contrast, the application of ABA (2.5 and 5 μM) enhanced further in the activities of these enzymes under Ni stress in comparison to the treatment of Ni alone. (Fig. 1A-B). Nevertheless, the CAT activity was decreased in Ni-stressed wheats, but the application of ABA (2.5-20 μM) overcame the adverse effects of Ni-stress, causing a notable increase in the activity of CAT of shoots tissues (Fig. 1C).

4 Discussion

In the present study, the symptoms of Ni toxicity on wheat plants are growth inhibition at the very early stage of their development. Shoot and root growth in wheat plants were decreased significantly when Ni concentrations reached 250 μM (Table 1). However, we found that exogenous ABA was able to ameliorate the toxic effect of Ni on the growth of wheat seedlings. Inhibition of growth in wheat seedlings might result from Ni-caused alterations of metabolic and biochemical processes [19]. The contents of MDA and O_2^- significantly increased at 250 and 500 μM Ni in both shoots and roots of plants (Table 1). These results showed that the oxidative stress and the peroxidation of membrane lipids were caused by Ni stress. Previous studies have demonstrated that MDA content can be induced in wheat contaminated with heavy metal such as Ni, Cu and Cd [20, 21].

Previous studies have demonstrated that ABA has great potential to enhance the tolerance to certain stresses including drought, saline–alkaline in different plants [22, 23]. Generally, low concentrations of ABA can improve plant resistance to adverse environments, and high concentrations of ABA can cause high levels of oxidative stress, leading to a decreased resistance to environmental stress [14, 24]. In this study, exogenous application of low concentrations of ABA (2.5 and 5 μM) significantly alleviated the deleterious effects of Ni on plant growth (Table 2). Previously, it was stated that ABA application alleviated the copper induced growth retardation in *Artemisia annua* [25]. However, this mitigation effect gradually reduced with increasing concentrations of ABA, and 20 μM ABA even inhibited even shoot growth.

In comparison to control plants, the content of proline was improved under Ni stress both by ABA treatment and treatment free plants (Table 2). The proline accumulation is essential to maintain osmotic adjustment under Ni stress.

It has been proposed that proline plays an important role in the antioxidative properties of heavy metal-stressed [26]. Additionally, Ni-stressed plants exhibited a significant reduction in soluble surgar. Exogenous application of ABA led to the increase in soluble surgar in shoots of plants (Table 2). Soluble surgar are known to contribute to the osmotic adjustment [24], thus high level of soluble surgar is beneficial for enhanced tolerance of the plants under Ni stress. This suggested that ABA treatment could elevate proline and soluble surgar to withstand Ni-induced injury.

Our results showed that the growth of wheat plants were decreased significantly under Ni (250 μM) stress, accompanying with the significant increase the contents of O_2^- and MDA (Table 2). Exogenous application of ABA reversed this adverse effect of Ni, causing a significant decrease in the O_2^- and MDA contents. Moreover, Ni toxicity in plants may be attributed to increasing lipid peroxidation leading to oxidative damage [27]. To prevent the Ni-induced oxidative damage on plants, plants have antioxidant defense mechanisms. In the present study, the activity of POD was increased in response to only Ni-stressed treatment and continually increase was observed by low concentrations of ABA treatment plus Ni. (Fig.1A). Similar trend was found for SOD (Fig. 1B). SOD catalyzes the conversion of O_2^- to O_2 and H_2O_2 [28]. Notably, results showed the content of O_2^- was at ABA treatment plus Ni lower than that of Ni alone, thus ABA enhanced the antioxidative system to scavenge the accumulation of O_2^-. In addition, Ni stress caused marked decrease in the activity of CAT, whereas ABA treatment induced a significant increase in the activity of CAT in shoots of plants (Fig. 1C) This is supported by the study of Jiang et al [29], who observed that ABA pretreatment enhanced the actions of SOD, CAT and ascorbate peroxidase enzymes in *Zea mays* seedling under abiotic stress. In fact, the results indicate that the increase in the activities of the antioxidant enzymes were not sufficient to protect cell membrane against Ni toxicity. However, this oxidative damage was alleviated by ABA treatment (Table 2). The application of ABA elevated enzymatic antioxidants in shoots of wheats, hence leading to alleviation of the oxidative damage as indicated by the lowered O_2^- and MDA levels.

5 Conclusion

In summary, ABA may attenuate Ni toxicity in wheat seedling exposed to Ni stress, which probably includes the regulation of the antioxidant system and the improvement of osmotic adjustment. This suggests that an appropriate concentrations ABA could be used as a potential growth regulator to alleviate Ni induced toxicity in plants.

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